The 8th International Electronic Conference on Medicinal Chemistry (ECMC 2022)
01–30 NOVEMBER 2022 | ONLINE

Anti-inflammatory and Anti-nociceptive properties of leaf fractions of *Sida linifolia* L. (Malvaceae) possibly mediated by peripheral and central mechanisms

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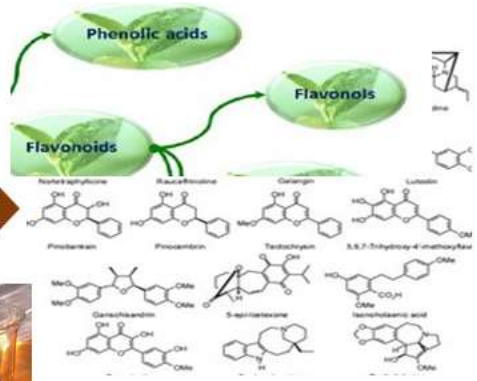
Title: Anti-inflammatory and Anti-nociceptive properties of leaf fractions of *Sida linifolia* L. (Malvaceae) possibly mediated by peripheral and central mechanisms

Sida linifolia L. (Malvaceae)

Extraction and fractionation to obtain ethyl acetate (EAF) and ethanolic (ETF) leaf fractions of *S. linifolia*.

GC-FID analysis



- Flavonoids
- Tannins
- Phenols
- Terpenoids
- Saponins

Induction with phlogistic agents

EAF and ETF effectively inhibited mice paw edema, hind paw licking and writhing comparable to standard drug (Aspirin)

Conclusion: These suggest that the leaf fractions of *S. linifolia* possess anti-inflammatory and analgesic potentials, which may follow peripheral and central mechanisms.

Abstract

Sida linifolia L., a common weed found in dry forest areas in West Africa and other parts of the world, is associated with several folkloric applications in Africa, including its use in assuaging painful whitlows and in malaria management; however, scanty or no scientific study has validated its bioactivities. Herein, we investigated the anti-nociceptive and anti-inflammatory mechanisms of ethanolic (ETF) and ethylacetate (EAF) fractions of *Sida linifolia* leaves. *In vivo* anti-inflammatory properties of the fractions were evaluated by edema induction with an intraperitoneal injection of freshly prepared carrageenan (0.1 ml of 0.01 g/ml) and 0.1 ml of undiluted fresh egg albumin into mice's hind paw, while hind paw licking and writhing were induced in mice using formalin (i.p.) (0.02 ml of 1 % v/v) and 0.6 % (v/v) (10 ml/kg bw) (i.p.) acetic acid, respectively, to assay for the anti-nociceptive potentials. Varying amounts of flavonoids, tannins, and other phenols, terpenoids, saponins, steroids, and alkaloids were detected in the fractions. The LD_{50} study showed no toxicity up to 5000 mg/kg body weight (*per oral*) EAF and ETF. Interestingly, oral administration of various concentrations (200, 400 and 600 mg/kg bw) of the fractions significantly ($p < 0.05$) inhibited all phases of edemogenesis, mice's hind licking, and writhing compared with control and were comparable with 100 mg/kg bw (*p.o*) aspirin. However, ETF showed non-significantly ($p > 0.05$) better anti-nociceptive and anti-inflammatory activities than EAF. These suggest that the leaf fractions of *Sida linifolia* possess anti-inflammatory and anti-nociceptive potentials, possibly mediated by peripheral and central mechanisms.

Keywords: NSAIDs; Anti-inflammation; Anti-nociceptive; Rheumatoid arthritis; Malaria; Whitlow

Introduction

Consistent exposure of the human system to exogenous and endogenous agents, capable of setting up chains of inflammatory events, could result in inflammatory disorders (Ammendolia et al., 2021). Inflammation results in pain, heat, redness, and swelling of the affected area due to increased vascular permeability and blood flow to the injured site. It also results in leucocyte recruitment, fluid exudation, lysosomal leakages, free radical production, and protein denaturation (Sarveswaran et al., 2017). Studies have expounded on the critical roles of inflammatory mediators such as histamine, serotonin, prostanoids, and transcription factors in inflammation (Ricciotti et al., 2011; Abdulkhaleq et al., 2018). Although inflammatory responses are projected to inactive, destroy, and remove invading pathogens or toxicants and proceed toward tissue repair (Medzhitov, 2010; Eming et al., 2014), prolonged inflammatory events result in several disease conditions (Crowson et al., 2013; Furman et al., 2019). The recent shift in research efforts to screening medicinal plants for alternative anti-inflammatory drug candidates is partly due to several adverse side effects of most synthetic steroidal and non-steroidal anti-inflammatory agents (Marcum and Hanlon, 2010).

The African communities are blessed with medicinal herbs and rely on these herbs for food and disease treatment (Sofowora et al., 2013; Lin et al., 2016). *Sida linifolia* L., a member of the Malvaceae family, is a medicinal plant in west tropical Africa (Akubue et al., 1983). The medicinal properties of most members of the *Sida* genus have been extensively studied (Rao et al., 1998; Ekpo and Etim, 2009; Palaksha and Ravishankar, 2012; Rodrigues et al., 2020); however, studies on the bioactivities of *Sida linifolia* is still limited. The traditional use of *S. linifolia* leaves in managing inflammatory diseases, warranted the present study. Hence, we investigated the anti-inflammatory and anti-nociceptive properties of ethanolic (ETF) and ethyl acetate (EAF) leaf fractions of *Sida linifolia*.

Results

Table 1: shows the results of the quantitative phytochemical composition of EAF and ETF

Phytochemical Composition	Concentrations (mg/g)	
	EAF	ETF
Flavonoids	1.377 ± 0.011[b]	1.180 ± 0.010[a]
Tannins	0.526 ± 0.002[a]	0.742 ± 0.001[b]
Other phenols	0.938 ± 0.010[b]	1.242 ± 0.006[b]
Cyanogenic compounds	0.351 ± 0.009[a]	0.374 ± 0.043[a]
Glycosides	0.255 ± 0.003[a]	0.444 ± 0.010[b]
Other saponins	0.231 ± 0.014[a]	0.436 ± 0.008[b]
Terpenoid	0.253 ± 0.007[a]	0.439 ± 0.007[b]
Steroids	0.176 ± 0.005[a]	0.542 ± 0.006[b]
Alkaloids	0.127 ± 0.007[a]	0.467 ± 0.003[b]

Results are presented as Mean ± SEM. Subsets in the same row with distinct alphabets as superscript are deemed significantly ($P < 0.05$) different.

Results

Table 2: Effect of *S. linifolia* leaf fractions on egg albumin-induced paw edema in mice

Groups	Mean oedema Volume (ml) (% edema inhibition) 1 h	2 h	3 h	4 h	5 h
Control	6.90 ± 0.10^{abC} ---	6.37 ± 0.07^{aA} ---	6.51 ± 0.05^{aA} ---	6.63 ± 0.04^{aA} ---	6.48 ± 0.06^{aA} ---
100 mg/kg bw (*p.o*) Aspirin	6.43 ± 0.17^{aA} (32.90 %)	6.12 ± 0.24^{aB} (40.25 %)	5.55 ± 0.09^{bC} (44.25 %)	3.69 ± 0.09^{cD} (66.68 %)	3.02 ± 0.10^{bE} (81.40 %)
200 mg/kg bw (*p.o*) EAF	5.73± 0.06^{bA} (34.74 %)	5.17 ± 0.11^{cB} (38.59 %)	3.79 ± 0.08^{dC} (52.61 %)	2.89 ± 0.06^{dD} (69.02 %)	2.66 ± 0.04^{cD} (74.94 %)
400 mg/kg bw (*p.o*) EAF	6.11±0.07^{aA} (38.92 %)	5.60 ± 0.13^{bB} (42.52 %)	4.82 ± 0.11^{cC} (49.27 %)	3.60 ± 0.10^{cD} (66.24 %)	3.14 ± 0.05^{bE} (75.66 %)
600 mg/kg bw (*p.o*) EAF	6.39±0.04^{aA} (33.50 %)	5.83 ± 0.08^{bB} (36.75 %)	4.50 ± 0.17^{cC} (47.39 %)	3.41 ± 0.10^{cD} (62.71 %)	3.05 ± 0.32^{bE} (70.61 %)
200 mg/kg bw (*p.o*) ETF	6.51 ± 0.09^{aA} (39.50 %)	5.84 ± 0.17^{bB} (44.06 %)	5.05 ± 0.13^{bcC} (51.06 %)	4.50 ± 0.06^{bD} (57.16 %)	3.29 ± 0.14^{bE} (78.27 %)
400 mg/kg bw (*p.o*) ETF	6.42±0.07^{aA} (37.41 %)	5.63 ± 0.07^{bB} (37.41 %)	4.92 ± 0.11^{cC} (48.65 %)	4.10 ± 0.12^{bD} (58.45 %)	3.09 ± 0.24^{bE} (77.80 %)
600 mg/kg bw (*p.o*) ETF	6.38 ± 0.07^{aA} (40.44 %)	5.63 ± 0.13^{bB} (45.93 %)	5.14 ± 0.10^{bcC} (50.26 %)	4.40 ± 0.91^{bD} (58.66 %)	3.36 ± 0.15^{bE} (74.84 %)

Results (Two-way ANOVA with Duncan *post hoc* test) are presented as Mean ± SEM (n=8). Figures in parentheses indicate % edema inhibition. Subsets of a column with different low case alphabets and rows with different upper case alphabets as superscript differ significantly ($p < 0.05$). Control = 10 mg/kg bw distilled water; bw= body weight; *p.o= per oral.*

Table 3: Effect of *S. linifolia* leaf fractions on carageenan-induced paw edema in mice

Groups	Mean oedema Volume (ml) (% edema inhibition) 1 h	2 h	3 h	4 h	5 h
Control	6.06 ± 0.10^{aC}	6.16 ± 0.07^{aBC}	6.26 ± 0.03^{aB}	6.30 ± 0.20^{aA}	6.05 ± 0.06^{aC}
	---	---	---	---	---
100 mg/kg bw (*p.o*) Aspirin	5.59 ± 0.07^{bA}	5.01 ± 0.10^{cB}	4.52±0.39^{bC}	3.99±0.12^{bcD}	3.29 ± 0.07^{bE}
	(45.73 %)	(51.09 %)	(56.64 %)	(64.78 %)	(77.80 %)
200 mg/kg bw (*p.o*) EAF	5.32 ± 0.15^{bcA}	4.90 ± 0.16^{bcB}	3.94 ± 0.23^{cC}	3.19±0.30^{cD}	2.83 ± 0.14^{aE}
	(38.72 %)	(42.03 %)	(52.60 %)	(66.58 %)	(72.73 %)
400 mg/kg bw (*p.o*) EAF	5.47 ± 0.08^{bcA}	5.22 ± 0.16^{bB}	4.51±0.07^{bC}	3.90±0.12^{bcD}	3.32 ± 0.08^{bE}
	(44.74 %)	(46.94 %)	(54.23 %)	(62.93 %)	(73.66 %)
600 mg/kg bw (*p.o*) EAF	5.64 ± 0.15^{bA}	5.22 ± 0.16^{bB}	4.85±0.11^{bC}	4.37±0.16^{bD}	3.09±0.12^{bE}
	(41.28 %)	(44.63 %)	(48.10 %)	(53.44 %)	(75.46%)
200 mg/kg bw (*p.o*) ETF	5.19 ± 0.11^{cA}	4.53 ± 0.14^{dB}	3.98±0.20^{cC}	3.18±0.23^{aD}	2.76±0.30^{aE}
	(40.12 %)	(45.64 %)	(52.15 %)	(64.65 %)	(74.99 %)
400 mg/kg bw (*p.o*) ETF	5.36 ± 0.14^{bcA}	4.92 ± 0.18^{cB}	4.43±0.10^{bC}	3.66±0.06^{bcD}	3.10±0.12^{bE}
	(43.85 %)	(47.77 %)	(52.94 %)	(63.92 %)	(75.50 %)
600 mg/kg bw (*p.o*) ETF	5.27 ± 0.06^{cA}	4.42 ± 0.07^{dB}	3.70±0.11^{cC}	3.23±0.12^{cD}	3.07±0.11^{bDE}
	(45.34 %)	(54.00 %)	(64.49 %)	(73.83 %)	(77.53 %)

Results (Two-way ANOVA with Duncan *post hoc* test) are presented as Mean ± SEM (n=8). Figures in parentheses indicate % edema inhibition. Subsets of a column with different low case alphabets and rows with different upper case alphabets as superscript differ significantly ($p < 0.05$). Control = 10 mg/kg bw distilled water; bw= body weight; *p.o= per oral*.

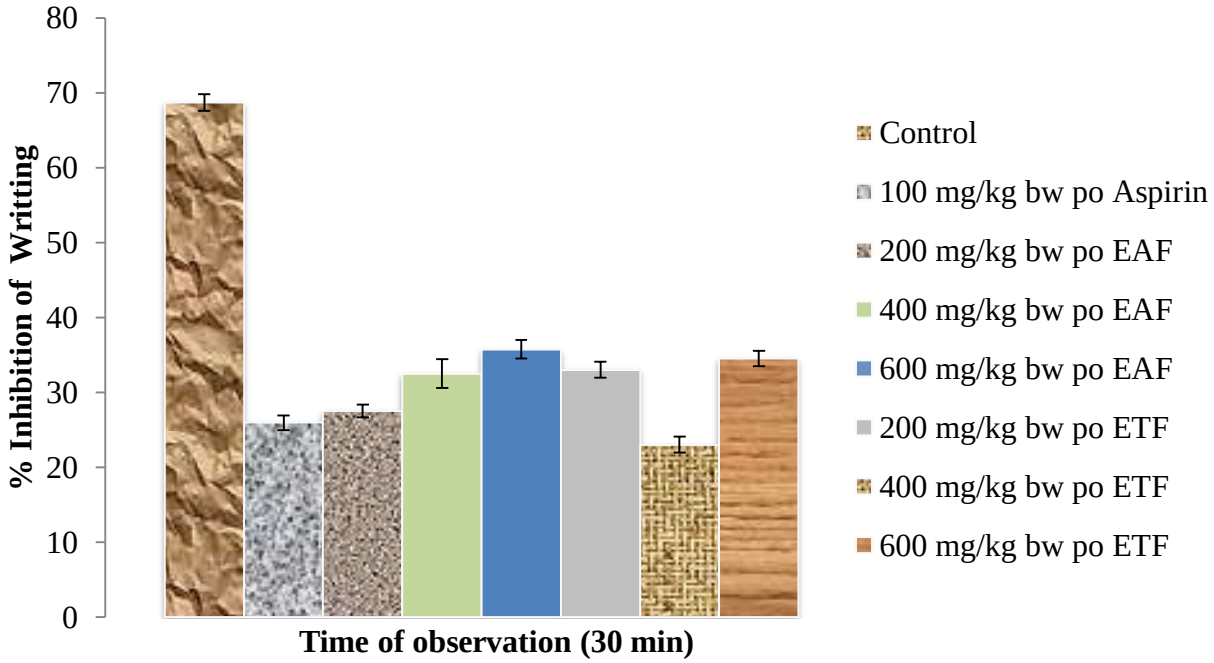


Figure 1. Effect of leaf fractions of *S. linifolia* on acetic acid induced writhing in mice. (n=8).

* Control = 10 mg/kg bw distilled water.

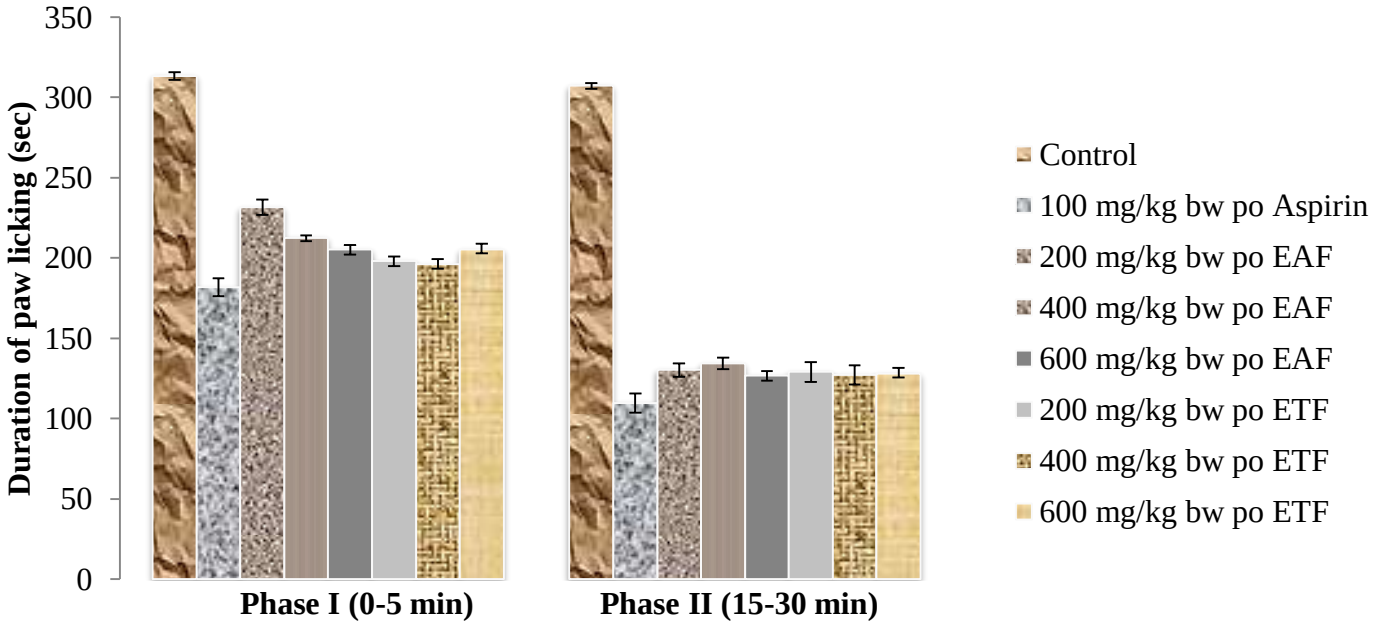


Figure 2. Effect of leaf fractions of *S. linifolia* on formalin-induced hind paw licking in mice. (n=8)

*Control = 10 mg/kg bw distilled water

Discussion

The acute toxicity (LD_{50}) study showed the safety of the fractions up to 5000 mg/kg bw (per oral), hence may be considered safe for oral administration (Anosike et al., 2012).

The phytochemical screening of *S. linifolia* leaf fractions revealed higher levels of flavonoids, tannins, and other phenols, and moderate levels of cyanogenic compounds, terpenoids, steroids, alkaloids, glycosides and other saponin. The amount of the various classes of phytocompounds present in the leaf fractions were higher in EAF compared with ETF.

From the result (Table 2), the fractions exhibited significant ($P < 0.05$) anti-inflammatory activities in the egg albumin model and were at par with 100 mg/kg bw p.o, aspirin. The maximum edema inhibitions (75.66 and 78.27 % inhibition) were produced by 400 mg/kg bw (p.o) EAF and 200 mg/kg bw (p.o) ETF, respectively, in the 5th hour, which did not differ ($P > 0.05$) with that (81.40 %) produced by aspirin at the same time. It could be that the fractions acted by inhibiting the production of histamine and serotonin, two primary inflammatory mediators implicated in egg albumin-induced edema (Akindele et al., 2015). Our result agrees with John-Africa et al. (2013), which reported a decrease in egg albumin-induced inflammation in mice pretreated with Sida *corymbosa* leaf extract.

The leaf fractions also exhibited potent inhibition of edema in carrageenan-challenged mice (Table 2). The plant leaf fractions showed a significant ($P < 0.05$) inhibitory effect on all three phases of carrageenan-induced edemogenesis with peak inhibition in the third phase. The activity of the fractions was similar to that observed with the reference drug (aspirin). The plant leaf fractions may have acted by inhibiting the release of histamine, serotonin, and kinin, which mediates the first phase (Georgewill et al., 2010) and prostaglandin synthesis that mediates the late phase (Ricciotti and FitzGerald, 2011). Our result aligns with Sutradhar et al. (2006).

Discussion Cont.

The leaf fractions were also potent in the writhing model. Pre-treatment with various doses of the leaf fractions produced a dose-dependent inhibitory effect on writhing syndrome caused by acetic acid and was comparable with aspirin (Figure 1). This result implies a significant peripherally mediated anti-nociceptive action of the fractions.

Furthermore, the observed bioactivity might be due to the association of the model with peripheral receptor activation, particularly the local peritoneal receptors on the surface of cells lining the peritoneal cavity (Zakaria et al., 2008). Our result corresponds with Azad et al. (2017) and Da Rosa (2018), which demonstrated the analgesic effects of S. *rhombifolia* and S. *tuberculata* extracts, respectively, using the acetic acid-induced writhing model.

The untreated formalin-challenged mice spent an extended time biting and licking the injected paw (Figure 2); however, groups pretreated with various doses of the fractions showed ($P < 0.05$) dose-dependent pain inhibition in all phases. The inhibitory effect of the plant fractions peaked in both phases, at the 400 mg/kg bw (p.o) EAF and 600 mg/kg bw (p.o) ETF dosage, and this did not differ significantly ($p > 0.05$) with the inhibition observed with 100 mg/kg bw, p.o, aspirin. The formalin test produces a distinct biphasic nociceptive reaction in animals (Hunskaar et al., 1985). Medications that act centrally, such as morphine, block both phases of the test, whereas drugs that act peripherally, such as aspirin, block mainly the late stage (Chan et al., 1983). Our result showed that the plant fractions suppressed ($p < 0.05$) nociceptive reflexes in both phases, supporting a peripheral mechanism of anti-nociception while simultaneously implying the participation of a central mechanism of action. Our findings agree with Bonjardim et al. (2011). The bioactivities of the fractions could be due to their rich composition of antioxidant and anti-inflammatory phytocompounds such as phenolics (Table 1).

Conclusion

These suggest that the leaf fractions of *S. linifolia* possess anti-inflammatory and analgesic potentials, which may follow peripheral and central mechanisms.

Acknowledgement

The authors appreciate the contributions of the entire staff of the Department of Biochemistry, University of Nigeria Nsukka and the various institutions consulted in the course of the study.