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Antioxidants, anti-proliferative, anti-inflammatory, anti-diabetic and anti-microbial effects of isolated compounds from *Swertia corymbosa* (Grieb.) Wight *ex* C.B. Clark – An *in vitro* approach

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Abstract

The present study, antioxidant, enzyme inhibitory, anti-inflammatory and antimicrobial activity of isolated compounds such as Decussatin (**1**), Gentiacaulein (**2**), Swertianin (**3**), 1,8-dihydroxy-2,6-dimethoxy xanthone (methylswertianin) (**4**) 8-hydroxy-1,2,4,6-tetramethoxyxanthone (**5**) and 1,2-dihydroxy-6-methoxyxanthone-8-O-β-D-xylopyranosyl (**6**) were investigated using an *in vitro* model. Results of antioxidant studies revealed that the compound **6** possessed an efficient 2,2-diphenyl-1-picryl-hydrazyl ($DPPH^{\bullet}$) (IC_{50} 07.19 ± 4.56 μmol/mL), 2,2-azinobis (3-ethylbenzothiozoline-6-sulfonic acid) ($ABTS^{\bullet+}$) (42.62 ± 0.25 mmol/L TE/g), superoxide (57.89 ± 3.45 μmol/mL), nitric oxide (18.45 ± 1.23 μmol/mL) and hydroxyl (12.13 ± 2.76 μmol/mL) radical scavenging activities, ferric reducing antioxidant power (14.76 ± 0.10 molar Fe (II)/g), metal chelating (213.85 ± 27.18 mg EDTA/g) ability. Compounds **6** and **3** exhibited significant anti-proliferative activity. Compound **6** displayed strongest antibacterial activity against *Streptococcus pneumoniae* and *Escherichia coli* with MIC value of 3.90 μg/mL and 21.21 ± 0.25 and 20.27 ± 0.11 mm zone of inhibition at 25 μg/mL concentration respectively. In the membrane stabilization and protein denaturation test **3** was the most potent with an IC_{50} value of 12.57, 18.75 μmol/mL respectively.

Keywords: *Swertia corymbosa*; Antioxidant; *In vitro* anti-diabetic; Anti-inflammatory; Anti-bacterial

1. Introduction

Natural compounds of plants are derived from the occurrence of biodiversity. Plants produce these compounds to enhance their survival and competitiveness. Due to their biological activities, many plant-derived compounds are used for the treatment of human illnesses or diseases. A World Health Organization (WHO) survey indicated that about 70–80% of the world's population relies on traditional medicine based mainly on plant materials for their primary healthcare [1]. Even though phenolic compounds are ubiquitous and rich in medicinal herbs and dietary plants, these compounds have gained much attention due to their antioxidant activities and use in medical practice as anti-inflammatory, choleretic, anti-tumoral and other agents [2–4]. Clinical evidence has demonstrated that chemoprevention by plant-derived phenolic compounds is an inexpensive, readily applicable, acceptable and accessible approach to various disease control and management [5]. The unprecedented structures of these molecules make them excellent synthetic targets and their potent activity against a broad number of therapeutic indications makes these natural products excellent drug lead candidates for new therapeutics [6].

Abbreviations: DPPH, 2,2-diphenyl-1-picrylhydrazyl; DMRT, Duncan's Multiple Range Test; BHT, butylated hydroxytoluene; ABTS, 2,2′azinobis (3-ethylbenzothiozoline-6-sulfonic acid) diammonium salt; TPTZ, 2,4,6-tripyridyl-S-triazine; EDTA, ethylenediamine tetraacetic acid; NMR, nuclear magnetic resonance; MTT, 3-(4,5-dimethylthiazol)-2,5-diphenyl tetrazolium bromide; HMBC, Heteronuclear Multiple Bond Correlation; TLC, Thin layer chromatography; ATCC, American type culture collection; MIC, minimum inhibition concentration; CFU, colony forming unit; SEM, standard error of measurement; HSQC, heteronuclear single quantum coherence; IC_{50}, half maximal inhibitory concentration; ANOVA, analysis of variance; TE, Trolox equivalents.

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Swertia corymbosa (Griseb.) Wight *ex* C.B. Clarke is a well known medicinal plant and all parts of this plant have been employed for the treatment of diarrhea, fever, jaundice, diabetic, inflammation and nervous disorders in Indian traditional systems of medicine. Medicinal value especially anti-diabetic property of *S. corymbosa* is well recorded in traditional literature [7]. In Yunani system, *S. corymbosa* is used as astringent tonic, stomachic, lessens inflammation, sedative to pregnant uterus and chronic fevers [7]. Recently pharmacological studies showed that its extensive biological activities such as antioxidant, anti-proliferative, anti-inflammatory, anti-diabetic, anti-convulsant, sedative, anxiolytic and anti-microbial activities of the extracts [7–12]. Phytochemical studies revealed the presence of alkaloid, flavonoids, phenols, xanthones which represented the main active components in *S. corymbosa* aerial parts [7]. Our recently published study showed that the xanthones in *S. corymbosa* display anti-inflammatory anti-nociceptive and anti-diabetic activities, but there has been very little information on antioxidant and medicinal properties of isolated xanthones from *S. corymbosa*. Hence, the present study attempts to investigate the antioxidant, anti-proliferative, anti-microbial, anti-inflammatoryand type II diabetic related enzyme inhibition properties of the isolated compounds using *in vitro* methods.

2. Materials and methods

2.1. Chemicals and reagents

All the antioxidant, anti-diabetic, antimicrobial chemicals and standards were obtained from Hi-media (Mumbai, India) and Sigma–Aldrich (St. Louis, MO, USA). Petroleum ether, chloroform, ethyl acetate and all the culture media were purchase from Hi-media. All other reagents used were of analytical grade.

2.2. Plant material and isolation

The aerial parts of *S. corymbosa* were collected from Vellingiri hills. The plantmaterial was authenticated by Dr. R. Ramachandran, Professor, Department of Botany, Bharathiar University, Coimbatore. Vouchers Specimen (No: 006144) of the plant material is deposited at the Department herbarium center, Department of Botany, Bharathiar University. The extraction and isolation of 1-hydroxy-3,7,8-trimethoxyxanthone (**1**), 1,7-dihydroxy-3,8-dimethoxyxanthone (**2**) and 1,2,8-trihydroxy-6 methoxy xanthone (**3**), 1,8-dihydroxy-2,6-dimethoxyxanthone (**4**), 8-hydroxy-1,2,4,6-tetramethoxyxanthone (**5**) and 1,2-dihydroxy-6-methoxyxanthone-8-O-β-D-xylopyranosyl (**6**) from *S. corymbosa* was performed as described in our previous study [8].

2.3. Antioxidant activity

2.3.1. Radical scavenging activity using DPPH$^{\bullet}$ method

The antioxidant activity of the compounds was determined in terms of hydrogen donating or radical scavenging ability, using the stable radical 2,2-diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$), according to the method of Blois [13]. A methanol solution of the sample compounds at various concentrations was added to 5 mL of 0.1 mmol/L methanolic solution of DPPH$^{\bullet}$ and allowed to stand for 20 min at 27 °C. The absorbance of the sample was measured at 517 nm. Methanol was served as blank and solution without compounds served as control. The percentage of free radical scavenging capacity of the compound was calculated and expressed as IC_{50} value. BHT and BHA were used as a standard antioxidant.

2.3.2. ABTS radical scavenging activity

The total antioxidant activity of the samples was measured by ABTS (2,2′-azinobis (3-ethylbenzothiozoline)-6-sulfonic acid) radical cation decolourization assay according to the method of Re et al. [14]. ABTS$^{\bullet+}$ was produced by reacting 7 mmol/L ABTS aqueous solution with 2.4 mmol/L potassium persulfate in the dark for 12–16 h at room temperature. Prior to assay, this solution was diluted in ethanol (about 1:89, v/v) and equilibrated at 30 °C to give an absorbance of 0.700 ± 0.02 at 734 nm. The stock solution of the sample compounds were diluted such that after introduction of 10 μL aliquots into the assay, they produced between 20 and 80% inhibition of the blank absorbance. After the addition of 1 mL of diluted ABTS solution to 10 μL of sample or Trolox (final concentration 0–15 μmol/L) in ethanol, absorbance was measured at 30 °C exactly 30 min after the initial mixing. Triplicate determinations were made at each dilution of the standard and the percentage inhibition was calculated against the blank (ethanol) absorbance at 734 nm and then was plotted as a function of Trolox concentration. The unit of total antioxidant activity (TAA) is defined as the concentration of Trolox having equivalent antioxidant activity expressed as mM/g sample compound.

2.3.3. Superoxide radical ($O_2^{\bullet-}$) scavenging activity

The ability to inhibit formazan formation by scavenging superoxide radicals by the compounds was studied by the method of Beauchamp and Fridovich [15]. Each 3 mL reaction mixture (50 mmol/L sodium phosphate buffer (pH 7.6), 20 μg riboflavin and 12 mmol/L EDTA and 0.1 mg NBT) with compounds in each test tube was illuminated for 90 s. Illuminated reaction mixture served as negative control, while the mixture without compounds in dark was taken as blank. Immediately after illumination, the absorbance was measured at 590 nm. The activity was compared to BHT and BHA. The percentage inhibition of superoxide anion generation was calculated and expressed as IC_{50} values.

2.3.4. Nitric oxide scavenging activity

The assay is based on the principle that sodium nitroprusside in aqueous solution at neutral pH spontaneously generates nitric oxide, which in turn reacts with oxygen to produce nitrite ions that can be estimated using Greiss reagent. Scavengers of nitric oxide compete with oxygen molecules that result in reduced production of nitrite ions. In the experiment, sodium nitroprusside (10 mmol/L) in phosphate buffered saline was mixed with different concentrations of samples/standard followed by incubation at room temperature for 150 min. The same reaction

mixture without the sample/standard served as the control. After the incubation period, 0.5 mL of Griess reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% N-(1-naphthyl) ethylenediamine HCl) was added and the absorbance of the formed chromophore by the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethylene-diamine dihydrochloride is measured spectrophotometrically at 546 nm [16]. The percent scavenging activity of the nitric oxide generated was measured by comparing the absorbance values of control and test preparations and the results were expressed in terms of IC_{50} values. BHA was used as a reference standard.

2.3.5. Ferric reducing antioxidant power (FRAP) assay

The antioxidant capacities of different compounds of samples were estimated according to the procedure described by Pulido et al. [17]. FRAP reagent (900 μL), prepared freshly and incubated at 37 °C, was mixed with 90 μL of distilled water and 30 μL of test sample or methanol (for the reagent blank). The test samples and reagent blank were incubated at 37 °C for 30 min in a water bath. The final dilution of the test sample in the reaction mixture was 1/34. The FRAP reagent was prepared by mixing 2.5 mL of 20 mmol/L TPTZ (2,4,6-tripyridyl-*s*-triazine) in 40 mmol/L HCl (Hydrochloric acid), 2.5 mL of 20 mmol/L $FeCl_3{\cdot}6H_2O$ and 25 mL of 0.3 M Acetate buffer (pH 3.6). At the end of incubation, the absorbance readings were taken immediately at 593 nm against the reagent blank using a spectrophotometer. Methanolic solutions of known Fe (II) concentration, ranging from 100 to 2000 μmol/L ($FeSO_4{\cdot}7H_2O$) were used for the preparation of the calibration curve. The parameter equivalent concentration was defined as the concentration of antioxidant having a ferric-TPTZ reducing ability equivalent to that of 1 mmol/L $FeSO_4{\cdot}7H_2O$. Equivalent concentration was calculated as the concentration of antioxidant giving an absorbance increase in the FRAP assay equivalent to the theoretical absorbance value of 1 mol/L concentration of Fe (II) solution.

2.3.6. Metal ion chelating activity

The chelating activity of compounds was determined by the method of Dinis et al. [18]. 800 μL of samples were added to 100 μL solution of 2 mmol/L $FeCl_2$. The reaction was initiated by the addition of 400 μL of 5 mmol/L ferrozine and incubated at room temperature for 10 min. Absorbance of the sample was then measured spectrophotometrically at 562 nm against the water (blank). The metal chelating capacities of the compounds were expressed as mg EDTA equivalents/g compound.

2.3.7. The inhibition of lipid peroxidation

A modified TBA reactive species assay by Banerjee et al. [19] was used to measure the lipid peroxide formed, using egg-yolk homogenates as lipid-rich media Ruberto et al. [20]. Malondialdehyde, a secondary product of the oxidation of polyunsaturated fatty acids, reacts with two molecules of TBA, yielding a pinkish red chromogen with an absorbance maximum at 532 nm. Egg homogenate (0.5 mL 10% in distilled water, v/v) and different concentrations of the test solution was mixed in a test tube and the volume was made up to 1 mL by adding distilled water. Finally, 0.05 mL $FeSO_4$ (0.07 mol/L) was added to the above mixture and incubated for 30 min, to induce lipid peroxidation. Thereafter, 1.5 mL of 20% acetic acid (pH adjusted to 3.5 with NaOH) and 1.5 mL of 0.8% TBA (w/v) (prepared in 1.1% SDS) and 0.05 mL 20% TCA were added, vortexed and then heated in boiling water bath for 60 min. After cooling, 5.0 mL of 1-butanol was added to each tube and centrifuged at 3000 r per minute for 10 min. The absorbance of the organic upper layer was measured at 532 nm:

$$\%\text{Inhibition} = 100\% \times (A_0 - A_s)/A_0$$

where A_0 is the absorbance of the control, and A_s is the absorbance of the tested sample.

2.3.8. Hydroxyl radical scavenging activity

The scavenging activity for hydroxyl radicals was measured according to the method [21] with slight modification. Reaction mixture contained 0.5 mL of 7.5 mmol/L $FeSO_4$, 0.5 mL 7.5 mmol/L 1,10-phenathroline and 2.5 mL of 0.2 mol/L phosphate buffer (pH 7.8), 0.5 mL of 30 mmol/L H_2O_2 and different concentration of test solution. The reaction was started by adding H_2O_2. After incubation at room temperature for 5 min the absorbance of the mixture at 536 nm was measured. Hydroxyl radical scavenging activity (%) = 100 × (*A* 562 (control) − *A* 562 (sample)/*A* (562 control)) and expressed in IC_{50} value.

2.4. Anti-proliferative activity

2.4.1. Cell lines and culture

The human cervical cancercell line (HeLa) HeLa (cervical), hepatocellular carcinoma (HepG2), neuroblastoma (IMR-32) and NIH 3T3 mouse embryonic fibroblasts were obtained from National Center for Cell Science (NCCS), Pune. The cancerous cell lines were maintained in Dulbecco's Modified Eagle's Medium (Sigma Aldrich Inc., USA) supplemented with 10% fetal bovine serum (Gibco BRL., USA) in a CO_2 incubator.

2.4.2. MTT assay

For screening experiment, the cells were seeded into 96 well plates with 100 μL of respective medium containing 10% FBS, at plating density of 10,000 cells/well and incubated at 37 °C, 5% CO_2, 95% air, and 100% relative humidity for 24 h prior to addition of compound. The compound was solubilized in DMSO and diluted in the respective serum free medium. After 24 h, 100 μL of the medium, containing the compound and camptothecin as standard at various concentrations (6.25, 12.50, 25, 50 and 100 μmol/L) was added and incubated at 37 °C, 5% CO_2, 95% air and 100% relative humidity for 48 h. Triplicate was maintained and the medium without compound served as negative control. After incubation, 15 μL of MTT (5 mg/mL) in PBS was added to each well and incubated at 37 °C for 4 h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100 μL of DMSO and then the absorbance measured at 570 nm using micro plate reader [22]. The % cell inhibition was determined using the following

formula and graph was plotted between % cell inhibition and concentration as follows:

%Cell viability = test absorbance/control absorbance × 100

IC_{50} (inhibition concentration) values were defined as the concentrations of compound required to reduce the absorbance to 50% of the control values.

2.5. Antimicrobial activity

2.5.1. Test bacteria

The antibacterial activity of isolated compounds 1–6 were evaluated against 6 pathogenic bacteria such as *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 29213), *Streptococcus pneumoniae* (ATCC 33400) *Klebsiella pneumoniae* (ATCC 10031) and *Bacillus subtilis* (ATCC 6633) procured from the Institute of Microbial Technology (IMTECH) Chandigarh, India. All the strains were stored in the appropriate medium before use.

2.5.2. Disc diffusion method

Disc diffusion method [23] was used for the evaluation of antibacterial activity of isolated compounds using 100 μL of suspension containing 10^8 CFU/mL of bacteria spread on the inoculated agar. A sterile cotton swab was dipped into the inoculums suspension to remove the excess of fluid. Whatmann filter paper discs (6 mm diameter) were prepared at the concentration of 25 μg/disc for isolated compounds and 10 μg/disc reference antibiotic (Ciprofloxacin). A disc prepared with only the corresponding volume of DMSO was used as negative control. The petriplates were then incubated and antimicrobial activity was evaluated by measuring the diameter of the zones of inhibition around the disc. The experiments were repeated in triplicate and the results were expressed as average values.

2.5.3. Minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of isolated compounds of *S. corymbosa* was determined using the microdilution assay in 96 well micro-plates [24]. Briefly, five hundred microlitres of each resuspended sample (1.0 mg/mL) in DMSO (2%). Serial two-fold dilutions were prepared from the stock solution to give concentrations ranging from 500 μg to 3.90 μg/mL of the isolated compounds for bacteria. The highest concentration of DMSO remaining after dilution (5%, v/v) caused no inhibition to bacterial growth. DMSO served as negative control. Streptomycin and Ciprofloxacin were served as positive controls. An aliquot of 100 μL standardized suspension of the test bacteria (10^8 CFU/mL) was transferred to a well of 96 plates. Then, 100 μL diluted samples were also added to each well and incubated at 37 °C for 24 h. The MIC was defined as the lowest concentration of samples which inhibited the visible growth of tested microorganisms. For further reconfirmation, 20 μL of MTT reagent (1 mg/mL) was added as an indicator for microbial growth to each well of the microtiter plates, followed by 20 min incubation at 37 °C. The reagent-suspension color will remain clear or yellowish indicating complete inhibition activity as opposed to dark blue for growth [25]. The MIC was recorded as the most repeatable minimum concentration of triplicate.

2.6. In vitro anti-inflammatory activity

2.6.1. Protein denaturation assay

The reaction mixture consisted of 2 mL of different concentration (6.25, 12.50, 25, 50 and 100 μmol/L) of isolated compounds **1–6** or standards diclofenac sodium and 2.8 mL phosphate buffer saline (pH 6.3) was mixed with 2 mL of egg albumin (form fresh hen's egg) and incubated at 37 °C for 15 min. Denaturation was induced by keeping the reaction mixture at 70 °C in a water bath for 10 min. After cooling, turbidity was measured spectrophotometrically 660 nm (Shimadzu, UV 1800). For negative control 2 mL of egg albumin and 2.8 mL phosphate buffer saline (pH 6.3) were used. Each experiment was done in triplicate and the average was taken. The percentage inhibition of protein denaturation was calculated by using the following formula [26]:

%Inhibition = absorbance of test sample

− absorbance of control/absorbance of control × 100.

The compound/standard drug concentration for 50% inhibition (IC_{50}) was determinate by plotting percentage inhibition with respect to control against treatment concentration.

2.6.2. Membrane stabilization assay

The principle concentrated in this method is stabilization of goat red blood cell membrane by hypo tonicity induced membrane lyses. Blood was collected 2 mL from healthy goat and was mixed with equal volume of sterilized Alsever's solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl in distilled water) and centrifuged at 3000 r per minute. The packed cells were washed with isosaline and a suspension in 10% (v/v). Reaction mixture (4.5 mL) contains 1 mL phosphate buffer, 2 mL hyposaline, 1 mL of different concentration of isolated compounds **1–6** (6.25, 12.50, 25 50 and 100 μmol/mL) and 0.5 mL RBC (Red blood cells) suspension. Diclofenac sodium was used as the reference drug. The reaction mixture was served as a control. The assay mixtures were incubated at 37 °C for 30 min and centrifuged at 3000 r per minute for 20 min and the supernatant solution was estimated using UV analysis at 560 nm. The percent GRBC membrane stabilization activity was calculated by the formula [27]:

% Inhibition = Control − Sample/Control × 100.

2.7. In vitro anti-diabetic activity

2.7.1. Inhibition assay for α-glucosidase

The inhibition of α-glucosidase assay is a modification of the method previously described by Kim et al. [28]. Five microlitre of the α-glucosidase solution (10 units/mL in 0.1 mol/L potassium phosphate buffer, pH 6.8) was mixed with 250 mL of the different compounds (12.5–62.5 μmol/mL) was mixed with

620 mL of 0.1 mol/L potassium phosphate buffer (pH 6.8). After 20 min of incubation at 37 °C, 10 mL of p-nitrophenyl glucopyranoside (pNPG, 10 mmol/L) was added and the mixture was re-incubated at 37 °C in a water bath for 30 min. The reaction was terminated by the addition of 650 mL of Na_2CO_3 solution (1 mol/L). Aliquots (0.3 mL) were withdrawn and mixed with distilled water (4.7 mL). The amount of released product (p-nitrophenol) was measured at 410 nm using a UV spectrophotometer to estimate the enzymatic activity. The IC_{50} value was defined as the concentration of the compound required to inhibit 50% of the α-glucosidase activity under the assay conditions:

$$\alpha\text{-Glucosidase inhibitory activity}(\%) = (A - B)/A \times 100$$

where *A* was the optical density of the reaction blank. The reaction blank mixture contained the same volume of the buffer solution instead of the sample; *B* was the optical density of the reaction in the presence of both α-glucosidase and compound sample.

2.7.2. Inhibition assay for α-amylase activity

α-Amylase inhibitory activity was assayed according to the procedure described by Wang et al. [29] with a slight modification. α-Amylase activity was determined using soluble starch(1%) as a substrate in 0.05 mol/L sodium phosphate buffer(pH 6.8) containing 1 mmol/L $CaCl_2$. Briefly, 400 μL of α-amylase solution (20 U/mL) was mixed with1.0 mL of sodium phosphate buffer (pH 6.8) and 200 μL of the different compounds (12.5–62.5 mg/mL). After incubation at 37 °C for10 min, 300 μL of the starch solution (1%) was added and the mixture re-incubated at 37 °C for 20 min. The reaction was terminated by adding 0.2 mL of dinitrosalicylic acid solution and boiled for 10 min in a boiling water bath. The total volume was made up to 8.0 mL with distilled water. The absorbance was measured at 540 nm against a control containing a buffer solution in place of the enzyme solution. Acarbose was used as a positive control. The assay was performed in triplicate. Inhibition (%) was calculated using the equation for α-glucosidase assay.

2.8. Statistical analysis

The results were expressed as mean ± SD and statistical analysis was carried out by analysis of variance (ANOVA) followed by Duncan's multiple tests. $P < 0.05$ was considered as indicative of significance as compared to the control group. All calculations were performed using SPSS version17.0 (SPSS Inc., Chicago, Illinois, USA).

3. Results

3.1. Structures of isolated compounds

The structure of 1-hydroxy-3,7,8-trimethoxyxanthone (**1**), 1,7-dihydroxy-3,8-dimethoxyxanthone(**2**) and 1,2,8-trihydroxy-6 methoxy xanthone (**3**), 1,8-dihydroxy-2,6-dimethoxyxanthone (**4**), 8-hydroxy-1,2,4,6-tetramethoxyxanthone (**5**) and 1,2-dihydroxy-6-methoxyxanthone-8-O-β-*D*-xylopyranosyl (**6**) (Fig. 1A) were previously reported [8,30].

3.2. Antioxidant capacity of the compounds

The bioactive compounds **1**–**6** obtained from petroleum ether and ethyl acetate extracts of *S. corymbosa* was tested for possible antioxidant activity by several complementary test systems namely DPPH, ABTS, OH, Nitric oxide radical, Super oxide radical scavenging activity, FRAP, metal chelating and lipid peroxidation inhibition.

3.2.1. DPPH radical scavenging activity

DPPH, a relatively stable organic radical with a characteristic strong absorbance band at 517 nm in visible spectroscopy (deep violet color) is used to evaluate the free radical scavenging ability of the investigated sample. The best known natural and synthetic antioxidant standards, *Viz.*, BHA and BHT were used as positive control for comparison. $DPPH^{\bullet}$ radical scavenging activity is a measure of non-enzymatic antioxidant activity. When an antioxidant scavenges the $DPPH^{\bullet}$ free radical by donating hydrogen, the purple color of the DPPH solution becomes light yellow. The decrease in the absorbance was taken as a measure of the extent of radical scavenging activity. A lower IC_{50} value corresponds with the higher antioxidant power.

In the present study, all the samples were able to interact intensively with DPPH and reduced the stable violet DPPH radical to the yellow DPPH-H, reaching their 50% reductive plateau ranging between 07.19 ± 4.56 and 91.12 ± 3.54 μmol/mL for compounds **1**–**6**. The reference antioxidant BHA recorded the highest scavenging efficiency toward DPPH radicals (25.58 ± 1.65 μmol/mL), followed by BHT (30.56 ± 2.90 μmol/mL). Among the isolated compounds (**1**–**6**) examined, the compound **3** (11.37 ± 3.76 μmol/mL) and compound **6** (07.19 ± 4.56 μmol/mL) possessed effective DPPH radical quenching capacity with their IC_{50} value less than 12 μmol/mL. Interestingly, these values were significantly lower ($P < 0.05$) that the standard antioxidants tested (Table 1) indicating their superior radical scavenging potential.

3.2.2. $ABTS^{\bullet+}$ Scavenging activity

The Trolox equivalent antioxidant capacity (TEAC) was measured using the improved ABTS radical cation decolorization assay. The decolorization of the ABTS cation radical is an anabiguous way to measure the TEAC of the test drugs. Since, TEAC is a measurement of the effective antioxidant activity of the compound; a higher TEAC value would imply greater antioxidant activity of the sample. This assay was calibrated with water-soluble α-tocophenol analog, Trolox. In the evaluation of the total antioxidant capacity by $ABTS^{\bullet+}$ method, all the tested compounds were able to quench $ABTS^{\bullet+}$ radical more efficiently with their TEAC values ranging between 12.14 ± 0.34and 42.62 ± 0.25 mmol/L Trolox equivalent/g compound (Table 1). In this context, the compound **6** and **3** were found to be best and potent scavenging of $ABTS^{\bullet+}$ radicals, as they were able to quench $ABTS^{\bullet+}$ radicals more readily than the other compound tested. The antioxidant activity

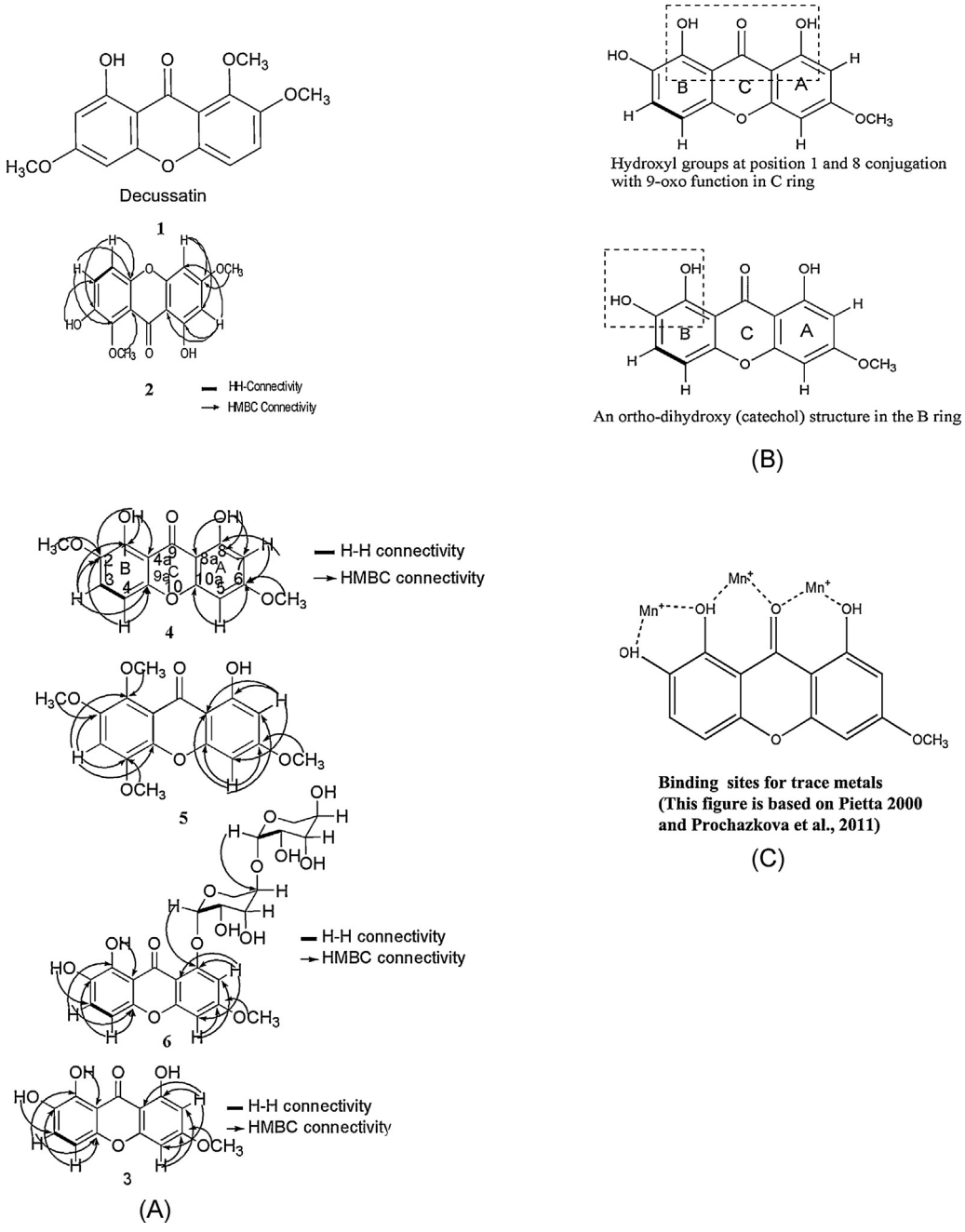

Fig. 1. Chemical structure of isolated compounds **1**–**6** from *Swertia corymbosa*. (A) HMBC and HH connectivity of the isolated compounds. (B) Hydroxyl groups and ortho-hydroxyl group of compound **3**. (C) Metal chelating activity (binding site of compound **3)** (proposal based on Pietta, 2000 [50] and Prochazkova et al., 2011 [51]).

for the compounds observed in the TEAC system is as follows **6** > **3** > **4** > **2** > **1** > **5** > BHA. Compound **5** and synthetic antioxidant standard BHA recorded the least activity among the sample investigated.

3.2.3. Hydroxyl radical scavenging activity

Hydroxyl radical is one of the most potent oxidants among the reactive oxygen species and is considered to be the initiator of lipid peroxidation. It induces several damage to the adjacent molecules and can be generated in biological cell through the Fenton reaction. The scavenging activity was dependent on the concentration. The same effects of the compound were observed in DPPH and ABTS scavenging activities. The IC_{50} valves were calculated for compound **6** (12.13 ± 2.76 μmol/mL) and compound **3** (25.13 ± 3.66 μmol/mL) revealed effective OH radical quenching capacity. Interestingly, these values were significantly lower ($P < 0.05$) than the standard antioxidants tested (Table 1) indicating their superior radical scavenging potential.

3.2.4. Nitric oxide radical scavenging activity

Nitric oxide (NO) is one of the most widespread signaling molecules and participates virtually in every cellular and organ function of the body. It is also a free radical with a single

Table 1
Antioxidant activity of isolated compounds (1–6) from *S. corymbosa*.

Compounds	DPPH IC_{50} (μmol/L)	TAA (mmol/L Trolox/g)	$O_2^{\bullet-}$ IC_{50} (μmol/L)	$OH^{\bullet}$ IC_{50} (μmol/L)	NO IC_{50} (μmol/L)	Lipid peroxidation IC_{50} (μM)	Metal chelating activity (mg EDTA/g)	FRAP (M Fe (II)/g)
1	76.55 ± 1.43^{f}	13.43 ± 0.38^{e}	171.24 ± 3.29^{f}	121.80 ± 1.20^{f}	89.19 ± 2.54^{e}	67.43 ± 2.99^{e}	40.34 ± 08.92^{f}	2.47 ± 0.15^{d}
2	57.12 ± 1.23^{e}	15.89 ± 0.31^{d}	105.11 ± 1.26^{d}	87.65 ± 2.18^{d}	35.19 ± 2.61^{c}	51.88 ± 5.76^{d}	57.54 ± 17.87^{e}	2.91 ± 0.34^{d}
3	11.37 ± 3.76^{b}	34.06 ± 0.31^{b}	87.99 ± 5.61^{c}	25.13 ± 3.66^{b}	23.49 ± 1.29^{b}	31.21 ± 4.61^{b}	121.34 ± 12.21^{b}	8.64 ± 0.18^{b}
4	60.31 ± 2.31^{e}	22.33 ± 0.24^{c}	137.81 ± 4.20^{e}	114.21 ± 7.44^{e}	97.67 ± 4.23^{f}	62.23 ± 7.34^{e}	76.12 ± 10.93^{d}	3.65 ± 0.13^{d}
5	91.12 ± 3.54^{g}	12.14 ± 0.34^{f}	193.42 ± 2.87^{g}	133.32 ± 5.31^{g}	196.45 ± 4.76^{g}	78.59 ± 4.67^{f}	43.89 ± 09.32^{f}	2.27 ± 0.16^{e}
6	07.19 ± 4.56^{a}	42.62 ± 0.25^{a}	57.89 ± 3.45^{b}	12.13 ± 2.76^{a}	18.45 ± 1.23^{a}	19.21 ± 3.45^{a}	213.85 ± 27.18^{a}	14.76 ± 0.10^{a}
BHA	25.58 ± 1.65^{c}	12.25 ± 0.32^{f}	32.74 ± 2.15^{a}	42.17 ± 1.98^{c}	41.55 ± 5.90^{d}	41.55 ± 5.90^{c}	97.31 ± 15.80^{c}	5.61 ± 0.19^{c}
BHT	30.56 ± 2.90^{d}		27.23 ± 1.76^{a}					2.13 ± 0.23^{f}

Values are mean of three replicate determinations ($n = 3$) ± standard deviation. Mean values followed by different superscripts in a column are significantly different ($P < 0.05$). Total antioxidant activity (mmolar Trolox equivalent/g compound). Metal chelating activity (mg EDTA/g compound). Concentration of substance having ferric-TPTZ reducing ability equivalent to that of molar Fe (II)/g compound.

unpaired electron. The plant products may have the property to counteract the effect of NO formation and considerable interest in preventing the ill effects of excessive NO generation in the human body. In the present study, antioxidant abilities of the isolated compounds **1–6** to scavenge. Nitric oxide radical generated under *in vitro* by sodium nitropursside was investigated. The calculated IC_{50} valve obtained from the plots of concentration dependent inhibition of NO are presented in Table 1. All the compounds used for the present study inhibited NO generation in a concentration dependent manner. The compounds registered excellent to moderate antioxidant activity with their IC_{50} ranging between 18.45 ± 1.23 and 196.45 ± 4.76 μM respectively. The highest measurable activity was found in the compound **6** (18.45 ± 1.23 μmol/mL) compound 3(23.49 ± 1.29 μmol/mL) and compound **2** (35.19 ± 2.61 μmol/mL).

3.2.5. Superoxide radical scavenging activity

The superoxide radical scavenging activity of compounds (**1–6**) is presented in Table 1. The ability of the compound to scavenge superoxide radicals generated in riboflavin-NBT-light system *in vitro* was assessed and IC_{50} under experimental condition was calculated. The scavenging ability of the compounds increased with increasing concentration. Compound **6** (57.89 ± 3.45 μmol/mL) showed maximum scavenging ability followed by compound **3** (87.99 ± 5.61 μmol/mL). The IC_{50} of the standard antioxidant BHA (32.74 ± 2.15 μmol/mL) and BHT (27.23 ± 1.76 μmol/mL) were remarkably higher than the compound **6**.

3.2.6. Metal chelating activity

The transition metal, iron is capable of generating free radicals from produced by Fenton reactions. An important mechanism of antioxidants activity is the ability to chelate and deactivate the transition metals. The ability of various compounds isolated from *S. corymbosa* to chelate metal iron (II) was investigated. Chelating activity against Fe^{2+} is expressed as mg EDTA equivalent per gram of compound. It can be seen from the data presented in Table 1, that all the compounds were capable of chelating metal iron. Among the different compounds evaluated, compound **6** exhibited higher chelating ability (213.85 ± 27.18 mg EDTA equivalent per gram of compound). The metal iron scavenging effect of various compound were in the order of **6** > **3** > BHA > **4** > **2** > **5** > **1**.

3.2.7. Ferric reducing antioxidant power (FRAP) assay

The FRAP assay measures the ability of antioxidants to reduce the ferric 2,4,6-tripyridyl-S-triazine complex [Fe (III)-$(TPTZ)_2]^{2+}$ to intensely blue colored ferrous complex [Fe(II)-$(TPTZ)_2]^{2+}$ in acidic medium. FRAP complex and the results are expressed as concentration of substance having ferric-TPTZ reducing ability equivalent to that of 1 mol/L concentration of Fe (II). The reduction capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The FRAP values for different compounds are depicted in Table 1. The compounds in general exhibited higher antioxidant capacities and the values ranged from 2.13 ± 0.23 to 14.76 ± 0.10 molar Fe(II)/g. Among the samples

experimented, compound **6** (14.762 ± 0.10 molar Fe (II)/g) registered much higher reducing ability comparable to that of the positive controls like BHT and BHA. All the compounds showed reducing power but not at the same level. The order of FRAP activity of various compound is as follows: **6** > **3** > BHA > **4** > **2** > **5** > **1** > BHT.

3.2.8. Lipid proxidation

Malondialdehyde, a secondary product of the oxidation of polyunsaturated fatty acids, reacts with two molecules of TBA, yielding a pinkish red chromogen with an absorbance maximum at 532 nm. Isolated compounds inhibited lipid peroxidation induced by ferrous sulphate in egg yolk homogenate. IC_{50} value of lipid peroxidation inhibition was recorded in the range of 19.21 ± 3.45–78.59 ± 4.67 μmol/mL. Among the isolated compounds (**1–6**) examined, the compound **6** (19.21 ± 3.45 μmol/mL) and compound **3** (31.21 ± 4.61 μmol/mL) possessed effective inhibiting capacity with the IC_{50} value less than 32 μmol/mL. Interestingly, these values were significantly lower ($P < 0.05$) that the standard antioxidants tested (Table 1) indicating their superior inhibition potential.

3.3. Anti-proliferative effect of compounds *1–6*

The compounds isolated from *S. corymbosa* were evaluated for the cytotoxic effect on HeLa (human cervical cancer cell line) HepG2 cells (Liver-Human hepatocellular carcinoma) NIH3T3 and Neuroblastoma (IMR32) cell lines using MTT assay. Cell lines were cultured with test compound at concentration in the range of 6.25, 12.5, 25.00, 50 and 100 μmol/mL for 72 h. The inhibitory effects of these compounds on cell growth was determined and their IC_{50} values were arrived (Table 2). When tested at different concentrations, all the compounds induced a dose dependent inhibition on cell growth.

In MTT assay, the compound **6** showed chemopreventive properties. It strongly and specifically inhibited the proliferation of HeLa, HepG2 and Neuroblastoma cell lines with the IC_{50} values of 7.3, 12.46 and 34.67 μmol/mL respectively. Furthermore, compound **3** was able to suppress proliferation of HeLa and HepG2 cell lines more effectively than Neuroblastoma cell with their IC_{50} values of 13.35, 25.56 and 41.56 μmol/mL respectively. The IC_{50} values of standard Camptothecin which inhibited the proliferation of HeLa, HepG2, Neuroblastoma and NIH3T3 cell lines were 5.76, 6.24 and 6.56 and 10.43 μmol/mL respectively and were remarkably higher than the tested compounds.

3.4. In vitro anti-inflammatory activity

In the present investigation, the *in vitro* anti-inflammatory effect of isolated compound **1–6** was evaluated against denaturation of egg albumin and membrane stabilization. The results are shown in Fig. 2. Among the compounds tested, compound 6 showed significant activity when compared to the standard diclofenac sodium. IC_{50} of protein denaturation by compound **6** was 10.45 μmol/mL.

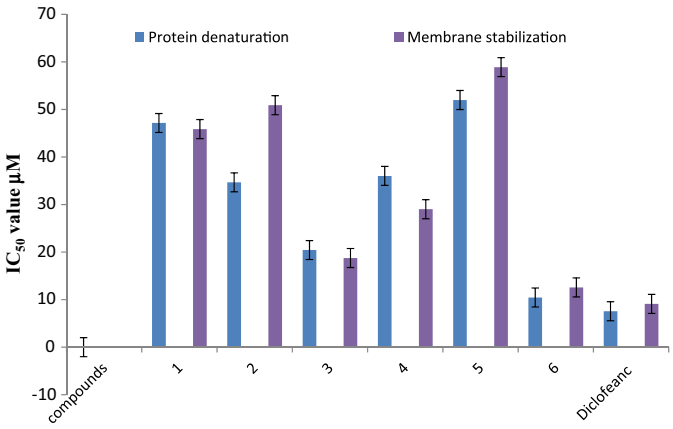

Fig. 2. *In vitro* anti-inflammatory activity of isolated compounds **1–6**. Values are mean of three replicate determinations ($n = 3$) mean ± standard deviation.

3.5. Antimicrobial assay

3.5.1. Disc diffusion method

The antimicrobial potential of these compounds (**1–6**) were evaluvated by measuring the diameter of the zone of inhibition against the pathogens. The results obtained in mm were compared with the standard Ciprofloxacin. The results showed that all the isolated compounds were capable of exhibiting antimicrobial activity. Among all these compounds, compounds 6 showed the maximum antimicrobial activity followed by compound **3**. The mean zone of inhibition of the isolated compounds for the tested microorganisms is tabulated in Table 3.

3.5.2. Antimicrobial activity (MIC)

The MIC value of the isolated compounds **1–6** is tabulated in Table 4.

4. Discussion

Free radical damage can be considered as one of the major causative factors involved in many diseases such as inflammation and dementia. It is well established that anti-oxidants are capable of preventing injury to blood vessel membranes, thereby optimizing blood flow to the heart and brain, defending against cancer-causing DNA damage and thus help lowering the risk of cancer, cardiovascular and various mental illnesses including Alzheimer's diseases [31,32]. Xanthones are closely related to the polyphenol family. Therefore, screening natural antioxidants from xanthones should be an effective pathway to discover lead compounds. In the present study, antioxidant capacity of all the isolated xanthones (**1–6**) was evaluated using the radical DPPH$^{\bullet}$, ABTS$^{\bullet+}$, $O_2^{\bullet-}$, OH$^{\bullet}$, NO, FRAP, metal chelating activity and the inhibition of lipid peroxidation assay. IC_{50} and equivalents (TE) per microliter of sample (mmol/L TE) was used for the evaluation of the activity. Of these tested compounds, compound **6** exhibited very strong antioxidant activity in a structural-dependent manner (Table 1). The second most active compound is **3**. The great radical scavenging activity of this compound is quite self-evident that it has a catechol moiety. Compound **6** is a dioxygenated xanthone with two free

Table 2
Anti-proliferative activity of isolated compound 1–6.

Compounds	HeLa IC_{50} (μmol/L)	HepG2 IC_{50} (μmol/L)	Neuroblastoma IC_{50} (μmol/L)	NIH3T3 IC_{50} (μmol/L)
1	50.15 ± 2.34^{f}	45.87 ± 2.27^{e}	53.67 ± 2.38^{d}	41.21 ± 1.21^{d}
2	34.67 ± 0.34^{d}	50.90 ± 2.23^{f}	56.21 ± 1.20^{d}	66.49 ± 0.87^{e}
3	13.35 ± 1.70^{b}	25.56 ± 1.09^{c}	41.56 ± 2.01^{c}	35.51 ± 1.33^{c}
4	27.45 ± 1.23^{c}	39.02 ± 2.21^{d}	42.16 ± 3.56^{c}	41.82 ± 2.17^{d}
5	49.56 ± 0.17^{e}	51.98 ± 1.30^{f}	58.90 ± 1.29^{d}	73.67 ± 1.09^{f}
6	7.3 ± 0.67^{a}	12.46 ± 1.55^{b}	34.67 ± 2.12^{b}	21.67 ± 2.12^{b}
Camptothecin	5.76 ± 1.00^{a}	6.24 ± 1.23^{a}	6.56 ± 0.32^{a}	10.43 ± 1.43^{a}

Values are mean of three replicate determinations ($n = 3$) ± standard deviation. Mean values followed by different superscripts in a column are significantly different ($P < 0.05$).

Table 3
Antimicrobial activity of the isolated compounds from aerial parts of *Swertia corymbosa* using agar disc diffusion method (zone of inhibition in mm) (25 μg/disc).

Micro-organisms	Inhibition zone diameters of the test compounds (mm)						
	1	2	3	4	5	6	Ciprofloxacin
E. coli (ATCC 25922)	10.27 ± 0.11	8.97 ± 0.23	13.47 ± 0.05	9.87 ± 0.05	7.07 ± 0.11	20.27 ± 0.11	21.77 ± 0.11
P. aeruginosa (ATCC 27853)	5.97 ± 0.19	6.17 ± 0.05	11.97 ± 0.05	10.07 ± 0.05	6.17 ± 0.05	15.97 ± 0.11	20.47 ± 0.17
S. aureus (ATCC 29213)	10.17 ± 0.25	8.97 ± 0.23	13.57 ± 0.11	9.67 ± 0.11	2.87 ± 0.11	17.37 ± 0.17	20.77 ± 0.11
S. pneumoniae (ATCC 33400)	4.97 ± 0.23	7.37 ± 0.05	15.27 ± 0.11	11.37 ± 0.11	4.47 ± 0.05	21.21 ± 0.25	21.07 ± 0.05
K. pneumoniae (ATCC 10031)	9.37 ± 0.78	8.87 ± 0.17	13.77 ± 0.05	11.97 ± 0.19	6.87 ± 0.11	15.77 ± 0.11	19.17 ± 0.11
B. subtilis (ATCC 6633)	10.17 ± 0.23	9.97 ± 0.11	15.47 ± 0.05	10.27 ± 0.05	7.97 ± 0.23	17.27 ± 0.17	22.67 ± 0.11

Values are mean ± standard deviation of three experiments in replicate. Ciprofloxacin standard antibacterial agent. 1-Hydroxy-3,7,8-trimethoxyxanthone (**1**), 1,7-dihydroxy-3,8-dimethoxyxanthone(**2**), 1,2,8-trihydroxy-6 methoxy xanthone (**3**), 1,8-dihydroxy-2,6-dimethoxyxanthone (**4**), 8-hydroxy-1,2,4,6-tetramethoxyxanthone (**5**) and 1,2-dihydroxy-6-methoxyxanthone-8-O-β-D-xylopyranosyl (**6**).

hydroxyls located at C-1 and C-2, and C-diglycoside at C-8; while compound **3** is also a trioxygenated xanthone with three free hydroxyls located at C-1, C-2 and C-8.

Generally, xanthones with higher oxygenation display better activity than those with less oxygenation. Glycosylated xanthones (**6**) is more active than those that are not (**1**–**5**). **6** is more active than **3** and **2** is more active than **5**. Antioxidant capacity of xanthones does not benefit from free hydroxyl group(s) located only at either C-1 or C-8 alone. Interestingly, **6** and **3** of the isolated compounds showed highest radical scavenging activities, which can be explained by the present of ortho-dihydroxy groups in their molecular structure [33–35]. The present study revealed that the hydroxyl group has strongest influence antioxidant activities, while methoxyl group has a weak influence (Fig. 1B and C).

Cancer diseases are one of the main causes of death worldwide. The discovery of new molecules from natural origin is a global trending currently for the less toxicity of natural products [36]. The anticancer properties of aerial parts methanol extract of *S. corymbosa* on HeLa (human cervical cancer), HepG2 cells (Liver-Human hepatocellular carcinoma) and Neuroblastoma (IMR32) cell lines have already been reported by Mahendran and Narmatha Bai [7]. MTT assay is most widely used to determine the cytotoxic potential of medicinal agents and other toxic materials. The reduction of yellow MTT to purple colored formazan takes place only when mitochondrial reductase enzymes are active and therefore the conversion can be directly related to number of viable cells. The cytotoxic effect of xanthone compounds after 48 h of treatment in HeLa, HepG2, IMR32 and NIH 3T3 cells was observed.

The results are presented in Table 2. Among these compounds, 1,2-dihydroxy-6-methoxyxanthone-8-O-β-D-xylopyranosyl (**6**) was found to have significant anti-proliferative activity. The presence of ortho-dihydroxy groups as well as

Table 4
Antimicrobial activity (MIC values μg/mL) of isolated compounds from aerial parts of *Swertia corymbosa*.

Micro-organisms	Minimum inhibitory concentration (MIC, μg/mL)							
	1	2	3	4	5	6	Ciprofloxacin	Streptomycin
E. coli (ATCC 25922)	250	62.5	7.81	15.62	500	3.90	3.90	1.90
P. aeruginosa (ATCC 27853)	62.5	15.62	3.90	31.25	125	7.81	1.90	3.90
S. aureus (ATCC 29213)	500	62.5	31.25	15.62	125	15.62	7.81	1.90
S. pneumoniae (ATCC 33400)	125	31.25	7.81	62.5	62.5	3.90	3.90	3.90
K. pneumoniae (ATCC 10031)	125	15.62	15.62	31.25	500	1.90	1.90	3.90
B. subtilis (ATCC 6633)	125	31.25	3.90	15.62	125	7.81	3.90	7.81

Values are mean of three experiments in replicate. Ciprofloxacin and Streptomycin standard antibacterial agent. 1-Hydroxy-3,7,8-trimethoxyxanthone (**1**), 1,7-dihydroxy-3,8-dimethoxyxanthone(**2**), 1,2,8-trihydroxy-6 methoxy xanthone (**3**), 1,8-dihydroxy-2,6-dimethoxyxanthone (**4**), 8-hydroxy-1,2,4,6-tetramethoxyxanthone (**5**) and 1,2-dihydroxy-6-methoxyxanthone-8-O-β-D-xylopyranosyl (**6**).

hydroxyl groups in their basic skeleton of xnathones is responsible of their wide range of activities [37,38]. It was shown that their cytotoxic activity could increase with the number and position of these groups in their structure [39,40]. This can explain to a certain degree the interesting antiproliferative activity of the ortho-dihydroxy groups (**3**, **6**) (Fig. 1b) and non ortho-dihydroxy group (**1**, **2**, **4** and **5**) reported in the present work. Different responses of xanthones by different cell lines have also been reported by Matsumoto et al. [41] who found that α-mangostin induced caspase-independent apoptosis *via* the mitochondrial pathway in colon cancer cells. In addition, gaudichaudione A, a cytotoxic xanthone, induced mitochondrial destabilization [42]. Therefore, although xanthones induced apoptosis *via* different pathways in different cancer cell types, in the present study, support that xanthones preferentially target the mitochondria for apoptosis induction.

The antibacterial screening data in Table 3 and 4 shown that 1,2-dihydroxy-6-methoxyxanthone-8-O-β-*D*-xylopyranosyl (**6**), 21.21 ± 0.25 and 20.27 ± 0.11 mm zone of inhibition at 25 μg/mL concentration tested against *S. pneumoniae* and *E. coli* with MIC of 3.90–15.62 μg/mL against most of the tested bacteria. Compared to it, the three compounds **1**, **2** and **5** are less active (MIC < 500 μg/mL), showing that the hydroxyl function and ortho-dihydroxy are important for its antimicrobial activity. The reduced activity of **1**, **2** and **5** is in agreement with the results of Dharmaratne et al. [43] who found that increasing the methoxy groups in xanthone skeleton their antimicrobial activity reduced drastically. The increased activity of the isolated compounds (**6**) can be explained by the electron delocalization over the whole molecules. This delocalization increases the lipophilic character of the molecule (**6**) and favors their permeation through the lipoid layer of bacterial membranes. The increased lipophilic character of the molecule seems to be responsible for their enhanced antibacterial activities [44]. It has been suggested that this molecule deactivate various cellular enzymes that play vital roles in the various metabolic pathways of these microorganisms. The activity of molecules **3** and **6** were tested against some human pathogenic bacteria, including Gram-positive bacteria (*S. aureus* and *S. pneumoniae*) and Gram-negative bacteria (*E. coli*, *P. aeruginosa, K. pneumonia* and *B. subtilis*) using the paper disc method (Table 3) and minimum inhibitory concentration (Table 4). The results obtained using these methods demonstrate that the tested molecules were highly active even at low concentrations. The results of the antibacterial screening indicate that compound **6** inhibits the growth of *S. pneumoniae*, *E. coli* and *K. pneumoniae*, but this compound is less active against the other tested bacteria.

In the present investigation, the *in vitro*-anti-inflammatory effect of isolated compound **1**–**6** was evaluated against denaturation of egg albumin and membrane stabilization. Among the compounds tested, compound **6** showed significant activity when compared to the standard diclofenac sodium. The production of auto antigens in inflammatory diseases may be due to *in vivo* denaturation of protein. The mechanism of denaturation possibly involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding [45]. From the result, it can be stated that the compound **6** was capable of controlling the production of auto antigen and thereby it inhibit the denaturation of proteins and its effect was compared with the standard drug diclofenac sodium.

The isolated compound **6** exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of GRBC. The erythrocyte membrane is analogous to the liposomal membrane [46,47] and its stabilization implies that the compound may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases which cause further tissue inflammation and damage upon extra cellular release [48]. Though the exact mechanism of the membrane stabilization by the test compound is not known yet; hypotonicity-induced hemolysis may arise from shrinkage of the cells due to osmotic loss of intracellular electrolyte and fluid components or interaction with membrane proteins [27,49]. The compound may inhibit the processes, which may stimulate or enhance the efflux of these intracellular components. On the basis of the above results it can be concluded that the isolated compounds had anti inflammatory activity.

5. Conclusion

In the present investigation, we have evaluated the antioxidant, anti-microbial, anti-proliferative and anti-inflammatory effect of xanthones isolated from *S. corymbosa*. Based on preliminary anti-proliferative activity, the active constituents **3** and **6** showed anti-proliferative effect *via* mitochondrial-mediated apoptosis induction by cell cycle arrest. Similarly, antiradical, anti-inflammatory and antibacterial activities were also performed and showed significant activity. Further studies are currently underway to assess the *in vivo* biological activities and to identify the molecular mechanism behind these biological activities.

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