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Irritant Contact Dermatitis Activity of some xanthones from *Swertia petiolata* D. Don

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ABSTRACT

Irritant contact dermatitis (ICD) of some xanthones from *Swertia petiolata* D. Don was investigated. Four xanthones, i.e., compounds 1-4 (swertianolin, norswertianin, bellidifolin and swertianin) were isolated from the n-butanol extract of methanol infusion of *Swertia petiolata*, purified by chromatography and re-crystallization and identified by comparative spectroscopic data. ICD was evaluated by open mouse ear assay and estimated by ID_{50}. Four of the xanthones exhibited mild ICD activities, compared with positive controlled euphorbium. Compound-3 (swertianin) and compound-4 (swertianolin) exhibited a little more intense reaction than other two isolated compounds (norswertianin and bellidifolin) with ID_{50} = 0.524 and 0.784 respectively.

Four xanthones (norswertianin, bellidifolin, swertianin and swertianolin) were isolated from n-butanol extract of methanol infusion of *Swertia petiolata*. These compounds had mild ICD action on mice's ears.

Keywords: Irritant contact dermatitis; xanthones; *Swertia petiolata*; ID_{50}.

INTRODUCTION

Many species of plant genus *Swertia* (Family–Gentianaceae) are widely distributed in the Himalayan region[1]. In China and in Northern India about 20 *Swertia* species had been utilized as canonical remedies for the treatment of hepatitis, pneumonia, vermifuge, dysentery, scabies, hypoglycemia and muscular spasm[2, 3]. Medicinal usage of many *Swertia* species including *Swertia petiolata* had been described in Indian

pharmaceutical codex, the British and the American pharmacopoeias[4]. *Swertia petiolata* was also known as *Ophelia ciliata* or *O. purpurascens* var. *ciliata* G. Don and commonly called 'Angulated Swertia' or 'Ciliated Ophelia'[1-4].

In Indo-Pak regions, *Swertia petiolata* had been used as canonical remedy for fever, asthma, liver disorders, anemia, as laxative and for hypoglycemia[1, 2, 4, 5] Many research workers found that the pharmacological and biological activities were due to xanthone types of phytochemical compounds, present in various *Swertia* species[6,7]. Antimicrobial[8, 9], antiviral[10], antifungal[11], antiprotozoal[12], hypoglycaemic[13], anti-inflammatory and analgesic[14-17] activities of these species had also been investigated.

The development about naturally-occurring xanthones had been outlined by Vieira and Kijjoa[18]. Few alkaloids, about 21 irridoid secoiridoid glycosides, primarily known as angustiamarins, angustiosides, nervosides and vegelosides along with lignans and lactones had also been separated and characterized[19]. Many *Swertia* species contained sawertiamarine, mangeferin[20-22], bitter secoiridoid glucosides, 'amaroswerin' and 'amaro-gentin'[23], xanthone glycoside (1,5-dihydroxy-3-methoxyxanthone-8-O-b-D-glucopyrano-side), 1,5,8-tri-hydroxy-3-methoxy-xanthone[24], 1-hydroxy-2,3,5,7-tetramethoxyxanthone, 1-hydroxy-3,5,8-trimethoxyxanthone, 1-hydroxyl-2,3,4,6-tetramethoxyxanthone, 1-hydr-oxy-2,3,4,7-tetramethoxyxanthone, 1,8-di-hydroxy-3,5-dimethoxyxanthone, 1,7-dihydr-oxy-3,8-dimethoxyxanthone, 1,3,5,8-tetrahydroxyxanthone, balanophonin, oleanolic acid, maslinic acid and sumaresinolic acid[25-27].

A number of *Swertia* species including *Swertia petiolata* have been found as weeds in wide area of Pakistan, including its northern regions[5]. The plants in these regions, grew

stronger during the harvesting season of other cereal crops and sometime cause a mild to severe irritation / allergic reaction on the hands and arms of local farmers during their removal from the crop fields.

Contact dermatitis (CD) is often an acute or chronic inflammation of skin induced by contact with certain chemical or physical agents. It is broadly categorized as either irritant contact dermatitis (ICD) or allergic contact dermatitis (ACD) [28, 29]. ICD is frequently caused by exposure to weak irritants such as soap, detergents and some plants and plant products. Many plants exhibited an effective defensive mechanism through CD and it indicated many clinical problem[29-31]. ICD is perceived by direct injury of the skin epidermal cells which activates the acquired immune system. Thus, giving rise to an inflammatory cutaneous reaction due to the external stimuli[29-32]. ICD from plants or plant products takes place through various mechanisms[31]. Many plants could provoke injuries by some mechanical means e.g., due to the presence of prickles, spines or thorns while other plants are capable of evoking an irritant skin reaction due to some irritant phytochemical compounds[31]. Such compounds vary from weak irritants, which involves a repeated exposure or some kind of skin abrasion to show their effects to the intense irritants which could provoke an inflamed reaction in microgram quantities (e.g., spurge, a resin present in various *Euphorbia* spp., of family 'Euphorbiaceae') [31]. ACD, on the other hand is characterized by a Type IV delayed immune response to an allergen[31]. It is very difficult to distinguish two types of dermatoses on the bases of clinical observations only[28-31]. Dermatitis could be a result after contact with living or processed plant materials and seemed in the patient at once or after some time of contact[29-32]. ICD is influenced by both the intrinsic (genetic) and extrinsic

(environmental) factors, both of which are significant in the pathogenesis of ICD, specially of the hand and arm dermatitis. For diagnosing and managing the ICD, the irritants (chemicals), their concentration, duration and amount of exposure must be considered. The skin eruptions due to ICD is well-define, typically confined to the area of contact with the irritant[29-33]. The mechanism of action of ICD will probably direct the understanding biochemistry of inflammation and pain production in mammalian skin[30]. Clinical display of irritant contact dermatitis is frequently of polymorphic nature, varying from simple dryness, cracking and hyperkeratosis of the skin to the inflammatory reaction, accompanied sometime with oedema, erythema, papules and vesicles[30, 31]. If the ICD is very strong, e.g., as with spurge (The resin present in various *Euphorbia* spp., of family Euphorbiaceae), there might be bullae, superficial necrosis or ulceration, along with pain[30, 31].

In spite of wide utilization of *S. petiolata* in folk medicine, in Pakistan no attempt had been made to evaluate any unhealthy effects of its phytochemical constituents on human beings or on animals. In the present communication, we report some of its irritant constituents extracted from different solvent extracts and evaluated on albino mice, followed by fractionation to isolate and characterize its active compounds, whose effectiveness was further evaluated by ID_{50}.

METHODOLOGY

General Experimental Procedures

Unless otherwise stated, the chemicals used were of analytical grades. Concentrations were carried out under reduced pressure at bath temperatures not exceeding 50°C. Melting points were determinated on Perfit apparatus with the

help of open capillary tubing and were uncorrected. UV spectra were measured on Hitachi-270-30 spectrophotometer in MeOH and IR spectra of the isolated compounds were obtained as KBr disc or as thin film on NaCl discs on a Pye-Unicam SP-8-400 spectrophotometer. ^{1}HNMR spectra were obtained in deuterated DMSO-d_6 solvent on Bruker NMR at 270 MHz using tetramethylsilane (TMS) as an internal standard. ^{13}CNMR spectra were carried out on Bruker AM-300 NMR, spectrometers with 75 MHz at 27±1.5°C and with 0.2-0.5 mM/ml sample concentrations, using 10 mm tubes and deuterated DMSO-d_6 as a solvent. Tetramethylsilane (TMS) was used as an internal reference. Chemical shifts were calculated for both ^{1}HNMR and ^{13}CNMR spectra in δ (ppm). EI and FD mass spectra were recorded on a Varian MAT-312 double focusing mass spectrometer using direct inlet method. FAB (positive) in glycerin, were carried on JEOL JMS-110 spectrometer. Column chromatography was conducted on silica gel 60 (70-230 mesh ASTM No. 7734 of E. Merck, Damstadt, Germany), monitoring its fractions by analytical TLC. The analytical and preparatory TLC were performed with silica gel $PF_{254+366}$ (from E. Merck, Damstadt Germany) on 10×20 or 20×20cm glass plates. Analytical TLC with 0.25 mm and preparatory TLC with 0.75 mm thickness was utilized, where the samples were applied as thin sports on analytical TLC and as narrow bands on preparatory TLC. Spots on chromatographs were visualized by a combination of UV fluorescence (with 254/365 nm UV light), I_2 vapors or ceric sulphate. {$Ce(SO_4)_2$ in conc. H_2SO_4 }[34]. The separated bands on preparatory TLC were scraped off and eluded with methanol.

Plant materials

5.45 kg of whole *Swertia petiolata* D. Don plants were collected from the waste and uncultivated areas of Hazara and Abbottabad at an altitude of nearly 100 to 900 meters in July / August 2019. These were authenticated by Prof. Dr. Zaheer-ud-Khan, Incharge herbarium, Department of Botany, Government College University, Lahore, Pakistan. A voucher specimen of the sample No. **P-cog. 0155** had been retained in the Herbarium Pharmacognosy Section, Faculty of Pharmacy, University of Central Punjab,Lahore. The whole plant material was dried in air at laboratory temperature. Aerial parts of the plant were separated, pulverized and stored in umbered glass bottles.

Extraction and isolation

5.20 kg of pulverized aerial parts of *Swertia petiolata* was extracted with methanol and the filtrate was dried to a residue (4.26kg). The dried residue was partitioned between light petroleum ether (40-60^{o}C), chloroform, EtOAc and n-butanol. Each solvent extract was concentrated under reduced pressure. 200g of the butanol extract was further subjected to column chromatography. It was incorporated with minimum amount of silica gel and pulverized. It was adsorbed on silica gel column and chromatographed in chloroform. The elution was carried out with increasing polarity of the column chromatographic system. It was increased slowly by increasing the amount of methanol in chloroform (Such as 95:5, 90:8, 90:10, 85:15, 80:20, 80:25, 70:30 v/v etc.). Based on the analytical TLC behavior, the column fractions with similar compounds were pooled. Pooled fractions were dried under reduced pressure.

Isolation of Compound-1.

Compound-1 was eluded from the silica gel column with $CHCl_3$/MeOH (95:5) after accumulating initial 60 fractions (50ml each) and by preparatory TLC (with $CHCl_3$/MeOH–90:10). It was obtained as yellow needles (85 mg, with 0.042% yield) after re-crystallization from hot CH_2Cl_2/MeOH (50:50). It had m.p. 335–336^oC. This compound appeared as a single spot on three-dimensional, three directional TLC (with $CHCl_3$/ MeOH–70:30, 80:20 and 90:10 used as developing solvent systems). HRMS: m/z: 260.0321 (mol. formula; $C_{13}H_8O_6$). EIMS: m/z (rel. int. %): 261 [M^++1] (16), 260 [M^+], (100), 231 (11), 203 (10), 186 (7), 152 (6), 116 (15), 79 (18), 69 (23). UV λ_{max} = 204, 239, 265 and 332nm. IR bands: 3452 (-OH), 3291 (-C-H), 1660 (ketonic -C=O), 1620, 1586 (C=C), 1472, 1282, 1142 (-C-O-), 826 and 804 cm^{-1}. ^{1}HNMR. δ: 6.24 (1H, d, J = 2.2H_z, H-2), 6.36 (1H, d, J = 2.2H_z, H-4), 6.84 (1H, d, J = 8.9H_z, H-5), 7.26 (1H, d, J = 8.8H_z, H-6), 9.35 (1H, s, OH at C-3), 11.16 (1H, s, OH at C-7), 11.71 (1H, s, OH at C-1 or C-8), 11.89 (1H, s, OH at C-1 or C-8). ^{13}CNMR. δ: 161.89 (C-1), 97.94 (C-2), 166.15 (C-3), 93.84 (C-4), 157.61 (C-4a), 105.72(C-5), 146.87(C-5a), 123.74 (C-6), 140.18 (C-7), 147.74 (C-8), 107.21(C-8a), 183.67 (C-9), 100.62 (C-9a) (Fig. 1). Compound-1 was identified after comparing its spectral data with the reported data and with CAS ID = 22172-15-2 as norswertianin[35, 36].

Isolation of Compound-2.

Compound-2 was eluded from the column with $CHCl_3$/MeOH (90:8) with further 61 to 92 fractions (50ml each) and by preparatory TLC (with $CHCl_3$/MeOH – 90:10). 1.51g (with 0.755% yield) of compound-2 was obtained as very light yellow crystalline mass

after re-crystallization from hot acetone/ethanol (50:50). It had m.p. 263–264°C. It appeared on TLC at hRf = 35 (with $CHCl_3$/MeOH–90:10) and gave single spot on three-dimensional, three directional TLC (with $CHCl_3$/MeOH – 70:35, 80:20 and 90:10 used as developing solvent systems). HRMS: m/z: 274.0477 (mol. formula; $C_{14}H_{10}O_6$). FDMS m/z: 274 [M^+]. EIMS: m/z (rel. int. %): 275 [M^++1] (19), 274 [M^+], (100), 245 (16), 231 (15), 217 (8), 203 (7), 137 (9), 123 (16), 69 (12). UV λ_{max} = 204, 256, 280, 332 and 391 nm. IR bands: 3452 (OH), 3302, 3254, 1654 (ketonic -C=O), 1626, 1612, 1592 (C=C), 1500, 1396, 1280, 1154 (-C-O-), 992 cm^{-1}. ^{1}HNMR. δ: 3.88 (3H, s, Ar-OMe), 6.40 (1H, d, J =2.4 Hz, H-2), 6.60 (1H, d, J = 2.5 Hz, H-4), 6.64 (1H, d, J = 8.6 Hz, H-7), 7.24 (1H, d, J = 8.4 Hz, H-6), 9.65 (1H, s, OH at C-5), 11.06 (1H, s, OH at C-1 or C-8), 11.91 (1H, s, OH at C-1 or C-8). ^{13}CNMR. δ: 162.65 (C-1), 97.12 (C-2), 166.25 (C-3), 92.15 (C-4), 156.38 (C-4a), 140.94 (C-5), 145.06 (C-5a), 121.10 (C-6), 112.42 (C-7), 149.34 (C-8), 111.90 (C-8a), 180.89 (C-9), 103.54 (C-9a), 56.00 (CH_3) (Fig. 1). Compound-2 was identified after comparing its spectral data with the reported data and with CAS ID = 2798-25-6 as bellidifolin[37].

Isolation of Compound-3.

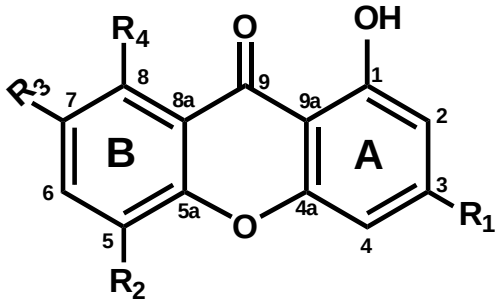
Compound-3 was eluded from the column with $CHCl_3$/MeOH (85:15) with further 93 to 124 fractions (50ml each) and by preparatory TLC, using $CHCl_3$/MeOH (80:20) as solvent system. 0.95g (yield = 0.475%). Light yellowish needles was obtained from hot MeOH. It had m.p. 220.5–221°C. This compound appeared on TLC at hRf = 43 ($CHCl_3$/ MeOH–80:20). This compound came out as single spot on three-dimensional, three directional TLC (with $CHCl_3$/MeOH – 60:35, 70:20 and 85:10, used as

developing solvent systems). HRMS: m/z: 274.0477 (mol. formula; $C_{14}H_{10}O_6$). FDMS m/z: 274 [M^+]. EIMS, m/z (rel. int. %): 275 [M^++1] (18), 274 [M^+], (100), 245 (18), 231 (11), 136 (6), 123 (11). UV λ_{max}= 238, 269 and 328 nm. IR bands: 3450 (OH), 3321, 3210, 1664 (ketonic -C=O), 1646, 1610, 1586 (-C=C), 1510, 1472, 1326, 1286, 1208 (-C-O-), 1171, 1156, 1092, 1062, 966 cm^{-1}. ^{1}HNMR. δ: 3.87 (3H, s, Ar-OMe), 6.36 (1H, d, J = 2.2 H_z, H-2), 6.58 (1H, d, J = 2.2 H_z, H-4), 6.80 (1H, d, J = 8.9 H_z, H-5), 7.26 (1H, d, J = 8.8 H_z, H-6), 9.38 (1H, s, OH at C-7), 11.62 (1H, s, OH at C-1 or C-8), 11.86 (1H, s, OH at C-1 or C-8). ^{13}CNMR. δ: 161.82 (C-1), 97.08 (C-2), 166.98 (C-3), 92.64 (C-4), 157.64 (C-4a), 105.82 (C-5), 147.05 (C-5a), 124.07 (C-6), 140.43 (C-7), 147.93 (C-8), 107.28 (C-8a), 184.15 (C-9), 101.65 (C-9a), 56.09 (CH_3) (Fig. 1). Compound-3 was identified after comparing its spectral data with the reported data and with CAS ID = 20882-75-1 as swertianin[37, 38].

Isolation of Compound-4.

Compound-4 was eluded from the column with $CHCl_3$/MeOH (80:20) with further 125 to 157 fractions (50ml each) and by preparatory TLC ($CHCl_3$/MeOH – 75:25). 1.91g (yield = 0.955%) of compound-4 was obtained. It came out as a pale yellow needles from hot CH_2Cl_2/MeOH – 25:75 mixture. It had m.p. 204.5–204ºC. Compound-4 appeared on TLC at hRf = 43 ($CHCl_3$/MeOH – 80:20). It gave a single spot on three-dimensional, three directional TLC (with $CHCl_3$/MeOH – 60:35, 70:20 and 85:10 used as developing solvent systems). HRMS: m/z: 274.0477 (mol. formula; $C_{14}H_{10}O_6$). FAB (positive) m/z: 437 [M^++1]. EIMS: m/z (rel. int. %): 274 [M^+-162)] (100), 273 (18), 259 (9), 245 (29), 244 (8), 231 (9), 217 (7), 203 (11), 152 (6), 137 (19), 123 (13), 60

(11). NOESY (H-H interactions), δ: 4.81 (H-1'), -3.42 (H-2'), 3.34 (H-5'), 7.12 (H-7), 3.42 (H-2'), -3.25 (H-3'), 3.52 (H-6'), 4.80 (H-1'), 7.12 (H-7), 7.26 (H-6), 3.86 (H; OCH_3), -6.36 (H-2), 6.57 (H-4), 7.12 (H-7), -7.26 (H-6). UV λ_{max} = 253, 276 and 326 nm. IR bands: 3450 (OH), 3210 (-C-H), 1656 (ketonic -C=O), 1612, 1576 (-C=C), 1495, 1310, 1281, 1162 (-C-O-) and 1082 cm^{-1}. ^{1}HNMR, δ: 3.18-3.52 (m, sugar-H), 3.16 (1H, dt, J = 5.3, 8.7 H_z, H-4'), 3.89 (3H, s, Ar-OMe), 4.81 (1H, d, J = 7.6 H_z, glu, H-1'; anomeric), 5.091 (1H, s, glu-OH at C-3'), 5.046 (1H, s, glu-OH at C-2'), 5.046 (1H, s, glu-OH at C-4'), 5.028 (1H s, glu-OH at C-6'), 6.36 (1H, d, J = 2.5 H_z, H-2), 6.58 (1H, d, J = 2.4 H_z, H-4), 7.12 (1H, d, J = 8.6 H_z, H-7), 7.26 (1H, d, J = 8.9 H_z, H-6), 10.06 (1H, s, OH at C-5), 13.08 (1H, s, OH at C-1). ^{13}CNMR, δ: 162.67 (C-1), 97.14 (C-2), 166.25 (C-3), 92.16 (C-4), 156.38 (C-4a), 140.96 (C-5), 145.10 (C-5a), 121.12 (C-6), 112.44 (C-7), 149.36 (C-8), 111.92 (C-8a), 180.84 (C-9), 103.56 (C-9a), 56.02 (CH_3), 103.20 (C-1'), 73.47 (C-2'), 76.06 (C-3'), 69.82 (C-4'), 77.35 (C-5'), 60.84 (C-6') (Fig. 1). The compound-4 was identified after comparison of their spectra data with the reported data and with CAS No = 23445-00-3 as swertianolin[35, 39, 40].



Compound = 1. $R_1 = R_3 = R_4 = OH$ $R_2 = H$

Compound = 2. $R_1 = OMe$ $R_2 = R_4 = OH$ $R_3 = H$

Compound = 3. $R_1 = OMe$ $R_2 = H$ $R_3 = R_4 = OH$

Compound = 4. $R_1 = OMe$ $R_2 = OH$ $R_3 = H$ $R_4 =$

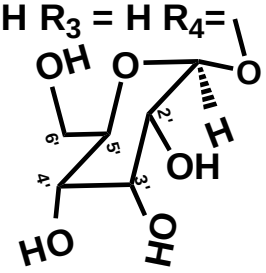


Fig. 1. Compounds isolated from aerial parts of *Swertia petiolata* D.Don

Animals

Albino mice weighing 5 to 10 g's were obtained from the Drug Testing Laboratory, Jail Road Lahore, Pakistan. The animals were housed in plastic cages on wooden shavings in an animal house in PCSIR Laboratories, Ferozepure Road Lahore. Eight or twelve mice were managed per cage in a laminar air flow room maintained at a temperature 29±1.5°C and relative humidity 45±3.2%. Palette food and de-ionized water were available *ad libitum*.

Irritant Contact Dermatitis Activity

10 mg of the test compound was dissolved in 10 ml of acetone to prepare 10 mg/10 ml (w/v) solution. It was further diluted according to the method of Evans and Schmidt[41] and Kinghorn and Evans[42]. Ten dilutions were prepared for the main assay. The main procedure for evaluating the irritant contact dermatitis on mouse ears had also taken up from Evans and Schmidt[41] and Kinghorn and Evans[42].

For the main assay, groups of 12 animals (n = 12) were used for each dilution. 5µl of one of the dilutions of testing compound was applied to the inner surface of one of the mouse's ear with the help of Drummond Microcaps (Drummond Scientific Co. USA). The other mouse's ear was kept as such, without testing material and considered as negative control. Nine other successive dilutions of the testing compound were employed similarly for other groups of animals. 10mg/10ml of euphorbium (a resin from *Euphorbia helioscopia*) [41, 42] in acetone was applied to another group of 12 animals as a positive control. Euphorbium was made chromatographically purified by column chromatography before use.

Each ear of the animal in experimental and in a positive controlled group was observed for redness after 30 minutes, then after every 15 minute intervals until two successive observations revealed that any further inflammation would not occur. Time of maximum redness was also detected. Number of ears elicited the degree of redness agreed with at least ++ intensity on Hecker's scale at peak irritancy[43] were detected and expressed in µg/10µl per ear. The animal's ears were also examined after 24, 48 and 72 hours to find out any persistent irritant effect of the test compounds. Number of red ears with at least ++ strength (denoted by IU — Irritant units on Hecker scale) [43] were recorded. If no redness was noticed, the procedure was repeated with higher

concentrations of the test solution on ears of another group of animals. Total number of red ears per dilution of the test compound were tabulated. ID_{50} (Irritant doses in 50 % individuals) were calculated and analyzed by probit analysis[44], using a computer program[45]. Number of inflamed mice ears induced by the isolated compounds and euphorbium (positive control), their ID_{50}, χ^2, time of ++ irritant reaction, upper and lower confident limits have been outlined in Table- 2.

Table 1. Irritant contact dermatitis (ICD) reaction of various solvent extracts from aerial parts of *Swertia petiolata* on mice ears.

Extracts	**Pet. ether**	**$CHCl_3$**	**EtOAc**	**n-butanol**	**Euph.**
ICD res.	±±	±±	±±	++	+++

Where:—

ICD res. = Irritant contact dermatitis response; Pet. ether = Petroleum ether (40-60^oC); $CHCl_3$ = Chloroform; EtOAc = Ethyl acetate; Euph. = Euphorbium.

n = 8 for each extract.

±± = Doubtful irritant reaction with very light and diffused inflammation.

++ = Prominent erythema (Irritant Units) [43]

+++ = Strong erythema with small vesicles and dense inflammation.

Table 2. Mice with positive irritant responses after testing with compounds-1 to -4.

Dose levels (µg/5µl)	Compound-1	Compound-2	Compound-3	Compound-4	Euphorbium
10	11*/12†	9/12	10/12	11/12	12/12
5.0	10/12	8/12	9/12	9/12	12/12
2.50	9/12	7/12	9/12	8/12	12/12
1.25	7/12	6/12	8/12	6/12	12/12
0.625	5/12	5/12	6/12	5/12	9/12
0.3125	3/12	3/12	5/12	4/12	8/12
0.15625	2/12	2/12	4/12	4/12	7/12
0.078125	1/12	1/12	3/12	2/12	6/12
0.0390625	1/12	0/12	2/12	1/12	3/12
0.0195312	0/12	——	1/12	0/12	1/12
µg/5 µl	0.857	1.583	0.524	0.784	0.088
S. D.	0.077	0.134	0.127	0.104	0.096

ID_{50}	χ^2	0.734	0.924	0.529	3.291	2.847
	t	2.5h	3.0h	1.5h	1.0h	20 min
	U.C.L	1.519	3.385	1.153	1.475	0.176
	L.C.L.	0.615	0.854	0.246	0.456	0.054
IU after	24 h	2.5	5	2.5	2.5	1.25
	48 h	5	⟩10	5	5	2.5

Where:—

Compound-1 = Norswertianin; Compound-2 = Bellidifolin; Compound-3 = Swertianin;

Compound-4 = Swertianolin. * = Number of animal ears reacted to the irritant compound.

† = Total number of animals' ears used; ID_{50} = Irritant dose in 50 % individuals;

S.D. = Standard deviation; χ^2 = Chi square; t = Time for maximum irritant reaction;

IU = Irritant units. U. C. L. = Upper confident limits; L.C.L. = Lower confident limits;

h = hours after application.

n = 12 for each dilution of the compound.

RESULTS AND DISCUSSION

Swertia petiolata as wild plants grown in crop fields, induced a mild to severe irritation on the hands and arms of indigenous farmers during their removal. Skins of the dorsal sides of hands and arms were involved and papular inflammatory eruptions were developed after about 20 to 24 hours. Such skin eruption was possibly due to some exudes from the plant. Skin eruption cleared up after about ten or eleven days. This type of reaction motivated us to carry out an investigation about the chemical nature of its harmful active compounds.

During preliminary irritancy assay on mice's ears, it was observed that the MeOH extract of aerial parts of *Swertia petiolata* was only the material which indicated an irritant reaction than other solvent extracts. MeOH extract was further fractionated into light petroleum ether (40-60^{o}C), $CHCl_3$, EtOAc and n-butanol extracts. The n-butanol extract demonstrated a positive irritant reaction than doubtful false irritant reactions of light petroleum ether (40-60^{o}C), $CHCl_3$ and EtOAc extracts (number of animal used for each extract was 8, i.e., n = 8) (Table-1).

The n-butanol extract was therefore further processed for column chromatography to isolate its active ingredient/s. Four of the principal compounds were isolated. These were purified by preparatory chromatographic and re-crystallization. The structures of these compounds were laid down by comparing their physical and spectral data with similar compounds [7, 35-40]. Their spectral data were based on EIMS, FDMS, HRMS, ^{1}HNMR and ^{13}CNMR assignments. These compounds were identified as xanthones (Fig.

1.).

Solvent extracts of plants were frequently a complex mixture of diverse phytochemical compounds. Some of them were able to cause irritant dermatitis and acted on human being and animal's skins by different mechanisms with different duration of action and effectivity[41, 42]. Mice ear tests were known to be good for assessing such reaction[41-43]. For comparing irritant contact dermatitis activity of xanthones from *S. petiolata*, the number of inflamed mice's ears were counted at the time of their peak irritancy. (Total number of animals used for each dilution of the isolated compound was 12, i.e., n = 12). The data was analyzed by a computer program[45] which enable us to compare the effectiveness of isolated xanthones, by means of ID_{50} that gave ample confidence because their limits were placed on upper and lower confident levels, along with standard deviation. Standard deviation also calculated the slop of probit regression line which suggested the overall 'shape' of tolerance curve[44]. The aim of χ^2 test was to find out whether the results of the assay after transformation, were suitably represented by the probit regression line[44].

All the isolated xanthones exhibited a mild irritant contact effects on the mice's ears when compared with the euphorbium reaction. Among all the four weak irritant xanthones, compound-3 (swertianin) and compound-4 (swertianolin) pointed out a little more intense reaction than two other compounds with ID_{50} = 0.524 and 0.784 respectively (Table 2).

In these compound there are probably three sites of action that were liable to be attached with the proteins of animal skin and displayed a mild irritation reaction within a short time. First site of action was probably the double bonded oxygen atom exocyclic to the middle ring (Fig. 1). Second sites of action were likely to be the presence of double bonds in 'A' and 'B' rings of the molecules. (Double bonds in these compounds were shown by strong UV absorption at λ_{max} 269nm for compound-3 and at λ_{max} 253nm for compound-4). The third sites of action were possibly the presence of 'OH' (that appeared in ^{1}HNMR at δ 9.38, 11.62 and 11.86 ppm for compound-3 and at δ 10.06 and 13.08 ppm for compound-4) while 'OCH_3' groups were indicated by ^{1}HNMR signals at δ 3.87 ppm for compound-3 and at 3.89 ppm along with NOESY at δ 3.86 ppm for compound-4 (Fig.1). Moreover, the compound-4 also possesses a sugar molecule whose ^{1}HNMR signals appeared at δ 3.18 to 3.52, 4.81, 5.091, 5.046 and 5.028 ppm. The sugar moieties in the molecule probably help for quick penetration in the animal skin [36, 38]. It probably takes a little less time for the appearance of irritant contact symptoms (Table-2).

Compound-1 (norswertianin) also demonstrated a mild contact irritant reaction with ID_{50} = 0.857 when compared with euphorbium. The molecule possess four exocyclic 'OH' moieties as revealed by ^{1}HNMR signals at δ 9.35, 11.16, 11.71 and 11.89 ppm along with the presence of double bonds in the molecules that were indicated by strong UV absorption at λ_{max} 239, which were probably the reactive sites and after penetration in animal skin, it was likely to form some kind of adduct with skin protein that resulted in a mild irritant reaction. Compound-2 (bellidifolin), on the other hand possesses three

reactive 'OH' groups as indicated by ^{1}HNMR signals at δ 9.65, 11.06 and 11.91 ppm and one 'OCH_3' moiety shown at δ 3.88 along with the double bonds in the molecule, demonstrated by strong UV absorption at λ_{max} 256nm that were probability the reactive sites in the molecule for skin proteins. This interaction constituted some kind of adduct and exhibited a mild irritant reaction (Table-2). The results further pointed out that the mild irritant reaction of these xanthones was continuous up to 24 or 48 hours (Table-2).

Although the mechanism of this activity was not clearly demonstrable, yet this phenomenon might occur due to different binding affinities of these xanthones to the active sites of animal's skin receptors. Moreover, the hyper-pigmentation of the skin was not observed. Probably its reason was that we had used only that much amounts of these compounds which at least gave ++ reaction on Hecker's scale[43]. Since, little quantities of these compounds were available to the animal skin, hyper-pigmentation was not visible.

We concluded that *Swertia petiolata* herb contained xanthones that exhibited not only mild acute irritant contact dermatitis activity but also a chronic persistent mild action. The low and repeated doses of these compounds, with controlled clinical conditions might lead to an anti-inflammatory and sedative action, as it was designated in folk medicines [1,2, 4-7]. For such purposes, oral administration of these xanthones in low and repeated doses might be a safer route than topical application. More work is needed to amplify this property, through the preparation of their derivatives that would possible

be lead to the structure-activity relationship of such important naturally originated molecules.

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