ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

PHYTOCHEMICAL SCREENING AND CHARACTERIZATION OF ACTIVE COMPONENT OF *SPATHODEA CAMPANULATA* (RAKTURA) FOR THEIR ANTI-ARTHRITIC ACTIVITY

Kiran Shandilya[1], Neena Arora*[2], Sadhna Goyel[3]

[1]*Research Scholar, Sri Sathya Sai College (Autonomous) for Women, Bhopal, Madhya Pradesh, India*
[2]*Dept. of Chemistry, Sri Sathya Sai College (Autonomous) for Women, Bhopal, Madhya Pradesh, India*
[3]*Dept. of Chemistry, Govt. Motilal Vigyan Mahavidyalaya, Bhopal, Madhya Pradesh, India*
**Corresponding author: aroraneena10@gmail.com*

ABSTRACT

S. campanulata, widely distributed in India is traditionally used as astringent, aphrodisiac, purgative, anthelmintic, depurative, febrifuge and expectorant. The plant is also used in rheumatism, dropsy, urinary diseases and jaundice. The purpose of this study is to evaluate the anti-arthritic activity of the methanolic extract of the flower of *S. campanulata* in experimental animal models. The powdered drug was subjected to successive solvent extraction, with solvents in increasing order of polarity to obtain the methanolic extract of the aerial parts of the plant. Isolation and characterization of plant extract were performed through UV, IR, NMR and Mass spectroscopy. Characterization approximately conformed the isolated compound was kaempferol. *S. campanulata* was evaluated for anti-arthritic action by Freund's adjuvant induced arthritis test in adult Albino rats (150-200 gm). Rats were injected 0.1 ml of complete Freund's adjuvant into the planter region of the left hind paw. Statistical analysis was performed using One way analysis of variance (ANOVA) followed by Bonferonni test. $P<0.05$ was considered statistically significant. The methanolic extract of in doses of 200 and 400 mg/kg showed 75.50% and 68.33% protection against increase in paw edema, respectively. *S. campanulata* showed dose-dependent action in all the experimental models.

Keywords: *S. campanulata*, Anti-arthritic, Freund's adjuvant induced, Paw edema.

1. INTRODUCTION

The goal of treatment for rheumatoid arthitic patient is to eliminate symptoms, slow disease progression, and optimize quality-of-life [1]. Therefore, before starting the treatment of RA certain goals must be kept in mind such as relief of analgesia, reduction of inflammation, protection of articular structure, maintenance of function, and control of systemic involvement [2]. Presently for the treatment of RA, strategies have changed from traditionally used non-steroidal anti-inflammatory drugs (NSAIDs) or disease modifying antirheumatic drugs (DMARDs) to novel biological agents, like TNF monoclonal antibody. Clinically, the treatment of RA includes five strategies. The foremost approach is the use of NSAIDs followed by mild doses of glucocorticoids to minimize the signs of inflammation as well as progression of disease. In chronic patients, the use of DMARDs such as methotrexate, sulfasalazine, gold salts or D-pencillamine can be included in the treatment. In certain cases, TNF-α neutralizing agents like infliximab, etanercept etc; IL-1 neutralizing agents like anakinra; and the drugs which interfere with T-cell activation such as abatacept can also be included in treatment of chronic cases. Finally, immunosuppressive and cytotoxic drugs such as cyclosporine, azathioprine, and cyclophosphamide are used for the treatment of chronic patients [3-5]. The above-mentioned therapeutic agents reduce the inflammation and joint destruction but their long-term risks are still unknown. However, long-term risks of drugs includes gastrointestinal ulcers, cardiovascular complications, hematologic toxicity, nephrotoxicity, pulmonary toxicity, myelosuppression, hepatic fibrosis, stomatitis, cirrhosis, diarrhea, immune reactions, and local injection-site reactions. Moreover, higher costs and side effects which include high risks of infections and melagnancies reguires continous monitoring [6].

S. campanulata is a monotypic genus in the flowering plant family Bignoniaceae. The single species it contains, *S. campanulata*, is commonly known as the African tulip tree, fountain tree, pichkari or Nandi flame. The tree grows between 7-25 m (23-82 ft) tall and

is native to tropical dry forests of Africa. It has been nominated as among 100 of the World's Worst invaders. *S. campanulata* grows commonly in several countries including Ghana, Nigeria, Uganda, Gabon, Cameroon and Senegal. The decoction from the leaves and stem bark of *S. campanulata* is traditionally used to cure some types of wounds including skin rashes, haemorrhoids and stomach ulcer. Other ethnomedicinal uses of *S. campanulata* include the treatment of malaria (leaves and stem bark); dyspepsia (stem bark and leaves); arthritis and fracture (leaves, root bark and fruit); toothache and stomach ache (stem bark). The decoction of *S. campanulata* bark is prepared by boiling until the water is half reduced, and is taken orally thrice a day for five days for the treatment of malaria. Likewise, Moreover, *S. campanulata* flowers and bark are used to treat fever, convulsion, bacterial infections, HIV, poor blood circulation, gastro-intestinal diseases, respiratory ailments, genital-urinary system disorders, filaria, gonorrhea, epilepsy and mental disorders [7-9].

2. MATERIAL AND METHODS

2.1. Selection, Collection and Authentication of Plant Material

Depending upon the literature survey report and traditional claim in various communities, the plant *Spathodea campanulata P. beauv* (Rugtoora, SC) was chosen for current work. Plant materials (Flowers parts) were collected from the local area of Bhopal (M.P.) India, where the people come to commercialize their raw material for financial benefit. Herbarium of both plants species were prepared graciously and submitted to Department of Botany, Saifia College of Science, Bhopal India, for authentication. Plants were authenticated by Dr. Zia-Ul-Hasan, Head, Department of Botany, Saifia College of Science, Bhopal, India. Plant authentication voucher numbers obtained were 392/Bot/Saifia/16 and 391/Bot/Saifia/16 for *N. arbor-tristis* and *S. campanulata* respectively.

2.2. Preparation of plant extracts

In present study, plant material was extracted by continuous hot percolation method using Soxhlet apparatus. Powdered material of *S. campanulata* was placed in thimble of soxhlet apparatus. Soxhlation was performed at 60°C using petroleum ether as non-polar solvent. Exhausted plant material (marc) was dried and afterward re-extracted with ethyl acetate and methanol solvent. For each solvent, soxhlation was continued till no visual colour change was observed in siphon tube and completion of extraction was confirmed by absence of any residual solvent, when evaporated. Obtained extracts was evaporated using rotary vacuum evaporator (Buchitype) at 40°C. Dried extract was weighed and percentage yield for each extract was determined using formula:

% Yield= (Weight of extract/Weight of plant material used) x100

Prepared extracts were observed for organoleptic characters (percentage yield, colour and odour) and were packed in air tight container and labelled till further use [10-11].

2.3. Qualitative Phytochemical Estimation of Extracts

Detailed phytochemical testing was performed to identify presence or absence of different phytoconstituents in *S. campanulata* flower extracts by using standard procedures [12-13]. The extracts prepared in Petroleum ether, Ethyl acetate and Methanol were subjected to following tests:

2.3.1. Isolation of Phytoconstituent by TLC and Column Chromatography

Isolation of pure component from selected fraction was done by following chromatography techniques.

2.3.1.1. Thin Layer Chromatography (TLC)

TLC is a primary, basic procedure used for separation of secondary metabolites. It has a wide application in area of natural product. It is quick, inexpensive and microscale technique used for analyzing a number of components in crude extract of plant material [14]. It is a common analytical technique which separates components on the basis of stationery and mobile phase, which forms the principle of TLC. It is a solid-liquid form of chromatography where the stationary phase is normally a polar absorbent and the mobile phase can be a single solvent or combination of solvents. Stationary phase is the silica gel coated on the aluminium plates and mobile phase is the solvent system.

TLC of all the extracts was done in four different solvent systems of different polarities ranging from lower to higher polarities. Following were the solvent systems;

1. Toluene: Ethyl formate: Formic acid (50: 40: 10)
2. Toluene/ Benzene: Acetone (90: 10)
3. Toluene: Chloroform: Acetone (40: 25: 35)
4. Butanol: Acetic acid: Water (40: 10: 50)

UV long (365 nm), UV short (254 nm); visible light and separated spots were marked.

R_f value or "retardation factor" or the "ratio-to-front" value was calculated and expressed as a decimal fraction.
Rf = distance spot travels/distance solvent travel

2.3.1.2. Column chromatography of Methanolic extract

Methanolic extract was having highest antioxidant potential, hence it was subjected to column chromatography on silica gel (60-120 mesh size) as the stationary phase. Silica gel was activated in oven at 100°C for 40 minutes to remove moisture content. The sample was weighed, binded with activated silica and dissolved in minimum amount of solvent. The weight of sample and details of column are as follows:

Weight of sample	1.006 gms
Weight after binding with silica gel	1.870 gms
Diameter of column	3.5 cm
Height of column	43 cm

Activated silica was mixed with pet ether solvent to make translucent slurry. The slurry was poured in a long cylindrical glass tube called column till it filled ¾ of its height. While pouring, slurry was continuously swirled so that lumps were not formed. The sides were tapped gently so that the slurry settled properly and air bubbles are excluded. The sample was poured gently on the silica gel to make an even surface. The solvent was poured alongside walls of column with the help of pipette so that sample layer was not disturbed. The column was eluted with solvents in pure form and also their ratios in gradient manner. The solvent used sequentially were Pet. ether, Chloroform, Ethyl acetate, Methanol and their ratio with increasing polarity. The fractions were collected until all the components were collected and labelled accordingly.

Length of silica gel/slurry	27 cm
Length of sample	0.4 cm

2.3.2. Ultra Violet -Visible Spectroscopy

UV Visible and associated techniques are most widely used for analytical and research work. This technique was used to measure the π-electron systems, conjugated unsaturation, aromatic compounds and conjugated non-bonding electron systems within the molecules. It requires radiation of high energy. UV region corresponds to 400-200 nm and visible region corresponds to 800-400 nm. The most important application of UV spectroscopy is extent of conjugation. Four types of transition occur between energy levels which account for UV/Visible spectra. The following table shows the transitions, wavelength range and examples

Table 1: Electronic transitions of molecular orbital

Transition	Wavelength range	Examples
$\sigma \rightarrow \sigma^*$	<200 nm	C-C, C-H
$n \rightarrow \sigma^*$	160-260 nm	H_2O, CH_3OH, CH_3OH
$\pi \rightarrow \pi^*$	200-500 nm	C=C, C=O, C=N, C≡C
$n \rightarrow \pi^*$	250- 600 nm	C=O, C=N, N=N, N=O

2.3.3. Infra-Red Spectroscopy

This technique was used for the detection of functional groups in pure compounds, and also for compound comparison. The IR region of wavelength 4000-650 cm^{-1} is useful for the study of organic compounds. The region between1500-600 cm^{-1} is fingerprint region and 4000-1500 cm^{-1} is functional group region. When organic compounds absorb electromagnetic radiation of IR region, it causes atoms to vibrate about the covalent bond. Radiation in this energy range corresponds to stretching and bending vibration of covalent bond. These molecular vibrations lead to a change in the dipole moment of molecule which gives rise to absorption bands in the infra-red. This is the basic principle behind IR spectroscopy.

2.3.4. Nuclear Magnetic Resonance Spectroscopy

The sample was prepared in sample tubes containing deutro chloroform ($CDCl_3$) as solvent, having a small amount of TMS, tetramethyl silane $(CH_3)_4Si$ as a reference compound. It is the most common reference compound added to the sample whose NMR spectrum is to be recorded. TMS gives a single sharp peak and this peak was used as a reference peak for all other peaks in the spectrum. The important features of a proton NMR spectrum are chemical shift, which is the distance of proton absorption from reference peak. relative peak size or area which is directly proportional to the number of hydrogens contributing to the peak, and spin-spin splitting, resulting in multiple peaks caused by nearby hydrogen atoms. These provide detailed information about the number and location of hydrogen atoms in a molecule. By incorporating information gained from carbon-13 magnetic resonance, chemists can often induce an unambiguous structure for a molecule whose molecular formula is known.

2.3.5. Mass Spectroscopy

This technique is used by chemists to characterize structure of organic compounds in two ways. Firstly, to measure the exact molecular weight, second to detect the

fragmentation pattern within the molecule, point at which it prefers to fragment. Mass spectrometry is different from other spectroscopic techniques, because the molecular information provided by the technique does not involve the absorption or emission of electromagnetic radiation. The basic principle of mass spectrometry is that molecules are ionized in gas phase and such ions are detected in a manner which is dependent on their molecular weight. The sample may be ionized in a number of ways depending on the nature of compound to be investigated. The most common technique used is electron impact (EI) mass spectrometry. The sample was bombarded by moving electron resulting in the formation of radical cation called molecular ion (M^+). The charged species was then detected.

The ionization technique used here was electrospray ionization (ESI). The ionized fragment was detected by virtue of molecular mass and its detection was indicated by a line on a chart called as mass spectrum. Mass spectrum records the masses of the fragments together with their relative abundance. The mass spectrum that is recorded shows the mass-to-charge ratio (m/z) along the horizontal axis and ion abundance along the vertical axis. For ions bearing a single positive charge, z equals 1, and the horizontal axis shows the masses of the fragments directly.

2.4. *In Vivo* Anti-Arthritic Activity

2.4.1. Animals

Animals were selected randomly from animal house of Pinnacle Biomedical Research Institute (PBRI), Bhopal, India and further divided into various treatment groups randomly and kept in propylene cage with sterile husk as bedding. Relative humidity of 30.7 % at 22±2°C and 12:12 light and dark cycle was maintained in the animal house and fed with standard pellets (Golden Feeds, New Delhi, India) and water was available *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. Animal experiments were approved by Institutional Animal Ethics Committee (IAEC) of Pinnacle Biomedical Research Institute (PBRI) Bhopal (Reg No. 1824/PO/ERe/S/15/CPCSEA). Protocol Approval Reference No. PBRI/IAEC/PN- 17101.

2.4.2. Acute Oral Toxicity

Healthy Wistar rats, starved overnight were subjected to acute toxicity studies to determine non-observable adverse effect dose level (NOAEL) by acute toxic class method of oral toxicity as per Organization for Economic Co-operation and Development 423 guidelines and three albino rats per step were selected. Two to four steps may be necessary to allow for making a judgment on the acute toxicity of the test substance depending on the mortality and/or the morbidity status of the animals. The test extracts were administered in fixed doses as shown in Figure using oral cannula. The rats (n=3) were administered SC extract in the limit test dose of 2000 mg/kg and observed incessantly for behavioural, neurological and autonomic profiles for 2hr and after a period of 24, 72hr and thereafter up to 14 days for any lethality, moribund state or death. The limit test was repeated in another group of rats (n=3) for corroboration and estimated LD50 determination.

2.4.3. Freund's adjuvant Induced Arthritis Model

Freund's adjuvant induced arthritis model [15] was used to assess the anti-arthritic activity of the methanolic extract of *S. campanulata* in Wister rats. Animals were randomly divided into six groups of six animals each (n=6). Group I animals received (0.01 ml *Freund's* adjuvant) served as an arthritic control, Group II animals received Indomethacine (10 mg.kg-1 p.o.) served as reference standard, Group III and IV animals received the crude methanolic extract of *S. campanulata* (200 and 400 mg/kg^{-1}). The paw volume is an indicator of arthritic condition to assess the anti-inflammatory and anti-arthritic activity of the *S. campanulata*. The extracts were given to the animal 30 minutes before the administration of freund's adjuvant and continued till 28^{th} day. Paw volume was measured on 0^{th}, 7^{th}, 14^{th}, 21^{th} and 28^{th} day by using electronic digital calipers. After 28^{th} days blood samples were collected by puncturing the retro-orbital plexus into heparanized vials and analysed for total leucocyte counts (TLC), differential leucocyte counts (DLC).

2.4.4. MIA-induced OA in rats

OA was induced with 2 mg of MIA in a total volume of 50 µl. Monosodium iodoacetate (Sigma Eldrich, Germany) was injected intraarticularly through the patellar ligament of the right knee, using a 26-gauge needle, while the rat was under anesthesia. The left knee joint (control) was injected with saline. Oral treatment with NA and SC extract (200 and 400 mg/kg given twice a day) was started on day 7 after MIA injection and was continued until the study was terminated on day 22. Another group of rats was administered Indomethacin

(10 mg/kg given twice a day) orally 30 minutes before each extract treatment. The control group was treated with vehicle alone. Each experimental group included six animals, and the experiments were performed 3 times. The diameter of the knees was measured every other day using digital calipers.

2.5. Statistical Analysis

The data is expressed as mean ± Standard Deviation (SD). Results were analyzed using one-way ANOVA followed by Bonferroni test. Differences were considered as statistically significant at $P < 0.05$, $P<0.001$ when compared with control.

3. RESULTS AND DISCUSSION

3.1. Percentage Yield

The plant material was extracted by Hot 'Soxhlation' and the percentage yield calculated by the formula

Yield (%) = (Weight of the residue obtained/Weight of the plant material taken) ×100

The yield of extract of *Spathodea campanulata* was found to be 3.30 % (petroleum ether), 5.3 % (ethyl acetate) and 11.7 % (methanol).

Table 2: Yield of crude extracts of *Spathodea campanulata* flower extract

S. No.	Solvent	% Yield
		Spathodea campanulata
1.	Pet. Ether	3.30
2.	Ethyl acetate	5.3
3.	Methanol	11.7

3.2. Qualitative Phytochemical Screening

Phytochemical testing of petroleum ether, ethyl acetate and methanolic extract of *Spathodea campanulata* was performed. The phytoconstituents were identified by chemical tests, which showed the presence of various constituents. The results of the extract of flower of *Spathodea campanulata* are shown in table. Hence the extracts have been selected for the pharmacological studies.

Table 3: Phytochemical screening of extract of *Spathodea campanulata* (SC)

S. No.	Experiment	Result		
		Pet Ether Extract of SC Flower	Ethyl acetate Extract of SC Flower	Methanolic Extract of SC Flower
1. Alkaloids				
1.1	***Mayer's reagent test***	*-ve*	*+ve*	*-ve*
1.2	Wagner's reagent test	*-ve*	*+ve*	*-ve*
1.3	*Hager's reagent test*	-ve	+ve	-ve
2. Carbohydrates				
2.1	*Molish's test*	*-ve*	*+ve*	*-ve*
2.2	*Barfoed's test*	*-ve*	*+ve*	*-ve*
3. Test for Reducing Sugar's				
3.1	*Fehling's test*	-ve	*+ve*	-ve
3.2	*Benedict's test*	-ve	*+ve*	-ve
4. Flavonoids				
4.1	*Alkaline reagent test*	*-ve*	*+ve*	*+ve*
4.2	*Shinoda test*	*-ve*	*+ve*	*+ve*
4.3	*Lead acetate test*	*-ve*	*+ve*	*+ve*
5. Glycoside				
5.1	*Borntrager test*	*-ve*	*+ve*	*-ve*
5.2	*Legal's test*	*-ve*	*+ve*	*-ve*
5.3	*Killer- Killiani test*	*-ve*	*+ve*	*-ve*
6. Tannin and Phenolic compound				
6.1	*Ferric chloride test*	*-ve*	+ve	*+ve*
6.2	*Lead Acetate test*	*-ve*	+ve	*+ve*
6.3	*Dilute Iodine solution*	*-ve*	+ve	*+ve*
7. Saponin				
7.1	*Faom Test*	*-ve*	*-ve*	*-ve*
8. Test for Proteins and amino acid				
8.1	Ninhydrin test	*-ve*	***+ve***	*+ve*
9. Test for Triterpenoids and Steroids				
9.1	*Salwonski Test*	*-ve*	*-ve*	*+ve*
9.2	*Libberman and Burchard's test*	*-ve*	*-ve*	*+ve*

Phytochemical estimation of methanolic extract of flower of *Spathodea campanulata* showed the presence of flavonoids, proteins and amino acids, tannins and phenolic compounds and triterpenoids and steroids. Ethyl acetate extract showed the presence of alkaloids, carbohydrates, reducing sugars, flavonoids, glycosides, tannins and phenolic compounds, protein and amino acids but in petroleum ether extract all phytoconstituents are absent.

3.3. Percentage yield of isolated compound

The methanolic extract was subjected to isolated through column chromatography and the percentage yield calculated by the formula

Yield (%) = (Weight of the residue obtained/Weight of the plant taken) × 100

The yield of isolated fractions from the extract of *Spathodea campanulata* was found to be 1.24 % (petroleum ether), 2.1 % (ethyl acetate) and 15.5 % (methanol).

Table 4: Yield of isolated fractions from the crude extracts of *Spathodea campanulata* flower extract

S. No.	Solvent	% Yield
		Spathodea campanulata
1.	Pet. Ether	1.24
2.	Ethyl acetate	2.1
3.	Methanol	15.5

3.4. Characterization of active component from *Spathodea campanulata* spectral interpretation

3.4.1. UV Spectra

UV data inform regarding unsaturation & kind of chromophore. The wavelength of absorption above 215 nm indicates presence of unsaturation and confirms presence of flavonoids. Presence of benzene is indicated by three absorption bands at 185, 204, 256 nm. The spectrum for compound isolated showed λ_{max} at 266 nm & 215 nm the compound may be benzene like compound.

3.4.2. IR Spectra

IR Spectrum -3526 broad strong peak O-H stretch, the stretch extended over 3412, 3317, 3222, 3035 may be hydroxyl stretch for acids. 1754: sharp medium intensity peak C=O stretch indicate presence of carbonyl group. 1671: sharp weak peak, C=C stretch presence of unsaturation. 1453: hydroxyl deformation vibration.1320: methyl bending. 1268: C-O Stretch of carbonyl. 1190: C-O-C ethereal stretch. 1069, 1028: out of plane bend (oop) of alkene 986, 864, 824 aromatic substitutions.

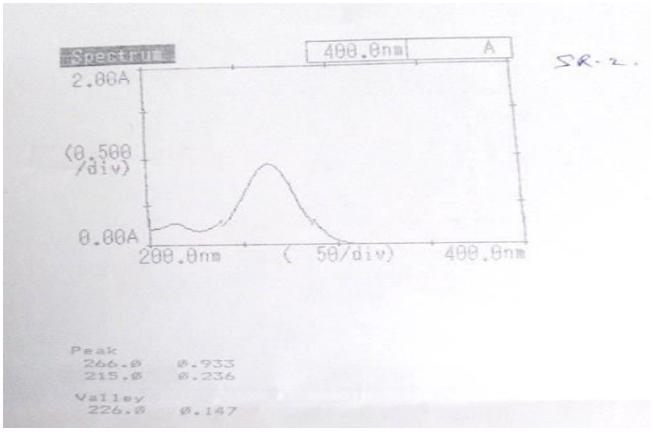


Fig. 1: UV Spectra active component from *Spathodea campanulata*

3.4.3. MASS Spectra

Mass spectrum 353.1914: most intense or base peak. 685.4332: second abundant peak. 633, 493, 437, 353, 301, 103: These fragments when coupled with phytochemical testing may be flavonoid compound since IR data supports broad stretch in Hydrogen stretching region 3536.

3.4.4. Nuclear magnetic resonance (NMR) Spectra

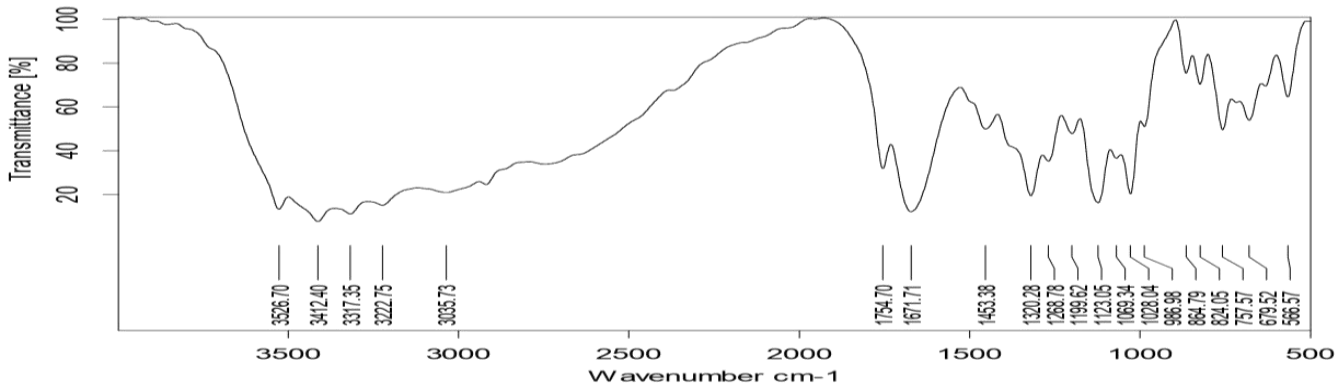
NMR data 0.5 - 1.5: multiplet CH_3 protons, 1.5 - 1.8: intense mutiplet CH_2 protons, 1.8 - 2.0: doublet CH_2 protons, 3.5 - 4.0: doublet may be of CH protons, 5.0 - 5.2: triplet O-H proton. 5.3: triplet of O-H proton, 5.5: multiplet alkene or hydroxyl proton, 7.0: intense peak of aromatic protons NMR data supports presence of polyphenolic multiplets showed coupling interaction.

3.4.5. Impression

All the data supports presence of benzene, sugars and hydroxyl groups. Given data supports the known flavonoid, confirm a selective flavonoid with literature survey of the selected plant & with chemical tests performed. Over all the data supports the isolated compound as flavonoids may be Kempferol.

3.4.6. Probable structure of isolated compound

Kaempferol is a natural flavonol, a type of flavonoid, found in a variety of plants and plant-derived foods including kale, beans, tea, spinach and broccoli. Kaempferol is a yellow crystalline solid with a melting point of 276-278°C. It is slightly soluble in water and highly soluble in hot ethanol, ethers, and DMSO.



Wavenumber	Abs. intensity	Rel. intensity	Width	Found if threshold <	Shoulder
3526.6950	0.132	0.105	470.3137	6.059832	0
3412.3965	0.077	0.932	1159.2672	99.630615	0
3317.3525	0.111	0.053	267.8745	2.668180	0
3222.7527	0.151	0.044	401.4724	1.782019	0
3035.7321	0.209	0.076	658.1123	2.248487	0
1754.6952	0.319	0.178	208.9722	11.736469	0
1671.7049	0.122	0.882	203.2410	93.873009	0
1453.3842	0.498	0.107	404.6203	7.192344	0
1320.2797	0.194	0.406	99.7682	39.320225	0
1268.7799	0.352	0.084	195.1212	2.899374	0
1199.6163	0.478	0.074	28.7262	7.236170	0
1123.0453	0.162	0.724	820.5291	56.538475	0
1069.3397	0.366	0.026	64.7315	1.699613	0
1028.0373	0.202	0.386	211.5563	21.684343	0
986.9832	0.510	0.076	106.8450	2.663068	0
864.7937	0.754	0.141	58.7197	8.588291	0
824.0496	0.704	0.175	28.0899	14.721720	0
757.5717	0.495	0.500	166.5620	53.165874	0
679.5161	0.539	0.178	127.5139	10.457522	0
566.5683	0.646	0.243	37.4363	20.447626	0

Fig. 2: IR Spectrum active component from *Spathodea campanulata*

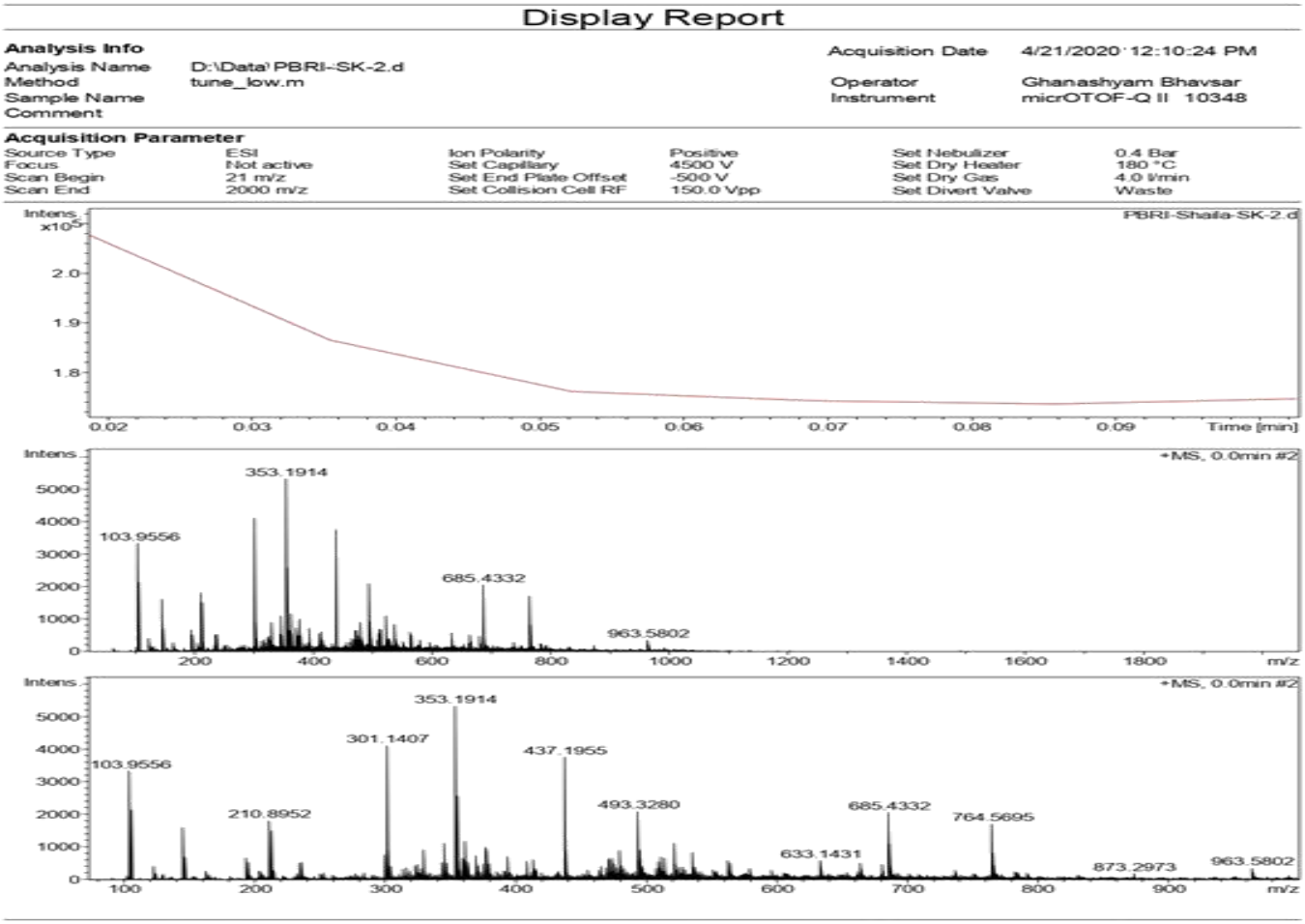


Fig. 3: Mass Spectrum chromatogram of active compound of *Spathodea campanulata* flower extract

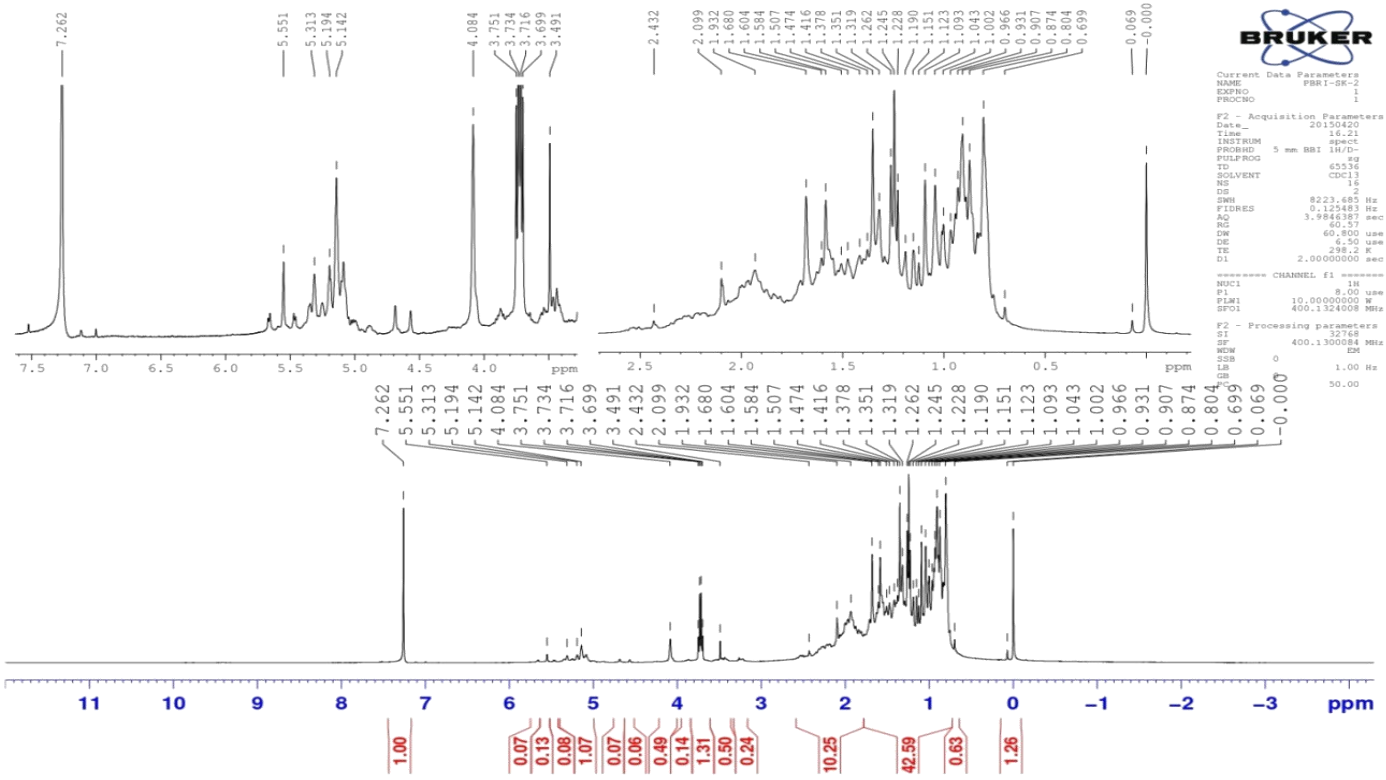


Fig. 4: NMR Spectra of the isolated compound from *Spathodea campanulata*

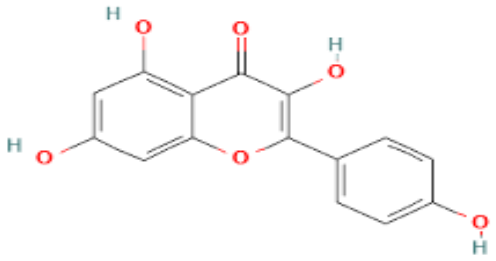


Fig. 5: Probable structure of isolated compound (Kaempferol)

3.5. Acute oral toxicity

Acute oral toxicity study of Methanolic extract of SC and Methanolic extract of NA according to OECD-423 guidelines in mice.

Acute oral toxicity study in Wistar albino rats shows no evidence of significant adverse effect or health risk toxic effects. According to the haematological, biochemical, and organ weight examinations, some parameters differed in rats but none of these appeared to be of toxicological significance, and were slightly higher or lower than those of the controls. Thus, it can be concluded that methanolic extract of SC is virtually non-toxic and showed no lethality at different dosages concentration from 5 mg/kg to 2000 mg/kg. The study results provide an experimental basis for SC extract to be safely used as ingredients of functional foods or pharmaceuticals.

Table 5: Acute oral toxicity study of Methanolic extract of SC according to OECD-423 guidelines

S. No.	Dose	Leathality
		Methanolic extract of SC
1	5 mg/kg	0/3
2	5 mg/kg	0/3
3	50 mg/kg	0/3
4	50 mg/kg	0/3
5	300 mg/kg	0/3
6	300 mg/kg	0/3
7	2000 mg/kg	0/3
8	2000 mg/kg	0/3

3.6. *IN-Vivo* anti-arthritic activity

Rheumatoid arthritis (RA) is a chronic inflammatory disorder characterized by the destruction of bone and cartilage of joints. It affects almost one percent of the world population and mostly at the age of 35-60. So nowadays, people move towards the nature-based medicines to treat RA due to lower toxicity and fewer side effects [16].

Rat adjuvant arthritis model has been widely employed for preclinical testing of anti-arthritic agents. It is a reliable method. The hallmarks of this model are it is easily measurable, with reliable onset and progression, marked bone resorption and proliferation. It allows us to study acute inflammatory reaction as well as immunological reaction that develop approximately 9 days later.

Augmentation in migration of total leukocyte, lymphocytes and monocytes/macrophages from blood into the synovial cavity influence the arthritic condition of joint and these mediators are responsible for the pain, destruction of cartilage and leads to severe disability. In this study, we evaluated the anti-arthritic activity of methanolic extract of the *S. campanulata* against complete freunds adjuvant (CFA) induced arthritic rats. Indomethacin (10 mg.kg-1 p.o.) was used as standard drug for this study it has been widely used in the treatment of RA.

3.6.1. Freund Adjuvant Model

Adjuvant induced arthritis in rats is an established model to study the physiological, biochemical and pharmacological aspects of arthritis. Freund's adjuvant induced arthritis has been used as a model of sub-chronic or chronic inflammation in rats and is of considerable relevance for the study of patho-physiological and pharmacological control of inflammatory processes, as well as the evaluation of analgesic potential or anti-inflammatory effects of drugs. CFA-induced arthritis is a very suitable model for testing anti-arthritic activity as it has a very high degree of validity. Paw swelling is an index of measuring the anti-arthritic activity. There was a significant increase in the paw volume of the arthritic control group induced with Complete Freund's Adjuvant on day 7, 14, 21 & 28 with 1.57, 2.23, 2.89 & 3.06 respectively.

The CFA-induced arthritis model is widely used for evaluating the anti-arthritic activity of drugs.As measurement of pawvolume is an important parameter in the evaluation of arthritis, the paw volume was recorded on 0^{th}, 7^{th}, 14^{th}, 21^{st} and 28^{th}day and the mean values of all the groups are presented in Table 34.The arthritic control group showed significant increase in paw diameter which indicates the sign of arthritis development. The result obtained from table showed that the paw diameter was increased up to 28^{th}day of adjuvant induction. With NA and SC extract treatment (200 and 400 mg/kg) there was significant ($P < 0.05$) decrease in paw diameter compared to arthritic control group on day 14which continued till day 28 which was found to be more effective than Indomethacin treatment.

Table 6: Anti-arthritic activity of *Spathodea campanulata* on rat Paw Diameter in mm

Groups	0 day	7 day	14 day	21 day	28 day
PositiveControl	0.63±0.030	1.57±0.039	2.23±0.044	2.89±0.051	3.06±0.040
Standard (Indomethacin)	0.59±0.05	1.15±0.04	1.33±0.03	1.09±0.03	0.61±0.04
Ext. B 200mg/kg (SC meth)	0.61±0.037	1.22±0.025	1.59±0.041	1.33±0.039	0.89±0.044
Ext. B 400 mg/kg (SC meth)	0.56±0.035	0.59±0.032	0.54±0.019	0.57±0.027	0.56±0.023

n = 6, Values are Mean ± SD Stats

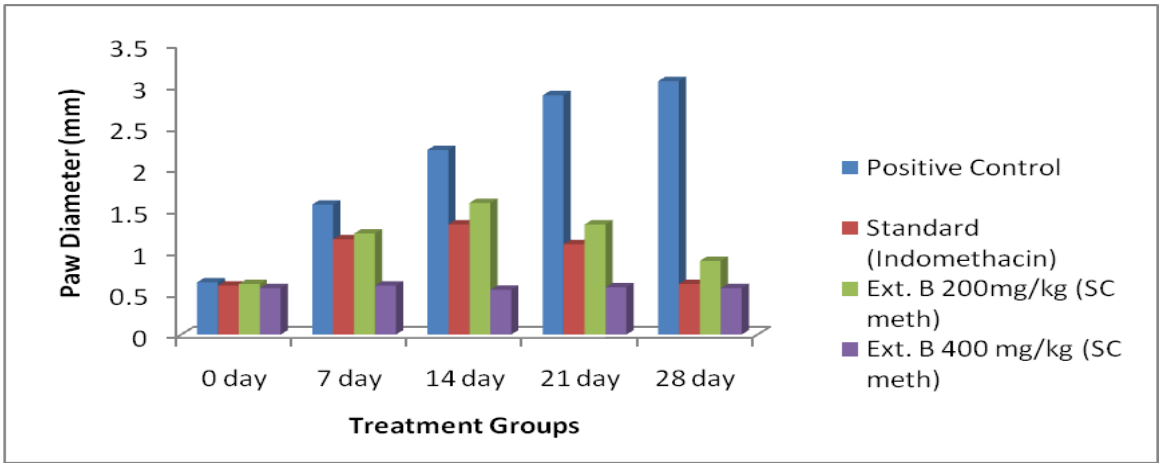


Fig. 6: Anti-arthritic activity of *S. campanulata* on rat paw diameter

Table 7: Anti-arthritic activity of *Spathodea campanulata* on ratHaematology (cell/mm^3)

Groups	TLC	DLC (Neutrophil count)
Positive Control	12268.33±37.071	4369.33±34.558
Standard (Indomethacin)	5780.83±33.67	2227.33±32.02
Ext. B 200mg/kg (SC meth)	6989.50±17.785	2823.50±28.829
Ext. B 400 mg/kg (SC meth)	5543.67±33.494	2076.83±32.102

n = 6, Values are Mean ± SD Stats

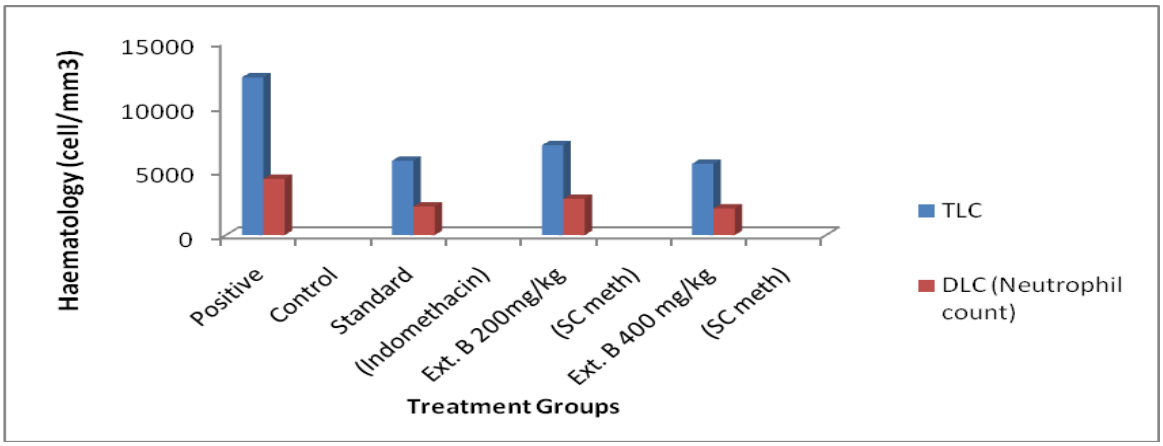


Fig. 7: Anti-arthritic activity of *Spathodea campanulata* on rat Haematology (cell/mm^3)

3.6.2. Osteoarthritis

Osteoarthritis (OA) is a musculoskeletal system disorder following mechanical and biological events that destabilize normal coupling between degradation and synthesis within articular cartilage [17]. Among many types of arthritis, OA is most commonly occurring joint degenerative disorder, characterized by chronic inflammation, pain, joint stiffness and loss of mobility.

3.6.3. Knee Joint Diameter

The effect of oral administration of SC extract (200 and 400 mg/kg) on knee joint diameter is shown in Table 8. Intra-articular injection of Monosodium iodoacetate produced increase in knee joint diameter on day 14 which continued up to day 22. With SC extract treatment (200 and 400 mg/kg) there was significant ($P < 0.05$) decrease in joint diameter compared to disease control group on day 18 which continued till day 22 which was found to be more effective than Indomethacin treatment. The knee joint diameter of animals treated with SC extract was comparable to positive control group on day 22.

A considerable part of the disability caused by arthritis conditions is joint damage. Accordingly, preventing and diminishing joint damage is an important treatment goal in early arthritis. Hence, reliable predictors of joint damage are required. The immunologically mediated complete Freund's adjuvant (CFA) arthritic model of chronic inflammation is considered as the best available experimental model of arthritis as it has been shown to share a number of clinical and immunological features with human arthritis. Method mimics the human pathophysiological state including chronic swelling in multiplejoints due to accumulation of inflammatory cells, joint cartilage erosion, bone destruction and used to investigate the activity of various potent anti-inflammatory and anti-arthritic agents. Therefore, the findings with this model are considered to have higher clinical reproducibility in arthritis.

In CFA model, macrophages play a central role. After activation they are capable of synthesizing mediators such as PGE2 and cytokines such as TNF-α and IL-1 and they induce the production of a variety of enzymes which initiate cartilage and bone destruction. It is also reported that damage to the cartilage in arthritic joint is associated with the cellular output of toxic agents such as nitric oxide and its oxidizing product (e.g. peroxynitrite), free radicals and products of hydrogen peroxide (e.g. hydroxyl radical and hypochlorous acid). Augmentation in migration of total leukocyte, lymphocytes and monocytes/macrophages from blood into the synovial cavity influence the arthritic condition

of joint and these mediators are responsible for the pain, destruction of cartilage and leads to severe disability. In this study, we evaluated the anti-arthritic activity of methanolic extract of the *S. campanulata*. Indomethacin (10 mg.kg-1 p.o.) was used as standard drug for this study it has been widely used in the treatment of RA.

Table 8: Anti-arthritic activity of *Spathodea campanulata* on rat Knee Diameter (mm)

Groups	14 day	16 day	18 day	20 day	22 day
	R - L	R - L	R - L	R - L	R - L
Positive control	1.90±0.022	3.15±0.043	3.97±0.035	4.49±0.047	4.92±0.043
Standard (Indomethacine)	0.82±0.028	1.51±0.038	1.68±0.019	1.26±0.014	0.93±0.018
Ext.B 200 mg/kg (SC meth)	1.47±0.045	2.41±0.031	2.99±0.023	2.66±0.024	2.20±0.018
Ext.B 400 mg/kg (SC meth)	1.27±0.019	2.05±0.030	2.55±0.034	2.24±0.027	1.76±0.019

n = 6, Values are Mean ± SD stats

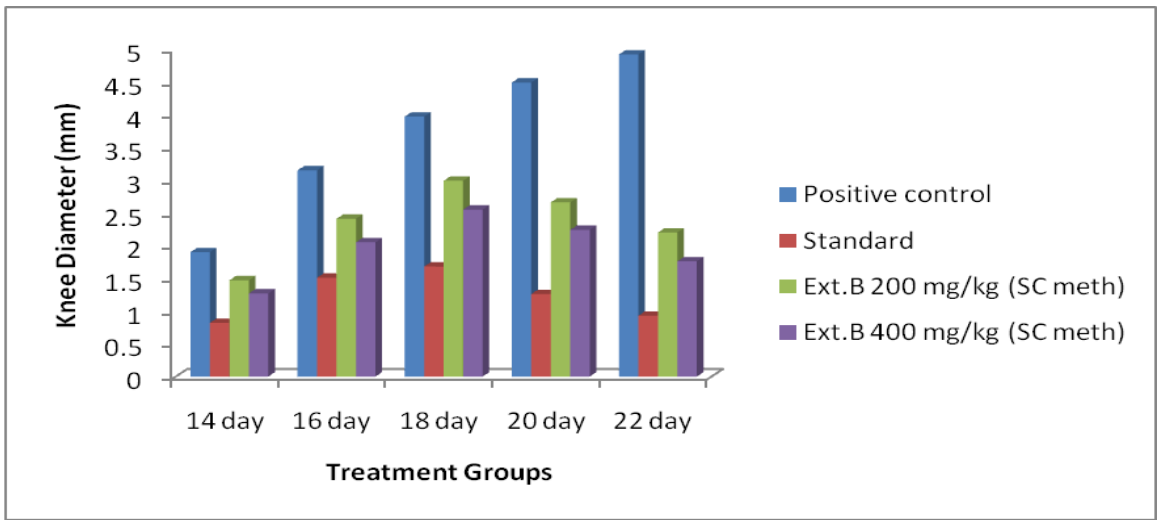


Fig. 8: Anti arthritic activity of *S. campanulata*

3.6.4. Effect on Paw Diameter

Saponins which are glycosides of bothtriterpenes and steroids are widely spread and have been reported to have been found in over seventy plant families[2]; both wild plants and cultivated crops [3, 4].

The CFA-induced arthritis model is widely used for evaluating the anti-arthritic activity of drugs. As measurement of paw volume is an important parameter in the evaluation of arthritis, the paw volume was recorded on 0^{th}, 7^{th}, 14^{th}, 21^{st} and 28^{th} day and the mean values of all the groups are presented in Table. The arthritic control group showed significant increase in paw diameter which indicates the sign of arthritis development. The result obtained from table showed that the paw diameter was increased up to 28^{th} day of adjuvant induction. With SC extract treatment (200 and 400 mg/kg) there was significant ($P < 0.05$) decrease in paw diameter compared to arthritic control group on day 14which continued till day 28 which was found to be more effective than Indomethacin treatment.

4. CONCLUSION

From the present experimental findings of both pharmacological and biochemical parameters observed from the current investigation, it is concluded that at the doses of 200 mg/kg and 400 mg/kg alcoholic extract of *S. campanulata* possesses potentially useful anti-arthritic activity since it gives a positive result in controlling inflammation in adjuvant induced arthritic model in rats. The drug is a promising anti-arthritic agent of plant origin in the treatment of inflammatory disorders.

Conflict of interest

None declared

5. REFERENCES

1. Vingsbo C, Sahlstrand P, Brun JG, Jonsson R, Saxne T, Holmdahl R. *Am J Pathol.,* 1996; **149(5):**1675-1683.
2. Vipin SP, Amit M. *International Journal of Pharmacy and Pharmaceutical Sciences,* 2011; **3(3):**210-212.

3. Laekeman AAG. *Pharmacognosy Journal,* 2016; **8(3):**243-246.
4. Taurog JD, Argentieri DC, McReynolds RA. *Methods Enzymol.,* 1988; **162:**339-355.
5. Thosani R, Pawar V, Giridhar R, Yadav MR. *J Pharm Bioallied Sci,* 2012; **S12-3:**2012.
6. Symmons D, Turner G, Webb R, Asten P, Barrett E, Lunt M. *Rheumatology (Oxford)*, 2002; **41:**793-800.
7. Suresh V. Senthilkumar N. *European Journal of Pharmaceutical and Medical Research,* 2018; **5(3):**295-300.
8. Adia MM, et al. *Journal of Ethnopharmacology,* 2014; **155:**580-588.
9. Adriana P, Jurandir PP, Dalva TF, Noemia KI, Raimundo Braz-Filho. *Agrarian Sciences, Londrina* 2007;**2 8(2):**251-256.
10. Agyare C., et al. *Journal of Ethnopharmacology,* 2009; **125:**393-403.
11. Kilimozhi D, Parthasarathy V, Amuthavalli N. *Int J Pharm Tech Res.,* 2009; **1(4):**1434-41.
12. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy; 23 ed., Nirali Prakashan: Pune: 2006; pp 493-497.
13. Komlaga G, et al. *Journal of Ethnopharmacology,* 2015; **172**:333-346.
14. Ramalingam K, Mathiyalagan A, Subramanian S, Bharathi Rajan UD. *J Biochem Technol.,* 2009; **1:** 108-113.
15. Verma A, Ahirwar AK. *International Journal of Research Studies in Biosciences,* 2015, **3(4):**18-22.
16. Zaheer Z, et al. *African Journal of Pharmacy and Pharmacology,* 2011; **5:**2226-2231.
17. Zhang W, Jones A, Doherty M. *Evidence Based Nursing*, 2005; **8:**21.