



Evaluation of Ulcer Protective Effects of Methanol Stem Bark Extract and Fractions of *Canarium schweinfurthii* Engl. (*Burseraceae*) on Wistar Rats

Abstract: In Nigeria, native medicinal plants like *Canarium schweinfurthii* are used to treat peptic ulcer disease, yet their safety and efficacy are undetermined. The study evaluated methanol extract and *Canarium Schweinfurthii* fractions on Wistar rats for ulcer protection. Methanol stem bark extract and fractions of *Canarium Schweinfurthii* underwent phytochemical analysis. Acute toxicity was assessed using a modified Lorke's method on healthy Wistar rats (200–240 g). The study involved four test groups: crude extract, n-hexane, ethyl acetate, and butanol fractions, with low (200 mg/kg) and high (400 mg/kg) doses given to groups of five rats (n=5). The antioxidant capacity was evaluated through 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) tests. Ulcer-protective effects of extracts and fractions were assessed using ethanol-induced and aspirin-induced ulcer models at varying doses. Additionally, gastrointestinal motility was analysed through charcoal transit tests to evaluate the inhibitory effects of the extracts. Data were examined with SPSS 27. Tannins, steroids, and alkaloids were absent from phytochemical screening. The crude extract and ethyl acetate fraction contained several phenols, flavonoids, proteins, and carbohydrates. The extract's LD50 exceeded 5000 mg/kg, indicating safety. The most antioxidant fraction was ethyl acetate, followed by butanol. Treatment had no significant ($p > 0.05$) pH alterations. Ethyl acetate and n-hexane fractions provided moderate ulcer protection (45.45%) in ethanol-induced models, but not statistically significant ($p > 0.05$). In aspirin-induced model, butanol fraction (400 mg/kg) showed effects comparable to omeprazole regarding gastric acid volume, total acidity, ulcer scores, and ulcer protection percentage. Omeprazole achieved an ulcer protection percentage of 85.7%, while the butanol (400 mg/kg) fraction provided 74.1%, indicating the protective efficacy of the plant's stem bark fraction. Conclusion. Our findings underscore the safety, abundant phytochemical composition, and ulcer-protective properties of the butanol fraction of *C. schweinfurthii* stem bark, justifying its local use as an alternative in the management of acid-induced ulcers.

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1.Introduction

Peptic ulcers constitute a significant global health issue, impacting millions each year due to causes such as *Helicobacter pylori* infection, extended nonsteroidal anti-inflammatory drug, (NSAID) usage, and stress-related mucosal injury, impacting approximately 10% of the total population historically [1],[2]. In Nigeria and West Africa, traditional medicine is crucial for addressing gastrointestinal problems, with plant extracts providing possible gastroprotective options in light of increasing antibiotic resistance and adverse effects of modern drugs[3]. *C. schweinfurthii* Engl. (*Burseraceae*), referred to as "African elemi" or "incense tree," is a medicinal tree indigenous to tropical African forests. Its stem bark decoctions have been utilised in ethnomedicine for centuries to address gastrointestinal issues, dysentery, ulcers, and inflammatory disorders[4].

In Nigeria and West Africa, traditional medicine predominates for the management of gastrointestinal disorders, utilising plant extracts as gastroprotective alternatives in response to antibiotic resistance and the adverse effects of synthetic drugs[5]. Communities depend on herbal cures for ulcers and associated conditions, as evidenced by ethnobotanical surveys that document plants with antiulcer properties. Previous investigation has proven that some of these medicinal plants protected rats from aspirin-induced ulcerogenesis, delayed intestinal transit, raised pH, and decreased both the volume and acidity of gastric secretions[6].

C. schweinfurthii flourishes in the forests of West and Central Africa, with stem bark decoctions traditionally used to treat ulcers, diarrhoea, pain, and inflammation. Phytochemical screening indicates an abundant presence of tannins, flavonoids, saponins, and terpenoids, compounds associated with gastroprotection through

antioxidant, anti-inflammatory, and mucosal barrier strengthening mechanisms. Moreover, previous research corroborates its antidiabetic, antihypertensive, and antimicrobial properties[7],[8] indicating extensive therapeutic potential.

Scientific anti-ulcer assays, such as ethanol-or aspirin-induced models in Wistar rats, are necessary due to the lack of scientific validation, despite anecdotal claims of ulcer healing. Establishing the anti-ulcer properties of *C. schweinfurthii* could facilitate the development of cost-effective, safe phytomedicines, integrating traditional knowledge with evidence-based pharmacology for Nigeria's economically disadvantaged populations. This study examined methanol stem bark extracts and fractions (n-hexane and ethyl acetate) to isolate active constituents and their effects on ulcer index, gastric pH, mucus production, and Wistar rat gastrointestinal motility. Our findings might lead to clinical trials, phytochemical standardisation, and the policy use of *Burseraceae* species for ulcer treatment.

2.Methods

Plant collection and experimental animal selection

Fresh barks of *C. schweinfurthii* were sourced locally from its natural habitat at Abagana town in Njikoka Local Government of Anambra State, Nigeria. The barks were collected in the month of March, in the morning, between 8 a.m. and 9 a.m. The plant barks were identified and authenticated by a botanist, Dr Chisom F. Iroka of the Department of Botany, Faculty of Biological Sciences, Nnamdi Azikiwe University, Awka, Nigeria, with a voucher number (NAUH-052).



Figure 1: *C. schweinfurthii* Plant (African Elemi) Leaves, Fruits, and Bark

Source: Culled from Chukunda et al. [9]

Healthy Wistar rats of both sexes weighing 200-240 g and 8 weeks old, obtained from the animal house of the Faculty of Pharmaceutical Sciences, Chukwuemeka Odumegwu Ojukwu University, Igbariam Campus, Anambra State, Nigeria, were used for the study. The rats were housed under standardized conditions and fed with pelletized top feed (grower feeds by Top Feeds Nigeria Limited) and water ad libitum. The rat cages underwent regular cleaning. Animals were maintained on a 12-hour light and 12-hour darkness cycle and acclimatized for one week before commencement of the experiment. The animals were handled in compliance with the National Research Council's Guide for the Care and Use of Laboratory Animals [10].

The bark of *C. schweinfurthii* was air-dried at room temperature, away from direct sunlight. After drying, they were pulverised to fine powder using a mechanical grinder. The powdered barks were stored in an airtight container to ensure their preservation for extraction. The extraction was carried out by maceration as described by Erhirhie and Ilodigwe [11]. A total of 1200 g of pulverised plant material was macerated in 3600 ml of methanol for 48 hours at room temperature with continual agitation. At the end of 48 hours, the mixture was filtered using muslin cloth, and the filtrate recovered was concentrated to a paste-like form using a water bath at 50°C. The

concentrated extract was stored at 4°C until needed for phytochemical analysis and evaluation of antioxidant and antiulcer activities. The percentage yield of the extract was determined using the formula:

$$\text{Percentage yield} = \frac{\text{Weight (g) of concentrated extract}}{\text{Weight (g) of powdered plant material}} \times \frac{100}{1}$$

Fractionation of the extracts

The crude extract was fractionated with n-hexane (2.5 L), ethyl acetate (2.5 L) and butanol (2 L) in order of increasing polarity. Approximately 70 g of crude extract was dissolved in 280 ml of distilled water and was added to a 1000 ml separating funnel with 500 ml of n-hexane. It was shaken by inversion and allowed to stand for about 15 minutes until two distinct layers (an n-hexane upper layer and a water lower layer) were obtained. The water layer was subjected to another phase of n-hexane fractionation repeatedly for 4 times. After the n-hexane phase, ethyl acetate and butanol fractionation were done following the same procedure. Various fractions were filtered and concentrated to dryness using a water bath at 50°C. The percentage yield for each fraction was calculated using the formula.

$$\text{Percentage yield} = \frac{\text{Weight (g) of dry fraction}}{\text{Weight (g) of crude extract}} \times \frac{100}{1}$$

Phytochemical Analysis

The whole methanol extract and various fractions were subjected to phytochemical analysis and screened for the presence of proteins, carbohydrates, phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids, and alkaloids using the methods of Sofowora [12], Evans & Trease [13], and Harborne [14], as described by Yadav & Agarwala [15]. The sample was prepared by dissolving 2 g of the extract and each of the fractions in 20 ml of distilled water.

Acute Toxicity Test

The acute toxicity study of the stem bark of *C. schweinfurthii* was carried out using modified Lorke's method [16]. The rats were divided into two phases.

Phase I: Nine animals were randomly divided into three groups of three animals each. Members of each of the three groups administered doses of 10 mg/kg, 100 mg/kg and 1000 mg/kg body weight of the crude extract, respectively. Administration of extracts was done orally with a cannula attached to a graduated syringe. The rats were observed over a period of 24 hours for signs of mortality and toxicity, such as behavioural profile (awareness, irritability, and anxiety), neurologic profile (spontaneous activity, pain response, and gait), autonomic profiles (evacuation and urination), and physical states such as lacrimation, anorexia, shocks, hair erection, drooling, and diarrhoea. In the absence of death in phase 1, phase 2 was initiated.

Phase 2: This phase involved the use of three animals. The animals were distributed into sample groups, with one animal per group. Based on the results of phase 1 tests, three doses, 1600, 2900, and 5000 mg/kg of the extract, were administered to the animals, respectively. The rats were observed over a period of 24 hours for signs of behavioural changes and mortality. The median lethal dose (LD_{50}), representing the dose of the extract that would be lethal to 50% of the population of animals, was calculated using the geometric mean of the highest non-lethal dose and the least lethal dose.

$LD_{50} = D_0 \times D_{100}$; where D_0 = the highest dose that gave no mortality, D_{100} = Lowest dose that produced mortality.

Invitro Antioxidant Assay: DPPH and FRAP Principle of 2,2-diphenyl-1-picrylhydrazyl (DPPH)

the DPPH antioxidant assay evaluates the capacity of substances to neutralize free radicals utilizing DPPH, a persistent purple radical exhibiting absorbance at 517 nm.

The DPPH free radical scavenging activities of the extract and fractions were carried out as described by Erhirhie *et al.* [17]. DPPH solution (0.6 mMol) was freshly prepared using methanol. The reaction mixtures contained 0.25 ml of various fold dilutions of samples. For ascorbic acid, 7.82, 15.63, 31.25, 62.5, 125, 250, 500 and 1000 µg/ml were used. The samples mixed with 0.25 ml of 0.6 mMol DPPH and 2 ml of methanol were incubated in the dark for thirty minutes at room temperature. This procedure was repeated for ascorbic acid. Thereafter, absorbance of the samples was measured at 517 nm using a spectrophotometer. Ascorbic acid was used as a standard. A tube containing 0.25 ml of DPPH solution and 2.25 ml of methanol served as a control. Assays were carried out in triplicate. The DPPH free radical percentage scavenging activities of samples and standard (ascorbic acid) were obtained using the formula below:

$$\text{DPPH scavenging activity} = 100 \times (\text{AC} - \text{AS})/\text{AC}$$

AC = Absorbance of control AS = Absorbance of sample

For ascorbic acid, a graph of percentage inhibition against concentration was plotted, and the concentration that caused 50% inhibition of DPPH radical (IC_{50}) was extrapolated using an equation. Low optical density (absorbance) values indicate high DPPH radical scavenging activity.

The Ferric Reducing Antioxidant Power (FRAP)

assay quantifies the potential of antioxidants to convert ferric ions (Fe^{3+}) into ferrous ions (Fe^{2+}), hence indicating the reducing power in samples such as plant extracts.

The FRAP assay was carried out following the method described by Habibur et al.[18]. Two hundred and fifty microlitres (0.25 ml) of various fold dilutions of samples as well as 7.82, 15.63, 31.25, 62.5, 125, 250, 500 and 1000 $\mu\text{g/ml}$ ascorbic acid were mixed with 0.625 ml of phosphate buffer and 0.625 ml of 1% potassium ferricyanide [$\text{K}_3\text{Fe}(\text{CN})_6$]. The mixtures were heated at 500°C for twenty minutes. Thereafter, 0.625 ml of 10% trichloroacetic acid (TCA) was added, and the mixtures were centrifuged at 3000 rpm for 5 minutes at room temperature. From the upper layer, 0.625 ml was pipetted and mixed with 0.625 ml of distilled water and 0.125 ml of 0.1% (w/v) ferric chloride (FeCl_3) solution. The absorbance of the mixture was measured at 700 nm against air using a spectrophotometer. Ascorbic acid was used as a standard. The tests were performed in triplicate.

The percentage inhibition was calculated using the formula below:

$$\% \text{ Inhibition} = (\text{Absorbance of sample} - \text{Absorbance of blank}) \times 100$$

For ascorbic acid, a graph of percentage inhibition against concentration was plotted, and the effective concentration (EC_{50}) was extrapolated using an equation. High optical density (absorbance) values indicate high FRAP activity

Study Design

Table 1. Study Design on Administration of Extract and Fractions of *C. schweinfurthii*

The experimental design included four test groups (crude extract, n-hexane, ethyl acetate and butanol fractions). The test animals were

given two different doses of the extract and fractions (200 mg and 400 mg). There were also negative and positive control groups. Each group had five animals ($n=5$). The study design can be summarized as follows:

Table 1. Study Design on Administration of Extract and Fractions of *C. schweinfurthii*

Groups	Wistar rats (n=5)	Treatments
1	5	10 ml/kg of 5% Tween 80 solution (negative control)
2	5	20 mg/kg of omeprazole (positive control)
3	5	200 mg/kg of whole methanol extract (WME)
4	5	400 mg/kg of whole methanol extract (WME)
5	5	200 mg/kg of n-hexane fraction.
6	5	400 mg/kg of n-hexane fraction.
7	5	200 mg/kg of ethyl acetate fraction
8	5	400 mg/kg of ethyl acetate fraction
9	5	200 mg/kg of butanol fraction
10	5	400 mg/kg of butanol fraction

Anti-Ulcer Screening Models

The anti-ulcer activity of the stem bark extract and fractions of *C. schweinfurthii* was assessed using two ulcer screening models: the ethanol and aspirin models.

Ethanol-Induced Ulcer Model: Based on the outcome of the acute toxicity test, with LD_{50} above 5000 mg/kg, two doses, 200 and 400

mg/kg, which were expected to be safe doses, were selected for the study similar to Neharkar and Gaikwad [19]. They were fasted for 24 hours with free access to water prior to the experiment, and were grouped and treated as contained in table 1 before ulcer induction. One hour after pre-treatment, an ulcer was induced with absolute ethanol (99%) orally at a dose of 1 ml/200 g. The animals were sacrificed one hour later with the aid of chloroform anaesthesia similar to Deshpande and Balekar [20]. Their stomachs were removed, washed and carefully inspected for ulcer severity using the ratings described by Dashputre and Naikwade [21]. The percentage ulcer protection was calculated as described by Bhajoni et al.[22].

Aspirin-Induced Ulcer Model: similar to ethanol model, the rats were fasted for 24 hours with free access to water prior to the experiment and were grouped and treated as contained in table 1 before ulcer induction. Thirty minutes after pre-treatment, an ulcer was induced with 300 mg/kg of aspirin. Four hours after aspirin administration, the animals were sacrificed with the aid of chloroform anaesthesia similar to Nitin Mahurkar and Sayeed [23]. Their stomachs were removed, washed and carefully inspected for ulcer severity using the rating described by Dashputre and Naikwade [21]. The percentage ulcer protection was calculated as described by Bhajoni et al.[22].

Parameters for the Evaluation of Anti-ulcer activity

1.Macroscopic Evaluation of the Stomach

The stomach was opened along the greater curvature, and the gastric contents were collected into a plain tube and centrifuged. The gastric volume was measured, and the contents were used for the determination of total acidity. The stomach was thereafter rinsed with normal saline to eliminate gastric contents and blood accumulation. The mucosal lesions were

examined using a 10x magnifier lens. The number of ulcers was calculated, and they were scored and given a severity rating as described by Dashputre and Naikwade [21].

2.Determination of pH of gastric juice

The pH of the stomach juice was measured via a pH meter. The pH meter electrode was meticulously inserted into the stomach juices and permitted to stabilise for several seconds, after which the pH of the fluid in the different groups was measured and documented accordingly. To guarantee accurate results, the electrode was cleaned with distilled water before to each test.

3. Determination of total acidity

This was determined by the method described by Dashputre and Naikwade [21]. with little modification. A volume of 1 ml of gastric juice was diluted with 9 ml of distilled water in a 50 ml conical flask, and 40 µl of phenolphthalein indicator was added to the mixture and titrated with 0.005 N NaOH until a permanent pink colour was observed. The volume of 0.005N NaOH consumed was recorded. The total acidity expressed as mEq/L was calculated using the following formula.

Total Acidity = $V_{NaOH} \times N \times 100$ mEq/L, where V is volume and N is normality.

4.Determination of gastric juice volume

The procedure was conducted according to the methodology outlined by Kumar et al., [24]. Each sample's gastric contents were drained into a separate tube, centrifuged for five minutes at 1000 rpm, and the gastric volumes were then recorded. The percentage ulcer protection was calculated similar to Abebaw et al., [25],

Ulcer Protection (%):

$$\frac{\text{Ulcer score of control} - \text{Ulcer score of test} \times 100}{\text{Ulcer score of control}}$$

Effects of extract and fractions on gastrointestinal motility

The animals were fasted for twenty hours prior to the experiment but with unrestricted access to water. The effect of different doses of crude extract and fractions of *C. schweinfurthii* stem bark on gastrointestinal motility was evaluated. The animals were treated orally as assigned in table 1. Thirty minutes post pre-treatment, each animal was administered 0.5 ml of charcoal meal (5% charcoal in 5% tragacanth mucilage) orally. The animals were slaughtered thirty minutes later, and their intestines were extracted and placed on a white sheet of paper. The distances travelled by the charcoal meal from the pyloric area to the caecum were measured using a metre rule, relative to the entire length of the intestine, and expressed as a percentage of the distance moved from the pylorus to the caecum for each specimen similar to Nwafor and Okoye [26].

Statistical Analysis

All experimental data were evaluated utilizing Windows SPSS version 27. The results for the analyzed parameters were expressed as mean $\pm$ standard deviation ($n = 5$). A one-way analysis of variance (ANOVA) was carried out, followed by Tukey's post hoc test. The mean results of the test groups were compared with those of the control group. P-values < 0.05 were considered statistically significant.

3. Results

Yield of Extract and Fractions

The percentage yields of the extract and fractions from *C. schweinfurthii* bark showed a yield of 9.13%w/w for the extract, 23.60% w/w for n-hexane fraction, 10.61%w/w for ethyl acetate fraction and 3.90 %w/w for butanol fraction. n-hexane fraction had the highest yield followed by ethyl acetate and then butanol fraction had the least yield (see Table 2 below).

Table 2: Percentage yield of the Extract and Fractions of *C. schweinfurthii* bark

Sample	Weight before extraction/fractionation	Weight after extraction /fractionation	Yield (%)
Extract	1200	109.5	9.13
n-hexane fraction	70	16.52	23.60
Ethyl acetate fraction	70	7.43	10.61
Butanol fraction	70	2.73	3.90
Water fraction	70	0.25	0.36

Phytochemical Analysis

The phytochemical screening of the crude bark extract and fractions of *C. schweinfurthii* revealed the presence of proteins, carbohydrates, phenols, flavonoids and terpenoids, with the crude extract and ethyl

acetate having higher levels than n-hexane and butanol fractions.

Tannins, steroids and alkaloids were absent in all samples. Saponins were absent in n-hexane fraction, while glycosides were absent in n-hexane and butanol fractions (Table 3)

Table 3: Qualitative phytochemistry of crude bark extract and fractions of *C. schweinfurthii*

S/no	Phytochemical	Test	Crude extract	n-hexane fraction	Ethyl acetate fraction	Butanol fraction
1	Proteins	Millon's test	++	+	++	+
		Ninhydrin test	++	+	++	+
2	Carbohydrates	Iodine test	++	+	+++	+
3	Phenols	Litmus test	++	+	+++	+
		Ferric chloride test	++	+	+++	++
4	Tannins	Geletin test	-	-	-	-
5	Flavonoids	Alkaline reagent test	+++	+	+++	++
		Shinoda test	+++	+	+++	++
6	Saponins	Frothing test	+	-	+	+
7	Glycoside	Keller-kilani test	+	-	++	-
8	Steroids	Liebermann-Burchard's test	-	-	-	-
9	Terpenoids	Salkowski's test	+	+	+	+
10	Alkaloids	Wagner's test	-	-	-	-

Key: “-” (Absent), “+” (Present in trace), “++” (Present in moderate level), “+++” (Present in abundant level).

Acute Toxicity Tests (LD₅₀)

The acute oral toxicity tests as detailed in Table 3 investigated the toxicity of the extract of *C. schweinfurthii*. The oral administration of all studied doses of the extract showed no physical signs of toxicity or mortality in both phases 1 and 2 of the acute toxicity test up to the highest dose of 5000mg/kg used in the study. Thus, the LD₅₀ (lethal dose for 50% of the population) was estimated to be above 5000 mg/kg.

Table 4: Acute toxicity test results of methanol bark extracts of *C. schweinfurthii*

Phase	Dose (mg/kg)	Signs of toxicity	Number of deaths
1	10	Nil	0/3
	100	Nil	0/3
	1000	Nil	0/3
2	1600	Nil	0/1
	2900	Nil	0/1
	5000	Nil	0/1

Comparison of summarised antioxidant results of DPPH scavenging activity versus FRAP activity

The antioxidant results for DPPH showed that ascorbic acid had the lowest concentration,



which caused 50% inhibition and neutralisation of free radicals (IC_{50}) with a value of 109.95 and therefore displayed the highest antioxidant activity. This was followed by the butanol fraction, the ethyl acetate fraction, the n-hexane fraction, and finally the crude extract. Typically, the lower the IC_{50} value of a sample, the greater the antioxidant activity.

The antioxidant results for FRAP showed that ascorbic acid had the lowest effective concentration of antioxidant substance required to reduce half of the ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) (EC_{50}) with a value of 118.55 $\mu g/ml$ and therefore displayed the highest antioxidant activity. This was followed by ethyl acetate (484.84 $\mu g/ml$), then butanol (735.46), crude extract (844.25 $\mu g/ml$) and n-hexane (2792.63). A lower EC_{50} signifies more potent antioxidant activity.

Amongst the four samples, the ethyl acetate fraction has the best antioxidant activity because it has the least EC_{50} for FRAP and the second least IC_{50} for DPPH. This is followed by the butanol fraction, the crude extract and, the least, n-hexane.

The antioxidant activity of the methanolic extracts and fractions showed inhibitory activity against the free radical DPPH and the ferric ion, but the inhibition is weak compared with the standard, ascorbic acid.

In comparison, the FRAP assay exhibited better antioxidant activity than the DPPH assay because the values of the EC_{50} for the extracts and fractions were all lower than the corresponding values for IC_{50} .

Table 5: Summary of antioxidant results

Sample	DPPH Scavenging activity: IC_{50} ($\mu g/ml$)	FRAP activity: EC_{50} ($\mu g/ml$)
Crude extract	4964.16	844.25
n-hexane fraction	3792.22	2792.63

Ethyl acetate fraction	854.06	484.84
Butanol fraction	804.32	735.46
Ascorbic acid	109.95	118.55

Anti-Ulcer Studies

The pH measurement of the samples

The pH of the various samples revealed the vehicle (5% Tween-80) had the highest value. This was followed by omeprazole, n-hexane fraction, butanol fraction, extract and ethyl acetate fraction, which had the least pH. The pH values recorded on the extract and fractions were concentration dependent, as higher concentrations led to lower pH (See Table 6).

Table 6: pH of various samples

Sample	Concentration (mg/ml)	pH
Ethanol	-	5.67
Vehicle: tween-80	-	6.56
Aspirin	300mg/10ml (equivalent to 30 mg/ml)	4.52
Omeprazole	20 mg/10 ml (equivalent to 2 mg/ml)	6.33
Extract: 200 mg/kg	300 mg/15 ml (equivalent to 20 mg/ml)	4.41
Extract: 400 mg/kg	600 mg/15 ml (equivalent to 40 mg/ml)	4.24
n-hexane: 200 mg/kg	300 mg/15 ml (equivalent to 20 mg/ml)	5.35
n-hexane: 400 mg/kg	600 mg/15 ml (equivalent to 40 mg/ml)	5.10
Ethyl acetate fraction: 200 mg/kg	300 mg/15 ml (equivalent to 20 mg/ml)	3.39



Ethyl acetate fraction: 400 mg/kg	600 mg/15 ml (equivalent 40 mg/ml)	3.08
Butanol fraction: 200 mg/kg	300 mg/15 ml (equivalent 20 mg/ml)	4.80
Butanol fraction: 400 mg/kg	600 mg/15 ml (equivalent 40 mg/ml)	4.74

Effects of extract and fractions of *C. schweinfurthii* on ethanol-induced ulcers in rats

Figures 2-5 shows the effects of extract and fractions of *C. schweinfurthii* on ethanol-induced ulcer and its measured parameters in wistar rats.

Ulcer scores: ulcer scores of animals treated with omeprazole were statistically significantly reduced (* $p < 0.05$) when compared to the control group. However, the ulcer scores of the crude extract, n-hexane, ethyl acetate and butanol fractions were reduced when compared to the control group but were not statistically significant ($p > 0.05$).

Ulcer protection: Ulcer protection was highest in ethyl acetate, followed by omeprazole, n-hexane, crude extracts, and butanol fractions.

Gastric volume: The gastric volumes of omeprazole, n-hexane (400 mg/kg), ethyl acetate (400 mg/kg) and butanol (200 mg/kg) were slightly lower than the gastric volume of the control group but were not statistically significant ($p > 0.05$). Gastric volumes of extract, ethyl acetate (200 mg/kg), hexane (200 mg/kg), ethyl acetate (400 mg/kg), and butanol (400 mg/kg) were slightly lower than the gastric volumes of the control but not statistically significant ($p > 0.05$).

Gastric pH: The gastric pH of omeprazole was significantly lower (* $p < 0.05$) than that of the control group. However, the gastric pH of other groups except ethyl acetate (200 mg/kg) was slightly higher than that of the control but not statistically significant ($p > 0.05$).

Total acidity: Omeprazole has the lowest total acidity when compared to the control, but it was not statistically significant ($p > 0.05$). Other groups, except the 400 mg/kg extract, have higher total acidity when compared to the control group but were not statistically significant ($p > 0.05$).

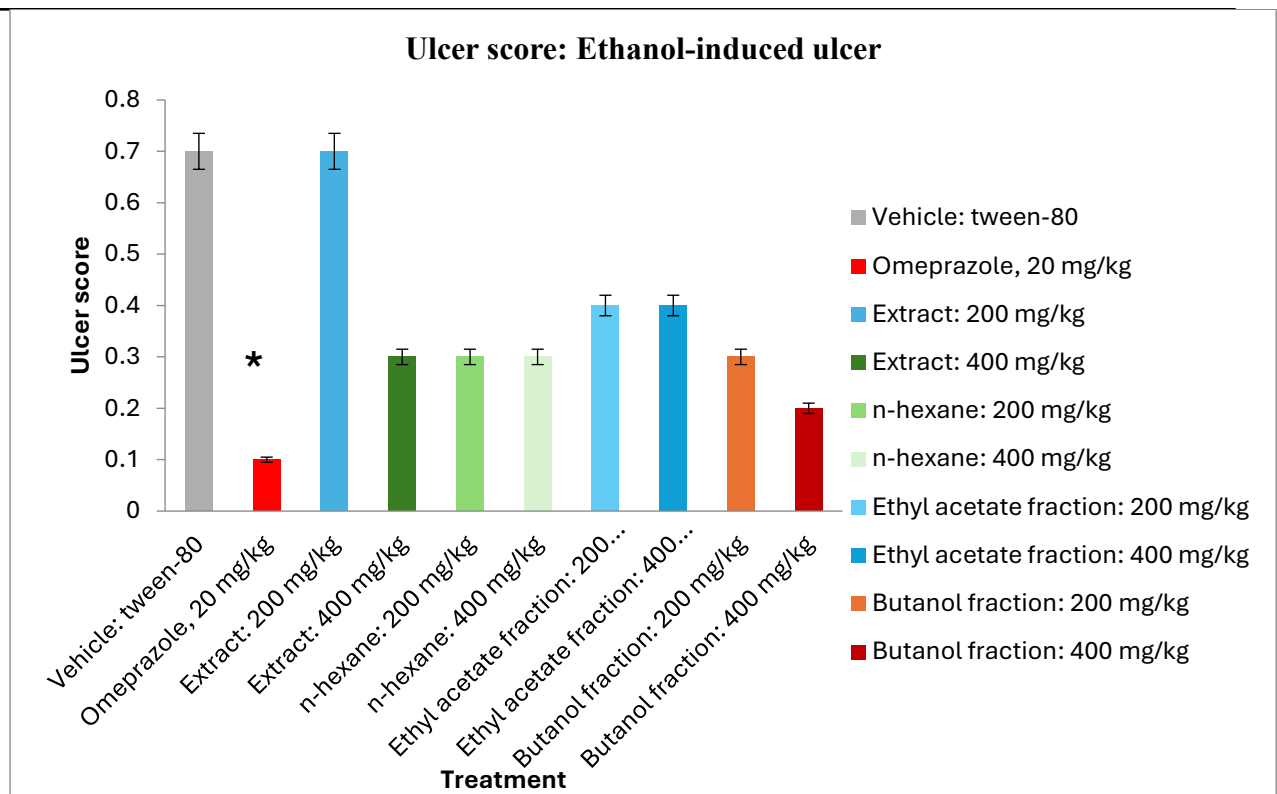


Fig. 2: Effects of extract and fractions of *C. schweinfurthii* on ethanol-induced ulcer in rats. Values are presented as mean ± Standard deviation, n =5. *P<0.05: Statistically significantly different from control

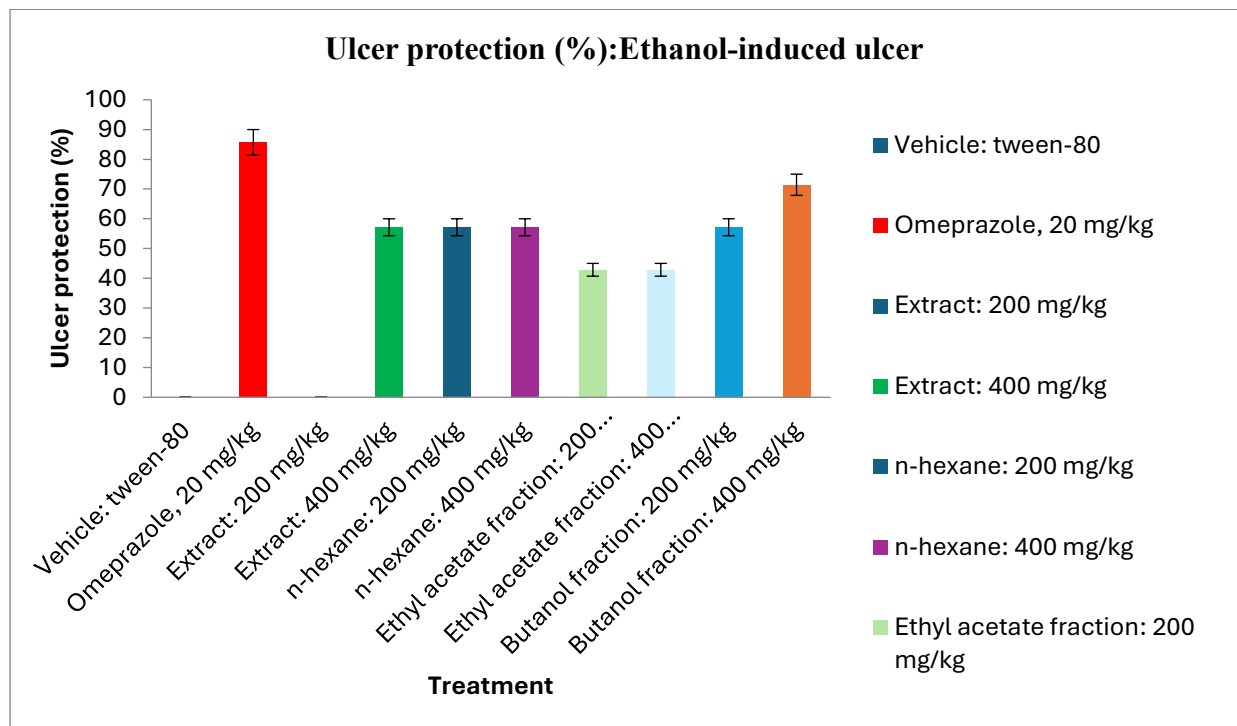


Fig. 3: Effects of extract and fractions of *C. schweinfurthii* on ethanol-induced ulcer in rats.

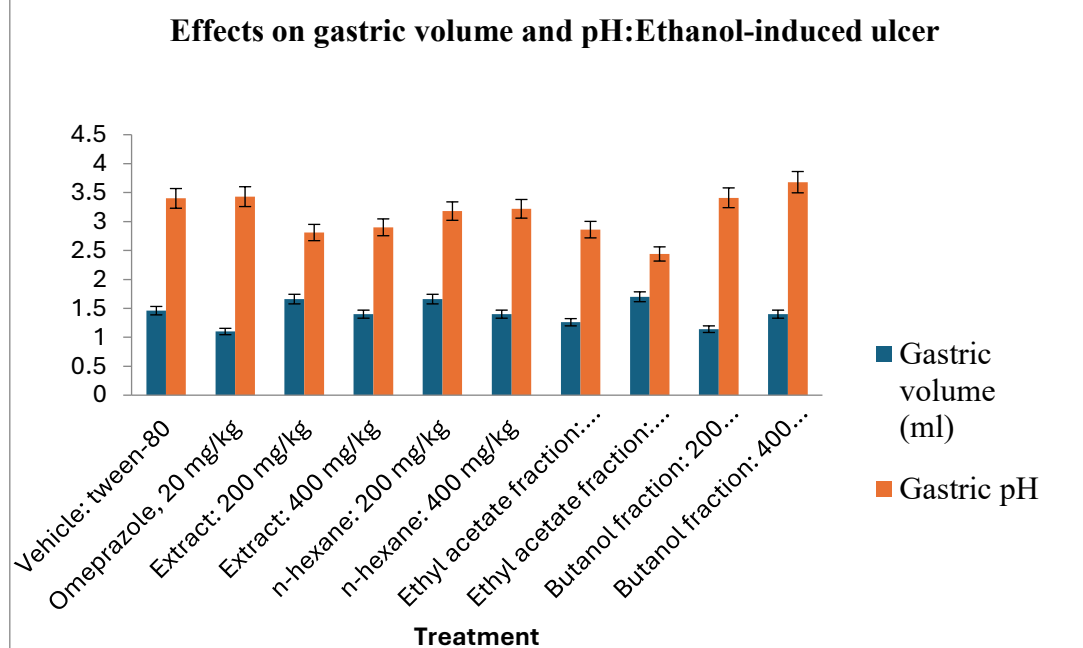


Fig. 4: Effects of extract and fractions of *C. schweinfurthii* on gastric volume and pH of ethanol-induced ulcer in rats. Values are presented as mean $\pm$ Standard deviation, n =5. ^{ns}P>0.05: Not statistically significantly different from control.

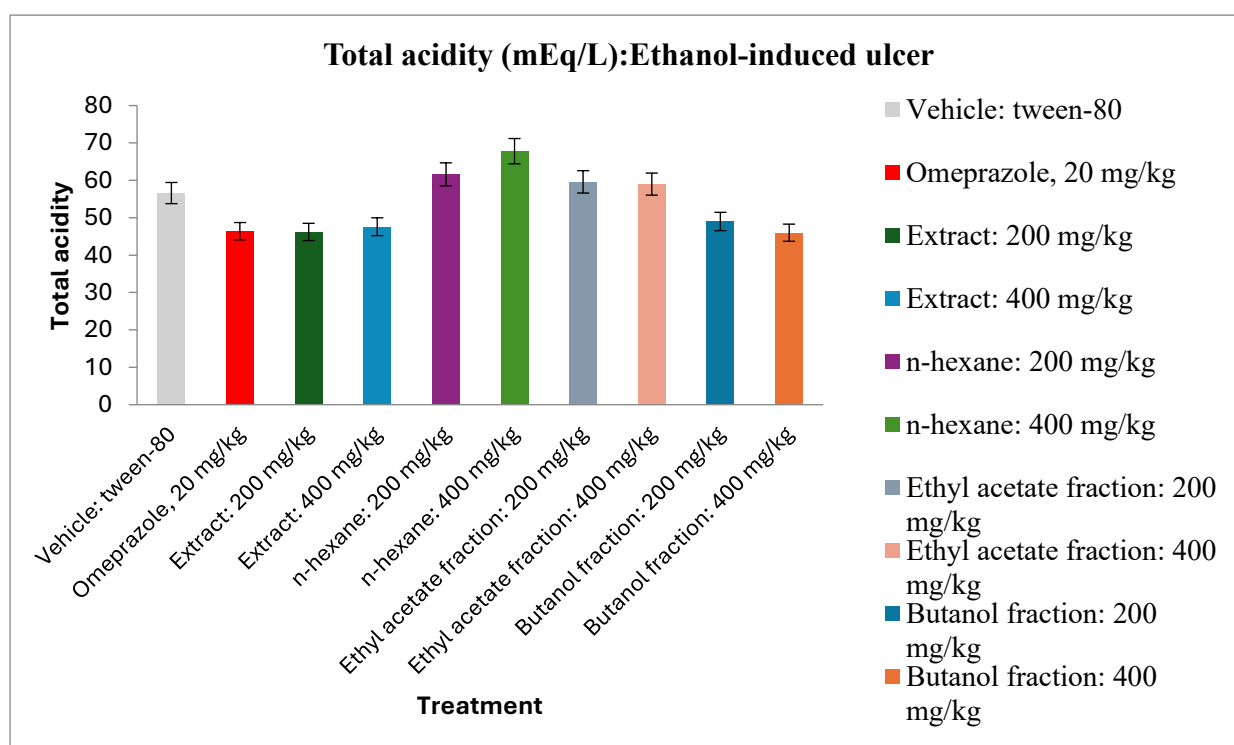


Fig. 5: Effects of extract and fractions of *C. schweinfurthii* on total acidity of ethanol-induced ulcer in rats. Values are presented as mean $\pm$ Standard deviation, n =5. ^{ns}P>0.05: Not statistically significantly different from control



Effects of extract and fractions of *C. schweinfurthii* on Aspirin-induced ulcer in rats.

Figures 6-9 shows the effects of extract and fractions of *C. schweinfurthii* on Aspirin-induced ulcer and its measured parameters in wistar rats.

Ulcer score: From figure 6, ulcer score of animals treated with Omeprazole was significantly lower ($*p<0.05$) when compared to control. Although ulcer score of extract and various fractions were slightly lower than that of control, they were not statistically significant ($p>0.05$).

Ulcer protection: Ulcer protection was highest in Omeprazole followed by butanol fraction, followed by n-hexane, extract 400 mg/kg and ethyl acetate fraction.

Gastric volume: Gastric volume of Omeprazole, ethylacetate -200 mg/kg and butanol 200 mg/kg were slightly lower than gastric volume of control group, but were not statistically significant ($p>0.05$). Gastric volume of extract, n-hexane fraction, ethyl acetate-400

mg/kg and butanol-400 mg/kg were slightly higher than gastric volume of control, but were not statistically significant ($p>0.05$).

Gastric pH: The gastric pH of omeprazole was significantly lower ($*p<0.05$) than that of the control group. However, the gastric pH of other groups except ethyl acetate-200 mg/kg was slightly higher than that of the control but was not statistically significant ($p>0.05$).

Total acidity: Omeprazole, crude extract and butanol fraction have lower total acidity when compared to control but were not statistically significant ($p>0.05$). Other groups have higher total acidity when compared to the control group, but it was not statistically significant ($p>0.05$).

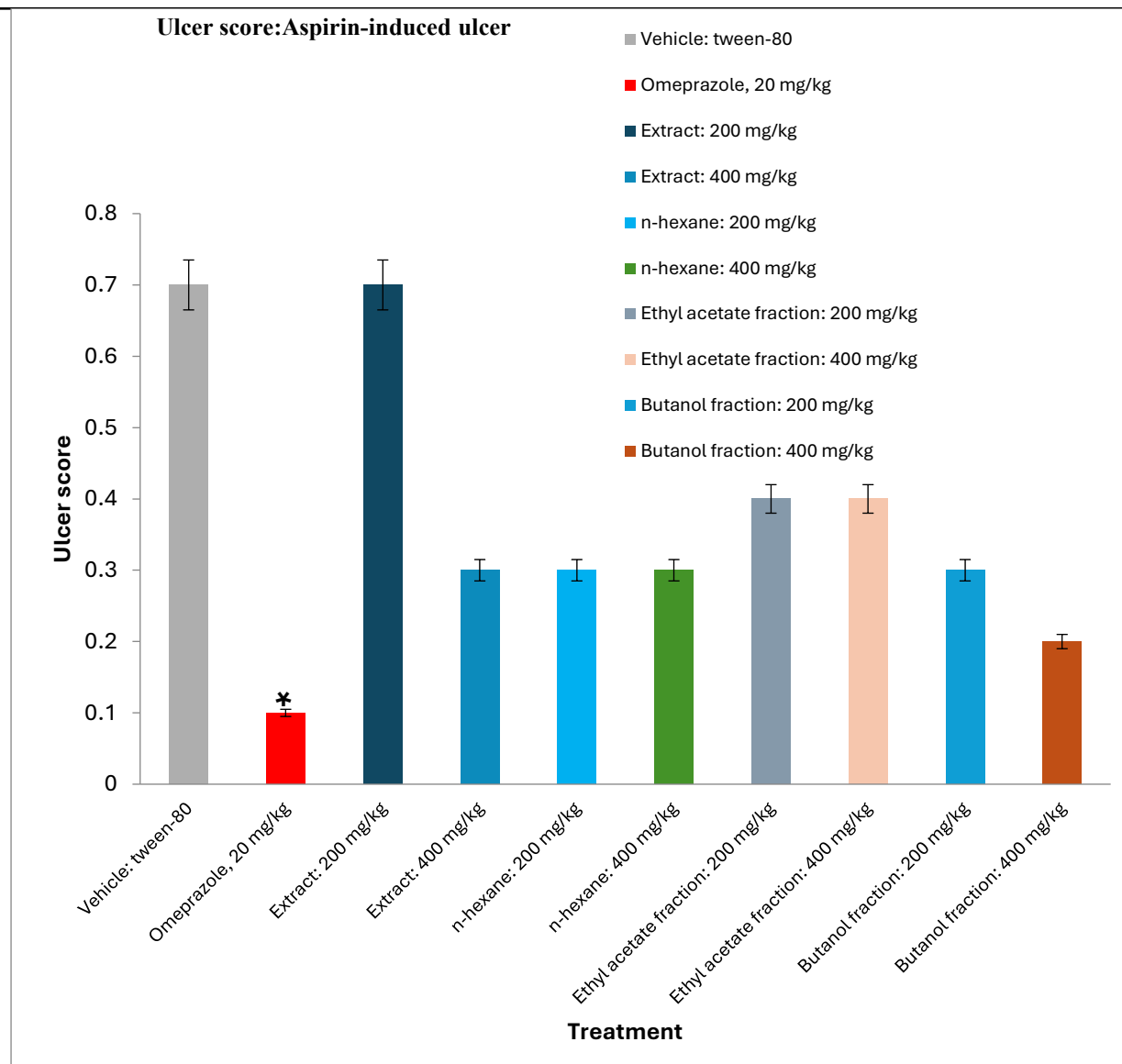


Figure 6. Effects of extract and fractions of *C. schweinfurthii* on aspirin-induced ulcer in rats. Values are presented as mean ± Standard deviation, n =5. *P<0.05: Statistically significantly different from control.

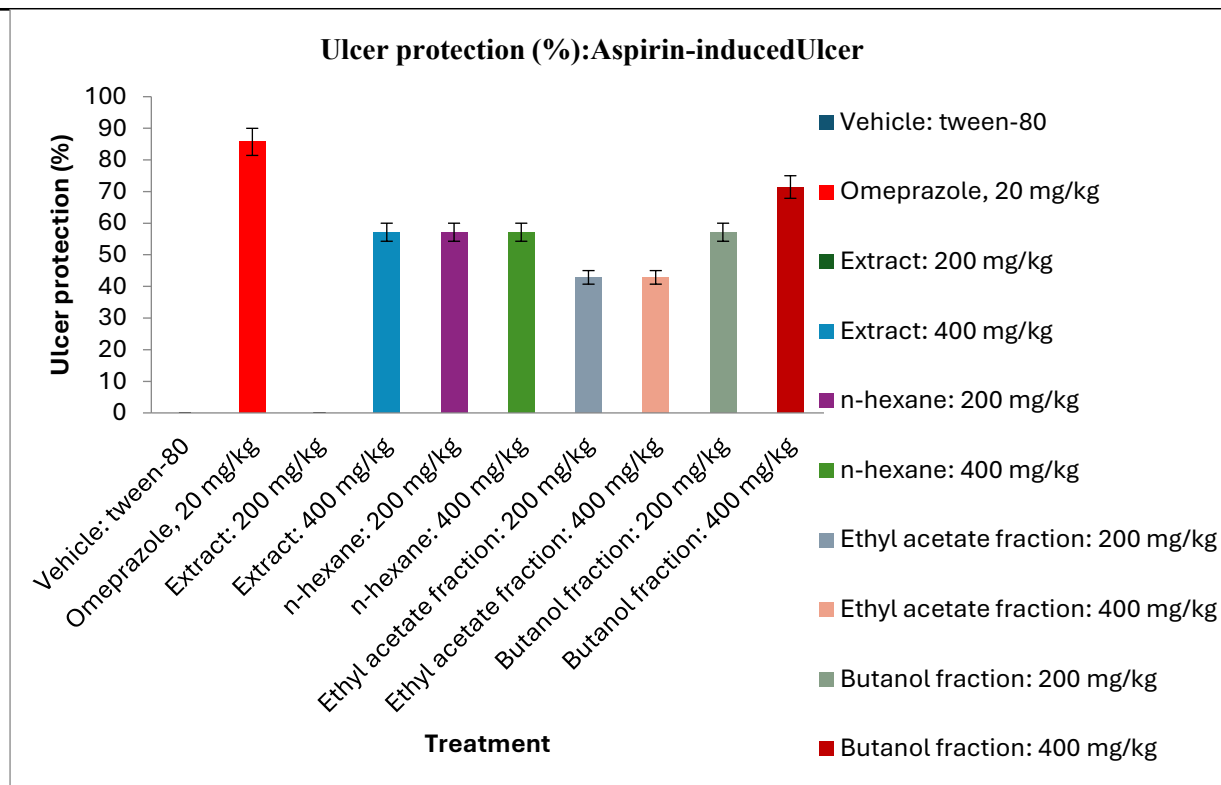


Figure 7. Effects of extract and fractions of *C. schweinfurthii* on aspirin-induced ulcer in rats.

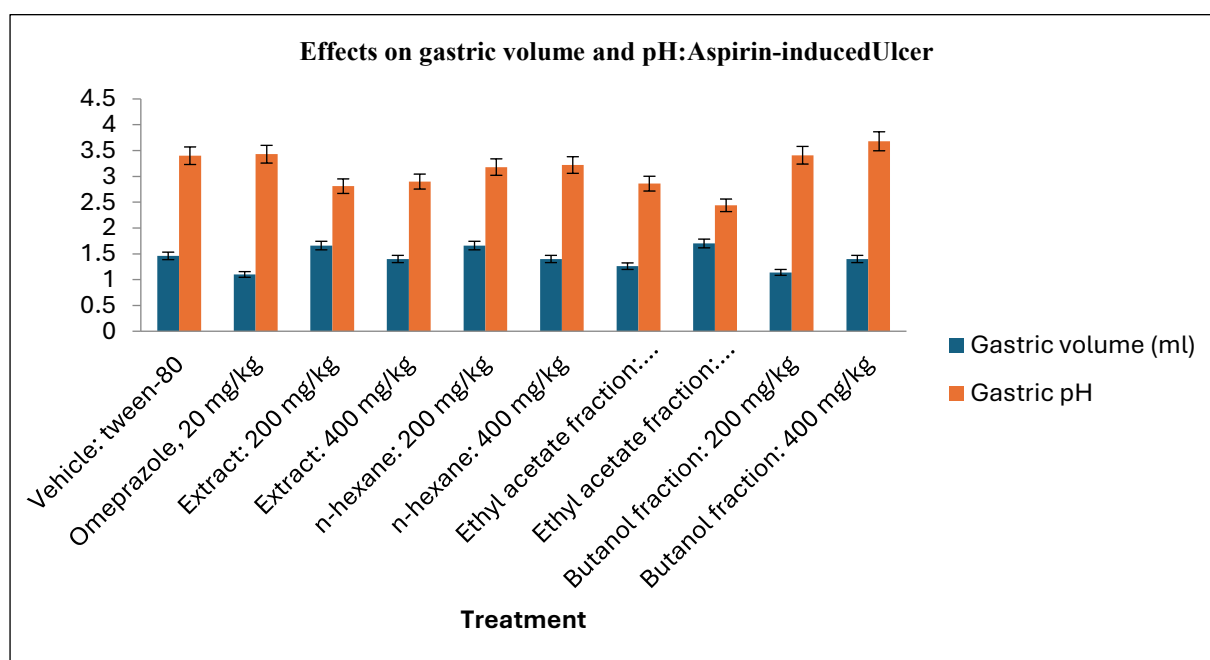


Figure 8. Effects of extract and fractions of *C. schweinfurthii* on gastric volume and pH of aspirin-induced ulcer in rats. Values are presented as mean $\pm$ Standard deviation, n =5. ^{ns}P>0.05: Not statistically significantly

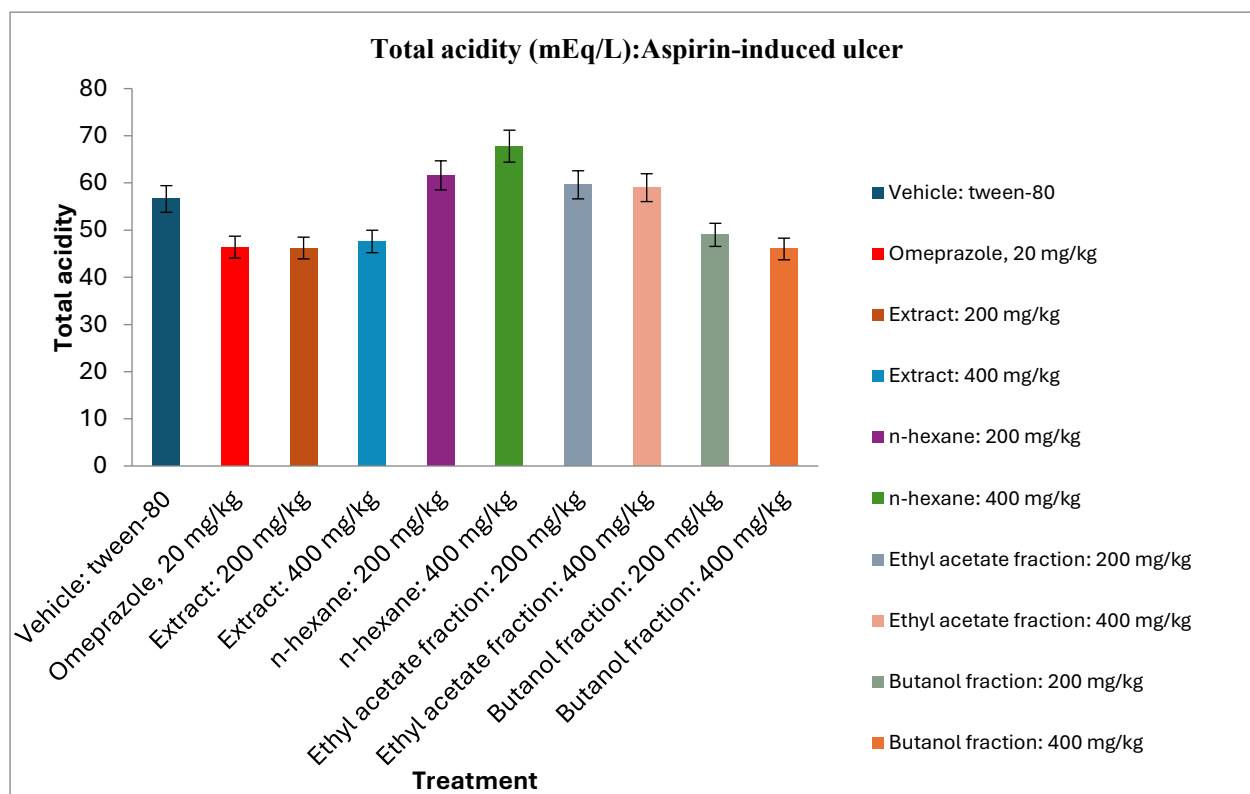


Figure 9. Effects of extract and fractions of *C. schweinfurthii* on total acidity of aspirin induced ulcers in Wistar rats. Values are presented as mean $\pm$ Standard deviation, $n = 5$. ^{ns} $P > 0.05$: Not statistically significantly different from control.

Effects of extract and fractions of *C. schweinfurthii* on gastrointestinal motility in rats.

In figure 10, atropine has a significantly ($*p < 0.05$) lower distance travelled by charcoal meal when compared to the control group. The distances travelled by the charcoal meal in the 400 mg/kg extract and the 400 mg/kg n-hexane were slightly lower than that of the control but were not statistically significant ($p > 0.05$).

On the other hand, the distance covered by the charcoal meal in other groups was slightly higher than that of the control but was not statistically significant ($p > 0.05$).

Atropine produced 52% inhibition against distance travelled by charcoal meal, extract 400 mg/kg (6.16%) and n-hexane 400 mg/kg (2.73%). Other groups have negative inhibition.

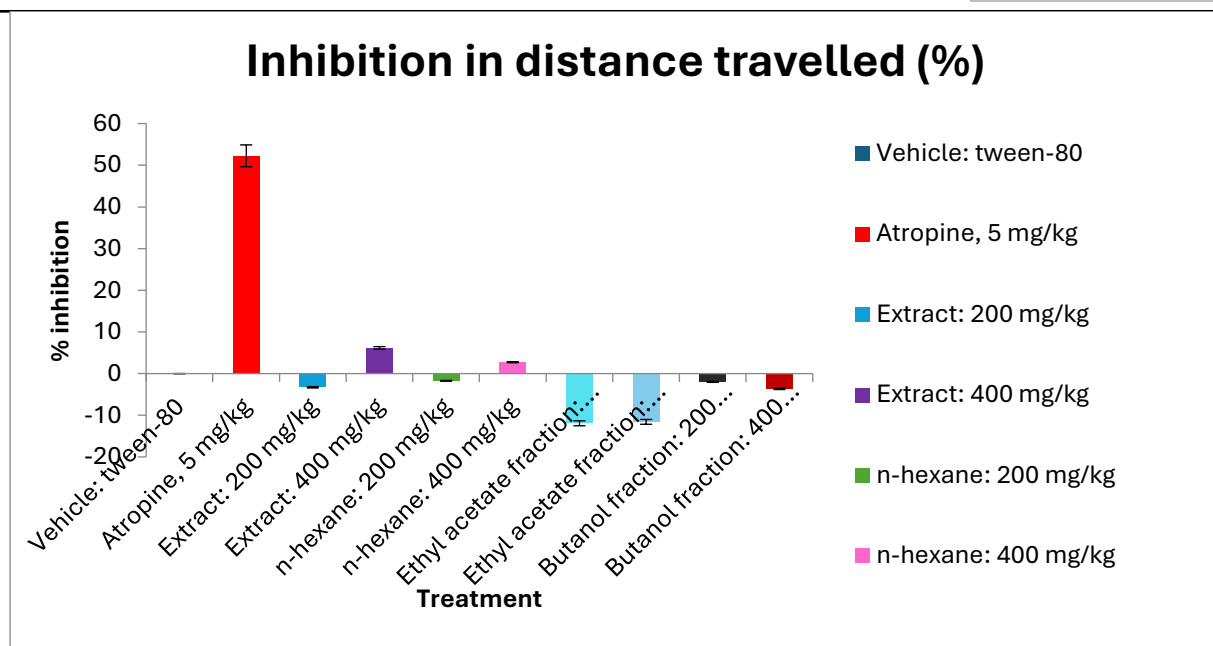


Figure 10: Effects of extract and fractions of *Canarium schweinfurthii* on gastrointestinal motility in Wistar rats. Values are presented as mean $\pm$ Standard deviation, $n = 5$. nsP>0.05: Not statistically significantly different from control.

4. Discussion

The study investigated the antiulcer properties of methanol extract and fractions of *C. schweinfurthii* on Wistar rats, revealing variations in extraction yields. The n-hexane fraction yielded 23%, while the water fraction yielded only 0.36%, indicating that solvent polarity significantly affects extraction efficiency, as supported by Nakamura et al. [27]. Non-polar solvents like n-hexane effectively extract non-polar compounds, while polar solvents extract polar compounds [28]. Phytochemical analysis showed the presence of proteins, carbohydrates, phenols, flavonoids, and terpenoids, but not tannins, steroids, or alkaloids. The crude extract and ethyl acetate fraction had higher concentrations of these compounds compared to n-hexane and butanol fractions. The varying concentrations are attributed to solvent polarity, with polar solvents extracting more polar phytochemicals [29].

Flavonoids and terpenoids contribute to gastric mucosal protection through antioxidant and

anti-inflammatory mechanisms [30]. Tannins, although absent in this study, are known to prevent ulcer development by forming protective layers [30]. The absence of tannins, steroids, and alkaloids contradicts findings by Kyewalabye *et al.* [7], potentially due to differences in plant origin and extraction methods. The acute oral toxicity study indicated no signs of toxicity or death at doses up to 5000 mg/kg, suggesting the safety of *C. schweinfurthii*'s stem bark for oral administration.

The study compared the antioxidant activity of different solvent fractions from *C. schweinfurthii* using DPPH and FRAP assays. The ethyl acetate fraction exhibited the highest antioxidant activity, followed by butanol, crude extract, and n-hexane, indicating that more polar solvents are richer in bioactive compounds like phenolics and flavonoids. Monthe *et al.* [31] supported these findings, noting significant antioxidant activity in hydroalcoholic extracts. Additionally, both low and high doses of the fractions showed acidic



pH levels, with the ethyl acetate fraction having a lower pH (3.08-3.39) compared to n-hexane (5.10-5.35), which is relevant for understanding gastroprotection. This aligns with findings on antiulcer activity and pH assessment by Mekonnen et al.[32].

The study on *C. schweinfurthii* extracts and fractions demonstrated a reduction in ulcer scores in rats induced by ethanol, although the results were not statistically significant ($p > 0.05$). The ethyl acetate and n-hexane fractions showed equal ulcer protection percentages of 45.45%, suggesting they contain more effective bioactive compounds. In contrast, the crude extract and butanol fraction exhibited moderate ulcer protection, indicating a lack of potent compounds or lower bioavailability [33].

The study indicates that both ethyl acetate and n-hexane fractions of *C. schweinfurthii* provide equal ulcer protection, highlighting the role of solvent polarity in extracting pharmacologically active compounds. Omeprazole markedly diminishes ulcer scores in aspirin-induced ulcer models owing to its powerful pharmacological properties, whereas the extracts and some fractions exhibit very minimal reductions. Nevertheless, butanol extract demonstrates ulcer scores that are more comparable to those of Omeprazole. The efficacy of omeprazole is ascribed to its function as a proton pump inhibitor, along with its supplementary anti-inflammatory and antioxidant characteristics.[34].

The extract and fractions of *C. schweinfurthii* exhibit some bioactive properties but are less potent than omeprazole in protecting against aspirin-induced ulcers. While omeprazole significantly reduces ulcer scores due to its targeted action on acid suppression and inflammation, the extracts show only mild effects that lack statistical significance [35]. While omeprazole achieved 85.7% ulcer

protection in aspirin-induced ulcers, the butanol fraction (400 mg/kg) recorded 71.4%, which is thought to work through indirect mechanisms like enhancing mucosal defences.[34]. The butanol fraction, rich in flavonoids and polyphenols, may improve gastric conditions by promoting mucus and bicarbonate release, but its effects are less direct and potent than those of omeprazole.

The study examined the effects of *C. schweinfurthii* extracts on gastrointestinal motility in rats, revealing that atropine significantly reduced the distance travelled by charcoal meal due to its anticholinergic properties, which slow intestinal motility Kumar *et al.* [36]. In contrast, the 400 mg/kg extract and n-hexane fraction showed only slight reductions in transit distance, which were not statistically significant ($p > 0.05$). The marginal inhibition percentages (6.16% for the extract and 2.73% for n-hexane) suggest a weaker anti-motility effect compared to atropine, potentially due to lower potency or different mechanisms of action related to phytochemical composition [37].

5. Conclusion

The study evaluated the antiulcer efficacy of methanol stem bark extract and its fractions from *C. schweinfurthii*, revealing varied yields with the n-hexane fraction at 23% and the aqueous fraction at 0.36%. Phytochemical study revealed the presence of proteins, carbohydrates, phenols, flavonoids, and terpenoids, however tannins, steroids, and alkaloids were not detected. The crude extract and ethyl acetate fraction exhibited elevated amounts of these chemicals attributable to solvent polarity. Acute toxicity assessments demonstrated that the stem bark is safe for oral ingestion. The ethyl acetate and butanol fractions exhibited superior antioxidant capabilities. In the two ulcer investigation models used in the study, the aspirin-induced



ulcer model of butanol fraction (400 mg/kg) consistently demonstrated comparable effects to omeprazole on gastric acid volume, total acidity, ulcer scores, and ulcer protection percentage. The ulcer protection percentages of omeprazole (85.7%) versus butanol fraction (74.1%) signal the protective ability of the plant's stem bark fraction. Our findings underscore the safety, abundant phytochemical composition, and ulcer-protective properties of the butanol (400 mg/kg) fraction of *C. schweinfurthii* stem bark, justifying its local use as an alternative in the management of acid-induced ulcerative conditions.

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Conflict of Interest. The authors declare there is no conflict of interest

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Ethics Statement

The number of animals used was in line with the Guide for the Care and Use of Laboratory Animals as approved by Nnamdi Azikiwe University animal research ethics committee with approval number NAU/AREC/2025/0140.

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