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Evaluation of Invitro Anti-Inflammatory Potential of Aqueous *Solanum Aethiopicup* (Garden Egg) Leaf Extract

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Abstract

Inflammation is a part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells or irritants. This study is aimed at evaluating the invitro anti-inflammatory potential of aqueous leaves extract of *Solanum aethiopicum* (Family: *Solanaceae*). In vitro anti-inflammatory potentials were evaluated using standard experimental protocols such as Inhibition of albumin denaturation, Anti-lipoxygenase activity, Membrane stabilization and Proteinase inhibitory action at different concentrations with aspirin and diclofenac used as the standard drug. Aqueous extract of *S.aethiopicum* leaves were tested for the presence of alkaloid, flavonoid, tannin, glycoside, steroids, phenol and terpenoids. The total phenol and flavonoid content were evaluated as well. The total phenol and flavonoid content were found to be (132±0.13 mg of Gallic acid/g of equivalent) and (146±1.12mg of Quercetin/g of equivalent). The IC_{50} values of the extract, diclofenac and aspirin (standard drug) in inhibition of albumin denaturation were 50.20 µg/ml and 31.54µg/ml; in Anti-lipoxygenase activity were 199µg/ml and 28.2µg/ml; in Membrane stabilization were 9.36µg/ml and 19.85µg/ml and Protein denaturation 714µg/ml and 23.5µg/ml. The Inhibition of membrane stabilization might be the possible mechanism by which the extract elicits its anti-inflammatory effect. The results obtained in the present study indicate that the aqueous extract of *Solanum aethiopicum* is a potent source of anti-inflammatory agents and this justified its uses in the treatment of various infections.

Keywords: Inflammation, Solanum aethiopicum, Antilipoxygenase, Membrane stabilization, Albumin denaturation.

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1. Introduction

Inflammation is a complex biological response of vascular tissues to harmful stimuli, pathogens, irritants characterized by redness, warmth, swelling and pain [1]. Inflammation is either acute or chronic inflammation. Acute inflammation, with exudation of fluid and plasma proteins as its main features, occurs very rapidly, and the process can last for few or several minutes to several days. Chronic inflammation occurs when the acute inflammatory process occurs repeatedly or continuously, with the process lasting for several weeks to months and even years [2]. The mechanisms of inflammation involve a series of events in which the metabolism of arachidonic acid plays an important role. It is metabolized by the Cyclooxygenase (COX) pathway to prostaglandins and thromboxane A2, whereas the 5-lipoxygenase (5-LOX) pathway to eicosanoids and leukotrienes (LT's), which are known to act as chemical mediators in a variety of inflammatory events [3]. Currently, available anti- inflammatory drugs block both enzyme activities and relief symptoms but they have serious side effects [4].Therefore, it is essential to administer anti- inflammatory drugs with lesser side effects.

Medicinal plants have the ability to synthesize a wide variety of phytochemical compounds as secondary metabolites. Many of the phytochemicals have been used to effectively treat the various ailments for mankind. Plants have a great potential for producing new drugs and are used in traditional medicine to treat chronic and even infectious diseases. In the present review an attempt has been made to investigate the anti-inflammatory activity of some medicinal plants [5].

Garden egg (*S. aethiopicum*) also known as African eggplant, Ethiopian eggplant or scarlet eggplant is a vegetable crop belonging to the family *Solanaceae.* The genus *Solanum* includes both the edible and non-edible species. The family is one of the largest and most important families of vegetables grown for their edible fruits [6]. They are native to sub-Saharan Africa and are essentially tropical in origin. *S. aethiopicum* is of high edible quality. The fruits can be eaten fresh without cooking and have a long history of consumption in West Africa [7]. Report has shown that *S. aethiopicum* possesses ulcer protecting properties against experimentally induced ulcers in rats. They are used to treat colic; severe pain resulting in periodic spasm in an abdominal organ and blood pressure [8]. Other reports on the pharmacological activity of the plant show that it has purgative [9], sedative and anti-diabetic effects [10], but none have reported its anti-inflammatory activity.

2. Materials and Methods

2.1 Collection and authentication of plant Leaves *of S. aethiopicum*:

Leaves of *S. aethiopicum* were purchased from Owode market in Ogun state, Southwest Nigeria. The leaves were identified and authenticated in the Department of Botany of University of Lagos, Lagos state by a botanist, Mr. G.I Nodza. With Voucher specimens No 8381 were deposited at the herbarium of Federal Polytechnic Ilaro,Ogun state.

2.2. Preparation of leave extract:

The leaves were selected and thoroughly washed in water to remove dirt and unwanted particles. It was air dried at room temperature for a month and reduced to coarse powder by grinding with a grinder. 200g of the sample was measured and macerated into 2000litres of distilled water and was transferred into a standard flask. It was shaken thoroughly and kept in fume cupboard for five days. The sample solution was filtered with a Whitman No 1. filter paper. The filtrate was freeze dried prior to analysis.

2.3. Test for phytochemicals:

Into a conical flask 2gm of the crude extract and 50 ml of solvent (distil water) was added. The flask was labeled and allowed to stand for 1hr, filtered using Whitman No.1 filter paper. Phytochemical analysis extract was carried out using the method described by [11].

2.3.1. Test for Phenol:

To $1cm^3$ of the extracts 2 drops of 5% $FeCl_3$ was added in a test tube. A greenish precipitate indicates the presence of phenol.

2.3.2. Test for Flavonoid:

To $3cm^3$ of the extract, $1cm^3$ of 10% NaOH was added. A yellow colouration indicates the presence of flavonoids.

2.3.3. Test for Sterols:

Into a test tube 5 drops of concentrated H_2SO_4 was added to $1cm^3$ of the extracts. Red colouration indicates the presence of steroids.

2.3.4. Test of Alkaloids:

In a test tube$1cm^3$ of 1%HCl was added to $3cm^3$ of the extracts. The mixture was heated for 20 minutes, cooled and filtered. The filtrate was used in the following tests: 2 drops of

Wagner's reagent was added to 1cm^3 of the extracts. A reddish-brown precipitate indicates the presence of alkaloids.

2.3.5. Test for Glycosides:

To 1cm^3 of the extracts10cm^3 of 50% H_2SO_4 was added, the mixture was heated in boiling water for 15 minutes. 10cm^3 of Fehling's solution was added and the mixture boiled. A brick red precipitate indicates the presence of glycosides.

2.3.6. Test for Tannins:

1cm^3 of freshly prepared 10% KOH was added to 1$cm^3$of the extracts. A dirty white precipitate indicates the presence of tannins.

2.3.7. Test for Saponins:

Frothing test: 2cm^3 of the extract in a test tube was vigorously shaken for 2 minutes. Frothing indicates the presence of saponins.

2.3.8. Test for Terpenoids:

Into a test tube, 5ml of extract of the sample is mixed with 2ml of CHCl3 in a test tube, 3ml of con. H2SO4 is carefully added to the mixture to form a layer. An interface with a reddish-brown coloration is formed if terpenoids constituent is present.

2.3.9. Estimation of Total Phenolic Content:

The total phenolic content of sample was estimated according to the method of [12]. The aliquots of the extract were taken in a test tube and made up to the volume of 1 ml with distilled water. Then 0.5ml of Folin-Ciocalteu reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) were added sequentially to the test tube. Soon after vortexing the reaction mixture, the tubes were placed in the dark for 40 min. and the absorbance was recorded at 725 nm against the reagent blank. Using Gallic acid monohydrate, a standard curve was prepared. The linearity obtained was in the range of 1-10 µg/ml. using the standard curve, the total phenolic content was calculated and expressed as Gallic acid equivalent in mg/g of extract.

2.3.10. Estimation of Total Flavonoid Content:

Total flavonoid content was measured by aluminium chloride colorimetric assay. 1ml of extracts or standard solution of Quercetin (500µg/ml) was added to 10 ml volumetric flask containing 4 ml of distilled water. To the above mixture, 0.3 ml of 5% NaNO2 was added. After 5 minutes, 0.3 ml of 10% AlCl3 was added. At 6th min, 2 ml of 1 M NaOH was added and the total volume was made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm. Total flavonoid content of the flower was expressed as percentage of Quarcetin equivalent per 100 g of fresh mass.

3. INVITRO ANTI INFLAMMATORY ASSAY

The plant extract was subjected to preliminary assessments using standard procedures to detect its anti-inflammatory activities.

3.1. Preparation of Extract

5mg of the plant extract was dissolved in distilled water to produce a solution of concentration 5mg/ml. The following assays were done using the method of [13] with slight modifications.

3.2. Antilipoxygenase Activity

A total volume of 200 µl assay mixture contained, 160 µl sodium phosphate buffer (100 mM, pH 8.0), 10 µl test extract (50 to 250 µg extracted material in 100 mM Tris's buffer pH 7.4) and 20 µl lipoxygenase enzyme. The contents were preincubated for 10 min at 25°C. The reaction was initiated by the addition of 10 µl linoleic acid solution as substrate. The change in absorbance was observed after 6 min at 234 nm. All reactions were performed in triplicates in 96-well microplate reader Spectra Max 190 (Molecular Devices, USA). The positive and negative controls were included in the assay. The percentage inhibition (%) was calculated by the following formula:

$$Inhibition\ (\%) = [(Abs\ of\ control - Abs\ of\ test\ sample)/Abs\ of\ control] \times 100$$

3.3. Protein Inhibitory Action

The test will be performed according to the method of [14] and [15] with modifications. The reaction mixture (1.5ml) containing 0.06mg trypsin, 0.5ml of 20mM TrisHCl buffer (pH7.4) and 0.5ml test sample of different concentrations of different solvents. The reaction mixture will be incubated at 37^0C for 5min and then 0.3ml of 1.5% (W/V) casein will be added. The mixture will be incubated for an additional 20 min, 0.2ml of 70% perchloric acid will be added to

terminate the reaction. Cloudy suspension will be centrifuged and the absorbance of the supernatant read at 210nm against buffer as blank. The experiment will be performed in triplicate. The percentage of inhibition of proteinase inhibitory activity will be calculated;
The % inhibition of the protein denaturation will be calculated by

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control- Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

3.4. Membrane Stabilization Activity

3.4.1. Preparation of Red Blood Cells (RBCs)

Suspension Fresh whole mammalian blood (10ml) was collected and transferred to heparinzed centrifuge tubes. The tubes were centrifuged at 3000rpm for 10min and were washed three times with equal volume of normal saline. The volume of the blood was measured and reconstituted as 10%v/v suspension with normal saline.

3.4.2. Heat Induced Haemolysis

The 2ml reaction mixture is consisted of 1ml of test extract at various concentrations and 1ml of 10% RBCs suspension, instead of drug only saline was added to the control test tube. Diclofenac sodium was taken as a standard drug. All the centrifuged tubes containing reaction mixture were incubated in a water bath at 560C for 30min. At the end of the incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500rpm for 5min and the absorbance of the supernatants was taken at 560nm. The experiment was performed in triplicate. % of membrane stabilization activity was calculated by the formula mentioned below:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control- Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

3.4.3. Inhibition of Albumin Denaturation

The 5ml of reaction mixture was comprised of 0.2ml of eggs albumin, 2.8ml of phosphate buffered saline (PBS, pH 6.4) and 2ml of varying concentration of extracts. Similar volume of double distilled water served a control. Then the mixture was incubated at 37 °C in incubator for about 15mins and then heated at 70 °C for 5mins. After cooling, their absorbance was measured at 660nm by using pure blank. Diclofenac sodium (standard drug) was used as reference drug and treated as such for determination of absorbance. The percentage inhibition of protein denaturation was calculated by the formula mentioned below:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control- Absorbance of Sample}}{\text{Absorbance of Control}} X\ 100$$

3.5. STATISTICAL ANALYSIS

The experimental data were expressed as mean ±SEM. The difference between the control and extract were compared using one-way analysis of variance (ANOVA) followed by Duncan (control vs test) using the SPSS software version 20.0. $P<0.05$ was considered statistically significant. The IC_{50} value was calculated using Microsoft Excel version 2016.

4. Results

4.1 Qualitative Phytochemical Screening

Table 1: Qualitative Phytochemical creening of *Solanum aethiopicum*.

Phytochemical test	***S. aethiopicum*leave extract**
Flavonoids	+
Steroids	+
Tannins	–
Alkaloids	+
Glycosides	+
Saponins	+
Terpenoids	+

Note:Key= + (present) - (absent)

The result of qualitative phytochemical screening of *S.aethiopicum*is presented in the table above. The leaves of *S.aethiopicum* were found to contain phenol, flavonoid, steroid and glycoside while tannin was found absent.

4.2. TOTAL PHENOLIC AND TOTAL FLAVONOID CONTENT

PLANT MATERIAL	**TOTAL PHENOLIC CONTENT**	**TOTAL FLAVONOID CONTENT**
S. aethiopicum	132 ± 0.13	146 ± 1.12

The total phenolic and flavonoid contents of the leaf extract were expressed as mg of garlic acid (GAE)/g of extract. The total phenolic content in Aqueous *S.aethiopicum* leaves

extract was 132± 0.13 GAE/g of the extract and total flavonoid content was 146±1.12GAE/g of extract.

4.3 INVITRO ANTI-INFLAMMATORY ACTIVITY

4.3.1 Anti-lipoxygenase activity

Table 2: Effect of aqueous *S.aethiopicum* leaves extract on anti-lipoxygenase

Concentration (µg/ml)	Percentage inhibition	
	Sample	Standard
50	19.6172^{a} ±0.419	82.75629^{c} ±0.450
40	18.48356^{a} ± 0.432	67.40437^{c} ±0.348
30	16.15015^{a} ±0.168	49.72505^{b} ±0.120
20	15.37209^{a} ± 0.408	36.35356^{b} ±0.024
10	11.17095^{a} ±0.01	26.49731^{b} ±0.418
IC_{50}	199	28.2

All statistical values are represented as mean ± SEM (in triplicates). All values are significant when compared to standard drug (Diclofenac). $p< 0.05$

Anti-lipoxygenase activity of the aqueous leaf extract of *S.aethiopicum* at a concentration of 50µg/ml demonstrated a stronger lipoxygenase inhibition of 19.6172±0.05% with an IC_{50} value of 199µg/ml. The standard drug (diclofenac) also demonstrated the highest lipoxygenase inhibition with an IC50 value of 28.2µg/ml when compared to leaf extract.

4.3.2 Membrane stabilization activity

Table 3: Effect of aqueous *S.aethiopicum* leaves extract on membrane stabilization

Concentration (µg/ml)	Percentage inhibition	
	Sample	Standard
50	69.127315^{b}±0.269	82.51644^{c}±0.269
40	63.47878^{a} ±0.897	72.38494^{b} ±0.179
30	62.044235^{a} ±0.956	69.33652^{a} ±0.06
20	58.93604^{a} ±0.598	63.269575^{a} ±0.69
10	58.87059^{a} ±0.006	61.835025^{a} ±1.225
IC_{50}	19.85	9.35

All statistical values are represented as mean ± SEM (in triplicates). All values are significant when compared to standard drug (Aspirin). $p< 0.05$. The plant extract and standard drug indicated that the high percentage erythrocyte stabilization was observed at a concentration 50µg/ml with a percentage inhibition of 82.5±0.269 also with an IC_{50} value of 9.35µg/ml compared to leaf extract 19.85µg/ml.

4.3.3 Inhibition of Albumin denaturation

Table 4: Effect of aqueous *S.aethiopicum* leaves extract on inhibition of albumin denaturation.

Concentration (µg/ml)	Percentage inhibition	
	Sample	Standard
50	$55.826495^{a} \pm 0.680$	$66.87573^{b} \pm 0.451$
40	$35.410315^{b} \pm 2.691$	$54.790155^{c} \pm 0.124$
30	$45.720985^{a} \pm 1.641$	$49.231535^{a} \pm 0.228$
20	$34.58382^{b} \pm 3.165$	$44.97304^{c} \pm 0.307$
10	$34.46659^{a} \pm 7.386$	$27.239155^{a} \pm 2.421$
IC_{50}	50.2	31.5

All statistical values are represented as mean ± SEM (in triplicates). All values are significant when compared to standard drug (Aspirin). $p< 0.05$

The result of inhibition of albumin denaturation indicated that the IC_{50} values of the standard were significantly higher than that of the extract which is 50.2µg/ml and 31.5µg/ml respectively. The standard drug significantly inhibited the activity of Albumin denaturation. However, it was discovered that at a concentration of 50µg/ml, the standard drug, aspirin, exhibited the highest percentage inhibition of Albumin denaturation at 66.86± 0.451.

4.3.4. Proteinase inhibitory action

Table 5: Effect of aqueous *S.aethiopicum* leaves extract on inhibition on proteinase inhibitory action.

Concentration (µg/ml)	Percentage inhibition	
	Sample	Standard
50	$22.52099^{a} \pm 0.283$	$65.49651^{c} \pm 0.112$
40	$50.36014^{b} \pm 0.409$	$59.22728^{c} \pm 0.213$
30	$50.22378^{a} \pm 0.014$	$52.0979^{a} \pm 0.350$
20	$38.98602^{a} \pm 0.385$	$50.3989^{b} \pm 0.091$
10	$27.32168^{a} \pm 0.091$	$41.34965^{b} \pm 0.301$
IC_{50}	714	23.5

All statistical values are represented as mean ± SEM (in triplicates). All values are significant when compared to standard drug (Aspirin). $p < 0.05$.

From the result of proteinase inhibitory Action, the standard drug (aspirin) exhibited a higher percentage inhibition of 65.496± 0.112 at a concentration of 50µg/ml with an IC_{50} value of 23.4µg/ml compared to that of the extract 714µg/ml. It was also observed that the standard shows a higher proteinase inhibition.

5. DISCUSSION

Plant based drugs used in the practice of traditional treatment of diseases including inflammation have become the focus of current research because they are cheap and have great therapeutic potential without much of the side effects associated with synthetic drugs [16]. The present study evaluated the anti-inflammatory activity of the aqueous extract of *S. aethiopicum and* showed its effectiveness in reducing inflammation in in-vitro inflammatory models. The analysis of the phytochemical constituents of plants aids the screening of their biological activities and has great interest in pharmaceutical companies for the production of new drugs.

The phytochemicals are the plants' secondary metabolites that help the plant to combat competitors, predators or pathogens [17]. The previous phytochemical studies showed that leaves of *S.aethiopicum* are rich on flavonoids especially Quercetin. Quercetin relaxes intestinal smooth muscle and inhibits the bowel contraction leading to anti-diarrheal effect [18] and reduces the capillary permeability in the abdominal cavity which promotes medicinal applications *of S.aethiopicum* leaves [19]. Other than that, flavonoids have biological activities such as anti-oxidant, anti-apoptotic, anti-aging, anti-carcinogenic, anti-inflammatory, anti-atherosclerosis, cardiovascular protection and improvement of endothelial function, inhibition for angiogenesis and cell proliferation activities [20]. Phenolic compounds contribute to analgesic, anti-inflammatory, anti-microbial, hepato-protective and antioxidant activities [19]. Moreover, phenols such as catechin and epichatechin decrease the cholesterol level prevent type 2 diabetes

and act as anti-oxidants [21]. The presence of steroids increases the pharmaceutical value of garden egg leaves in such a way that the steroids increase the protein synthesis and thus promotes the growth of muscle and bones [22]. Further, Glycosides such as Saponins can reduce the cholesterol levels in the body [23]. Phytochemical screening of the plants revealed that the fruits contained alkaloids, flavonoids, sterols, saponins, cardiac glycosides. Further it has been reported that flavonoids and saponins exerted profound stabilizing effect on lysosomal membrane both in vitro and in vivo while tannins and saponins possess ability to bind cations there by stabilizing erythrocyte membranes and other macromolecules [24].

The denaturation of proteins is one of the causes of inflammation. In certain rheumatic diseases, the production of auto-antigens may be due to denaturation of proteins [25]. Anti-inflammatory drugs are known to inhibit the denaturation of proteins [26]. Non-steroidal anti-inflammatory drugs are the major pharmacological agents used for the anti-inflammatory and pain-relief management due to their capacity in inhibiting protein denaturation [27]. Denaturation of the protein involves the disruption of secondary, tertiary and quaternary structure of the molecules and finally leads to cell death. It occurs due to stress such as high level of salt, high temperature and high level of acidity. From the findings, there was significant ($p<0.05$) inhibition of protein denaturation in standard at concentration of 50µg/ml showed better activity than extract. From the results of the present study it can be stated that *S.aethiopicum* may control the production of auto-antigens by preventing in-vitro denaturation of proteins in rheumatic diseases and the IC_{50} value obtained at the inhibition of protein denaturation, indicated that the standard has higher inhibitory effect than the aqueous extract. This result is supported by the findings of [28] on the in vitro anti-inflammatory potential of pharmacophores.

Stabilizing effect of heat and saline induced erythrocyte lysis is a very good index of anti-inflammatory activity. The membrane of RBC is similar to that of lysosomal membrane. In inflammatory condition, stabilizing the lysosomal membrane helps to prevent the release of lysosomal constituents [29] of activated neutrophil such as proteases and bactericidal enzymes which cause further tissue inflammation and damage upon extra cellular release. In the study there was significant ($p<0.05$) membrane stabilization effect of both standard and extract and percentage inhibition of membrane stabilization produced by standard concentrations50µg/ml showed better activity than extract with an IC_{50} of 9.35 and 19.85.Activities of flavonoids and alkaloids have also been reported to modulate cellular activities of inflammatory related cells by stabilizing their membranes, thus preventing de-gradation and therefore impairing lysosomal enzyme release of arachidonic acid, elactase and glucoronidase [30].

Lipoxygenases (LOXs) are a family of non-heme iron-containing enzymes that have been implicated in the metabolism of arachidonic, linoleic and other polyunsaturated fatty acid into biologically active metabolites which are known mediators of inflammatory and immune response. The aqueous extract of the leaves showed a lesser Anti-lipo-oxygenase ability when

compared with the standard drug diclofenac. The IC_{50} of anti-lipoxygenase of the extract *S. aethiopicum* was found to be 199 and the result of standard diclofenac 28.2 which showed that the standard drug inhibits compared to extract. The result of this study revealed that Inhibition of Anti-Lipoxygenase enzyme was concentration dependentand also suggest that *S. aethiopicum* has a significant anti-inflammatory activity.

6. CONCLUSION

From this study, results indicate that the aqueous extract of S. aethiopicum leaves have high potent anti-inflammatory activities. The extract was able to reduce the activities of stabilizing membrane of erythrocyte. These activities may be due to the strong occurrence of secondary metabolite such as alkaloids, flavonoids, terpenoids, steroids and phenols.

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