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Phytochemical, Antibacterial, Antiinflammatory And Analgesic Studies On Swertia Tetragona Edgew

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Abstract: *Objective: To evaluate whole plant extracts of Swertia tetragona Edgew for antibacterial, anti-inflammatory and analgesic activity. Methods: Hydroacholic extract of powdered S. tetragona Edgew whole plant was prepared by Soxhlet apparatus using Ethanol: Water in a ratio of 80:20. The aqueous extract was prepared using cold maceration technique. The phytochemically analysis, HPTLC fingerprinting and pharmacological evaluation were carried out using standard procedures.Results: Phytochemical analysis revealed the presence of alkaloids, flavonoids, proteins, saponins and sterols. The phytochemical analysis was supported by HPTLC fingerprinting, which showed 12 peaks representing the phytochemical constituents present. The pharmacological evaluation revealed that both extracts possess good antiinflammatory activity (depicted by %* inhibition *of inflammation by 70.00 %, 72.10% and 74.07 % for hydro-alcoholic extract and 56.00 %, 59.00 % and 66.66 % for aqueous extract. The standard acelofenac decreased inflammation by 62.00 %, 64.05 % and 66.66 % under identical experimental conditions 1,2 and 3 hours post treatment) and analgesic activity (depicted by delay in reaction time in seconds by 8.5, 9 and 8.7 by hydro-alcoholic extract; 8.1, 7.8 and 7.5 by aqueous extract). Under identical experimental conditions standard acelofenac delayed reaction time by 9.5, 9.5 and 8.5 seconds when tested by tail flick, tail clip and hot plate methods respectively. The antibacterial evaluation revealed that hydro-alcoholic extract exhibited antibacterial potential, which was more for gram positive bacteria than gram negative.Conclusion: Both extracts of S. tetragona Edgew have anti-inflammatory and analgesic potential. However, antibacterial potential was only shown by hydroalcoholic extract.*

Key words: *Swertia tetragona, phytochemical analysis, antiinflammatory activity, analgesic activity*

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I. Introduction

Natural resources such as plants have always been considered as a reliable and diverse source of molecules to be used as medicines. Use of botanicals in medicines, dietary supplements, and functional food is common worldwide. According to W.H.O. estimation, around 80% of the world's population depends on alternative system of medicines for their primary health care. The plants are the major constituents of various alternative systems of medicines used worldwide since ancient times. The biggest advantage of herbal medicine lies in the fact that these contain multiple constituents having varied pharmacological activity and can be thus used for multiple diseases. There are many examples of plants which have shown multiple actions (Chirata, tulsi, Rawulfia, Ajuga, Artemisia, Seneceo being some of such examples). There has been a tremendous increase in the search for newer drugs of plant origin in the past decade with a hope for finding the ideal remedies for some crippling inflammatory diseases as rhematoid arthritis, ankylosing spodylitis, gout etc. Also despite the availability of many allopathic medicines (steroidal and nonsteroidal anti-inflammatory drugs) for the treatment of chronic inflammation and/or acute pain, research is on for finding newer anti-inflammatory agents that affect only the aberrant, uncontrolled inflammation by modifying inflammatory response without interfering the normal inflammatory process, which is an essential part of body's vital defense mechanism to its major environmental insults or invading microorganisms.

II. Inflammation

Inflammation is a complicated biological phenomenon, which in short can be defined as reaction of tissues to injury in higher animals. The pathophysiological study of the disease shows complex vascular, lymphatic and local tissue reactions at the site of injury. Essentially, it is a normal and necessary defense and repair response of the body. The basic symptomatic features of inflammation are swelling, heat, pain, redness and subsequent reduction in structural and functional organization [1]. The basic difference between acute and chronic inflammation lies in the duration of inflammatory process. Acute inflammation lasts for a week or two, whereas chronic inflammation lasts for months, sometimes years or whole life and is associated with increased

activity of cellular components characterized by three phases, which are interrelated and merged one into another. Antibiotic toxicity to host organs, tissues and cells can be cured/ prevented or antagonized with herbs [2]. Herbal molecules are safe, can overcome the resistance produced by the pathogens since they are in combined form or in pooled form of more than one molecule in the protoplasm of the plant cell. Some herbs have antibacterial and antifungal properties which can be useful clinically [3]. Some *in vitro* studies have shown that herbal medicine can overcome the clinical drug resistant strains and different serotype strains of infection [4]. The past record of rapid, wide spread emergence of resistance to newly introduced antimicrobial agen ts indicates that even new families of antimicrobial agents will have a short life expectancy [5]. For this reason, researchers are increasingly turning their attention to herbal products, looking for new leads to develop better drugs against multi drug resistant microbe strains [6]. Many plants have been found to cure urinary tract infections, gastrointestinal disorders, respiratory diseases and cutaneous infections [7,8]. These evidences contribute to support and quantify the importance of screening natural products. *Swertia* species are used in several countries for treatment of a variety of diseases including the inflammatory diseases and bacterial infections [9, 10]. *Swertia chirata* is reported to be effective against both Gram-positive and Gram-negative bacteria (although the activity was shown to be more towards Gram-positive bacteria), and *Swertia corymbosa* has been observed to inhibit the growth of *Staphylococcus aureus* and *Salmonella typhi. S.corymbosa, S.angustifolia, S.lawii and S.densifolia* have shown antihypoglycemic and antioxidant potential [11]. *Swertia petiolata* is locally also used in ophthalmic infections and relief of pain. In our earlier studies we found the plant to possess antibacterial [12] and antiinflammatory and analgesic activity [13].The present work was undertaken to phytochemically evaluate another species of *swertia* found in vale of Kashmir: *Swertia tetragona* Edgew and to explore its anti-inflammatory, analgesic and antibacterial potential. *S. tetragona* derives its name based on its 4-angled stem. It grows in moist situations on rocky slopes, shady grassy places at an altitude of 1900-3100 mt. It is found in India (Jammu & Kashmir, Uttar Pardesh, Himachal Pardesh and Punjab), Pakistan and Nepal. In Jammu & Kashmir, it is found in Pahalgam, Aharbal, Banamarg and Gilgit regions. Very limited data is available about its constitution and pharmacological activity. In our earlier studies we found the plant to possess antihepatotoxic potential [13]. Earlier Bhat and co workers [14] reported hypoglycemic activity of its hydro-alcoholic extract.

III. Materials And Methods

Identification and collection of herb:

For the present study, whole plant of *S. tetragona* was collected in late July from Aharbal area at 3100 mts. Collection was done at fruiting stage. The collection and identification was done in presence of consultant taxonomist, Department of Pharmaceutical Sciences, University of Kashmir, Srinagar. Specimen of the herb was preserved under voucher number ST/012/13 in the Department of Pharmaceutical Sciences, University of Kashmir, Srinagar for further reference.

Physico-chemical characterization:

Physico-chemical parameters of the plant extract were evaluated as per standards laid down by World Health Organization (Quality control methods for medicinal plant materials, [15] and determined as per the standards and methods laid down by [16]. These included:

Loss on drying:

It is the loss of weight in % w/w resulting from water and volatile matter of any kind that can be driven off under specified conditions. Loss on drying was estimated as per the method described in Indian Pharmacopoeia (1996). Accurately weighed (2 g and 10 g) of the air dried drug was placed in the tarred crucibles (also weighed) and put in oven, maintained at 105°C for five hours. The crucibles were then taken out, cooled and again weighed. The percentage loss was then calculated with reference to air-dried drug.

Extractive values:

The extractive value (% yield obtained) was determined in the 50% alcoholic medium, which was used in this study. Alcohol dissolves most substances such as glycosides, resins, alkaloids, and the water dissolves water-soluble substances in the extract. Both cold and hot extractive values were determined. For cold extraction, the air-dried crude powder of the drug (20g) was macerated with 100 ml solvent (50% hydro-alcoholic) in a closed flask for 24 hours, shaking frequently and allowed to stand for 24 hours. It was filtered rapidly, taking precaution against the loss of solvent. The filtrate was evaporated to dryness in a tarred flat bottom dish, dried at 105°c to constant weight, and weighed. For hot extraction, the powdered herb (20g) was packed in a Soxhlet apparatus separately in 50% hydro-alcoholic solution. Each extract was evaporated to dryness and the extractive value was calculated.

Ash values:

This parameter was used for the determination of inorganic material such as carbonates, silicates, oxalates and phosphates. Heating causes the loss of organic material in the form of CO_2 leaving behind the inorganic components. Ash value is an important characteristic of a drug and with the help of this parameter, quality and purity of the drug can be tested. To determine the total ash, the ground drug (1 g) was incinerated in a silica crucible at temperature not exceeding 450°C until free from carbon. The ash was then cooled and weighed to get the total ash content. Acid insoluble ash and water insoluble ash values were determined as follows: Acid insoluble ash – The ash was boiled with 25 ml dilute HCl (6N) for five minutes. The insoluble matter collected on an ash less filter paper was washed with hot water and ignited at a temperature not exceeding 450°C to a constant weight. For determining the water insoluble ash, the ash of the selected herb was dissolved separately in distilled water and the insoluble part collected on an ashless filter paper and ignited at 450°C to constant weight. By subtracting the weight of insoluble part from that of the ash, the weight of the soluble part of ash was obtained.

Determination of Ph:

pH of 1% and 10% solution was determined. Accurately weighed (1 g) amount of the extract was dissolved in appropriate volume of distilled water to make 1% or 10% solution. The pH of the filtrate was determined using glass electrode.

Tests for sterolsSalkowaski reaction:

Few mg of the residue of each extract were taken in 2 ml of chloroform and 2 ml concentrated sulphuric acid was added from the side of the test-tube. The test-tube was shaken for few minutes. Development of red colour in the chloroform layer indicated the presence of sterols.

Liebermann's test:

To a few mg of the residue in a test-tube, few ml of acetic anhydride was added and gently heated. The contents of the test-tube were cooled. Few drops of concentrated sulphuric acid were added from the side of the test-tube. A blue colour gave the evidence of the presence of sterols.

Test for alkaloids:

Few mg of residue of the extract was mixed in 5 ml of 1.5% v/v hydrochloric acid and filtered, addition of a saturated aqueous solution of picric acid (Hager's reagent) resulted in an orange yellow precipitate, indicating the presence of alkaloids.

Test for saponins (Foam Test)

Few mg of the test residue was taken in a test tube and shaken vigorously with a small amount of sodium bicarbonate and water. A stable, characteristic honeycomb like froth indicated the presence of saponins.

Test for tannins:

The test residue of each extract was taken separately in water, warmed and filtered. A 5% w/v solution of ferric chloride in 90% alcohol was prepared. Few drops of this solution were added to a little of the above filtrate. Dark green or deep blue colour indicated tannins.

Test for flavonoids:

A small quantity to test residue was dissolved in 5 ml ethanol (95% v/v) and reacted with few drops of concentrated hydrochloric acid and 0.5 g of magnesium metal. The pink, crimson or magenta colour, developed within a minute or two, indicated flavonoids.

Test for proteins

Proteins were detected using the xanthoproteic test, in which a little amount of residue in water forms a yellow colour complex, which indicates the presence of proteins. Aromatic amino acids (derivatives of benzene) can undergo reactions that are characteristic of benzene and benzene derivatives. Nitration of benzene ring with nitric acid is commonly referred to as the xanthoproteic test, when used to identify the presence of an activated benzene ring, because of the yellow color product; Greek, *xanthos:* yellow. Briefly, mixing a little amount (3-5 mg) of residue with 0.5 ml of concentrated nitric acid and 2.0 ml of distilled water gives yellow color, which indicates proteins.

Test for sugars:

Presence of sugars was tested using Barfoed's test. The reagent was prepared by dissolving 13.3 gm of crystalline neutral copper acetate in 200 ml of 1% acetic acid solution. The test residue was dissolved in water and heated with a little of the reagent. Appearance of red precipitate of cuprous oxide formed within two minutes indicated the presence of monosaccharides.

IV. Characterization Of Phytoconstituents By Hptlc Method

Preparation of the extract:

Test material was washed, dried and crushed in mixer grinder. Crushed material was subjected to extraction in a Soxhlet apparatus at 60-70ºC for 6 hours continuously in 50 % ethanol. The extracted material was evaporated to dryness under reduced pressure at 40-50^{0}C. The dried extract was diluted with methanol (2.0 mg/ml) and filtered through membrane filter (pore size: 0.45 micron) before loading onto the HPTLC plate. 10 µl of the filtrate was loaded on the HPTLC plate with the help of an automatic sampler for analysis.

Chromatographic Conditions:

A Camag HPTLC system equipped with an automatic TLC sampler Linovet 5, TLC scanner 3 and integrated software Win Cats version 3.0 was used for the analysis. HPTLC was performed on a pre-coated silica gel HPTLC plate of 0.20 mm layer thickness. Sample was applied to the plate as 8 mm wide bands in different volumes of standard solution with an automatic TLC sampler (Linovet 5) under a flow of N2 gas, 10 mm from the bottom and 10 mm from the side. The space between two spots was 6 mm. The linear ascending development was carried out in a CAMAG twin trough chamber (10 cm x 10 cm), which was pre saturated with the mobile phase for 30 min at room temperature. The length of the chromatogram run was 8 cm. Toluene: ethyl acetate: formic acid mix (70:20:5) was used as mobile phase. After the development, plate was dried in oven at 60^{0}C for 5 min. For subsequent development, after drying post chromatographic derivatization was carried out in sulphuric acid followed by heating at 130^{0}C for 10 min. For finger printing analysis, plate scanning was performed in absorption mode at various wavelengths under computerized CAMAG TLC scanner 3.1.

Preparation of extracts for Pharmacological evaluation:

Shade dried whole plant was crushed in a mixer grinder. The weighed crushed material was subjected to extraction in Soxhlet apparatus at 60-70ºC for 6 hours continuously in hydro methanol (20:80). The extracted material was evaporated to dryness under reduced pressure at 40-50ºC. The material was also cold extracted with water for 24 hours with occasional stirring.

Experimental model:

Inbred pathogen free adult male Wistar rats (180-200gm body weight), in an environmentally controlled room with a 12 h light-dark cycle at constant room temperature (20-24ºC) and relative humidity 15 %, were used throughout the study. The animals were acclimatized for one week prior to start of the experiment. A maximum of six rats were kept in polypropylene cages. Animals had free access to pellet diet (Hindustan lever Ltd, Bombay, India) and water ad libitum. Guidelines issued by the CPCSEA for the care and use of laboratory animals were followed.

V. Experimental Design

Evaluation of the anti-inflammatory activity:

Animals were divided into five groups: Group I (Normal Control) received normal saline, Group II rats (Inflammation model) were injected carrageenan, while Group III and IV, (Experimental rats) were injected carrageenan and given Swertia tetragona extracts (aqueous and hydro-alcoholic respectively). Group V (standard group) rats were injected carrageenan and treated with Aceclofenac (5 mg/kg body weight). The doses of the extracts administered to rats were 1gm/kg body weight for aqueous extract and 200 mg/ kg for hydroalcoholic extract. The extracts were given orally 1 hour prior to carrageenan insult. The measurement of hind paw volume was carried out 1, 2 and 3 hours post carrageenan injection using plethysmometer. Injection of 0.1 ml 1% carrageenan solution (prepared by suspending 0.1g carrageenan in 10 ml normal saline) into hind paw was used to induce inflammation. Percent inhibition was calculated as follows: % inhibition =Vc-Vt/ Vc X 100Where Vc is the volume of oedema following carrageenan injection, and Vt is the volume of oedema in extract/drug treated group. Volume of oedema was derived by taking the difference inthe volume of left hind paw receiving carrageenan injection, minus the volume of the right hind paw, which was not given carrageenan injection.

Analgesic Activity:

Male rats (Wistar strain) were divided into 4 groups. In addition to the control rats (group I), experimental rats from Group II and III received, respectively, the aqueous and the hydro-alcoholic extracts of S. Petiolata (1g/kg and 200mg/kg body weight respectively) whereas Group IV received Aceclofenac (5 mg/kg body weight). Analgesic activity of each extract was determined using standard pharmacological methods (tail flick method, hot plate method and tail clip method).

Experimental design for analgesic activity:

Analgesic activity of the extracts was determined by the following methods:

a) Tail flick method
b) Hot plate method
c) Tail clip method

Tail flick method [17]:

The animals were divided into 4 groups, each group containing six animals. Group-I received normal saline and acted as control, group-II & III received aqueous and hydro-alcoholic extracts of *S. tetragona*, whereas group-IV received aceclofenac (5mg/kg body weight). Two hours post administration of the extracts and standard drug, the animals were held in a position with the tail portending out on the coil of analgesiometer. A current of 5 volts was applied and the time taken by the animals to withdraw (flick) the tail was taken as the reaction time.

Hot plate method [18]:

The animals 2 hours post treatment were placed on a hot plate, maintained at 55°c. The reaction time (the time between placing the animals on the hot plate and licking of the fore or hind paws by the animals) was noted. The mean increase in the reaction time in treated groups was compared with that recorded in control animals to determine the analgesic activity.

Tail clip method [19]:

An artery clip with thin rubber sleeves was applied to the base of rat-tail to produce a painful stimulus. Control animals make a continuous effort to dislodge the clip by biting it. Analgesia makes the animals indifferent to the pain produced by clip presser. Two-hour post treatment, the artery clip was applied to the base of tail, and the reaction time was noted. The mean increase in the reaction time in treated group was compared with that recorded in control animals to determine analgesic activity.

VI. Assessment Of Antibacterial Activity

Preparation of extract:

100 gm Shade dried and powdered whole plant of *Swertia tetragona* was first soxhlet extracted with petroleum-ether (60:80) for 3 days and subsequently using chloroform and hydro-methanol (20-80). The extracts were dried under reduced pressure using a rotary flash evaporator.

Bacterial culture:

The bacterial cultures were grown on nutrient agar. The agar plates were earlier sterilized in an autoclave and then stored at 4°C in sterile conditions. Bacillus subtilis, Staphylococcus aureus, E. coli and Pseudomonas aeruginosa were cultured in appropriate broths at 30°C overnight.

VII. Antibacterial Assay

Assay for antibacterial activity:

The in-vitro antibacterial activity of the herb extracts was studied by disc diffusion method [20]. All the extracts (3mg /ml) were tested against Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa. The activity of extracts was compared with ampicillin and cefutaxim which were used as antibacterial standards. The extracts prepared from Swertia tetragona were dissolved in dimethyl formamide:water (0.5 ml :4.5 ml), which was earlier evaluated and ascertained not to interfere with the assay. The filter paper discs (Whatman No.1 filter paper discs ϕ, 6mm), were dipped in respective extracts (concentration of each extract 3mg/ml) and dried. Soaked and dried discs were placed on the prepared Petri plates (previously inoculated agar) and incubated at 37°C for 24 hours. Clear inhibition zones around the discs indicated antibacterial activity. The assay was carried out in triplicate. The strength of activity was classified as strong for inhibition zone diameters (inhibition diameter) ≥ 15.0 mm(++++), moderate for diameters ranging from10-14.5mm(+++) and weak for diameters 5-10mm(+).

VIII. Results

Physico-chemical examination of the extracts:.

Yeild: The percent yield was 3.2gm (chloroform), 4.4gm (petroleum-ether) and 14.2gm (hydro-alcohol) for 500 gmsof powder. The extracts were stored in airtight containers and refrigerated.

Table 1: showing Physico-chemical analysis of *Swertia tetragona* Edgew.

Physical analysis		
S.NO	Particulars	Result
1	Loss on drying at 105°C	7.78 %
2	Total ash	13.169 %
3	Acid insoluble ash	5.658 %
4	Alcohol soluble extractive	2.63 %
5	Water soluble extractive	2.53 %
6	pH of the extract at 21.4^0C	6.41
Chemical analysis		
1	Alkaloids	Present
2	Flavonoids	Present
3	Proteins	Present
4	Saponins	Present
5	Sterols	Present
6	Sugars (monosacharides)	Absent
7	Tannins	Present

HPTLC finger printing of the *Swertia Tetragona* extract.

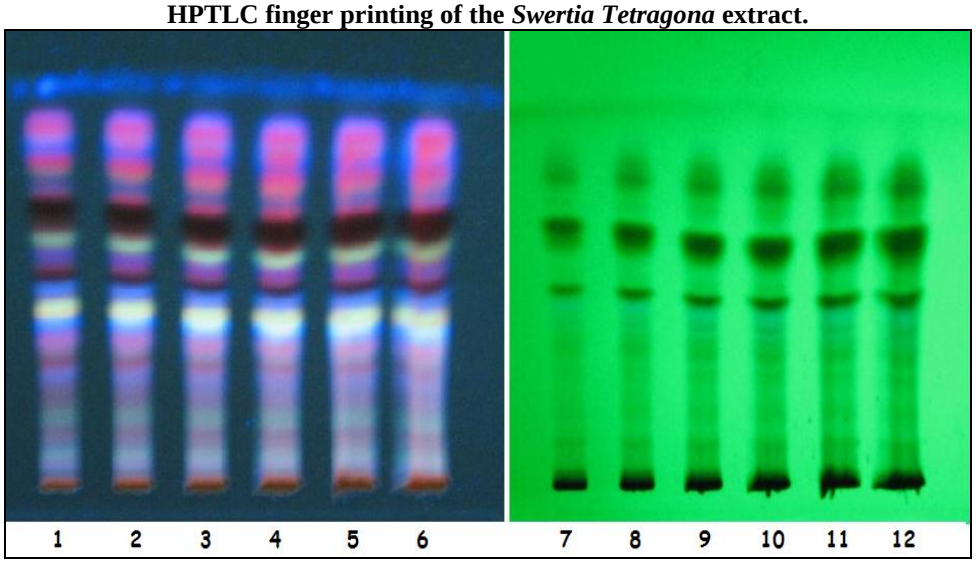

Figure 1: Photographic images of HPTLC analysis of S. tetragona.Tracks (1-6) run at different volumes (1.0-6.0µl) (Bands 1-6 observed under fluorescent light at 366 nm, and 7-12 under UV at 254 nm)

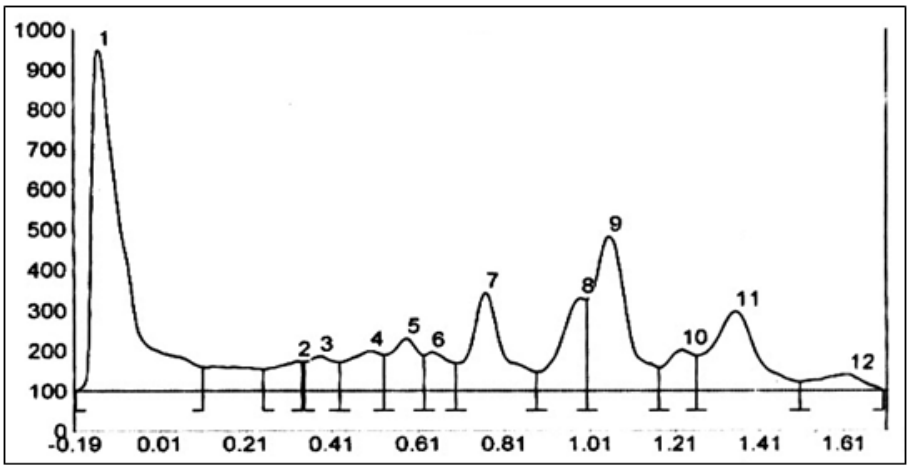

Figure 2: HPTLC chromatogram of Swertia tetragona showing prominent peaks representing the constituents present

Table 2: showing the anti-inflammatory activity of aqueous and hydro-alcoholic extracts of *S. Tetragona.*

Group		Percent reduction in paw volume		
S.NO	Type of treatment	1 hr post treatment	2 hr post treatment	3 hr post treatment
1	S.T (H/A)	70.00 ± 1.34	72.10± 1.76	74.07± 1.15
2	S.T (Aq)	56.00 ± 1.89	59.00± 1.24	66.66± 1.51
3	Aceclofenac	62.00± 0.97	64.05± 1.11	66.66± 0.90
4				

Results are expressed as Mean ± S.E of 6 animal's .S.T (H/A): S. tetragona hydro alcoholic extract; and S.T (Aq): S. tetragona aquous extract**.**

Table 3: showing Analgesic activity of of aqueous and hydro-alcoholic extracts of Swertia tetragona Edgew.

Type of treatment	Increase in the reaction time (in seconds)		
	Tail flick method	Tail clip method	Hot plate method
S.T (H/A)	8.5 ± 0.52	9.0± 0.71	8.7± 0.67
S.T (Aq)	8.1± 0.77	7.8± 0.89	7.5± 0.72
Aceclofenac	9.5± 0.43	9.5± 0.52	8.5± 0.31

Results are expressed ad Mean ± SE of 6 animals. S.T (H/A): S. tetragona hydro alcoholic extract; and S.T (Aq): S. tetragona aqueous extract.

Table 4: Showing Antimicrobial activity of various fractions prepared from the whole plant of S. tetragona

Type of microorganism	Petroleum ether (3 mg/ml)	Chloroform	Hydro-methanol	Amoxicillin	Cefutaxim
Bacillus subtilis	+	+	+++	++++	++++
Staphylococcus aureus	+	+	+++	++++	++++
Pseudomonas aeruginosa	++	++	+++	+++	++++
Escherichia coli	++	++	+++	+++	+++

The experiment was performed in triplicate, and the diameter of the zone of inhibition was measured in mm; Diameter >4 mm = +, 5-10 mm = ++, 10 – 15 mm = +++, > 15 mm = ++++. The zones of inhibition were 13.7, 14.1, 11.2 and 11.5 (for Bacillus subtilis,Staphylococcus aureous,, Pseudomonas aeruginosa and Escherichia coli)in case of Hydromethanol.petroleumether and chloroform extractsshowed only marginal activity.The zones of inhibition after 24 h incubation were measured. Data is presented in Table 4. The hydro-alcoholic extract prepared from Swertia tetragona showed strong antibacterial activity against all the strains of Gram-positive and Gram-negative bacteria. Petroleum ether and chloroform extracts showed only marginal activity. The antimicrobial activity was stronger against Gram-positive bacteria, Bacillus subtilis and Staphylococcus aeruginosa and lesser against Gram-negative bacteria, E. coli and Pseudomonas aeruginosa. However, in comparison to amoxycillin and cefutaxim, the antimicrobial activity of herb extracts was lower.

IX. Discussion

Results presented in Table 2 and 3 provide clear evidence suggesting the anti-inflammatory and analgesic role of Swertia tetragona collected from the Kasmhir valley. Significant alleviation of pain and inflammation was demonstrated by the extract which was comparable to aceclofenac, the standard anti-inflammatory and analgesic drug. The findings are in accordance with the earlier studies on other Swertia species, particularly Swertia chirata, which is widely used in India to treat fever, inflammatory diseases and malaria [21, 22] and Swertia petiolata [12].Identification of a particular plant species and time of its collection are very important as then only one can be sure of the effectiveness of the plant. The phytochemical analysis is of paramount importance for a plant drug for determining the active constituent(s) useful in therapy. The marker analysis of phytoconstituents is also helpful in phytoequivalence studies, including such issues as pharmacokinetics and other parameters by studying the absorption, distribution, metabolism, and elimination of the drug in vivo. To rationalize the use of botanicals, a need based and novel concept of markers is gaining momentum. In this regard, marker analysis of the plant materials as well as their extracts is recommended by various organizations for the evaluation of medicinal products. In this study, we developed the characteristic fingerprints of the plant S. tetragona. which is used traditionally in treatment of various diseases. The characteristic fingerprint of this plant together with analytical specifications by conventional methods may help to establish the identity and standardization profiles of this plant as raw material as well as in formulations of effective herbal dosage forms. The extract showed 12 peaks in HPTLC chromatographic analysis representing phytoconstituents present (Figure 1). Physico-chemical analysis of the extract showed the presence of alkaloids, flavonoids, proteins, saponins, and sterols. We are laying down the phytochemical standards of S.tetragona with

regard to its purity and other parameters. Previous studies on other Swertia species also reported the presence of flavonoids [23-25], terpenoids, alkaloids [13, 26-28], irridoids [29-31]. These important molecules may serve as markers for the plant material/extract and may be useful for identifying and quantifying the plant material in formulations. Inhibition of carrageenan-induced inflammation is one of the most suitable test procedures to screen antiinflammatory agents. Oedema formation in paw is the result of a synergism between various inflammatory mediators that increase vascular permeability and/or mediators that increase blood flow. The development of carrageenan–induced oedema is biphasic, the first phase is attributed to the release of histamine, 5-HT, and kinins, while the second phase is related to the release of prostaglandins [32-34]. Both steroids and non-steroidal anti-inflammatory drugs (like aspirin and indomethacin) inhibit biosynthesis of prostaglandins. Steroids inhibit conversion of membrane phospholipids to arachidonic acid and thus block formation of leukotrienes, prostacyclin, PGE_2 and related prostaglandins whereas non-steroidal antiinflammatory drugs irreversibly inactivate cycle-oxygenase. Moreover, so far very few antiinflammatory agents have been shown to have direct antaqqistic action against exogenous prostaglandins. Oxygen derived free radicals and oxidants have been shown to play an important role in various forms of inflammation [35, 36]. In order to identify the compounds responsible for the biological activities observed, many compounds as Xanthones, mangiferin, α-mangostin, isomangostin, α-mangostin triacetate, besides some other compounds like ursolic acid have been separated from the extracts of S. chirata, and reported to be antiinflammatory [37, 38].Several reports are there which suggest a strong role of ursolic acid in antiinflammatory action. It not only inhibits human leucocyte elastase, but also 5-lipoxigenase and cycloxygenase activity [39, 40]. The mechanismof antiinflammatory activity of ursolic acid have been attributed to inhibition of histamine release from mast cells [41-43] and to inhibition of complement activity [44]. Moreover, ursolic acid exhibited strong inhibitory activity on the production of nitric oxide in macrophages [45]. Polyphenols were demonstrated as possessing in vivo anti-inflammatory properties too [46, 47]. Irridoids and seco-irridoids could also contribute to anti-inflammatory effect [48, 49]. Flavonoids also have been known to possess anti-inflammatory and antioxidant activities. To conclude it can be assumed that either the anti-inflammatory and analgesic activity of S. tetragona Edgew is due to one of these compounds or many of them may be acting synergistically at different levels of inflammation. This also gathers support from the fact that hydro-alcoholic extract of the herb showed more anti-inflammatory activity than its aqueous extract, which may be due to the presence of more anti-inflammatory phytoconstituents in hydro-alcoholic extract than in aqueous extract.For thousands of years, natural products have been used in traditional medicine all over the world before theintroduction of antibiotics and other modern drugs. The antimicrobial efficacy attributed to some plants in treating diseases has been beyond belief. It is estimated that local communities have used about 10% of all flowering plants on Earth to treat various infections, although only 1% have gained recognition by modern scientists [50]. Owing to their popular use as remedies for many infectious diseases, searches for plants containing antimicrobial substances are frequent [51] Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids and flavonoids, which have been found in vitro to have antimicrobial properties [52]. A number of phytotherapy manuals have mentioned various medicinal plants for treating infectious diseases because of their availability, fewer side effects and reduced toxicity [53]. There are several reports on the antimicrobial activity of different herbal extracts [54-57]

Swertia tetragona (hydro-alcoholic extract) exhibited good antibacterial activity as compared to petroleum-ether and chloroform extract, which showed only marginal activity against all the gram positive and gram negative strains tested. Moreover, the activity as assessed by the area of zone of inhibition was more in case of gram positive bacteria as compared to gram negative bacteria. This is in accordance with the earlier studies performed on the other species of Swertia. S. purpurascens showed positive activity against selected test microorganisms [58]. S. chirata extracts were found to be effective against Gram-positive and Gram-negative bacteria; the activity being more pronounced against the former type of organisms. In another study, the aqueous, MeOH, $CHCl_3$, and hexane extracts of S. corymbosa were tested in vitro for their antimicrobial properties. Maximum inhibitory activity was observed against Staphyllococcus aureus and Salmonella typhi [59]. Studies on Swertia petiolata earlier also revealed its antibacterial potential [13]. Several xanthones and their D-glucosides have been shown to be antimicrobial [60], swertiamarin, isolated from S. japonica, exhibited antibacterial activity against Staphyllococcus aureus [61]. The xanthones and their D-glucosides, swertianolin, norswertianolin, swerchirin, amarogentin and swertiamarin in earlier studies [62]. Anupam, 2012 have been shown to possess the antimicrobial activity. It has been proposed that the mechanism of the antimicrobial effect involves the inhibition of various cellular processes, followed by an increase in plasma membrane permeability and finally ion leakage from the cells [63]. The antimicrobial potency of plants is believed to be due to tannins, saponins, phenolic compounds, essential oils and flavonoids [64]. Thus it can be assumed that the activity exhibited by swertia tetragona can be either due to one of these compounds or due to all these constituents which are soluble in water and alcohol acting in synergism.

X. Conclusion

It can be concluded that the herb Swertia tetragona Edgew possesses potential antiinflammatory, analgesic and antibacterial activity. Thus the herb looks promising as far the herbal medicine are concerned. The herb has the potential to be formulated in dosage forms for various types of inflammatory diseases besides having antibacterial potential. However isolation and characterization of various molecules and their screening for pharmacological action will be fruitful in the development of newer molecules with lesser side effects.

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