International Journal of Herbal Medicine
Available online at www.florajournal.com

I J H M International Journal of Herbal Medicine

E-ISSN: 2321-2187
P-ISSN: 2394-0514
IJHM 2018; 6(2): 01-06
Received: 01-01-2018
Accepted: 02-02-2018

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Phytochemical investigation, *in vitro* antioxidant and anti-inflammatory evaluation of stem extracts of *Solanum erianthum*

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Abstract
Solanum erianthum stem extracts were screened for antioxidant and anti-inflammatory activities. The antioxidant properties of the extracts were evaluated using the DPPH and Ferric Reducing Antioxidant assays. The total phenolic content of each of the extracts was obtained as milligram Gallic acid equivalent per gram of extract (mg GAE/g). The *in vitro* anti-inflammatory property of the extracts was determined using the membrane stabilization assay. In the DPPH assay, the IC_{50} values of the extracts ranged between 0.304 and 0.729. The ferric reducing activity of the extracts ranged between 2.468 and 28.614 mg Ascorbic acid equivalent/g of sample. The total phenolic contents of the extracts ranged between 0.484 and 3.340 mg GAE/g sample. The ethylacetate extract showed a membrane-stabilizing activity higher than that of Indomethacin at 0.1 mg/ml and 0.5 mg/ml. This study justified the use of the plant in the treatment of various ailments by traditional medical practitioners in South-western Nigeria.

Keywords: Antioxidant, Anti-inflammatory, standard, IC_{50}, Phytochemicals

1. Introduction

Oxidative stress is one of the among major causative factors in induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression and neurodegenerative diseases [1]. In healthy individuals, the production of free radicals is balanced by the antioxidative defense system; however, oxidative stress is generated when equilibrium favors free radical generation as a result of a depletion of antioxidant levels [2]. A potent scavenger of these species may serve as a possible preventive intervention for free radical-mediated diseases. Recent studies showed that a number of plant products including polyphenolic substances (e.g., flavonoids and tannins) and various plant or herb extracts exert antioxidant actions [3].

Inflammation is part of normal host response to infection and injury. Although inflammation helps clear infection, and along with repair, makes wound healing possible, both inflammation and repair have considerable potential to cause harm [4]. Prolonged presence of inflammatory agents may lead to chronic inflammation. Chronic inflammation is abnormal and the excessive reactive oxygen/nitrogen species and lipid peroxidation products formed facilitate carcinogenesis and other chronic diseases [5, 6]. Several medicinal herbs have been shown to inhibit inflammation as well as antioxidant activity. There is very little information in literature on the antioxidant and anti-inflammatory properties of the stem of this plant. The current study investigates the stem extracts of *Solanum erianthum* for antioxidant and anti-inflammatory properties.

The leaves and root of *Solanum erianthum* are used for the treatment of various diseases by traditional medical practitioners in South-western Nigeria. The leaf extracts and volatile oils have been reported to possess antimicrobial and anticancer properties in breast and prostate tumor cells [7, 8]. Solavetivone, isolated from the root of the plant possesses fungitoxic, antimicrobial and weak cytotoxic activities [9].

2. Materials and methods

2.1 Preparation of Extract

The stem of the plant was dried under mild sunlight for several days. After drying, samples were ground and subjected to successive extraction using hexane, ethylacetate and methanol. The hexane, ethylacetate and methanol extracts were coded as Seshe, Sesee and Sesme respectively.

2.2 Phytochemical Screening of the Extracts

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 [10].

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 [10].

Test for tannins: Two (2) drops of 5% $FeCl_3$ was added to 1ml of the extract. A dirty green precipitate indicated positive test [10].

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 [10].

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. 10ml Fehling's solution A and B (5 ml each) were added and boiled. Brick red precipitate indicated positive test [11].

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 [12].

Test for Phenols: Equal volumes of extract solution and $FeCl_3$ were mixed. A deep bluish green solution confirmed the presence of phenols [12].

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 [12].

Test for sterols (Salkowski's test): Two (2) ml of conc. H_2SO_4 was added 2 ml of extract solution. A red precipitate indicated steroidal ring [13].

Terpenoid (Salkowski) test: 0.2g of the extract sample was mixed with 2ml of chloroform ($CHCl_3$) and conc. H_2SO_4 (3ml) 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 [13].

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 [14].

2.3 Antioxidant Activity of Extracts

2.3.1 DPPH (2, 2-diphenyl-1-picrylhydrazyl) Free Radical Scavenging Assay

The radical scavenging properties of the extracts was determined as described by Brand-Williams *et al.* [15] 1 ml 0.3 mM DPPH in methanol was added to1 ml of varying concentrations of the sample extract/standard (Ascorbic acid) and allowed to react. The mixture was vortexed and incubated in the dark for 30 min and the absorbance was measured at 517nm 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:

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 a graph of inhibition percentage against extract concentration.

2.3.2 Ferric Reducing Antioxidant Power Assay (FRAP)

This was carried out as described by Ahmed *et al* [16]. 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 1ml of FRAP working reagent. Absorbance measurement was taken at 593nm 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 x 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 Rabeta and Nur Faraniza [17]. 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. 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 x 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 *In vitro* Anti-inflammatory Assay (Membrane Stabilization Assay)

The membrane stabilizing properties of the extracts was used as a measure of their anti-inflammatory activity based on the procedure described by Oyedapo *et al* [18]. 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:

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

The blood control represented 100% lysis.

3. Results and Discussion

3.1 Phytochemical Screening of the Extract

The results of the phytochemical screening of the extracts are shown in Table 1. The extracts contain a broad range of phytochemicals.

Table 1: Phytochemical Screening of Extracts

	Seshe	Sesee	Sesme
Tannins	-	+	-
Glycosides	-	-	+
Resin	-	+	+
Saponins	+	+	-
Phlobatannins	-	-	-
Flavonoids	+	-	+
Sterols	-	-	-
Phenols	+	+	+
Carbohydrates	-	-	+
Alkaloids	+	+	-
Terpenoids	-	+	-

+ - Present; - - Absent

Plants' secondary metabolites have been reported to possess a broad range of medicinal properties [19-23]. The presence of different phytochemicals in the extracts could be responsible for their antioxidant and anti-inflammatory properties.

3.2 Antioxidant Activity

3.2.1 DPPH Scavenging Activity

The DPPH scavenging activity of the standard (Ascorbic acid) and the extracts are shown in Tables 2a and 2b respectively. The percentage DPPH inhibition increased with extract concentration.

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	

Table 2b: DPPH Assay: Determination of IC_{50} Value of *S. erianthum* Stem Extracts

Extract	Conc. (mg/ml)	Absorbance			Mean Absorbance	% Inhibition	IC_{50}
		A1	A2	A3			
Seshe	1	0.311	0.226	0.320	0.286	63.140	0.729
	0.5	0.447	0.439	0.419	0.435	43.871	
	0.25	0.561	0.61	0.678	0.616	20.473	
	0.125	0.719	0.679	0.68	0.693	10.624	
	0.0625	0.746	0.744	0.754	0.748	3.484	
Sesee	1	0.101	0.109	0.095	0.102	86.882	0.172
	0.5	0.099	0.099	0.219	0.139	82.065	
	0.25	0.277	0.273	0.306	0.285	63.183	
	0.125	0.430	0.450	0.43	0.437	43.656	
	0.0625	0.509	0.542	0.509	0.520	32.903	
Sesme	1	0.106	0.108	0.159	0.124	83.957	0.304
	0.5	0.177	0.199	0.221	0.199	74.323	
	0.25	0.309	0.309	0.351	0.323	58.323	
	0.125	0.482	0.5	0.528	0.503	35.054	
	0.0625	0.587	0.597	0.597	0.594	23.398	

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.

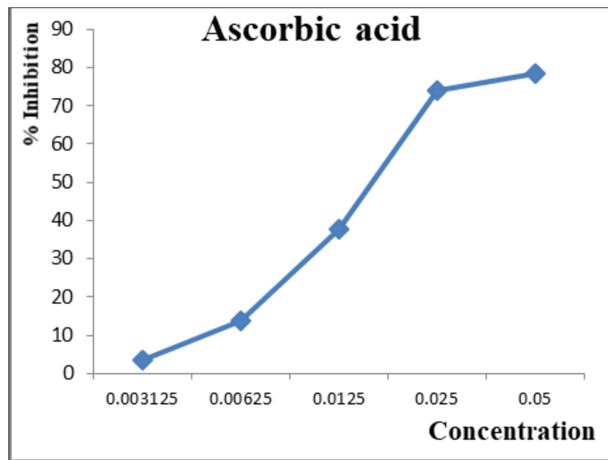

Fig 1a

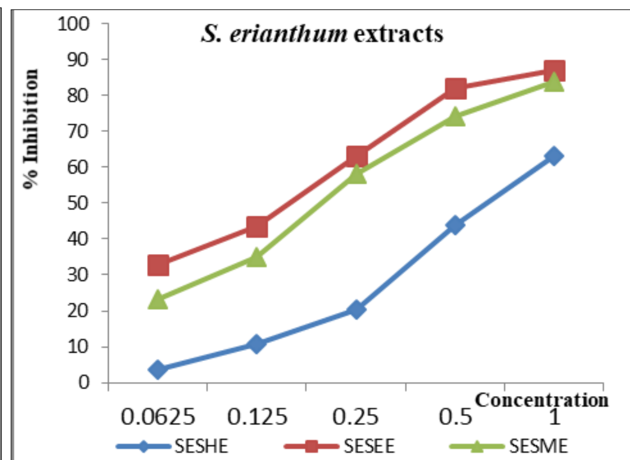

Fig 1b

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

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 [24]. 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 Figure 2.

Table 3a: Ferric Reducing Antioxidant Potential of Standard (Ascorbic acid)

Conc(mg/ml)	Absorbance			Mean
	A1	A2	A3	
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

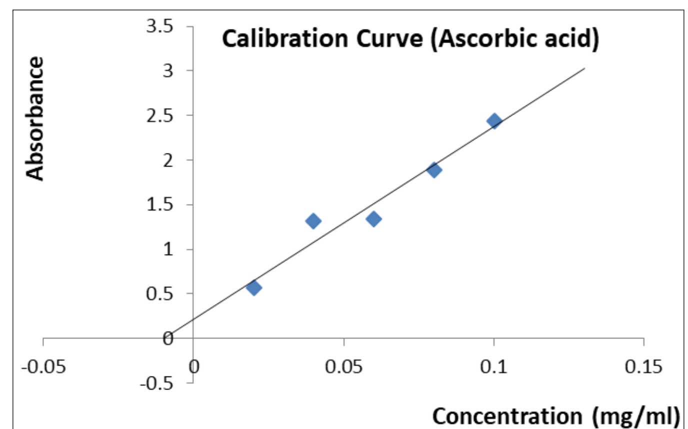

Fig 2: FRAP Assay: Graph of Absorbance against Concentration

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

Table 3b: Ferric Reducing Antioxidant Potential of Extracts

Extract	Absorbance			Mean Absorbance	mg/ml ascorbic acid equivalent	mg ascorbic acid equivalent / g of the sample
	A1	A2	A3			
SESHE	0.294	0.249	0.264	0.269	0.002	2.468
SESEE	0.791	0.932	0.778	0.834	0.029	28.614
SESME	0.520	0.544	0.630	0.565	0.016	16.158

The results show that the ethylacetate extract, Sesee has the highest ferric reducing antioxidant property.

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.

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

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.

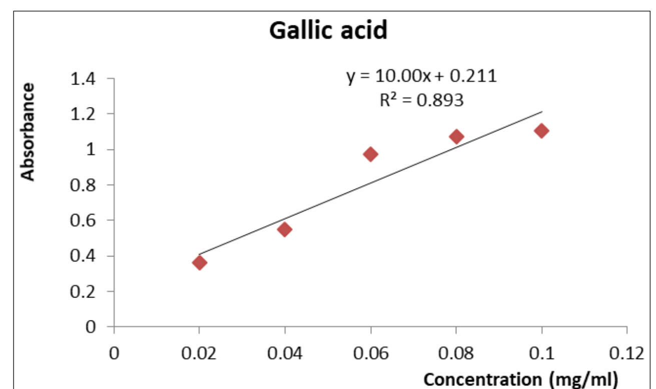

Fig 3: Plot of Gallic Acid absorbance against Concentration

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

Table 4b: Total Phenol Content of Extracts

Extract	Average Mg GAE/g Sample	Standard Deviation	Standard Error of Mean
Seshe	37.859	0.954	0.551
Sesee	101.240	5.784	3.340
Sesme	129.065	0.838	0.484

The conventional DPPH and FRAP assays used in antioxidant studies are simple, rapid, highly reproducible and low cost techniques [26-29]. A comparison of the IC_{50} values of the extract to that of the standard shows that SESEE had the highest DPPH scavenging property. Tannins, alkaloids, saponins, flavonoids and phenols are examples of phytoconstituents that have been reported to exhibit DPPH scavenging properties [30-32]. Similarly, SESEE showed the highest activity in the FRAP assay. Furthermore, this study demonstrated that there is a direct correlation between the phenolic content of the extracts and their antioxidant properties (with SESEE possessing the highest phenolic content also showing the highest DPPH scanvenging and ferric reducing properties). Previous studies indicate that the antioxidant activity of plant materials closely correlated with the content of their phenolic contents [33]. Phenolics are well recognized as potential antioxidants and free radical scavengers due to their hydroxyl groups [34-35].

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 [25]. The percentage membrane stability of the extracts and the standard (Indomethacin) measured at different concentrations are shown in Table 5.

Table 5: Membrane-stabilizing Activity of *S. erianthum* Stem extracts

Conc (mg/ml)	Mean Percentage Stability ± Stand. Deviation			
	Indomethacin	Seshe	Sesee	Sesme
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
0.1	69.12 ± 6.65	42.86 ± 15.40	91.14 ± 0.60	41.90 ± 2.68
0.2	75.02 ± 6.04	54.33 ± 1.24	86.86 ± 0.42	30.10 ± 1.57
0.3	75.55 ± 2.16	65.68 ± 4.45	57.61 ± 0.60	35.34 ± 10.91
0.4	73.51 ± 1.88	61.99 ± 1.37	30.15 ± 0.17	50.75 ± 1.69
0.5	52.65 ± 1.18	78.06 ± 2.87	97.31 ± 0.39	47.08 ± 5.98

Fig. 4 shows the trends in membrane stability with concentration. The extract, SESEE showed an anti-inflammatory property higher than that of the standard drug at 0.1 mg/ml and 0.5 mg/ml. The anti-inflammatory activity of SESHE was high than that of the standard drug at 0.5 mg/ml while the activity of SESME was lesser than that of the standard drug at all test concentrations.

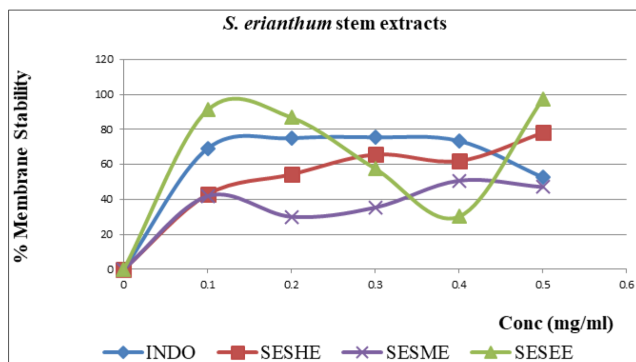

Fig 4

Some plant extracts have been reported to protect and stabilize red blood cells exposed to heat induced stress [36] or both hypotonic and heat induced stress [18, 37]. The high anti-inflammatory activities displayed by SESEE and SESME could be due to the presence of compounds possessing anti-inflammatory properties in the extracts [38]. 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 [39].

4. Conclusion
The stem of *Solanum erianthum* has demonstrated considerable antioxidant and anti-inflammatory activities. Further studies, however, has to be carried out in order to identify the compounds responsible for the observed medicinal properties.

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