

Original Research Article

PHYTOCHEMICAL AND ANTI-ULCER PROPERTIES OF *ALLOPHYLUS AFRICANUS* P BEAUV. (SAPINDACEAE) LEAF

ABSTRACT

Introduction: Ulcer has remained a persistent discomforting illness commonly caused by the bacteria *Helicobacter pylori* and prolonged or excessive use of anti-inflammatory pain killers like aspirin. While existing cases are difficult to treat and, treat new cases are being diagnosed. Current treatments do not seem to be giving much hope to patients. Natural product and plant-based preparation are offering some measure of hope. *Allophylus africanus* has been reported to be effective in traditional medicine as an anti-ulcer agent. **Objectives of the study:** this research aims to evaluate the antiulcer properties of the crude methanol extract of the leaf of the plant. **Methodology:** the plant material was extracted using the cold maceration method. The acute toxicity was investigated according to Lorke's method, while antiulcer activity was evaluated using stress and ethanol models, Omeprazole (200 mg/ml) was the reference drug. Phytochemical analysis of the crude extract was carried out using standard methods. **Results:** The crude extract is non-toxic at a below 5000 mg/kg dose. Preliminary phytochemical tests showed the presence of alkaloids, Saponins, tannins, flavonoids, carbohydrates, proteins, terpenoids etc. There was no significant difference between the activity of the standard drug (omeprazole 20mg/kg) and the crude extract (500 mg/kg) at ($P \leq 0.05$). **Conclusion:** *A. africanus* has ulcer prevention activity and its use in the treatment of ulcer in ethnomedicine is justified.

Keywords: Pharmacognostic evaluation, Anti-Ulcer, *Allophylus Africanus*

INTRODUCTION

"The use of herbs as medicine is the oldest form of healthcare known to humanity and has been used in all cultures throughout history. Early humans recognized their dependence on nature for a healthy life, and since that time, humanity has depended on the diversity of plant resources for food, clothing, shelter, and medicine to cure myriads of ailments" (Babu *et al.*, 2017). "However, man's remedies for ailments or health care delivery before this century depended solely on organized crude drugs from medicinal plants prepared from various parts of the plant, such as roots, leaves, seeds, fruits and bark" (Jain, 1991; Jain, 1999).

Ulcers are caused as a result of an imbalance between aggressive and defensive factors. Ulcers are crater-like sores (generally $\frac{1}{4}$ to $\frac{3}{4}$ inch in diameter, but sometimes 1-2 inches in diameter) that form in the lining of the stomach (called gastric ulcers), just below the stomach at the beginning of the small intestine in the duodenum (called duodenal ulcers) or less commonly in the esophagus (called esophageal ulcers).

"It can also be termed as an open sore of the skin or mucus membrane, often characterized by the sloughing of inflamed dead tissues" (Chan and Graham, 2004). "Ulcers are lesions on the skin's surface or a mucous membrane characterized by a superficial tissue loss. They are most common on the skin of the lower extremities and in the gastrointestinal tract, although they may be encountered at almost any site" (Vishnoi *et al.*, 2017). "Many types of ulcers exist such as mouth ulcers, peptic ulcers, esophageal ulcer, and genital ulcer, of which peptic ulcer is the most common in society. Peptic ulcers are erosion of the lining of the stomach or the duodenum. They may further be

classified based on the site of ulceration: gastric ulcer and duodenal ulcer" (Vishnoi *et al.*, 2017).

"*Allophylus africanus* is a species of the genus *Allophylus* of the family Sapindaceae. It is commonly known as African false current, ebe/ukpe (esan tribe in Edo state), akanro, akaraesu (in Yoruba), akaito (in Igbo) and karki (in Hausa). It is a shrub with small and creamy-yellow flowers" (Burkill, 1985). "The fruit is fleshy, red to black when ripe (Burkill, 1985). *Allophylus africanus* mostly grows in riverine thicket, open wood land and forest edges, often associated with mounds, at 960-1540m. Its flowering time is usually from December to March. It is widely distributed throughout tropical Africa, extending to the Eastern Cape South Africa" (Burkill, 1985). *Allophylus africanus* has been reported to be used not just for medicinal purposes but also as food and horticulture (Burkill, 1985). "In ethnomedicine, the leaves are used to treat various ailments such as arthritis, rheumatism, gout, haemorrhoids, dysentery, venereal diseases and malnutrition" (Burkill, 1985).

Roots and twig are used as chewing stick for dental and oral healthcare and diarrhoea treatment. *Allophylus africanus* is reported to demonstrate biological activities such as antibacterial, anti-oxidant (Sofidiya *et al.*, 2012) and anti-malarial activity (Oladosu *et al.*, 2013). "Some chemical components reported from *Allophylus africanus* leaves are tannins, saponins, flavonoids and carbohydrates" (Oladosu *et al.*, 2013). "The boiled bark, root and leaves are used in aches, fever and rheumatic pains" (Sofidiya *et al.*, 2007). Twigs and root are chewed for teeth cleaning, toothache and diarrhea in Edo State, Nigeria (Idu *et al.*, 2009). In Senegal, the leaves of *Allophylus africanus* are used in arthritis management, and eye treatment, as febrifuges, in malnutrition, gout, venereal diseases and insanity. Leaf and root are used in dysentery, menstrual cycle disorders, dropsy and edema. The root is used as a lactation stimulant, abortifacients and ecboics, while the fruit is used in heart conditions, haemorrhoids and eaten as food.

The decoction of leaves and powder of roots are used as an appetite stimulant. In Obollo-Afor in Udenu local government of Enugu State, the leaf of *Allophylus africanus* is used in conjunction with *Uvaria chamea* for the management of stomach ulcers.

MATERIALS AND METHODS

Collection, Identification and Preparation of the Plant Material

The leaves of *Allophyllus africanus* (Sapindaceae) were collected from Onuiyi, Nsukka, Enugu State, Nigeria. The plant material was identified following the taxonomic keys illustrated in the Flora of West Tropical Africa Vol. II (Hutchinson and Dalziel, 1968) and other relevant literature and authenticated by Mr Felix Nwafor, a plant taxonomist in the Department of Pharmacognosy and Environmental Medicines, University of Nigeria, Nsukka. The herbarium sample was deposited in the Herbarium of the same Department, and voucher number (PCG/UNN/0112) was assigned. The plant material was dried under shade at room temperature. The properly dried leaves were ground to powdered form using a local hand mill, sieved and stored in an air-tight container until use.

Extraction Procedure

A 1000 g of the powdered leaf was extracted by maceration in 7.5 L of methanol for 48 h with intermittent agitations. The leaf powder was exhaustively extracted with repeated washings with fresh portions of methanol. The methanol extract (ME) was recovered after evaporation of the filtrate *in vacuum* at 40 °C using a rotary evaporator. The methanol extract (ME) was fractionated using n-hexane, ethylacetate and methanol. A 200 g each of the crude extracts was initially dissolved in methanol: water (80:20) v/v mixture and sequentially extracted with solvents of increasing polarity starting with n-hexane, followed by ethyl acetate, methanol as prescribed by Harborne (1998). The fractions

were concentrated using a rotary evaporator. The dried fractions were weighed and stored properly.

Preliminary Phytochemical Screening

Preliminary phytochemical tests for detecting various secondary metabolites present in both crude extracts were carried out using standard methods. These secondary metabolites include alkaloids—saponins, tannins, flavonoids, carbohydrates, proteins, terpenoids etc.

Experimental Animals

The experimental animals (adult mice and Swiss albino rats) were acquired from the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka. Adult mice weighing 24g - 36 g were used to determine the lethal dose of the plant drug. Adult Swiss albino rats (100g – 300 g), also of either sex, were used for ulcer prevention studies. They were acclimatized to laboratory conditions for 14 days before they were used for the experiments.

Acute Toxicity Studies (LD₅₀)

The acute toxicity test was carried out using Lorke's method (Lorke, 1983) and Duffus models (Duffus, 1993). The administration was oral, using a feeding needle (Hassan *et al.*, 2007). For each crude extract, nine mice were divided into three groups of three each. Each animal group was administered different doses (10, 100 and 1000mg/kg) of the test drug. The animals were observed for 24 hours to monitor their behavior and mortality.

A second phase involved using another nine mice, which were distributed into three groups of three animals each. The animals were administered higher doses (1600, 2900 and 5000 mg/kg) of test substance (s) and then observed for 24 hours for behavior as well as mortality.

The formula calculated the LD₅₀:

$$LD_{50} = (D_0 \times D_{100})$$

D₀ = Highest dose that gave no mortality,

D^{100} = Lowest dose that produced mortality.

Ulcer Protective Test.

Thirty adult albino rats were distributed randomly into five groups of 6 each and deprived of food for 24 hours, but the water was allowed ad libitum. The weights of rats were taken. A 0.5 ml/animal physiological saline was administered orally (Mukherjee, 2002). After 30 minutes, the plant extract (150 mg/ml) was administered in doses of 250mg/kg and 500 mg/kg to groups 1 and 2, respectively. Omeprazole (20 mg/ml) was given as the reference drug to group 3 at 250 mg/kg. Distilled water was administered to the 4th group as a negative control. Acute gastric mucosal lesions were induced in different groups of rats using absolute ethanol (Sigma – Aldrich, Germany) administered at a dosage of 2.5 ml/kg, bd and the animals were killed with ether (Sigma- Aldrich, Germany) after 1 hour.

The stomachs were removed, washed with water and opened along the greater curvature (Aguwa and Ramanujam, 1984). Their inner surface was examined, the gastric lesions were quantified, the mean ulcerative index and percentage inhibition was calculated as Okabe et al. (1976) described,. Then, the procedure was repeated using the fractions.

Statistical Analysis

Data obtained were analysed using Microsoft Excel (2010) and IBM SPSS Statistics Version 20. Analysis of Variance (ANOVA) was used to test for significant differences at $P \leq 0.05$. In contrast, Duncan's multiple range test (DMRT) was used to compare the means of the treatment groups with the control for both the antimicrobial and anti-ulcer tests.

RESULTS.

Table 1: Result of the phytochemical screening of the crude extract of *A. africanus* leaves

Constituent	Inference
Flavonoids	+
Alkaloids	+
Saponins	+
Tannins	+
Hydrolysis test for glycosides	+
Steroids	+
Terpenoids	+

Key: + means Present

Table 2. Result of acute toxicity test on the crude extract

Dose	No of death	Inference
10	-	No mortality
100	-	No mortality
1000	-	No mortality
1600	-	No mortality
2900	-	No mortality
5000	-	No mortality

Table 3: Result of peptic ulcer inhibition of crude extract and fractions of the leaf of *A. africanus* on an ethanol-induced ulcer in rats.

TREATMENT	TOTAL ULCER SCORE		ULCER INHIBITION		PERCENTAGE ULCER INHIBITION (%)	
	250 mg/kg	500 mg/kg	250 mg/kg	500 mg/kg	250 mg/kg	500 mg/kg
The crude extract (ME)	10.40 ± 1.72 ^{cd}	8.40 ± 1.57 ^d	21.40 ± 2.80 ^{ab}	23.40 ± 1.50 ^a	52.20 ± 10.31 ^{bc}	73.80 ± 4.69 ^b
MF	6.00 ± 0.95 ^d	6.60 ± 0.93 ^d	25.80 ± 1.59 ^a	25.20 ± 1.69 ^a	81.06 ± 3.03 ^a	79.08 ± 3.18 ^a
EAF	23.40 ± 2.73 ^b	16.00 ± 2.47 ^c	8.40 ± 2.54 ^c	15.60 ± 3.09 ^b	26.41 ± 8.26 ^d	48.21 ± 8.56 ^c
NHF	28.00 ± 2.76 ^{ab}	16.20 ± 3.80 ^c	4.00 ± 1.70 ^d	15.60 ± 3.09 ^b	12.84 ± 5.50 ^{de}	50.06 ± 10.33 ^c
Drug	11.20 ± 1.39 ^{cd}		20.60 ± 1.12 ^{ab}		64.63 ± 3.96 ^{bc}	
Control (Ethanol)	31.80 ± 1.39 ^a		0.00 ± 0.00 ^d		0.00 ± 0.00 ^e	

Means with different letters as superscripts along the columns and across rows are significantly different at $P \leq 0.05$

This means that same letters as superscripts along the columns and across rows are NOT significantly different at $P \leq 0.05$

KEY:

MF: Methanol fraction
 EAF: Ethylacetate fraction
 NHF: n-Hexane fraction

Table 4: Result of peptic ulcer inhibition of crude extract and fractions of the leaf of *A. africanus* on a stress-induced ulcer in rats

TREATMENT	TOTAL ULCER SCORE		ULCER INHIBITION		PERCENTAGE ULCER INHIBITION (%)	
	250 mg/kg	500 mg/kg	250 mg/kg	500 mg/kg	250 mg/kg	500 mg/kg
The crude extract (ME)	4.50 ± 2.09 ^b	0.20 ± 0.20 ^b	15.70 ± 3.37 ^a	20.00 ± 3.94 ^a	78.96 ± 10.43 ^a	98.82 ± 1.18 ^a
MF	0.40 ± 0.04 ^b	0.60 ± 0.60 ^b	19.80 ± 4.00 ^a	19.60 ± 4.13 ^a	97.65 ± 2.35 ^a	96.00 ± 4.00 ^a
EAF	0.40 ± 0.40 ^b	3.00 ± 3.00 ^b	19.80 ± 3.84 ^a	17.20 ± 5.38 ^a	98.26 ± 1.74 ^a	82.35 ± 17.65 ^a
NHF	3.50 ± 2.62 ^b	0.00 ± 0.00 ^b	16.70 ± 4.85 ^a	20.20 ± 3.89 ^a	79.65 ± 17.56 ^a	100.00 ± 0.00 ^a
Drug	0.00 ± 0.00 ^b		20.20 ± 3.89 ^a		100.00 ± 0.00 ^a	
Control (Ethanol)	20.20 ± 3.89 ^a		0.00 ± 0.00 ^b		0.00 ± 0.00 ^b	

Means with different letters as superscripts along the columns and across rows are significantly different at P ≤ 0.05

This means with same letters as superscripts along the columns and across rows are NOT significantly different at P ≤ 0.05

KEY:

MEF: Methanol fraction
EAF: Ethylacetate fraction
NHF: n-Hexane fraction

UNDER PEER REVIEW

Table 5. Results showing ulcer inhibition effect

Result of peptic ulcer inhibition of crude extract and fractions of the leaf of *A. africanus* on ethanol-induced ulcer in rats.

TREATMENT	TOTAL ULCER SCORE		ULCER INHIBITION		PERCENTAGE ULCER INHIBITION (%)	
	250 mg/kg	500 mg/kg	250 mg/kg	500 mg/kg	250 mg/kg	500 mg/kg
Crude extract (ME)	10.40 ± 1.72 ^{cd}	8.40 ± 1.57 ^d	21.40 ± 2.80 ^{ab}	23.40 ± 1.50 ^a	52.20 ± 10.31 ^{bc}	73.80 ± 4.69 ^b
MF	6.00 ± 0.95 ^d	6.60 ± 0.93 ^d	25.80 ± 1.59 ^a	25.20 ± 1.69 ^a	81.06 ± 3.03 ^a	79.08 ± 3.18 ^a
EAF	23.40 ± 2.73 ^b	16.00 ± 2.47 ^c	8.40 ± 2.54 ^c	15.60 ± 3.09 ^b	26.41 ± 8.26 ^d	48.21 ± 8.56 ^c
NHF	28.00 ± 2.76 ^{ab}	16.20 ± 3.80 ^c	4.00 ± 1.70 ^d	15.60 ± 3.09 ^b	12.84 ± 5.50 ^{dc}	50.06 ± 10.33 ^c
Drug	11.20 ± 1.39 ^{cd}		20.60 ± 1.12 ^{ab}		64.63 ± 3.96 ^{bc}	
Control (Ethanol)	31.80 ± 1.39 ^a		0.00 ± 0.00 ^d		0.00 ± 0.00 ^e	

Means with different letters as superscripts along the columns and across rows are significantly different at $P \leq 0.05$
 Means with same letters as superscripts along the columns and across rows are NOT significantly different at $P \leq 0.05$
 KEY: MF: Methanol fraction, EAF: Ethylacetate fraction, NHF: n-Hexane fraction

Result of peptic ulcer inhibition of crude extract and fractions of the leaf of *A. africanus* on stress-induced ulcer in rats

TREATMENT	TOTAL ULCER SCORE		ULCER INHIBITION		PERCENTAGE ULCER INHIBITION (%)	
	250 mg/kg	500 mg/kg	250 mg/kg	500 mg/kg	250 mg/kg	500 mg/kg
Crude extract (ME)	4.50 ± 2.09 ^b	0.20 ± 0.20 ^b	15.70 ± 3.37 ^a	20.00 ± 3.94 ^a	78.96 ± 10.43 ^a	98.82 ± 1.18 ^a
MF	0.40 ± 0.04 ^b	0.60 ± 0.60 ^b	19.80 ± 4.00 ^a	19.60 ± 4.13 ^a	97.65 ± 2.35 ^a	96.00 ± 4.00 ^a
EAF	0.40 ± 0.40 ^b	3.00 ± 3.00 ^b	19.80 ± 3.84 ^a	17.20 ± 5.38 ^a	98.26 ± 1.74 ^a	82.35 ± 17.65 ^a
NHF	3.50 ± 2.62 ^b	0.00 ± 0.00 ^b	16.70 ± 4.85 ^a	20.20 ± 3.89 ^a	79.65 ± 17.56 ^a	100.00 ± 0.00 ^a
Drug	0.00 ± 0.00 ^b		20.20 ± 3.89 ^a		100.00 ± 0.00 ^a	
Control (Ethanol)	20.20 ± 3.89 ^a		0.00 ± 0.00 ^b		0.00 ± 0.00 ^b	

Means with different letters as superscripts along the columns and across rows are significantly different at $P \leq 0.05$
 Means with same letters as superscripts along the columns and across rows are NOT significantly different at $P \leq 0.05$
 KEY: MEF: Methanol fraction, EAF: Ethylacetate fraction, NHF: n-Hexane fraction

Discussion

This study was undertaken to study the anti-ulcer potentials of the crude extract and fractions of the leaf of *A. africanus*. The qualitative

phytochemical screening of the crude drug sample showed that most of the tested chemicals were present as shown in Table 1. The result showed that the leaves of *A. africanus* are rich in bioactive compounds, while the differences observed in their occurrence could be attributed to the fact that different solvents vary in their extractability based on the nature of the phytochemicals present. It could be suggested, based on this result, that the sample contained more polar compounds.

These phytochemicals are known to be of various therapeutic importance, and their presence supports the ethnomedicinal uses and pharmacological activities previously reported for this species (Chavan and Gaikwad, 2016). For example, alkaloids have been accorded multiple biological activities, including antimicrobial, anti-malarial, anti-inflammatory and antihyperglycemic activities. Conversely, flavonoids are known for their antioxidant, anti-cancer, anti-allergic, anti-inflammatory and antimicrobial activities (Arya and Patni, 2013). In addition, tannins have anti-tumor, anti-viral, wound healing and anti-parasitic activities, while steroids are often associated with sex hormones (Akaneme, 2008; Arya and Patni, 2013; Nweze and Nwafor, 2014).

The acute toxicity study of the crude extract of the leaves of *A. africanus* at the limit dose of 5000 mg/kg indicated that no mortality was observed in all test animals during the study period (Table 2). Furthermore, no overt behavioral or physical signs of toxicity were discerned at this dose. The observation of no sign of morbidity as well as mortality at this high dose led to the inference that the LD₅₀ of the drug sample could be greater than 5000 mg/kg and thus is relatively safe for consumption and in herbal formulations.

This study revealed a significant anti-ulcer effect of the crude extract and fractions from the leaves of *A. africanus* in experimental models of gastric lesion induced ethanol and lesions stress. ethanol (at 250 mg/kg)

and stress produced visible gastric ulcers in the experimental rats (Tables 3 and 4, respectively).

“However, ethanol caused more severe gastric mucosal ulceration. This could be either by a direct effect of the ethanol solution on the gastric epithelium or indirectly by the release of vasoactive products from the mast cells, resulting in the release of mediators such as histamine” (Goulart *et al.*, 2005). Endogenous histamine formation and its release from mast cells in the gastric mucosa also have been implicated in the pathogenesis of gastric ulcers produced by acute stress. Levine and Senay (1968) further showed that “stress increases histidine decarboxylase activity in the gastric mucosa and that the degree of increase correlated positively with the number and severity of lesions”. For the ethanol-induced ulcer, the crude extract (at 250 mg/kg) produced a comparable antiulcer inhibition effect as the control drug but had higher activity than the control drug at 500 mg/kg. Methanol fraction (at both 250 mg/kg and 500 mg/kg) had a significantly ($P \leq 0.05$) higher ulcer inhibition effect than the control and other fractions (Tables 4 and 5). This result agrees with Goulart *et al.* (2005) who reported the anti-ulcer potentials of aqueous ethanolic extract from *Kielmeyera coriacea* Mart. (family Calophyllaceae) on ethanol-induced gastric ulceration in rats. Mesia-Vela (2007) also documented that the aqueous extract of the aerial parts of *Scoparia dulcis* L. (family Plantaginaceae) reduced gastric hypersecretion and ulcer in rodents. Other plants with ulcer inhibition potentials include *Amomum subulatum* Roxb (family Zingiberaceae), *S. dulcis*, *Jasminum grandiflorum* Linn (family Oleaceae), *Davilla rugosa* Poir (family Dilleniaceae), *K. coriacea*, *Larrea divaricate* Cav. (family Zygophyllaceae), *Qualer grandiflora* Mart. (family Vochysiaceae), *Mammea Americana* Linn. (family Guttiferae) *A. occidentale*, *O. sanctum*, *A. indica* (Kumar *et al.*, 2011).

CONCLUSION

The drug sample exhibited impressive ulcer inhibition activities comparable to commercial antibiotics. Furthermore, the LD₅₀ was higher than 5000 mg/kg and is considered safe for consumption and local healthcare delivery. These facts have justified the use of *A. africanus* for local healthcare management in Nigeria. However, further works are recommended to explore other pharmacological actions inherent in the plant. Isolate and characterize the chemical compounds responsible for these actions.

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