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Free Radical Scavenging and Inflammation Counteracting Properties of Ethylacetate Fraction of *Sida linifolia* †

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Abstract: *Sida linifolia* L., is traditionally used in a number of diseased conditions including the relief of uncomfortable teething and the prevention of malaria. The aim of this study was to investigate the antioxidant and anti-inflammatory properties of *Sida linifolia*. For the in vitro anti-inflammatory tests, platelet aggregation, albumin denaturation, protease, and phospholipase A_2 were performed. Then for the in vivo studies of the same, egg albumin and carrageenan induced models were employed. The total antioxidant capacity (TAC), 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferric reducing power (FRAP), and nitric oxide (NO) assays were used in the in vitro antioxidant assessment, and the reference standards for the antioxidant tests were butylated hydroxytoluene (BHT), gallic acid, and ascorbic acid, whereas the anti-inflammatory studies employed aspirin and prednisolone as standards. Every parameter was calculated using conventional methods. In the fraction, there were varying concentrations of terpenoids, saponins, steroids, alkaloids, flavonoids, tannins, and other phenols. The EALFSL displayed robust, concentration-dependent anti-inflammatory effects, which were comparable to those of the reference drugs (Aspirin/Prednisolone). The EALFSL fraction's IC_{50} values ranged from 0.93 to 1.20 mg/mL which was less active than those of BHT (0.30), ascorbic acid (0.32–0.50), and gallic acid (0.47). The outcomes additionally demonstrated that EALFSL has a significant level of concentration-dependent antioxidant activity. These suggest that the ethylacetate leaf fraction of *Sida linifolia* possessed anti-inflammatory and antioxidant effects which could be attributed to phytochemicals contained in it.

Keywords: *Sida linifolia*: NSAIDs; anti-inflammation; antioxidants; anti-malaria; phytochemicals

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1. Introduction

Oxidative stress is an oxidative imbalance caused by an inability to detoxify reactive metabolites created by the generation of reactive oxygen species (ROS) during cellular metabolism. The increase in free radicals produced by biological oxidation disrupts the structure and functions of intracellular proteins, causes membrane damage due to polyunsaturated fatty acid peroxidation with lipids, nucleic acid base modifications and chromosome changes (DNA single-strand and double-strand breaks, DNA and protein cross-links), and causes cell death and oxidative damage to cellular structures and components such as polysaccharide depolymerization and carcinogenesis (Kiran et al., 2023).

Inflammation is one of the body's defence systems in reaction to potentially damaging physicochemical and microbiological irritants (Ammendolia et al., 2021). Due to increased vascular permeability of the affected area, it often manifests as painful episodes, heat, edema, and redness of the infected area. The most common consequence is fluid exudation and tissue function loss. As a result, neutrophils and other leucocytes get drawn to the inflammatory environment and begin to behave aggressively, resulting in

lysosomal leakage, protein denaturation, and cell death. Chronic and increasing inflammation can cause tissue damage and organ failure (Sarveswaran et al., 2017).

Sida linifolia L. is a common weed found in West Africa and other parts of the world. It belongs to the Malvaceae family and the genus Sida, which contains over 180 species and has a variety of ethnomedical applications (Dinda et al., 2015; Saensouka et al., 2016).

The anti-inflammatory, antinociceptive, and antioxidant activities of *Sida linifolia*'s ethanol leaf fraction have been examined (Nwankwo et al., 2023). The aim of this study was to look into the phytochemicals found in the ethylacetate fraction of *Sida linifolia* leaves and to discover feasible mechanisms explaining the plant's anti-inflammatory and antioxidant capabilities.

2. Results

Standards: DPPH- butylated hydroxytoluene (BHT); FRAP- ascorbic acid; TAC- gallic acid; NO- ascorbic acid

3. Discussion

The phytochemical analysis of Sida linifolia's ethylacetate leaf fraction (EALFSL) (Table 1) revealed a range of quantities of flavonoids, phenols, tannins, cyanogenic compounds, glycosides, saponin, terpenoids, steroids, and alkaloids. According to studies by Barbosa-Filho et al. (2006) and Farooq et al. (2022), medicinal plants' high levels of flavonoids, tannins, terpenoids, phenols, and steroids are what give them their anti-inflammatory properties.

Table 1. Phytochemical screening of EALFSL.

Phytochemical composition	Concentration (mg/g)	
	EALFSL	**ELFSL**
Flavonoids	1.377 ± 0.011 ^{b}	1.180 ± 0.010 ^{a}
Phenols	0.938 ± 0.010 ^{b}	2.242 ± 0.006 ^{a}
Tannins	0.526 ± 0.002 ^{a}	0.742 ± 0.001 ^{b}
Cyanogenic compounds	0.351 ± 0.009 ^{a}	0.374 ± 0.043 ^{a}
Glycosides	0.255 ± 0.003 ^{a}	0.444 ± 0.010 ^{b}
Terpenoids	0.253 ± 0.007 ^{a}	0.439 ± 0.007 ^{b}
Saponins	0.231 ± 0.014 ^{a}	0.436 ± 0.008 ^{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.

Anti-inflammatory properties of the EALFSL in vitro (Table 2). At 1.0 mg/mL, EALFSL exhibited the highest level of membrane stabilizing action (57.49 0.25) against hemolysis brought on by hypotonicity. The activity of the fraction (39.030.31) at 0.2 mg/mL is extremely comparable to that of the conventional medication (39.260.65) at the same concentration.

Table 2. In vitro anti-inflammatory activities of EALFSL.

Treatments/Conc. (mg/mL)	Hypotonicity-Induced Hemolysis Inhibition (%)	Heat-Induced Hemolysis Inhibition (%)	Platelet Aggregation Inhibition (%)	Protease Inhibition (%)	Phospholipase A_2 Activity inhibition (%)	Protein Denaturation (%)
EALFSL (0.2)	39.03 ± 0.31 ^{a}	40.05 ± 0.43 ^{a}	37.19 ± 0.17 ^{a}	40.55 ± 1.00 ^{a}	32.99 ± 0.34 ^{a}	31.91 ± 0.31 ^{a}
" (0.4)	40.58 ± 0.23 ^{b}	53.04 ± 0.23 ^{c}	40.97 ± 0.40 ^{b}	44.16 ± 0.99 ^{b}	41.38 ± 0.99 ^{b}	36.79 ± 0.23 ^{b}
" (0.6)	46.13 ± 0.27 ^{c}	57.30 ± 0.26 ^{d}	46.89 ± 0.28 ^{d}	53.03 ± 0.33 ^{d}	46.94 ± 0.59 ^{c}	42.14 ± 0.41 ^{c}

" (0.8)	50.81 ± 0.14 ^{d}	67.76 ± 0.14 ^{e}	51.40 ± 0.17 ^{e}	57.72 ± 0.26 ^{e}	52.61 ± 1.67 ^{d}	51.63 ± 0.35 ^{d}
" (1.0)	57.49 ± 0.25 ^{e}	74.97 ± 0.30 ^{f}	55.94 ± 0.21 ^{f}	62.45 ± 0.19 ^{f}	54.20 ± 0.50 ^{d}	56.10 ± 1.02 ^{e}
Aspirin (0.2)	39.26 ± 0.65 ^{a}	45.27 ± 0.13 ^{b}	45.52 ± 0.84 ^{c}	48.72 ± 0.23 ^{c}	ƥ 41.61 ± o.51 ^{b}	41.12 ± 0.28 ^{c}

Data are represented as means ± S.E.M; (n = 3). Down the column, values with different letters superscripts are significantly ($p < 0.05$) different; ƥ: signifies that prednisolone served as reference drug.

EALFSL had the strongest inhibitory effect at 1.0 mg/mL and the least effective effect at 0.2 mg/mL for heat-induced hemolysis, platelet aggregation, and protease. The conventional drug (Aspirin) exhibited values that were much higher than the findings for EALFSL at 0.2 mg/mL.

The anti-inflammatory activity values of the EALFSL, which were concentration-dependent, were equivalent to those of the reference drug (Aspirin/Prednisolone), across all test parameters, according to the results of the in vitro investigation.

Prednisolone was used as the control drug in both tests, but it had a similar general pattern of inhibition of phospholipase A2 activity and protein denaturation.

EALFSL may be able to reduce inflammatory cascades because of its ability to inhibit phospholipase A2 in a concentration-dependent manner, which was comparable to the reference drug (Prednisolone). The inhibition of arachidonic acid release, which is required for the synthesis of pro-inflammatory mediators, may have contributed to the fraction's anti-inflammatory actions (Coutinho and Chapman, 2011). As with the reference drug aspirin, EALFSL demonstrated a strong dose-dependent decrease of protein denaturation. This might suggest that they have the ability to stop inflammatory cascades (Raju et al., 2019). In a concentration-dependent manner, the EALFSL demonstrated high inhibitory potentials against protease activity, showing its anti-inflammatory properties. Studies have shown that when leucocytes manufacture proteases in excess or when there is lysosomal leakage, proteases play critical roles in a range of pathogenic and inflammatory disorders (Bermudez-Humarán et al., 2015; Enechi et al., 2019). The EALFSL showed high inhibitory potentials against protease activity, indicating its anti-inflammatory effects, in a concentration-dependent manner. According to studies, proteases play crucial roles in a variety of pathogenic and inflammatory diseases when leucocytes produce them in excess or when there is lysosomal leakage (Bermudez-Humarán et al., 2015; Enechi et al., 2019).

The development of mice paw edema was significantly ($p < 0.05$) decreased by EALFSL, precisely like the reference drug (aspirin), in each of the three phases of the carrageenan inflammatory model, with the greatest effect in the third phase (Table 3). Inflammation mediators are released during the first phase of edemogenesis in the carrageenan model, and the excellent inhibition of edema formation demonstrated by EALFSL during this phase (1–2 h) suggests that the fraction likely inhibited this release (Georgewill and Georgewill 2010; Georgewill et al., 2010).

Table 3. Effect of EALFSL on carrageenan-induced paw edema in mice.

	Mean Edema Volume (mL) (% Edema Inhibition)				
Treatments	**1 h**	**2 h**	**3 h**	**4 h**	**5 h**
Control	6.06 ± 0.10 ^{aA} -	6.16 ± 0.07 ^{aA} -	6.26 ± 0.03 ^{aA} -	6.34 ± 0.19 ^{aA} -	6.05 ± 0.06 ^{aA} -
EALFSL (200 mg/kg)	5.32 ± 0.15 ^{cA} (38.72%)	4.90 ± 0.16 ^{cB} (42.03%)	3.94 ± 0.23 ^{cC} (52.60%)	3.19 ± 0.30 ^{cD} (66.58%)	2.83 ± 0.14 ^{cE} (72.73%)
" (400 mg/kg)	5.47 ± 0.08 ^{bcA} (44.74%)	5.22 ± 0.16 ^{bB} (46.94%)	4.51 ± 0.07 ^{bC} (54.23%)	3.90 ± 0.12 ^{bcD} (62.93%)	3.32 ± 0.08 ^{bE} (73.66%)

" (600 mg/kg)	5.64 ± 0.15 ^{bA} (41.28%)	5.22 ± 0.16 ^{bB} (44.63%)	4.85 ± 0.11 ^{bC} (48.10%)	4.37 ± 0.16 ^{bD} (53.44%)	3.09 ± 0.12 ^{bE} (75.46%)
Aspirin (100 mg/kg)	5.59 ± 0.07 ^{bA} (45.73%)	5.01 ± 0.10 ^{bcB} (51.09%)	4.52 ± 0.39 ^{bC} (56.64%)	3.99 ± 0.12 ^{bcD} (64.78%)	3.29 ± 0.07 ^{bE} (77.82%)

Results are presented as Mean ± SEM (n = 4). Figures in parentheses indicate inhibition (%) of edema progression. 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 of distilled water.

In mice induced with carrageenan but not given any therapy (control), edema development peaked (5.27 0.05 mL) at 4 h following injection. EALFSL had a time- and concentration-dependent partial edema blocking effect. The highest percentage inhibition (75.46%) and lowest percentage inhibition (38.72%) were recorded at 5 h following treatment with 600 mg/kg of EALFSL. The traditional drug, aspirin, demonstrated a 77.82% inhibition at 5 h following therapy at a dosage of 100 mg/kg.

Table 4 shows how EALFSL affected mice with egg-albumin-induced edema. In mice induced with egg-albumin, edema formation peaked (6.85 0.14 mL) at 1 h following injection. 600 mg/kg of EALFSL showed the least amount of edema inhibition after 1 h and 400 mg/kg of EALFSL showed the highest percentage of edema inhibition after 5 h of therapy. At a dose of 100 mg/kg, the standard drug (aspirin) gave 81.36% inhibition five hours following therapy.

Table 4. Effect of EALFSL on egg albumin-induced paw edema in mice.

	Mean Edema Volume (mL) (% Edema Inhibition)				
Treatments	**1 h**	**2 h**	**3 h**	**4 h**	**5 h**
Control	6.85 ± 0.14 ^{abA}	6.37 ± 0.07 ^{aAB}	6.51 ± 0.05 ^{aA}	6.63 ± 0.04 ^{aA}	6.48 ± 0.06 ^{aAB}
EALFSL (200 mg/kg)	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 ^{cD} (69.02%)	2.66 ± 0.04 ^{cD} (74.94%)
" (400 mg/kg)	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 ^{bD} (66.24%)	3.14 ± 0.05 ^{bE} (75.66%)
" (600 mg/kg)	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 ^{bD} (62.71 %)	3.05 ± 0.32 ^{bE} (70.61%)
Aspirin (100 mg/kg)	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 ^{bD} (66.68 %)	3.02 ± 0.10 ^{bE} (81.36%)

Results are presented as Mean ± SEM (n = 4). Figures in parentheses indicate inhibition (%) of edema progression. 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 of distilled water.

According to Akindele et al. (2015), the EALFSL also suppressed egg-albumin-induced edemogenesis, raising the possibility that it could reduce inflammation. Aspirin also significantly reduced the edema caused on by the injection of egg albumin and was comparable to the fraction, as would be expected from a potent cyclooxygenase inhibitor.

The EALFSL was able to effectively scavenge DPPH, nitric oxide radicals, TRAC, and FRAP, according to the reactive and free radical scavenging potential experiment (Table 5). The EALFSL showed excellent antioxidant and free radical scavenging potentials, which were concentration-dependent. The IC50 value for its DPPH scavenging activity

was 1.20 mg/mL. The standard (BHT)'s IC50 value of 0.30 mg/mL showed stronger inhibitory effects.

Table 5. Antioxidant capacity of EALFSL.

	Percentage inhibition			
Treatments/Conc. (mg/mL)	**DPPH**	**FRAP**	**TAC**	**NO**
EALFSL (0.2 mg/mL)	19.37 ± 0.68 [b]	21.31 ± 1.12 [a]	23.54 ± 0.28 [a]	35.02 ± 0.61 [a]
" (0.4 mg/mL)	15.15 ± 0.73 [a]	27.00 ± 0.76 [b]	32.70 ± 0.85 [b]	38.23 ± 0.44 [b]
" (0.6 mg/mL)	26.61 ± 0.35 [c]	28.48 ± 0.97 [b]	36.97 ± 0.49 [c]	41.01 ± 0.39 [c]
" (0.8 mg/mL)	35.31 ± 0.48 [d]	34.60 ± 0.92 [c]	44.94 ± 0.42 [d]	43.29 ± 0.44 [d]
" (1.0 mg/mL)	50.07 ± 0.80 [e]	61.18 ± 2.01 [d]	56.49 ± 0.20 [e]	51.22 ± 0.37 [e]
Standards (0.2 mg/mL)	45.06 ± 0.61 [e]	29.75 ± 1.67 [b]	37.05 ± 0.48 [c]	45.65 ± 0.47 [d]

Values are represented in mean ± Standard error of the Mean. Down the column values with different letter super scripts are significantly ($p < 0.05$) different.

While ascorbic acid had a value of 0.32 mg/mL for scavenging nitric oxide, EALFSL had an IC_{50} of 1.04 mg/mL. The Total Antioxidant Capacity (TAC) IC_{50} value for EALFSL was 0.93 mg/mL vs. 0.47 mg/mL for the reference (gallic acid). Compared to ascorbic acid, which has an IC_{50} value of 0.50 mg/mL, EALFSL has a FRAP IC_{50} value of 0.88 mg/mL.

EALFSL generally showed excellent, concentration-dependent antioxidant potentials. The reference drugs showed a comparable level of activity, although they were more effective. The EALFSL was discovered to have much better DPPH and nitric oxide scavenging capacities when compared to BHT and ascorbic acid. It has a remarkable high reducing power and antioxidant capacity, both of which were dose-dependent, making it comparable to ascorbic acid and gallic acid. The antioxidant potential of EALFSL has been shown to be caused by its phenol concentration.

The IC_{50} value ranges (in mg/mL) for EALFSL (0.93–1.20) show that it has a very strong antioxidant capacity, but it is less effective than BHT (0.30), ascorbic acid (0.32–0.50), and gallic acid (0.47).

4. Conclusions

The results of the current study showed that the ethylacetate leaf fraction of *S. linifolia* (EALFSL) had anti-inflammatory and antioxidant properties. These effects may have been caused by mechanisms, such as the inhibition of prostaglandin, serotonin, and histamine kinin release. The range of phytochemicals present in the plant fraction may be the source of the aforementioned qualities. The particular mechanisms of action of the various biologically active compounds linked to the proven anti-inflammatory and antioxidant potentials of EALFSL as well as their separation, characterisation, and possibly structural elucidation, could be the subject of future research.

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Conflicts of Interest:

References

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1. Author 1, A.B.; Author 2, C.D. Title of the article. *Abbreviated Journal Name* **Year**, *Volume*, page range.
2. Author 1, A.; Author 2, B. Title of the chapter. In *Book Title*, 2nd ed.; Editor 1, A., Editor 2, B., Eds.; Publisher: Publisher Location, Country, 2007; Volume 3, pp. 154–196.
3. Author 1, A.; Author 2, B. *Book Title*, 3rd ed.; Publisher: Publisher Location, Country, 2008; pp. 154–196.
4. Author 1, A.B.; Author 2, C. Title of Unpublished Work. *Abbreviated Journal Name* year, *phrase indicating stage of publication (submitted; accepted; in press)*.
5. Author 1, A.B. (University, City, State, Country); Author 2, C. (Institute, City, State, Country). Personal communication, 2012.
6. Author 1, A.B.; Author 2, C.D.; Author 3, E.F. Title of Presentation. In Proceedings of the Name of the Conference, Location of Conference, Country, Date of Conference (Day Month Year).
7. Author 1, A.B. Title of Thesis. Level of Thesis, Degree-Granting University, Location of University, Date of Completion.
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