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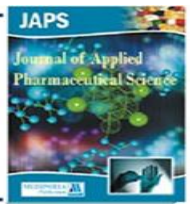

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Pharmacological Study of a Siddha Holistic Herb *Sivakaranthai - Sphaeranthus Amaranthoides* Burm for Analgesic and Anti-Inflammatory activities

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ABSTRACT

Sphaeranthus amaranthoides Burm belonging to the family Asteraceae is a rejuvenator herb of *Siddha* system having Tamil name '*Sivakaranthai*'. The present studies deals with a detailed pharmacological including pharmacognostical study particularly on leaf and infloresence of *Sphaeranthus amaranthoides*. The morphological characters of leaf and inflorescence observed by double staining. It revealed the stomatal index of lower surface with 42-48 and upper surface with 30-36/mm^2 and presence of leaf resin canals. Inflorescences are cone shaped and its axis is siphonate. *Sivakaranthai* leaf powder (SLP) subjected to quality control test and by phytoconstituents estimation, appreciable presence of calcium, ferrous, tannin, proteins and phenols known. The results of elemental concentration level indicated the presence of toxic metals within the tolerance level. *In vitro* antibacterial activity evaluation confirmed the good anti-microbial activity at the dilution of 50 µl/disc against bacterial strain such as *Streptococcus mutans, Staphylococcus aureus, Escherchia coli Klebsiella pneumonia, Pseudomonas aeruginosa*. Rats found safe up to a maximum dose of 2000mg/kg body weight in acute toxicity study following OECD 423 guidelines. The analgesic and anti-inflammation activities evaluation were done by tail flick hot water immersion method and by Carrageenan induced acute hind paw oedema method on Wistar albino rats, respectively. The SLP has shown very weak analgesic and moderate anti-inflammatory action rather than standard Indomethacin and no adverse effects produced. In the light of above results, it concluded that *Sphaeranthus amaranthoides* might use in any suitable formulation in which anti-microbial property and micronutrients needed.

Keywords: *Sphaeranthus amaranthoides*, pharmacognosy, phytoconstituents, anti-bacterial, acute toxicity, analgesic, anti-inflammation.

INTRODUCTION

As per Bentham and Hooker Classification, *Sphaeranthus amaranthoides* belongs to Plant kingdom, Dicotyledon class, Gamopetalae sub class, Inferae series, Asterales order, Asteraceae family. It's a weed of paddy field of Southern India particularly in Tanjore, Thirunelveli, Southern Mysore and Travancore area (Nadkarne 2010 & Murugesha 2008). *Features of this herb*: Low annuals with spreading branches. Stem – erect, glabrous, sometimes as thick as the little finger, but short, branches, not winged and 8 to 12 inches. Leaves - 2 to 4 inches, linear – oblong narrowed at the base, decurrent, obtuse, serrulate, alternate and toothed. Heads – ½ to 1 inches, clusters, small, solitary ovoid in terminal which are usually involucrate by a bracts imbricating spinescent, sub sessile on a common receptacle.

Outer Flora few or many fertile, slender, minutely 2 – 3 toothed. Disc Flora – +(o) solitary or few, fertile or sterile, tube thickened, limb 4 – 5 toothed. Involucre – narrow, Bracts - narrow, acute, dry, unequal Receptacle – small, naked. Anther – bases sagittate. Auricles – acute or tailed, style arms of filiform. Achenes - oblong, subcompressed Pappus – o. This plant is known for the treatment of eczema, blood disorders and helminthiasis (Krithikar, 1971). Swarnalatha *et al*., (2009) attributed the Phenolic compound in this, responsible for its antibacterial and antidiarrhoeal activities. In Siddha system of medicine, Kalpa drug plays a vital role among therapeutics. Kalpa drug meant for rejuvenation therapy eradicates the infective organisms from our body and prevents further by developing an immune response. It also prevents the destruction and aging of the soft parts, muscles, nerves, bones, bone marrow, blood cells etc. *Sphaeranthus amaranthoides* belongs to one of the Kalpa drugs, which been used as an ingredient in certain Siddha polyherbal formulation possessing antioxidant property. Eventhough, the entire part of this herb possess therapeutic value, leaves has more value. The plant has astringent and mild hot taste and hot potency. These tastes get biotransformation into hot taste after absorption. The plant has aromatic, astringent, stomachic, antispasmodic, emmenagogue and diuretic properties. *Theran* and *Agathiar Gunavagadams* have illustrated its use in treating *Vadha* diseases, Eczema, vomiting, abdominal discomfort, increase the semen consistency and make the body shine (Murugesha 2008). The juice of leaves has the properties of detoxification of Mercury. The effects of this drug while consuming is illustrated only in the Siddha literature under Tamil name *Sivakaranthai* and so much praised as a holistic herb by *Bhogar* (one among eighteen Siddhars) in *Bhoga munivar Karpam* 300 (Murugesha 2008). Hence, great interest has been created towards this Kalpa herb. Up to date no pharmacognosy and pharmacological study been systematically conducted to evaluate the anti-inflammatory and anti-nociceptive action of *Sphaeranthus amaranthoides*, supporting traditional uses of this plant in Siddha medicine. Hence, the present study was undertaken to evaluate macroscopic and microscopic features of leaf and inflorescence, physico chemical parameters, *in vitro* antimicrobial activity, *in vivo* acute toxicological study and *in vivo* anti- inflammatory, and analgesic activity.

MATERIALS AND METHODS

Plant collection

The leaves and inflorescences of *Sivakaranthai (Sphaeranthus amaranthoides)* were collected during the season of March from the Paddy fields around Shankaran Koil area, Thirunelveli dt., Tamil Nadu, India. The identification was authenticated by the botanist Dr. Sasikala, Dept of Botany, Central Research Institute for Siddha, Chennai.

Drugs and Chemicals

Indomethacin and fine chemicals used in efficacy experiments were obtained from Sigma Chemicals Company, St. Louis, U.S.A. Other analytical grade chemicals were obtained from S.D Fine Chemicals Ltd., Mumbai.

Morphological studies

Free hand as well as microtome sections of the leaves and inflorescences of *Sivakaranthai* were double stained. Alcoholic safranin (0.5%) counter stained with 0.25% fast green. All slides, after staining in safranin were dehydrated by employing graded series of ethyl alcohol (30%, 50%, 70%, 90% and absolute alcohol) and stained fast green in clove oil and xylol-alcohol (50-50) and passed through xylol and mounted in DPX mountant. For studying stomatal number and stomatal index, clearing of leaves were done by using 5% sodium hydroxide along with chlorinated soda solution supplemented with gentle heat. Quantitative microscopy was carried out and values were determined as per the procedure given in Wallis (2005). Photomicrographs were taken with the help of Nikon Eclipse E200 Microscope.

Preparation of test drug

Sivakaranthai leaf powder (SLP) was prepared by the method described in pg no.229, Siddha Materia Medica - Medicinal Plants Division (Murugesha 2008). The dried leaves of *Sivakaranthai* were made into very fine powder by grinding in pulverizer and filtered through the mesh of the sieve size no. 125 and preserved in an air tight container. The expiry period of SLP has been for 3 months. It should be used within that period.

Physico-chemical analyses

The shade dried part of *Sphaeranthus amaranthoides* was subjected for determination of physicochemical parameters according to standard methods adapted by WHO. TLC of SLP was carried out according to the method described in "The Ayurvedic Pharmacopoeia of India". The plate was developed in Toluene: Ethyl acetate 5: 1.5. The plate was subsequently visualized in UV 254 and 366 nm. The plate was then dipped in Vanillin - Sulphuric acid (Spray reagent) and heated in air oven at 105ºC till the spots appeared.Aqueous extract of SLP was used for the qualitative analysis of acidic/basic radicals and phytochemical constituents based on Sofowora (1993); Trease and Evans (2002). Quantitative estimation of heavy metal contents were done by Atomic Absorption Spectrometer (AAS) – Make: Varian, Australia

Anti-bacterial activity

This activity was carried out by paper disc diffusion method following Ashok Rathan (2000). The average number of viable *Streptococcus mutans, Staphylococcus aureus, Escherchia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* organisms per ml of the stock suspensions were determined by means of the surface viable counting technique (Miles *et al*., 1938).

Experimental animals

Wistar strain albino rats of weighing 120-175g of either sex were used for the pharmacological and toxicological studies. The animals were kept under standard conditions and fed on standard pelleted diet (Hindustan Lever Pvt Ltd., Bangalore) and tap water ad libitum. The experimental protocol was approved by IAEC/XIV/19/CLBMCP/24-07-07 of CL. Baid Metha College of

Pharmacy. Acute oral toxicity was conducted as per the OECD guidelines 423-2001 (Acute Toxic Class Method) and Ecobichon (1997).

Study on analgesic action

Analgesic effect of SLP was determined by the tail flick method described by Sewell and Spencer (1976). Wistar albino rats of either sex, 120-150 g were randomly distributed into 5 groups of 6 animals each. The animals were fasted for 24 h before the experiment and water was given ad libitum. Group I were treated with Normal saline at 1ml/100g,b.wt/p.o as control. Group II were treated with standard drug Indomethacin 10 mg/kg/p.o. Group III, IV, V animals were treated with test drug SLP at the dose of 250, 500, 1000 mg/kg/p.o respectively suspended well in butter. One to two centimeter of the tail of rats was immersed in warm water kept constant at 55ºC. The reaction time was the time taken by the rats to deflect their tail. The first reading was not considered and the reaction time was taken as the index of anti-nociception and was determined before and at 30, 60, 90, and 120 min after the administration of drugs. The maximum reaction time was fixed at 10 sec. The percentage maximum possible analgesia (MPA) was calculated as.

$$\%MPA = \frac{\text{Test Reaction Time} - \text{Control Reaction Time}}{10 - \text{Control Reaction Time}} \times 100$$

Study on anti-inflammatory action

The anti-inflammatory activity was assessed by Carrageenan induced hind paw inflammation method described by Winter *et al.*, (1962). Albino wistar rats of either sex of 150-175 g were used. Five groups were taken with the distribution of six animals in each group and the purpose of each group is same as in analgesic action. The paw volume was measured plethysmometrically immediately after injection (0 h) and then, every hour till 3 h. The difference between the initial and subsequent reading gave the actual oedema volume. The percentage of an oedema and percentage of an oedema inhibition were calculated using the formulae: % Oedema = [(F-I)/I] 100. Where, F – Paw volume after 1, 2 and 3h after Carrageenan injection, I – Initial paw volume. % Oedema Inhibition = (1-Vt/Vc) 100. Where, Vt – Mean paw volume of drug treated group, Vc – Mean paw volume of control group.

Statistical analysis

All in vivo experimental results have been expressed as mean $\pm$ standard deviation. Analysis were done using students''t' test by SPSS software.

RESULTS AND INFERENCES

Macroscopic characters

Leaf: Linear-oblong, narrowed at base, deccurrent, obtuse and serrulate, upto 10cm long and 2cm broad (Figure-1). ***Inflorescence*****:** Flower heads reddish, in clusters, ovoid or cone like, subsessile, 2.5cm long, tips of bracts spinescent.

Fig.1: *Sphaeranthus amaranthoides* Burm.

Microscopic Characters

The leaf is dorsiventral in construction (Figure 2-A) as revealed from the transverse section of the lamina. The upper epidermis is made up of nearly barrel shaped cells. The simple glandular trichomes are 3 to 5 cells tall and club shaped. The epidermis is followed by 1 cell deep compactly arranged palisade mesophyll. Below this comes the spongy mesophyll, which is less chlorophyllous. The spongy tissue is composed of slightly lobed nearly isodiametric globular cells (Figure 2-B). Along the line where the palisade and spongy mesophyll meet, are placed occasional vascular bundles. The vasculature is collateral and is always accompanied by a single resin canal on the abaxial side. The lower epidermis is similar to the upper epidermis in structure excepting that the cells are smaller. The stomatal index value is 42 to 48/mm^2 for lower surface and 30 to 36/mm^2 for upper surface. The midrib shows arcuate collateral vascular bundles in the centre with 1 to 3 resin canals located on the abaxial side. One larger vascular bundle situated in the centre and a smaller one occurs next to this. The ground tissue is parenchymatous (Figure 2-C,D,E). The adaxial foliar epidermis is composed of cells with slightly wavy margins (Figure 3-A). Clothing and short glandular trichomes occur. The abaxial foliar epidermis is similar to the adaxial epidermis in structure excepting that the epidermis cells are wavy margined (Figure 3-B). The stomata are of the ranunculaceous (anomocytic) as well as of the cruciferous (anisocytic) type, present on both surfaces.

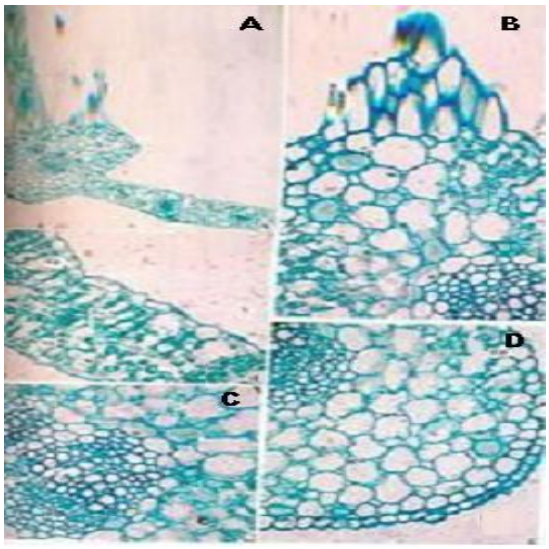


Fig. 2: (A) T.S. of leaf, (B) T.S. of lamina, (C) T.S. of midrib showing vascular bundles, (D) & (E) T.S of midrib – A portion enlarged.

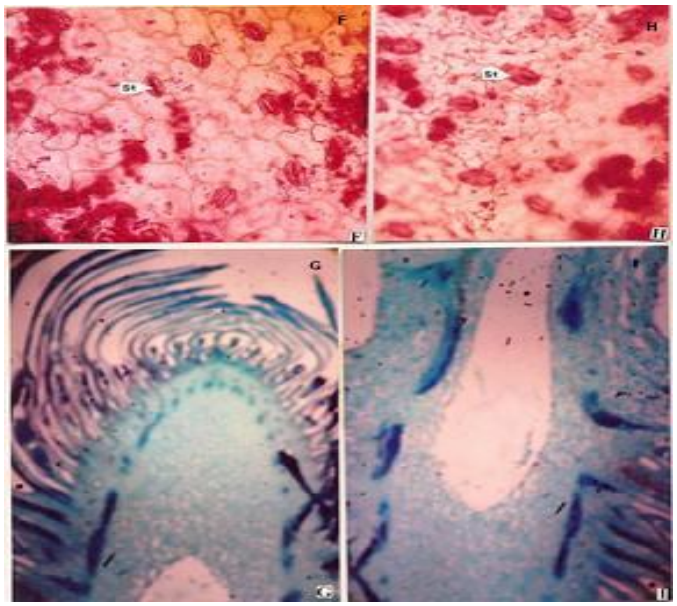
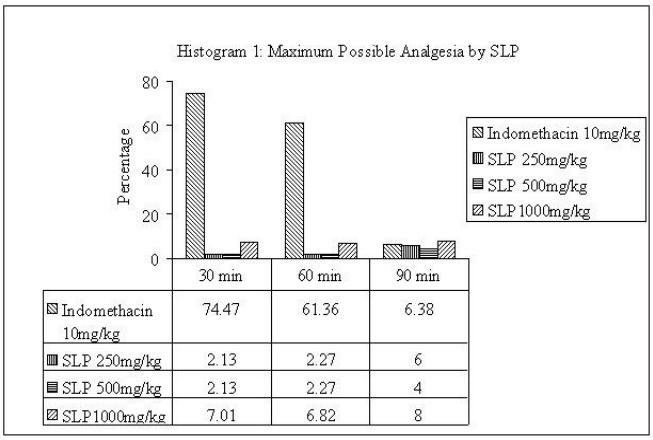
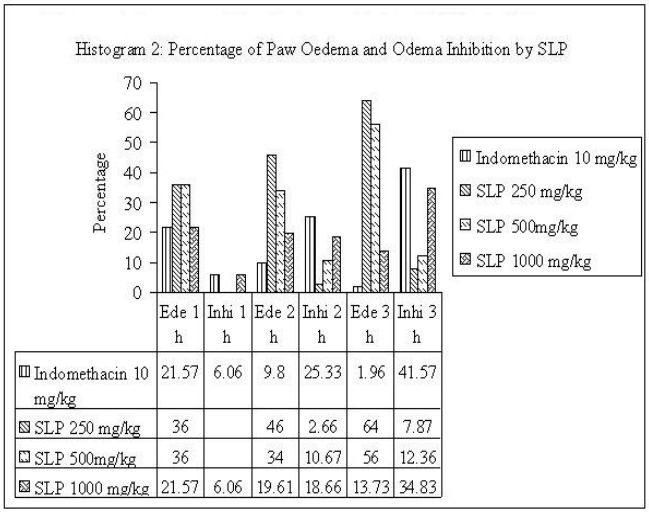


Fig. 3: F - Adaxial foliar epidermis, G - Abaxial foliar epidermis, H - T.S. of upper portion of inflorescence, I - T.S. of lower portion of inflorescence.
Abbreviations used in Figure 2 & 3: T.S -Transverse section, Ep-Epidermis, P-Parenchyma, Pa-Palisade tissue, Rc-Resin canal, Sp-Spongy tissue, St-Stoma, V Vessel, Vb-Vascular bundle.

The clothing trichomes occur mostly along the veins in the leaf, more on the abaxial surface of the leaf. These trichomes are long, unbranched, made up of 4 to 6 cells, the basal 1 or 2 being shorter, while the terminal is the longest with a very acute tip. Occasionally, these tip cells are topped by a glandular unicellular gland. The glandular trichomes are abundant on the abaxial and adaxial epidermis of the lamina. These trichomes are composed of 3 to 6 cells in a file, the top most being glandular functionally. The transverse section of inflorescence is conical in shape. The individual heads are clearly visible in longitudinal section of young inflorescence particularly the male flowers. The inflorescence axis is siphonate (Figure 3-C,D).



	30 min	60 min	90 min
Indomethacin 10mg/kg	74.47	61.36	6.38
SLP 250mg/kg	2.13	2.27	6
SLP 500mg/kg	2.13	2.27	4
SLP1000mg/kg	7.01	6.82	8



	Ede 1 h	Inhi 1 h	Ede 2 h	Inhi 2 h	Ede 3 h	Inhi 3 h
Indomethacin 10 mg/kg	21.57	6.06	9.8	25.33	1.96	41.57
SLP 250 mg/kg	36		46	2.66	64	7.87
SLP 500mg/kg	36		34	10.67	56	12.36
SLP 1000 mg/kg	21.57	6.06	19.61	18.66	13.73	34.83

Abbreviations used in Histograms: SLP-Sivakaranthai leaf powder, Ede-Edema, Inhi-Inhibition.

Physico-chemical parameters

The pH value, Loss on drying value at 105ºC, Total Ash value at 550ºC, Acid insoluble Ash value, Water soluble extractive value and Alcohol soluble extractive value have been reported in Table 1. Under ultraviolet light at 254 nm (Figure 4), it shows major spots at R_f 0.11 (Dark green), 0.19 (Blue), 0.20 (Pale green), 0.50 (White), 0.60 (Greenish yellow) and 0.78 (Greenish yellow). Under ultraviolet light at 366 nm (Figure 4), it shows major spots at R_f 0.03 (Pink), 0.11 (Violet), 0.14 (Pink), 0.19 (White), 0.20 (Pink), 0.27 (Dark pink), 0.32 (Pink), 0.40 (White), 0.50 (White), 0.57 (Dark pink), 0.60 (Dark pink), 0.70 (Dark pink), 0.78 (Dark pink) and 0.98 (Pink). With spray reagent under visible light, it shows major spots at R_f 0.11 (Yellow), 0.20 (Grey), 0.50 (Grey), 0.57 (Grey), 0.60 (Grey), 0.70 (Grey) and 0.78 (Grey).

Table. 1: Physicochemical parameters of *S. amaranthoides.*

S.No	Parameter	Values (% w/w)
1	Foreign organic matter	<1%
2	pH	5.95
3	Loss on drying	0.38%
4	Total ash	11.16%
5	Acid insoluble ash	12.10%
6	Water soluble ash	9.86%
7	Ethanol soluble extractive	29.66%
8	Water soluble extractive	36.2%

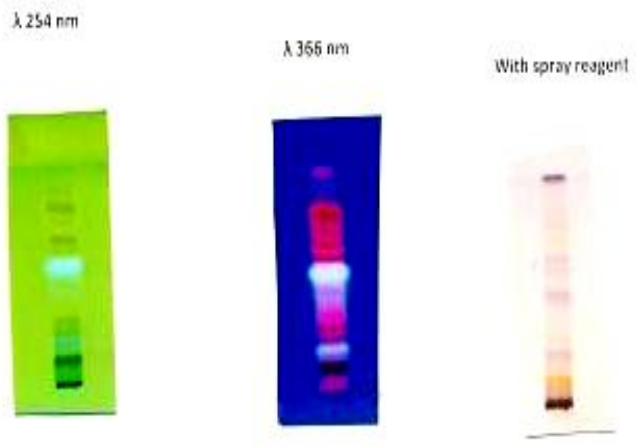


Fig. 4: Thin layer chromatography study of *Sphaeranthus amaranthoides.*

Qualitative and Quantitative screening

The acid radicals present in SLP are Sulphate, Chloride and Phosphate. The basic radicals present in SLP are Calcium and Ferrous iron. The secondary metabolites present in SLP are displayed in Table 2. The concentration of Mercury, Arsenic, Lead and Cadmium is found to be under the permissible limit.

Table.2: Preliminary Phytochemicals screening.

Plant Constituents	Test / Reagent	AQE*
Calcium	Ammonium oxide	++
Ferrous iron	Conc.HNO_3 and Ammonium thiocynate	++
Sulphate	Barium chloride	+
Chloride	Silver nitrate	+
Phosphate	Ammonium molybdate and Conc.HNO_3	+
Starch	Weak Iodine	+
Reducing sugars	Fehling's test	+
Carbohydrates	Molisch's test	+
Steroids	Libermann-Burchard test	++
Alkaloids	Dragendorff's and Mayer's reagent	++
Saponins	Foam test	+
Tannins	1%Ferric chloride	++
Phenol	Ferric chloride test	++
Flavonoids	Shinoda's test	++
Proteins	Biuret test	++
Amino acids	Ninhydrin's test	++
Unsaturated compounds	Potassium permanganate	++

*Aqueous extract of *Sphaeranthus amaranthoides* leaf powder.
The nil inference of other biochemical analyses are not indicated in this above table

Antibacterial evaluation

Table 3 shows that SLP exhibits good antibacterial activity in 10 µl / disc against *Escherchia coli,* but in 50 µl / disc SLP displays good antibacterial activity against *Streptococcus mutans, Staphylococcus aureus, Klebsiella pneumoniae and Pseudomonas aeruginosa* when compared to standard.

Table. 3: SLP sensitivity against bacterial strains.

Organism	Standard Drug Ciprofloxacin 50 mcg/disc	Test drug (SLP*µl/disc) Zone of inhibition in mm		
		10µl	25µl	50µl
S. mutans	31	13	17	19
S. aureus	30	14	16	20
E. coli	30	17	21	24
K. pneumoniae	30	12	15	19
P. aeruginosa	28	11	14	17

Acute oral toxicity evaluation

SLP at the dose of 2000mg/kg/po did not exhibit mortality and did not show any signs of acute toxicity and behaviour changes. As per OECD 423 guidelines, the dose is said to be "Unclassified" under the toxicity scale.

Analgesic activity evaluation

The results of table 4 show that SLP exhibits no significance ($P<0.01$) when compared to control and standard in Tail flick method. At 1000mg/kg dosage, SLP exhibits analgesic response of 9 % after 30 min. This minimum effect is found to decline after 120 min. This might be due to the delayed absorption and lesser content of the phytoconstituents responsible for the analgesic activity particularly flavonoids which are reported to have analgesic activity through inhibition of enzyme prostaglandin synthetase, more specifically the endoperoxidase (Ramaswamy *et al*., 1985). It indicates that *Sphaeranthus amaranthoides* has weak analgesic activity.

Table. 4: Effect of SLP on hot tail flick test.

Groups	Initial	30 min	60 min	90 min	120 min
Control	5.0 ± 0.2	5.3 ± 0.3	5.6 ± 0.3	5.0 ± 0.4	5.1 ± 0.2
Indomethacin (10 mg/kg)	5.4 ± 0.9	8.8 ± 1.3**	8.3 ± 0.9*	5.3 ± 0.9	4.0 ± 0.5
SLP* (250mg/kg)	5.2 ± 0.7	5.4 ± 0.8	5.7 ± 0.8	5.3 ± 0.2	5.1 ± 0.6
SLP* (500mg/kg)	5.1 ± 0.7	5.4 ± 0.2	5.7 ± 0.5	5.2 ± 0.8	4.6 ± 0.3
SLP* (1000mg/kg)	5.5 ± 0.7	5.7 ± 0.3	5.9 ± 0.2	5.4 ± 0.2	4.8 ± 0.2

Values are expressed as mean ± S.E.M of reaction time in seconds (n=6); $p<0.01$, **$p<0.05$ as compared to control.
*Sivakaranthai leaf powder.

Anti-inflammatory activity evaluation

The results of table 5 points that the test drug SLP is a dose dependent anti-edematogenic effect on paw edema induced by carrageenan. The control group reflects edema which is increasing throughout the experimental period producing maximum edema at 3 hrs (64.81%). The standard drug Indomethacin (10 mg/kg, p.o.) reveals maximum inhibition of 97.14% at 3 hrs. The formulation SLP at doses of 250 mg/kg, 500 mg/kg, and 1000 mg/kg produce as maximum inhibition of 42.86%, 95.24% and 95.24% after two hours of carrageenan injection as compared to the standard drug Indomethacin which gives 76.19% inhibition during the same period. The study suggests that all the doses of SLP did not suppress the edema, produced by carrageenan which may be related to the inhibition of inflammatory mediators such as histamine, serotonin and 5-Hydroxytryptamine.

However, at 1000 mg/kg SLP has significant effect on the second phase of the acute inflammation which is prostaglandin synthesis, as it produces 95% inhibition during the second hour of the experiment. Such, observed anti-inflammatory activity may be associated to the flavonoids present in the raw material (Alcaraz *et al*., 1988 & Williamson, 2001).

Table. 5: Effect of SLP on carrageenan induced acute inflammation.

Groups	Paw volume in ml			
	0 hr	1hr	2 hr	3hr
Control	0.54 ± 0.03	0.66 ± 0.03	0.75 ± 0.12	0.89 ± 0.06
Indomethacin (10 mg/kg)	0.51 ± 0.01	0.62 ± 0.02	0.56 ± 0.04*	0.52 ± 0.02*
SLP* (250mg/kg)	0.5 ± 0.0	0.68 ± 0.04	0.73 ± 0.08	0.82 ± 0.04
SLP* (500mg/kg)	0.5 ± 0.0	0.68 ± 0.02	0.67 ± 0.08	0.78 ± 0.03
SLP* (1000mg/kg)	0.5 ± 0.01	0.62 ± 0.08	0.61 ± 0.03*	0.58 ± 0.06*

Values are expressed as mean ± S.D (n=6); $p<0.05$ as compared to control.
*Sivakaranthai leaf powder

DISCUSSION

Sphaeranthus amaranthoides belonging to Asteraceae family is a small procumbent herb, with stems rooting and pubescent with appressed hairs, leaves palmately 3-foliolate.[3] In *Bhoga munivar Karpam* 300, *Sivakaranthai* powder is indicated as *Kalp*a (Rejuvenation) medicine and illustrated that when *Sivakaranthai* powder is given with milk, honey or butter for 2 months, it gives relief from *vatha* diseases. Since *Sivakaranthai* is one of the *Kalpa* drug, it should possess anti-oxidant property and *Bhogar* indicates *Sivakaranthai* Powder for dermatitis having anti-histamine property. Further, it was used in Alchemy processes such as binding the Mercury and its compounds (Murugesha 2008). It has unique pharmacognostical characters such as stems – branches not winged; leaves linear, oblong, narrowed at the base, obtuse and serrulate; in lamina, vasculature is collateral and is accompanied by a single resin canal on the abaxial side; the lower epidermis differs from upper epidermis whose cells are smaller; Stomatal Index – 42 to 48/mm^2 for lower surface, for upper surface stomatal index – 30 to 36/mm^2; Midrib shows an arcuate collateral vascular bundles in the centre with 1 to 3 resin canals located in abaxial side.

Due to resin canal presence, the leaves have a more aromatic odour. Adaxial epidermis is made up of slightly wavy margin cells. The glandular trichomes are abundant on abaxial and adaxial epidermis. Infloresence – head is reddish and in cluster and cone, axis is siphonate. The foreign organic matters present in the sample were less than 1%. The pH value at 25ºC found to be 5.95 leading to state that, it is acidic in nature might be due to more content of tannic acid in it. The loss on drying test indicates that only 0.019 g (0.38%) of water and volatile component would be lost when 1 g of plant material kept at 105ºC. This moisture content helps to prevent degradation. So, low or high moisture content compromises the quality of drug and affects its efficacy. This less value of moisture content could prevent bacterial, fungal and yeast growth. Ash values are useful to judge the identity and purity of crude drug for the purpose of adulteration. Total ash value of the plant material indicates the amount of minerals and earthy material attached to the plant. The sample of *Sphaeranthus amaranthoides* analysed during the studies reflects the following ash values: total ash, 11.16%; acid insoluble ash, 12.10% and water soluble ash, 9.86%. The acid insoluble ash value indicates the amount of siliceous matter present in the plant material. The water soluble extractive value is indicating the presence of sugar, acids and inorganic compounds which the ethanol soluble extractive values indicate the presence of polar constituents.

Chromatography is used for the isolation and identification of various components present in the drug. Thin layer chromatography is particularly valuable for the qualitative determination of small amounts of impurities. The present TLC study done on this plant material clearly establishes the presence of different component. The R_f value of developed spots seen in Figure 4 can be used as standard for *Sphaeranthus amaranthoides* in future, since TLC for this plant has not been conducted past.

The phytochemical screening of the drug is a very sensitive aspect in the process of standardization and quality control because the constituents vary quantitatively and qualitatively not only from plant to plant but also in different samples of the same species depending upon atmospheric factors and storage conditions. The preliminary phytochemical screening of aqueous extract of SLP showed the presence of Tannins, Tannic acids, Alkaloids, Steroids, Flavonoids, Proteins, Amino acids, Phenols and Glycosides as secondary metabolites. Starch, Reducing sugar and Saponins were found traceable. Minerals like Calcium and Ferrous iron were also present in aqueous extract of SLP. The presence of phenolics, flavonoids and alkaloids pointed out sufficient exhibits for ample biological activities. Atomic absorption Spectroscopy study has shown that the presence of heavy metals in SLP is much below the WHO/FDA permissible limits. Swarnalatha *et al*., (2009) reveal the ethanolic extract of *Sphaeranthus amaranthoides* has good antibacterial and antidiarrhoeal activity in rats. The present studies also declare that SLP has significant antibacterial activity. Most of the common bacterial strains were highly sensitive to *Sphaeranthus amaranthoides*. The LD_{50} of SLP as per OECD guideline falls under class four with no signs of acute toxicity at the maximum dose of 2000mg/kg. Any changes in normal behavioral pattern or signs and symptoms of toxicity and mortality were not observed up to this dose level. Generally, analgesia is defined as a state of reduced awareness to pain and analgesics are substances which decrease pain sensation by increasing threshold to pain stimuli. The present study reveals SLP has no significant analgesic activity when tested by the tail immersion method on Wistar albino rats. SLP treatment increase the response latency of Tail flick, when compared to control ($p<0.001$). So, *Sivakaranthai* powder possesses weak analgesic property.

The carrageenan-induced paw edema model in rats is known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents, which primarily inhibit the cyclooxygenase involved in prostaglandin synthesis (Siebert *et al*., 1994). Carrageenan-induced hind paw edema is the standard experimental model of acute inflammation.. Carrageenan is sulphated polysaccharides obtained from seaweed (Rhodoplyceae) and by causing the release of histamine, serotonin, bradykinin and prostaglandins, it produces inflammation and oedema. High doses of Carrageenan (>500µg) can cause continuous extravasation parallel to the edema increase

due to continuous presence of damaging stimulus and maximum edema reaches within 3 hours.

The anti-edematogenic drug Indomethacin is effective with high doses of carrageenan. The activity is related to its effect upon plasma exudation (Zanin *et al*., 1978). The dose 1000 mg/kg of SLP produced a significant inhibition of carrageenan induced paw edema at +2h. Therefore, it can be inferred that the inhibitory effect of leaf powder of *Sphaeranthus amaranthoides* on carrageenan induced inflammation could be due to inhibition of the enzyme cyclooxygenase and subsequent inhibition of prostaglandin synthesis. It remains delayed anti-inflammatory activity in acute exudative phase of inflammation. The delayed response for its anti-inflammatory activity clearly reveals that the mechanism of action may be attributed by PG synthetase inhibition rather than inhibiting the mediators of early phase of inflammation. So, the experimental pharmacological studies establish *Sivakaranthai* powder as weak analgesic and moderate anti-inflammatory drug.

CONCLUSION

The present study on pharmacognosy, physicochemical parameters and preliminary phytochemical analyses provides important information which may be helpful in authentication and adulteration for standardization of *Sphaeranthus amaranthoides*. Further, the experimental pharmacological studies conclude that *Sivakaranthai* powder has weak analgesic and moderate anti-inflammatory action in animal model at 1000mg/kg dosage. Even more than 2000 mg/kg, no toxicity has produced in animal model; it is safe to use SLP at 2 – 4 g dosage which is prescribed in the literature to achieve the particular therapeutic value in human adult. Moreover, these studies add the scientific knowledge to *Sphaeranthus amaranthoides* for the development of formulations for treating various diseases using this herb.

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