

Phytoconstituents, Antioxidant Potential and Antiulcer Activity of *Ficus Exasperata*

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<https://doi.org/10.56201/ijccp.vol.12.no6.2026.pg47.56>

Abstract

Medicinal plants continue to play an essential role in global healthcare and drug discovery. According to the world health organization, nearly 80% of the world Population depend on plant-based medicine for primary healthcare. Plants contain numerous bioactive phytochemicals-flavonoids, alkaloids, tannins, saponins and phenols-that promote mucosal protection, inhibit acid secretion and facilitate ulcer healing. This study investigated the phytochemical, antioxidant activity, and antiulcer potential of *ficus exasperata* leaves. The phytochemical constituents were determined by standard methods, the antioxidant activity was ascertained by the DPPH assay, while the antiulcer potential was assessed by an ethanol-gastric induced model in wister rats. Bioactive constituents identified in the methanol extract included alkaloids, flavonoids, glycoside, phenols and tannins. The extract exhibited a total phenolic (29.5mgGAE/g) and flavonoid (122.8mg QE/g) contents, contributing to a strong antioxidant activity in the DPPH assay, with an IC₅₀ of 1.90 µg/mL, compared to 1.30 µg/mL for ascorbic acid. The extract demonstrated significant gastroprotection with the 200mg/kg dose showing the highest ulcer inhibition (44.10%). These finding show that *Ficus exasperata* possesses promising anti-ulcer properties at moderate dose supporting its ethnomedicinal uses in managing gastric ulcers.

Keywords: *Ficus exasperata*, Phytochemical, Ulcer, Antioxidant.

Introduction

Ulcer disease is a major health challenge affecting populations across the globe. Ulcers are lesions that develop on the skin or mucous membranes, typically characterized by the shedding of inflamed and dead tissue (Mukheet *et al.*, 2019). Peptic ulcer in particular, refers to injury of the digestive tract caused by acid peptic thus resulting in mucosal break reaching the submucosa (Jan, 2016). The peptic ulcer comprises of the gastric (found in the stomach) and the duodenum ulcer (found in the duodenum). *Helicobacter pylori* is the primary cause of peptic ulcers which is associated with about 70% and 90% of individual with gastric ulcers

and duodenal ulcers respectively. In addition, factors including alcohol consumption, cocaine, tobacco and amphetamine use, chronic administration of nonsteroidal anti-inflammatory drugs (NSAIDs), fasting, Zollinger–Ellison syndrome and cancer treatment with angiogenesis inhibitors have been reported to induce peptic ulcer. The common symptoms of peptic ulcer include pain in the upper abdomen, a feeling of full stomach, loss of appetite, weight loss, dizziness, breathing problem as well as stool that is black and tarry (Shipa *et al.*, 2022). Proton Pump Inhibitors (PPIs) amongst others are the most commonly used conventional medications in the treatment of peptic ulcer worldwide. However, the cost and associated adverse effects including headache, diarrhea, constipation, abdominal discomfort as well as certain vitamins deficiency have created a need to explore new, less expensive therapeutic approaches with fewer or no side effects (Albarri *et al.*, 2018). Medicinal plant has been an integral resource for traditional medicine for centuries. Medicinal plants have been used by humans for its therapeutic value since the dawn of human civilization to alleviate various health problems such as prevention of cardiovascular diseases, inflammation disorders, or reducing the risk of cancer amongst others (Škrovánková *et al.*, 2012; Mensah *et al.*, 2019). A wide range of different human diseases have been treated by a rich source of bioactive molecules provided by natural products (Riaz *et al.*, 2023). The remarkable number of reports on the therapeutic properties of medicinal plants combined with long-term experience in folk medicine has led to a growing interest in the use of natural products (Tlili and Sarikurku, 2020). *Ficus Exasperata* is a terrestrial afro-tropical shrub or deciduous plant commonly known as sand paper tree owing to the rough surface of the leaves. It is widely spread in West Africa in all kinds of vegetation (Adebayo *et al.*, 2009). It is called “Ewe ipin” in Yoruba, Anwerenwa in Igbo, and Ameme in Edo (Bafor *et al.* 2009). The leaf extract from *F. exasperata* is reported to have diverse uses. For instance, the south-western people of Nigeria use the decoction and the infusion of *F. exasperata* leaves for the management, control, and treatment of hypertension, diabetes mellitus, and certain cardiovascular dysfunction (Odiba *et al.*, 2012). The leaf extract of *F. exasperata* is also taken to suppress stomach ache, treat peptic ulcer, and as an antidote to poison. On account of the promising attributes of *F. exasperata* particularly in alleviating stomach ache-related disorders and promoting wound healing, the present study is thus aimed at phytochemical screening and antioxidant activity of the methanol extract in Wistar rat.

Materials and Methods

Sample Collection.

Fresh leaves of *Ficus exasperata* were collected from their natural habitat in Evhuabogun community Ikpoba-Okha Local Government Area, Edo State, Nigeria. The plant was identified and authenticated as *F. exasperata*, at the Department of Plant Biology and Biotechnology, University of Benin, Benin City, where a voucher number of UBH-F319 was deposited.

Plant preparation and extraction

The leaves of the plant were air-dried at ambient temperature ($30 \pm 2^\circ\text{C}$) for 28 days. The dried leaves were pulverized using an electric blender into fine powder after which a total of 500g of the powdered leaf was macerated in 500 ml of methanol at room temperature and allowed to stand for 72 hours. The extract was concentrated in rotary evaporator at 40°C and dried in a vacuum oven.

Extract characterization

Phytochemical Screening

The crude extract of *F. exasperata* leaves were screened for the presence of secondary metabolites including; alkaloids, saponins, cardiac glycosides, flavonoids, terpenoids, steroids, phenols, and tannins using standard methods (Tiwari *et al.*, 2011; Zohra *et al.*, 2012).

Determination of Polyphenolic Content

Total Phenol

Total phenol content in the extracts was determined by the method previously described by Kim *et al.* (2003). The extract solution (0.5 mL) of concentration 1 mg/mL was added to 4.5 mL of distilled water, and 0.5 mL of Folin - Ciocalteu's reagent (previously diluted with water 1: 10, v/v) was added to the solution. After mixing, they were maintained at room temperature for 5 minutes followed by the addition of 5 mL of 7% sodium carbonate and 2 mL of distilled water. After mixing the samples, the samples were incubated at room temperature for 30 minutes. The absorbance was measured by a spectrophotometer at 750nm. The total phenolic content was expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE/g extract). The standard curve was prepared using gallic acid in six different concentrations (12.5, 25, 50, 75, 100 and 150 mg/L).

Total Flavonoid

Total flavonoid content was estimated using the method previously described by Ebrahimzadeh *et al.* (2008). Briefly, 0.5 mL of extract sample (1 mg/mL) was mixed with 1.5 mL of methanol and then, 0.1 mL of 10 % aluminum chloride was added, followed by 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water. The mixture was incubated at room temperature for 30 minutes. The absorbance was measured by a spectrophotometer at 415 nm. The results were expressed as milligrams quercetin equivalent per gram of extract (mg QE/g extract). The standard curve was prepared by quercetin in six different concentrations (12.5, 25, 50, 75, 100 and 150 mg/L).

Determination of antioxidant activity

(DPPH Radical Scavaging Assay:- This was done on the crude extract of *Ficus exasperata* by the method described by Jane *et al.*, 2008. A solution of 0.1mM DPPH in methanol was prepared and 1.0mg/mL of this solution was mixed with 3.0mL of extract in methanol containing 0.001-0.2mg/mL of the extract. The reaction mixture was mixed thoroughly and left in the dark at room temperature for 30mins. A spectrophotometer was used to measure the absorbance at 517nm. Ascorbic acid was used as a reference standard. The scavaging ability of the extract was calculated using the formula.

DDPH radical scavaging activity% = $\{A_o - A_t / A_o\} \times 100$ where A_o = Absorbance of DPPH radical + methanol

A_t = Absorbance of DPPH radical + sample extract standard

Experimental design

Animals

Twenty-five (25) Wistar rats of either sex weighing between 80 - 250 g were obtained and kept in the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Edo state, Nigeria. They were allowed to acclimatize for a period of 2 weeks and provided with a standard pellet feed and while maintaining high sanitary and normal lighting conditions. Animal care followed international OECD guidelines (2001), with ethical approval granted by the Faculty's Animal Use and Ethics Committee.

Ethical approval was obtained from the animal use and ethics committee of the Faculty of Pharmacy, University of Benin

Ethanol induced ulcer

The experiment followed the method of Noor *et al.* (2006) with slight modifications. After 48 hours of fasting, Wistar rats (n = 25) were divided into five groups (n = 5 per group): three groups received 100, 200, and 400 mg/kg of *Ficus exasperata* methanol extract, one received Omeprazole (30 mg/kg, positive control), and another received 1 mL distilled water (negative control). All treatments were given orally, followed by 1 mL of ethanol 60 minutes later to induce ulcers. After 30 minutes, the animals were sacrificed using chloroform. Stomachs were excised, opened along the greater curvature, rinsed with saline, and examined. Ulcer areas were measured with a ruler, and lesions counted. The ulcer areas (UA) was determined as the total sum of gastric lesion for each stomach in the group. The percentage protection was calculated according to the formula;

$$\text{Percentage protection} = \frac{(UA_{\text{control}} - UA_{\text{pretected group}})}{UA_{\text{control}}} \times 100$$

Results and Discussion

Qualitative phytochemical constituents

Table 1: Phytochemical screening of *F. exasperata*

Phytochemical	Result
Alkaloids	+
Anthraquinones	-
Carbohydrate	+
Flavonoids	+
Glycosides	+
Phenolic	+
Phytosterols	-
Protein	-
Reducing sugars	+
Saponins	-
Tannins	+

Phytochemical screening was used to evaluate the constituents of the plant extracts, and their predominance, along with the search for bioactive constituents that may be helpful in the production of therapeutic drugs (Silva *et al.*, 2016). In this current study, the phytochemical tests carried out on the methanolic extract of the leave of *F. exasperata* revealed the presence of alkaloids, flavonoids, carbohydrates, and cardiac glycosides, tannins, phenols and reducing sugars. However, anthraquinones and saponins were absent. The therapeutic potential of *F. exasperata* may be attributed to the presence of these phytochemicals. Flavonoids are known to combat free radicals to reduce oxidative stress in the body while also regulating cellular activity for effective metabolism (Ullah *et al.*, 2020). Tannins possess antiviral, antibacterial, and antitumor activity (Bhattacharya *et al.*, 2016). Alkaloids are core framework in many pharmaceuticals and are documented to be bioactive agents used as anti-inflammatory substances and activities (Heinrich *et al.*, 2021). Cardiac glycosides are naturally occurring phytochemical with potential for inhibition of Na⁺/K⁺-ATPase and treatment of congestive heart failure (Škubník *et al.*, 2021).

Total Phenol and Flavonoid

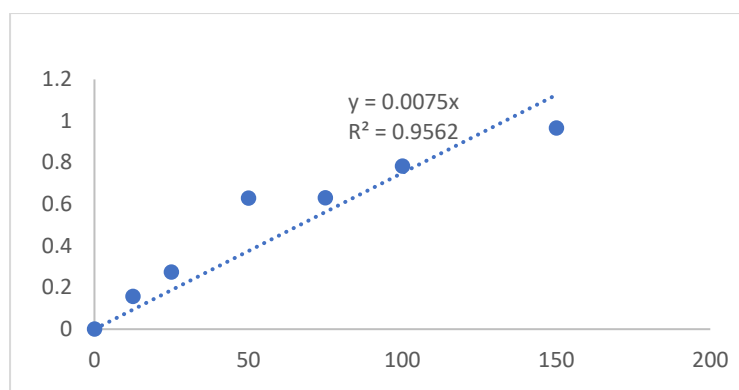


Figure 1: Gallic acid standard calibration curve for the quantification of the total phenolic content.

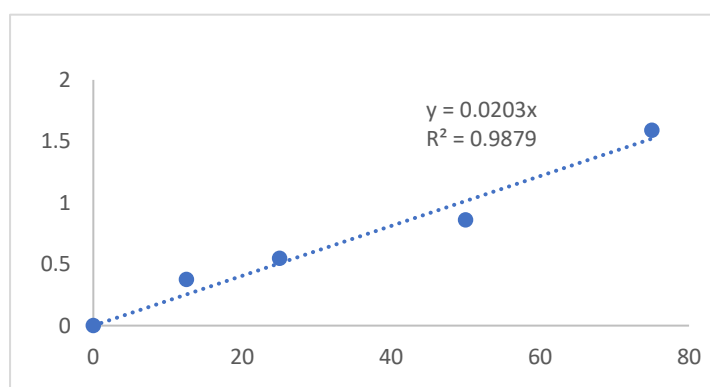


Figure 2: Quercetin standard calibration curve for the quantification of the total flavonoid content.

Table 4: Quantification analysis of *F. exasperata*

Phytochemical	Observed values
Total Phenolic Content (mg GAE/g extract)	29.5 ± 2.09
Total Flavonoid Content (µg QE/g extract)	122.8 ± 15.23

The total phenolic content of *F. exasperata* was calculated using a linear equation for gallic acid standard ($y = 0.0075x$; $R^2 = 0.9562$) (Figure 1), and the total flavonoid content of *F. exasperata* was calculated using a linear equation for quercetin standard ($y = 0.0203x$; $R^2 = 0.9879$) (Figure 2). Using the equations obtained from the calibration curves, the total phenolic content and total flavonoid content of *F. exasperata* were 67.77 ± 2.09 mg gallic acid equivalent/g dry weight and 283.50 ± 15.23 mg quercetin equivalent/g dry weight, respectively as presented in Table 4. The phenolic and flavonoid compounds are naturally occurring compounds with substantial antioxidant capacity that can be found in various parts of plants. Phenolic and flavonoid compounds neutralize reactive oxygen species by forming stable phenoxyl radicals, thereby interrupting oxidative chain reactions and protecting the body from oxidative damage. Their antioxidant properties contribute to cardio-protective, anti-cancer, anti-diabetic, anti-aging, and neuro-protective effects (Pandey and Rizvi, 2009).

DPPH Radical Scavenging Activity

The antioxidant activity of *Ficus exasperata* was evaluated through its ability to reduce DPPH radicals. The interaction between antioxidant compounds and DPPH occurs as a result of hydrogen atom or electron transfer, converting DPPH into 1,1-diphenyl-2-picrylhydrazine (Sreelatha and Padma, 2009; Rahman *et al.*, 2015). This reduction is accompanied by a visible color change from deep purple to pale yellow, indicating radical scavenging activity (Akar *et al.*, 2017). The antioxidant activity of the methanol extracts of *F. exasperata* and ascorbic acid against DPPH assay were tested at various concentrations (1 to 200 µg/ ml), and the results presented in Figure 3.

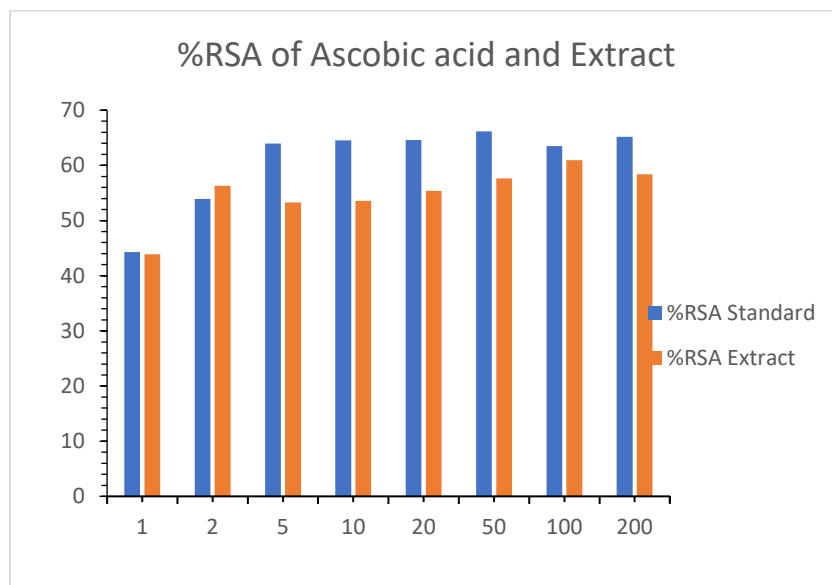


Figure 3: DPPH radical scavenging activity of methanol extract of *F. exasperata*

As shown in Figure 3, the percentage radical scavenging activity (%RSA) of both the standard and the extract increased with increasing concentration, indicating an almost concentration-dependent antioxidant effect. At 1 µg/mL, both the standard and the extract exhibited strong activity, with %RSA values of 44.31% and 43.89%, respectively. This suggest that both the standard and the extract contain efficient radical scavenging components. Across the tested concentrations, the standard consistently exhibited higher %RSA, reaching about 66% at 50 µg/mL, whereas the extract attained about 61% at 100 µg/mL before stabilizing. The IC₅₀ values further supported this difference in potency, with the standard at 1.30 µg/mL and the methanol extract at 1.90 µg/mL. According to Tariq *et al.* (2022), low IC₅₀ values correspond to high antioxidant activity; thus, the standard demonstrated greater efficacy, although *F. exasperata* extract also showed appreciable free radical scavenging capacity. Comparable findings were reported by Trinh *et al.* (2020) for Asteraceae medicinal plants.

Anti-ulcer activity of *Ficus exasperata*

The percentage inhibition of methanol extract of the leaves of *F. exasperata* against the ethanol-induced gastric ulcer in rats is shown in Fig. 4.

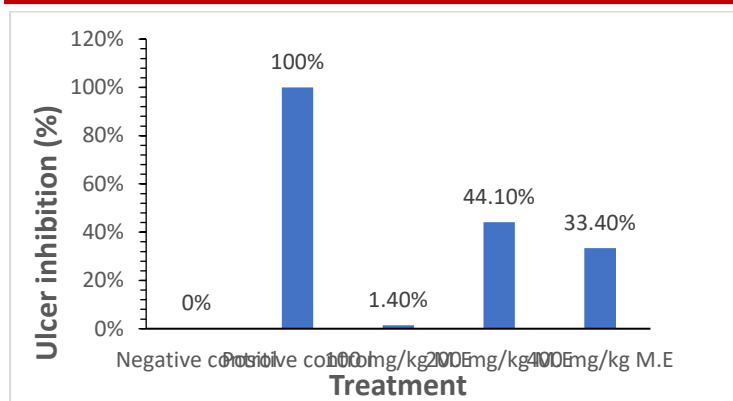


Figure 4: Effect of methanol extract of *F. exasperata* on the degree of protection against ulceration in ethanol ulcerated rats.

Ethanol-induced gastric ulcer is a widely used animal model for studying peptic ulcer disease, as it mimics acute ulcers in humans and helps evaluate the cytoprotective and antioxidant effects of test agents (Shawon and Gautam, 2012; Arab *et al.*, 2015; Hernandez-Munoz *et al.*, 2000; Takagi *et al.*, 1970). Ethanol disrupts the mucosal protective layer, allowing pepsin and hydrochloric acid to damage the gastric membrane (Takagi *et al.*, 1970; Sener *et al.*, 2004). In this study, oral administration of absolute ethanol caused severe gastric mucosal damage in the negative control rats. From the antiulcerogenic test conducted, it was observed that the negative control (water) showed no gastroprotective effect. The positive control (omeprazole) produced complete gastroprotection (100%), confirming its strong protective property and validating the experimental model. Among the extract-treated groups, the 100 mg/kg dose exhibited only minimal gastroprotective effect (1.40%), whereas the 200 mg/kg dose provided maximum gastroprotection effect (44.10%), suggesting optimal activity at this level. However, the effect decreased at 400 mg/kg (33.40%), indicating that higher concentrations may not enhance protection.

Conclusion

The methanol extract of *F. exasperata* showed the presence of various secondary metabolites in the plant which indicates medicinal properties while its carbohydrate content suggests high energy value. The antioxidant potential of the plant compared favourably in the standard ascorbic acid and gastroprotection of the Wistar rat was observed at the moderate dose. These findings corroborate the traditional use of the extract in ulcer treatment.

Conflict of interest

No conflict of interest was declared by the authors.

Acknowledgement: we wish to appreciate the following technical officers for their supports Mr. Amayo and Mr. Clinton.

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