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Original Research Article

Phytochemical Analysis and Anti-Inflammatory Activity of *Solanum dasyphyllum* Schumach. & Thonn. Leaves Methanol Extract and its Fractions

Oladapo E. Oyinloye[1,2], Abayomi M. Ajayi[1]* Olusegun G. Ademowo[1,3]

[1]*Department of Pharmacology and Therapeutics, Faculty of Basic Medical Sciences, University of Ibadan, Oyo-State, Nigeria*
[2]*Department of Pharmacology and Toxicology, Faculty of Pharmacy, Olabisi Onabanjo University, Ago-Iwoye, Ogun-State, Nigeria*
[3]*Institute for Advanced Medical Research and Training, College of Medicine, University of Ibadan, Oyo-State, Nigeria*

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ABSTRACT

Solanum dasyphyllum (SD) leaf is used in ethnomedicine for treatment of gastrointestinal diseases, gout, swelling, pain and fever-related illnesses. There is however paucity of phytochemical and pharmacological reports on the leaves. Hence, this study was designed to investigate the phytochemical profile and anti-inflammatory activity of SD crude extract and fractions. Powdered SD leaf was extracted with 80% methanol (MESd), and partitioned sequentially into n-hexane (SdHXF), dichloromethane (SdDMF), ethyl-acetate (SdEAF), and aqueous fractions (SdAQF), respectively. Phytochemical screening and thin layer chromatography (TLC) was carried out on extract and fractions. Anti-inflammatory activity of extract/fractions were investigated using membrane stabilizing assay, carrageenan-induced rat paw oedema and hyperalgesia models.
Phytochemical screening revealed appreciable amount of alkaloids, anthraquinones, saponins, tannins, phenols and flavonoids in MESd while TLC spots revealed 13 points for SdHXF, 7 in SdDMF and 10 in SdEAF, respectively. The MESd and SdEAF showed significant anti-inflammatory activities in membrane stabilizing and in carrageenan-induced paw oedema and hyperalgesia models. In conclusion, bioassay guided evaluation of anti-inflammatory activity of *Solanum dasyphyllum* leaf demonstrated that the ethylacetate fraction demonstrated the most pronounced anti-inflammatory activity. Further study is needed to characterize bioactive constituents and demonstrate its mechanisms of anti-inflammatory activity.

Keywords: *Solanum dasyphyllum*, Bioactivity, Ethylacetate fraction, Anti-inflammatory.

Introduction

Historically, vegetables have served as medicinal plants of proven value with immense therapeutic potential. Traditional medicine practice in different African countries have used extracts of different plants for the treatment of a wide variety of disorders including acute and chronic inflammation.[1] The biological activity of a medicinal vegetable is closely related to the plant phytochemicals. Hence, identifying and characterizing crude vegetal herbal extracts combines ethnopharmacological knowledge and use of bioactivity models.[2]
Numerous studies using crude plant extracts with reported anti-inflammatory effects in whole animal models (e. g carrageenan-induced rat paw oedema) usually demonstrate gross anti-inflammatory activities; however, these studies typically fall short of identifying possible intracellular targets.[3] To identify the active components and elucidate structures and mechanisms of action, crude extracts are expected to be fractionated.[4] Phytoactive molecules in crude extracts are soluble in solvents of differing polarity. Fractionation thus makes use of separation techniques using solvent of increasing polarity such as n-hexane, dichloromethane, ethylacetate, and butanol.[5]
The fractionation procedure improves the capacity to separate pure molecules.[5] However, it can often result in the loss of activity in the whole moiety. As a result, if fractionation reduces the native extracts' activities or health advantages, the extracts should be standardized (active components).[6] The *Solanum* species (egg plants) have served as medicinal foods in various traditional medicine vegetables in Nigeria.[7] There are about 25 species which have been the subject of various investigations due to their importance in the sub-Saharan African traditional medical systems.[8] They are used in indigenous medicine for several conditions including asthma, allergic rhinitis and inflammatory arthritis of different forms.[7] *Solanum dasyphyllum* Schumach.& Thonn, is a semi-woody undershrub member of the Solanaceae family that is naturalized in the tropical Western African region and is closely related to S. macrocarpon.[9,10] Solanum dasyphyllum leaves is being used as food, while leaf-saps are used as medicine in treatment of gastrointestinal diseases, gout, swelling (inflammation), pain and infections.[10,11] *Solanum dasyphyllum* (SD) leaf is used in ethnomedicine for the treatment of fever-related illnesses. There is however paucity of phytochemical and pharmacological reports on the leaves of *S.dasyphyllum*. Hence, this study aimed to investigate the phytochemical profile and anti-inflammatory activity of SD crude extract and fractions.

*Corresponding author. E mail: yomexj@yahoo.com
Tel: +2348103861380

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Materials and Methods

Plants extract fractionation
The Leaves of *Solanum dasyphyllum* were collected from Akungba, Akoko in Ondo state of Nigeria in June 2016. The plant sample was identified by Mr. Chukwuma, E.C. of Forestry Research Institute of Nigeria (FRIN) Ibadan and a voucher specimen deposited with herbarium voucher number (F.H.I. 109799). The plant name was supplied on 2012-04-18 as *Solanum dasyphyllum* Schumach. & Thonn., according to Plant List (http://www.theplantlist.org/). Fresh *S.*

dasyphyllum leaves were air dried and ground into powder at room temperature. Dry powder weighing three kilograms were soaked for three days in 15 liters of 80% methanol. The mixture was stirred intermittently and allowed to stand at room temperature. The mixture was filtered using muslin cloth and concentrated in vacuo using a rotary evaporator (Buchi Rotavapor -124) under reduced pressure and 40^{o}C temperature. The resultant crude methanol extract of *Solanum dasyphyllum* (MESd) was concentrated to dryness in vacuum oven at 40^{o}C with resultant yield of 12.5%. Fractionation of methanol extract of *Solanum dasyphyllum* (MESd) was carried out using n-hexane, dichloromethane, and ethyl acetate by liquid-liquid partitioning. The process was repeated thrice in order to get adequate quantity for each fraction. The n-hexane, dichloromethane, ethyl acetate, as well as the aqueous residue fractions were concentrated in vacuo using a Rotary evaporator (Buchi Rotavapor -124) and dried to constant weight.

Phytochemical analysis

Qualitative and quantitative phytochemical screening of MESd leaves

Qualitative phytochemical screening of secondary metabolites (flavonoids, alkaloids, saponins, cyanogenic glycosides, tannins and reducing sugars) in the crude extract was carried out utilizing conventional protocol from Sofowora, [12] Evans, [13] and Harborne, [14] Identified secondary metabolites were subsequently quantified using standard analytical methods (AOAC)

Thin layer Chromatography (TLC) of fractions

TLC was performed on a 10×20 cm TLC sheets coated with 0.2 mm layers of silica gel N-HR/UV254 (Macherey-Nagel, Ref. 804023). After application of the extract, fractions and the standard solution (5 µl) the sheet was developed in paper-lined all-glass previously left for a preconditioning in the developing chamber for at least 30 min. The mobile phases used were: toluene: ethyl acetate (3:2).[15]

Experimental animals

Wistar rats of both sex weighing 150 -180g were obtained from the Central Animal House of the College of Medicine, University of Ibadan, Ibadan, Nigeria. The animals were acclimatized in the experimental animal laboratory of the Department of Pharmacology and Therapeutics for one week before the commencement of each experiment. The animals were housed in polypropylene plastic cages with wood shavings as beddings, under natural ventilation, and at room temperature of 28 ± 2°C. They were allowed to access water *ad libitum* and fed with commercial rodent chow (Vital feeds Ltd, Jos, Nigeria).Experimental procedures were performed following the approved guidelines of University of Ibadan, Animal Care and Use Research Ethics Committee (ACUREC) with ethical no UI-ACUREC/APP/2016/021

In vitro evaluation of anti-inflammatory effects of MESd and its fractions by erythrocytes membrane stabilizing assay

Membrane stabilizing assay in rat erythrocytes using method by Ajayi *et al*.[16] was used. Blood was collected from healthy Male Wistar rats by cardiac puncture. Same volume of sterilized Alsever's solution was mixed with the blood. The blood and Alsever's solution mix was centrifuged for 10 min at 3000 rpm, before washing the resulting packed cells with sodium phosphate buffer (0.1M, pH 7.2) and then suspended in 10% (V/V) phosphate buffer saline (PBS).

The assay mixture consists of hyposaline (2mL), sodium phosphate buffer at pH 7.2 (1mL), RBC suspension (0.5 mL) and 1 mL of varying concentrations of extract or fractions (0.5, 1.0, 1.5, 2.0 mg/mL) made up to 5mL with isosaline solution. Similar assay mixtures as described above were made for the normal control and drug control, except that the extract was replaced with distilled water in normal control and the drug control lacked erythrocyte suspension. Each sample was prepared in triplicate. The assay mixtures were incubated in a water bath for 30 min at 56^{o}C. After cooling under running water, the assay mixture was centrifuged for 10 min at 4000rpm. Erythrocyte lysis was determined by the release of haemoglobin. The supernatants were collected and the absorbance of released hemoglobin was read at 560 nm using a UV Spectrophotometer (752N INESA, China).

The percentage membrane stability was estimated using the expression:

$$\% \text{ Membrane Stability } = \frac{C - T}{C} \times 100$$

Where,

C - Absorbance of Control

T - Absorbance of Test Sample

In vivo evaluation of anti-inflammatory effects of MESd and its fractions in carrageenan- induced rat paw oedema

The carrageenan-induced rat paw oedema test was used in predicting the efficacies of anti-inflammatory agents that act by inhibiting the mediators of acute inflammation. Inflammation was induced in the hind foot of Wistar rats of both sexes weighing between 150 and 180g using the method described by Winter *et al.*[17] The crude extract was screened for anti-inflammatory activity. In this experiment, the rats were divided into six groups (n = 5). The test groups of rats received 250, 500 and 1000 mg/kg of extracts orally, 1 h before carrageenan was administered subcutaneously into the right hind paw. The control and standard reference groups received 10 mL/kg vehicle, and 10mg/kg indomethacin, respectively. After every hour, paw volumes were measured with digital Ugo Basile plethysmometer (Comerio VA, Italy) till the fifth hour after carrageenan injection. Similarly, the n-hexane (SdHXF), dichloromethane (SdDMF), ethyl-acetate (SdEAF), and aqueous fractions (SdAQF) each at 250 mg/kg dose were screened in carrageenan-induced paw oedema as described above.

Evaluation of anti-hyperalgesia effects of MESd and its fractions in carrageenan-induced hyperalgesia in rats

Hyperalgesia (mechanical) was measured for evaluating the analgesic activity using Randall-Selitto test. Pain perception at the site of inflammation is based on the pressure imposed on the inflamed paw. This pain perception is measured using Ugo-Basile 37215 Analgesy-meter (Comerio VA, Italy) which constantly exerts a constantly increasing force on the rat paw. The method described by Vongtau *et al*.[18] was used for the experiment. Briefly, thirty rats grouped as described in the experiment above (2.7) were each assessed by placing on a plinth under the pusher of the analgesy-meter. As soon as the rat showed a nociceptive response the stimulus was terminated and force threshold readings (g) recorded. Readings was taking at 0 and 120 min after treatment.

Statistical analysis

Data were expressed as mean ± standard error of mean. All data entered and managed with Microsoft Excel® spreadsheet (Microsoft *Corporation*, Redmond, and W.A, USA). Graphs were constructed using either Microsoft Excel or Graph Pad Prism® software version 5. (GraphPad Software,San Diego, CA, USA). Statistical analysis was done using analysis of variance (ANOVA), significant main effects were further analyzed by *post hoc* test using Newmann keuls or Bonferroni's multiple comparison test to compare the treatment groups, $*p < 0.05$ was denoted for statistical significant difference among treatments.

Results and Discussion

There is an emerging interest in the pharmacological evaluation of various plants used in Nigeria traditional systems of medicine. However, to understand therapeutic value of plants used in the treatment of ailments, there is need to assess their phytochemical content and pharmacological activity. Thus, in these studies, attempts were made to carry out bioassay guided evaluation of anti-inflammatory activity of methanol leaf extract of *Solanum dasyphyllum* and its fractions.

The efficacy of extract is known to be affected by solvents and extraction procedures. Components that are soluble in a particular solvent are selectively extracted based on polarity of the solvents used

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for the extraction. The percentage yield of *Solanum dasyphyllum* leaves were 12.5, 8.12, 3.25, 2.02 and 11.31% for methanol, hexane, dichloromethane, ethylacetate and aqueous fraction respectively. The preliminary qualitative and quantitative screening of methanol extract of *S.dasyphyllum*was carried out and the results are presented in the Table 1. The following phytoconstituents viz., flavonoids (0.0063), alkaloids (1.284), saponins (0.395), cyanogenic glycosides (0.016), tanins (0.037) and reducing-sugar (0.863) were quantified. The phytochemical screening of the MESd showed presence of alkaloids, anthraquinones, saponins, tannins, phenols and flavonoids. The extractive values of liquid-liquid partitioning revealed the solubility and polarity particulars of the metabolites in the leaves extracts.[19]

Number of different compounds soluble in different extracting solvents was determined by the TLC technique. The result of thin layer chromatographic analysis revealed thirteen, seven, ten, two and eleven spots with hexane, dichloromethane, ethylacetate, aqueous fractions and crude extract of *S. dasyphyllum*, respectively when developed with toluene: ethyl acetate (3:2) as solvent system (Figure 1). The TLC of extract can be a guide for the identification of the chemical constituents and the standardization of the extract and fractions. [20] Membrane-stabilizing properties of compounds are specifically linked to inhibition of phospholipase, the enzyme that triggers formation of inflammatory mediators in early phase of inflammation.[21] The membrane stabilizing assay has been standardized as a simple *in vitro* model for screening of drugs or extracts with potential anti-inflammatory activity.[16] In this study, MESd at the concentration of 0.5, 1.0, 1.5 and 2.0 mg/mL were found to have stabilizing effect of 15.9%, 18.8%, and 23.2% on rat erythrocytes respectively (Figure 2A). The stabilizing effect of MESd (1.0 -2.0 mg/mL) were significant ($p<0.05$) in comparison with negative control, but not MESd 0.5 mg/mL (Figure 3A). Indomethacin (0.5 mg/mL) significantly inhibit rat erythrocytes destabilization with stabilizing effect of 60.9%. From the results it was observed that MESd fractions (SdHXF, SdDMF, SdEAF and SdAQF) have significant ($p<0.0001$) membrane stabilizing effect on rat erythrocytes, at 0.25, 0.5 and 1.0mg/mL. The stabilizing effect of indomethacin 0.5 mg/mL was higher than all the fractions at various concentrations used except SdEAF 1.0 mg/mL that showed 99.9% stabilizing effect as compared with indomethacin 0.5 mg/mL 69.9%. (Figure 2B).The membrane stabilizing effectof the methanol extract and its fractions depict a potential to prevent release of inflammatory mediators in late phase of inflammation. Anosike *et al*.[22] use the membrane stabilizing assay to demonstrate the anti-inflammatory properties of the solanum fruit extract. *In vivo* bioactivity of MESd and its fractions were investigated in a carrageenan-induced rat paw oedema model. The results of carrageenan-induced paw oedema test of MESd (250.500 and 1000mg/kg), showed anti-inflammatory effect by decreasing paw volume in dose dependent manner (Figure 3A). Administration of MESd (250, 500, 1000 mg/kg) and indomethacin (10mg/kg) significantly showed 34.6, 15.1, 20.2 and 46.9% inhibition of paw oedema formation, respectively (Figure 3B). Result showed that MESd fractions (SdHXF, SdDMF, SdEAF and SdAQF) administered at 250 mg/kg p.o, all have significant ($p < 0.05$) anti-inflammatory effect by a decreasing paw volume compared with negative control (Figure 3C). The area under the curve showed that SdHXF, SdDMF, SdEAF, SdAQF and indomethacin(10 mg/kg), produced 34.0, 36.1, 44.0, 41.9 and 69.0% percentage inhibition of oedema formation respectively (Figure 3D). Furthermore, results of the carrageenan-induced hyperlagesia showed an increase in paw withdrawal latency in carrageenan-induced inflamed hind paw of rat pre-treated with MESd (250.500 and 1000mg/kg oral) at 60min after pre-treatment, when compared with negative control suggesting peripheral analgesic effect. The pain threshold (g) increases significantly ($p<0.05$) at 120 min in MESd 500, 1000 mg/kg and indomethacin 10 mg/kg. The anti-hyperalgesic effects of fractions SdHXF, SdDMF, SdEAF and SdAQF as shown in table 2 revealed that only SdEAF (250 mg/kg) produced significantly ($p<0.05$) profound increase in paw withdrawal latency at the 120 min. Carrageenan model of inflammation evaluates the aspects of acute inflammation that is attributable to mediators involved in vascular changes.

Three distinct phases of discharge of inflammatory mediators are involved in carrageenan-induced inflammation; first phase involving serotonin and histamine (within 0- 2 h), second phase involves kinins within 3 h, and in the third phase involves prostaglandins ingreater than 4 h.[23,24]

Table 1: Phytochemical Screening of crude methanol Extract of *Solanum dasyphyllum* leaves

Phytochemical groups	Phytochemical Components	
	Qualitative assay	Quantitative assay (%)
Flavonoids	+	0.0063
Alkaloids	+	1.284
Saponins	+	0.395
Cyanogenic glycosides	+	0.016
Tannins	+	0.037
Reducing sugar	+	0.863

Qualitative and Quantitative analysis of *Solanum dasyphyllum*
+ = Present

Table 2: Antihyperalgesic effect of MESd and its fraction in carrageenan-induced hyperalgesia in rats

Treatment	Dose (mg/kg)	Paw withdrawal threshold (g)[a]		Percentage inhibition
		0 min	120 min	
Control	10 mL/kg	270.25 ± 2.88	120.75 ± 1.18	
MESd	250	385.00 ± 1.72	131.00 ± 0.39	7.82
MESd	500	361.25 ± 1.97	373.75 ± 1.83*	67.70
MESd	1000	302.5 ± 1.42	343.25 ± 0.96*	64.80
SdHXF	250	325.75 ± 1.49	226.25 ± 0.21*	46.63
SdDMF	250	306.25 ± 1.02	187.00 ± 0.43*	35.43
SdEAF	250	312.50 ± 1.36	240.00 ± 0.52*	49.69
SdAQF	250	289.50 ± 2.30	203.25 ± 0.43*	40.35
Indomethacin	10	356.25 ± 0.97	328.75 ± 0.99*	63.30

[a]Values are mean ± SEM (n=5). * $p< 0.05$ vs control using one-way ANOVA followed by Newman keuls post hoc test. MESd- Methanol extract, SdHXF-hexane fraction, SdDMF-dichloromethane fraction, SdEAF-ethylacetate fraction, SdAQF- aqueous fraction

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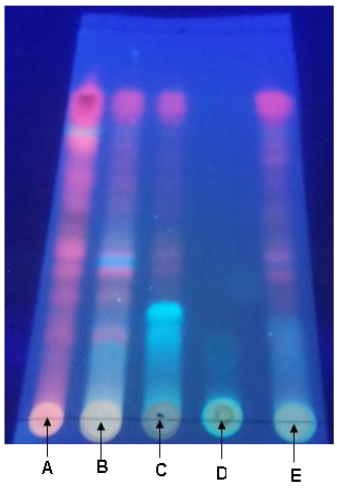

Plant extract & fractions	No of Visible bands	Rf Values
A = Hex	13	0.06,0.17,0.3,0.35, 0.38,0.41,0.42,0.47, 0.57 , 0.61, 0.65, 0.73 and 0.76
B = DCM	7	0.08,0.14,0.27,0.35,0.38,0.73 AND 0.76
C = ETOAc	10	0.05,0.14,0.15,0.18,0.32,0.56,0.59,0.65,0.68, and 0.73
D = AQUE	2	0.14 and 0.18
E = Crude extract	11	0.05, 0.11, 0.14, 0.32, 0.37, 0.39, 0.59, 0.62, 0.65, 0.73 and 0.77)

Hex - hexane, DCM - dichloromethane, ETOAc - ethyl acetate, AQUE - aqueous extract

Figure 1: Thin layer chromatography of MESd and its fraction under the UV 365 nm wavelength

This present investigation revealed that methanol extract (MESd) and all fractions of *S. dasyphyllum* showed anti-edematous activity at first hour, while maximum activity was seen at 5 h, thus the anti-inflammatory activity might be mediated via inhibition of prostaglandins and bradykinin in the three phases of inflammation, with 250 mg/kg of MESd and SdEAF possessing maximum activities. In conclusion, bioassay guided evaluation of anti-inflammatory activity of *Solanum dasyphyllum* leaf demonstrated that the ethylacetate fraction of the methanol extract presented with most pronounced anti-inflammatory activity. Further study is needed to characterize bioactive constituents and demonstrate mechanisms of anti-inflammatory activity.

Conclusion

The methanol extract of *T. globiferus* contains some phytochemicals with antidepressant activity, and was practically nontoxic using the OECD guidelines. The extract ameliorated anhedonia by reducing the immobility time in tail suspension test but not in the forced swim test. The extracts activity was shown not to be due to locomotor stimulation. The methanol extract of *T. globiferus* possesses antidepressant activity. This provides justification for the use of the plant in the management of depression in traditional medicine.

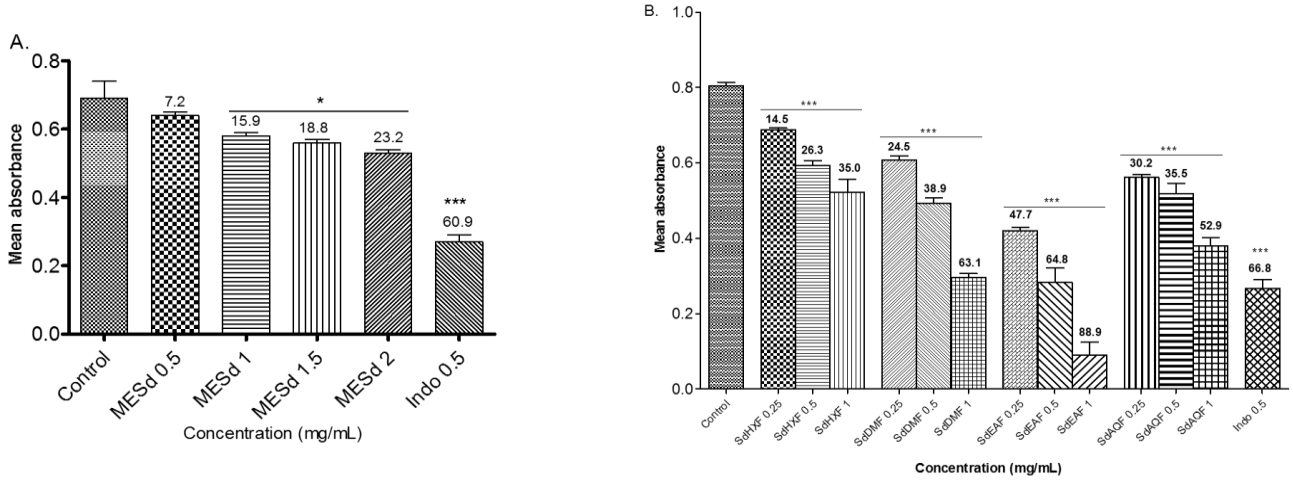

Figure 2: *In vitro* membrane stabilizing activity of methanol extract and fractions of *S. dasyphyllum* (Sd). Values are expressed as the Mean ± S.E.M for measurements in triplicate.
Significant difference denoted by *p< 0.05 compared to control analysed by one-way ANOVA followed by post hoc Newman-Keuls Multiple Comparison Test. MESd- Methanol extract, SdHXF- hexane fraction, SdDMF-dichloromethane fraction, SdEAF-ethylacetate fraction, SdAQF-aqueous fraction.

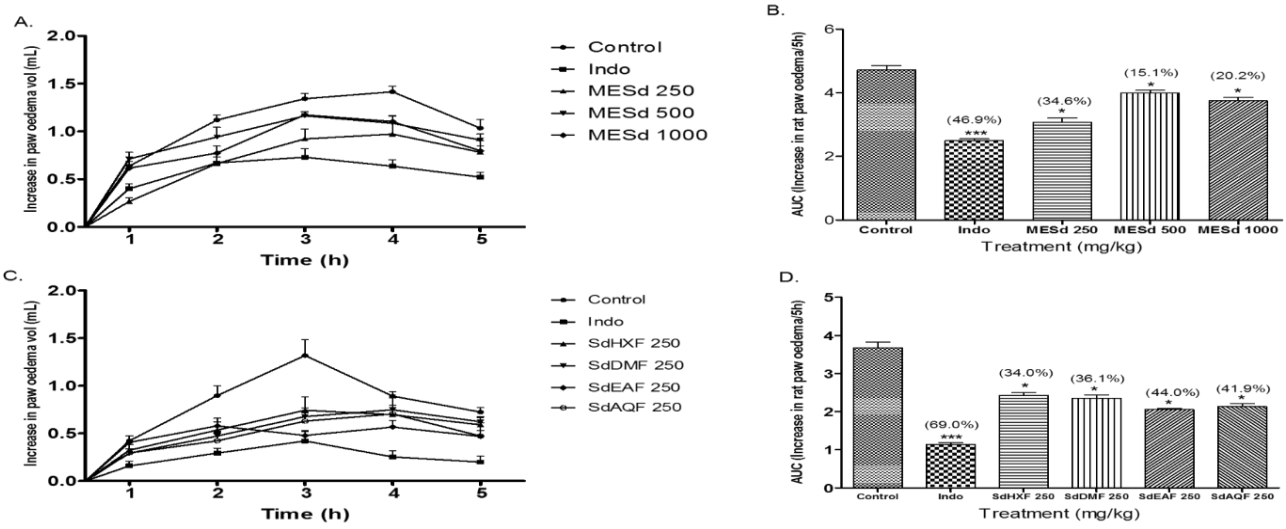

Figure 3: Anti-inflammatory activity of methanol extract and fractions of *S. dasyphyllum* (Sd) leaves in carrageenan-induced paw oedema in rats.
Data are expressed as Mean ± SEM (n= 5 rats). Significant difference denoted by *p< 0.05 compared to control analysed by one-way ANOVA followed by post hoc Newman-Keuls Multiple Comparison Test. MESd- Methanol extract, SdHXF- hexane fraction, SdDMF-dichloromethane fraction, SdEAF-ethylacetate fraction, SdAQF- aqueous fraction.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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