Section A-Research Paper
ISSN 2063-5346

In vitro and in vivo evaluation of anti-inflammatory activity and free radical scavenging potential of flowers extract from Sphaeranthus indicus

Madhvi Singh Chauhan[1*], Dr. Swati Yadav[2]

*Corresponding Author

Madhvi Singh Chauhan

[1]Research Scholar, Department of Botany, Dr. A. P. J. Abdul Kalam University, Indore MP 452010

[2]Professor, Department of Botany, Dr. A. P. J. Abdul Kalam University, Indore MP 452010

Email ID: *madhvisinghchauhan25@gmail.com*

Abstract

Sphaeranthus indicus L. is a medicinal plant widely used in Indian traditional system of medicine to cure various illnesses. Geographically, it is distributed throughout India, Sri Lanka, Africa and Australia. It grows in rice fields, dry waste places and cultivated lands in tropical parts of India and survives up to 1200 m altitude. The present study highlights the antioxidant and anti-inflammatory activities from *Sphaeranthus indicus* flowers methanolic and ethyl acetate extract. The *in vitro* antioxidant activity of ethyl acetate and methanolic extract of the flowers was assessed against 1,1-diphenyl-2-picrylhydrazyl (DPPH) and superoxide radical scavenging assay method using standard protocols. The anti-inflammatory activity was evaluated using protein denaturation assay, membrane stabilization assays and carrageenan induced paw edema method on wistar albino rats. The activities of both flowers extract against DPPH and superoxide radical scavenging assay method were concentration dependent. Furthermore, the anti-inflammatory activity was revealed by inhibition of protein denaturation and red blood cell membrane stabilization at concentrations of 100-800 µg/ml and 100-500 µg/ml, respectively. Acute toxicity of the extract (1200 mg/kg) was examined in wistar rats for 14 days. Both extract up to 1200mg/kg did not produce any toxic effects. The studied specimen showed expected dose dependent result in *invivo* anti-inflammatory assay. Methanolic extracts of plant at a dose of 200mg/kg showed highly significant anti-inflammatory activity ($P<0.01$) as compared to control group. It showed maximum percentage reduction in paw edema at 180 min. Methanolic extract of *Sphaeranthus indicus* at the dose of 200mg/kg body weight showed percentage of inhibition of paw edema at 180 min 0.548%. This finding have proved that methanolic extract of *Sphaeranthus indicus* potent anti-inflammatory activity possibly due to

presence of good quantity flavonoid and phenolic content, also have good anti-oxidant property than ethyl acetate extract. Further, investigation pertaining isolation and characterization of active ingredient may provide an insight regarding its phytochemical activity.

Keywords: Sphaeranthus indicus, Antioxidant, Anti-inflammatory activities, DPPH, Superoxide radical scavenging assay, Carrageenan induced paw edema

Introduction

Inflammation is a protective response that eliminates the initial cause of cell injury, weakening, destroying, or neutralizing harmful agents, along with the removal of damaged tissue, and new tissue generation. Inflammation is defined as a hosts' defense response to injury, tissue ischemia, autoimmune reactions, or infectious agents. Signs and symptoms can be prolonged for days or weeks, for instance, in bronchitis, pharyngitis, appendicitis, and dermatitis. Chronic inflammation is detrimental to the system as it represents inflammatory reactions occurring for an extended period [1]. Unabated inflammation can cause chronic diseases such as cancer, diabetes, asthma, heart attack, and arthritis [1]. Inflammation has been described as a response activated by harmful stimuli and other injurious conditions. Moreover, the relationship between oxidative stress induced by free radicals and the inflammatory response has been reported by numerous studies [2]. It is postulated that free radicals and other reactive species generated in living organisms are involved in diseases such as tumors, hepatitis, liver injury, and immunodeficiency disorders [3]. The plants produce several secondary metabolites during their physiological activities like, alkaloids, poly-phenols, phenols, flavonoids, flavonol, terpenoids, and carotenes etc. These secondary metabolites act as therapeutic and prophylactic agents for human health against several diseases and also help in improving the immunity [4]. Isolation, characterization and synthesis of these bioactive phytochemicals are an emerging area of research [5]. These secondary metabolites are potent source of anticancer [6], antioxidant [7], antiviral [8], anti-inflammatory [9] and antimicrobial [10] agents. The active biomolecules are not only distributed differentially on various parts of plant, but also show variation in different developmental stages of the plant. They also exhibit qualitative and

quantitative variations provincially and geographically in their secondary metabolite content and are therefore worth investigating in various regions [11, 12]. *Sphaeranthus indicus* Linn (Asteraceae) popularly known as 'Gorakmundi' is cultivated all over India for its medicinal values [13]. Preliminary phytochemical screening of plant revealed the presence of flavonoids, carbohydrates, alkaloids, gums and mucilage [14]. The herb *Sphaeranthus indicus* is much branched, strongly scented, and erect with branched tapering roots. It is used to treat fever, epilepsy, mental illness,

hemicranias, jaundice, hepatopathy, gastropathy, hernia, diabetes, pectoralgia, cough, hemorrhoids, leprosy, helminthiasis, dyspepsia and skin diseases [15]. Several scientific reports are available on *Sphaeranthus indicus* exhibiting hypotensive, peripheral vasodilatory, cathartic, antimicrobial, nematocidal, larvicidal, antiinflammatory, immunomodulatory, anxiolytic, neuroleptic, antioxidant, antihyperglycemic, analgesic, antipyretic, mast cell stabilizing action , renoprotective, hepatoprotective, antiviral, macrofilaricidal, sedative, bronchodilatory and antihyperlipidemic activities[16]. However, to the best of our knowledge, no study had been carried out on the antioxidant and anti-inflammatory potentials of methanolic and ethyl acetate flowers extract of *Sphaeranthus indicus*. Therefore, the aim of this study was to investigate the antioxidant and anti-inflammatory potentials of methanolic and ethyl acetate flowers extract of *Sphaeranthus indicus* using *in vitro* and *invivo* methods.

Materials and Methods

Plant material

The Pinnacle Biomedical Research Institute (PBRI), near the Bharat Scout and Guides Campus, Shanti Marg, Shyamla Hills Road, Depot Chouraha, Bhopal, Madhya Pradesh 462003, India, collected the flowers of *Sphaeranthus indicus*. Botanist Dr. Saba Naaz from the Saifia College of Science in Bhopal's Department of Botany carried out the plant's identification and authentication. For future use, a voucher specimen with the number 310/Saif./Sci./Clg/Bpl for *Sphaeranthus indicus* was conserved in the department of botany at Saifia College of Science, Bhopal.

Chemical reagents

The Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Chem. Ltd. (Mumbai, India), and SRL Pvt. Ltd. (Mumbai, India) provided all the chemicals used in this work. The investigation only employed analytical-grade compounds.

Hot soxhlet extraction method

This technique involved gathering, correctly washing, and properly rinsing the blossoms of *Sphaeranthus indicus*. They were mechanically pulverised after being shade-dried. The plant material from *Sphaeranthus indicus*, either whole or coarsely powdered, was successively extracted using solvents such as petroleum ether, ethyl acetate and methanol in increasing polarity order for various lengths of time. The Soxhlet apparatus' chamber was filled with powder using a "thimble" design. The solvent used for extraction was heated in flasks, and its vapours were then condensed in a condenser. The powder is extracted by touch when the condensed extractant is dropped into the thimble holding it. The liquid inside the chamber syphon drops into the flask when the liquid level in the chamber reaches the top of the syphon tube. This procedure was continued until an

evaporated drop of solvent from the syphon tube did not leave any residue. The resulting extract was filtered, dried by concentration, weighed, and stored for later use [17]. The following formula is used to determine the extract's yield.

$$\textbf{Yield (\%)} = \frac{\text{Weight of the residue obtained} \times 100}{\text{Weight of the plant material taken}}$$

Antioxidant activity

DPPH radical scavenging activity

For DPPH assay, the method of Gulçin *et al.,* 2006 [18] was adopted. A solution of 0.1mM DPPH (4mg/100ml) in methanol was prepared and 1 ml of this solution was mixed with 1 ml of different concentrations of the different extracts. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. Ascorbic acid was used as reference standard while methanol was used as control. Reduction of the stable DPPH radical was used as a marker of antioxidant capacity of *Sphaeranthus indicus* extracts. The change in colour was measured at 517 nm wavelength using methanolic solution as a reference solution. This was related to the absorbance of the control without the plant extracts. The percentage inhibition of free radical DPPH was calculated from the following equation: % inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%. All the tests were carried out in triplicates. Though the activity is expressed as 50% inhibitory concentration (IC50), IC50 was calculated based on the percentage of DPPH radicals scavenged. The lower the IC50 value, the higher is the antioxidant activity.

Superoxide radical scavenging activity

The reason behind this assay was the ability to prevent the reduction of nitro blue tetrazolium (NBT) in the NBT system [19]. For calculation of superoxide dismutase activity, a method developed by Martinez et al. was used with a little modification [20]. Each 3 ml reaction mixture comprised of 50 mM sodium phosphate buffer, pH 7.8, 13 mM methionine, 2 mM riboflavin, 100 mM EDTA, NBT (75 mM) and 1 ml sample solution. The formation of blue colour formazan was accompanied by perceptive increase in absorbance after 10 min lighting from a fluorescent lamp at 560 nm. The entire reaction assembly was surrounded within a box, covered with aluminium foil. Tubes with reaction mixture were kept in the dark which served as blanks.

% inhibition = [(Absorbance of control – Absorbance of test sample)/ (Absorbance of control)] × 100

***In-vitro* anti-inflammatory activity**

Protein denaturation assay

The *in-vitro* anti-inflammatory activity was determined by protein denaturation assay using bovine

serum albumin as the protein. Proteins are required for structural integrity of our body. Denaturation is phenomenon that involves transformation of well defined, folded structure of protein formed under physiological condition, to an unfolded state under non-physiological condition. When we heat bovine albumin (protein) solution, it leads to denaturation which increases solubility of the protein upon cooling its solubility decreases hence turbidity increases which can be measured spectrophotometrically. Denaturation of tissue proteins is one of the well-documented causes of inflammatory diseases. Production of auto antigens may be due to denaturation of proteins in vivo. Agents that can prevent protein denaturation therefore, would be worthwhile for anti-inflammatory drug development. Bovine albumin was weighed and 1% solution was made by dissolving initially in Dimethylformamide then diluted with phosphate buffer pH 7.4. Similarly extracts were weighed and diluted to get concentration range of 100- 800µg/ml of extract solution in phosphate buffer for all the extracts.1 ml of extracts solutions were mixed with 1 ml of albumin solution. The mixture was incubated for 15 minutes at 27º C. It was then kept for 10 min at 60 º C and then cooled and spectrophotometric determinations were made at 660nm. % Inhibition was calculated by the formula. (Absorbance of Control- Absorbance of Test) / Absorbance of Control * 100. Results were compared with Diclofenac sodium as standard.

Hypotonic solution-induced haemolysis or membrane stabilizing activity

This test was done according to the method described by Shinde et al., 1999 [21] with slight modifications. The test sample consisted of stock erythrocyte (RBC) suspension 0.030ml mixed with 5ml of hypotonic solution (154mM NaCl in 10mM Sodium Phosphate Buffer at pH 7.4) containing Herbal Preparation (*Sphaeranthus indicus*) ranging from concentration 100-500µg/ml. The control sample consisted of 0.030ml RBC suspension mixed with hypotonic buffered solution alone. The standard drug acetylsalicylic was treated similar as test at 50-250µg/ml concentrations. The experiment was carried out in triplicate. The mixtures were incubated at 10 minutes at room temperature, centrifuged for 10 minutes at 3000rpm and absorbance of the supernatant was measured spectrophotometrically at 540 nm. The percentage inhibition of haemolysis or membrane stabilization was calculated by following equation.

$$\% \text{ Inhibition of haemolysis} = 100 \times [A\ 1 - A\ 2 / A\ 1]$$

Where:

A 1 = Absorbance of hypotonic buffered solution alone

A 2 = Absorbance of test /standard sample in hypotonic solution.

In-vivo anti-inflammatory activity

Acute toxicity test

Acute toxicity study of the prepared flowers extracts was carried out according to the Organization

for Economic Co-Operation and Development (OECD) Guidelines-423. The animals were fasted for 4 h, but allowed free access to water throughout. As per the OECD recommendations, the starting dose level should be that which is most likely to produce mortality in some of the dosed animals; and when there is no information available on a substance to be tested in this regard; for animal welfare reasons, three animals of single sex are used for each step. Animals were divided randomly into a control and six treatment groups, each group consisting of five rats. Control group received only the vehicle & each treatment group received orally the methanol and ethyl acetate extracts of the studied plant in a dose of 100, 200, 400, 800 and 1200 mg/kg. Animals were kept under close observation for 4 hours after administering the extract and then they were observed daily for three days for any change in general behavior and/or other physical activities [22].

Animals

In the present investigation the Wistar rats (150-180 gm) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2°C, 55-65%). Rats received standard rodent chow and water *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. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India. The registration number for the Institutional Animal Ethical Committee is (Reg. No. 1824/PO/RcBi/S/15/CPCSEA), and the animal experiment proposal number is IAEC- PBRI/IAEC/PN-21118.

Grouping of animals

Animals were further divided in four groups with six animals in each group

Group I: Normal control (Carrageenan 1%w/v)

Group II: Positive control (indomethacin 10mg/kg, i.p.)

Group III: Rats received *S. indicus* ethyl acetate extract (200mg/kg of bw)

Group IV: Rats received *S. indicus* methanolic extract (200mg/kg of bw)

Carrageenan induced rat paw edema

The anti-inflammatory activity of the test compounds were evaluated in Wistar rats employing the method. Animals were fasted overnight and were divided into control, standard and different test groups each consisting of five animals. The different test extracts were administrated to the animals in the test groups at the dose of 200mg/kg by oral route. Animals in the standard group received Indomethacin at the dose of 10 mg/kg, by oral route. Control group animals were received 1% DMSO at the dose of 10ml/kg body weight. Thirty minutes after administration of the respective

ISSN 2063-5346

drugs, all the animals were challenged with 0.1 ml of 1% carrageenan in the sub planter region of left hind paw. Paw volume was measured by using digital plethysmometer before administration of carrageenan and after 30, 60, 120 & 180 min intervals. The efficacy of different drug was tested on its ability to inhibit paw edema as compared to control group [23]. The percentage of inhibition of paw-edema is calculated by

$$\% \text{ inhibition of paw edema} = \frac{C-T}{C} \times 100$$

Where, C = increase in paw volume of control group; T = increase in paw volume after administration of extracts

Statistical evaluations

All results are expressed as mean ±S.D. Statistical evaluation was done using one-way analysis of variance (ANOVA), followed by Student's *t-test.*

Results and Discussions

After completing each successive Soxhlet extraction step, the crude extracts were concentrated on a water bath by totally evaporating the solvents to obtain the real extraction yield. In phytochemical extraction, the percentage yield of extraction is crucial for assessing the standard extraction efficiency for a certain plant, other portions of the same plant, or various solvents utilized. Table 1 shows the yield of extracts made from plant flowers using petroleum ether, ethyl acetate and methanol as solvents. DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method is an antioxidant assay based on electron-transfer that produces a violet solution in methanol. This free radical remains stable at room temperature and gets decreased in the presence of an antioxidant molecule, which give rise to colorless methanol solution**.** The scavenging activity of extracts and standard on the DPPH radical expressed as IC50 value of methanol was 20.69, ethyl acetate was 65.98 and ascorbic acid was 11.80. IC_{50} value of methanolic extract was effective and close to ascorbic acid which is a well-known antioxidant Table 2. Superoxide that is the one-electron reduced form of molecular oxygen, is a precursor of other ROS such as hydrogen peroxide, hydroxyl radical, and singlet oxygen that have the potential of reacting with biological macromolecules and there by inducing tissue damages and also it has been implicated in initiating oxidation reactions associated with aging Table 3. 90 % inhibition was shown by standard drug Diclofenac sodium at the concentration of 50 µg/ml. similar treatment was given with extracts. The methanolic extract of *Sphaeranthus indicus* showed dose dependent inhibition of protein denaturation. In concentrations as high as 800µg/ml the % inhibition was found to be 88.94 %.Inhibition of protein denaturation up to 79% at concentrations 800µg/ml were found in ethyl acetate extracts of *Sphaeranthus indicus* Table 4. For hypotonic solution induced hemolysis, at

concentration range of 100–500µg/ml, the extract showed significant inhibitory effect against RBCs hemolysis. The inhibition of hemolysis was found to be dose dependent, increasing with increased concentration of the extract in the medium and was comparable with that obtained for acetylsalicylic Table 5 & 6. No adverse effect or mortality was detected in albino rats up to 1200 mg/kg bw of methanol and ethyl acetate extracts of *Sphaeranthus indicus.* 24 hr observation periods basing on which the respective doses are selected for further study Table 7 & 8. During the search for anti-inflammatory efficacy of selected extract of the plant using Carrageenan induced rat paw edema method, it was quite evident that, a gradual increase in paw volume was observed after carrageenan administration and which reached maximum time period and then declined. The standard drug Indomethacin at a dose level of 10mg/kg body weight showed highly significant activity ($P< 0.01$) as compared to control group at 30, 60, 120 and 180 min. Methanolic extracts of *Sphaeranthus indicus* at a dose of 200mg/kg showed highly significant anti-inflammatory activity ($P<0.01$) as compared to control group at 30, 60, 120 and 180 min respectively. The ethyl acetate extracts also showed significant activity ($P<0.01$) at 30, 60, 120 and 180 min. The standard drug Indomethacin at a dose of 10mg/kg body weight inhibited the development of edema significantly from 30 min onwards. It showed maximum percentage reduction in paw edema at 180 min. Methanolic extract of *Sphaeranthus indicus* at the dose of 200mg/kg body weight showed percentage of inhibition of paw edema at 180 min 0.548% Table 9.

Table 1: Results of % yield from the various extracts acquired from *Sphaeranthus indicus*

S. No.	**Solvent**	**% Yield (w/w)**
1.	Pet. ether	2.0%
2.	Ethyl acetate	0.90%
	Methanol	6.5%

Table 2: DPPH assay of ascorbic acid, ethyl acetate and methanolic extract of *Sphaeranthus indicus*

S. No.	Conc. (µg/ml)	Ascorbic acid (% Inhibition)	Ethyl acetate Extract (% Inhibition)	Methanolic Extract (% Inhibition)
1.	**20**	55.04	36.46	50.44
2.	**40**	59.65	40.71	55.44
3.	**60**	65.13	49.73	58.65
4.	**80**	75.75	53.81	64.88
5.	**100**	84.25	60.35	71.66
IC 50 Value		**11.80**	**65.98**	**20.69**

Table 3: Superoxide radical scavenging activity of extract of *Sphaeranthus indicus*

Superoxide radical scavenging activity			
Concentration	**% Inhibition**		
	Ascorbic acid (std.)	**Ethyl acetate extracts**	**Methanolic extracts**
20 µg/ml	22.67	16.33	20.33
40 µg/ml	45.33	36.00	41.67
60µg/ml	55.00	44.67	51.33
80µg/ml	70.67	54.33	63.33
100µg/ml	83.00	65.33	74.00

Table 4: Protein denaturation assay of extracts of *Sphaeranthus indicus*

Concentration in µg/ml	Absorbance at 660 nm of methanolic extract	%Inhibition (n=3) by methanolic extract	Absorbance at 660 nm of ethyl acetate extract	% Inhibition (n=3) by ethyl acetate extract
100	0.69	30.65±1.2	0.73	26.13±0.010
200	0.45	54.77±1.05	0.52	47.03±0.8
400	0.33	66.83±0.9	0.42	57.08±0.7
600	0.25	74.87±1.03	0.25	74.27±0.7
800	0.11	88.94±1.2	0.2	79.29±0.8

Table 5: *In-vitro* anti-inflammatory effect of standard evaluated by hypotonic solution – hemolysis

Concentration	Absorbance of Sample	% Inhibition of hemolysis
50	0.802	34.744
100	0.738	39.951
150	0.68	44.670
200	0.571	53.539
250	0.427	65.256

Table 6: *In-vitro* anti-inflammatory effect of sample *Sphaeranthus indicus* evaluated by hypotonic solution –hemolysis

Concentration	Absorbance of Sample	% Inhibition of hemolysis
100	1.045	14.972

200	0.986	19.772
300	0.733	40.358
400	0.608	50.529
500	0.558	54.597

Table 7: Acute toxicity study on *Sphaeranthus indicus*

Group	Extract				
	No. of rats	**Death**	**Dose difference (mg)**	**Mean death**	**Dose difference X death**
Group I	6	0	0	-	-
Group II	6	0	100	-	-
Group III	6	0	200	-	-
Group IV	6	0	400	-	-
Group V	6	0	800	-	-
Group VI	6	0	1200	-	-

Table 8: Signs and symptoms of *Sphaeranthus indicus* extract toxicity on rats

Group (Dose)	Signs & symptoms (No. of animals)	Score
Group I	Iritability (0) Laboured breathing (0) Staggering (0) convulsion (0) Death (0)	Normal
Group II	Iritability (0) Laboured breathing (0) Staggering (0) convulsion (0) Death (0)	Good/Normal activity seen
Group III	Iritability (0) Laboured breathing (0) Staggering (0) convulsion (0) Death (0)	Good/Normal activity seen
Group IV	Iritability (0) Laboured breathing (0) Staggering (0) convulsion (0) Death (0)	Good/Normal activity seen
Group V	Iritability (0) Laboured breathing (0) Staggering (0) convulsion (0) Death (0)	Good/Normal activity seen
Group VI	Iritability (0) Laboured breathing (0) Staggering (0) convulsion (0) Death (0)	Poor/Normal activity seen

Table 9: Evaluation of anti-inflammatory activity of methanol and ethyl acetate extracts of *Sphaeranthus indicus* (% of paw edema volume)

Group	Dose	Paw edema volume in ml as measured by mercury displacement at				
		0 min	**30 min**	**60 min**	**120 min**	**180 min**
I	0.1 ml	0.529 ± 0.004	0.596 ± 0.006	0.745 ± 0.004	0.850 ± 0.006	1.21 ± 0.032
II	10 mg/kg	0.426 ± 0.004	0.468 ± 0.009	0.520 ± 0.008	0.527 ± 0.002	0.419 ± 0.007
III	200 mg/kg	0.424 ±	0.480 ±	0.534 ±	0.590 ±	0.419 ±

		0.003	0.008	0.006	0.007*	0.007*
IV	200 mg/kg	0.391 ± 0007	0.427 ± 0.005	0.488 ± 0.001	0.522 ± 0.002*	0.548 ± 0.001*

P values: * * P<0.01; * P< 0.05

Conclusion

The results described in this study demonstrate that the *Sphaeranthus indicus* is presented as a promising source of molecules with antioxidant and anti-inflammatory activities and corroborates ethanopharmacological data. Furthermore, they encourage the continuation of experimental works to identify the compounds responsible for this activity.

References

1. D. Laveti, M. Kumar, R. Hemalatha, et al., Anti-inflammatory treatments for chronic diseases: a review, Inflamm. Allergy - Drug Targets 12 (2013) 349e361.
2. M. Allegra, Antioxidant and anti-inflammatory properties of plants extract, Antioxidants (Basel) 8 (2019), 549.
3. Sinmisola, B.M. Oluwasesan, A.P. Chukwuemeka, Blighia sapida K.D. Koenig: a review on its phytochemistry, pharmacological and nutritional properties, J. Ethnopharmacol. 235 (2019) 446e459.
4. Kang S, Min H. Ginseng, the 'Immunity Boost': The Effects of Panaxginsengon immune System. J Ginseng Res. 2012;36:354–68.
5. Mohammadi A, Nazari H, Imani S, Amrollahi H. Antifungal activities and chemical composition of some medicinal plants. J MycoMed. 2014;24:e1–8.
6. Cragg GM, Newman DJ. Plants as source of anti-cancer agents. J Ethnopharmacol. 2005;100:72–9.
7. Krishnaiah D, Sarbatly R, Bono A. Phytochemical antioxidants for health and medicine – A move towards nature. Biotechnol. Mol. Biol. Rev. 2007;1(4):97–104.
8. Orhan DD, Özçelik B, Özgen S, Ergun F. Antibacterial, antifungal, and antiviral activities of some flavonoids. Microbiol Res. 2010;165:496–504.
9. Bazylko A, Granica S, Filipek A, Piwowarski J, Stefańska J, Osińska E, Kiss AK. Comparison of antioxidant, anti-inflammatory, antimicrobial activity and chemical composition of aqueous and hydroethanolic extracts of the herb of Tropaeolummajus L. Ind Crops Prod. 2013;50:88–94.
10. Saranraj P, Sivasakthi S. Medicinal Plants and its Antimicrobial Properties: A Review. Global J Pharmacology. 2014;8(3):316–27.

11. Ali BH, Blunden G, Tanira MO, Nemmar A. Some phytochemical, pharmacological and toxicological properties of ginger (Zingiber officinale Roscoe): A review of recent research. Food ChemToxicol. 2008;46:409–20.
12. Capasso A. Antioxidant action and therapeutic efficacy of Allium sativum L. Molecules. 2013;18:690–700.
13. Warrier P K, Nambiar V P K & Raman Kutty C, in Indian Medicinal Plants, Vol. I, (Orient Longman, Hyderabad, India) 2004, 180.
14. Kokate C K, Practical pharmacognosy, 1st ed, (Vallabh Prakashan, New Delhi, India) 1986a, 15.
15. Kirtikar KR, Basu BD. ICS. Indian medicinal plants. In: Blatter E, Caius JF, Mhaskar KS, editors. vol. 2. 2nd ed. Allahabad India: Lalit Mohan Basu; 1981. p. 1346–8.
16. Tandon D, Gupta AK. Comparative assessment of antimicrobial and antioxidant activity between whole plant and parts of Sphaeranthus indicus Linn.(Asteraceae). Clinical Phytoscience. 2020; 6(1):1-5.
17. Pradhan A, Jain P, Pal M, Chauhan M, Jain DK. Qualitative and quantitative determination of phytochemical contents of hydroalcoholic extract of Salmalia malabarica. Pharmacologyonline 2019; 1:21-6.
18. Gulçin I, Elias R, Gepdiremen A, Boyer L. Antioxidant activity of lignans from fringe tree (Chionanthus virginicus L.). Eur Food Res Technol 2006; 223: 759-767.
19. Beauchamp C, Fridovich I. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem 1971; 44(1):276-87.
20. Martinez CA, Loureiro ME, Oliva MA, Maestri M. Differential responses of superoxide dismutase in freezing resistant Solanum curtilobum and freezing sensitive Solanum tuberosum subjected to oxidative and water stress. Plant Sci 2001; 160:505-15.
21. Shinde U A, Phadke AS, Nair AM, Mugantiwar AA, Dikshit VJ and Saraf VO (1999).Membrane stabilizing activity –a possible mechanism of action for the anti-inflammatory activity of *Cedrus deodara* wood oil. *Fitoterapia*70 (3) 251-257.
22. Guideline Document on Acute oral Toxicity Testing, Series on Testing and Assessment No. 423. Paris: Organization for Economic Co-Operation and Development, OECD Environment, Health and Safety Publications; 1996. Available from: http//www.oecd.org/ehs.
23. Winter CA, Risley EA., Nuss GW. Carrageenan-induced edema in the hind paw of rat as an assay for anti-inflammatory activity. Proc Soc Exp Biol Med, 1962; 111: 544-547.