IN VIVO EVALUATION OF ANTIULCER ACTIVITY OF FRACTIONS OF METHANOL EXTRACT OF *SIDA ACUTA* (BURM. F.) LEAVES

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NOVEMBER, 2025

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NOVEMBER, 2025

CERTIFICATION

This is to certify that this work was carried out by **OHANADO IKECHUKWU KINDNESS** in the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin City.

…… ……

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Dr. Osayemwenre Erharuyi Date

(Project Supervisor)

…….. ……

Dr. Vincent Imieje Date

(Head of Department)

DEDICATION

This work is dedicated to Almighty God and my beloved family, for their unwavering support which has been my strength and motivation throughout my journey in Pharmacy school and to those who have lost their lives to peptic ulcer.

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This work was inspired and sustained by a number of entities worthy of note; First and foremost, I wish to thank God Almighty for His Grace to start and finish this work.

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ABSTRACT

Peptic Ulcer Disease (PUD) management faces rising challenges from antibiotic resistance and side effects of synthetic drugs. The plant *Sida acuta* Burm. f. is traditionally used in African Traditional medicine for gastrointestinal ailments. Preliminary studies confirmed its anti-ulcerogenic and antioxidant potential. This study aims to evaluate the *in vivo* curative anti-ulcer activity of the fractions of the crude methanol extract of *S. acuta* leaves and to identify the most potent fraction and its possible mechanism of action.

The study involved methanol extraction and subsequent fractionation of the extract using solvents of increasing polarity: n-Hexane, Dichloromethane, Ethyl acetate and Aqueous methanol. Phytochemical screening and antioxidant efficacy tests (DPPH, FRAP) were carried out to confirm the extract's components. The *in vivo* anti-ulcer activity was assessed in Wistar rats using the ethanol-induced ulcer model over five days. Groups received 100 or 200 mg/kg of each fraction, or 40 mg/kg Omeprazole (standard). Ulcer severity was determined via the Mean Ulcer Index (MUI) and percentage ulcer inhibition.

Qualitative analysis confirmed Alkaloids, Carbohydrate, Saponins, Terpenoids, Phenols, and Flavonoids. The crude methanol extract, containing significant levels of Total Phenol (38.61 mg GAE/g) and Total Flavonoid (20.70 mg QE/g), exhibited potent antioxidant activity, confirmed by the DPPH (IC_{50} of 32.26µg/ml) and FRAP (0.21 mM FeSO4 equivalent/g) assays. The *in vivo* anti-ulcer study, using the ethanol-induced ulcer model in Wistar rats, showed the standard treatment group achieved 73.95% ulcer inhibition. Among the fractions, the n-Hexane fraction exhibited the superior anti-ulcer potential, achieving 61.5% inhibition at 100 mg/kg. The DCM, EtOAC, and Aqueous-Methanol fractions showed moderate to low inhibition (39.70% to 57.07%).

The dominant activity concentrated in the non-polar n-Hexane fraction suggests that the primary anti-ulcer mechanism of *S. acuta* is rooted in cytoprotection (mucosal stabilization) rather than the polar antioxidant effects.

This finding successfully validates the traditional use of the plant in treatment of gastrointestinal ailments and identifies the n-Hexane fraction as the primary candidate for future bioassay-guided isolation of novel anti-ulcer compounds.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1. Natural Products and Their Place in Health Care

Modern medicine is a continuous process with regard to the search of effective therapeutic agents, and natural products continue to play a central role in the process of new drug discovery and development process. Natural products are defined as chemical compounds formed through biological activity, and derived from plants, fungi, animals, and microorganisms, and therefore are an unparalleled source of chemical diversity and biologically pre-tested structures (Newman and Cragg, 2024; Thomford *et al.,* 2018). Their contribution to the healthcare of the global population is twofold: on one hand, they have become the basis of traditional healthcare systems employed by a large part of the global population, and on the other hand, they have become the direct or indirect source of an enormous number of modern pharmaceuticals (Thomford *et al.,* 2018; Wu *et al.,* 2024). The significant importance of natural products in medicine in the past was determined by the discovery of key therapeutics, such as morphine (an opium poppy), quinine (Cinchona bark), and artemisinin (Artemisia annua) (Thomford *et al.,* 2018). This has had a lasting impact into the present day, as around one-third of all the Food and Drug Administration (FDA)-approved medications in the last 20 years are either derived directly or semi-synthetically out of natural products (Thomford *et al.,* 2018). Their complex and unusual chemical scaffolds are of great value, especially in the context of the development of treatments of complex diseases such as cancer and infectious diseases, in which a large portion (up to 60-80%) of existing agents owe their existence to a natural product (Thomford *et al.,* 2018; Wu *et al.,* 2024). The modern natural product research trend has been primarily predetermined by the significant health issues in the world, the most important of which is the growing crisis of Antimicrobial Resistance that now requires a sharp influx of new antibiotics (Thomford *et al.,*

2018; Wu *et al.,* 2024). In addition, the current technological innovations, such as High-Throughput Screening (HTS), state-of-the-art chromatographic separation, and computer algorithms, such as Artificial Intelligence (AI) and bioinformatics, are reinvigorating natural product drug discovery. Already, such combined tools are essential, offering unprecedented specificity to quick isolating, structural clarifying, and mechanistic knowledge of bioactive compounds, making natural resources a sustainable and fundamental route to innovating healthcare in the future (Kam *et al.,* 2023; Wu *et al.,* 2024).

1.2. Medicinal Plants as Source of Phytochemicals

Historically, medicinal plants have been the ultimate biochemical factories of the vast majority of therapeutic agents and this fact remains relevant in current drug discovery (Savickiene & Raudone, 2024). This complexity of the chemical structure i.e. the existence of phytochemicals is intrinsically connected to the therapeutic effect of these plants and the non-nutritious, biologically active compounds are characterized mainly as secondary metabolites (Lal *et al.,* 2024; Thomford *et al.,* 2018). The secondary metabolites are produced in pathways that are obligatory to for the plant to stay in her ecological niche unlike primary metabolites, which are essential to support basic plant life functions such as growth and photosynthesis (Savickiene and Raudone, 2024). This pressure of evolution has led to an early astounding number of structurally varied compounds, which have exclusive molecular attributes that in most cases demonstrate greater affinity to intricate biological targets than a host of synthetic molecules (Lal *et al.,* 2024). These phytochemicals fall mainly in four largest categories, which share different pharmacological effects:

- **Alkaloids**: Nitrogen-based compounds of which the most common are of the type of a basic nature, featured by strong physiological action, e.g., analgesic (e.g., morphine), antimicrobial, and anti-malarial (Thomford *et al.,* 2018).

- **Terpenoids (including Triterpenoids):** The isoprene units are the building blocks of this large category, which includes essential oils and structural components such as steroids. They possess high anti-inflammatory, anti-oxidant, and anti-cancer qualities (Lal *et al.,* 2024). An example of these is pentacyclic triterpenes, which form the active basis of new treatments of rare wound conditions (Chihomvu *et al.,* 2024).
- **Phenolic Compounds:** These have aromatic rings with a hydroxyl group attached to them and are the most prevalent group and include lignans, phenolic acids, and flavonoids. They are very effective antioxidants and metal chelators and have a protective effect against cardiovascular disease, cancer, and age-related disorders (Lal *et al.,* 2024).
- **Flavonoids**: This is a subclass of phenolic compounds commonly referred to as flavonoids and are characterized by high levels of anti-inflammatory, antimicrobial and antioxidant activities. They are known to give plants their rich colours and are widely researched on in relation to their therapeutic use in the treatment of chronic illnesses (Jain *et al.,* 2022).

  Modern drug discovery starts with the phytochemical profiling of medicinal plants, which uses state-of-the-art analytical methods, including chromatography and mass spectrometry, to fractionate, identify, and characterize these bioactive substances in a systematic manner. This is the fundamental initial phase of justifying traditional use and changing a plant extract into a quantifiable, clinically significant treatment, and is typically directed by established ethnomedical information (El-Gharib and Abdo, 2025). There is an enormous potential of new drug discoveries based on medicinal plants more so in overcoming new health challenges where synthetic libraries have failed (Savickiene and Raudone, 2024).

1.3. Review of *Sida acuta* Burm. f.

1.3.1. Taxonomy

Sida acuta Burm. f., commonly `known as broom weed or spiny-head **s**ida, is a plant of considerable ethnobotanical and pharmacological importance across tropical regions (Paarakh *et al.,* 2023). Belonging to the Malvaceae family, the genus *Sida* is notably large and complex, encompassing approximately 200 species found across tropical and subtropical environments (Thomford *et al.,* 2018). The specific epithet *acuta* refers to the acute or pointed apex of its leaves.

Sida acuta is classified under the following botanical hierarchy:

Kingdom: Plantae

Class: Dicotyledonae

Order: Malvales

Family: Malvaceae

Genus: Sida

Specie: *Sida acuta*

Common names: Wire weed, broomweed, sida, stubborn grass

Local names: Udo (igbo), Isekutu or iyeye (yoruba), Nsukere (efik), Kalkashin kwado (hausa)

Fig 1.1: Leaves and flower of *Sida acuta* (Julien *et al.,* 2012)

1.3.2. Botanical Description

Sida acuta is usually characterized as an upright, branched, perennial subshrub or shrub, usually having a height of 0.5 to 1.5 meters, and usually woody at the base with a thick and deep taper root (Paraakh *et al.,* 2023; Odewo *et al.,* 2025). The leaves are simple, alternate, usually lanceolate or linear-lanceolate in shape, and have sharp and regularly serrated margin and acute (pointed) apex (Thomford *et al.,* 2018). They can be described as having a large venation, and can possess sparsely scattered stellate (star-shaped) hairs (Paarakh *et al.,* 2023). The flowers are small, but most commonly yellow, and they occur singly or in small groups within leaf axils. They are are bisexual, actinomorphic (radially symmetrical), and with five petals which are joined only slightly at the base (Thomford *et al.,* 2018). The fruit is a schizocarp, splitting into 5-8 single seed mericarps when mature. The peculiarity is that every mericarp is birostrate, and two rigid and pointed awns (spines) are located at the top (Paarakh *et al.,* 2023; Thomford *et al.,* 2018).

1.3.3. Geographical Distribution

Although considered the native of the Central American and Mexican area, *Sida acuta* has obtained a pantropical distribution, or, in other words, being located all over the tropical and subtropical areas of the globe (Thomford *et al.,* 2018). It grows in South Asia, the Americas, and is a widespread phenomenon throughout the African continent, such as West Africa, where nations such as Nigeria use the plant extensively traditionally (Paarakh *et al.,* 2023; Thomford *et al.,* 2018).

1.3.4. Ecology and Growth

It is a species whose natural habitat is the seasonally dry tropical biome and which is highly adaptable and resilient (Royal Botanical Gardens, 2024). It can be ecologically found, among others, in disturbed habitats, like; roadsides and waste places, farm lands (where it is commonly thought a ubiquitous weed), saw edges and empty open fields (Paarakh *et al.,* 2023; Thomford *et al.,* 2018). It can thrive well at sea level up to 1500 meters, highly rainy regions, and will look well in locations that are full-sun and fully exposed (Thomford *et al.,* 2018). The plant reproduces by seed majorly.

1.3.5. Cultivation

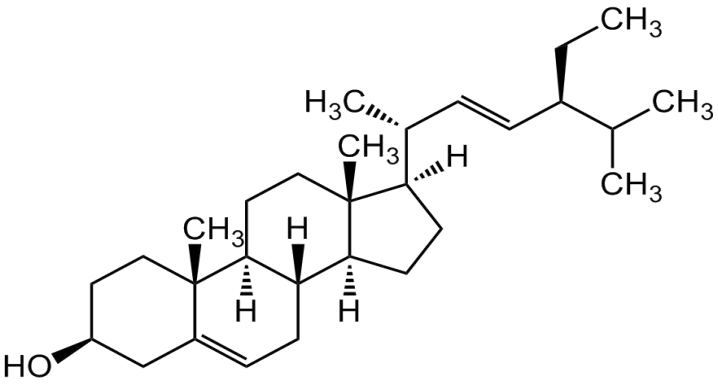
Though *Sida acuta* is considered a weed in the agricultural setting throughout the world (Thomford *et al.,* 2018), its cultivation in Africa and Nigeria in particular is not necessarily formalized in large scale farms to the purpose of commercial interests. Instead, it is commonly cultivated as an opportunity, or harvested wild since it thrives well in most of the neglected lands and farmlands across the areas (Odewo *et al.,* 2025). In Nigeria, the plant is commonly found and harvested in the wild to satisfy its wide range of application in traditional medicine in treating ailments like high blood pressure, gonorrhea, malaria, and

general inflammatory diseases, and it is culturally and medically significant in the area (Paarakh *et al.,* 2023).

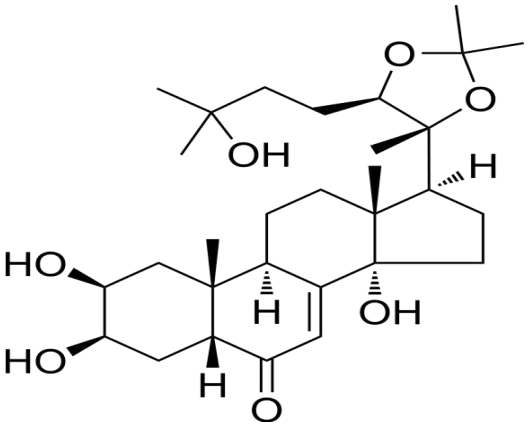
1.3.6. Phytochemistry

Sida acuta is a rich source of numerous phytochemicals. Alkaloids are significantly present which are probably the most researched compounds in *S. acuta*. The plant is also said to have indoloquinoline alkaloids, the most common ones being cryptolepine, and their derivatives quindoline and cryptolepinone (Paarakh *et al.,* 2023). There are also other alkaloids, such as b-phenethylamines, quinazolines, carboxylated tryptamines, and choline and betaine (Thomford *et al.,* 2018). Cryptolepine is of great importance as its anti-malarial and cytotoxic properties are already known (Paarakh *et al.,* 2023). Present are phenolic compounds and flavonoids. These families occur in large amounts, and their presence brings about the high antioxidant ability of the plant (Raimi-Oyekanmi *et al.,* 2016 Paarakh *et al.,* 2023). Research has also measured excessive amounts of total flavonoids and phenolics, both of which are associated with its anti-inflammatory and anti-free radical effects(El-Gharib and Abdo, 2025). Certain compounds such as scopoletin (a coumarin), and other flavonoids and phenolic acids were isolated (Thomford *et al.,* 2018). Saponins (a category of triterpenoid glycosides), terpenoids, and steroids (β-sitosterol and ecdysterone) are all active substances that are regularly reported in the extracts of the leaves, stem, and root (Raimi-Oyekanmi *et al.,* 2016; Paarakh *et al.,* 2023). Such compounds are usually related to anti-inflammatory, hepatoprotective and anti-ulcerogenic effects (Paarakh *et al.,* 2023). Other Constituents such as tannins, cardiac glycosides, traces of phlobatannins and lignans have also been recommended as other important phytochemicals (Raimi-Oyekanmi *et al.,* 2016; El-Gharib and Abdo, 2025). The diverse and abundant phytochemical composition of *Sida acuta,* especially high concentration of alkaloids, flavonoid and terpenoids compounds, offers a

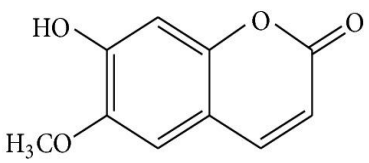
solid chemical explanation of its use as an anti-ulcer agent and explains the interest of the scientific community in the therapeutic potential.



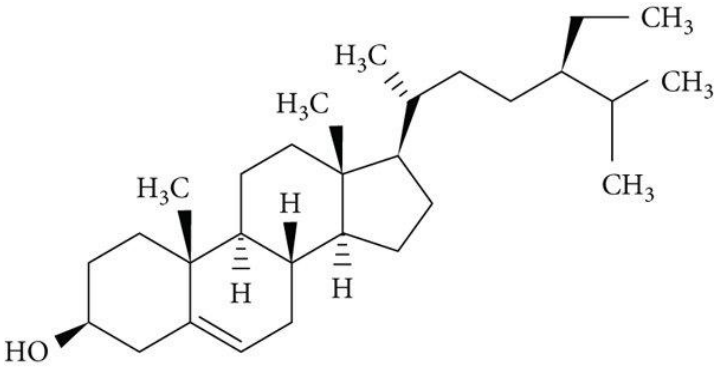
Stigmasterol



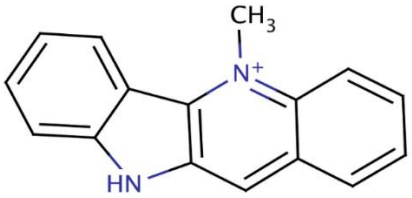
Ecdysterone



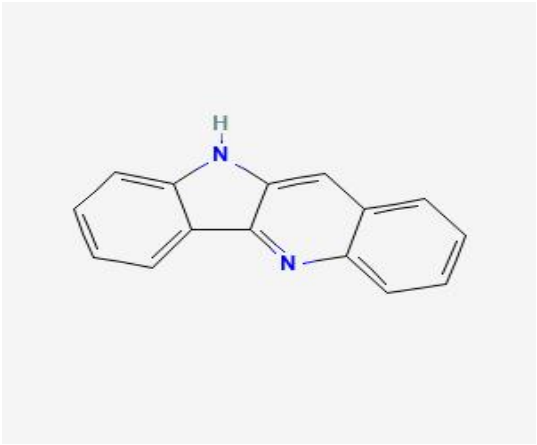
Scopoletine



β-Sitosterol



Cryotolepine



Quindoline

Figure 1.2: Some chemical compounds isolated from *S. acuta*

1.3.7. Ethnomedicinal uses of *S. acuta*

The ease of its distribution in tropical and subtropical locations is directly attributed to the large and diverse ethnobotanical history of *Sida acuta*. In traditional African, Asian, and American medicine, nearly every part of the plant is used, such as the aerial, roots, and leaves (Paarakh *et al.,* 2023; Pimple, 2024). This historical application is the main basis of justification of the pharmacological studies in modern times such as the present research on its anti-ulcer property.

Gastrointestinal and Anti-Ulcer Uses: It is important to note that the plant has been traditionally mentioned in the treatment of ulcers and other digestive diseases in many countries, such as Central America and Nigeria (Paarakh *et al.,* 2023; Malairajan *et al.,* 2006). Oil is usually smeared on leaves or used as a poultice on external visible sores and decoctions are taken as internal ulcers (Malairajan *et al.,* 2006). It is a strongly prized root as a stomachic to help digest and even cure chronic bowel issues (Pimple, 2024).

Anti-infective and Anti-Inflammatory Uses: *S. acuta* is among the most commonly mentioned herbal remedies against fever (antipyretic) and malaria across the West African region and other malaria endemic areas, such as Nigeria (Pimple, 2024; Paarakh *et al.,* 2023). They are used as a paste or in combination with oil and applied to the skin to treat wounds, skin diseases, and scabies and also to treat sores, as they are traditionally known to have wound healing and antimicrobial effects (Konate *et al.,* 2023; Malairajan *et al.,* 2006).

Reproductive and Urinary Health: It is traditionally applied as a diuretic and demulcent in gonorrhoea, urinary infections, and leucorrhoea (Pimple, 2024; Malairajan *et al.,*2006). The plant is reported to have aphrodisiac effects and is suggested to be effective in the treatment of azoospermia (low sperm count) and oligospermia; it is being used as a decoction of the leaves (Paarakh *et al.,* 2023; Pimple, 2024).

1.3.8. Pharmacological Significance and Activity of *Sida acuta*.

The wide-ranging ethnomedical application of *Sida acuta* has triggered serious scientific research justifying a diverse range of pharmacological actions. The pre-clinical research has already verified the plant to treat a variety of conditions, and the identified effect is most frequently associated with the high content of secondary metabolites (Jiménez-Ferrer *et al.,* 2025; Paarakh *et al.,* 2023).

- **Anti-Ulcer and Gastroprotective Activity:** The fundamental importance of *Sida acuta* to the project is that it has been shown to have anti-ulcer (ulcer-preventing) and ulcer-healing (gastroprotective) activities through multiple in vivo animal models. The extract has shown:
  Anti-Secretory Mechanism: Decrease in the level of gastric acid secretion, gastric volume, and free acidity in the models of pylorus ligation (Kukarnil and Kale, 2010).

  Cytoprotective Mechanism: Mucosal barrier protection against chemical insults, which are explained by the effect of flavonoids, tannins, and triterpenoids (Kukarnil and Kale, 2010)

  Anti-Helicobacter pylori Effect: The bacterial load of H. pylori was reduced significantly in the mouse stomach tissue, and tissue repair and improvement of the healing of ulcers were observed (Ekwealor *et al.,* 2020).
- **Hepatoprotective Activity:** The root extracts have high hepatoprotective properties against chemically-induced liver damage and prevent the increase in serum hepatic enzyme levels (ALT, AST, ALP) and total bilirubin (V. R. S. K. A. H. R. J. R., 2009). The move has a robust association with the fact that the plant has a high antioxidant activity (V. R. S. K. A. H. R. J. R., 2009).

- **Anti-inflammatory and Analgesic Activity:** The extracts possess significant anti-inflammatory activity, shown by inhibiting protein denaturation (Shankar *et al.,* 2021). The presence of phytochemicals like flavonoids and terpenoids helps to inhibit inflammatory mediators and pathways, which is critical in ulcer pathology where inflammation is a key feature (Jiménez-Ferrer *et al.,* 2025). Furthermore, analgesic (pain-relieving) properties have been reported, validating its traditional use for rheumatism and body aches (Paraakh *et al.,* 2023).
- **Antioxidant Activity Activity:** *S. acuta* exhibits potent antioxidant activity, scavenging free radicals such as DPPH and H_2O_2 in a concentration-dependent manner (Shankar *et al.,* 2021). The richness of phenolics and flavonoids in the extract underpins this action, which is vital for preventing oxidative stress damage associated with chronic diseases, including the pathogenesis of gastric lesions (Paraakh *et al.,* 2023).
- **Antimicrobial and Antimalarial:** Scientific evidence strongly supports the traditional use of *S. acuta* as an anti-infective agent. It shows significant antimicrobial activity against a broad spectrum of Gram-positive and Gram-negative bacteria (Malairajan *et al.,* 2006). Its anti-plasmodial activity against *Plasmodium falciparum* has been well-documented, with the activity often traced to its alkaloid content, specifically cryptolepine (Adesina., 2020).
- **Antidiabetic and Hypoglycemic Activity:** The leaf and root aqueous and methanolic extracts have been shown to have a strong antihyperglycemic effect on diabetic animals. Extracts reduce Fasting Blood Glucose (FBG), have a beneficial effect on glucose tolerance (OGTT), and can possess a beneficial effect on key carbohydrate-digesting enzymes like alpha-amylase and alpha-glucosidase (Panda *et al.,* 2015; Ukpo *et al.,* 2020; IJPC, 2020).
- **Safety Profile:** Acute toxicity studies often confirm that crude extracts of *S. acuta* exhibit a high degree of safety, with no observed mortality at high doses (e.g., up to 2000mg/kg in rats), supporting its safe use in traditional medicine (Paraakh *et al.,* 2023).

1.4. Peptic Ulcer Disease (PUD)

PUD is a common and potentially severe gastrointestinal complication which is a break in the inner lining of the gastrointestinal (GI) tract extending to the muscularis mucosa and usually occurs in the stomach or in the proximal duodenum (Ghonge *et al.,* 2025; Malik *et al.,* 2024). PUD is a dysfunction of the dainty balance between the obnoxious elements (e.g., gastric acid, pepsin) and defensive (e.g., mucus, bicarbonate, blood flow, prostaglandins) components of the gastroduodenal mucosa (Brzozowski and Bilski, 2025).

1.4.1. Types of Peptic ulcer disease

The main classification of PUD is based on its anatomic location:

Gastric Ulcer (Stomach Ulcer): It occurs in the stomach lining. Several hours after food consumption, pain can also be reported to be aggravated when food enters the stomach and activates acid production in the stomach (Malik *et al.,* 2024).

Duodenal Ulcer: It is located in the duodenum, which is the first part of the small intestines. Suffering is usually 2-3 hours after a meal or can disturb the sleep of the patient at night since the effect of buffering of food has passed (Ghonge *et al.,* 2025).

1.4.2. Etiology

The two significant scientifically proven factors with which PUD development is overwhelmingly related are:

- **Helicobacter pylori (*H. pylori*) Infection:** It is a Gram-negative bacterium and a major etiologic agent, especially with duodenal ulcers. H. pylori infects the gastric mucosa, synthesizing urease, which releases ammonia locally neutralizing acid and permitting the bacteria to survive, leading to chronic inflammation. This damage and inflammation mediated by cytokines eventually undermines the mucosal wall (Malik *et al.,* 2024).

- **Use of Non-Steroidal Anti-inflammatory Drug (NSAID):** The second commonest cause of PUD, especially gastric ulcers, is chronic use of Non-Steroidal Anti-inflammatory Drug (NSAID), including aspirin and ibuprofen. The major mechanism of NSAIDs ulcerogenic activity is by systemic inhibition of the Cyclooxygenase-1 (COX-1) enzyme. The production of prostaglandins, which are important in upholding the defenses of the stomach, such as the mucus secretion and mucosal circulation, are usually facilitated by COX-1 (Malik *et al.,* 2024).

1.4.3. Risk Factors Associated with PUD

In addition to the main causes of mentioned above, several risk factors could predispose individuals to peptic ulcer (Kishore *et al.,*2011). Gastric ulcers are more common in older persons. Some individuals may be genetically predisposed to developing the disease, making them more susceptible. The use of some medications such as corticosteroids can increase the risk of having PUD. Environmental factors such as, lifestyle choices, living conditions can also impact ulcer risk.

1.4.4. Signs and Symptoms

Typical symptoms include:

- Epigastric Pain: It is the most typical symptom, which is commonly referred to as a dull, gnawing, or burning pain in the upper abdomen (Mayo Clinic, 2024).
- Feeling too full soon while eating a meal
- Bloating
- Belching
- Nausea and vomiting
- Gastrointestinal bleeding or perforation as seen in complicated PUD (Mayo Clinic, 2024; Malik *et al.,* 2024).

1.4.5. Diagnosis and Treatment

Diagnostic methods include:

Endoscopy (Upper GI) which enables one to look at the ulcer directly, biopsy to exclude malignancy, and sample H. pylori testing (Vakil, 2024). H. pylori Testing entails diagnostic non-invasive tests such as Urea Breath Tests or Stool Antigen Tests that are usually performed to determine the presence of infection (Cleveland Clinic, 2025).

Conventional Treatment include:

Acid Suppression: The current cornerstone is the Proton Pump Inhibitors (or PPIs) (e.g., Omeprazole) and they act by potently inhibiting the secretion of acid (Johns Hopkins Medicine, 2023). Less potent are Histamine-2 Receptor Antagonists (H2RAs) (e.g., Famotidine), which are also used as well as antacids to neutralize stomach acid (Mayo Clinic, 2024). Eradication of H. pylori Triple or quadruple therapy is used, which includes a PPI and two or three antibiotics (e.g., Clarithromycin, Amoxicillin) during 7 to 14 days (Malik *et al.,* 2024).

Mucosal Protection: Cytoprotective agents (such as Sucralfate) give a protective coating to the ulcer crater and promote the local defense mechanisms (Mayo Clinic, 2024).

The drawbacks of standard treatment, including the rise in cases of antibiotic resistance to H. pylori, the possibility of side effects caused by using PPI over prolonged periods of time, and the affordability of combination therapies allow noting the necessity to continue the search of novel, safe, and effective anti-ulcer agents (Ghonge *et al.,* 2025).

1.4.6. Curative Gastric ulceration Models

The testing of anti-ulcer agents especially using natural products scientifically depends on good animal models. The proposed models usually emulate the different pathogenic processes that cause Peptic Ulcer Disease (PUD) in humans (Satapathy et al, 2024).

- **Ethanol-Induced Gastric Ulcer Model:** It is the most commonly employed model of acute screening of natural products (Beiravand, 2024). It replicates the acute human gastric injury through severe haemorrhagic necrosis, oxidative stress, and mucosal injuries (Saini *et al.,* 2024; Shin *et al.,* 2024). It is superior in determining the compounds that possess cytoprotective and anti-oxidant properties (Taher *et al.,* 2024).
- **NSAID-Induced Model:** This is a model that emulates the clinical reality of ulcers that occur because of Non-Steroidal Anti-inflammatory Drugs (NSAIDs) by systemically obstructing the synthesis of prostaglandins, thereby undermining the protective mucosal lining (Satapathy *et al.,* 2024).
- **Pylorus Ligation (Shay) Model:** This is one of the most important curative models in which the pylorus is surgically ligated and does not empty acid, resulting in the accumulation and autodigestion of gastric wall. It cannot be done without testing agents of anti-secretory activity (Ahmed *et al.,* 2022).
- **Helicobacter pylori Infection Model:** The models required are special so that antimicrobial effect of an anti-ulcer agent can be confirmed against the bacterial cause of PUD (Patil *et al.,* 2025). The applicability of this model to the present investigation is proven by the efficacy of *Sida acuta* in reducing the burden of H. pylori (Ekwealor *et al.,* 2020).

All these models enable researchers to identify the precise mechanism of action, i.e. cytoprotection, acid neutralization, antimicrobial effect etc., of an extract or its fractions that cause an anti-ulcer effect.

1.5. Justification of the Study

The fact that, to date, new, effective and cost-efficient anti-ulcer therapeutics are still required is a direct result of the challenges that have been facing the traditional treatment of Peptic Ulcer Disease (PUD). The fundamental reasons, which lead to the in vivo testing of anti-ulcer activity of fractions of crude methanol extracts of *Sida acuta* leaves include:

- **Shortcomings of Conventional Therapies:** PPIs Conventional PUD treatments are increasingly challenged by developing resistance to antibiotics working on H. pylori and long-term use of these drugs poses the additional risk of bone fracture (Ghonge *et al.,* 2025; Malik *et al.,* 2024). This puts a desperate situation of safe, bio-based alternatives.
- **Scientific Evidence of *Sida acuta*:** *S. acuta* has a good ethnopharmacological foundation due to the long history of its use in gastrointestinal diseases, ulcers, and diarrhea (Paraakh *et al.,* 2023; Pimple, 2024). Its antioxidant and anti-inflammatory effects have already been confirmed in preliminary research, which showed that it has anti-ulcer, anti-secretory, cytoprotective, and anti-H. pylori activity (Ekwealor *et al.,* 2020; International Journal of ChemTech Research, 2015).
- **Research Gap (Fractionation):** The activity of the crude extract is known, but it is not known which specific compounds or fractions contribute to the activity of the crude extract. This work fills this important gap by separating and analysing the fractions of the crude methanol extract. This is required to focus the bioactive principles, lower the toxicity and to determine the particular chemical class that can be an effective novel drug lead to the standardization of a safe, effective phytomedicine.

1.6. Aim of the Study

The aim of the study was to assess the curative antiulcer effect of the different fractions of the crude methanol extracts of the leaves of *Sida acuta* Burm. f.

1.7. Specific Objectives of the Study.

The specific objectives of the study were to:

- Fractionate the crude methanol extract of *Sida acuta* Burm. f. Leaves.
- perform phytochemical screening to determine and quantify the phytochemical components of *S. acuta* leaf extract.
- evaluate the antioxidant activity of the crude extract using different models;
- To assess and compare the curative anti ulcer potential of the fractions of the crude methanol extract of leaves of *S. acuta* using the ethanol-induced ulcer model.

CHAPTER TWO

MATERIALS AND METHODS

2.1. Materials

2.1.1. Glassware and Laboratory consumables

Measuring cylinder (10 mL, 50 mL, 100 mL, 2000 mL), conical flasks (250 mL, 500 mL), beakers (100 mL, 250 mL, 500 mL), separating funnel, glass jars, round-bottom flasks, stirrer, funnel, test tubes, glass jars, spatula, glass bottles (All procured from Pyrex®), porcelain dish, test tube rack, test tube holder, sample bottles, markers, gloves, cotton wool, nose masks, masking tape, retort stand and clamp, aluminium foil paper, filter paper, forceps, pipettes, and micropipette tips, syringes (1 mL and 5 mL), orogastric tube, cages, animal feed, forceps, surgical scissors, stainless plate, cardboard, thumb push pins, hand lens.

2.1.2. Reagents

The reagents used in this research, all of analytical grade, include: methanol (99.8%) (Loba Chemie®), dichloromethane, ethyl acetate, n-hexane, distilled water, hydrochloric acid (Molychem®), glacial acetic acid (GHTECH®), concentrated sulphuric acid (Molychem®), diluted sulphuric acid, Dragendorff's reagent, Hager's reagent, Wagoner's reagent, Mayer's reagent, sodium hydroxide (Loba Chemie®), sodium acetate trihydrate(Merck®), gelatin (Kermel®), ferric chloride hexahydrate (Xi'an tian mao chemicals®), ascorbic acid (Sigma Aldrich®), 10 mM TPTZ (2,4,6- tripyridyl-s-triazine) (Molychem®), DPPH (2,2-diphenyl-1-picrylhydrazyl) (Molychem®), Quercetin (Aldrich®), Benedict's reagent, and alpha naphthol, ferrous sulphate (Molychem®), tween-80.

2.1.3. Equipment

Rotary evaporator coupled to temperature-controlled water bath (Bibby Scientific Limited®), UV-Visible spectrophotometer (PG Instruments LTD®), weighing balance (Ohaus®), mechanical grinder, drying oven, refrigerator

2.1.4. Plant material

Harvested leaves of *Sida acuta*

2.1.5. Study centres

The study was carried out in the following centres: Department of Pharmacology and Toxicology, University of Benin, Benin City. Natural Product Research Laboratory, Department of Pharmaceutical chemistry, University of Benin, Benin City.

2.2. METHODS

2.2.1. Sample Collection and Preparation

Fresh leaves of *Sida acuta* was harvested from Ekosodin community in Ovia North-east, Benin City in on 12 September, 2025 and identified by a taxonomist Prof. Henry of the Department of Plant Biology and Biotechnology, University of Benin and a voucher number of UBH- S454 was issued.

The leaves were air dried away from direct sunlight for 5 days; the dried leaves were subsequently ground into a dry fine powder giving a total weight of 1kg. The powder was subsequently stored in an air-tight container and labelled properly while awaiting further analysis.

2.2.2. Extraction

Extraction was carried out on the plant sample to separate the compounds present from the dried leaves. The powdered sample 1kg (1000g) was weighed accurately, 500g each into a Mason jar and macerated with 2.5 litres each of methanol for 6 days. The sample was stirred with a glass rod every 24 hours for the 6 days maceration period and the solid remnant was filtered with a filter paper. The combined extract was concentrated in a vacuum rotary evaporator at 40ºC and air-dried at room temperature for 8 days to obtain the solid crude extract. The sample was adequately covered during this process to avoid contamination of the extract. Based on the original weight of the sample, the dry mass obtained was weighed and the percentage yield was calculated.

2.2.3. Phytochemical Screening

For phytochemical screening, an aqueous extract was made from the powdered plant sample by adding 5 g of the powdered sample to a beaker containing 75 mL of distilled water. The mixture was boiled for 30 minutes at 100ºC for 30 minutes. The extract was filtered while it was still hot and the filtrate was used to test for secondary plant metabolites in accordance with standard protocols.

2.2.3.1. Qualitative Tests for Secondary Metabolites

General Test for Alkaloids

- 2 mL of the filtrate was shaken with 2 drops of Dragendorff's reagent.

  An orange precipitate indicated the presence of alkaloid

- 2 mL of the filtrate was shaken with 2 drops of Wagner's reagent

  A brown precipitate indicated the presence of alkaloids

- 2 mL of the filtrate was shaken with 2 drops of Hager's reagent

  A yellow precipitate indicated the presence of alkaloids

- 2 mL of the filtrate was shaken with 2 drops of Mayer's reagent

  A milky precipitate indicated the presence of alkaloids

Test for Carbohydrates

- **Molisch's Test**

  Two drops of 1% alcoholic alpha naphthol to 2 ml of the filtrate followed by slow addition of concentrated sulphuric acid at a slanted position. A purple ring at the interface

  indicated the presence of sugars.

Test for Reducing sugars

- **Fehling's Test**

  1 mL each of Fehling's A and B solution to 2ml of the filtrate. The mixture was subsequently heated in a boiling water bath for 5 minutes.

  A brick-red precipitate was indicative of reducing sugars.

Test for Saponins

- **Frothing Test:**

  10 mL of distilled water were added to 1mL of filtrate and then forcefully agitated for a minute

  A stable froth which persisted for 5 minutes indicated the presence of saponins.

Test for Tannins

- **Gelatin Test**

2 mL of 1% gelatin solution to 2mL of the filtrate

Formation of a buffy white precipitate indicated the presence of tannins.

Test for Terpenoids

- **Salkowski's Test**

5 mL of the filtrate was mixed with 2 mL of chloroform and concentrated H_2SO_4 was carefully added (drop wise) to form a layer.

A reddish-brown ring at the interface was an indicator for the presence of terpenoids

Test for Phenols

To 2 mL of the filtrate, 5 mL of distilled water was added, followed by two drops of 5% ferric chloride solution and observed for green colour change which was indicative of the presence of phenols.

Test for Flavonoids

Few drops of lead acetate solution were added to 2 mL of the filtrate.

2.2.3.2. Quantitative Tests for Secondary Metabolites

- **Total Phenol Content**

The total phenol content of the plant extract was evaluated using the method described by Kim *et al.,* 2003. 4.5 mL of deionized distilled water was mixed with 0.5 mL of Folin Ciocalteu's reagent (which had been diluted with water 1:10, v/v) and then added to 0.5 ml of 1000 μg/mL extract solution. The tubes were shaken and allowed to sit at room temperature

for 5 minutes then 5 mL of 7% sodium carbonate and 2 mL of deionized distilled water were added. The samples were mixed and then allowed to sit at room temperature for 90 minutes. A spectrophotometer was used to measure the absorbance at 750 nm. Milligrams of gallic acid equivalents (GAE) per gram of extract (mg GAE/g extract) was the unit of measurement used to represent the total phenolic content. Gallic acid was used to create the standard curve in six different strengths (12.5, 25, 50, 75, 100 and 150 mg/L) and triplicate readings were taken (Kim *et al.,* 2003).

- **Total Flavonoid**

The method described by Ebrahimzadeh et al. (2008) was used to estimate the total flavonoid contents. Methanol (1.5 mL) and 0.5 mL of extract sample (1 mg/mL) were mixed together and then 0.1 mL of 10% aluminium chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water were added sequentially. The mixture was incubated for 30 minutes at room temperature and a spectrophotometer was used to measure the absorbance at 415 nm. Milligrams quercetin equivalents (QE) per gram of extract (mg QE/g extract) were used to express the results. Six distinct strengths of quercetin (12.5, 25, 50, 75, 100, and 150 mg/L) were used to create the standard curve and all readings were done in triplicates (Ebrahimzadeh *et al.,* 2010).

2.2.4. Antioxidant Screening

The antioxidant activity of the crude methanolic extract of *Sida acuta* was evaluated using Two different models; 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay and Ferric Reducing Antioxidant Power (FRAP) Assay.

- **DPPH Assay**

The scavenging activity of the crude methanol extract on DPPH radical was evaluated using the method described by Jain *et al.,* (2008). Extract (3.0 mL) in methanol containing 0.01 to 0.2 mg/mL of the extract was mixed with 1.0 mL of a solution of 0.1 mM DPPH in methanol. The mixture was thoroughly shaken and was allowed to sit in the dark at room temperature for 30 minutes. Using spectrophotometry, the mixture's absorbance was determined at 517 nm. The reference standard was ascorbic acid. The ability to scavenge DPPH radical was calculated by the following equation:

DPPH radical scavenging activity (%) = [(A0−A1)/(A0)] × 100

Where; A0 was the absorbance of DPPH radical + methanol, A1 was the absorbance of DPPH radical + sample extract /standard. The 50% inhibitory concentration value (IC_{50}) is the effective concentration of the sample that can scavenge 50% of the DPPH free radical (Jain *et al.,* 2008).

- **FRAP Assay**

The FRAP assay was done according to Benzie and Strain (1996) with some modifications. Stock solutions were prepared containing 20 mM $FeCl_3.6H_2O$ solution, 10 mM TPTZ (2, 4, 6- tripyridyl-s-triazine) solution in 40 mM HCl, 300 mM acetate buffer (3.1 g $C_2H_3NaO_2.3H_2O$ and 16 mL $C_2H_4O_2$), and pH 3.6. 100 mL of acetate buffer, 10 mL of TPTZ solution, and 10 mL of $FeCl_3.6H_2O$ solution were combined to create the working solution, which was then heated to 37°C before use. 1.5 mL of 0.1 mg/ml of the crude extract was measured into a test tube and 3 ml of FRAP working solution was added, it was incubated in the dark for 30 minutes and the absorbance of the reaction mixture was measured at 593 nm. For construction of the calibration curve, five concentrations of $FeSO_4.7H_2O$ (0.1, 0.4, 0.8, 1, 1.2, 1.5 mM) were used and the absorbance values were measured as for sample solutions and triplicate readings were taken (Benzie & Strain, 1996).

2.2.5. Solvent-Solvent Partitioning (Fractionation)

Extract (60 g) was weighed into a mortar and dissolved with a 1:1 mixture of methanol and water (200 mL of water and 200 mL of methanol) The sample was vigorously triturated and allowed to dissolve properly before being transferred into a separating funnel held by a retort stand and clamp. n-Hexane was then introduced into the separating funnel and was gently mixed and allowed to settle. The n-Hexane fraction was at the top of the resultant two layers and was collected and covered in a clean glass jar. The procedure was repeated until the n-hexane layer was clear. The solvents dichloromethane and ethyl acetate were used for fractionation following the same procedure as for n-Hexane. The remaining fraction was used as the aqueous methanol fraction. All fractions were stored in sterile, sealed glass jars. The fractions were subsequently concentrated at 40°C in a rotary evaporator and the resultant dried mass was weighed and their percentage yield calculated

2.2.6. Animals

50 adult Wistar rats of average weight 180 g were procured from an accredited animal facility and housed in plastic cages with wood shavings as bedding. The rats were acclimatized to the laboratory conditions for a period of three weeks, during which they were provided with standard rodent pelletized finisher feeds and *ad libitum* access to water. Care was taken to maintain a standard temperature of 25 ± 2°C throughout the acclimatization period. The wood shavings in the cages were replaced daily to ensure cleanliness and hygiene, and the rats were closely monitored to ensure their well-being and adaptation to the new environment. All experimental procedures were conducted in strict adherence to the guidelines outlined in the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH publications No. 86-23, revised in 2011). Ethical approval was requested from the Faculty of Pharmacy Ethical Committee.

2.2.6.1. Experimental Design

Following the acclimatization phase, the rats were individually weighed using a precision weighing balance, and markings were applied for easy identification. Subsequently, the rats were divided into experimental groups, with each group comprising five rats. The experimental groups included the Hexane group (administered dose of 100 mg/kg, 200 mg/kg, of the n-hexane fraction), the DCM group (administered doses of 100 mg/kg, and 200 mg/kg, of Dichloromethane fraction), the EtOAC group (administered doses of 100 mg/kg, and 200 mg/kg, of Ethyl acetate fraction), the Aqueous methanol group (administered doses of 100 mg/kg, and 200 mg/kg of aqueous methanol fraction), standard treatment group (administered 40 mg/kg of Omeprazole), and a negative control group (non-treatment group). The rats were then distributed evenly among ten cages, with each cage housing rats for an experimental group and marked distinctively for easy identification.

2.2.6.2.Preparation of Fractions into Administrable Dosage Forms

To prepare the plant fractions into administrable dosage forms suitable for oral administration, a 5mL solution was formulated each day, pre-calculated, and determined to be sufficient to cover the five-day treatment duration for ameliorating induced ulceration. Each millilitre (ml) of the solution was standardized to contain 25 mg or 50 mg of the respective plant extract depending on the dose.

2.2.6.3. Preparation of 25 mg/mL and 50 mg/mL of Hexane fraction into Oral Dosage Form

Considering the calculated doses for the n-hexane fraction administered to the experimental groups (100 mg/kg and 200 mg/kg), a total volume of 5 mL per day was determined based on the weight of the rat in kilograms (kg). Multiplying this volume by the total treatment

duration of five days yielded a total volume of 25 mL with each ml containing 25mg and 50 mg of the fraction for the respective doses.

The n-hexane fraction of dose 100 mg/kg was formulated into a suspension using 10% tween-80 as the emulsifying agent. Initially, 125 mg of the n-hexane fraction was weighed using an analytical balance into a porcelain dish then 0.5 mL of tween-80 was added and the mixture was triturated thoroughly. Distilled water (1 mL) was added to dissolve the fraction. Gradually, graded amounts of distilled water (1 mL and 2 mL) were added to the solution and mixed thoroughly. The resultant solution was then transferred into a pre-calibrated plain bottle additional amount of distilled water was added to bring the volume up to 5 mL. For the 200 mg/kg dose the same procedure was repeated using a weight of 250 mg of then-hexane fraction.

2.2.6.4. Preparation of 25 mg/mL and 50 mg/mL of Dichloromethane (DCM) fraction into Oral Dosage Form

Considering the calculated doses for DCM fraction administered to the experimental groups (100 mg/kg and 200 mg/kg), a total volume of 5ml per day was determined based on the weight of the rat in kilograms (kg). Multiplying this volume by the total treatment duration of five days yielded a total volume of 25 mL with each ml containing 25 mg and 50 mg of the fraction for the respective doses.

The DCM fraction of dose 100 mg/kg was formulated into a suspension using 10% tween-80 as the emulsifying agent. Initially, 125 mg of dichloromethane fraction was weighed using an analytical balance into a porcelain dish then 0.5 mL of tween-80 was added and the mixture was triturated thoroughly. Distilled water (1 mL) was added to dissolve the fraction. Gradually, graded amounts of distilled water (1 mL and 2 mL) were added to the solution and

mixed thoroughly. The resultant solution was then transferred into a pre-calibrated plain bottle additional amount of distilled water was added to bring the volume up to 5 mL. For the 200 mg/kg dose the same procedure was repeated using a weight of 250 mg of the DCM fraction.

2.2.6.5. Preparation of 25 mg/mL and 50 mg/mL of Ethyl acetate (EtOAC) fraction into Oral Dosage Form

Considering the calculated doses for the Et fraction administered to the experimental groups (100 mg/kg and 200 mg/kg), a total volume of 5ml per day was determined based on the weight of the rat in kilograms (kg). Multiplying this volume by the total treatment duration of five days yielded a total volume of 25 mL with each ml containing 25 mg and 50 mg of the fraction for the respective doses.

The ethyl acetate fraction of dose 100 mg/kg was formulated into a suspension using 10% tween-80 as the emulsifying agent. Initially, 125 mg of the EtOAC fraction was weighed using an analytical balance into a porcelain dish then 0.5 mL of tween-80 was added and the mixture was triturated thoroughly. Distilled water (1 mL) was added to dissolve the fraction. Gradually, graded amounts of distilled water (1 mL and 2 mL) were added to the solution and mixed thoroughly. The resultant solution was then transferred into a pre-calibrated plain bottle additional amount of distilled water was added to bring the volume up to 5 mL. For the 200 mg/kg dose the same procedure was repeated using a weight of 250 mg of the EtOAC fraction.

2.2.6.6. Preparation of 25 mg/mL and 50 mg/mL of Aqueous methanol fraction into Oral Dosage Form

Considering the calculated doses for the aqueous methanol fraction administered to the experimental groups (100 mg/kg and 200 mg/kg), a total volume of 5 mL per day was determined based on the weight of the rat in kilograms (kg). Multiplying this volume by the total treatment duration of five days yielded a total volume of 25 mL with each ml containing 25 mg and 50 mg of the fraction for the respective doses.

The aqueous methanol fraction of dose 100 mg/kg was formulated into a suspension using 10% tween-80 as the emulsifying agent. Initially, 125 mg of the aqueous methanol fraction was weighed using an analytical balance into a porcelain dish then 0.5 mL of tween-80 was added and the mixture was triturated thoroughly. 1mL of distilled water was added to dissolve the fraction. Gradually, graded amounts of distilled water (1 mL and 2 mL) were added to the solution and mixed thoroughly. The resultant solution was then transferred into a pre-calibrated plain bottle additional amount of distilled water was added to bring the volume up to 5 ml. For the 200 mg/kg dose the same procedure was repeated using a weight of 250 mg of the aqueous methanol fraction.

2.2.7. Dosing Calculations and Dosing Table

2.2.7.1. Fraction Dose(s) Calculation

Using the formula, **volume to be administered (mL)** $= \frac{\boldsymbol{weight(kg)\times dose(mg/kg)}}{\boldsymbol{stock\ solution(mg/mL)}}$,

different volumes based on the individual Wistar rat weight in their various groupings 100 mg/kg and 200 mg/kg, were obtained.

Using an example of the weights represented in table 7 which shows the weight of rats in 100 mg/kg group of n-hexane, the volume of n-hexane fraction to be administered is calculated below

- Rat marking: one tail marked (black), Rat weight: 0.155kg, n-hexane fraction dose:100 mg/kg/day, Stock solution 25mg/ml therefore volume to be administered = 0.155×100/25 = 0.62 mL

The calculation was then repeated for the remaining rats in the 100 mg/kg n-hexane group and this was also repeated for the 200 mg/kg n-hexane group and the volume to be administered was obtained respectively.

The whole process was then repeated for the DCM (100 mg/kg, 200 mg/kg), EtOAC (100 mg/kg, 200 mg/kg) and aqueous methanol groups (100 mg/kg and 200 mg/kg) respectively.

2.2.7.2. Ethanol Dose(s) Calculation

Using a dose of 2.5mg/kg, the volume of ethanol to be administered for ulcer induction on days 1 and 3 was calculated for all the rats across each group, n-hexane groups (100 mg/kg and 200 mg/kg), DCM groups (100 mg/kg and 200 mg/kg), EtOAC groups (100 mg/kg and 200 mg/kg), aqueous methanol groups (100 mg/kg and 200 mg/kg), and negative control group respectively. This was done by multiplying their individual weight by 2.5.

An example is shown below using a weight sample from table 2.3 which shows the weight of rats in the control group (non-treatment). The volume of ethanol to be administered is calculated below

Rat marking: One tail marked (blue), Rat weight: 0.188kg, ethanol dose: 2.5mg/kg, volume to be administered = 0.188×2.5 = 0.47mL.

This calculation was then repeated for the other groups and the volume to be administered was recorded.

2.2.7.3. Omeprazole Dose(s) calculation

Using a recommended dose of 40mg/kg and a stock solution of 10mg/ml, the volume of Omeprazole to be administered was calculated based on their various weights. An example is shown below using a weight sample from table 2.3 which shows the weight of rats in the Omeprazole group. The volume of Omeprazole to be administered is calculated below

-Rag marking: One tailed (black), Rat weight: 0.229kg, dose: 40mg/kg, stock solution 10mg/ml.

Volume to be administered = 0.229 x 40/10 = 0.916mL

2.2.8. Ulcer Induction

Ulcer induction followed the standard experimental procedures for inducing ulceration in Wistar rats. Prior to ulceration induction, all rats were subjected to a 16-hour fasting period by withdrawing their food from the cages to ensure the clearing of the GIT and accuracy of the ulcerative effect. Ulcer was induced in all the rats on days 1 and 3 with absolute ethanol at a dose of 2.5 mg/kg. The rats were arranged according to their assigned groupings, and the calculated volume of ethanol, as outlined in Tables 2.1, 2.2, and 2.3, was administered to each rat with the aid of an orogastric tube and a 1 mL syringe. To minimize errors in administration, the ethanol was administered sequentially, one cage at a time. Following the administration of ethanol the rats were allowed to rest for a period of one hour to facilitate the onset of the ulcerative effect.

2.2.9. Treatments

One hour after the administration of the calculated dose of ethanol to all rats, the treatment process commenced. The rats were treated according to their respective groups, which included the n-hexane groups (100 and 200 mg/kg respectively), DCM groups (100 and 200 mg/kg respectively), EtOAC groups (100 and 200 mg/kg respectively), and the aqueous

methanol group (100 and 200 mg/kg respectively). Tables 2.1, 2.2, and 2.3 provides details of the volumes administered per group to each rat. All glassware and apparatus utilized in this section were thoroughly washed and cleaned.

2.2.9.1. Treatment with Fractions

The freshly prepared 5mL of the stock solutions of the 100mg/kg and 200mg/kg respective doses of the n-hexane fraction was thoroughly shaken. An orogastric tube connected to a 1mL syringe was then employed to draw out the calculated volumes, as specified in Table 2, for administration to the rats in the n-hexane groups (100 and 200 mg/kg respectively). The administration sequence commenced with the lowest dose of 100 mg/kg/day, followed by 200 mg/kg/day. To ensure accuracy and prevent errors such as overdosing or missed doses, a tally marking system was implemented. The entire procedure was repeated for four consecutive days at the same time. The entire process was carried out for the other fractions.

2.2.9.2. Treatment with Omeprazole

A vial of Omeprazole injection was reconstituted with 4mL of water for injection. Using a 1mL needle syringe the appropriate volume to be administered doors to the rats in the Omeprazole group was collected and administered carefully to the rats intraperitoneally once daily. The procedure was repeated daily for four consecutive days.

2.2.10. Post Treatment Operations

2.2.9.3. Animal Sacrifice

Upon completion of the five-day treatment period, the rats were fasted. On the following day, they were transferred to the pharmacology laboratory. To induce anaesthesia, each rat was introduced into a glass jar containing cotton wool soaked in chloroform, one at a time

according to their respective groups. An abdominal incision was performed using surgical blades, scalpels, and scissors, while surgical hand gloves were worn to maintain sterility and ensure safety during the procedure. The stomach was carefully identified and collected, and the stomach content was rinsed out gently with normal saline to remove any food residue and blood that may obscure the ulcer. Each stomach was then laid out and stretched on a cardboard paper to examine the degree of ulceration. The length of ridges of ulceration was measured with a metre rule and used to calculate the ulcer indices of the rats which was used to deduce the %Ulcer inhibition of the fractions.

The formula used as described by Goel and Bhattacharya (1991) are expressed as follows

$$\textbf{Mean Ulcer Index (MUI)(mm)} = \frac{\sum \textbf{Ulcer index of rats in group}}{\textbf{number of rats in group}}$$

$$\textbf{\%Ulcer Inhibition} = \frac{\textbf{MUI(control)} - \textbf{MUI(treatment)}}{\textbf{MUI(control)}} \textbf{ X 100}$$

Representative isolated stomachs were selected from each group for storage and analysis. The selected stomachs were placed in red-covered plain universal bottles containing formal-saline solution to preserve tissue integrity. This sacrificing procedure was conducted for all rats across all experimental groups.

2.2.9.4. Slide preparation and Histopathology

The dissected organs were fixed in 10 percent formal saline. Fixed tissues were completely dehydrated in ascending concentrations of alcohol (70, 90, 96 and 100%).

The tissues were placed in xylene to remove the alcohol, impregnated and embedded with molten paraffin wax. They were allowed to solidify before sectioning into 4 µm using a microtome (Leica RM 2235, UK), the 4µm sections were placed on slides and stained with hematoxylin-eosin dye (Bancroft and Gamble, 2006). Stained slides were viewed using an

optical photomicroscope (Olympus 230 V 50/60 He, Germany) and camera (Eakins 12Mega pixels, UK) at ×40, ×100 and ×400 magnification.

CHAPTER THREE

RESULTS

The results of the investigations into the dried powdered leaves of *Sida acuta* are presented in this section using text, graphics and tabular formats.

3.1. Organoleptic Properties

The result presented in the table below summarizes the organoleptic characteristics of the plant sample

Table 3.1: Organoleptic Properties of the Powdered Leaves of *Sida acuta*

Features	Observation
Colour	Green
Taste	Rough
Odour	Pungent
Texture	Slightly bitter

3.2. Weight and Percentage Yields of Methanol extract and Fractions of *Sida acuta*

The percentage yield of the extract and fractions was calculated based on the weight of sample used in extraction and the weight of sample used in solvent-solvent partitioning and is shown in **Table 3.2** below:

Table 3.2: Weight and percentage yield of extract and fractions of *S. acuta*

Extraxt/Fractions	Weight (g)	Percentage (%)
Extract	73.32	7.33
N-Hexane	20.03	33.38
Dichloromethane	1.67	2.78
Ethyl acetate	1.69	2.81
Aqueous Methanol	24.48	40.8

3.3. Phytochemical Screening

Table 3.3 highlights the phytochemical constituents (Secondary metabolites) determined through qualitative tests.

Table 3.3: Phytochemical constituents of powdered leaves of *Sida acuta*

Phytoconstituent	**inference**
Alkaloids	Present
Carbohydrate	Present
Reducing sugars	Present
Saponins	Present
Tannins	Absent
Terpenoids	Present
Phenols	Present
Flavonoids	Present
Anthraquinones	Absent

Quantitative Tests for Secondary Metabolites

- **Total Phenol content**

Figure 3.1 shows the calibration curve obtained using Gallic acid as standard

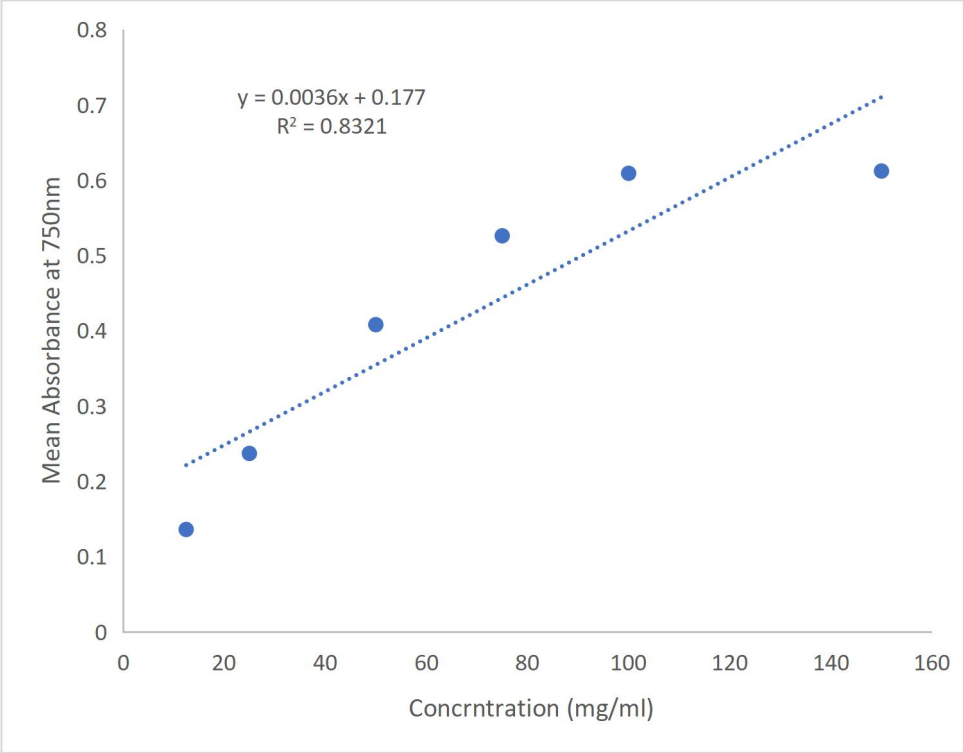


Figure 3.1: Gallic acid Calibration curve

From The standard calibration curve (**Figure 3.1)** the total phenol was calculated using the formula:

$y = 0.0036x + 0.1770$

$x = (y - 0.1770)/0.0036$

Where:

- y is the absorbance of the extract.
- x is the Total phenol content (in mg GAE/g extract)

- **Total Flavonoid content**

  Figure 3.2 shows the calibration curve using Quecertin as standard

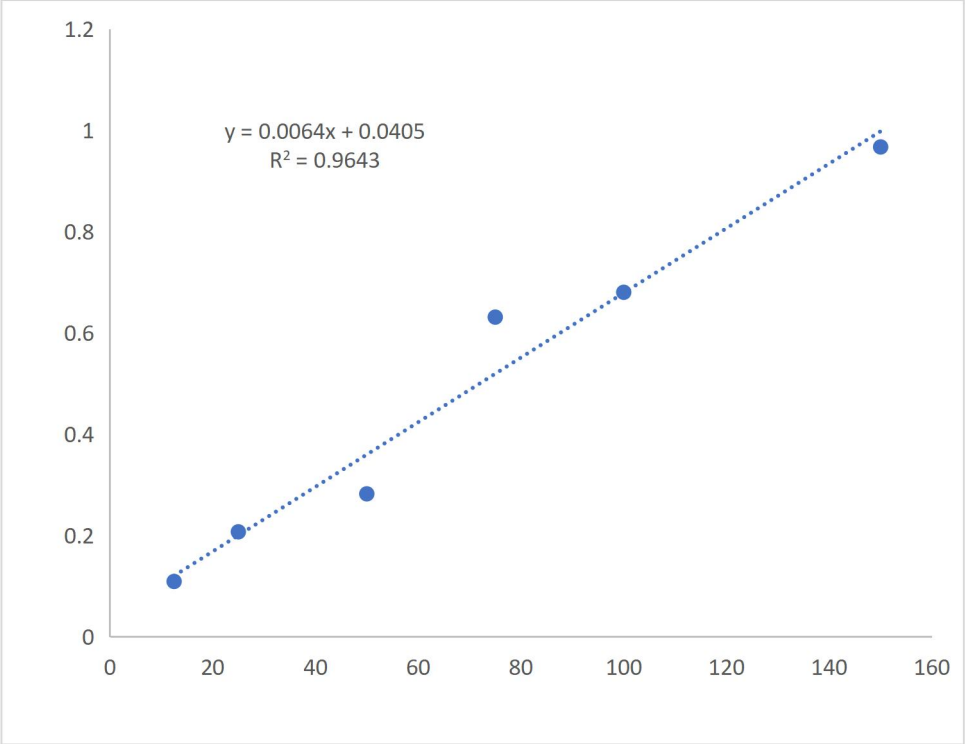


Figure 3.2: Quercetin Calibration curve

From the standard calibration curve (**Figure 3.2)** the total flavonoid was calculated using the formula:

$y = 0.0064x + 0.0405$

$x = (y - 0.0405)/0.0064$

Where:

- y is the absorbance of the extract
- x is the Total flavonoid content (in mg QE/g extract)

Table 3.4: Extrapolated Total Phenol and Flavonoid content of the extract

Sample / **Parameter**	***Sida acuta***
Total Phenol (mg GAE/g extract)	38.61
Total Flavonoid (mg QE/g extract)	20.70

3.4. Antioxidant activity

- **DPPH Radical Scavenging Activity**

  Figure 3.3 and **Figure 3.4** highlights the curves obtained from the DPPH assay of the standard (Ascorbic acid) and methanol extract of leaves of *Sida acuta* respectively.

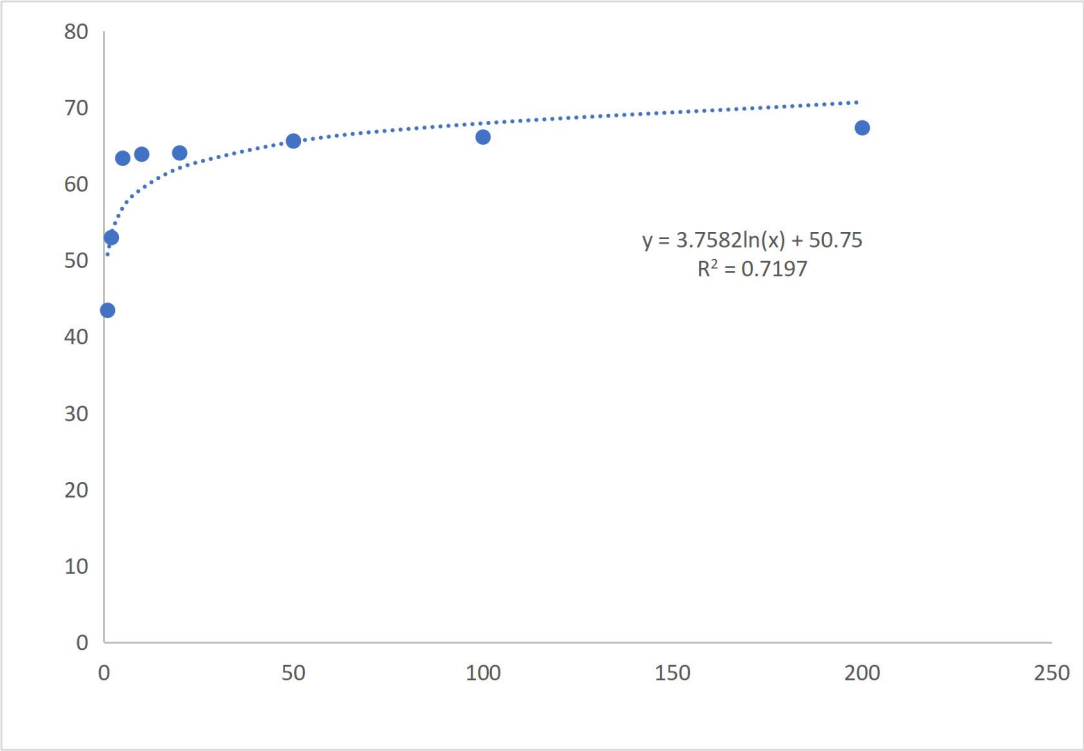


Figure 3.3: DPPH Radical Scavenging Activity of Ascorbic acid

The IC_{50} of the standard (Ascorbic acid) was calculated using the logarithmic equation;

y = 3.7582In(x) + 50.75

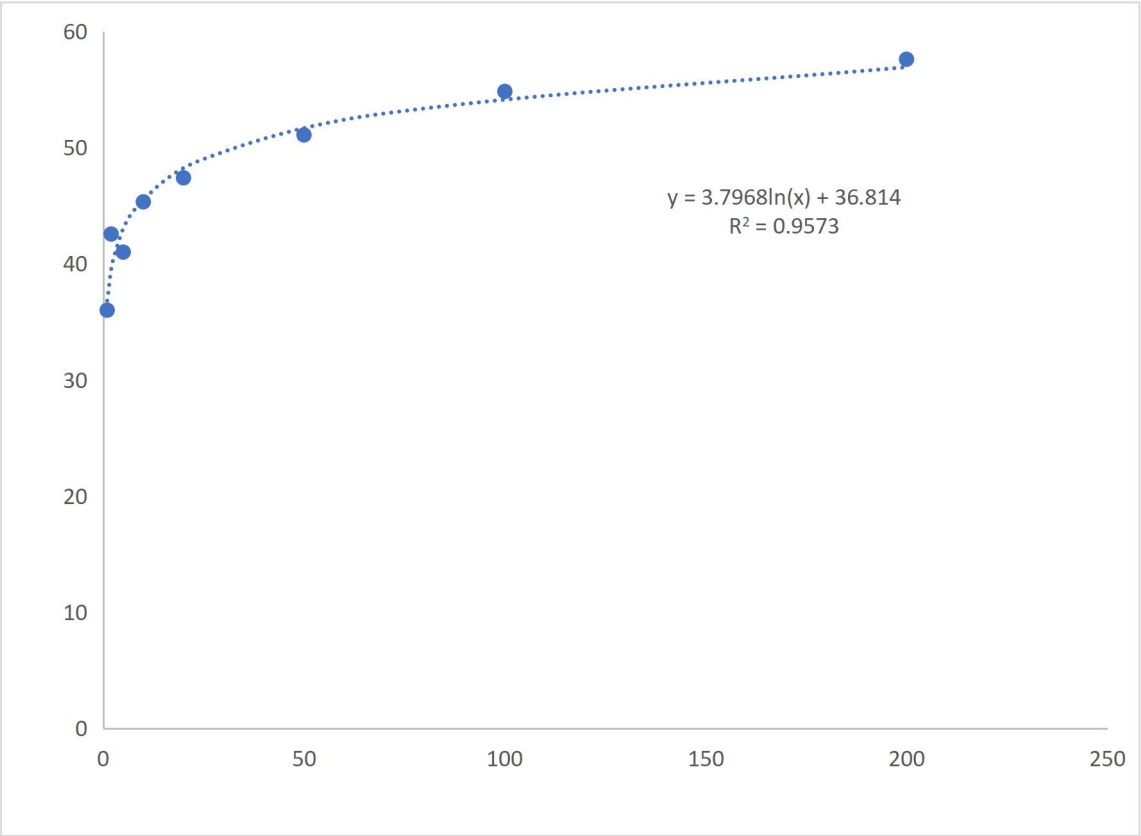


Figure 3.4: DPPH Radical Scavenging Activity of Methanol extract of Leaves of *S. acuta*

The IC_{50} of the Crude extract was calculated using the logarithmic equation;

y = 3.7968In(x) + 36.814

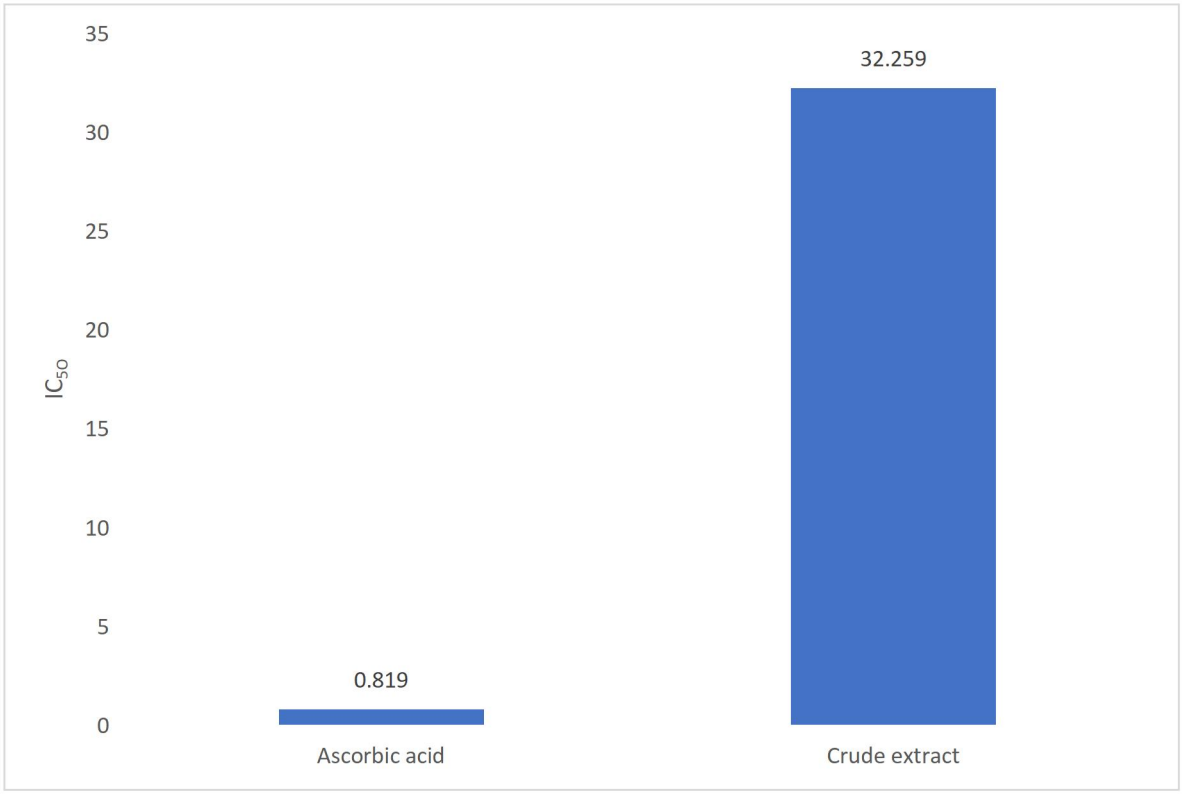


Figure 3.5: Comparison of IC50 of Ascorbic acid and the crude extract

- **FRAP ASSAY**

Figure 3.5 shows the calibration curve using Ferrous Sulphate heptahydrate as standard

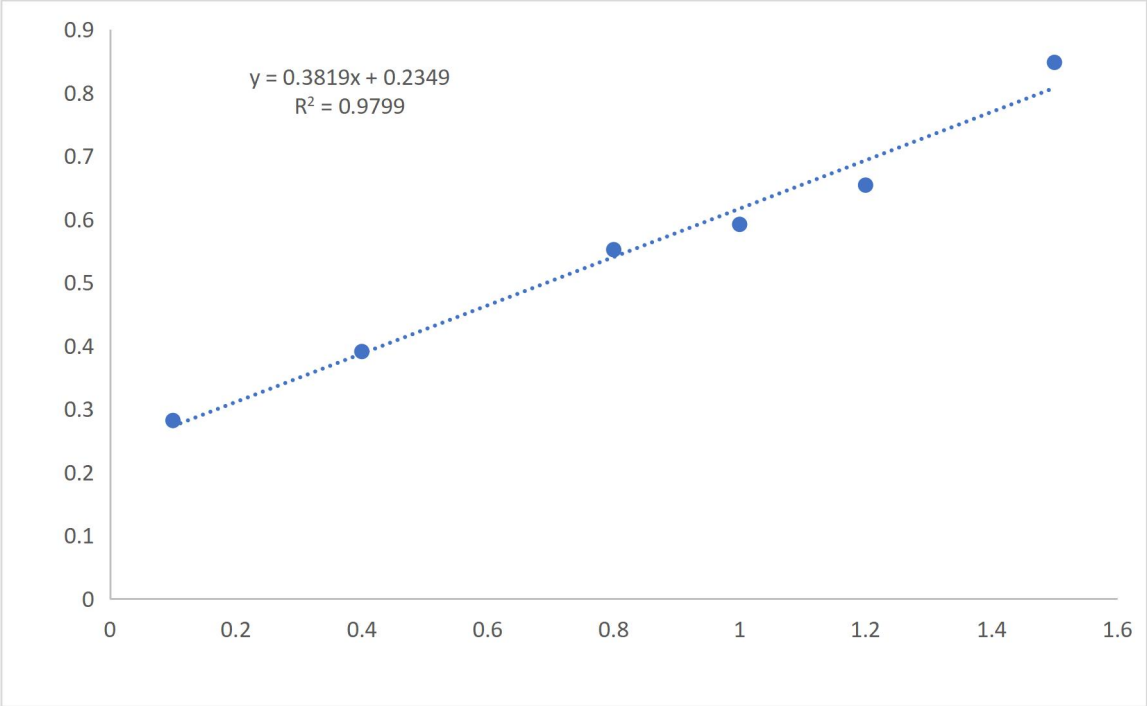


Figure 3.6: Ferrous Sulphate Calibration curve

From the standard calibration curve in **Figure 3.6**, the FRAP was calculated using the formula:

$y = 0.3819x + 0.2349$

$x = (y - 0.2349)/0.3819$

- y is the absorbance of the extract
- x is the FRAP ability in mM $FeSO_4$ equivalent per gram)

Table 3.5: Extrapolated FRAP of the extract

Sample / Parameter	*Sida acuta*
Absorbance	0.315
Concentration (mM FeSO4 equivalent/g)	0.21

3.5.Anti ulcer activity

Table 3.6 highlights the mean ulcer index (MUI) and percentage ulcer inhibition of the various fraction groups and that of the negative control and treatment groups

Table 3.6: MUI and %Ulcer inhibition of various treatment groups

Groups	**MUI (mm) ± SEM**	**%Ulcer Inhibition**
Hex 100mg/kg	31.40 ± 1.17	61.5
Hex 200mg/kg	32.60 ± 1.57	59.55
DM 100mg/kg	40.60 ± 1.21	49.63
DCM 200mg/kg	37.60 ± 1.72	53.35
EtOAC 100mg/kg	40.20 ± 0.58	50.12
EtOAC 200mg/mg	34.60 ± 1.12	57.07
Aq-Meth 100mg/kg	44.60 ± 1.72	44.67
Aq-Meth 200mg/kg	48.60 ± 0.81	39.70
Control	80.6 ± 1.72	00.00
Standard treatment (Omeprazole)	21.00 ± 0.71	73.95

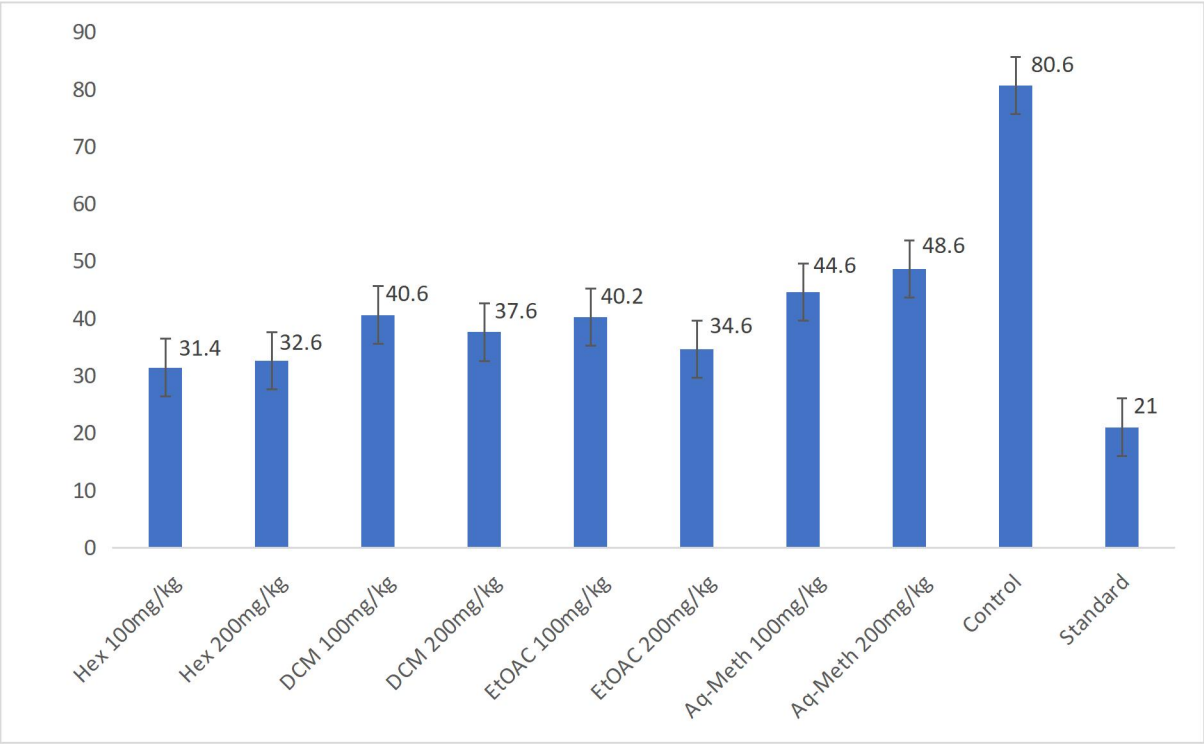


Figure 3.7: Chart of MUI of Treatment groups

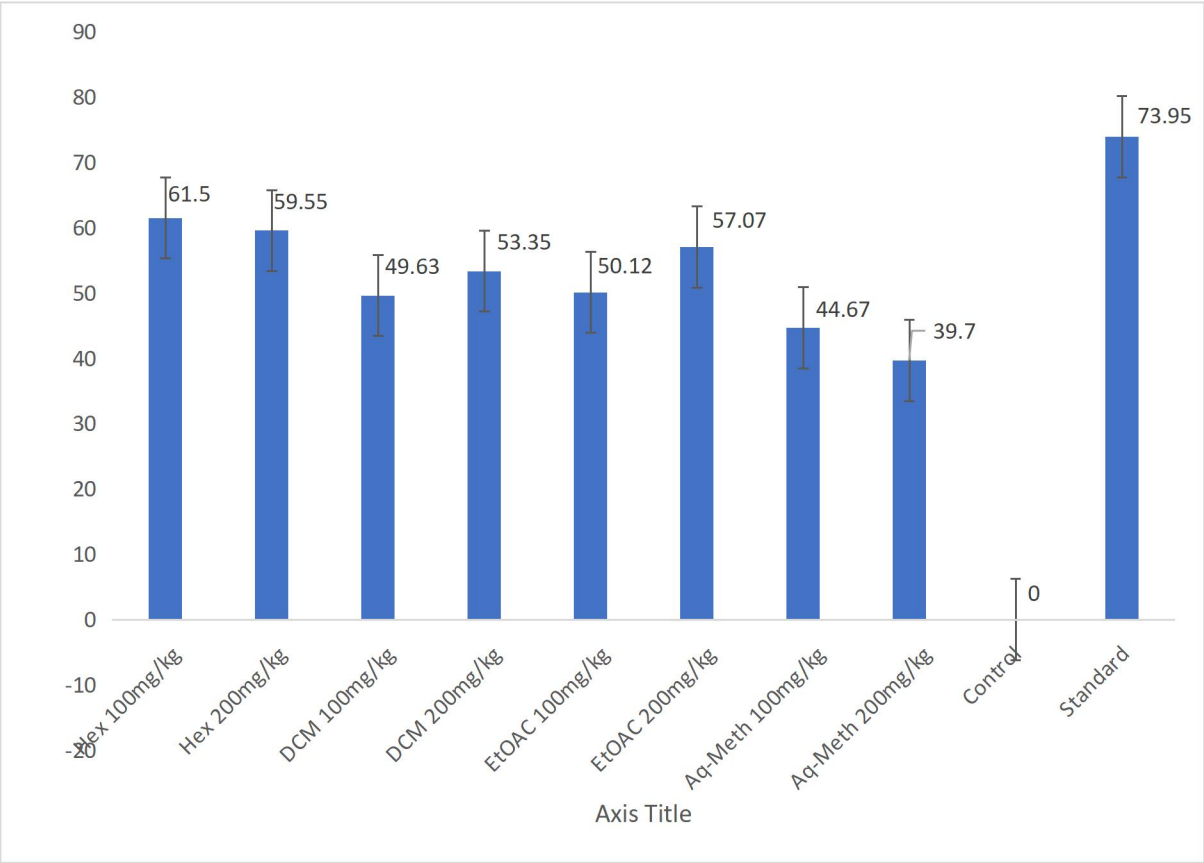


Figure 3.8: Chart of %Ulcer inhibition of treatment groups

Histology Result

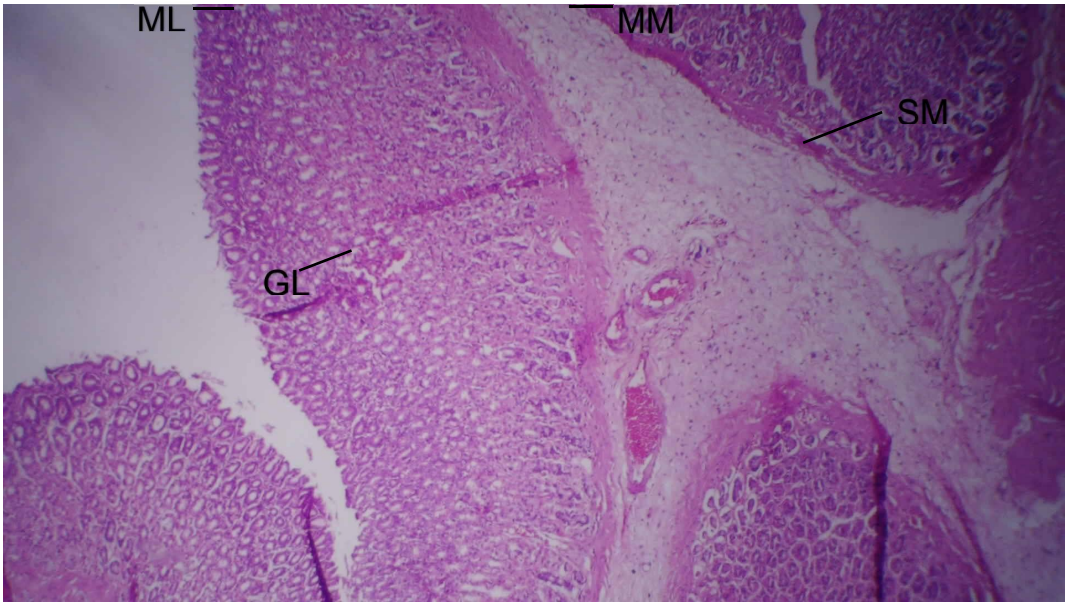


Figure 3.9: Rat stomach healthy show: normal architecture: pitted mucosal membrane (ML), glands (GL), muscularis mucosa (MM) and submucosa (SM): H&E 40 X

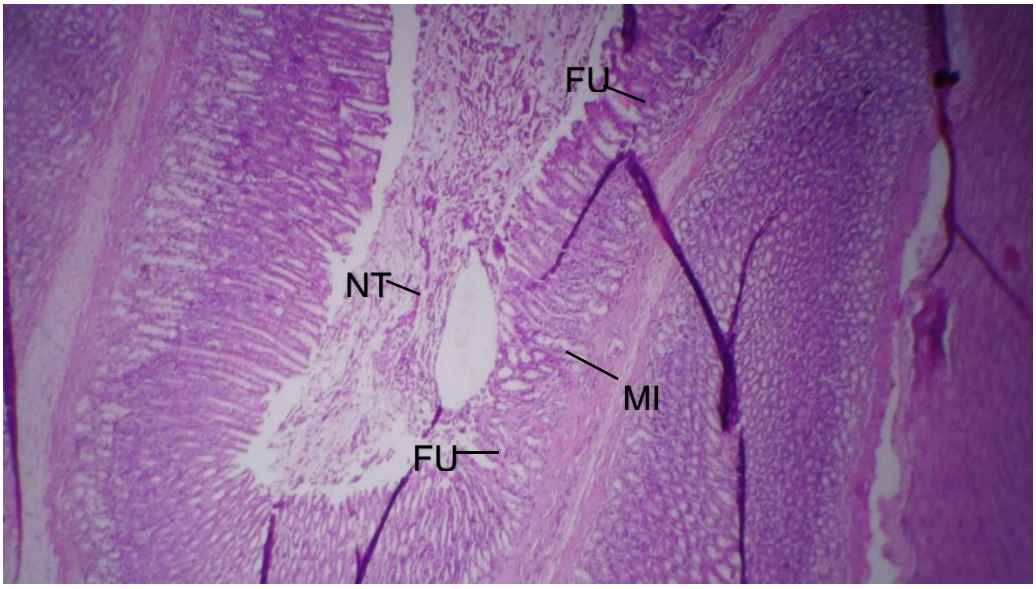


Figure 3.10: Rat stomach induced ulcer show: marked luminal necrotic tissue debris (NT), focci of funnel-shaped ulcer (FU), mucosal infiltrates of inflammation (MI): H&E 40 X

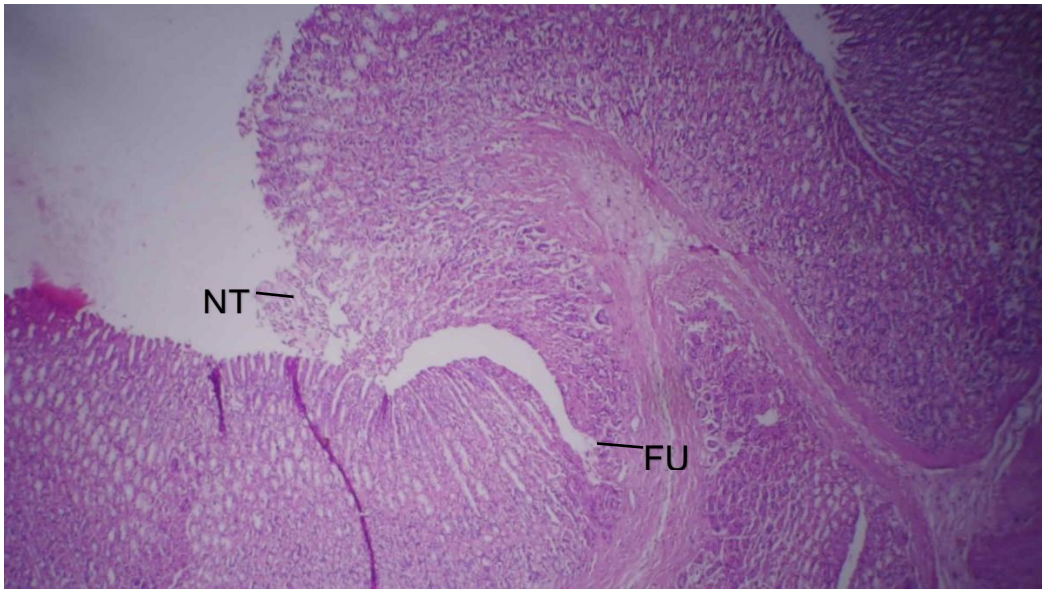


Figure 3.11: Rat stomach induced and given 100 mg/kg Hexane fraction methanol leaf

extract show: mild luminal debris (NT), focal funnel-shaped ulcer (FU): H&E 40 x

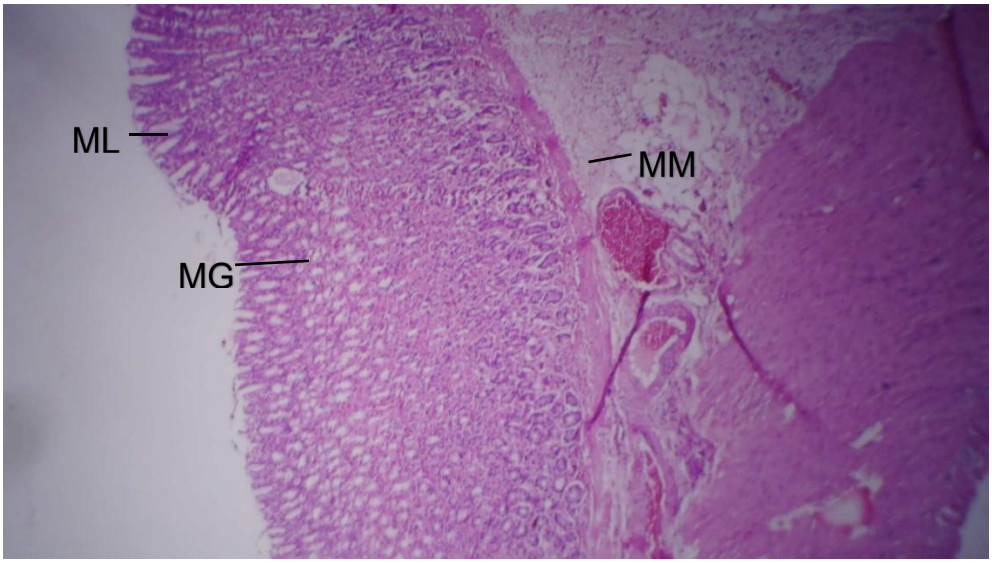


Figure 3.12: Rat stomach induced and given 200 mg/kg Hexane fraction methanol leaf extract show: normal mucosal membrane (ML), glands (MG) and muscularis mucosa (MM): H & E 40 X

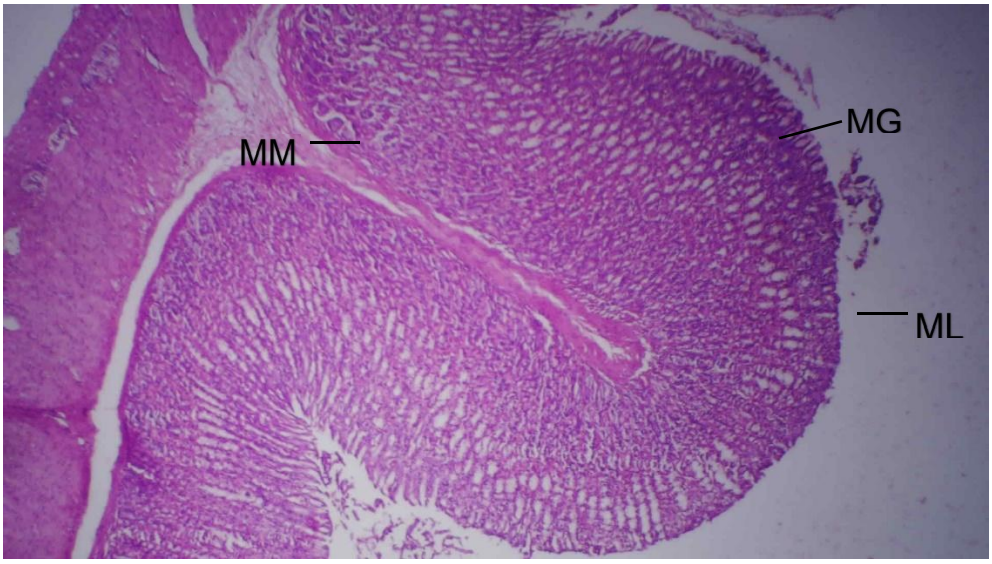


Figure 3.13: Rat stomach induced and given 100 mg/kg Ethylacete fraction methanol extract show: normal mucosal membrane (ML), glands (MG) and muscularis mucosa (MM): H&E 40 X

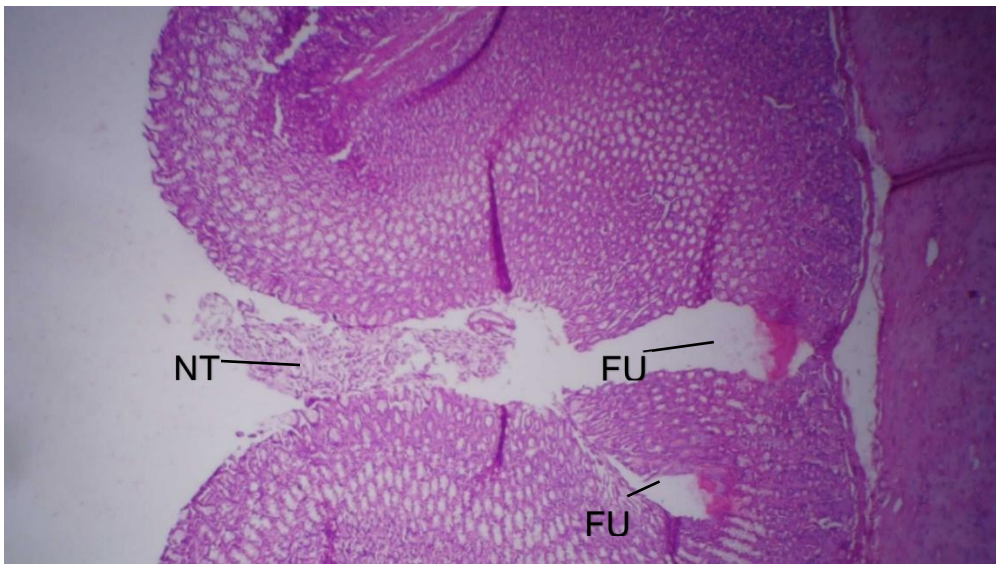


Figure 3.14: Rat stomach induced and given 200 mg/kg Ethylacete fraction methanol extract show: moderate luminal necrotic tissue debris (NT), foci of flask-shaped ulcer (FU): H&E 40 X

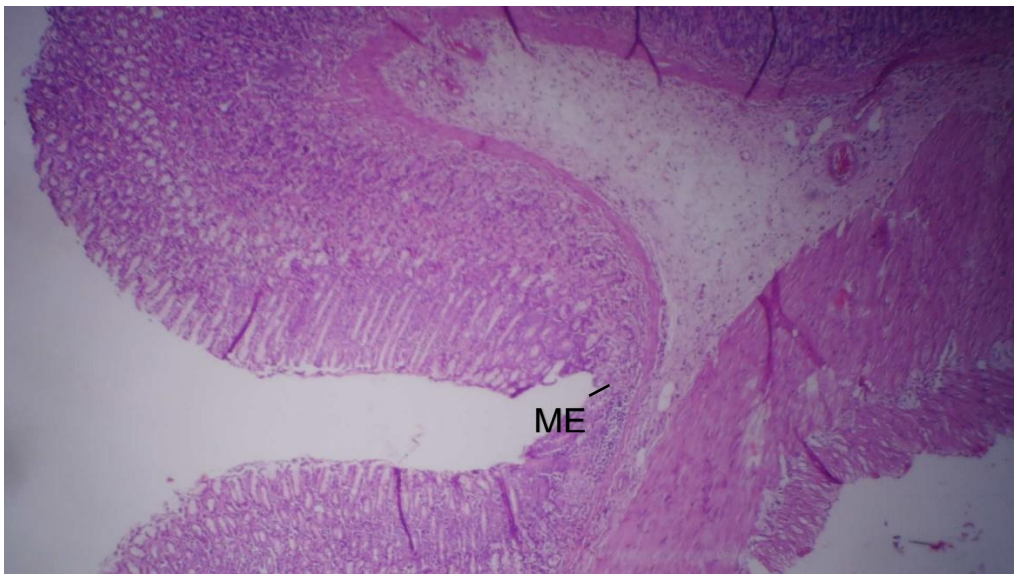


Figure 3.15: Rat stomach induced and given 100 mg/kg Dichloromethane fraction methanol extract show: Focal mucosal erosion (ME): H&E 40 X

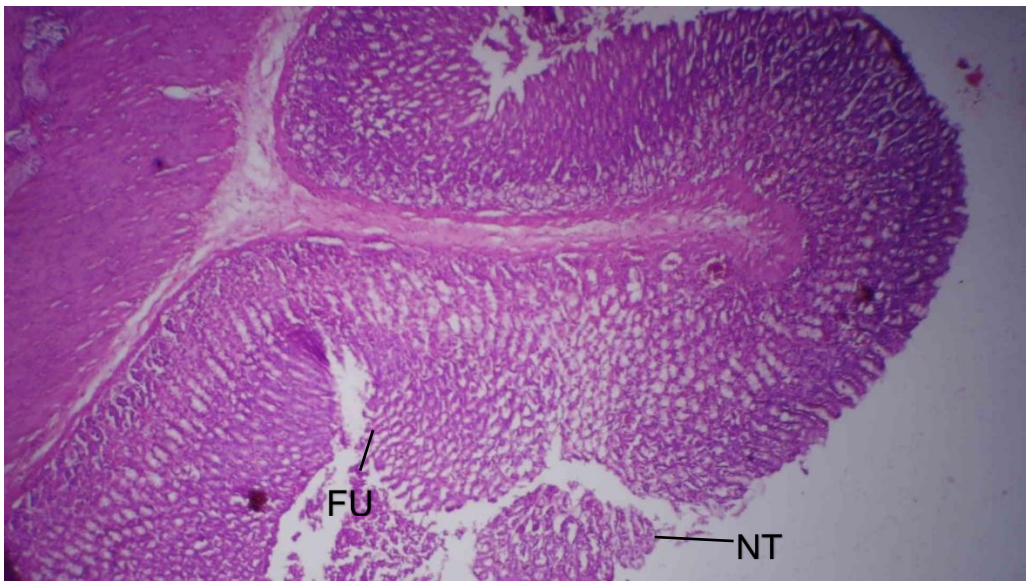


Figure 3.16: Rat stomach induced and given 200 mg/kg Dichloromethane fraction extract show: marked luminal necrotic tissue debris (NT) and flask-shaped ulcer (FU): H&E 40 X

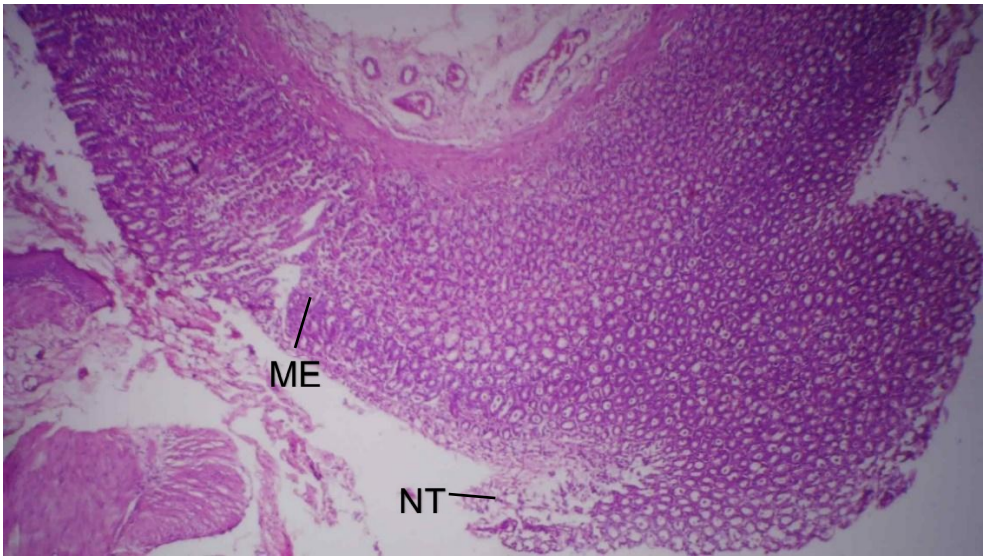


Figure 3.17: Rat stomach induced and given 100 mg/kg aqueous methanol fraction, methanol extract show: moderate luminal necrotic tissue debris (NT) and mucosal erosion (ME): H&E 40 X

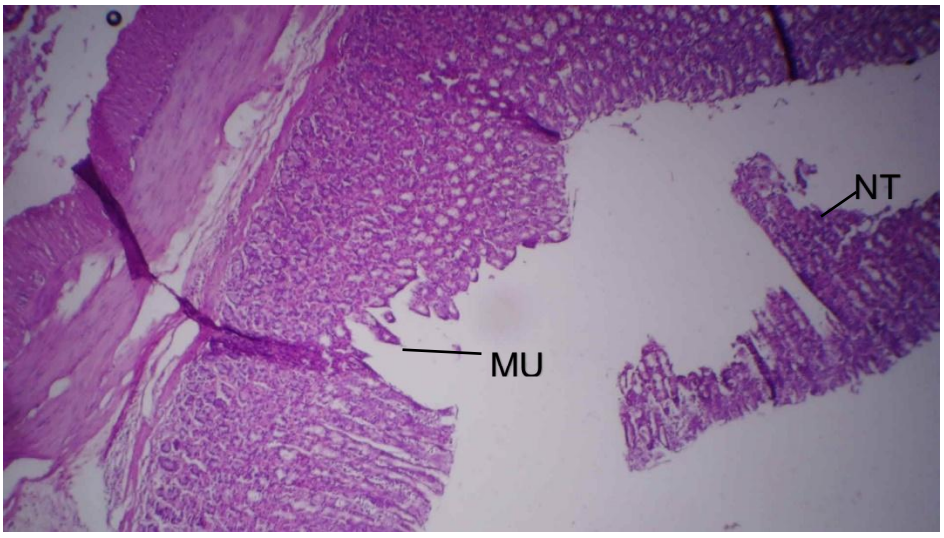


Figure 3.18: Rat stomach induced and given 200 mg/kg aqueous methanol fraction, methanol extract show: luminal necrotic tissue (NT) and irregularly-shaped

ulcer (MU): H&E 40 X

CHAPTER 4

DISCUSSION

4.1. Organoleptic Properties, Extraction and Fractionation Yields.

Organoleptic analysis, utilizing the senses (sight, smell, touch, taste), was performed on the plant sample. These descriptive tests are essential for preliminary identification when laboratory equipment is unavailable. The sample exhibited typical plant characteristics, but the bitter taste strongly suggests the presence of alkaloids. This finding is currently empirical and requires further experimental validation before definitive inferences can be drawn.

The *Sida acuta* leaf material was extracted then further fractionated in the preparation stages of the study offering the chemical foundation of the observed pharmacological effects. The extraction yield is calculated to be 7.33%

The solvent-solvent partitioning process gave a highly polar Aqueous Methanol (Aq-Meth) fraction of 40.8%, which confirmed that most of the extractable compounds in the crude methanol extract were highly polar (Thomford *et al.,* 2018). On the other hand, the medium-polarity fractions, which were Dichloromethane (DCM) and Ethyl Acetate (EtOAC), had low yields (2.78% and 2.81%, respectively). The goal of fractionation, which is to separate the less abundant, selective chemical classes of the total bulk of polar materials was obtained. This separation is important to determine the exact compounds that cause the biological activity because, in most cases, the concentration of active principles may be higher in the resultant fractions (Savickiene and Raudone, 2024).

4.2. Phytochemical constituents

The qualitative and quantitative analysis of phytochemicals in the extracts was determined by performing a qualitative and quantitative phytochemical profile analysis. The presence of many bioactive secondary metabolites, among which there were Alkaloids, Saponins, Terpenoids, Carbohydrates, Phenols, and Flavonoids were confirmed by the qualitative screening of the powdered leaves. These varying compounds exist simultaneously, which is a solid pharmacological explanation of the extensive ethnomedicinal uses of the plant (Lal *et al.,* 2024; Thomford *et al.,* 2018); alkaloids have analgesic, anti-inflammatory, anti-malarial properties, saponins possess immune boosting, antilipidemic and antimicrobial properties, terpenoids have anti-inflammatory, anticancer and antimicrobial properties, carbohydrates possess cardiotonic effects, phenols are antioxidants with anti-inflammatory, antimicrobial, and anticancer properties, and flavonoids are known for their antioxidant activity. Tannins are also conspicuously absent as lots of conventional anti-ulcer vegetation depends on the astringent and protein-precipitating effect of tannins to be active (Bello *et al.,* 2021). This implies that the gastroprotective effect of *Sida acuta* depends on other chemical classes.

Quantitative analysis established a significant level of concentration of the major antioxidant constituents: Total Phenol Content (38.61 mg GAE/g extract) and Total Flavonoid Content (20.70 mg QE/g extract). These elevated concentrations of the compounds confirm the prior results with *Sida acuta* and directly confirm the identified antioxidant and cytoprotective potential of the plant (Paraakh *et al.,* 2023; Konate *et al.,* 2023)

4.3. Antioxidant Activity

The antioxidant property of *Sida acuta* crude methanol extract was strongly affirmed by two complementary tests, that is, the DPPH Radical Scavenging Activity and the Ferric Reducing Antioxidant Power (FRAP) assay. This two-fold technique gives a total of the capacity of the

extract to neutralize the reactive species, and one of which is the ability of the extract to react to the stable 2,2-diphenyl-1-picrylhydrazyl radical and provides a vitally important IC50 value. The comparison between the IC_{50} values of the crude extract and standard (ascorbic acid) confirms that the crude methanol extract possessed high radical scavenging capacity that could be visually compared to the standard (Ascorbic acid). This mopping up of free radicals' ability is the direct argument in favour of the traditional application of the plant as a gastroprotective agent (Paraakh *et al.,* 2023). Oxidative stress is a significant factor contributing to the pathogenesis of gastric ulcers, particularly models with acute agents, such as ethanol, in which there is reactive oxidative species production resulting in severe mucosal injury (El-Gharib and Abdo, 2025; Ghonge *et al.,* 2025). This antioxidant potential of the crude methanol extract is strongly related to the high concentration of phenolics and flavonoids, which explains why the FRAP assay value of crude methanol extract was determined to be 0.21mM $FeSO_4$ E/g. This ferric ion reduction capacity and free radical scavenging is one of the basic mechanisms of action of gastroprotective agents (El-Gharib and Abdo, 2025). The occurrence of oxidative stress and the formation of Reactive Oxygen Species (ROS) is an important aspect of the pathogenesis of gastric ulcers, especially the models that are produced under the influence of acute drugs, such as ethanol (Taher *et al.,* 2025). Hence, the inherent antioxidant property of the *Sida acuta* extract validates its medicinal efficacy in ulceration due to oxidative effects.

4.4. Anti-Ulcer Activity

The in vivo anti-ulcer efficacy testing provided empirical validation for the traditional use of *Sida acuta* against gastric lesions, successfully achieving the core objective of the study. The experimental design utilized an acute ulcer induction model (the ethanol-induced method), with Omeprazole serving as the synthetic standard treatment group. The negative control

group exhibited the highest Mean Ulcer Index (MUI) of 80.6mm and 00.00% inhibition, confirming the successful induction of gastric mucosal injury.

The treatment group demonstrated highly potent efficacy, achieving 73.95% ulcer inhibition. This result is comparable to the high inhibition percentages often reported for standard synthetic anti-ulcer drugs, in pre-clinical screening models (Kukarnil and Kale, 2010; Malairajan *et al.,* 2015).

A critical objective of this study was to compare the activities of the resultant fractions. The results clearly showed that the non-polar n-Hexane fraction exhibited the highest efficacy among the fractions, achieving an inhibition of 61.5% at 100 mg/kg and 59.55% at 200 mg/kg. The finding that the n-Hexane fraction concentrated the activity validates the study's approach, demonstrating that fractionation successfully isolated the most potent chemical group from the bulk of the crude extract (Savickiene & Raudone, 2024).

The superior activity of the non-polar n-Hexane fraction is particularly noteworthy, given the high concentration of polar antioxidants in the crude extract (Thomford *et al.,* 2018). The n-Hexane fraction typically concentrates terpenoids, steroids, and lipophilic alkaloids, suggesting that the primary anti-ulcer mechanism of *Sida acuta* is rooted in cytoprotection rather than solely acid neutralization or antioxidant effects (Ekwealor *et al.,* 2020).

The lipophilic compounds concentrated here, such as terpenoids confirmed in the qualitative screening, are hypothesized to integrate into the gastric mucosal cell membranes, enhancing their structural integrity and preventing their lysis by corrosive agents (El-Gharib and Abdo, 2025; Ghonge *et al.,* 2025). Furthermore, these compounds are often known to stimulate mucosal blood flow and enhance the synthesis of the defensive mucus layer, which are key defense factors in preventing ulcer formation (Lal *et al.,* 2024).

While the n-Hexane fraction demonstrated superior efficacy, the anti-ulcer results for the Dichloromethane (DCM) and Ethyl Acetate (EtOAC) fractions provide crucial context by further isolating the dominant mechanism of action. These two fractions generally contain compounds of medium polarity, such as less polar flavonoids, certain alkaloids, and intermediate terpenoids, bridging the chemical space between the non-polar n-Hexane and the highly polar Aqueous-Methanol fractions (Savickiene & Raudone, 2024).5.4.

The DCM and EtOAC fractions exhibited moderate anti-ulcer activity, suggesting that their chemical constituents contribute to the overall gastroprotection of the crude extract, albeit less significantly than the n-Hexane fraction. The DCM fraction showed inhibition ranging from 49.63% (at 100 mg/kg) to 53.35% (at 200 mg/kg). The EtOAC fraction showed inhibition ranging from 50.12% (at 100mg/kg) to 57.07% (at 200mg/kg). The moderate inhibition observed in these fractions is still therapeutically significant when compared to the complete absence of inhibition in the control group. The activity of the EtOAC fraction, which often concentrates glycosylated flavonoids and moderately polar phenolic acids, supports a mechanism involving both antioxidant defense and anti-inflammatory action (Lal *et al.,* 2024). Flavonoids, being potent antioxidants, help mitigate the oxidative damage induced in the acute ulcer model, while simultaneously possessing confirmed anti-inflammatory properties that reduce mucosal damage (El-Gharib and Abdo, 2025). The role of the DCM and EtOAC fractions, therefore, appears to be supportive: their constituents contribute an auxiliary effect through anti-inflammatory and antioxidant activities, supplementing the primary cytoprotective action provided by the less-polar components (Ekwealor *et al.,* 2020).

In contrast, the highly polar Aqueous-Methanol fraction showed the least inhibition, ranging from 39.70% to 44.67%. This result indicates that while the polar compounds (phenols and flavonoids) contribute to the overall anti-ulcer activity through the previously confirmed

strong antioxidant action, the non-polar agents are the dominant players in direct tissue protection in the model used (Taher *et al.,* 2024). The differences in activity across fractions highlight the significance of the multi-target approach employed by *Sida acuta*.

Histopathological Findings

Sections of rat stomach given baseline feed and water randomly show normal gastric tissue architecture with well-defined mucosal membrane displaying its characteristic pitting, lamina propria supporting gastric mucosal glands, muscularis mucosa and submucosa. Similar findings were observed in sections of rat stomach induced ulcer and treated with 200mg/kg body weight of hexane fraction, 200 mg/kg body weight of ethylacetate fraction of methanol leaf extract of Sida acuta. Sections of rat stomach induced and treated with 100mg/kg body weight of dichloromethane fraction of methanol leaf extract of Sida acuta show a localized area of superficial mucosal sloughing (erosion), with a greater part of the stomach appearing normal.

However, sections of rat stomach induced ulcer show devitalized mucosa, ulcerated mucosa, and marked necrotic tissue debris in the gastric lumen resulting from the damaged gastric mucosa. Similar findings were observed in sections of rat stomach induced and treated with 100mg hexane fraction, 200mg, ethylacetate fraction, 200mg dichloromethane fraction, and graded doses of aqueous methanol fraction of methanol leaf extract of Sida acuta. This implies that these fractions of the extracts could not mitigate the injury induced on the gastric mucosa by the ulcerogenic agent.

Thus, there was selective potency in the fractions of the methanol leaf extract of Sida acuta anti-ulcer activity in the stomach of adult Wistar rats.

CHAPTER FIVE

CONCLUSION

The study revealed that the tested plant sample contain bioactive compounds such as Alkaloids, Saponins, Phenols, Terpenoids, Carbohydrates and Flavonoids which contributes to the medicinal properties. The plant extract was found to possess substantial amount phenols and flavonoids. Subsequent investigation showed that the sample possesses potent antioxidant and free radical scavenging activities with IC_{50} 32.259µg/ml using the DPPH assay. The FRAP assay showed that the plant extract has moderate antioxidant activity**.**

The n-Hexane fraction of the methanol extract expressed greater antiulcer potential with the highest %ulcer inhibition when compared with other fractions. The DCM and EtOAC fractions showed moderate antiulcer potential while the Aqueous methanol fraction had the least antiulcer potential by having the lowest %ulcer inhibition.

The findings suggests that the non-polar bioactive compounds in the plant play a major role in the gastroprotective effects of the plant as well as supporting the traditional use of *Sida acuta* in the treatment of peptic ulcers

Recommendations

To translate these promising findings into a standardized and clinically relevant phytomedicine, the following recommendations are made for future research:

- **Bioassay-Guided Isolation:** The most potent fraction (n-Hexane) should be subjected to detailed bioassay-guided chromatographic isolation to separate and purify the specific, active compounds (e.g., individual terpenoids or lipophilic alkaloids) responsible for the anti-ulcer activity.

- **Structural Elucidation:** The chemical structure of the isolated compound(s) should be definitively determined using spectroscopic techniques such as NMR and Mass Spectrometry.
- **Mechanistic Studies:** Further *in vivo* studies should be conducted specifically using the n-Hexane fraction to confirm the precise mechanism of action (e.g., measuring gastric mucus content, endogenous prostaglandin levels, and H^+/K^+ ATPase pump activity) to precisely define its cytoprotective and/or anti-secretory profile.
- **Toxicity Profiling:** Although initial safety is often suggested by traditional use, comprehensive sub-acute and chronic toxicity studies on the purified active fraction are mandatory to determine its safety for long-term therapeutic application.

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APPENDIX

SUPPLEMENTARY DATA

Appendix 1 Absorbance of Gallic acid

Gallic acid (µg/ml)	ABS1	ABS2	ABS3	ABS	Standard deviation
12.5	0.173	0.131	0.104	0.136	0.035
25	0.232	0.237	0.242	0.237	0.005
50	0.415	0.418	0.392	0.408	0.014
75	0.523	0.534	0.520	0.526	0.007
100	0.602	0.609	0.615	0.609	0.007
150	0.610	0.608	0.618	0.612	0.005

Appendix 2 Absorbance of Quercetin

Quercetin acid (µg/ml)	ABS1	ABS2	ABS3	Standard deviation
12.5	0.122	0.113	0.093	0.109
25	0.232	0.203	0.187	0.207
50	0.114	0.355	0.377	0.282
75	0.637	0.665	0.591	0.631
100	0.661	0.693	0.690	0.680
150	0.969	0.968	0.964	0.967

Appendix 3 Absorbance and DPPH RSA of Ascorbic acid

Ascorbic acid(µg/ml)	ABS1	ABS 2	ABS3	Mean ABS	Standard deviation	%RSA
1	0.332	0.335	0.324	0.327	0.004	43.42
2	0.269	0.268	0.279	0.272	0.006	52.94
5	0.214	0.208	0.214	0.212	0.004	63.32
10	0.217	0.215	0.195	0.209	0.012	63.84
20	0.201	0.212	0.210	0.208	0.006	64.01
50	0.201	0.212	0.210	0.199	0.011	65.57
100	0.193	0.202	0.193	0.196	0.005	66.09
200	0.184	0.182	0.201	0.189	0.010	67.30

Appendix 4 Absorbance and DPPH RSA of *S. acuta*

Crude extract (µg/ml)	ABS1	ABS2	ABS3	Mean ABS	Standard deviation	%RSA
1	0.367	0.371	0.372	0.370	0.003	36.00
2	0.333	0.333	0.330	0.332	0.002	42.56
5	0.341	0.344	0.337	0.341	0.003	41.00
10	0.314	0.319	0.314	0.316	0.003	45.33
20	0.305	0.302	0.305	0.304	0.001	47.40
50	0.275	0.284	0.289	0.283	0.007	51.09
100	0.262	0.258	0.263	0.261	0.002	54.84
200	0.242	0.247	0.246	0.245	0.003	57.61

Appendix 5 FRAP of Ferrous Sulphate Heptahydrate

Ferrous sulphate (mM)	ABS1	ABS2	ABS3	Mean ABS	Standard Deviation
0.1	0.293	0.277	0.277	0.282	0.009
0.4	0.381	0.401	0.392	0.391	0.010
0.8	0.546	0.542	0.568	0.552	0.014
1	0.590	0.601	0.584	0.592	0.009
1.2	0.648	0.661	0.652	0.654	0.007
1.5	0.899	0.822	0.824	0.848	0.044

Appendix 6 Dosing Tables

Table 2.1: The calculated volumes of n-hexane and DCM fractions to be administered

S/N	Rat Markings	Rat weight (kg)	Volume of ethanol administered (mL)	Volume of n-hexane fraction administered (mL)	Volume of DCM fraction administered (mL)
1 (Hex 100mg/kg)	Black tail (1 mark)	0.1550	0.39	0.62	
2	Black tail (2 marks)	0.1772	0.44	0.71	
3	Black tail (3 marks)	0.1560	0.39	0.62	
4	Black tail (4 marks)	0.1623	0.41	0.65	
5	Black tail (5 marks)	0.1681	0.42	0.67	
6 (Hex 200mg/kg)	Green tail (1 mark)	0.2076	0.52	0.83	
7	Green tail (1 mark)	0.2100	0.53	0.84	
8	Green tail (1 mark)	0.2074	0.52	0.83	
9	Green tail (1 mark)	0.1945	0.49	0.78	
10	Green tail (1 mark)	0.2035	0.51	0.81	
11 (DCM 100mg/kg)	Blue tail (1 mark)	0.1400	0.35		0.56
12	Blue tail (2 marks)	0.1370	0.34		0.55
13	Blue tail (3 marks)	0.1464	0.37		0.59

14	Blue tail (4 marks)	0.1334	0.33		0.53
15	Blue tail (5 marks)	0.1301	0.33		0.52
16 (DCM 200mg/kg)	Green tail (1 mark)	0.1958	0.49		0.78
17	Green tail (2 marks)	0.1279	0.32		0.51
18	Green tail (3 marks)	0.1313	0.33		0.53
19	Green tail (4 marks)	0.1507	0.38		0.60
20	Green tail (5 marks)	0.1391	0.35		0.56

Table 2.2: The calculated volumes of EtOAC and AQ-Meth fractions to be administered

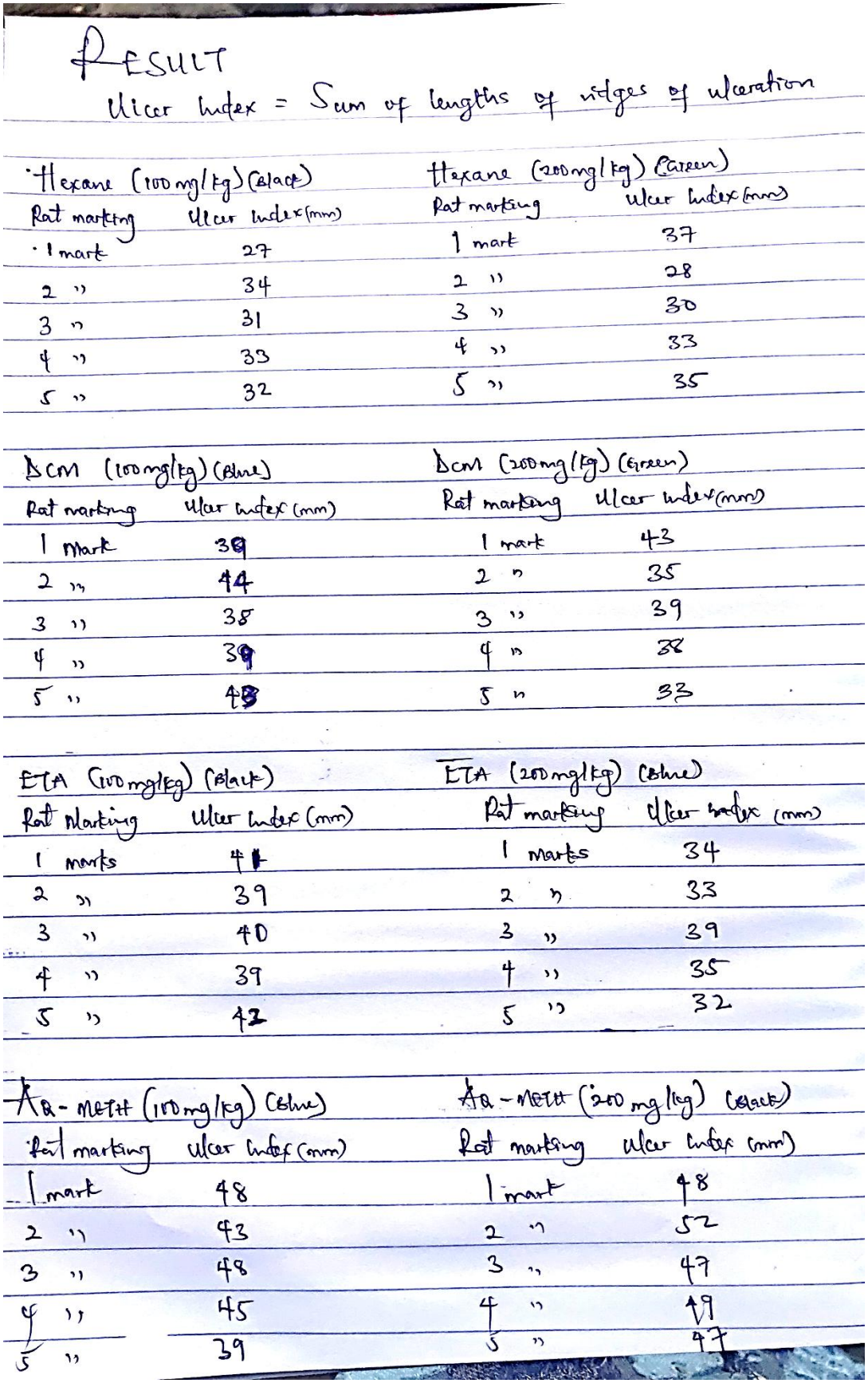
S/N	Rat Markings	Rat weight (kg)	Volume of ethanol administered (mL)	Volume ofEtOAC fraction administered (mL)	Volume of AQ-Methanol fraction administered (mL)
1 (EtOAC 100mg/kg)	Black tail (1 mark)	0.1241	0.31	0.50	
2	Black tail (2 marks)	0.1266	0.32	0.51	
3	Black tail (3marks)	0.1176	0.29	0.47	
4	Black tail (4 marks)	0.1259	0.31	0.50	
5	Black tail (5 marks)	0.1278	0.32	0.51	
6 (EtOAC 200mg/kg)	Blue tail (1 mark)	0.1168	0.29	0.47	
7	Blue tail (2 marks)	0.1257	0.31	0.50	
8	Blue tail (3 marks)	0.1148	0.29	0.46	
9	Blue tail (4 marks)	0.1300	0.33	0.52	
10	Blue tail (5 marks)	0.1169	0.29	0.47	
11 (AQ-Meth 100mg/kg)	Blue tail (1 mark)	0.1947	0.49		0.78
12	Blue tail (2 marks)	0.164.2	0.41		0.66
13	Blue tail	0.1528	0.38		0.61

	(3 marks)			
14	Blue tail	0.1635	0.41	0.65
	(4 marks)			
15	Blue tail	0.1463	0.37	0.59
	(5 marks)			
16 (AQ-Meth 200mg/kg)	Black tail (1 mark)	0.19558	0.49	0.78
17	Black tail (2 marks)	0.1937	0.48	0.77
18	Black tail (3 marks)	0.1924	0.48	0.77
19	Black tail (4 marks)	0.1784	0.45	0.71
20	Black tail (5 marks)	0.1939	0.48	0.78

Table 2.3: The calculated volumes of Omeprazole to be administered

S/N	Rat Markings	Rat weight (kg)	Volume of ethanol administered (mL)	Volume of Omeprazole administered (mL)
1 (Control)	Blue tail (1 mark)	0.1876	0.47	
2	Blue tail (2 marks)	0.2197	0.54	
3	Blue tail (3 marks)	0.1767	0.44	
4	Blue tail (4 marks)	0.1703	0.43	
5	Blue tail (5 marks)	0.2345	0.6	
6 (Treatment)	Black tail (1 mark)	0.2291	1.15	0.92
7	Black tail (2 marks)	0.2620	1.31	1.05
8	Black tail (3 marks)	0.2652	1.33	1.06
9	Black tail (4 marks)	0.2700	1.35	1.08
10	Black tail (5 marks)	0.2351	1.18	0.94

Appendix 7 Ulcer indices of treatment groups



RESULT

Ulcer index = Sum of lengths of ridges of ulceration

Hexane (100 mg/kg) (black)

Rat marking	Ulcer index (mm)
1 mark	27
2 "	34
3 "	31
4 "	33
5 "	32

Hexane (200 mg/kg) (Green)

Rat marking	Ulcer index (mm)
1 mark	37
2 "	28
3 "	30
4 "	33
5 "	35

DCM (100 mg/kg) (Blue)

Rat marking	Ulcer index (mm)
1 Mark	39
2 "	44
3 "	38
4 "	39
5 "	43

DCM (200 mg/kg) (Green)

Rat marking	Ulcer index (mm)
1 mark	43
2 "	35
3 "	39
4 "	38
5 "	33

ETA (100 mg/kg) (Black)

Rat Marking	Ulcer index (mm)
1 marks	41
2 "	39
3 "	40
4 "	39
5 "	43

ETA (200 mg/kg) (Blue)

Rat marking	Ulcer index (mm)
1 marks	34
2 "	33
3 "	39
4 "	35
5 "	32

AQ - METH (100 mg/kg) (Blue)

Rat marking	Ulcer index (mm)
1 mark	48
2 "	43
3 "	48
4 "	45
5 "	39

AQ - METH (200 mg/kg) (Black)

Rat marking	Ulcer index (mm)
1 mark	48
2 "	52
3 "	47
4 "	49
5 "	47

Control group		Treatment (Omeprazole)	
Rat marking	Ulcer index (mm)	Rat marking	Ulcer index (mm)
1 mark	81	1 mark	19
2 "	83	2 "	22
3 "	79	3 "	21
4 "	85	4 "	23
5 "	75	5 "	20