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# Phytochemical screening and *in vitro* evaluation of anti-inflammatory and antioxidant potentials of leaf extracts of *Sida linifolia* L. (Malvaceae)

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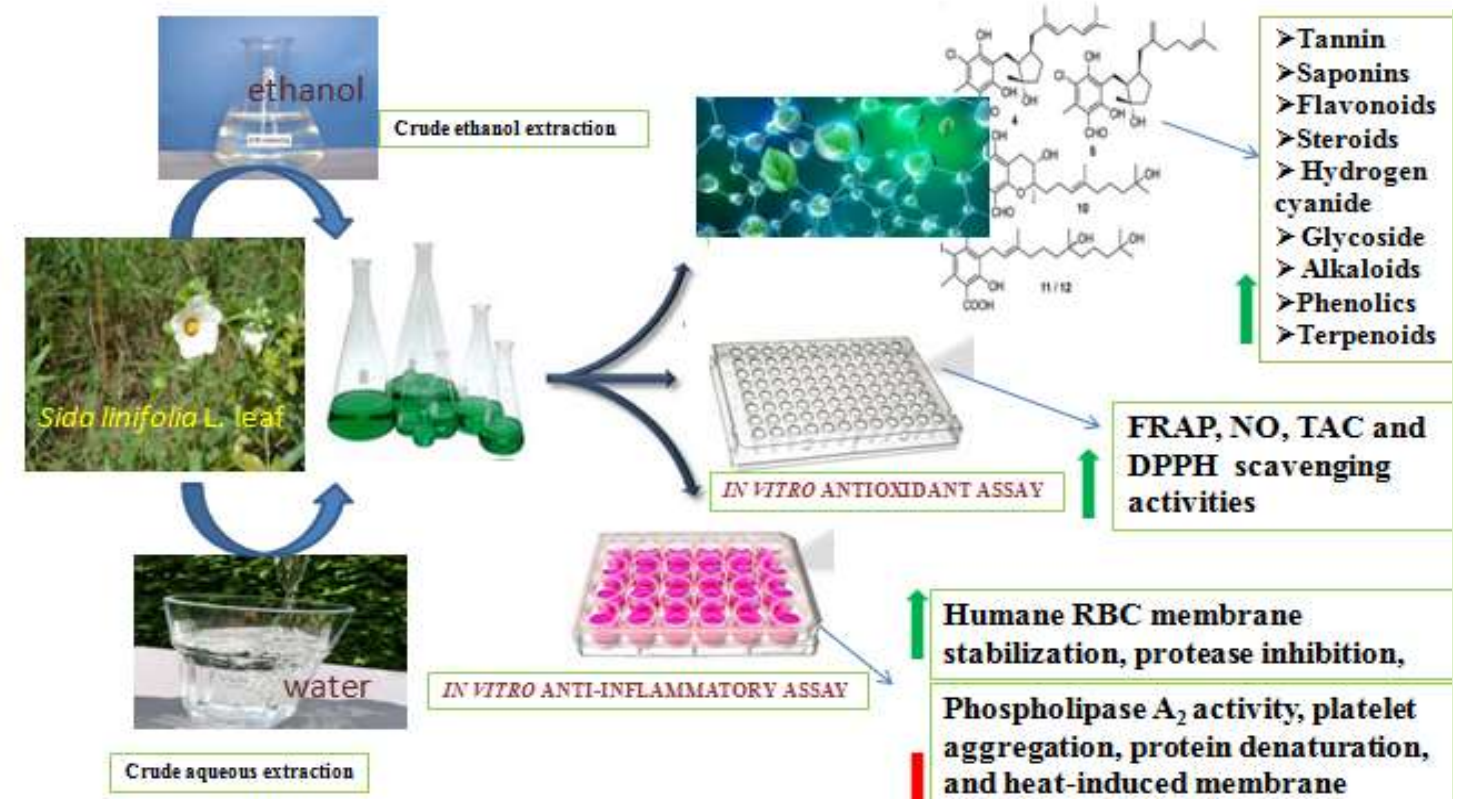
# Phytochemical screening and *in vitro* evaluation of anti-inflammatory and antioxidant potentials of leaf extracts of *Sida linifolia* L. (Malvaceae)




Conclusion: *Sida linifolia* leaf crude extracts possess anti-inflammatory and antioxidant properties.

**Abstract**

This study aimed to ascertain the *in vitro* anti-inflammatory and antioxidant potentials of crude aqueous extract (CAE) and crude ethanol extract (CEE) of *Sida linifolia* leaf. The assayed *in vitro* anti-inflammatory parameters were phospholipase $A_2$ activity, platelet aggregation, membrane stabilization, protease inhibition, protein denaturation, and heat-induced membrane hemolysis. Also, antioxidant properties of the extracts were determined using ferric reducing antioxidant power (FRAP) assay, nitric oxide radical (NO) scavenging assay, total antioxidant capacity (TAC) assay, and 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) scavenging assay. Aspirin was used as a reference drug for the anti-inflammatory test, while ascorbic acid, gallic acid, and butylated hydroxytoluene (BHT) served as reference drugs. All parameters were determined following standard procedures. Phytochemical analysis of the extracts revealed appreciably high amount of tannins, saponins, flavonoids, steroids, hydrogen cyanide, glycoside, alkaloids phenolics, and terpenoids. At the various concentrations (0.2, 0.4, 0.6, 0.8, and 1.0 mg/ml), both CAE and CEE exhibited significantly ($p < 0.05$) high percentage inhibition of inflammation as the standard drug and increased with concentrations. However, CEE was significantly ($p < 0.05$) more potent, compared to CAE at all concentrations. The *in vitro* antioxidant assay revealed that both extracts demonstrated considerably high antioxidant potentials and increased with dosage. The ranges of $IC_{50}$ values (in mg/ml) for CAE (0.64-0.92) and CEE (0.51-0.62) were close to that of gallic acid (0.47), ascorbic acid (0.32-0.50) and BHT (0.3). The overall antioxidant potentials of the plant extracts were strongest in CEE. These suggest that *Sida linifolia* leaf possess anti-inflammatory and antioxidant properties.

**Keywords:** Anti-inflammation; NSAIDs; Antioxidants; Phytochemicals.

## Introduction

Inflammation is one of the multifarious arrays of defensive mechanisms deployed by the immune system against invading pathogens. It usually present with excruciating pains and represents the cause of high mortality associated with disease conditions such as Rheumatoid arthritis and Alzheimer's disease (Arulselvan *et al*., 2016). It is usually manifested in increased vascular permeability, proteins denaturation, and distortion of cellular membrane integrity and may result in tissue damage upon prolonged and exaggerated inflammatory regime (Sarveswaran *et al*., 2017). Also, elevated levels of reactive oxygen species (ROS) within the inflammation milieu may further aggravate the inflammatory process by initiating chains of oxidative reactions that destroys membrane macromolecules, including lipids and proteins (Verma, 2016). The plant, *Sida linifolia* is prevalent in West Tropical Africa and is commonly found in dry forest areas. It is a member of the *Sida* genus which consists of several species of erect perennial shrubs including *Sida rhombifolia*, *S. cordifolia* and *S.acuta* which are known for their various medicinal applications (Palaksha and Ravishankar, 2012; Rodriguez *et al*., 2020). However, there remains a dearth of knowledge of the pharmacological potency of some species of the plant, including *Sida linifolia*. The little knowledge we have about this plant, *Sida linifolia* is that it is used ethnomedicinally in the treatment of inflammatory diseases such as whitlow and also in the treatment of malaria. Due to the ethical issues associated with the use of animals at the preliminary stage of drug discovery, the study thus, employed *in vitro* assays to investigate the bioactive compounds, anti-inflammatory and antioxidant properties of *Sida linifolia* leaf extracts.

## Results

### Evaluation of quantitative phytochemical composition of extracts of *Sida linifolia* leaf

The result of the quantitative bioactive ingredient present in the crude aqueous (CAE) and crude ethanol extract (CEE) of *Sida linifolia* leaf showed appreciably high amounts of important phytochemicals such as, flavonoids, phenolics, tannins, hydrogen cyanide, saponin, steroids, glycosisde, alkaloids and terpenoids. This is presented in Table 1.

Table 1: the results of the quantitative phytochemical composition of CAE and CEE

| | **Concentrations (mg/g)** | |
|---|---|---|
| **Phytochemical Components** | **CAE** | **CEE** |
| Tannin | $1.058 \pm 0.003^{b}$ | $0.733 \pm 0.002^{a}$ |
| Saponin | $0.549 \pm 0.006^{b}$ | $0.418 \pm 0.007^{a}$ |
| Flavonoids | $1.637 \pm 0.021^{b}$ | $1.166 \pm 0.016^{a}$ |
| Steroids | $0.652 \pm 0.005^{b}$ | $0.593 \pm 0.017^{a}$ |
| Hydrogen cyanide | $0.481 \pm 0.008^{b}$ | $0.302 \pm 0.008^{a}$ |
| Glycosisde | $0.622 \pm 0.003^{b}$ | $0.375 \pm 0.005^{a}$ |
| Alkaloids | $0.623 \pm 0.004^{b}$ | $0.471 \pm 0.003^{a}$ |
| Phenols | $1.441 \pm 0.010^{b}$ | $1.043 \pm 0.006^{a}$ |
| Terpenoid | $0.587 \pm 0.003^{a}$ | $0.590 \pm 0.005^{a}$ |

Mean ± SEM in triplicate. Subsets with dissimilar alphabets as superscript in the same column are considered significantly ($p < 0.05$) different.

Table 2A-2F: the anti-inflammatory properties of leaf extracts of *Sida linifolia*

**A**

| Treatments | % Membrane stabilization | | |
|---|---|---|---|
| | Concentration (mg/ml) | CAE | CEE |
| Extracts | 0.2 | 49.64 ± 0.16[a] | 41.60 ± 0.69[a] |
| | 0.4 | 51.90 ± 0.23[b] | 48.40 ± 0.04[b] |
| | 0.6 | 55.45 ± 0.33[c] | 52.06 ± 0.63[c] |
| | 0.8 | 58.55 ± 0.13[d] | 58.40 ± 0.26[d] |
| | 1.0 | 63.68 ± 0.53[e] | 67.38 ± 0.07[e] |
| Standard | 0.8 | 83.53 ± 0.63[g] | 83.53 ± 0.62[f] |
| | 1.0 | 76.35 ±0.48[f] | 76.35 ± 0.48[g] |

**B**

| Treatments | % Protein denaturation | | |
|---|---|---|---|
| | Concentration (mg/ml) | CAE | CEE |
| Extracts | 0.2 | 44.24 ± 0.36[a] | 40.43 ± 0.27[a] |
| | 0.4 | 52.51 ± 0.30[b] | 49.59 ± 0.29[b] |
| | 0.6 | 54.13 ± 0.49[c] | 57.61± 0.51[c] |
| | 0.8 | 56.03 ± 0.18[d] | 62.20 ± 0.78[d] |
| | 1.0 | 64.23 ± 0.51[f] | 66.40 ± 0.29[e] |
| Standard | 0.8 | 57.30 ± 0.09[e] | 57.30 ± 0.09[c] |
| | 1.0 | 69.73 ± 0.47[g] | 69.73 ± 0.47[f] |

**C**

| Treatments | % Platelet aggregation | | |
|---|---|---|---|
| | Concentration (mg/ml) | CAE | CEE |
| Extracts | 0.2 | 36.12 ± 0.23[a] | 35.09 ± 0.17[a] |
| | 0.4 | 40.63 ± 0.38[b] | 38.79 ± 0.23[b] |
| | 0.6 | 44.22 ± 0.17[c] | 43.87 ± 0.24[c] |
| | 0.8 | 46.62 ± 0.29[d] | 51.39 ± 0.20[d] |
| | 1.0 | 66.13 ± 0.10[e] | 63.96 ± 0.17[f] |
| Standard | 0.8 | 83.53 ± 0.63[g] | 83.53 ± 0.62[f] |
| | 1.0 | 76.35 ±0.48[f] | 76.35 ± 0.48[g] |

**D**

| Treatments | % Proteinase Inhibition | | |
|---|---|---|---|
| | Concentration (mg/ml) | CAE | CEE |
| Extracts | 0.2 | 49.25 ± 0.29[a] | 44.73 ± 0.69[a] |
| | 0.4 | 54.51± 0.75[b] | 46.18 ± 0.31[b] |
| | 0.6 | 58.37 ± 0.52[c] | 47.55 ± 0.19[c] |
| | 0.8 | 59.63 ± 0.30[c] | 53.40 ± 0.21[d] |
| | 1.0 | 62.12 ± 1.52[d] | 64.86 ± 0.27[f] |
| Standard | 0.8 | 62.92 ± 0.17[d] | 62.92 ± 0.17[e] |
| | 1.0 | 66.44 ± 0.13[e] | 66.44 ± 0.13[g] |

**E**

| Treatments | % Phospholipase $A_2$ | | |
|---|---|---|---|
| | Concentration (mg/ml) | CAE | CEE |
| Extracts | 0.2 | 45.35 ± 1.08[a] | 43.88 ± 1.09[a] |
| | 0.4 | 46.83 ± 0.60[a] | 46.94 ± 1.04[a] |
| | 0.6 | 54.20 ± 0.60[b] | 51.47 ± 1.20[b] |
| | 0.8 | 57.14 ± 0.39[c] | 56.12 ± 1.23[c] |
| | 1.0 | 58.16 ± 0.39[c] | 59.98 ±0.60[d] |
| Standard | 0.8 | 58.83 ± 0.59[c] | 58.84 ± 0.59[cd] |
| | 1.0 | 67.11 ± 1.18[d] | 67.11 ± 1.18[e] |

**F**

| Treatments | % heat-induced membrane haemolysis | | |
|---|---|---|---|
| | Concentration (mg/ml) | CAE | CEE |
| Extracts | 0.2 | 45.35 ± 1.08[a] | 35.82 ± 0.69[a] |
| | 0.4 | 46.83 ± 0.60[a] | 51.30 ± 0.47[b] |
| | 0.6 | 54.20 ± 0.60[b] | 66.48 ± 0.13[c] |
| | 0.8 | 57.14 ± 0.39[c] | 82.60 ± 0.66[f] |
| | 1 | 58.16 ± 0.39[c] | 75.01± 0.51[d] |
| Standard | 0.8 | 67.57 ± 0.20[d] | 67.57 ± 0.20[c] |
| | 1 | 76.69 ± 0.20[e] | 76.69 ± 0.20[e] |

*Mean ± SEM in triplicate. Subsets with dissimilar alphabets as superscript in the same column are considered significantly ($p < 0.05$) different.

**Antioxidant properties of leaf extracts of *Sida linifolia***

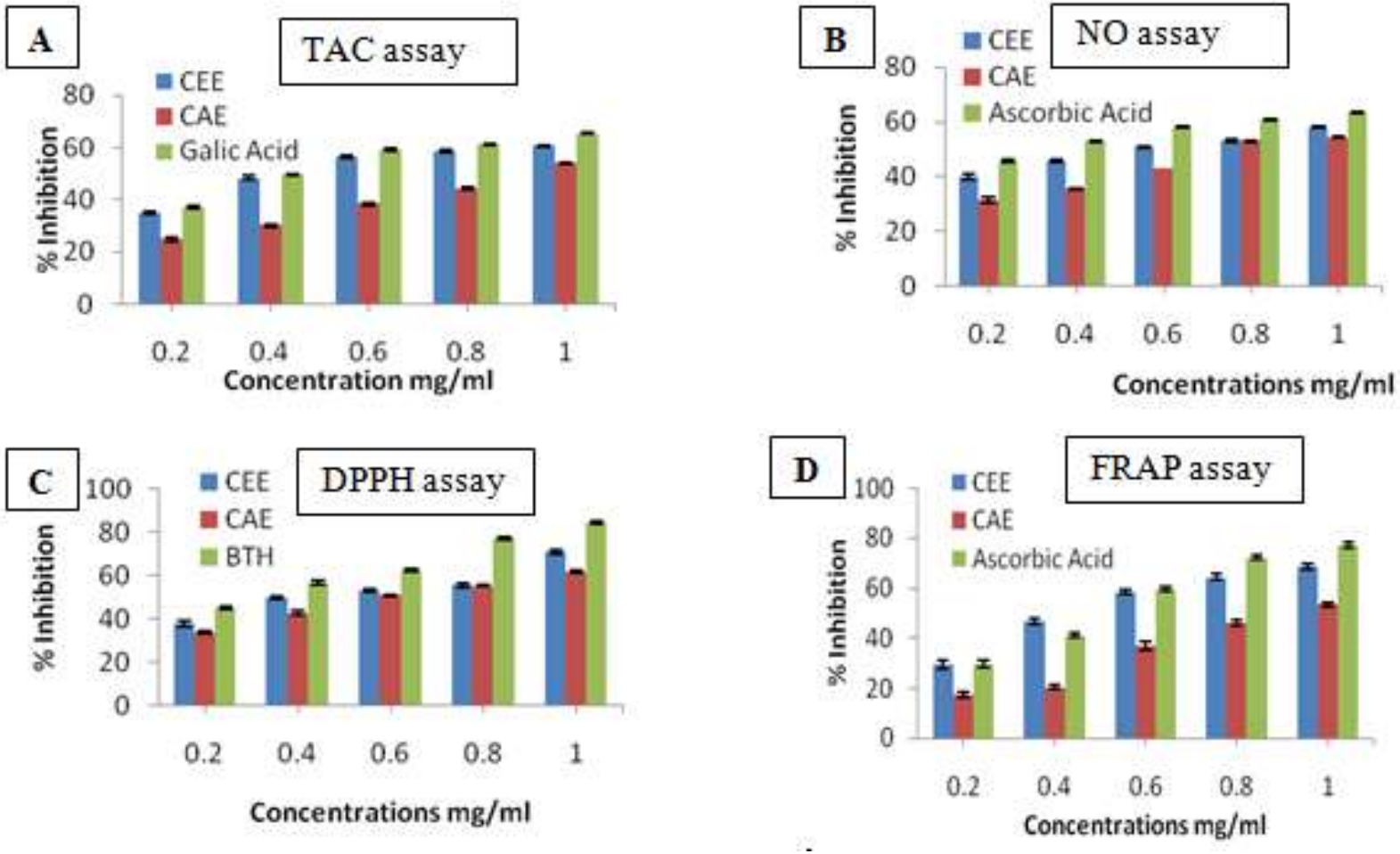



**Figure 1A- 1D:** the antioxidant activities of leaf extracts of *Sida linifolia* (CAE and CEE) compared to standards.

**Discussion**

Non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin, indomethacin, and ibuprofen, are frequently prescribed as anti-inflammatory agents worldwide. However, they are not without several side effects (Parvin *et al*., 2015). These informed the need to expedite research for alternative drug candidates from natural sources.

The phytochemical screening (Table 1) of the leaf extracts of *Sida linifolia* revealed that CAE and CEE are rich in secondary metabolites such as tannins, saponins, flavonoids, steroids, hydrogen cyanide, glycoside, alkaloids phenolics, and terpenoids. This gives insights into the pharmacological properties of the plant.

The result of the *in vitro* anti-inflammatory studies showed that both CAE and CEE exhibited varied percentage (%) membrane stabilization (Table 2A) in a concentration-dependent manner, akin to the reference drug. These indicate the anti-inflammatory properties of the plant extracts and showed a close relationship between the plant extracts and the standard drug as anti-inflammatory agents. From the result, it is apparent that the biological activity of the crude extracts could be optimized to match the reference drug if purified. The observed membrane stabilizing effect of *S. linifolia* leaf extracts could be due to its considerably high composition of bioactive principles with known antioxidant properties (Cory *et al*., 2018). In the same vein, Yoganandam *et al*. (2010) concluded that the erythrocyte membrane is analogues to lysosomal membrane therefore the ability of pharmacological agents to inhibit haemolysis of erythrocyte reflect to their lysosomal membrane stabilizing potentials.

**Discussion Cont.**

The result also showed that CAE and CEE exhibited concentration-dependent inhibition of protein denaturation (Table 2B), akin to the reference drug, which suggest their potentials to attenuate inflammatory process. This agrees with the work of Raju *et al*. (2019) which submitted that proteins denaturation results in inflammation, and agents that could inhibit this process would thus be good candidate for anti-inflammatory formulations. Both extracts of *S. linifolia* leaf effectively inhibited $CaCl_2$-induced aggregation of platelets (Table 2C) in a manner that is proportional to the concentration, and this was akin to the reference drug. Platelets whether attached to blood vessel or circulates in the blood stream are component blood cells essential in the formation blood clot. However, they liberate several inflammatory mediators that play roles in intensifying inflammation cascades such as leukocyte mobilization and endothelial responses to a variety of inflammatory stimuli (Hosseinzadegan and Tafti, 2017). Both extracts *S. linifolia* leaf were effective in inhibiting proteinase activity (Table 2D) following a dose dependent trend, as the reference drug, thus indicating their anti-inflammatory potentials. Bermúdez-Humarán *et al*. (2015) documented that proteinase play key roles in various pathologic conditions resulting in inflammation upon excessive release by immune cell or lysosomal leakage. The observed concentration dependent inhibitory effect of CAE and CEE on phospholipase $A_2$ activity as shown in the result (Table 2E), suggests that the extracts prevented the liberation of fatty acids from the membrane lipid bilayers, needed for the production of pro-inflammatory mediators (Coutinho and Chapman 2011). Some steroidal anti-inflammatory agents also follow similar mechanism of action, and are based on their ability to inhibit phospholipase A2 activity (Vane and Botting, 1998).

**Discussion Cont.**

Also, the high steroid composition of CAE and CEE implies that the extracts follow similar mechanism and could be a good candidate for managing inflammatory diseases. The result also showed that both extracts inhibited heat-induced membrane hemolysis (Table 2F) in a concentration-dependent manner, as the reference drug. This implies that extracts from *S. linifolia* leaf could use to manage inflammatory diseases.

The *in vitro* antioxidant assay (Figure1A-D) showed that both extracts (CAE and CEE) demonstrated significantly ($p < 0.05$) high ferric reducing antioxidant power (FRAP), nitric oxide radical scavenging activities, total antioxidant capacity (TAC), and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities in concentration-dependent manner. The observed antioxidant properties of the plant extracts could be due to the rich phenolic and flavanoid contents of the leaf extracts. Oxidative stress is ensued when the generation of free radicals overwhelms the antioxidant mechanisms that keep them in check (Reuter *et al*., 2010). The ranges of $IC_{50}$ values (in mg/ml) for CAE (0.64-0.92) and CEE (0.51-0.62) were close to that of gallic acid (0.47), ascorbic acid (0.32-0.50) and BHT (0.3). The overall antioxidant potentials of the plant extracts were strongest in CEE.

## Conclusions

The results of the present study suggest that crude aqueous and ethanol extracts of *Sida linifolia* leaf possess antioxidant and anti-inflammatory properties and this could be due to their rich phytochemical composition. Further studies are warranted to elucidate the mechanism by which the bioactive principles of this plant confer ant-inflammatory and antioxidant activities, observed in the present study.

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