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Antioxidant and antiulcer activities of ethanol leaf extract and fractions of solanum anomalum

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ABSTRACT

Solanum anomalum leaf is used in Ibibio traditional medicine for the treatment of various ailments including diabetes mellitus, malaria and ulcer. The ethanol leaf extract and fractions of *Solanum anomalum* were investigated for *in vitro* antioxidant activity using various models; 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, Ferric Reducing Power assay (FRAP) and Nitric oxide (NO) scavenging assay and antiulcer activity using indomethacin, ethanol and histamine-induced ulcer models in rats. The leaf extract and fractions exhibited significant antioxidant activity with the hexane and dichloromethane fractions demonstrating higher antioxidant potentials. The leaf extract (70-210 mg/kg) was found to significantly ($p<0.05$ -0.001) inhibit ulcers induced by indomethacin, ethanol and histamine in a dose-dependent fashion. These results suggest that the leaf extract of *Solanum anomalum* possess antioxidant and antiulcerogenic potentials which are due to the activities of the phytochemical constituents.

Keywords: *Solanum anomalum*, antioxidant, antiulcer, medicinal plant

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INTRODUCTION

Solanum anomalum Thonn. ex Schumach. (family *Solanaceae*) is a shrub growing up to 2 metres tall. The stem, branches and midribs of the leaves are usually armed with prickles up to 5 mm long. The edible fruits are gathered from the wild and consumed locally. Both the fruits and the leaves are used medicinally. The plant is sometimes cultivated or semi-cultivated for its fruits. It is found in West tropical Africa - Sierra Leone to southern Nigeria, Cameroon and DR Congo. It is Known as 'childrens' tomatoes', they are more commonly used as a condiment in soups and sauces and the fruits are eaten raw or cooked.[1] The sap from the leaves and fruits is drunk, or taken by enema 1-2 times daily, as a treatment for leprosy and gonorrhoea.[1] The fruits are used as a laxative and digestive.[1] They are also served ground up in soups and sauces as an appetizer for sick persons, sometimes mixed with fruits of Parkia.[1] The crushed fruits are applied to maturate inflammations on fingers or toes.[1] The fruit juice is applied to sores on the ears to alleviate pain.[2] Offor and Ubengama[3] reported the antidiabetic activity of the fruit of this plant. The anti-inflammatory activity of the leaf extract has been reported.[4] Although there is a little information on the biological activity of the leaves of this plant, We report in this study the antioxidant and antiulcer activities of the leaf extract and fractions of the plant.

MATERIALS AND METHODS

Plants collection

The plant material *Solanum anomalum* (leaves) were collected in compounds in Uruan area, Akwa Ibom State, Nigeria in August, 2018. The plant was identified and authenticated by a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. Hebarium specimen was deposited at Department of Pharmacognosy and Natural Medicine Herbarium.

Extraction

The plant parts (leaves) were washed, and air-dried on laboratory table for 2 weeks. The dried leaves were pulverized using electric grinder. The powdered leaves was divided into two parts; one part (1.5 kg) was macerated in 50% ethanol for 72 hours. While the other part was successively and gradiently macerated in n-hexane, dichloromethane, ethyl acetate and methanol to give the corresponding fraction of these solvents. The liquid filtrates obtained were concentrated and evaporated to dryness in vacuo at 40°C using rotary evaporator. The crude extract and fractions were stored in a refrigerator at -4°C until they were used for the experiments reported in this study.

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Animals

Swiss albino male rats (125-160 g) used for these experiments were gotten from Animal house of Department of Pharmacology and Toxicology, University of Uyo. The animals were housed in standard cages and were maintained on a standard pelleted feed (Guinea feed) and water *ad libitum*. Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics Committee, University of Uyo.

In-vitro antioxidant studies:

Determination of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

DPPH free radical scavenging of the leaf extract and fractions of *Solanum anomalum* and ascorbic acid prepared in methanol at concentrations of 20, 40, 60, 80 and 100 µg/mL were evaluated according to the method of Shekhar and Anju.[5] 1mL of DPPH was added to 3 mL of the solutions prepared with the leaf extract and fractions (ethyl acetate, n-hexane, n-butanol, and dichloromethane) and ascorbic acid and stirred for 1 minute. Each mixture was kept in the dark for 30 minutes and the absorbance (A_s) was measured at 517 nm. The assays were carried out in triplicates and the results expressed as mean values ± standard deviations. Lower absorbance of the reaction mixture indicated higher free radical activity. A similar procedure was repeated with methanol as blank, which served as control. The percent DPPH scavenging effect was calculated using the following equations:

1. DPPH scavenging effect (%) or Percent inhibition= $[(A_0 - A_s) / A_0] \times 100$.
2. Where A_0 is the absorbance of control reaction and A_s is the absorbance in the presence of test or standard sample (ascorbic acid).[5]

Ferric Reducing Power assay (FRAP)

This was determined using the method of Oyaizu.[6] Various concentrations 20, 40, 60, 80, and 100 µg/mL of the leaf extract and fractions of *Solanum anomalum* and ascorbic acid (1 mL) was mixed with 1 mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 minutes. After which 1 mL of 10% trichloroacetic acid was added and the resulting mixture centrifuged at 650 rpm for 10 minutes. The mixture (4 mL) was then mixed with 4 mL of de-ionised water and 1 mL of 0.1% ferric chloride and the absorbance was measured at 700 nm. A similar procedure was repeated with methanol as blank, which served as control. Higher absorbance indicates higher reducing power. The assays were carried out in triplicates and the results were expressed as mean values ± standard deviations.

Nitric oxide (NO) scavenging assay

Nitric oxide generated from sodium nitroprusside (SNP) was measured according to the modified method of Marcocci *et al.*[7] Three milliliters (3 mL) of SNP in phosphate buffered saline (pH 7.4) was added to 2 mL of different concentrations of leaf extract and fractions of *Solanum anomalum* and ascorbic acid (20, 40, 60, 80, and 100 µg/mL) the resulting solutions was then incubated at 25°C for 60 minutes. A similar procedure was repeated with methanol as blank, which served as control. To 3 mL of the incubated sample, 5 mL of Griess reagent (1% sulphonamide in 2% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride) was added. The absorbance of chromophore (purple azo dye) that were formed during the diazotisation of nitrite ions with sulphanilamide and subsequent coupling with naphthyl ethylenediamine dihydrochloride was measured at 540 nm. The assays were carried out in triplicates and the results were expressed as mean values ± standard deviations.[7] The percentage inhibition was calculated according to the following equation.[8]

%inhibition=$(1-A_1/A_o) \times 100$. Where A_1 = Absorbance of the extract or standard. A_o = absorbance of the control

Evaluation of antiulcer activity

Indomethacin-induced ulcer

Thirty male adult albino rats were used for the experiment. They were randomly divided into five groups of six rats each. Food was withdrawn 24 hours and water 2h before the commencement of experiment.[9] Group 1 (control) received only indomethacin (Sigma, 60 mg/kg p.o. dissolved in 5% Na_2CO_3); Groups 2-4 were pretreated with leaf extract (70, 140 and 210 mg/kg p.o. respectively) of *Solanum anomalum* dissolved in distilled water and administered as aqueous suspension; Group 5 received cimetidine (100 mg/kg p.o. dissolved in 50% Tween 80). One hour later, groups 2-5 were administered with indomethacin. Four hours after indomethacin administration, animals were killed by cervical dislocation. The stomachs were removed and opened along the greater curvature. The tissues were fixed with 10% formaldehyde in saline. Macroscopic examination was carried out with a hand lens and the presence of ulcer lesion was scored.[10] Ulcer index (UI) and preventive ratio (PR) of each of the groups pretreated with extract were calculated using standard methods.[11,12] Ulcer index represents the degree of lesion or ulceration caused by the ulcerogen, while preventive ratio is the protective potential of the extract/drug.

Ethanol-induced gastric ulceration

The procedure was similar to that used in indomethacin induced ulceration. Thirty rats were randomly assigned into five groups of six rats each based on their body weight. Food was withdrawn 24 hours and water 2h before the commencement of experiment.[9] Group 1 (control) received only ethanol (2.5 ml/kg p.o), Groups 2-4 were pretreated with *Solanum anomalum* leaf extract (70, 140 and 210 mg/kg p.o. respectively) dissolved in distilled water and administered as aqueous suspension; Group 5 received propranolol (40 mg/kg p.o. dissolved in distilled water). One hour later, groups 2- 5 were administered with ethanol. Four hours after ethanol administration, animals were killed by cervical dislocation. The stomachs were removed and opened along the greater curvature. The tissues were fixed with 10% formaldehyde in saline. Macroscopic examination was carried out with a hand lens and the presence of ulcer lesion was scored.[10]

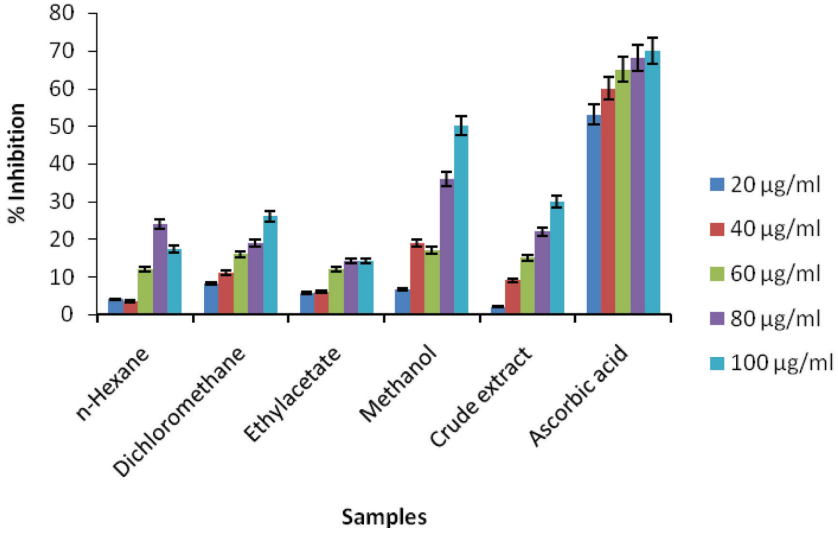

Figure 1 Antioxidant activity of leaf extract and fractions of *Solanum anomalum* against DPPH free radical

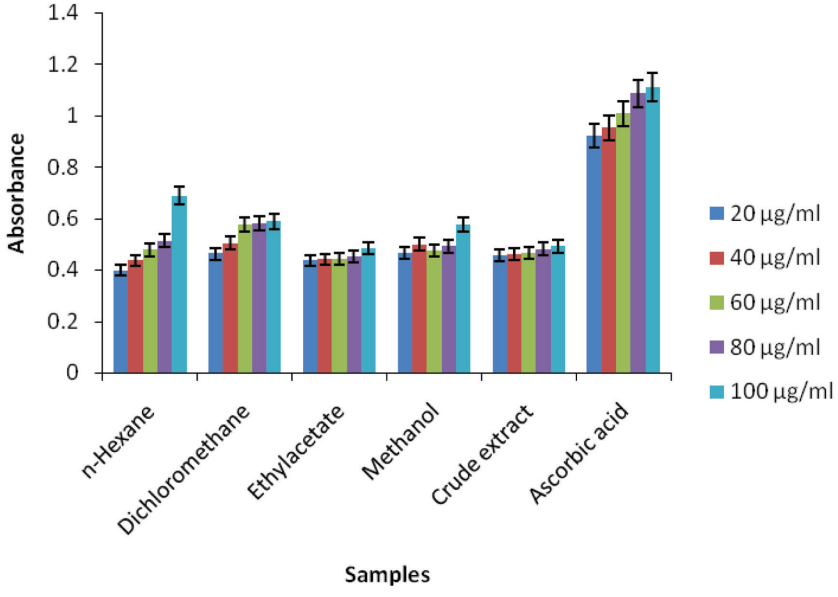

Figure 2 Effect of *Solanum anomalum* on FRAP

Histamine-induced gastric ulceration in rats

Thirty adult albino rats of both sexes weighing 120-160 g were used for the experiment. They were randomized into five groups of six rats each. Food was withdrawn 24 hours and water 2 h before the commencement of experiment.[9] Group 1 (control) received only histamine acid phosphate (Sigma, 100 mg/kg i.p. dissolved in distilled water);[13] Groups 2-4 were pretreated with leaf extract of *S. anomalum* (70, 140 and 210 mg/kg p.o. respectively) dissolved in distilled water and administered as aqueous suspension; Group 5 received cimetidine (100 mg/kg p.o. dissolved in 50% Tween 80), 1 hour prior to histamine administration. One hour later, groups 2-5 were administered with histamine acid phosphate (100 mg/kg, i.p). 18 hours after histamine administration, animals were killed by cervical dislocation. The stomachs were removed and opened along the greater curvature. The tissues were fixed with 10% formaldehyde in saline. Macroscopic examination was carried out with a hand lens and the presence of ulcer lesion was scored. Ulcer indexes (UI) and preventive ratio (PR) of each of the groups pretreated with the extract were calculated using standard methods.[11,12]

Statistical Analysis

Data obtained from this work were analyzed statistically using ANOVA (One-way) followed by a post test (Tukey-kramer multiple comparison test). Differences between means were considered significant at 5% level of significance ($p \leq 0.05$).

RESULTS

Effect of extract and fractions on DPPH free Radicals

The extract and fractions of *S. anomalum* were able to scavenge DPPH free radical via hydrogen donating activity at different concentrations. The scavenging activity increased in a concentration dependent fashion. However, methanol fraction had the highest inhibitory activity though not compared to control (ascorbic acid) (Figure 1).

Effect of extract and fractions on Reducing Power assay

The extract and fractions demonstrated their potential to reduce Fe^{3+} to Fe^{2+}. The result showed that the reducing power of the extract and fractions were concentration dependent. The n-hexane fraction followed by dichloromethane fraction had the highest reducing capacity though their effects were not comparable to that of the standard drug, ascorbic acid (Figure 2).

Table 1 **Effect of ethanol leaf extract of *Solanum anomalum* on indomethacin-induced ulcer**

Treatment	Dose mg/kg	Ulcer Indices	Preventive Ratio
Control normal	60	12.16 ±0.48	-
Indomethacin			
Cimetidine	100	0.55 ±0.01[c]	95.47
Crude extract	70	7.10±1.45[a]	41.61
	140	5.22 ± 0.56[b]	57.07
	210	3.34 ± 0.84[c]	72.53

Data are expressed as MEAN ± SEM, Significant at [a]p < 0.05,[b]p<0.01,[c]p<0.001, when compared to control. (n=6).

Table 2 **Effect of ethanol leaf extract of *Solanum anomalum* on ethanol-induced ulcer**

Treatment	Dose mg/kg	Ulcer Indices	Preventive Ratio
Control normal	60	6.15 ±0.64	-
Propranolol	40	1.08 ±0.16[b]	82.43
Crude extract	70	3.16±1.32	48.61
	140	2.15 ± 0.66	65.04
	210	1.78 ± 0.13[a]	71.05

Data are expressed as MEAN ± SEM, Significant at [a]p < 0.05,[b]p<0.01,[c]p<0.001, when compared to control. (n=6).

Table 3 **Effect of ethanol leaf extract of *Solanum anomalum* on histamine-induced ulcer**

Treatment	Dose mg/kg	Ulcer Indices	Preventive Ratio
Control normal	60	5.84 ±0.22	-
Cimetidine	100	0.00 ±0.00[c]	100
Crude extract	70	2.12±1.01[a]	63.69
	140	1.08 ± 0.14[b]	81.50
	210	0.54 ± 0.12[c]	90.75

Data are expressed as MEAN ± SEM, Significant at [a]p < 0.05,[b]p<0.01,[c]p<0.001, when compared to control. (n=6).

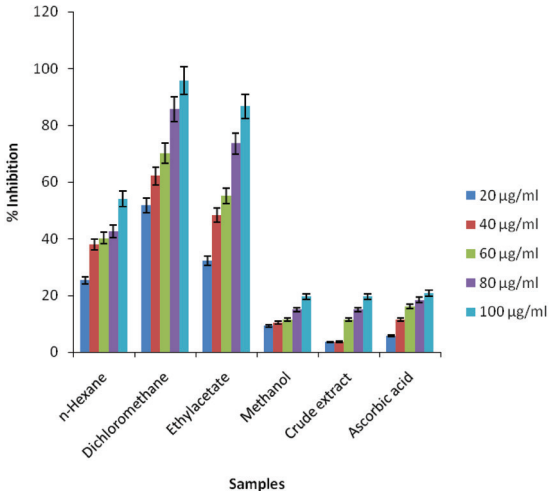

Figure 3 Effect of *S. anomalum* on Nitric oxide generation

Effect of extract and fraction on Nitric oxide assay

The extract and fraction considerably reduced generation of nitric oxide. The DCM fraction exhibited the highest NO scavenging ability followed by ethyl acetate fraction. The activity of the DCM fraction was higher than that of the standard drug, Ascorbic acid. (Figure 3).

Indomethacin-induced gastric ulceration

The leaf extract (p.o.) pretreatment on indomethacin induced gastric ulceration showed a dose dependent reductions in ulcer indices in pretreated groups relative to control. These reductions were statistically significant (p<0.05) compared to control. (Table 1). The effect was lower when compared to that of the standard drug, cimetidine.

Ethanol-induced gastric ulceration

Pretreatment of rats with leaf extract of *Solanum anomalum* significantly protected the animals from ethanol–induced ulcer (Table 2). This protection was significant (p<0.01) and dose-dependent as shown in the reduction of ulcer indices relative to control.

Histamine–induced ulceration

Administration of the leaf extract to rats significantly (p<0.001) reduced histamine-induced gastric ulceration in a dose-dependent fashion compared to control (Table 3). The effect of the extract was lower than that of the standard drug, cimetidine.

DISCUSSION

The leaves of *Solanum anomalum* is used in traditional medicine in the treatment various gastrointestinal disorders including ulcer. For this reason, the antioxidant and antiulcer activities of the leaf extract and fractions were evaluated for in vitro antioxidant activity using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, Ferric Reducing Power assay (FRAP) and Nitric oxide (NO) scavenging assay. While antiulcer activity was evaluated using indomethacin, ethanol and histamine–induced ulcer models. Indomethacin is known to cause ulcer especially in an empty stomach[14] and mostly on the glandular (mucosal) part of the stomach[10,15] by inhibiting prostaglandin synthetase through the cycloxygenase pathway.[16] Prostaglandins function to protect the stomach from injury by stimulating the secretion of bicarbonate and mucus, maintaining mucosal blood flow and regulating mucosal turn over and repair.[17,18]

Suppression of prostaglandins synthesis by indomethacin result in increased susceptibility of the stomach to mucosal injury and gastroduodenal ulceration. The extract was observed to

significantly reduce mucosal damage in the indomethacin–induced ulcer model, suggesting the possible extract mobilization and involvement of prostaglandin in the anti- ulcer effect of the extract. Administration of ethanol has been reported to cause disturbances in gastric secretion, damage to the mucosa, alterations in the permeability, gastric mucus depletion and free radical production.[19] This is attributed to the release of superoxide anion and hydroperoxy free radicals during metabolism of ethanol as oxygen derived free radicals has been found to be involved in the mechanism of acute and chronic ulceration in the gastric mucosa.[20] It was observed in this study that the extract significantly reduced ethanol induced ulcer. This may be due to cytoprotective effect of the extract via antioxidant effects. The extract and fractions were observed in this study to exhibit significant antioxidant activity. Ethanol is also reported to cause gastric mucosal damage by stimulating the formation of leukotriene C4 (LTC4).[21] The gastroprotective effect of the extract may in part be due to the suppression, by the extract of lipoxygenase activity.[10]

Histamine-induced ulceration is known to be mediated by enhanced gastric acid secretion as well as by vasospastic action of histamine.[22] The inhibition of ulcer due to histamine by the extract may be due to its suppression of histamine-induced vasospastic effect and gastric secretion. Okokon *et al.*,[4] reported that the leaf extract contains flavonoids, terpenes, saponins, alkaloids and cardiac glycosides among others. Flavonoids such as quercetin have been reported to prevent gastric mucosal lesions in various experimental models[23] by increasing the amount of neutral glycoproteins.[23] Flavonoids have been reported to protect the gastric mucosa from damage by increasing the mucosal prostaglandin content and by inhibiting histamine secretion from mast cells by inhibition of histidine decarboxylase. Free radical scavenging ability of flavonoids has been reported to protect the gastrointestinal tract from ulcerative and erosion lesion.[24] Saponins, especially triterpenes type have been implicated in antiulcer activity mediated by formation of protective mucus on the gastric mucosa and also protect the mucosa from acid effects by selectively inhibiting PGF2α.[25] However, the extract has been reported to contain alkaloids, flavonoids, cardiac glycosides, tannins, saponins, and terpenes.[4] The antiulcer activity observed in this study maybe due to the antioxidant activities of these phytochemical compounds.

CONCLUSION

The results of the present study show that leaf extract displays antioxidant and gastroprotective activities as demonstrated by significant inhibition of the formation of ulcers induced through the three different ulcer models. This supports its use in the treatment of gastrointestinal disorders in traditional medicine.

CONFLICT OF INTEREST DECLARATION

The authors declare no conflict of interest.

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