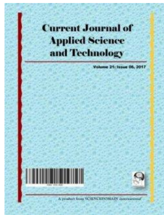

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Phytochemical Investigation, *in vitro* Antioxidant and Anti-inflammatory Evaluation of Leaf Extracts of *Solanum erianthum*

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: *Solanum erianthum* has been used in the treatment of various diseases by traditional medical practitioners in south-western Nigeria. The leaf extracts of the plant have been shown to possess antimicrobial activities. However, very little exists in literature on the antioxidant and anti-inflammatory properties of the plant. This study examines leaf extracts of *Solanum erianthum* for antioxidant and anti-inflammatory activities.
Place and Duration of Study: Department of Chemistry, Federal University of Technology Akure, Nigeria between September 2015 and January 2017.
Methodology: Leaf extracts were subjected to successive extraction using hexane, ethylacetate and methanol. The extracts were screened for the presence of phytochemicals using standard procedures. The DPPH and Ferric Reducing Antioxidant Assays were used to evaluate the antioxidant properties of the extracts. The DPPH scavenging properties of the extracts were assessed by comparing their IC_{50} values with that of the standard (Ascorbic acid). The ferric reducing properties of the extracts were determined as the concentration that gave a ferric reducing

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ability equivalent to that of ascorbic acid. The total phenolic content of the extracts was measured as milligram Gallic acid equivalent per gram of extract (mg GAE/g). The membrane stabilizing properties of the extracts were determined and used as a measure of their *in vitro* anti-inflammatory activities. The anti-inflammatory properties of the extracts were compared with that of the standard drug (Indomethacin).

Results: Phytochemical screening showed that the extracts contain flavonoids, alkaloids, tannins, glycosides, saponins, terpenoids, phlobatannins, sterols and carbohydrates. In the DPPH assay, the extracts had IC_{50} values ranging between 0.379 and 2.025 compared to the ascorbic acid with IC_{50} value of 0.017. The ferric reducing properties of the extracts ranged between 1.187 and 6.743 mg ascorbic acid equivalent/g of sample. The methanol extract showed the highest DPPH and ferric reducing antioxidant properties. The phenolic contents of the extracts ranged between 3.039 and 4.196 mg GAE/g sample. In the in vitro anti-inflammatory assay, the ethylacetate extract, showed a membrane stabilizing property higher than that of Indomethacin at 0.2 mg/ml and 0.5 mg/ml. The anti-inflammatory activities of the hexane and methanol extracts are lesser than that of Indomethacin at all the test concentrations.

Conclusion: The results showed that the leaf extracts of *Solanum erianthum* possess a broad range of medicinal properties. This could justify the use of the plant in the treatment of various diseases by traditional medical practitioners in Southwestern Nigeria.

Keywords: Solanum erianthum; antioxidant; anti-inflammatory; DPPH; membrane-stabilizing; standard.

1. INTRODUCTION

Oxygen, an indispensable element for life, is used by cells to generate energy. Free radicals, reactive oxygen species (ROS) as well as reactive nitrogen species (RNS), are by-products of cellular redox process. These species can be toxic or beneficial. The delicate balance between their two antagonistic effects is clearly an important aspect of life. At low or moderate levels, ROS and RNS exert beneficial effects on cellular responses and immune function. At high concentrations, they generate oxidative stress, a deleterious process that can damage all cell structures [1-4]. Reactive oxygen species (ROS) play important role in degenerative or pathological processes such as aging, cancers, coronary heart diseases, Alzheimer's disease, neurodegenerative disorders, atherosclerosis, cataracts and inflammation [5]. Antioxidants provide protection against the damage caused by ROS. Antioxidants interfere with the oxidative processes by scavenging free radicals, chelating free catalytic metals and by acting as electron donors [6]. Antioxidants have been employed as supplements in slowing or preventing the development of complications associated with diseases [7]. However, many synthetic antioxidants have been known to elicit toxic and/or mutagenic effects, which have shifted the attention towards natural antioxidants [8]. In the search for sources of natural antioxidants, many scientists and researchers have attracted attention to the bioactive compounds isolated from plants species used in the preparation of folk remedies [9]. Indeed, there have been many reports of plant extracts and different types of phytochemicals especially phenolic compounds as secondary metabolites from plants, which were shown to have a very effective antioxidant activity [2-3,10].

Inflammation, often characterized by redness, edema, fever, pain, and loss of function is a part of body's response to harmful stimuli [11-12]. It may also be associated with 'flu-like' symptoms such as fever, fatigue, cold, loss of appetite and muscular stiffness [13]. Acute inflammation lasts for a short time, usually ceasing upon the removal of injurious stimulus. Chronic inflammation arises when the injurious agent is persistent leading to a progressive shift in the type of cells present at the site of inflammation and may last for many days, months or years [11]. Chronic inflammation is characterized by dominating presence of macrophages in the injured tissue. Although, these cells are powerful defensive agents in the body, the toxins they release (including reactive oxygen species) are injurious to the organisms' own tissues as well as invading agents. Consequently, chronic inflammation is always accompanied by tissue destruction.

Solanum erianthum belongs to the family '*Solanaceae*'. It is called '*ewuro-igbo*' in parts of Southwestern part of Nigeria. The leaves and the

root are used by traditional medical practitioners for the treatment of veneral diseases, leprosy, dermatological problems, headache, leucorrhoea, piles, wound, among others. The ethylacetate and chloroform extracts of the leaves show high sensitivity to *Vibrio cholerae*, *Salmonella typhi*, and *Serratia marcescens* and less sensitivity to *Pseudomonas aeruginosa* [14]. *Solanum erianthum* leaf volatile oil demonstrated potent inhibitory activity against Hs 578T and PC-3 human breast and prostate tumor cells respectively. In addition, the *Solanum* essential oils exhibited significant antimicrobial activity (19.5–625 µg/mL) [15]. However, very little information exists in literature on the antioxidant and antioxidant activities of the leaves of this plant. The current study screens the leaf extracts of *S. erianthum* for antioxidant and anti-inflammatory activities.

2. METHODOLOGY

2.1 Sample Collection and Extract Preparation

The leaf samples were collected from an uncompleted building in Osogbo, Osun State, Nigeria. The plant sample was identified at the Herbarium of the University of Ibadan, Nigeria. The leaves were dried under mild sunlight for several days. After drying and subsequent grinding, 1000g each of both dried plant parts were subjected to successive extraction using hexane, ethylacetate and methanol [16]. This approach leads to the distribution of the non-polar, medium polar and polar compounds in the plant leaves into the hexane, ethylacetate and methanol extracts respectively. The extracts were exposed to open air to allow the solvents to evaporate. The hexane, ethylacetate and methanol extracts were coded as SELHE, SELEE and SELME respectively.

2.2 Phytochemical Screening of the Extracts

Phytochemical screening of the extracts was carried out using standard procedures. Details of the procedures are given below

Test for tannins: Two (2) drops of 5% $FeCl_3$ was added to 1ml of the extract. A dirty green precipitate indicated positive test [17].

Test for glycosides: Ten (10) ml of 50% H_2SO_4 was added to 1ml of extract in a test tube, this mixture was heated in boiling water for 5 minutes. 10 ml Fehling's solution A and B (5 ml each) were added and boiled. Brick red precipitate indicated positive test [18].

Test for resins: Two and a half (2.5) ml of Copper (II) Sulphate solution was added to 2.5 ml of the extract. The resulting solution was shaken vigorously and allowed to settle. A green colour indicated positive test [17].

Test for saponins (Frothing test): Two (2) ml of extract in water was vigorously shaken in test tube for two minutes. Frothing indicated positive test [17]

Test for phlobatannins: Five (5) ml of distilled water was added to 5 ml of extract solution and boiled with 1%HCl for two minutes. A deep green colour indicated positive test [17].

Test flavonoids: Two (2) ml of the extract solution was heated with 10 ml of ethyl acetate on a water bath and cooled. The layers were allowed to separate and a colour of ammonia layer (red colouration formed) indicated positive test [19].

Test for sterols (Salkowski's test): Two (2) ml of conc. H_2SO_4 was added 2 mlof extract solution. A red precipitate indicated steroidal ring [20].

Test for Phenols: Equal volumes of extract solution and $FeCl_3$ were mixed. A deep bluish green solution confirmed the presence of phenols [19].

Test for carbohydrate. (Fehling test): Five (5) ml of the mixtures of equal volume Fehling solution A and B were added to 2 ml of the extract in a test tube. The resultant mixture was boiled for two minutes. A brick red precipitate of copper oxide indicated a positive test [19].

Test for alkaloids: One (1) ml of conc. H_2SO_4 was added to 3 ml of the extract, then treated with few drops of Wagner reagent. Reddish brown precipitate indicated positive test [21].

Terpenoid (Salkowski) test: 0.2 g of the extract sample was mixed with 2 ml of chloroform ($CHCl_3$) and conc. H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface was formed to indicate positive result for the presence of terpenoids [20].

2.3 Determination of Antioxidant Activity

2.3.1 DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging assay

The hydrogen or radical scavenging properties of the extract from the plant sample was determined by the stable radical DPPH (2, 2-diphenyl-1-picrylhydrazyl radical) method as described by [22]. To 1 ml of varying concentrations of the sample extract/standard (Ascorbic acid) was added 1 ml 0.3 mM DPPH in methanol and allowed to react. The mixture was vortexed and incubated in the dark for 30 min and the absorbance was measured at 517 nm against a DPPH negative control containing only 1 ml of methanol in place of the sample extract.

The percentage inhibition of the DPPH scavenging activity was calculated using the equation below:

$$\text{DPPH \%Inhibition} = [1-(A_{517nm\ sample}/A_{517nm\ control})] \times 100$$

Where;

$A_{517nm\ sample}$ is the absorbance of the sample (extract/standard) at 517nm.
$A_{517nm\ control}$ is absorbance of the negative control at 517nm.

Sample concentration providing 50% inhibition (IC_{50}) was obtained from the graph by plotting inhibition percentage against extract concentration.

2.3.2 Ferric reducing antioxidant power assay (FRAP)

This was carried out as described by [23]. A 300 mmol/L acetate buffer of pH 3.6, 10 mmol/L TPTZ [2, 4, 6 tri-(2-pyridyl)-1, 3, 5-triazine] and 20 mmol/L $FeCl_3.6H_2O$ were mixed together in the ratio of 10:1:1 respectively, to give the working FRAP reagent. A 50 µl aliquot of the sample extract at 1 mg/ml and 50 µl of the standard solutions of ascorbic acid (0.02, 0.04, 0.06, 0.08, 0.1 mg/ml) was added to 1 ml of FRAP working reagent. Absorbance measurement was taken at 593 nm exactly 15 min after mixing against reagent blank containing 1 ml of the FRAP working reagent and 50 µl of methanol.

All measurements were taken at room temperature and the reducing power was expressed as equivalent concentration which is defined as the concentration of antioxidant that gave a ferric reducing ability equivalent to that of the ascorbic acid standard (AAE).

$$AAE = c \times v/m$$

Where,

AAE= Ascorbic acid equivalent of sample extract (mg AAE/g of sample); c= concentration of ascorbic acid established from the standard calibration curve in mg/ml; v= volume of the sample extract in ml; m= weight of the sample extract in g

2.3.3 Determination of total phenol

This was carried out using procedures described by [24]. To a mixture of 0.1 mL of sample extract (1 mg/mL) or standard and 0.9 mL of distilled water was added 0.2 mL Folin's reagent. The mixture was vortexed. After 5 min, 1.0 mL of 7% (w/v) Na_2CO_3 solution was added and the solution was further made up to 2.5 mL by the addition of 0.3 mL distilled water, before it was finally incubated for 90 min at room temperature. The absorbance against a reagent blank containing 1 mL of methanol in place of the sample was measured spectrophotometrically at 750 nm. Gallic acid at different concentrations of 0.1, 0.08, 0.06, 0.04 and 0.02 mg/mL was used as the standard [14]. The total phenolic content of the extracts was expressed as mg gallic acid equivalent per gram of extract (mg GAE/g) as shown below;

$$C = c \times v/m$$

Where: C = total phenolic compound in gallic acid equivalent (mg GAE/g); c = concentration of gallic acid established from the calibration curve in mg/mL; v = volume of the extract in mL; m = weight of the extract in gram

2.4 Determination of *In vitro* Anti-inflammatory Properties of Extracts

The membrane stabilizing properties of the extracts was used as a measure of their anti-inflammatory activity based on the procedure described by [25]. The assay mixture consisted of hyposaline (1 ml), 0.1 M phosphate buffer, pH 7.4 (0.5 ml), varying concentrations of the extract (0-0.5 mg/ml), varying concentration of normal saline and 0.5 ml of 2% (v/v) erythrocyte suspension in a total volume of 3 ml. The control

was prepared as above without the drug while the drug control (3 ml) lacked erythrocyte suspension. The standard anti-inflammatory drug for the assay was Indomethacin. The reaction mixtures were incubated at 56°C for 30 min. The absorbance of the released haemoglobin was read at 560 nm against reagent blank. The percentage membrane stability was estimated using the expression:

$$\text{Percentage membrane stability} = 100 - \frac{\text{Abs}_{\text{test drug}} - \text{Abs}_{\text{drug control}}}{\text{Abs}_{\text{blood control}}} \times 100$$

The blood control represented 100% lysis.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening

The results of the phytochemical screening of the extracts are as shown in Table 1. The results showed that all the extracts contain tannins, resin, saponins, sterols, phenols and alkaloids.

Table 1. Phytochemical screening of extracts

	SELHE	SELEE	SELME
Tannins	+	+	+
Glycosides	+	-	-
Resin	+	+	+
Saponins	+	+	+
Phlobatannins	-	+	+
Flavonoids	+	+	-
Sterols	+	+	+
Phenols	+	+	+
Carbohydrates	+	-	-
Alkaloids	+	+	+
Terpenoids	+	-	+

+ - Present; - -Absent

The distribution of the phytoconstituents between the different extracts arose from the differences in the polarity of the solvents used in carrying out the extraction (hence, largely non-polar compounds are in the hexane extract; medium polar compounds in the ethylacetate extract and polar compounds in the methanol extract). Plants' phytoconstituents are responsible for a broad range of pharmacological activities displayed by plants. For example, flavonoids have been known to possess anti-inflammatory, antioxidant, cardioprotective, antimicrobial, and anticancer effects [26]; saponins are known anti-nutritional phytochemicals that have been known to reduce the uptake of nutrients including cholesterol and glucose at the gut suggesting possible uses in the treatment diabetes and cardiovascular-related diseases; and alkaloids have been known to exhibit muscle relaxant [27], antioxidant [28], anticancer [29], antimicrobial and amoebicidal activities [30]. The presence of different phytochemicals in the leaf of this plant could be responsible for its usefulness in the treatment of various diseases by traditional medical practitioners.

3.2 Antioxidant Activity

3.2.1 DPPH scavenging activity

Tables 2a and 2b show the DPPH scavenging activity of the standard (Ascorbic acid) and the extracts at different concentrations. Generally, percentage DPPH inhibition is found to increase with concentration.

The IC_{50} values of the standard and the extracts were obtained from the plot of percentage inhibition against the different concentrations. The plots for the standard and the extracts are shown in Figs 1a and 1b respectively.

A comparison of the IC_{50} values of the extracts with that of the standard showed that SELME possess the highest DPPH inhibiting property while SELEE had the least.

Table 2a. Determination of IC_{50} value of ascorbic acid

Conc. (mg/ml)	Absorbance			Mean Absorbance	% Inhibition	IC_{50}
	A1	A2	A3			
0.05	0.195	0.128	0.163	0.162	78.255	0.017
0.025	0.148	0.161	0.271	0.193	74.049	
0.0125	0.449	0.457	0.483	0.463	37.852	
0.00625	0.612	0.712	0.604	0.643	13.736	
0.003125	0.646	0.758	0.755	0.720	3.400	

A1, A2 and A3 are the first, second and third absorbance readings respectively

Table 2b. DPPH assay: determination of IC_{50} value of *S. erianthum* Leaf Extracts

Extract	Conc. (mg/ml)	Absorbance			Mean absorbance	% Inhibition	IC_{50}
		A1	A2	A3			
SELHE	1	0.409	0.453	0.309	0.390	47.606	1.050
	0.5	0.529	0.556	0.59	0.558	25.056	
	0.25	0.656	0.615	0.684	0.652	12.528	
	0.125	0.691	0.657	0.726	0.691	7.204	
	0.0625	0.709	0.699	0.724	0.711	4.609	
SELEE	1	0.504	0.524	0.653	0.560	24.787	2.025
	0.5	0.639	0.655	0.65	0.648	13.020	
	0.25	0.726	0.702	0.703	0.710	4.653	
	0.125	0.700	0.732	0.719	0.717	3.758	
	0.0625	0.719	0.736	0.731	0.729	2.192	
SELME	1	0.15	0.147	0.145	0.147	80.224	0.379
	0.5	0.225	0.328	0.307	0.287	61.521	
	0.25	0.352	0.44	0.455	0.416	44.206	
	0.125	0.466	0.511	0.536	0.504	32.304	
	0.0625	0.534	0.561	0.598	0.564	24.251	

A1, A2 and A3 are the first, second and third absorbance readings respectively

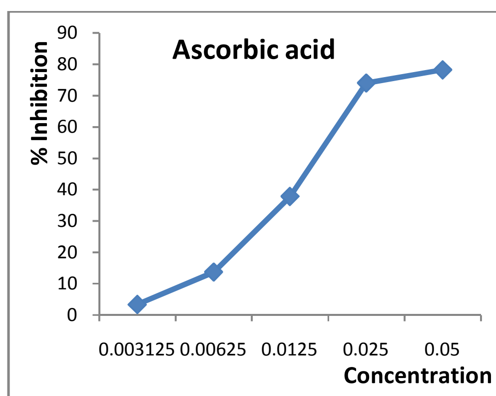

Fig. 1a

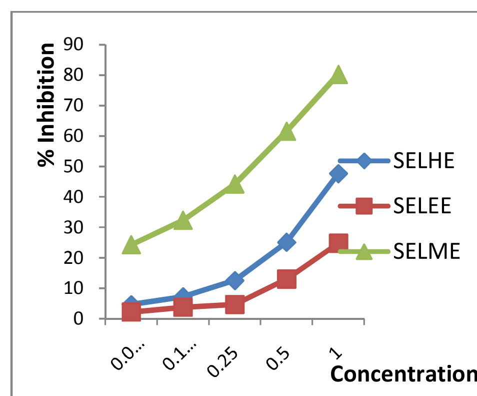

Fig. 1b

Fig. 1a & 1b. Graph of % Inhibition against different Concentrations of Ascorbic acid and Extracts respectively.

The DPPH radical scavenging assay (an inhibition assay) is a rapid, simple and convenient method to investigate the ability of the phenolic components in extracts to act as donors of hydrogen atoms or electrons [31-32]. In addition to phenolics, numerous plant constituents have been demonstrated to exhibit free radical scavenging or antioxidants activity. For example, tannins, alkaloids, saponins, flavonoids and phenols have been reported to exhibit DPPH scavenging properties [33-35]. The presence of these metabolites could be responsible for the varying degree of DPPH scavenging properties exhibited by these extracts. In the three extracts tested, the observed activities were concentration-dependent, increasing with concentration of the extracts.

3.2.2 Ferric reducing antioxidant potential

The ferric reducing antioxidant assay measures the reducing ability of antioxidants against oxidative effects of reactive oxygen species. Increasing absorbance indicates an increase in reductive ability [36]. Table 3a shows the ferric reducing activity of Ascorbic acid at different concentrations.

The calibration curve obtained from the plot of the mean absorbance against the different concentrations is shown in Fig. 2.

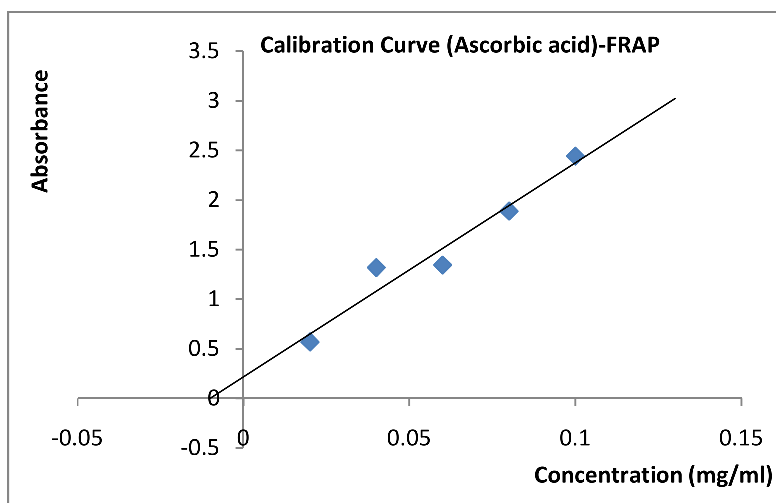

Fig. 2. FRAP assay: Graph of absorbance against concentration

The ferric reducing properties of the extracts relative to the standard are as shown in Table 3b.

The results showed that SELME had the highest ferric reducing property while SELHE had the least. The results show that the extract SELME is more effective in extracting compounds with ferric reducing ability than the other extracts. FRAP assay is a simple and quick method for assessing the antioxidant properties. In addition, the reaction is highly reproducible. Higher FRAP values give higher antioxidant capacity because FRAP value is based on reducing ferric ion, where antioxidants are the reducing agent [37].

3.2.3 Determination of total phenol

The total phenolic content was determined as the Gallic acid equivalence/gram of sample. The absorbance of Gallic acid at different concentrations is shown in Table 4a.

The calibration curve (of gallic acid) was obtained from a plot of absorbance against concentration (Fig. 3). The gallic acid equivalence of the extract (mg/ml) was obtained from the calibration curve.

The total phenol contents of the extracts are measured in triplicates. The average phenolic contents (mg GAE/g sample) are shown in Table 4b.

The extracts, SELME and SELEE possess the highest phenolic contents. The radical scavenging activity of these fractions correlated reasonably well with their phenolic contents which according to the earlier report of [38] that the antioxidant activity of plant materials closely correlated with the content of their phenolic contents. Phenolics are well recognized as potential antioxidants and free radical scavengers due to their hydroxyl groups [39-40]. Under the basic reaction conditions, a phenol loses an H+ ion to produce a phenolate ion, which reduces Folic-Ciocalteu reagent. The change is monitored spectrophotometrically. As phenolics (including many flavonoids) contain polar phenolic hydroxyl group/s, their high extraction into methanol is quite reasonable. Similarly, many flavonoids are known to possess aprotic, ether(e.g. methoxy) group(s) rather than protic, hydroxyl groups [23]. This may be responsible for the high presence of phenolics in the ethylacetate and hexane extracts.

Table 3a. Ferric reducing antioxidant potential of standard (Ascorbic acid)

Conc	Absorbance			Mean
(mg/ml)	A1	A2	A3	absorbance
0.02	0.476	0.645	0.581	0.567
0.04	1.677	1.145	1.13	1.317
0.06	0.915	1.521	1.594	1.343
0.08	1.618	2.054	1.99	1.887
0.10	2.414	2.456	2.456	2.442

A1, A2 and A3 are the first, second and third absorbance readings respectively

Table 3b. Ferric reducing antioxidant properties of the extracts

Extract	Absorbance			Mean absorbance	mg/ml ascorbic acid equivalent	mg ascorbic acid equivalent / g of the sample
	A1	A2	A3			
SELHE	0.295	0.208	0.221	0.241	0.001	1.187
SELEE	0.298	0.305	0.309	0.304	0.004	4.089
SELME	0.361	0.369	0.354	0.361	0.007	6.743

A1, A2 and A3 are the first, second and third absorbance readings respectively

3.3 *In vitro* Anti-inflammatory Activity

The membrane stabilizing activity of red blood cell membrane exhibited by some drugs, serves as a useful *in vitro* method for assessing the anti-inflammatory activity of various compounds [41]. The percentage membrane stability of the extracts and the standard (Indomethacin) measured at different concentrations are shown in Table 5.

Table 4a. Absorbance of gallic acid at different concentrations

GAE(mg/ml)	A1	A2	Average
0.1	1.095	1.110	1.103
0.08	1.083	1.058	1.071
0.06	0.978	0.969	0.974
0.04	0.534	0.566	0.550
0.02	0.365	0.360	0.363

A1 and A2 are the first and second absorbance readings respectively

Table 4b. Total phenol content of extracts

EXTRACT	AVERAGE mg GAE/g sample	Standard deviation	Standard error of mean
SELHE	41.624	5.264	3.039
SELEE	83.012	7.269	4.196
SELME	86.477	5.903	3.408

Table 5. Membrane-stabilizing activity of *S. erianthum* leaf extracts

Conc (mg/ml)	Mean percentage stability ± Stand. deviation			
	Indomethacin	SELHE	SELEE	SELME
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ±0.00	0.00±0.00
0.1	69.12 ± 6.65	37.43 ± 3.05	3.12 ± 0.59	8.39 ±1.24
0.2	75.02 ± 6.04	84.73 ± 2.56	0.57 ± 0.20	40.06 ± 0.87
0.3	75.55 ± 2.16	69.48 ± 2.36	28.50 ± 7.70	24.95 ± 1.66
0.4	73.51 ± 1.88	54.98 ± 0.93	28.36 ±1.85	31.06 ±1.38
0.5	52.65 ± 1.18	99.00 ± 0.38	11.63 ± 1.31	2.07 ± 1.24

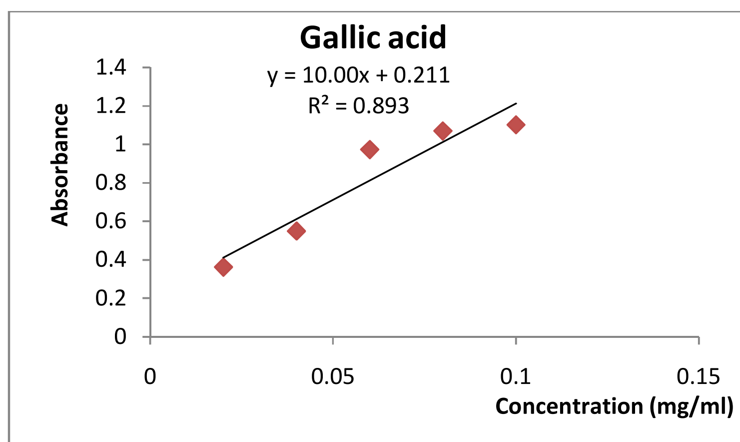

Fig. 3. Plot of gallic acid absorbance against concentration

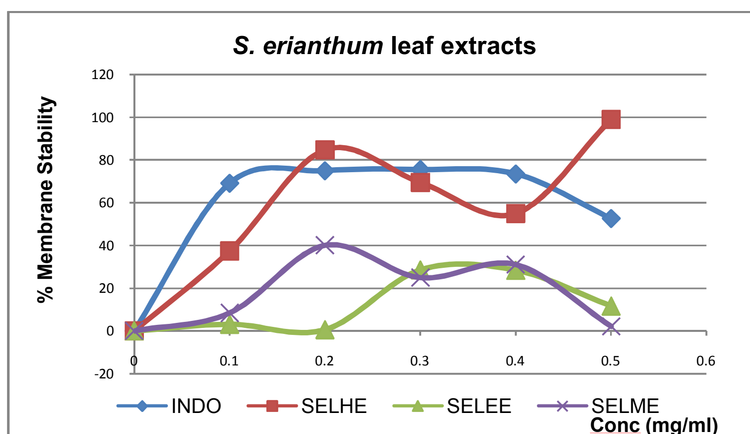

Fig. 4. Plot of percentage membrane stability against extract concentration

Above Fig. 4 shows the trends in membrane stability with concentration. The extract, SELEE showed an anti-inflammatory property higher than that of the standard drug at 0.2 mg/ml and 0.5 mg/ml. The anti-inflammatory activities of SELEE and SELME are lesser than that of the standard drug at all test concentrations.

Several herbal derived drugs have been demonstrated to contain principles that possess ability to facilitate the stability of biological membranes when exposed to induced lyses. Some plant extracts have been reported to protect and stabilize red blood cells exposed to heat induced stress [42] or both hypotonic and heat induced stress [25,43]. The extract, SELHE, exhibited a biphasic mode of protection possess greater activity than the standard drug (Indomethacin) at 0.2 mg/ml and 0.5 mg/ml. The high anti-inflammatory activity observed must be due to the presence of membrane-stabilizing compounds in the extracts [44]. Flavonoids and saponins detected in the hexane extract could be responsible for the high anti-inflammatory activity of the hexane extract [45]. Compounds with membrane stabilizing potentials are well known for their ability to interfere with the early phase of inflammatory reactions by preventing the release of phospholipase that triggers the formation of inflammatory mediators [46].

4. CONCLUSION

The current study has validated the use of the leaves of this plant in the treatment of various diseases by traditional medical practitioners in Southwestern Nigeria. Further studies however need to be carried out in order to isolate and characterize the compounds responsible for the observed medicinal properties.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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