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HPLC characterization and anti-ulcer effects of methanol seed extract and fractionated components of *Telfairia occidentalis* in rodents.

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ABSTRACT

***Background**:* This study aimed to characterize the secondary metabolites and evaluate the ulcer-protective activity of Telfairia occidentalis seed extract and its fractionated components in rodents.

***Methods**:* The seed coverings were removed, and the seeds were dried and milled into a coarse powder, which was macerated with 70% methanol and filtered after 72 h. The resultant filtrate was concentrated *in vacuo*, and a portion was partitioned with dichloromethane, ethyl acetate, and n-butanol using a separating funnel. The acute oral toxicity (LD_{50}) of the methanol extract was determined following the OECD protocol. Phytochemical constituents were profiled using high-performance liquid chromatography (HPLC). Ulcer-protective effects were evaluated in rats using ethanol-, indomethacin-, and histamine-induced ulceration models.

***Results**:* The LD_{50} value was estimated at 5000 mg/kg. Phytochemical analysis identified flavonoids, alkaloids, steroids, tannins, saponins, terpenoids, phenols, and glycosides. The methanol extract and its fractions provided significant ($p \leq 0.05$) protection against ulcers induced by ethanol, indomethacin, and histamine, with effects comparable to standard drugs such as propranolol and cimetidine.

***Conclusion**:* The methanol seed extract and fractionated components of *T. occidentalis* exhibited notable ulcer-protective properties, with the n-butanol fraction (NBT) showing the highest activity.

***Keywords**:* Anti-ulcer, HPLC, partitioned components, seed, *Telfairia occidentalis*

1. INTRODUCTION

Telafairia occidentalis Hook. f., a member of the Cucurbitaceae family, is a widely consumed vegetable in Nigeria, valued for its nutritional, culinary, and medicinal properties, particularly in the southern region [1,2]. Most plant parts are used in ethnomedicine, notably for treating abdominal pain–related disorders [2,3]. Reported pharmacological activities include; antiplasmodial [4–6], antioxidant, antidiabetic [7], haematological, anti-inflammatory [8,9], and antinociceptive [10] effects. While the antiulcer activity of the stem extract has been documented [11], no studies have investigated the potential of the seeds in ulcer protection. This study, therefore, aimed to profile the methanol extract and solvent-partitioned fractions of the seeds for phytoconstituents and to assess their ulcer-protective effects in rats.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Biological Materials

Albino Wistar rats (*Rattus norvegicus*), albino mice (*Mus musculus*).

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2.1.2 Chemicals and Reagents
Ethanol (Sigma), indomethacin (Sigma), histamine acid phosphate (Sigma), propranolol, cimetidine, dichloromethane, ethyl acetate, *n*-butanol. All solvents and reagents used were of analytical grade.

2.1.3 Equipment and Apparatus
High-performance liquid chromatography (HPLC) system, hand lens, rotary evaporator.

2.2 Methods
2.2.1 Preparation of Extract and Fractions
Fresh fruits of *Telfairia occidentalis* were collected from the Medicinal Plant Garden, Department of Pharmacognosy and Natural Medicine, University of Uyo, Uyo, Akwa Ibom State, Nigeria. The fruits were opened with a knife, and the seeds were separated from the pulp. The seed coats were removed, and the seeds were shade-dried for 10 days. The dried seeds were pulverized using a hammer mill, and 1200 g of the powder was macerated with 70% aqueous methanol. The mixture was filtered after 72 h and concentrated *in vacuo* at 40 °C. A portion of the methanol extract (100 g) was partitioned successively with dichloromethane, ethyl acetate, and *n*-butanol to obtain the respective fractions. All extracts were concentrated and stored at 4 °C until use.

2.2.2 High-Performance Liquid Chromatography (HPLC) Analysis
HPLC analysis was carried out using an Agilent 1100 dual binary pump system, HP CTO-10AS column oven, and HP Prominence SPD-20A UV/Vis detector. A C-12 normal phase column (Phenomenex, Gemini 5 µm, 200 mm × 4.8 mm i.d.) was employed. The mobile phase consisted of acetic acid–acidified deionized water (pH 2.8, solvent A) and acetonitrile (solvent B), with a flow rate of 0.8 mL/min. Column equilibration was performed with 5% solvent B for 20 min before each injection. The column temperature was maintained at 38 °C, and a 20 µL sample volume was injected.
Detection was performed at 280 nm. Identification and quantification of phytochemicals were based on comparison of retention times and peak areas with external standards, and calibration curves were constructed accordingly. The gradient elution program was as follows:
0–5 min, 5–9% solvent B;
5–15 min, 9% solvent B;
15–22 min, 9–11% solvent B;
22–38 min, 11–18% solvent B;
38–43 min, 18–23% solvent B;
43–44 min, 23–90% solvent B;
44–45 min, 90–80% solvent B;
45–55 min [12,13].

2.2.3 Handling of Laboratory Animals
Adult mice (25–30 g) for acute toxicity study and adult rats (180–220 g) for anti-ulcer experiments were obtained from the Animal House Unit, Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Nigeria. Animals were acclimatized for seven days in standard cages and fed standard pellet diets. Food was withheld for 24 h and water for 2 h before the commencement of the experiment.

2.2.4 Animal Ethics
Animal handling was carried out in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication, 1996) and was approved by the Faculty of Pharmacy Ethics Committee, University of Uyo, Nigeria.

2.2.5 Determination of Median Lethal Dose (LD_{50})
The median lethal dose (LD_{50}) of the methanol seed extract of *T. occidentalis* was determined according to OECD guidelines [14]. Adult mice (three groups, three mice per group) weighing 25–30 g were used. Group 1 received 300 mg/kg of the extract orally, with no mortality recorded. This was followed by administration of 2,000 mg/kg to the remaining two groups at 24-h intervals.

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2.2.6 Anti-Ulcer Study

2.2.6.1 Ethanol-Induced Ulceration

Rats were randomized into nine groups (n = 5). Group 1 received 97% ethanol (2.5 mL/kg) only. Groups 2–4 received 500, 1,000, and 1,500 mg/kg of methanol seed extract of *T. occidentalis*, respectively. Groups 5–8 received 1,000 mg/kg of the partitioned fractions, while Group 9 received propranolol (40 mg/kg). One hour later, all animals were given 97% ethanol intragastrically via an orogastric cannula. Five hours after the start of the experiment, rats were sacrificed by cervical dislocation under diethyl ether anesthesia. The stomachs were excised, opened along the greater curvature, rinsed with running water, fixed in formalin, and examined with a hand lens for ulcer lesions, which were scored as earlier described [15].

2.2.6.2 Indomethacin-Induced Ulceration

The procedure was similar to that described in the ethanol model, except that indomethacin (0.2 g dissolved in 5% Na_2CO_3 in 20 mL distilled water) was used as the ulcerogen, and cimetidine (100 mg/kg) was used as the reference drug [16,17].

2.2.6.3 Histamine-Induced Ulceration

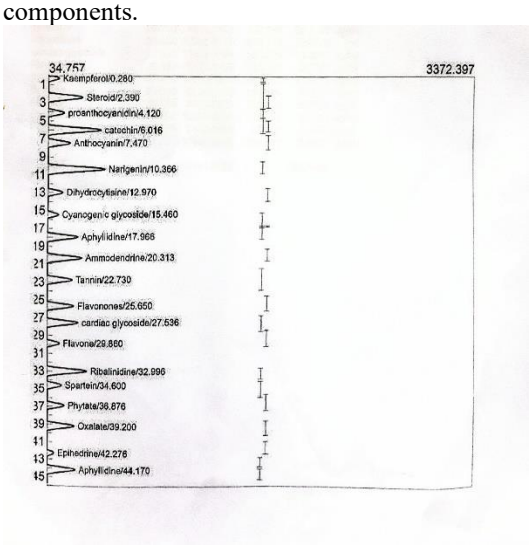
The procedure was similar to previously described methods, except that histamine acid phosphate (Sigma, 100 mg/kg) was administered intraperitoneally to induce ulcer lesions. Animals were allowed 18 h for lesion development before sacrifice [11].

2.3 Data Analysis

Results are expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way ANOVA followed by a post hoc test. Percentage ulcer preventive ratios were also calculated. Differences were considered statistically significant at $p \leq 0.05$. Data analysis was carried out using Primer statistical software (Primer-E Ltd., UK).

3. RESULTS

The result of HPLC chromatogram of the methanol seed extract and fractions of *T. occidentalis* are represented in Figures 1-5 while the result of chemical profiling of the methanol seed extract and partitioned fractions of *T. occidentalis* is presented in Table 1. Tables 2-4 captured the antiulcer responses of the extract and fractionated components.



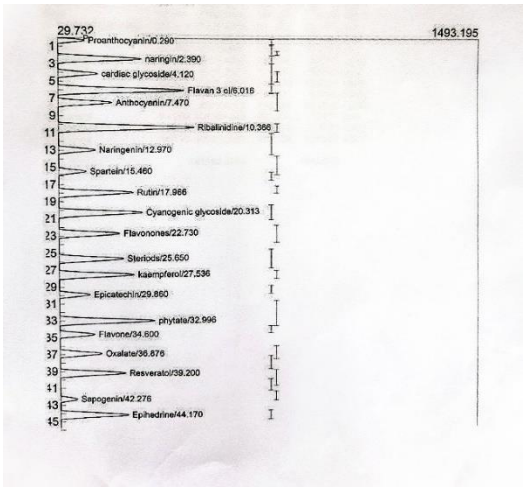


Figure 1: HPLC chromatogram for methanol extract and DCM components

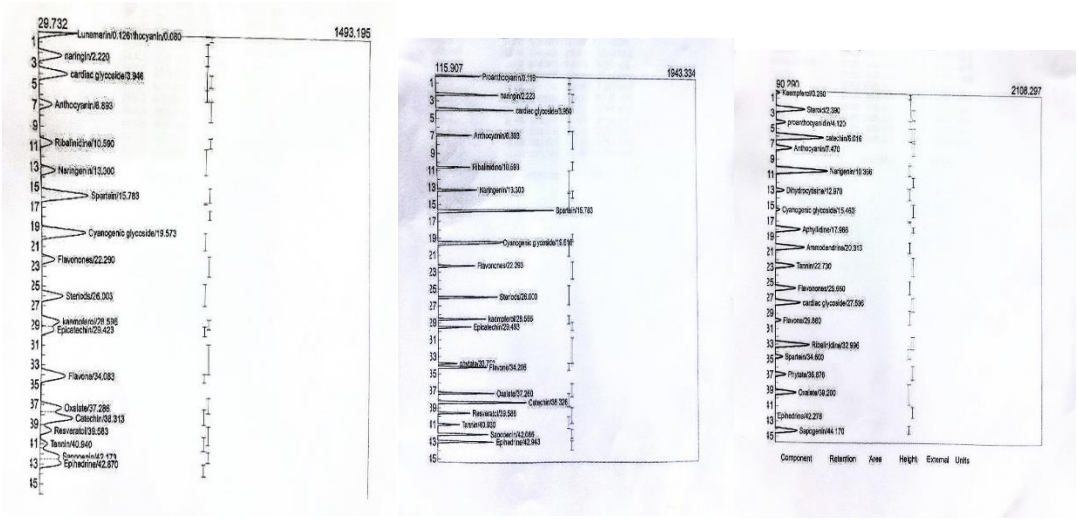


Figure 2: HPLC chromatograms of BUT, EA and AQ components

Table 1: HPLC characterization of methanol extract and fractions of *T. occidentalis* seed

Compounds	Concentration of Constituents (µg/mL)				Chemical class
	Methanol	DCM	ETH	BUT	AQ	
Kaempferol	4.37	5.30	3.98	2.81	2.37	Flavonoid
Steroids	15.76	17.20	11.72	11.55	15.76	steroid
proanthocyanidin	5.52	3.24	6.90	0.66	8.16	tannins
Catechin	15.81	-	2.06	2.18	23.35	flavonoid
Epicatechin	-	8.22	5.73	6.13	-	flavonoid
Anthocyanidin	7.36	7.25	7.70	3.86	3.90	flavonoid
Narigenin	17.08	15.30	-	-	13.57	flavonoid
Cyanogenic glycoside	7.83	17.15	9.91	16.85	8.70	glycoside
Ammodendrine	22.36	-	-	-	6.25	Alkaloid
Tannin	26.12	-	2.00	1.89	9.81	Tannin
Flavonones	12.81	8.21	10.18	4.05	8.72	flavonoid
Cardiac glycosides	14.73	3.97	10.15	4.99	14.22	terpenoid
Flavone	6.58	3.61	7.36	5.64	3.61	flavonoid
Ribalinidine	18.02	8.40	9.30	1.85	10.19	Alkaloid
Naringenin		2.64	3.12	2.05		flavonoid
Spartein	-	8.90	28.66	22.83	4.20	alkaloid
Rutin	-	7.04	8.78	-	-	flavonoid
Epihedrine	4.67	13.54	11.19	7.77	1.95	steroid
Sapogenin	-	5.73	7.39	9.70	18.03	saponin
Lunamarin	-	-	-	5.28	-	alkaloid
Naringin	-	-	12.43	8.15	-	flavonoid
Resveratol	-	-	6.70	3.20	-	phenol

DCM= dichloromethane, ETH= ethyl acetate, NBT= normal butanol, AQ=aqueous

Table 2: Effect of extract/fractions of *T. occidentalis* seed on ethanol ulcer model

Treatments	Mean ulcer score	Percentage Preventive Ratio
Control ethanol 2.5 mL/kg	4.83 ± 0.04	-
T. occidentalis 500 mg	2.67±0.40*	45%
T. occidentalis 1000 mg	1.33±0.03*	72%
T. occidentalis 1500 mg	0.33 ±0.02	93%
DCM fraction 1000 mg/kg	1.67 0.02*	65%

ETH fraction 1000 mg/kg	3.00± 0.00	38%
NBT fraction 1000 mg/kg	0.33 ± 0.01*	93%
AQ fraction 1000 mg/kg	2.67 ± 0.02*	45%
Propranolol 40mg/kg	0.33 ± 0.01*	93%

DCM= dichloromethane, ETH= ethyl acetate, NBT= normal butanol, AQ=aqueous, n=5

Table 3 Effect of extract/fractions of *T. occidentalis* seed on indomethacin ulcer model

Treatments	Mean ulcer score	Percentage Preventive Ratio
Control indomethacin 60 mg/kg	12.07 ± 0.94	-
T. occidentalis 500 mg	3.33±0.12*	72%
T. occidentalis 1000 mg	2.50 ±0.11*	79%
T. occidentalis 1500 mg	1.25 ±0.10*	89%
DCM fraction 1000 mg/kg	1.68 ± 0.12*	86%
ETH fraction 1000 mg/kg	3.33± 0.12*	72%
NBT fraction 1000 mg/kg	2.08 ± 0.11*	83%
AQ fraction 1000 mg/kg	4.18 ± 0.12*	65%
Cimetidine 100mg /kg	0.86 ± 0.11*	93%

DCM= dichloromethane, ETH= ethyl acetate, NBT= normal butanol, AQ=aqueous, n=5

Table 4: Effect of extract/fractions of *T. occidentalis* seed on histamine ulcer model

Treatments	Mean ulcer score	Percentage Preventive Ratio
Control histamine 100 mg/kg	4.83± 0.04	-
T. occidentalis 500 mg	0.83±0.02*	83%
T. occidentalis 1000 mg	0.50 ±0.02*	89%
T. occidentalis 1500 mg	0.33±0.01*	93 %
DCM fraction 1000 mg/kg	1.33 0.02*	72%
ETH fraction 1000 mg/kg	1.67± 0.02*	65%
NBT fraction 1000 mg/kg	0.83 ± 0.01*	83%
AQ fraction 1000 mg/kg	1.67 ± 0.02*	65%
Cimetidine 100mg /kg	0.33 ± 0.01*	93%

DCM= dichloromethane, ETH= ethyl acetate, NBT= normal butanol, AQ=aqueous, n=5

4.DISCUSSION

The acute toxicity study showed that no mortality occurred in mice administered 300 mg/kg of the methanol seed extract of *T. occidentalis* within 24 hours. Similarly, 2000 mg/kg produced no deaths in two sets of three mice. In line with OECD guidelines, the LD_{50} was extrapolated to be 5000 mg/kg, with experimental doses of 500 mg/kg (low), 1000 mg/kg (medium), and 1500 mg/kg (high) for the crude extract, and the medium dose for fractions. HPLC analysis (Table 1) identified 22 major compounds: flavonoids (10), alkaloids (4), tannins (2), steroids (2), saponin (1), terpenoid (1), phenol (1), and glycoside (1). Key flavonoids included kaempferol, proanthocyanidin, rutin, and anthocyanidin, while ammodendrine, ribalinidine, sparteine, and lunamarin represented the alkaloids. These classes are well-documented for anti-ulcer activity [11, 17]. Alkaloids dissolve well in gastric acid, protecting the mucosa [18]; flavonoids enhance protective mechanisms while suppressing aggressive factors in the gastrointestinal tract [19–21]; and saponins form a protective layer over the mucosa [22–24]. The combined presence of these metabolites likely explains the observed anti-ulcer effects [25]. Anti-ulcer experiments (Tables 2–4) showed that the methanol seed extract protected against ethanol-, indomethacin-, and histamine-induced ulcers in a dose-dependent manner, with effects comparable to propranolol and cimetidine, and statistically significant ($p \leq 0.05$) versus the normal control. The NBT fraction consistently showed the highest protection, suggesting a broad mechanism of action [15]. Ethanol induces ulcers by damaging the gastric mucosa, promoting inflammatory infiltration, and triggering oxidative stress via malondialdehyde formation and glutathione depletion [26, 27]. The extract and fractions may protect by blocking these pathways. Indomethacin causes ulceration through prostaglandin and COX-1 inhibition, reduced mucus and bicarbonate secretion, and increased gastric acid influx [28, 29]; the observed protection suggests interference with this

cascade. Histamine-induced ulceration results from excessive gastric acid secretion, disrupting mucosal integrity; the extract and fractions may prevent this process [30].

5. CONCLUSION

The methanol seed extract and fractions of *T. occidentalis* contained structurally diverse metabolites that confer protection against ulcers induced by ethanol, indomethacin, and histamine. The NBT fraction was consistently the most active and holds promise for drug development.

DELARATIONS

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Conflict of Interest: None.

Authors' Contributions:

Uwemedimo F. Umoh – Conceptualization and Supervision; Ekikere E. Ubengama – Manuscript Drafting; Edikan U. Udofia – Collection and Preparation of Extract; Ogechi I. Obasi – Fractionation of Extract; Uduakabasi K. Okonna and Esther S. Umanah - Anti-ulcer Study; Joy E. Etefia – HPLC Data Collation; Jude E. Okokon – Manuscript Vetting and Editing

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