

Original Research Article

Phytochemical screening, antioxidant, antiulcer and toxicity studies on *Desmodium adscendens* (Sw) DC Fabaceae leaf and stem

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Abstract

Purpose: To assess the phytochemical profile, toxicity, as well as the antioxidant, and antiulcer activities of the methanol extracts of *Desmodium adscendens* stem and leaf.

Methods: Maceration procedure was employed in the preparation of the methanol extracts. Phytochemical characterization of the extracts was carried out according to standard methods. In vitro antioxidant activity was evaluated using 2, 2-diphenyl-1-picrylhydrazyl (DPPH I) and ferric reducing antioxidant power assay (FRAP). Antiulcer activity was investigated using ethanol-induced ulcer model, while toxicity was assessed by observing the mice for mortality.

Results: Phytochemical analysis indicate the presence of glycosides, alkaloids, tannins, flavonoids, and saponins in the stem and leaf. Methanol extracts of the plant exhibited antioxidant activity, with DPPH assay results showing median inhibitory concentration (IC_{50}) of 87.59 (leaf), 108.87 (stem), 28.52 (alpha-tocopherol), and 5.05 $\mu\text{g/mL}$ (ascorbic acid). The FRAP assay results for the stem and leaf extracts were 1483 and 1953 $\mu\text{M Fe}^{2+}/\text{g}$ dry plant, respectively, while for ascorbic acid it was 3463 $\mu\text{MFe}^{2+}/\text{g}$. The extracts showed significant antiulcer activity, with 14.27 and 15.18 % ulceration inhibition for the leaf extract, and 12.31 and 13.36 % for the stem extract at administered doses of 100 and 200 mg/kg, respectively. Cimetidine and omeprazole (standards) showed ulceration inhibition of 5.53, and 8.26 % at 5.7 and 0.57 mg/kg doses, respectively.

Conclusion: The methanol extracts of *Desmodium adscendens* stem and leaf offer significant protective activity against ethanol-induced gastric ulceration in rats, and the activity may be related to their antioxidant effect.

Keywords: Antioxidant, Antiulcer, Toxicity, *Desmodium adscendens*

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INTRODUCTION

Desmodium adscendens (Family Fabaceae) is a climbing woody stem plant which grows in an uncultivated land on the West Coastal region of Africa. The species of *Desmodium adscendens*

(*D. adscendens*) is frequently found in Nigeria, Cameroon, Zimbabwe, and tropical America. It is a sole runway hedge, preferring damp locations and it is found in forest areas and on the border of savannas [1].

Ethnomedical studies on *D. adscendens* in India showed a broad spectrum of activity which includes antileishmanial, antioxidant, immunomodulatory, antiulcer, cardio-protective, antidiabetic, anti-amnesia, antiviral, and hepatoprotective. [2]. Five phenolic compounds identified as caffeic acid, quercetin, *p*-coumaric acid, epicatechin, and rutin have been isolated and characterized from *Desmodium adscendens* leaves.[3]. Also, compounds that have been isolated from the leaf include phenylethylamines, indole-3-alkyl amines, tetrahydroiso-quinolones, and triterpenoid saponins [4]. The major compounds reported in the volatile oil of the leaves were phytone (14.72 %), caryophyllene oxide (11.32 %), esdesma (7.41 %), geraniol (5.42 %), linalool (5.33 %), palmitic acid (5.06 %), α -caryophyllene (4.76 %), scytalone (3.83 %), β -ionone (3.47 %), 2,2-dimethyl-hexanale (3.37 %), pelargonaldehyde (3.26 %), hyperforine (3.27 %), 2-pentyl furan (2.71), oleic acid (2.68 %), and 4- azidoheptane (2.02%) [5]. A literature research on the safety of *D. adscendens* extracts on renal, and hepatocytes cells in addition to the protection against oxidative stress had been investigated. The report had shown that the concentrations of *D. adscendens* (1 and 10 mg/ml) were safe for HEPG2 and LLC-PK1 and also offers protection against oxidative stress in LLC-PK1 cells [6]. *D. adscendens* leaf are largely combination of flavonoids with a total polyphenol of 11.1 mg of gallic acid equivalent (GAE)/g dry weight (dw), 12.8 mg of catechin equivalent (CE)/g dw, while the total tannin and anthocyanin compounds were 0.39 mg CE/g dw and 0.0182 mg CgE/g dw respectively [4]. Although *Desmodium adscendens* plant has gained popularity in ethnomedical uses as an antiulcer agent in Nigerian traditional medicine, there has been no empirical evidence of the antiulcer activity. As a result, the current research was focused on evaluating the gastroprotective effect of the methanol extracts of *D. adscendens* stem and leaf on ethanol-induced gastric ulceration, and also the antioxidant activity.

EXPERIMENTAL

DPPH (2, 2-diphenyl-1-picrylhydrazyl), Ferric 2,4,6-tripyridyl-s-triazine (Fe III TPTZ), Iron(III)chloride hexahydrate (FeCl₃.6H₂O), rutin and gallic acid were ordered from Sigma-Aldrich, USA. The reference drugs used include cimetidine, omeprazole, ascorbic acid, and alpha-tocopherol. Analytical type reagents and chemicals utilized in this research were ordered from May & Baker LTD, England. Double beam UV-Visible Spectrometer (PG Instruments Ltd,

T80+, S/N 151885-01-0094) was utilized in measuring the absorbance.

Animals

Male Albino rats (180 – 190 g), and Swiss albino mice (18 -20 g) were used in the study. The animals were acquired from the Animal care house at the Physiology department of the College of Medicine, Lagos University Teaching Hospital, Idi araba campus, Lagos, Nigeria. They were harboured in a dirt free cage with the rats separated from the mice and preserved in a well ventilated room. They fed on standard pellet diet (Livestock Nigeria brand) and tap water was made available *ad libitum*. The animals were maintained under standard condition of (23 ± 1° C), on a light/day cycle for 12 h and the cage cleaned frequently. The study procedure was in compliance with the nationally accepted guidelines for laboratory animal use and care and consent was given by Health Research Ethics Committee (HREC), of the College of Medicine, University of Lagos, Nigeria, with approval number CMUL/HREC/07/17/212. The Guide for the Care and use of Laboratory Animal was followed for the research [7].

Collection of plant material

D. adscendens leaves and stems were gathered from Ibadan, Nigeria. The materials were authenticated by Mr. Adeleke at the Pharmacognosy department, University of Lagos, and a sample was kept at the herbarium of Pharmacognosy department, University of Lagos, Nigeria with the voucher number PCGH164. The samples were dried at ambient temperature and milled into a uniformly dried powder.

Preparation of extracts

Dried powdered leaf sample (200 g) was immersed in 1000 mL of 96 % (w/v) methanol for 3 days. The extract was sieved through Whatman filter paper no. 42 (125 mm) and the filtrates collected were dried using a rotary evaporator at 40 °C. It was suitably labelled and kept in a cool place until use. The same process was replicated for the stem sample.

Phytochemical screening

The qualitative phytochemical examination of the extracts of *D. adscendens* stem and leaf was conducted by using standard methods [8, 9]. The extracts were examined for the following phytochemicals; flavonoids, glycosides,

alkaloids, tannins, and saponins using appropriate reagents.

Determination of antioxidant activity

DPPH radical scavenging activity

The scavenging activity of *D. adscendens* extracts against 2, 2-diphenyl-1-picrylhydrazyl radical was ascertained by reading the UV absorbance at 517 nm, as stated by the modified procedures of Amaeze *et al*, and Brand-Williams *et al* [10, 11]. Different concentrations of the extracts (0.02, 0.04, 0.06, 0.08 and 0.1 mg/mL) were prepared with methanol, using alpha-tocopherol, and ascorbic acid as standards. For each prepared solutions, 2 ml was transferred into test tubes and added to it was 0.5 ml of 1 mM 2, 2-diphenyl-1-picrylhydrazyl solution in methanol. The tests were done in triplicates. The prepared solution was agitated and allowed to rest for 15 min at ambient temperature, and the absorbance was measured spectrophotometrically at 517 nm [12]. DPPH inhibitory activity (D) was computed as in Eq 1. Reduction in absorbance measurement indicates the extent of antioxidant activity of the solutions.

$$D (\%) = \{(A_0 - A_1)/A_0\}100 \dots\dots\dots (1)$$

where A_0 is blank absorbance, A_1 is test sample absorbance and D is the DPPH inhibitory activity.

Total antioxidant assay (FRAP assay)

An adapted method of Benzie and Strain [13] was assumed for the ferric reducing antioxidant power (FRAP) assay. The principle involved relies on the capability of the test sample to decrease the ferric tripyridyltriazine (Fe [III]-TPTZ) complex to ferrous tripyridyltriazine (Fe [II]-TPTZ) at low a pH. Fe [II]-TPTZ has an intense blue colour which was taken at 593 nm. Newly prepared FRAP solution (1.5 mL), containing 25 mL of 300 mM acetate buffer at pH 3.6, 2.5 mL of 10 mM 2,3,6-TPTZ in 40 mM HCl, and 2.5 ml of 20 mM Iron(III)chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) solution was transferred to 1 mL of the extracts and absorbance taken at 593 nm [14]. Calibration plot was linear between 50 - 300 μM and it was prepared with $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ solution. Results are indicated in μM Fe (II)/g dry plant material and were juxtaposed with that of ascorbic acid.

Total phenolic content

The phenolic content of the extract of *D. adscendens* was determined on the authority of Folin and Ciocalteu method [15] with slight

modifications. The calibration curve was derived by adding gallic acid in ethanol (1 mL, 0.025 mg/mL) to 5 ml Folin-Ciocalteu reagent (on ten folds dilution) and Na_2CO_3 (4 mL, 0.7 M). Absorbance was determined at 765 nm. One ml of the extracts in ethanol (5 mg/ml) was added to the reagent, mixed and after 2 h the absorbance was read to confirm the phenolic content. Each test was done in triplicate. The result was exhibited as gallic acid equivalent (GAE) mg/g, using the equation derived from the calibration curve: $y = 11.523x + 0.678$, $R^2 = 0.999$.

Total flavonoid content

The flavonoid content was studied with the procedure of Miliauskas *et al* [16]. To 2 ml of the extracts in methanol (concentrations of 0.1 and 1 mg/mL), was added 2 ml of 2% AlCl_3 in ethanol and the absorbance was read at 420 nm after 1h of incubation at room temperature. The concentrations of rutin prepared in methanol (0.01, 0.02, 0.04, 0.08 and 0.10 mg/mL) were used to establish the calibration plot. Total flavonoid content was calculated as rutin equivalent (RE) in mg/g using the equation derived from the calibration curve: $y = 0.5451 x + 0.0203$, $R^2 = 0.9205$.

Toxicity studies

Acute toxicity study was conducted by using gradient doses of each extracts in albino mice. They were splitted into four groups, each of seven mice each, the stem extract were administered orally in graded doses (5,000, 10,000, 15,000 and 20,000 mg/kg body weight). They were observed at various time intervals (30 min, 1, 2, 3 and 4 h) for toxic symptoms and mortality.

Sub-acute toxicity was conducted by a single administration of the dose to the animals with daily observation and weighing of the mice for one week [17].

Anti-ulcer activity study

Ulcer was established by administering ethanol, and the route of administration of prepared solutions was by oral. The adult male albino rats were made to abstain from food for 36 h prior to the administration of ethanol. The animals were split into seven groups, each made up of four rats except for the control group which contains three rats:

Group I: animals were administered distilled water only and this represents the control group

Group II: received cimetidine (5.7 mg/kg) as reference drug

Group III: received Omeprazole 0.57 mg/kg) as reference drug

Group IV: Received methanol extract (100 mg/kg) of *D. adscendens* leaf

Group V: Received methanol extract (200 mg/kg) of *D. adscendens* leaf

Group VI: Received methanol extract (100 mg/kg) of *D. adscendens* stem

Group VII: Received methanol extract (200 mg/kg) of *D. adscendens* stem.

After about 30 min, all animals were orally given 1 ml of ethanol and thereafter kept in a specially built cage to prevent coprophagia. They were afterwards decapitated after 1 h of administration; the stomach was incised along the greater curvature and cleansed in physiological saline solution to remove debris. It was laid flat and gastric mucosa lesions immediately traced on acetate paper using a magnifying lens. Ulcer protection (U) was calculated as in Eq 2.

$$U (\%) = \{(U_c - U_t)/U_c\}100 \dots\dots\dots (2)$$

where U_c is the control ulcer index and U_t is the ulcer index of disease tested control group [18].

Statistical analysis

The data are presented as mean \pm standard error of mean (SEM). Comparisons were done between the treated groups by two-way analysis of variance (ANOVA) using SPSS. The level of statistical significance was set at $p < 0.05$.

RESULTS

Phytochemical evaluation of the *D. adscendens* stem and leaf unveiled the confirmation of flavonoids, glycosides, saponins, tannins, and alkaloids.

DPPH assay data showed inhibitory activity ranging from 3.17 – 91.37 % for leaf extract, 2.87 – 61.65 % for stem extract, 43.93 – 92.58 % for alpha tocopherol, and 95.97 - 97.97 % of ascorbic acid (Figure 1) with IC_{50} of 87.59, 108.87, 28.52 and 5.05 $\mu\text{g/mL}$ respectively. The FRAP assay of the *Desmodium adscendens* stem was 1483 $\mu\text{M Fe}^{2+}/\text{g}$, 1953 $\mu\text{M Fe}^{2+}/\text{g}$ for the leaf extract, while the ascorbic acid had a value of 3463 $\mu\text{M Fe}^{2+}/\text{g}$.

Total phenolic contents were derived from the regression equation of the calibration plot of gallic acid ($y = 11.523x + 0.6775$, $R^2 = 0.998$) and expressed as gallic acid equivalent (GAE). The phenolic content of the leaf and stem of *D. adscendens* were 10 and 24 mg/g of plant material, respectively. The correlation coefficient (R^2) between the DPPH inhibition and total phenolic content were 0.997 and 0.986 for leaf and stem respectively. Total flavonoid content was calculated from the regression equation of the calibration plot of rutin ($y = 0.5451x + 0.0203$, $R^2 = 0.92$) and expressed as rutin equivalent (RE). Total flavonoid contents were determined as 2.76 and 2.58 mg/g of powdered plant extract for leaf and stem of *D. adscendens* respectively. Acute toxicity studies on the stem extract showed the death of two mice at the highest dose of 20 g/kg, which accounts for 28.57 % mortality, while no death was observed at lower doses. The sub-acute oral toxicity revealed death at 15 and 20 g/kg, which account for 14.29 and 42.84 % of dead mice respectively.

Pre-treatment with cimetidine and omeprazole were discovered to inhibit the ethanol induced intestinal mucosal lesion in rats. The methanol extracts of *D. adscendens* leaf and stem revealed a dose-dependent antiulcer activity. The rats treated with extracts at 100 and 200 mg/kg, doses respectively, showed significant ($p < 0.05$) reduction in the number of ulcer index. The results showed 14.27 and 15.18 % ulceration inhibition at doses of 100 and 200 mg/kg, respectively for *D. adscendens* leaf extract, while the *D. adscendens* stem extract showed 12.31 and 13.36 % ulceration inhibition at doses of 100 and 200 mg/kg respectively. However, cimetidine showed ulceration inhibition of 5.53 % at a dosage of 5.7 mg/kg, while omeprazole showed ulceration inhibition of 8.26 % at a dosage of 0.57 mg/kg.

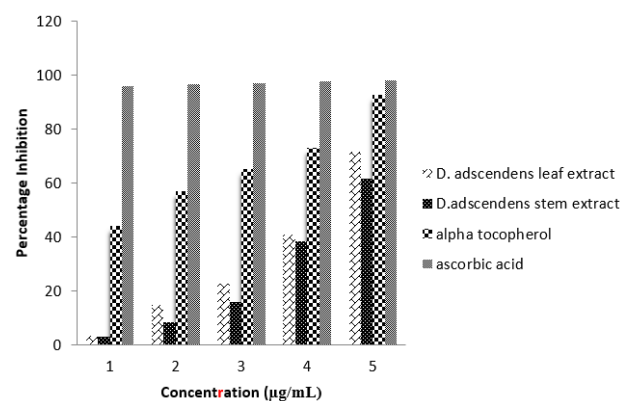


Figure 1: DPPH free radical scavenging activity of methanol leaf and stem extracts of *D. adscendens* as well as alpha-tocopherol and ascorbic acid

DISCUSSION

Preliminary phytochemical evaluation confirmed the presence of flavonoids, glycosides, alkaloids, tannins, and saponins in extracts of the stem and leaf of *D. adscendens*. Phytochemical quantification showed the phenolic content of *D. adscendens* leaf and stem as 10 mg GAE/g and 24 mg GAE/g of plant material, respectively, while the flavonoid content was 2.76mg/g and 2.54mg/g for leaf and stem extracts respectively. This is similar to the results from the work of Konan *et al* [19] who found total phenolics (3.76 mg GAE/g) and (2.15 mg GAE/g) for the leaf and stem; also 0.077 and 0.02 % of flavonoids were found in the leaf and stem.

The scavenging property of *D. adscendens* stem and leaf extract may be ascribable to the phenolic compounds that can supply the necessary component as a radical scavenger. The IC₅₀ value for the leaf (87.59 µg/mL) was lower than the stems extract (108.87 µg/mL), which reflects a good demonstration of its antioxidant activity. While the research of Konan *et al* demonstrates that the ethyl acetate extracts of the leaf of *D. adscendens* has IC₅₀ 260 µg/mL for radical scavenging property based on DPPH assay [19]. Free radicals are often generated as a result of biological reactions or from exogenous factors, its implication in the progression of some disease conditions is well established and has been published [20]. The antioxidant property of *D. adscendens* stem and leaf could be related to the high amount of flavonoid and phenolic compounds in this plant extract [21].

Ethanol-induced intestinal ulcer is a suitable model for exploring the gastroprotective effects of herbal drugs. The causes of peptic ulcer are still unknown in most cases; nevertheless, it has been recommended that it results from a variation between some destructive elements and the preservation of mucosal cohesion through the internal defense mechanisms. To restore this balance, different therapeutic agents like H₂-blockers, proton pump inhibitors, and plant based substances have been used [22]. *D. adscendens* stem and leaf extracts used in this study to evaluate the anti-ulcerogenic in ethanol induced ulcers in rats showed a significant dose dependent ulceration inhibition activity at 100 and 200 mg/kg respectively. This could arise from a cytoprotective effect of the extracts through anti-oxidant effects. The anti-ulcer effect of *D. adscendens* stem and leaf could arise from two factors: reductions in gastric acid release and gastric cytoprotection. The remarkable increase in the anti-ulcer effect of *D. adscendens* leaf and stem extracts might be accredited to the

confirmation of saponins, glycosides, tannins, and flavonoids compounds. Flavonoids are in the group of cytoprotective substance where its anti-ulcer efficacy has been studied [23]. Hence, the anti-ulcer activity of *D. adscendens* may be assigned to its flavonoids content.

It is well known that ethanol causes disorder in gastric secretion, destruction of the mucosa alters the permeability of the gastric membrane, gastric mucus depletion, and free radical production. This has been accredited to the release of free hydroperoxy and superoxide anion radicals produced during metabolism of ethanol. Free radicals derived from oxygen have been indicated in the mechanism of action of acute and chronic ulceration in the mucous membrane of the gastrointestinal tract [24]. Ethanol induction of gastric ulcer which causes the development of necrotic features of tissue lesion and haemorrhage, may arise from inactivity in gastric blood flow. The alcohol rapidly infiltrates the intestinal mucous membrane and causes destruction to the plasma and cell membrane, which leads to increased intracellular membrane permeability to sodium and water.

Furthermore, an enormous intracellular increase of calcium correlates with a vital pace in the development disease state of gastric mucosal lesion [22]. It is indicated that active phytochemical compounds would be able to stimulate mucus, bicarbonate and the prostaglandin secretion and, counteracts the distorting activity of reactive oxidants in the stomach and intestinal lumen [25]. Hence, this research recommends that the methanol extract of *D. adscendens* stem and leaf may be used to forestall gastric lesions. Thither is a demand to identify specific molecular species present in the extracts which are responsible for its gastroprotective effect, nevertheless, this would warrant further investigations.

CONCLUSION

The findings of this study indicate that methanol extracts of *Desmodium adscendens* stem and leaf are rich in phenolics and flavonoids, with high antioxidant activities, and also may offer a new gastroprotective treatment against ethanol-induced ulcers.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Gloria A. Ayoola conceived and designed the study/ experiment. Oluwatosin O. Johnson and Samuel O. Eze collected and analysed the data. Oluwatosin O. Johnson and David K. Adeyemi wrote the manuscript. All authors have read and approved the manuscript for publication.

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