Phytochemical Screening and Anti-Ulcerogenic Properties of n-Hexane, Ethyl-Acetate and Aqueous-methanol Fractions on Indomethacin and Aspirin Induced Ulcer in Rats: A Comparative study

ABSTRACT

Harungana madagascariensis is a plant abundant in several phytochemicals that can be extracted, purified, and packaged for the aim of promoting optimum health of humans. The extraction processes were carried out following standard extraction methods. The fresh leaves of *H. madagascariensis* were air-dried, milled into coarse powder and macerated in a mixture of methanol and chloroform (2:1) for 48 hours. The solution was filtered and partitioned with 20% distilled water of the total volume of the filtrate to obtain two layers that were separated using a separation funnel. The upper layer was designated methanol extract and the lower layer designated chloroform extract. The methanol extract was further fractionated into n-hexane, ethyl-acetate and aqueous-methanol fractions. Preliminary phytochemical analyses was conducted which revealed the presence of diverse secondary metabolites including alkaloids, flavonoids, phenolic compounds, tannins, saponins and terpenoids, steroids in varying concentrations. With the highest concentration of the phytochemical recorded in the aqueous-methanol fractions. The acute toxicity test carried out showed no lethality or behavioural change at 5000mg/kg. The ethyl acetate and aqueous-methanol fractions exhibited better anti-ulcerogenic effect against both indomethacin and aspirin induced gastric ulcer than that obtained for the n-hexane fraction. Comparatively, the aqueous-methanol fraction had the highest anti-ulcerogenic activity than ethyl-acetate and n-hexane fractions. The results showed that the various fractions had significant anti-ulcer activity and could be used for the effective treatment of ulcer and inflammatory related diseases which could be due to their abundant phytochemical constituents.

Keywords: *Harungana Madagascariensis*; n-hexane; ethyl-acetate; aqueous-methanol, anti-ulcerogenic

1. INTRODUCTION

One of the most prevalent chronic gastrointestinal disorders in the modern era is gastric ulcers. It is currently a widespread global health issue that affects many people worldwide and continues to be a leading cause of morbidity and mortality. Inflamed lesions or excavations of the mucosa and tissue lining the gastrointestinal tract are typical of gastric ulcer disease. Gastric ulcers are caused by damage to the mucous membranes that typically shield the oesophagus, stomach, and duodenum from gastric acid and pepsin (Flasherud, 2020). An imbalance between mucosal defensive factors like bicarbonate, prostaglandin, nitric oxide, peptides, and growth factors and harmful factors like acid and pepsin is thought to be the patho-physiology of this gastrointestinal disorder (Choi *et al*., 2024). The modern approach is to control gastric ulceration by inhibiting gastric acid secretion, to increase gastro-protection, to increase epithelial cell proliferation or to stop apoptosis for effective ulcer healing process (Zaib *et al*., 2023; Mohamed & Farid 2024). Though different classes of drugs are used in the treatment of gastric ulcer but most of the drugs are exhibits serious side effects like arrhythmias, impotence, stomach ulcer, osteoporosis, and Cushing syndrome and suppressed immune function (Tarnawski & Ahluwalia, 2021). Alternative approach in recent days is the research of natural herbs from ayurvedic or traditional medicinal system. The use of Phyto-constituents as drug therapy to treat major ailments has proved to be clinically effective and less relatively toxic than the existing drugs and also reduces the offensive factors serving as a tool in the prevention of gastric ulcer (Farzaei *et al*., 2015). In this modern era also 75-80% of the world populations still use herbal medicine mainly in developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. The chemical constituents present in the herbal medicine or plant are a part of the physiological functions of living flora and hence they are believed to have better compatibility with human body (Bee *et al*., 2022). Natural products from plants are a rich resource used for centuries to cure various ailments. The use of natural medicine in the treatment of various diseases like gastric ulcer is an absolute requirement of our time. Therefore, alternative approach in recent days is the research of medicinal plants from traditional medicine. The use of phyto-constituents as drug therapy to treat major ailments has proved to be clinically effective and relatively less toxic than the existing drugs and also reduces the offensive factors serving as a tool in the prevention of peptic ulcer (Kandunuri, 2023; Chaachouay & Zidane, 2024).

Harungana madagascariensis is a plant species membership of the Hypericaceae family. Different plant parts (bark, leaves, fruits, roots) of *H. madagascariensis* have been used in traditional medicine over many years in various countries for the treatment of several diseases including jaundice, dysentery, typhoid fever, diarrhea, hemorrhoids, anemia, as well as some skin and heart problems (Happi *et al*., 2020). *H. madagascariensis* displayed a wide range of pharmacological activities including anti-plasmodial (Happpi *et al*., 2020), anti-protozoal (Tankeo *et al*., 2016), anti-anaemic (Seriki & Otoikhila, 2022), vasodilatory (LIorent-martinez *et al*., 2020), analgesic and anti-inflammatory (Njan *et al*., 2015), anti-diabetic (Ezike *et al*., 2022), antioxidant (Kandunuri, 2023), antibacterial and antifungal activities (Longo *et al*., 2022 ;Asogwa *et al*., 2023). This study is set out to compare the

phytochemical composition and anti-ulcerogenic properties of n-hexane, ethyl-acetate and aqueous-methanol fractions *Harungana Madagascaeiensis.*

2. MATERIALS AND METHODS

Materials

Collection of Plant Material

The leaves of *Harungana madagascariensis* were used for this study. They were collected from Adada River in Nsukka, Enugu State and identified by Mr. Alfred Ozioko of Bioresource Development and Conservation Programme (BDCP) Research Centre, Nsukka, Enugu State Nigeria.

Methods

Preparation of plant extract

The fresh leaves of *Harungana madagascariensis* were air-dried, milled into coarse powder and macerated in a mixture of methanol and chloroform (2:1) for 48 hours. The solution was filtered with Whatman No.1 filter paper and fractioned with 20% distilled water of the total volume of the filtrate to obtain two layers that were separated using a separation funnel. The upper layer was designated methanol extract and the lower layer designated chloroform extract. They were concentrated differently using a rotary evaporator at an optimum temperature range of 45–50^{O}C.

Fractionation of the methanol extract into different fractions

Solvent partitioning of the methanol extract was done by using the protocol designed by Kupchan and Tsou 1979 and modified version of Wagenen *et al.* 1993. Fractionation was carried out using n-hexane, ethylacetate, and 20% aqueous methanol (v/v). Methanol extract (20 g) was weighed and dissolved in 250 ml of 20% aqueous methanol (v/v) to form a stock solution. Then, 250 ml of n hexane was added to the solution and poured into a separating funnel. The mixture was allowed to stand for 20 min for proper separation, and the upper part was collected in a beaker. The aqueous methanol part was washed repeatedly with n hexane, after which the different n-hexane fractions were collected. The above procedure was repeated using ethyl acetate. At the end, ethyl-acetate fractions were collected and concentrated. The fractions were concentrated using rotary evaporator and used for anti-ulcer tests.

Qualitative Evaluation of the Plant's Phytochemical Constituents

Qualitative phytochemical analysis of n-hexane, ethyl-acetate and aqueous-methanol fraction of *H. madagascariensis* were carried out according to the method of Harborne (1998) and Trease and Evans (1989) to identify its active constituents.

Test for reducing sugar

Fehling Test: Sample (0.5g) of each of chloroform and methanol extract was dissolved in distilled water and filtered. The filtrates were heated with 5 ml of equal volume of Fehling's solution A and B. Formation of red property of cuprous oxide was an indication of the presence of reducing sugar.

Test for Carbohydrate

Molisch's Test: One gramme (1g) each of the samples (chloroform and methanol) were boiled with 2 ml of distilled water and filtered. To the filtrates, few drops of alpha (α-) naphtol solution (molisch's reagent) were poured down the side of the test tubes to form a lower layer. A purple interfacial ring indicated the presence of carbohydrate.

Test for Steroid

A measured volume, 9 ml of ethanol was added to 1g of the samples and refluxed for a few minutes and filtered. The filtrates were concentrated to 2.5 ml on a boiled water bath, and 5 ml of hot water was added. The mixture was allowed to stand for 1 hour, and the waxy matter filtered off. Chloroform (2ml) and 1 ml of concentrated sulphuric acid were added to each extract, formation of reddish-brown ring at interface indicated the presence of steroids.

Test for Saponins

A known quantity, 0.1g of the extracts were boiled with 5ml of distilled water for 5 minutes. The mixture was filtered while still hot.

Frothing Test: A known quantity, 1 ml of the filtrates was diluted with 4 ml of distilled water. The mixture was shaken vigorously and then observed on standing for a stable foam.

Test for tannins

A quantity, (0.1g) each of chloroform and methanol extracts was added in 10 ml of deionised water, filtered and then treated with 3 drops of ferric chloride. A greenish-brown precipitate indicated the presence of tannins

Test for Alkaloids

Wagner's Test

The samples (0.5 g) was mixed with 5ml of 1% hydrochloric acid, boiled for five minutes and then filtered. To 1 ml of the filtrates was added 1ml of Wagner's reagent. Reddish brown precipitate indicated the presence of alkaloids.

Mayer's Test

The samples (0.5 g) were mixed with 5 ml 1% hydrochloric acid, boiled for five minutes and then filtered. To 1 ml of the filtrates were added 1ml of Mayer's reagent and creamy white precipitate indicated the presence of alkaloids.

Flavonoids (Ammonium Test Method)

To 1 g of samples was added 10ml ethyl acetate, heated for 3 minutes, filtered and cooled. Then the filtrates (4 ml) were shaken with 1 ml of dilute ammonia solution. An intense yellow coloration at the upper layer indicated the presence of flavonoids.

Test for glycosides

The samples (0.5 g) were mixed with 10ml of distilled water, boiled for 5 minutes and then filtered. The filtrates (2 ml) were mixed with one hundred micro litres (100 µl) of concentrated hydrochloric acid, followed by few drops of aqueous ammonia to make the solution alkaline. Five hundred micro litre (500 µl) of Fehling solutions A and B was added, boiled for 5-10 minutes and observed for a change of brick red which indicated the presence of glycoside.

Test for Phenols

Distilled water (2 ml) was added to each of 0.1 g of chloroform and methanol extracts, followed by addition of sodium carbonate (0.5 ml) and Folin Ciocalteau's reagent (0.5 ml). Formation of green colour indicated the presence of phenols.

Test for Cardiac glycosides

Glacial acetic acid (2 ml) and few drops of 5% ferric chloride were added to each of 0.1 g samples of methanol and chloroform extracts. This was under layered with 1 ml of conc. Sulphuric acid. Formation of brown ring at interface indicated the presence of cardiac glycosides.

Quantitative Phytochemical Analysis of the Fractions

Quantitative phytochemical screening of the fraction (n-hexane, ethyl acetate and aqueous-methanol) of *H. madagascariensis* leaf extract were done using the method of Harbone (1998) and Trease and Evans (1989).

Determination of Total phenols

Folin Ciocalteau reagent (1/10 dilution) and 1.5 ml of Na_2CO_3 2% (w/v) were added to each of the fractions (n-hexane, ethyl acetate and aqueous-methanol) were mixed. The blend was incubated in the dark room temperature for 15 minutes. The absorbance of blue-coloured solutions was measured at a wavelength of 765 nm.

Test for Flavonoids

Exactly 0.5 g of each of the fraction was mixed with 2 ml of distilled water and subsequently with 0.15 ml of 5 % $NaNO_2$ solution. After 6 minutes, 0.15 ml of 10 % AlCl3 solution was added and allowed to stand for 6 minutes. Then, 2 ml of 4 % NaOH solution was added to the mixture. Afterward, water was added to bring the final volume to 5 ml, and then the mixture was thoroughly mixed and allowed to stand for another 15 minutes. The absorbance of the mixture was measured at a wavelength of 510 nm against water blank. The analysis performed in triplicate.

Test for Tannins

A known weight, 0.5 g of each of the fraction was mixed with FolinCiocalteau's reagent (0.5 ml), followed by the addition of saturated Na2CO3 solution (1 ml) and distilled water (8 ml). the reaction mixture was allowed to stand for 30 minutes at room temperature. The supernatant was obtained by centrifugation and the absorbance was measured at a wavelength of 725 nm using UV-visible spectrophotometer. The analysis was performed in triplicate.

Test for Terpenoids

A quantity, (0.5 g) of each of the fraction was macerated with 50 ml of ethanol and filtered. To the filtrates (2.5 ml), 2.5 ml of 5 % aqueous phosphomolybdic acid solution was added and 2.5 ml of concentrated H_2SO_4 was gradually added and mixed. The mixture was left to stand for 30 minutes and then made up to 12.5 ml with ethanol. The absorbance was taken at a wavelength of 700 nm. The analysis was performed in triplicate.

Test for Steroids

Each of 0.5 g of the fractions was macerated with 20 ml of ethanol and filtered. Chromagen solution (2 ml) was added to 2 ml of the filtrates and the solution left to stand for 30 minutes. The absorbance was read at a wavelength of 550 nm. The analysis was performed in triplicate.

Test for Alkaloids

A known weight, 0.5 g of each fractions was macerated with 20 ml of ethanol and 20 % H_2SO_4 (1: 1 v/v). The filtrates (1 ml) were added to 5 ml of 60 % H_2SO_4. A volume, 5 ml of 0.5 % formaldehyde in 60 % H_2SO_4 was mixed with the mixtures after 5 minutes and allowed to stand for 3 hours. The absorbance was read at a wavelength of 565 nm. The analysis was performed in triplicate.

Test for Glycosides

Each of 0.5 g of the fractions was macerated with 50 ml of distilled water and filtered. Alkaline pirate solution (4 ml) was added to 1 ml of the filtrate. The mixture was boiled for 5 minutes and allowed to cool. The analysis was performed in triplicate.

Test for Reducing Sugar

Each fraction (0.5 g) was macerated with 20 ml of distilled water and filtered. To 1 ml of the filtrates, Alkaline copper reagent (I ml) was added. The mixture was boiled for 5 minutes and allowed to cool. Then 1 ml of phosphomolybdic acid reagent and 2 ml of distilled water were added and the absorbance read at a wavelength of 420 nm. The analysis was performed in triplicate.

Anti-ulcerogenic tests

Indomethacin- induced ulcer

This determination was carried out using the method of Urushidani *et al.*, (1979). Twenty adult rats randomly divided into 5 groups of 4 rats each were deprived of food for 18 hours and orally with normal saline and varying doses of the (n-hexane, ethyl acetate and aqueous-methanol). The extracts and drugs used were freshly prepared as a suspension in 3% tween 80 and administered orally to the animals in 5 ml/kg doses. Group 1(normal control) was administered 3 % tween 80 (2 ml/kg). Groups II, III and IV were treated with 100, 200, and

400 mg/kg of the respectively. Group V (reference group) was administered 100 mg/kg cimetidine, a standard anti-ulcer drug.

Thirty minutes later, 50 mg/kg of indomethacin was administered (p.o) to the rats. After 8 hours, each animal in the groups was sacrificed by chloroform anaesthesia and the stomach removed and opened along the greater curvature, rinsed with water and pinned flat on a board. Erosions formed on the glandular portions of the stomach were counted and the ulcer index calculated as described by Main and Whittle (1975). The ulcer was usually counted and scored 0= no ulcer; 1= superficial ulcer; 2 = deep ulcer; 3= perforations. The sum of all the lesions/ulcers in all the animals for each group (total ulcer score) was used to calculate the ulcer index. The percent ulcer inhibition was calculated relative to control as follows:

$$\%\ \text{ulcer inhibition}\ (\%\,\text{U.I}) = 1 - \frac{\text{Ut}}{\text{Uc}}\ \text{X}\ 100$$

Where Ut and Uc represents the ulcer index of the treated and that of the control group respectively.

Aspirin induced ulcer

Ulcer was induced according to the method of Akah *et al*. (1993). The animals were fasted for 24 hours but had access to water. The drug and vehicle were respectively administered p.o as stated above. Thirty minutes later, an aqueous suspension of aspirin was given orally to the rats at a dose of 200 mg/kg. After 8 hours, the animals were killed and the stomach removed and opened along the grater curvature. The stomach was rinsed in water, pinned flat on a board, examined with a hand lens (x10) and scored for ulcer. The total ulcer scores and ulcer indices for the groups were obtained and used to calculate the percent ulcer inhibition as above.

STATISTICAL ANALYSIS

The biochemical data obtained from the study were analyzed using a statistical program SPSS, version 22. The results were expressed as Mean ± SD. A one-way ANOVA was employed for comparison among the groups followed by Dunnet's Post-Hoc Multiple Comparisons tests. *P=0.05* was considered as statistically significant.

3. RESULTS

Qualitative phytochemical analysis of n-hexane, ethyl acetate and aqueous-methanol fractions of *Harungana madagascariensis* leaves

The phytochemical compositions of n-hexane fraction showed the presence of phenolics, saponins, terpenoids, reducing sugar and glycosides in moderate amounts, flavonoids, carbohydrates, alkaloids, tannins, and steroids in trace amounts. The ethyl acetate fractions revealed the presence of phenolics, flavonoids, tannin, saponin, reducing sugar and terpenoids in moderate quantity, carbohydrates, alkaloids, steroids and glycosides at low amount. The aqueous-methanol fraction showed the presence of phenolics and flavonoids in high quantity, tannin, reducing sugar and glycoside in moderate amount and carbohydrate, alkaloids, saponin, steroids and terpenoids in low amounts (Table 1).

Table 1: Qualitative phytochemical analysis of n-hexane, ethyl acetate and aqueous methanol fractions

Phytochemicals	n-Hexane	Ethylacetate	Aqueous-methanol
Phenolics	++	++	+++
flavonoids	+	++	+++
Carbohydrate	+	+	+
Alkaloids mayer	+	+	+
Wagner	ND	+	+
Dragendorf	+	+	+
Tannin	+	++	++
Saponin	++	++	+
Steroids	+	+	+
Terpenoids	++	++	+
Reducing sugar	++	++	++

Glycosides	++	+	++

Key: + low concentration
++ Moderate concentration
+++ High concentration
ND Not Detected

Quatitative phytochemical analysis of n-hexane, ethyl acetate and aqueous-methanol fractions of methanol extract of *Harungana madagascariensis*leaves

Table 2 shows the results of the quantitative phytochemical constituents of the fractions. The n-hexane fraction had a high amount of phenols (1465 ± 2.4 mg/100g) and reducing sugar (1698 ± 1.6 mg/100g); moderate concentration of tannins (355.5 ±2.2 mg/100g), Flavonoids (967.76±1.6 mg/100g), terpenoids (360.8±4.2 mg/100g); and trace amounts of Carbohydrate (80.98±1.8 mg/100g), steroids (70.37 ±4.6 mg/100g), glycosides (71.19±2.1mg/100g) and alkaloids (20.2±1.0 mg/100g). The ethylacetate fraction showed a high amount of phenolics (1594.6±1.5 mg/100g) and flavonoids (1690.5±1.1 mg/100g), reducing sugar (2314.9±1.7 mg/100g);moderate amount of tannin (825.5±3.7 mg/100g), and terpenoids (394.28±3.1 mg/100g); and trace amounts of steroids (88.59±4.3mg/100g), glycosides (69.73±2.2 mg/100g) and alkaloids (78.93±1.3 mg/100g). While the aqueous-methanol fraction showed a high amount of phenolics (4806.5±1.1 mg/100g), flavonoids (1911.7±3.6 mg/100g) and reducing sugar (2374±2.3mg/100g); moderate amount of tannin (909.9±0.9 mg/100g), terpenoids (274.38±2.7mg/100g) and glycosides (477.82±2.0 mg/100g); and trace amounts of steroids (91.03±1.1 mg/100g), carbohydrates (80.46±2.4 mg/100g) and alkaloids (48.93±1.1 mg/100g).

Table 2: Quatitative phytochemical analysis n-hexane, ethylacetate and aqueous-methanol fractions of methanol extract of *Harunganamadagascariensis*leaves.

Phytochemicals	n-Hexane mg/100g (Mean ± SD)	Ethylacetate mg/100g (Mean±SD)	Aqueous-methanol mg/100g (Mean±SD)
Phenolics	1465.1±2.4	1594.6±1.5	4806.5±1.1
Tannins	355.5±2.2	825.5±3.7	909.9±0.9
Steroids	70.37 ±4.6	88.59±4.3	91.03±1.1
Terpenoids	360.8±4.2	394.28±3.1	274.38±2.7
Reducing sugar	1698.8±1.6	2314.9±1.7	2374±2.3
Glycosides	71.19±2.1	69.73±2.2	477.82±2.0
Flavonoids	967.76±1.6	1690.5±1.1	1911.7±3.6
Carbohydrate	80.98±1.8	75.24±2.4	80.46±2.4
Alkaloids	20.2±1.0	78.93±1.3	48.93±1.1

Acute toxicity study (LD_{50}) of the n-hexane, ethyl-acetate and aqueous-methanol fractions of *H. madagascariensis*leaves

The acute toxicity test (LD_{50}) of the n-hexane, ethyl-acetate and aqueous-methanol fractions was indeterminable as there was no death recorded and no obvious toxicological signs at a dose of 5000mg/kg body weight. The acute toxicity test of the fractions indicated that the plant is not toxic and is an indication that the leaves are safe for human and animal consumption (Table 3).

Table 3: Phase I and II of the acute toxicity (LD50) test of chloroform and methanol extracts of *Harunganamadagascariensis* leaves

Dosage mg/kg body weight		Mortality		
Groups	Dose (mg/kg)	n-hexane	ethyl-acetate	aqueous-methanol
Phase 1				
Group 1	10	0/3	0/3	0/3
Group 2	100	0/3	0/3	0/3
Group 3	1000	0/3	0/3	0/3
Phase II				
Group 4	1600	0/3	0/3	0/3
Group 5	2900	0/3	0/3	0/3

Group 6	5000	0/3	0/3	0/3

Effect of methanol extract fractions (n-hexane, ethyl acetate and aqueous- methanol) on Indomethacin-induced ulcers in rats.

Groups treated with n-hexane fraction had significant reductions ($p<0.05$) in the ulcer formed as is shown from the significantly reduced ulcer lesion indices (ULI) obtained for 100, 200 and 400 mg/kg b.w of the fraction respectively, when compared with that of the control. The following percentage inhibitions of ulcer, 45.7, 43.2 and 46.5% were obtained for the various doses of the n-hexane fraction respectively (Table 4). While the ethyl-acetate fraction treated rats showed significantly reduced ulcer lesion indices (ULI) of 1.93 ± 0.2, 1.83 ± 0.3 and 1.77 ± 0.4 obtained for the 100, 200 and 400 mg/kg b.w respectively, as compared to the 4.3 ± 3.5 ulcer index obtained for the control. The percentage ulcer inhibitions of 55, 57.4 and 58.9 % were obtained for the ethyl acetate fraction at 100, 200 and 400 mg/kg b.w respectively. However, the aqueous-methanol fraction treated rats showed significantly($P<0.05$) reduced ulcer lesion indices (ULI) of 1.80 ± 0.2, 1.63 ± 0.5 and 1.37 ± 0.3 obtained for the different doses, as compared to the 4.3 ± 3.5 ulcer index obtained for the control. The percentage ulcer inhibitions of 58.1, 62.0 and 68.2 % were obtained for the aqueous-methanol fraction. Comparatively, the ulcer lesion indices of groups treated with 100, 200, and 400 mg/kg b.w of the aqueous-methanol fraction were significantly ($p<0.05$) lower than those of n-hexane and ethyl acetate fractions at equivalent doses. Thus, the percentage inhibitions produced by aqueous-methanol fractions were higher than those obtained for n-hexane and ethyl acetate fractions.

Table 4: Effect of methanol extract fractions (n-hexane, ethyl acetate and aqueous-methanol) on Indomethacin-induced ulcers in rats.

Treatment	Dose (mg/kg)	Ulcer Index	Percentage (%) inhibition
Control (3% tween 80	2 ml	4.3^{d}	-
n-Hexane	100	2.33 ± 0.3^{b}	45.7
	200	2.4 ± 0.4^{c}	43.4
	400	2.3 ± 0.1^{b}	46.5
Ethyl-acetate	100	1.93 ± 0.2^{b}	55.0
	200	1.83 ± 0.3^{b}	57.4
	400	1.77 ± 0.4^{b}	58.9
Aqueous-methanol	100	1.80 ± 0.2^{a}	58.1
	200	1.63 ± 0.5^{a}	62.0
	400	1.37 ± 0.3^{a}	68.2
Cimetidine	100	1.2 ± 0.7^{a}	72.1

Values are expressed as mean ± SD (n=4).Means with different superscript are significantly different at ($P<0.05$), while mean values with the same superscript are not significantly different ($P>0.05$).

Effect of methanol fractions (n-hexane, ethyl acetate and aqueous-methanol) on Aspirin-induced ulcers in rats.

Groups treated with n-hexane fraction had significant reductions ($p<0.05$) in the gastric ulcer formed as is shown from the significantly reduced ulcer lesion indices (ULI) of 2.53 ± 0.3, 2.33 ± 0.3, and 2.20 ± 0.3 obtained for 100, 200 and 400 mg/kg b.w of the fraction respectively, when compared with that of the control (4.67± 0.6). The percentage inhibitions of ulcer, 45.8, 50.0 and 52.9 % were obtained for the various doses of the n-hexane fraction; 100, 200 and 400 mg/kg b.w respectively. Also the group treated with ethyl acetate fraction showed significantly reduced ulcer lesion indices (ULI) of 2.28 ± 0.5, 1.67 ± 0.6 and 1.77 ± 0.4 for different doses respectively, as compared to the 4.67 ± 6.4 ulcer index obtained for the control but not dose dependently. The aqueous-methanol fraction showed significantly($P<0.05$) reduced ulcer lesion indices (ULI) of 1.90± 0.8, 1.70 ± 0.1 and 1.50 ± 0.1 obtained for the 100, 200 and 400 mg/kg b.w respectively, as compared to the 4.67 ± 6.4 ulcer index obtained for the control dose dependently. The percentage ulcer inhibitions of 59.3, 63.6 and 67.9 % were obtained for the aqueous-methanol fraction at 100, 200 and 400 mg/kg b.w respectively. Comparatively, the ulcer lesion indices of groups treated with 100 and 400 mg/kg b.w of the aqueous-methanol fractions were significantly ($p<0.05$) lower than those of n-hexane and ethyl acetate fractions at equivalent doses. Thus, the percentage inhibitions produced by aqueous-methanol fraction were higher than those obtained for n-hexane and ethyl acetate fractions (Table 5).

Table 5: Effect of methanol extract fractions (n-hexane, ethyl acetate and aqueous-methanol) on Aspirin-induced ulcers in rats.

Treatment	Dose (mg/kg)	Ulcer Index	Percentage Inhibition (%)
Control (3% tween 80)	2 ml	4.67^{c}	-
n-Hexane	100	2.53 ± 0.3^{b}	45.8
	200	2.33 ± 0.3^{b}	50.0
	400	2.20 ± 0.3^{b}	52.9
Ethyl-acetate	100	2.28 ± 0.5^{ab}	51.5
	200	1.67 ± 0.6^{ab}	64.3
	400	1.77 ± 0.4^{b}	62.2
Aqueous methanol	100	1.90 ± 0.8^{a}	59.3
	200	1.70 ± 0.1^{a}	63.6
	400	1.50 ± 0.1^{a}	67.9
Cimetidine	100	1.03 ± 0.0^{a}	76.4

Values are expressed as mean ± SD (n=4).Means with different superscript are significantly different at ($P<0.05$), while mean values with the same superscript are not significantly different ($P>0.05$).

4. DISCUSSION

The result of this study revealed the presence of many phytochemicals of *Harungana madagascariensis* leaf extract such as flavonoids, phenols, glycosides and tannins. The analysis of *Harungana Madagascariensis* fractions provides important information about the phytochemical profile of the plant. This information can be used to identify potential medicinal or therapeutic components and to also understand the plant's chemical makeup. In Addition, analysis of the fractions can be used to identify and quantify the active phytochemical compounds, which can be useful for the standardization and quality control of plant [10]. In this study, the qualitative and quantitative analyses of *H. madagascarienssis* were determined comparatively for the various fractions (n-hexane, ethyl-acetate and aqueous-methanol)

In this study, Table 1 shows the qualitative analysis of the different fractions of *H. madagascarienssis*. The constituents that have been identified in fractions include flavonoids, alkaloids, cardiac glycosides, steroids, tannins, phenols, carbohydrate and saponins in different concentration across the various fractions. However, alkaloid was not detected in n-hexane fraction using wagner's method.

The qualitative analysis of *H. madagascarienssis* presents the difference in constituents of fractions due to the difference in the solvent used. In comparison to aqueous-methanol fraction, it extracted a greater number of phytochemicals than the other fractions (n-hexane and ethyl-acetate) (Table 1). This may be attributed to the impact of the polar nature of the solvent used on the plant. The result of this study corroborates the reports of several studies. Kuete, & Seukep, (2023) revealed the presence of carbohydrates, proteins, phenols, flavonoids, saponins, glycosides, alkaloids, terpenoids, and steroids in ethanol extracts of *H. Madagascariensis* during their study on the application of *Harungana Madagascariensis* leaf extracts as a source of antibacterial agent. These phytochemicals contained in *H. Madagascariensis* might be responsible for the anti-ulcerogenic and other medicinal properties possessed by the plant[20].

In quantitative phytochemical analysis of the fractions as presented in Table 2, the aqueous-methanol fraction had the most phenols. The result shows that a higher concentration of the phenols was recovered using aqueous-methanol fraction. Phenolic compounds comprise one (Phenolic acids) or more (polyphenols) aromatic rings with attached hydroxyl groups in their structures. Their antioxidant capacities are related to these hydroxyl groups in their structure. They constitute one of the groups of phytochemicals acting as antioxidants and inhibit specific enzymes that cause inflammatory disorder. Many flavonoids were shown to have

anti-oxidative activity, free radical scavenging capacity, hepato-protective, and anti-inflammatory activities. Their activities are structure dependent. The high flavonoids content obtained in this study supports its ethnomedicinal uses in treating infection and inflammatory related disorders (Shipa *et al*., 2022). The presence of these biological active compounds suggests that the plant could serve as a potential source of drugs and its secondary metabolites could exert some biological activities when taken by humans.

Relatively high concentrations of phytochemicals were found in the fractions. Comparactively, the aqueous-methanol fraction had higher concentrations and better activity which may be due to their polar nature.

The fractions (n-hexane, ethyl acetate and aqueous methanol) exhibited anti-ulcerogenic effect against both indomethacin and aspirin induced gastric ulcers (Table 4 and 5) with highest inhibition obtained at 400 mg/kg of aqueous-methanol comparable with that obtained for cimetidine, the reference anti-ulcer drug used. Chronic administration of non-steroidal anti-inflammatory drugs (NSAIDs) such as Aspirin, during the course of anti-inflammatory therapy, is often associated with the development of adverse gastrointestinal disorders such as gastric erosions, gastric or duodenal ulceration and other severe complications such as gastro-intestinal haemorrhage or perforation that often limited their wide spread clinical use (Elisha *et al*., 2016)Aspirin and Indomethacin are known to induce gastric ulcer by inhibition of prostaglandins which are cytoprotective to gastric mucosa, particularly due to the inhibition of cyclooxygenase pathway of arachidonic acid metabolism resulting in excessive production of leukotrienes and other products of 5-lipoxygenase pathway (Tai & McAlindon, 2021). In the stomach, prostaglandins play a vital protective role, stimulating the secretion of bicarbonate and mucus; maintain mucosal blood flow, and regulating mucosal cell turnover and repair(Asadu *et al*., 2025).Thus, the suppression of prostaglandins synthesis by aspirin and Indomethacin result in increased susceptibility to mucosal injury and gastric ulceration. Recently, reactive oxygen species (ROS) have also shown to play a critical role in the development of pathogenesis in acute experimental gastric lesions induced by NSAIDs (Bjarnason *et al*., 2018; Setshedi 2020). The search for alternative anti-inflammatory therapies devoid of deleterious effects on the integrity of gastric mucosa makes the research of natural products with simultaneous anti-inflammatory and anti-ulcerogenic activity important. In this study, the significant increase in ulcer index and in percentage of ulcerated mucosal surface area following oral administration of Indomethacin and Aspirin in the ulcerated rats may be attributed to either free radical formation or inhibition of prostaglandins synthesis. The fractions produced strong anti-ulcerogenic activities against Indomethacin and Aspirin and caused significant reduction in the ulcerative lesion index which is comparable with that obtained for Cimetidine. Reduction of the Indomethacin and Aspirin induced ulcer shown by this plant could be attributed to the high flavonoid content of the plant. In this work, result of the phytochemical analysis of fractions showed that the plant contains Phenols and Flavonoids in abundance and this could be the active constituents exerting the anti-ulcerogenic effect.

5. CONCLUSION

Results from this study, thus suggest that the plant could alleviate irritation and inflammation on the mucosal lining and thus reduce intestinal bleeding and ulcer most especially in aqueous-methanol fraction.

Consent for publication

Not Applicable in this section

Ethics approval and consent to participate

In adherence to the International and University standards for conducting research with animals, written ethical approval was obtained and preserved by the authors

Availability of data and material

The data analysed during the current study are available from the corresponding author on reasonable request

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