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Profiling of Bioactive Compounds, Antioxidant Properties and Inhibitory Potentials of Ethanolic Leaf Fraction of *Sida linifolia* L. (Malvaceae) on Enzymes Implicated in the Pathology of Diabetes, Inflammation, and Neurological Disorders

Chaired by Dr. Alfredo Berzal-Herranz and Prof. Dr. Maria Emília Sousa

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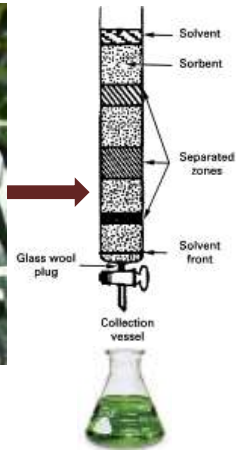
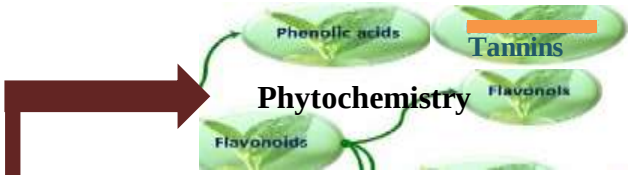
Abstract

Sida linifolia L. is a weed ubiquitously found in Africa with several folkloric applications. Traditional healers in the Southeastern part of Nigeria employ the alcoholic concoction of *S. linifolia* leaves as antidepressants, anti-malaria, antihypertensive, anti-abortifacients, and for managing painful whitlow; however, these claims lack scientific validation. The present study aimed to explore the phytochemical profile of the plant, *S. linifolia* with special emphasis to its antioxidant and inhibitory actions on enzymes linked to inflammation, diabetes, and neurological disorders. Phytochemical profiling and *in vitro* antioxidant and enzyme inhibition assays were employed to assess the pharmacological profile of *S. linifolia* ethanolic leaf fraction (SLELF). Preliminary phytochemical screening of SLELF revealed appreciable amounts of total phenolics (91.64 ± 7.61 mg GAE/g), total tannins (62.44 ± 3.86 mg TAE/g), and total flavonoids (27.35 ± 1.48 mg QE/g) present in SLELF. Results of HPLC analysis of SLELF revealed rich composition in bioactive compounds such as ellagic acid, quercetin, ferulic acid, 3,4-dimethoxy benzoic acid, gallic acid, 4-methoxy cinnamic acid, sinapic acid, vanillic acid, and chlorogenic acid. Enzymatic antioxidants (catalase and superoxide dismutase), non-enzymatic antioxidants (reduced glutathione (GSH), Vit A, C, and E), elemental minerals (Cu, Mn, Zn, Cr, Fe, and Ca), and γ-aminobutyric acid (GABA) were present in SLELF in appreciable levels. At various concentrations (0.2 – 1.0 mg/ml), SLELF exhibited potent and concentration-dependent hydrogen peroxide (H_2O_2) and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic) acid (ABTS) radical scavenging activities and exerted moderate inhibitory actions on enzymes associated with inflammation (cyclooxygenase-2 (COX-2) and lipoxygenases (LOXs), diabetes (α-amylase, α-glucosidase), and neurological disorders (butyrylcholinesterase (BChE) and γ-aminobutyric acid transaminase (GABA-T), compared to respective standards (ascorbic acid, acarbose, indomethacin, galanthamine, and vigabatrin). Perhaps, the observed potent pharmacological activities of SLELF could be anchored to its phytoconstituents. Furthermore, the slightly higher ranges of IC_{50} values (0.57 – 0.87 mg/ml) of SLELF compared to standards (0.44 – 0.68 mg/ml) suggest moderation in enzyme inhibition that may preclude adverse side effects. This study lends credence to the folklore claims of *S. linifolia* leaves and reveals its potential as a possible source of bioactive compounds for medicinal and pharmaceutical exploration.

Keywords: Phytocompounds; Antioxidant; Anti-diabetic; Anti-inflammatory; Neuromodulatory; *Sida linifolia*

Sida linifolia L. leaves





Zn, Cr, Mn, Ca, Fe, Cu
Vit A, C, E, GSH, CAT, and SOD

GABA content

Ethanolic leaf fraction of *S. linifolia* (SLELF)

In vitro free radical scavenging potential of SLELF

ABTS$^{\bullet+}$ and H_2O_2 Neutralization

In vitro anti-diabetic potential of SLELF

α-amylase and α-glucosidase Inhibition

In vitro anti-inflammatory potential of SLELF

COX-2 and LOXs Inhibition

In vitro neuromodulatory potential of SLELF

BChE and GABA-T Inhibition

Conclusion: The ethanolic leaf fractions of *S. linifolia* contain bioactive principles and exhibits antioxidant properties and inhibitory potential on enzymes linked to inflammation, diabetes, and neurological disorder

Introduction

Disease states are brought on by the human body's repeated exposure to oxidants and hazardous substances that can come from either endogenous or exogenous sources and change physiological status and normal homeostasis (Sharifi-Rad *et al.*, 2020). When reactive oxygen and nitrogen species exceed the antioxidant systems that have been put in place to control their oxidative activities, oxidative stress results, which manifests as the oxidative destruction of cellular and subcellular entities (Jomova *et al.*, 2023). The increasing body of evidence linking oxidative stress to the pathogenesis of numerous health abnormalities, such as inflammatory illnesses, diabetes, and neurological disorders, may be the cause of the recent upsurge in research on antioxidants derived from natural sources (Vona et al., 2021). Moreover, recent data connecting synthetic drugs to a number of unfavorable side effects has spurred an increase in the quest for natural sources of drug candidates that are less toxic and have better efficacy-ideally generated from plants (Najmi *et al.*, 2022). Some of these phytoactive principles, such as polyphenolics, vitamins, trace minerals, enzymes, peptides, and non-proteinogenic amino acids, have been reported to exhibit good antioxidant, anti-inflammatory, anti-diabetic, and neuromodulatory potentials (Choi *et al.*, 2022). Herein, the inhibitory potential of *S. linifolia* on enzymes implicated in some disease pathology was investigated and related to its phytoconstituents.

Results

High-Performance Liquid Chromatography (HPLC) Profile of Polyphenolic and Flavonoids Composition of SLELF

Presented in Table 1 are the polyphenolic compounds identified in SLELF using HPLC techniques. From the result, ellagic acid (5.46 ppm), quercetin (3.37 ppm), and ferulic acid (1.17 ppm) were detected in SLELF at varying concentrations. The HPLC chromatogram (shown in Figure 1) displayed three peaks representing ellagic acid (which retained at 1.607 min), quercetin (which retained at 3.098 min), and ferulic acid (which retained at 1.607 min).

Furthermore, Table 2 also shows the flavonoids identified in SLELF using HPLC techniques. From the result, different concentrations of flavonoids such as ellagic acid (1.23879 ppm), quercetin (0.616178 ppm), chlorogenic acid (0.056642 ppm), vanillic acid (0.053052 ppm), 3,4-dimethoxybenzoic acid (0.020722 ppm), gallic acid (0.012173 ppm), sinapic acid (0.001245 ppm), 4-methoxy cinnamic acid (0.000302 ppm), and one unidentified compound (0.000144 ppm) were detected in SLELF. The chromatogram (Figure 2) showed nine peaks representing different flavonoids with their retention time and peak area.

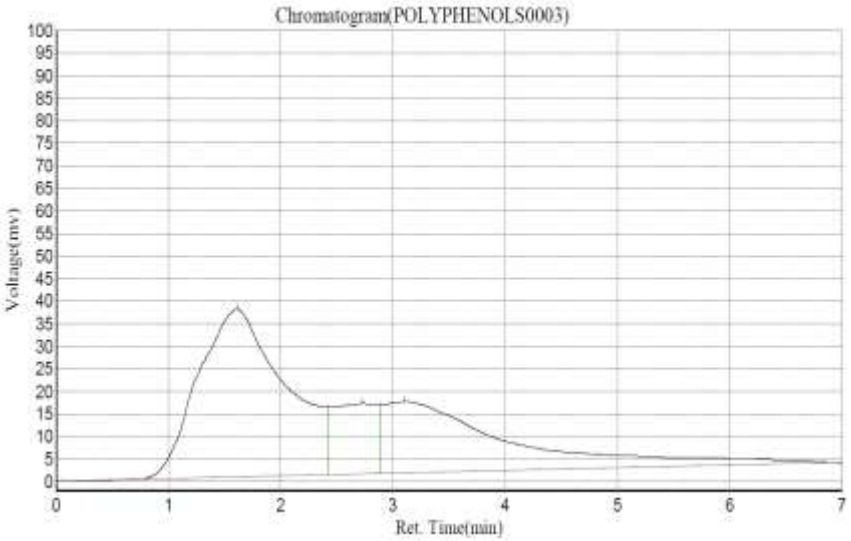


Figure 1: HPLC chromatogram of polyphenolic compounds in SLELF

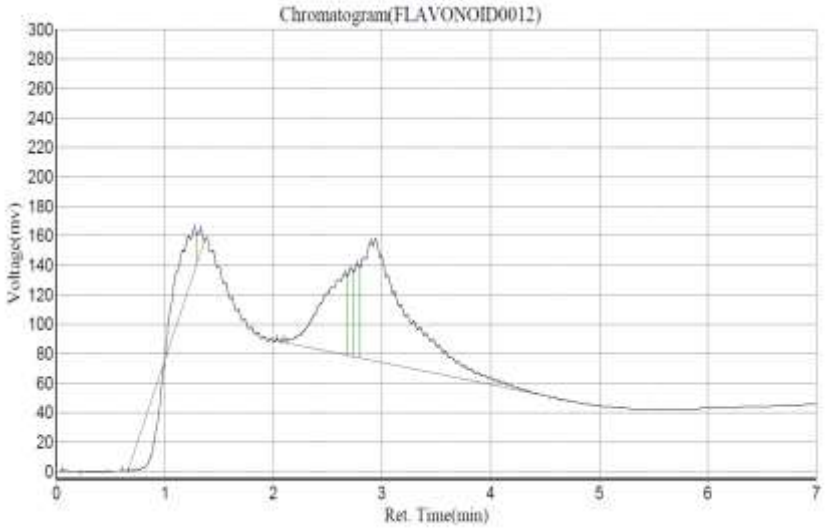


Figure 2: HPLC chromatogram of flavonoids in SLELF.

Table 1: HPLC Profile of Polyphenolic and Flavonoids Composition of SLELF

PeakNo	Peak ID	Retention Time (min)	Peak Height	Peak Area	Conc. (ppm)
Phenolics					
1	Ellagic acid	1.607	37242.449	2001188.750	5.46
2	Quercetin	3.098	15813.879	1232989.500	3.37
3	Ferulic acid	2.732	15614.772	428381.125	1.17
Flavonoids					
1	Ellagic acid	2.657	94729.250	1909336.125	1.23879
2	Quercetin	2.898	79751.523	2346381.250	0.616178
3	Chlorogenic acid	2.773	62934.246	215689.000	0.056642
4	Vanillic acid	2.707	58791.965	202020.719	0.053052
5	3,4-Dimethoxybenzoic acid	1.273	28171.299	78909.594	0.020722
6	Gallic acid	1.332	14966.476	46353.305	0.012173
7	Sinapic acid	2.098	1979.141	4741.750	0.001245
8	4-Methoxycinnamic acid	2.040	981.600	1148.800	0.000302
9	Unidentified	0.615	192.667	550.200	0.000144

Table 2: Phytochemical composition of SLELF

Phytoconstituents	Content/Activity
Total phenolics (mg GAE/g)	91.64 ± 7.61
Total tannins (mg TAE/g)	62.44 ± 3.86
Total flavonoids (mg QE/g)	27.35 ± 1.48
GSH (µg/g)	17.80 ± 0.23
GABA (pg/g)	153.06 ± 2.21
SOD (U/g)	192.59 ± 8.23
Catalase (U/g)	363.41 ± 11.23

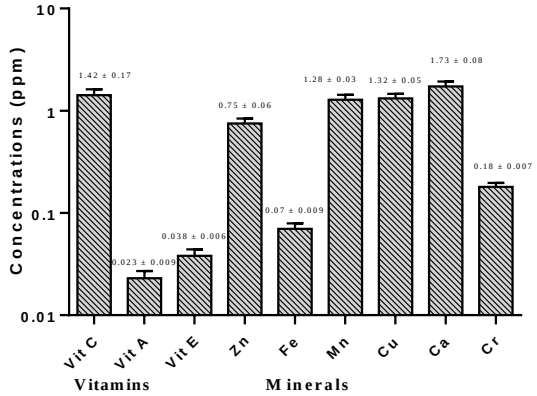


Figure 2: Antioxidant vitamins and mineral composition of SLELF

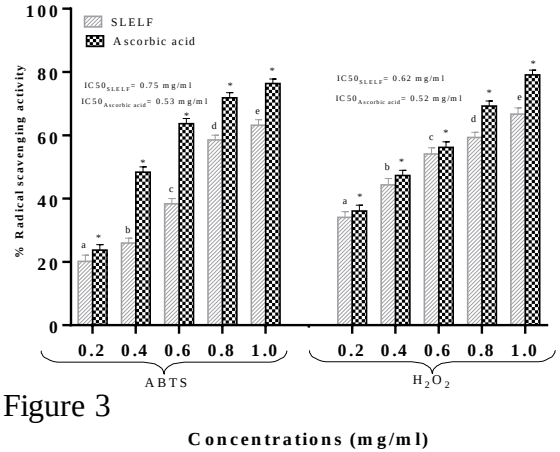


Figure 3

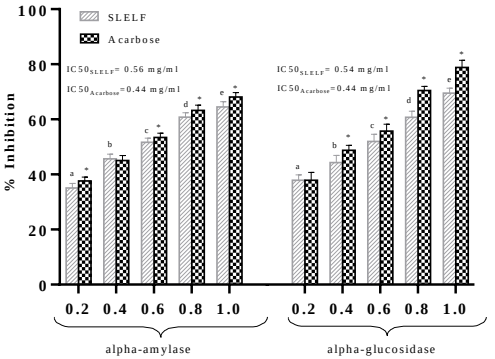


Figure 4

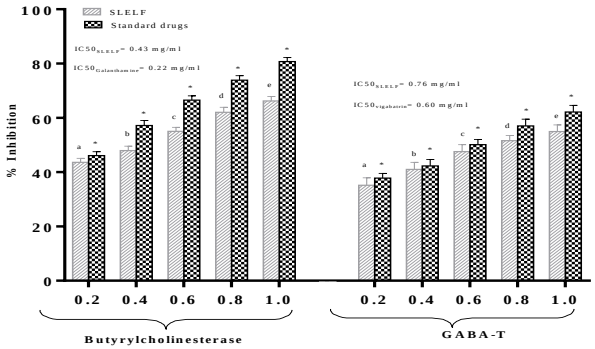


Figure 5

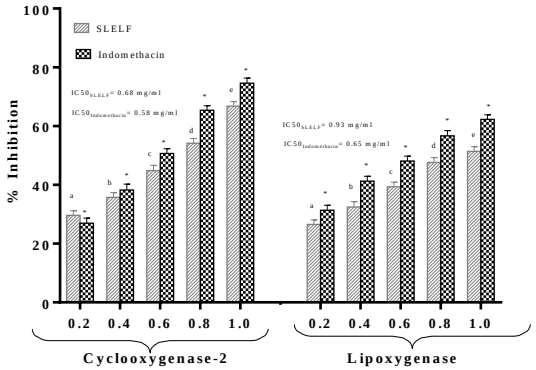


Figure 6

Discussion

The present study was designed to replicate the alcoholic decoction of the *Sida linifolia* plant, as used by traditional healers in African folklore medicine. Polar fractions are usually rich in phenolics because of their polar chemistry. The enrichment of polyphenolics in polar fractions also influenced our choice of plant fraction. Moreover, the choice of leaf fraction aligns with Debalke *et al.* (2018) and Nwankwo *et al.* (2023), which demonstrated excellent pharmacological activities using the aerial parts of *Sida rhombifolia* and ethanolic leaf fraction *S. linifolia* in their studies, respectively.

Results of the phytochemical analysis of SLELF revealed appreciable amounts of total phenolics (91.64 ± 7.61 mgGAE/g), total tannins (62.44 ± 3.86 mgTAE/g), and total flavonoids (27.350 ± 1.48 mgQE/g). This result corresponds with de Oliveira *et al.* (2012), which reported relatively higher levels of total phenolics (88.31 ± 2.66 mgGAE/g) in the ethyl acetate leaf fraction of *Sida rhombifolia*. Subramanya *et al.* (2015) recorded appreciable amounts of total phenolic, total tannins, and total flavonoids in very close relatives of the study plant, including *S. rhombifolia* and *Sida cordifolia*. Our result showed a considerable amount (17.80 ± 0.23 µg/g) of reduced glutathione (GSH) in SLELF, indicative of its potent antioxidant capacity. Our findings agree with Malar *et al.* (2014), which reported a moderate level (9.0 ± 0.2 µg/ml) of GSH in the ethanolic leaf extract of *Lepidium sativum*, as well as excellent antioxidant properties of the extract *in vitro*.

Furthermore, appreciable amounts of vitamin C (1.42 ± 0.17 ppm), vitamin E (0.038 ± 0.003 ppm), and vitamin A (0.023 ± 0.009 ppm) were recorded in SLELF. A similar study by Nwankpa *et al.* (2015) reported a relatively higher amount of Vit C (2.427 ppm) and a lower level of Vit E (0.185 ppm) in the ethanolic leaf extract of *S. acuta.* In addition, varied amounts of pharmacologically relevant minerals such as Ca (1.73 ± 0.05 ppm), Cu (1.32 ± 0.07 ppm), Mn (1.28 ± 0.06 ppm), Zn (0.75 ± 0.06 ppm), Cr (0.18 ± 0.03 ppm) and Fe (0.07 ± 0.03 ppm), were also detected in SLELF.

Our data revealed that SLELF exhibited appreciable and concentration-dependent $ABTS^{\cdot+}$ radical quenching and hydrogen peroxide pro-oxidant neutralizing potentials in a concentration-dependent manner akin to ascorbic acid. This could be due you the rich antioxidant phytochemicals present in the leaf fraction

Discussion Cont.

From the results, SLELF was effective against α-amylase and α-glucosidase. The inhibitory action of SLELF was directly proportional to concentration and was on par with the standard drug, acarbose. Inhibitory actions of SLELF on enzymes linked to diabetes pathologies could be anchored to its rich composition in phytochemicals known for regulating sugar metabolism via their suppressive actions on these enzymes (Chukwuma et al., 2022).

In the present study, SLELF suppressed COX-2 and LOXs activity in a concentration-dependent manner, akin to indomethacin. The observed inhibitory actions displayed by SLELF could further be used to explain the folkloric use of the plant extract in managing painful whitlows. Moreover, the rich phytochemistry observed in the leaf fraction may justify the potent pharmacological properties recorded. Our result aligns with the work of Preethidan *et al.* (2013), which recorded potent lipoxygenase inhibitory activity of isolated compounds from six members of *Sida* species, namely, *S. acuta*, *S. cordata*, *S. mysorensis*, *S. alnifolia*, *S. cordifolia*, and *S. rhomboidea*.

Interestingly, from the data obtained, SLELF moderately inhibited BChE activity following a concentration-dependent trend and was on par with the conventional antipsychotic drug galanthamine. In the same vein, a recent study by Kundo *et al.* (2021) reported a strong correlation between phenolics, flavonoids, and proanthocyanidin content with cholinesterase inhibition activities. Naringenin found in the leaf fraction has been reported to exhibit effective anti-BChE activity. Furthermore, it was observed from the result, that SLELF moderately inhibited GABA-T activity in a concentration-dependent fashion and was on par with a conventional GABA-T inhibitor, vigabatrin. The anti-GABA-T effect of the leaf fraction could be attributable to its rich phenolic compounds and other phytoconstituents. In relation to our findings, phytochemicals such as flavonoids, alkaloids, phenolic acids, saponins, and terpenes have been shown to demonstrate good anxiolytic effect in several animal models by exhibiting potent binding affinity with GABA-A receptors (Fedotova et al., 2017). Several phenolic acids, including chlorogenic acid, sinapic acid, cinnamic acid, vanillic acid, ferulic acid, and gallic acid, have been associated with anxiolytic and anti-depressant activities (Cordeiro et al., 2022). Moreover, the bioactive compounds present in famous anxiolytic plants such as *Ilex paraguariensis*, *Ginkgo biloba*, and *Matricaria recutita* were phenolic compounds, chlorogenic acid, theobromine, caffeic acid, caffeine, gallic acid, and rutin (Augšpole et al., 2018), some of which were detected in SLELF. Moreover, an appreciable amount of GABA was detected in SLELF, suggesting its neuromodulatory potential.

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