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Comparative phytochemical studies and anti-inflammatory activities of *Desmodium velutinum* (Willd) and *Desmodium scorpiurus* Desv. (Family Papilionaceae) growing in Nigeria

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Members of the genus *Desmodium* are widespread in the sub-tropics, tropics and used in traditional medicine, as part of recipes for treating fever, pains, cough and dysentery. In this study, phytochemical screening and comparative anti-inflammatory activities of the methanol extracts, hexane and ethyl acetate fractions of the leaves of *Desmodium velutinum* and *Desmodium scorpiurus* were investigated using the egg-albumin induced oedema and protein denaturation methods. The presence of flavonoids, saponins, tannins and sterols were detected in the leaves of *D. velutinum* and *D. scorpiurus* while, the extracts and fractions exhibited anti-inflammatory activities not significantly different ($p<0.05$) from the activities of the reference drug (Diclofenac Sodium). The hexane fractions of *D. velutinum* and *D. scorpiurus* exhibited the most pronounced effects of 76.9 and 68.2% respectively at a dose of 2000 µg/ml in the *in vitro* model; 27.2 and 24.5% respectively in the *in vivo* model. This study has been able to report the anti-inflammatory activities of the leaves of *D. velutinum* and *D. scorpiurus*.

Key words: *Desmodium velutinum, Desmodium scorpiurus,* anti-inflammatory activities, papilionaceae, *in vivo*, *in vitro*.

INTRODUCTION

The genus *Desmodium* is a member of the Papilionaceae family with more than three hundred and fifty (350) species widely spread and occurring throughout the sub-tropics and tropics (Lenne and Stanton, 1990). Traditionally, members of the genus *Desmodium* are used in the treatment of rheumatism, pyrexia, dysentery, cough, malaria and hepatitis while in the traditional Chinese medicine, most species of *Desmodium* are used in the treatment of fever, for neutralizing toxins, inhibiting pains and suppressing cough (Ma et al., 2011).

Previous biological studies have reported the antibacterial, hepatoprotective, anti-inflammatory, diuretic,

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antipyretic analgesic and choleretic activities of *Desmodium* species (Liu et al., 2013) while, several flavonoids, alkaloids, terpenoids, steroids and a number of volatile oils have been characterized and isolated from about fifteen *Desmodium* species (Liu et al., 2013; Ma et al., 2011). However, most of the *Desmodium* species in Asia and Africa are yet to be fully studied in details.

D. velutinum is a perennial erect or sub-shrub which grows to about 3 m in height. It is used in folklore medicine for treating diarrhea, dysentery, stomachache (Setyowati-Indorto et al., 1999) and fever (Anowi et al., 2012) while, *D. scorpiurus*, commonly called Tick trefoil or Scorpion tick trefoil is a weak erect plant, used traditionally for the treatment of constipation, cough, convulsion, veneral infections and ringworm (Ndukwe et al., 2006). Though *Desmodium velutinum* and *Desmodium scorpiurus* are not documented for treating rheumatism and inflammation, the earlier reports of members of the genus *Desmodium* as part of recipes for treating inflammation (Liu et al., 2013) has led to investigating the anti-inflammatory activities and phytochemical analysis of these two species: *D. velutinum* and *D. scorpiurus* found growing in Nigeria.

MATERIALS AND METHODS

Plant collection and authentication

The leaves of *D. velutinum* and *D. scorpiurus* were collected from Eruwa near Ibadan in Oyo State in August, 2012 and authenticated at the Forestry Research Institute of Nigeria by Mr Chukwudi where, voucher specimens with voucher numbers FHI 108953 and 109646 were deposited.

Drying and extraction

The leaves of *D. velutinum* and *D. scorpiurus* were dried under shade and powdered in a miller. The powdered leaves were extracted by percolating in 80% methanol. They were filtered and the filtrates concentrated under reduced pressure to dryness after which a solvent-solvent partitioning was carried out successively with hexane and ethyl acetate to give the hexane and ethyl acetate fraction.

Phytochemical analysis

Phytochemical screening of the leaves of *D. velutinum* and *D. scorpiurus* were carried out using standard procedures (Evans, 2009).

Animals

Wistar albino male rats weighing between 130 and 150 g obtained from the Animal House of the University of Ibadan were allowed to acclimatize for two weeks before use and allowed12 h Light and 12 h dark cycle at 25°C. Animals were handled according to the International standard guiding the handling of animals and fed with standard pellets and allowed water *ad libitum*.

Anti-inflammatory activities

In vivo assay

The egg-albumin model was used in the *in vivo* anti-inflammatory study as the phlogistic agent (Anosike et al., 2012). The initial diameters of the right hind paws were taken and rats grouped into five groups (n=5).

Group A – Rats were administered 200 mg/kg of extract or fraction
Group B – Rats received 100 mg/kg of extract or fraction.
Group C- Rats were administered 50 mg/kg of extract or fraction.
Group D – Rats were administered 5 mg/kg of Diclofenac (Cataphlam, Novartis)
Group E- Rats were administered 1 ml/kg of distilled water.

30 min after administering the extract, fraction or standard drug, 0.1 ml of egg albumin was injected into the right hind paw of each rat by sub plantar administration. Following the injection of the egg albumin, the hind paws of the rats were measured at an interval of 1 h for 6 h (0 to 6 h). The percentage inhibition of the edema was calculated as described by Jain and Khanna (1981).

$$\% \text{ Inhibition of oedema} = \frac{I_o - I_1}{I_o} \times 100$$

I_o = Change in paw circumference in control group; I_1 = change in paw circumference in treated groups.

In vitro anti-inflammatory assay

The *in vitro* anti-inflammatory assay was carried out using the method adopted from Sangita et al. (2012). 0.2 ml of egg albumin (from fresh hen's egg) was added to 2.8 ml phosphate buffer saline (PBS, pH 6.4) and 2 mls of the extract/fraction or standard drug using five doses (2000, 1000, 500, 250 and 125 µg/ml) while, equal volume of distilled water served as control. The varying mixtures were incubated at 37±2°C for 15 min after which, the mixtures were heated in a temperature regulated water bath at 70°C for 5 min. After cooling, the absorbance of the mixtures was read in the spectrophotometer at 660 nm and the vehicle used as blank. The percentage inhibition of protein denaturation was calculated as:

% PI = 100 X $[1 - V_t]/V_c$
V_t = absorbance of test sample
V_c = absorbance of control.

Statistical analysis

The results are expressed as percentage inhibition (%) and data analyzed by using the one way analysis of variance (ANOVA) ($p<0.05$).

RESULTS AND DISCUSSION

The phytochemical screening of the leaves of *D. velutinum* and *D. scorpiurus* revealed the presence of flavonoids, saponins, tannins and sterols showing

Table 1. Phytochemical screening of *D. velutinum* and *D. scorpiurus* Leaves.

Qualitative analysis	*D. velutinum*	*D. scorpiurus*
Flavonoids	+	+
Saponins	+	+
Tannins	+	+
Alkaloids	-	-
Cardiac glycosides	-	-
Anthraquinones	-	-
Resins	+	-
Sterols	+	+

+ = Present; - = Absent.

similarities in the secondary metabolites they possessed (Table 1).

The phytochemical analysis of *D. velutinum* used in this study is however similar to the one collected from the South east region of Nigeria (Anowi et al., 2012). Furthermore, there were similarities in the secondary metabolites of the leaves of *D. scorpiurus* and *D. velutinum* as well as those *Desmodium* species previously studied (Ma et al., 2011; Liu et al., 2013).

The egg-albumin model used in this study is one of the models adopted for determining anti-inflammatory and anti- oedematogenic activities of medicinal plants (Hira et al., 2013). The model induces inflammation by producing histamine, serotonin and bradykinin in the first phase and the release of prostaglandins and nitric oxide in the second phase by showing peak inflammaton at 3 h (Seibert et al.,1994).

The methanol extracts, hexane and ethyl acetate fractions of *D. velutinum* and *D. scorpiurus* were investigated for their anti-inflammatory potentials. In the *in vivo* anti-inflammatory study, the activities of the methanol extract of *D. velutinum* leaf at 200 and 100 mg/ml were not significantly different ($p>0.05$) from each other but more pronounced than that of the reference drug (Diclofenac sodium) when compared with the untreated group. The methanol extract at 200 and 100 mg/ml exhibited a reduction in oedema of 28.8 and 32.1% at 2 h respectively while, the hexane fraction of the leaf of *D. veluti num* at 200 and 100 mg/kg exhibited same anti-inflammatory effect by reducing the oedema by 27.2% at 6 h (Tables 2 and 3). The ethyl acetate fraction exhibited a weak anti-inflammatory effect of 13.2 and 6.8% at 200 and 100 mg/ml after 6 h (Table 4).

The methanol extract of the leaf of *D. scorpuirus* exhibited dose dependent anti-inflammatory activities at all doses (200, 100 and 50 mg/ml) tested. The methanol extract at 200 and 100 mg/ml exhibited high anti-inflammatory effects of 27.1 and 21.9% 6 h after extract administration when compared to the untreated group (Table 5). The hexane fraction 6 h after administration at 200 and 100 mg/kg exhibited a reduction of 24.5 and 18.1% in the oedema and the activity of the ethylacetate was the weakest with a reduction of 18.1 and 14.3% at 6 h. This effect is similar with that exhibited by the ethylacetate fraction of the leaf of *D. velutinum.* In the *in vitro* assay, the methanol extracts, hexane and ethyl acetate fractions of *D. velutinum* and *D. scorpiurus* exhibited dose-dependent activities.

The hexane fractons of *D. velutinum* and *D. scorpiurus* exhibited significant ($p<0.05$) anti-inflammatory activities of 76.9 and 68.6% at a dose of 2000 µg/ml respectively (Figures 1 and 2).

This study shows that the anti-inflammatory activities exhibited by the leaves of *D. velutinum* and *D. scorpiurus* are mainly in the hexane fraction, and could probably be due to non-polar compounds present in the leaves. In addition, the anti-inflammatory activities exhibited by the leaves of *D. velutinum* and *D. scorpiurus* are similar to some other species of *Desmodium* previously reported: *D. gangeticum* (Govindarajan et al., 2001) and *D. triflorum* (Lai et al., 2010). The methanol extracts and fractions of *D. velutinum* and *D. scorpiurus* lowered the paw oedema from the 2nd to 6th h (Tables 6 and 7). This is similar to the effect of the methanol extract of *D. triflorum* at the 3rd to 6th h at doses of 0.5 and 1 g/kg (Shang-Chih et al., 2009). The activities of the extracts and fractions of *D. velutinum* and *D. scorpiurus* were similar to the effect of the reference drug used, (Diclofenac Sodium) which reduces inflammation by inhibiting the synthesis of prostaglandin (Della et al., 1986) through inhibition of the cyclooxygenase in the arachidonic acid pathways (Skoutakis et al., 1988; Chen et al.,1995).

The activities of flavonoids, tannins, sterols and saponins present in the leaves of *D. velutinum* and *D. scorpiurus* have been attributed to anti-inflammatory activities, and previous studies have reported the potentials of flavonoids and other phenolic compounds in exerting anti-inflammatory activities by inhibiting the enzymes involved in the mediators of inflammation (Sawadogo et al., 2006) or by inhibiting the migration of leukocyte, reducing serum lysozyme levels and nitric acid (Wu et al., 2006).

The potentials of the leaf extracts and fractions of *D. velutinum* and *D. scorpiurus* to reduce paw oedema from the second hour shows the ability of the compound(s)

Table 2. *In vivo* anti-inflammatory activities of methanol extract of *D. velutinum* leaf in egg albumin induced rats.

Dose mg/kg	0 min	1 h	2 h	3 h	4 h	5 h	6 h
50	2.93±0.07	2.90±0.06, 8.22%	2.70±0.06, 3.7%	2.63 ± 0.09, 12.3%*	2.43±0.09, 17.1%*	2.33±0.09. 17.7%*	2.27±0.07, 14.3%*
100	3.03±0.09	2.67±0.07, 15.5%	2.37 ± 0.03, 32.1%*	2.33 ± 0.03, 22.3%*	2.23 ± 0.03, 23.8%*	2.20±0.06. 22.3%*	2.10±0.06, 20.8%*
200	2.87±0.03	2.50±0.00, 20.9%	2.23 ± 0.03, 28.8%*	2.20 ± 0.00, 26.7%*	2.17 ± 0.03, 25.9%*	2.10±0.06. 25.8%*	2.07±0.03, 21.9%*
Control (Untreated)	3.37±0.07	3.16±0.06	3.13 ± 0.03	3.00 ± 0.03	2.93 ± 0.06	2.83±0.03	2.65±0.03
Diclofenac Sodium	3.10±0.06	2.93±0.15 7.3%	2.90 ± 0.16, 7.4%	2.63 ± 0.03, 12.3%	2.57 ± 0.03, 19.1%	2.37±0.09. 16.3%	2.17±0.09, 18.1%

Percentage decrease of anti-inflammatory effects compared to the untreated group,*p<0.05.

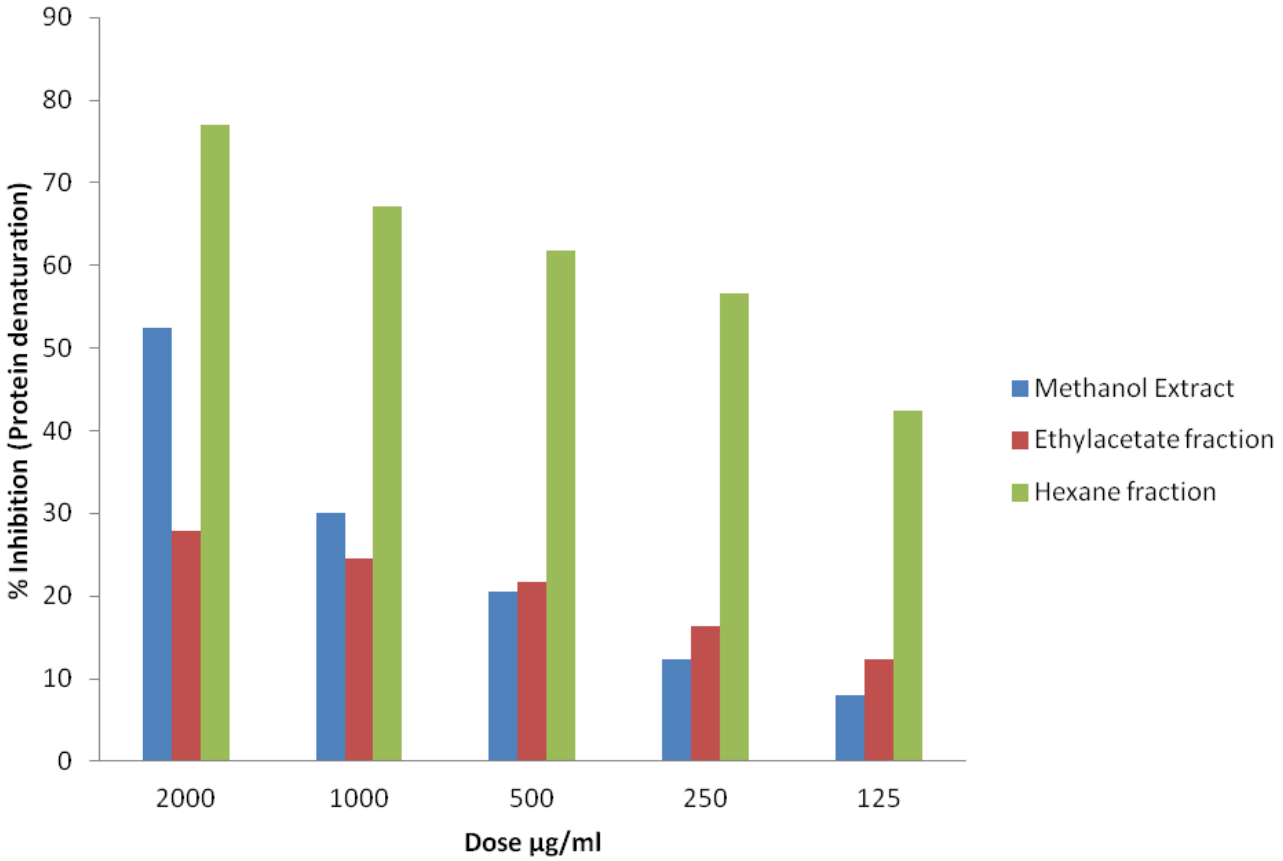

Figure 1. Effect of the methanol extract, hexane and ethyl acetate fractions of *D. velutinum* leaf against protein denaturation.

Table 3. *In vivo* anti-inflammatory activities of hexane extract of *D. velutinum* leaf in egg- albumin induced rats.

Dose mg/kg	0 min	1 h	2 h	3 h	4 h	5 h	6 h
50	3.13±0.07	3.10±0.07, 1.9%	2.90±0.06, 7.3%	2.73±0.03, 9.0%*	2.60±0.06, 11.3%*	2.40±0.09, 15.2%*	2.23±0.07, 15.8%*
100	3.00±0.06	2.77±0.03, 12.3%	2.67±0.03, 14.7%	2.47±0.03, 17.7%*	2.27±0.03, 22.5%*	2.17±0.03, 23.3%*	1.93±0.03, 27.2%*
200	3.03±0.09	2.70±0.06, 14.6%*	2.47±0.03, 21.1%*	2.40±0.06, 20.0%*	2.23±0.09, 23.9%*	2.10±0.06, 25.8%*	1.93±0.03, 27.2%*
Control (Untreated)	3.37±0.07	3.16±0.06	3.13±0.03	3.00±0.03	2.93±0.06	2.83±0.03	2.65±0.03
Diclofenac Sodium	3.10±0.06	2.93±0.15, 7.3%	2.90±0.16, 7.4%	2.63±0.03, 12.3%	2.57±0.03, 19.1%	2.37±0.09, 16.3%	2.17±0.09, 18.1%

Percentage decrease of anti-inflammatory effects compared to the untreated group, *p<0.05.

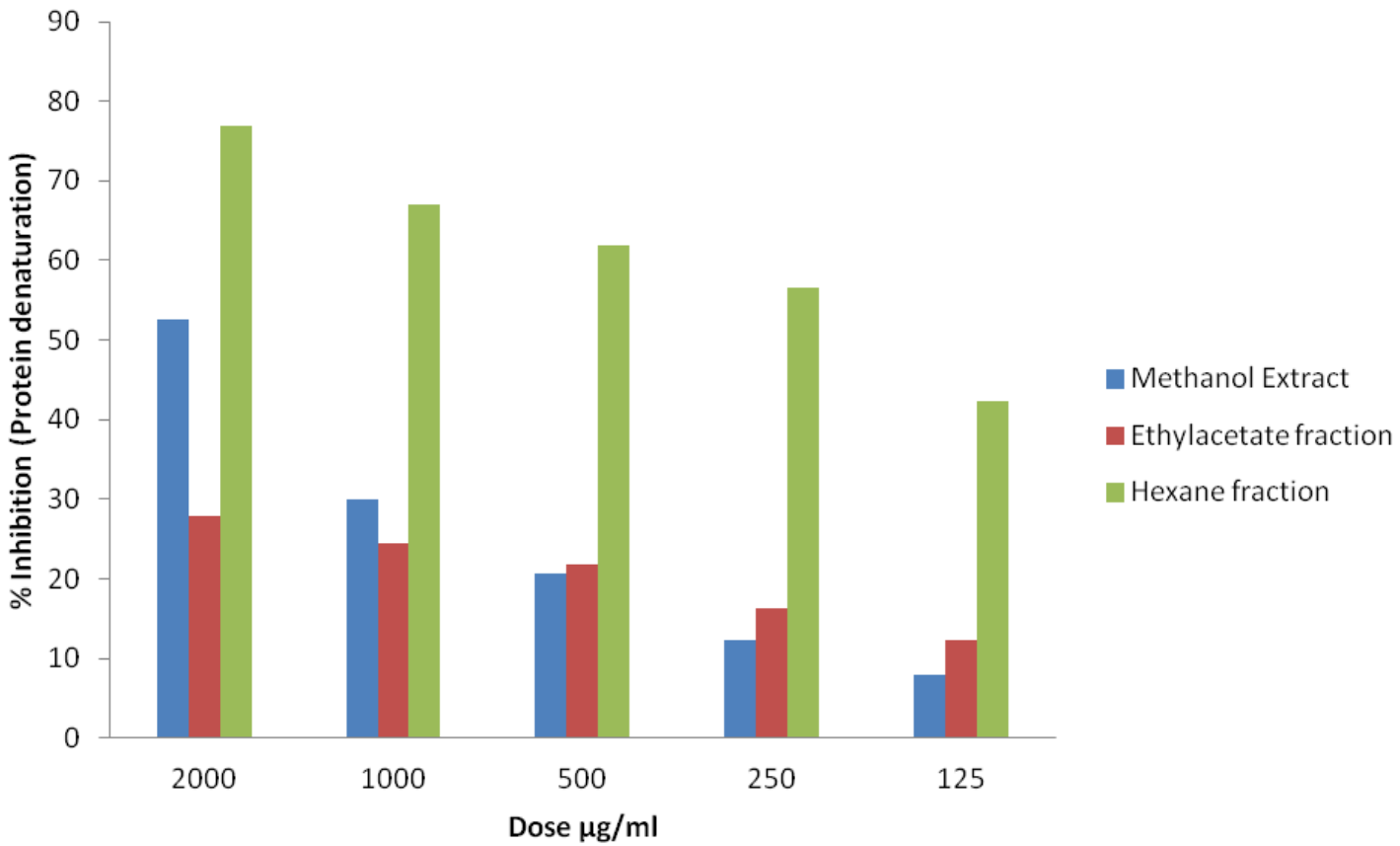

Figure 2. Effect of the methanol extract, hexane and ethyl acetate fractions of *D. scorpiurus* leaf against protein denaturation.

Table 4. *In vivo* anti-inflammatory activities of ethylacetate extract of *D. velutinum* leaf in egg- albumin induced rats.

Dose mg/kg	0 min	1 h	2 h	3 h	4 h	5 h	6 h
50	3.17±0.15	3.03±0.17,4.1%	3.03±0.17, 3.2%	2.87±0.09, 4.3%	2.83±0.12, 3.4%	2.67±0.07, 5.7%	2.57±0.07, 3.0%
100	3.30±0.06	3.03±0.08, 4.1%	2.93±0.03, 6.4%	2.83±0.03, 5.7%	2.73±0.03, 6.8%	2.53±0.06, 11.8%	2.47± 0.06, 6.8%
200	3.17±0.03	2.87±0.09, 9.2%	2.77±0.08, 11.5%*	2.63±0.03, 12.3%*	2.53±0.09, 13.7%*	2.43±0.07, 14.1%*	2.30± 0.10, 13.2%*
Control (Untreated)	3.37±0.07	3.16±0.06	3.13±0.03	3.00±0.03	2.93±0.06	2.83±0.03	2.65± 0.03
Diclofenac Sodium	3.10±0.06	2.93±0.15, 7.3%	2.90±0.16, 7.4%	2.63±0.03, 12.3%	2.57±0.03, 19.1%	2.37±0.09, 16.3%	2.17± 0.09, 18.1%

Percentage decrease of anti-inflammatory effects compared to the untreated group, *p<0.05.

Table 5. *In vivo* Anti-inflammatory activities of methanol extract of *D. scorpiurus* leaf in egg- albumin induced rats.

Dose mg/kg	0 min	1 h	2 h	3 h	4 h	5 h	6 h
50	3.10±0.15	3.07±0.19, 2.8%	2.93±0.18, 6.4%	2.67±0.13, 11.0%*	2.60±0.15, 11.3%*	2.53±0.14, 10.6%*	2.37±0.09, 10.6%*
100	3.07±0.07	2.83±0.03, 10.4%	2.80±0.06, 10.5%*	2.67±0.13, 11.0%*	2.57±0.03, 12.3%*	2.27±0.06, 19.8%*	1.93±0.07, 21.9%*
200	3.10±0.10	2.83±0.07, 10.4%	2.73±0.07, 12.8%*	2.43±0.13, 19.0%*	2.33±0.03, 20.5%*	2.23±0.07, 21.2%*	2.07±0.09, 27.1%*
Control (Untreated)	3.37±0.07	3.16±0.06	3.13±0.03	3.00±0.03	2.93±0.06	2.83±0.03	2.65±0.03
Diclofenac Sodium	3.10 ±0.06	2.93±0.15, 7.3%	2.90±0.16, 7.4%	2.63±0.03, 12.3%	2.57±0.03, 19.1%	2.37±0.09, 16.3%	2.17±0.09, 18.1%

Percentage decrease of anti-inflammatory effects compared to the untreated group, *p<0.05.

Table 6. *In vivo* anti-inflammatory activities of ethyl acetate extract of *D. scorpiurus* leaf in egg- albumin induced rats.

Dose mg/kg	0 min	1 h	2 h	3 h	4 h	5 h	6 h
50	3.07±0.07	3.07±0.07 2.8%	2.90± 0.06, 7.4%	2.87±0.07, 4.3%*	2.73±0.07, 6.8%*	2.57±0.09, 9.2%*	2.57±0.09, 3.0%*
100	2.97±0.03	2.83±0.03 10.4%	2.77±0.06, 11.5%*	2.63±0.13, 12.3%*	2.43±0.03, 17.1%*	2.30±0.06, 18.7%*	2.27±0.03, 14.3%*
200	2.97±0.03	2.73±0.03 8.1%	2.63±0.03, 15.9%*	2.50 ±0.06, 16.7%*	2.27±0.07, 22.5%*	2.20±0.00, 23.2%*	2.17±0.03, 18.1%*
Control (Untreated)	3.37±0.07	3.16±0.06	3.13±0.03	3.00±0.03	2.93±0.06	2.83±0.03	2.65±0.03
Diclofenac Sodium	3.10±0.06	2.93±0.15, 7.3%	2.90±0.16, 7.4%	2.63±0.03, 12.3%	2.57±0.03, 19.1%	2.37±0.09, 16.3%	2.17±0.09, 18.1%

Percentage decrease of anti-inflammatory effects compared to the untreated group, *p<0.05.

Table 7. *In vivo* anti-inflammatory activities of hexane extract of *D. scorpiurus* leaf in egg- albumin induced rats.

Dose mg/kg	0 min	1 h	2 h	3 h	4 h	5 h	6 h
50	2.90±0.06	2.83±0.09, 10.4%	2.80±0.10, 10.5%	2.63±0.12, 12.3%*	2.53±0.12, 13.7%*	2.43±0.12 ,14.1%*	2.40±0.12, 8.3%*
100	3.10±0.06	2.83±0.09, 10.4%	2.83±0.09, 9.6%*	2.73±0.09 9.0%*	2.67±0.09 8.9%*	2.47±0.03, 12.7%*	2.17±0.03, 18.1%*
200	2.93±0.12	2.70±0.10, 14.6%	2.67±0.09, 14.7%*	2.53±0.09, 15.7%*	2.37±0.09 19.1%*	2.17±0.03, 23.3%*	2.00±0.06, 24.5%*
Control (Untreated)	3.37±0.07	3.16±0.06	3.13±0.03	3.00±0.03	2.93±0.06	2.83±0.03	2.65±0.03
Diclofenac Sodium	3.10±0.06	2.93±0.15, 7.3%	2.90±0.16, 7.4%	2.63±0.03, 12.3%	2.57±0.03, 19.1%	2.37±0.09, 16.3%	2.17±0.09, 18.1%

Percentage decrease of anti-inflammatory effects compared to the untreated group,*p<0.05.

in the leaves to inhibit production of serotonin and bradykinnin in the first phase as well as inhibit the release of prostalglandins and nitric oxide responsible in the second phase of inflammation.

Conclusion

This study has therefore been able to further justify and report the anti-inflammatory effect of some other members of the genus Desmodium
apart from those previously studied.

Conflict of Interests

The authors have not declared any conflict of interests.

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