**CHAPTER ONE**

**INTRODUCTION**

**1.1 Background of the Study**

*Cnidicollus anconitifolius*, also known as "Grewia mollis" or "Ancestor's Lantern" is a plant belonging to the family Malvaceae. It is widely distributed in tropical Africa, and its leaves, roots, and bark have been traditionally used to treat various diseases such as fever, inflammation, and diarrhoea. However, there is limited scientific evidence to support the traditional uses of this plant.

Inflammation is a physiological response of the body's immune system to injury, infection, or stress. The inflammatory process involves a complex interplay between various immune cells, cytokines, and signalling pathways. Although inflammation is essential for the body's defense mechanism, chronic inflammation can lead to various diseases such as arthritis, asthma, and cancer (Majdalawieh et al., 2017). Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used to treat inflammation-related diseases. However, long-term use of NSAIDs is associated with adverse effects such as gastrointestinal bleeding and kidney damage (Majdalawieh et al., 2017). Therefore, there is a need to develop new drugs from natural sources with fewer side effects.

Cnidicollus anconitifolius is a plant belonging to the family Malvaceae. It is widely distributed in tropical Africa, and its leaves, roots, and bark have been traditionally used to treat various diseases such as fever, inflammation, and diarrhoea (Adeyemi et al., 2016). However, there is limited scientific evidence to support the traditional uses of this plant. Therefore, the present study aims to evaluate the phytochemical composition and anti-inflammatory activities of the methanolic leaf extract of Cnidicollus anconitifolius on albino rats.

Phytochemicals are natural compounds found in plants that have various biological activities. Several studies have shown that phytochemicals possess anti-inflammatory, antioxidant, and antimicrobial activities. Therefore, the phytochemical composition of plants can be used as a basis for developing new drugs for the treatment of inflammation-related diseases.

Methanol is a widely used solvent for the extraction of plant constituents. Methanolic extracts of plants have been shown to possess various biological activities. Therefore, the methanolic leaf extract of *Cnidicollus anconitifolius* is expected to possess anti-inflammatory activities due to its rich phytochemical composition.

**1.2 Problem Statement**

Inflammation-related diseases pose a significant public health problem worldwide, and there is a need to develop new drugs with fewer side effects. Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used to treat inflammation-related diseases, but their long-term use is associated with adverse effects such as gastrointestinal bleeding and kidney damage. Therefore, there is a need to identify new drugs that can treat inflammation-related diseases without causing significant adverse effects.

*Cnidicollus anconitifolius* is a medicinal plant that is traditionally used to treat inflammation-related diseases. However, there is limited scientific evidence to support its traditional uses. Therefore, there is a need to evaluate the anti-inflammatory activities of the plant to determine its potential as a source of new drugs.

Furthermore, the phytochemical composition of the methanolic leaf extract of *Cnidicollus anconitifolius* has not been fully characterized. Therefore, there is a need to determine the phytochemical composition of the extract to identify the active compounds responsible for its anti-inflammatory activities. In addition, the acute toxicity of the extract has not been determined. Therefore, there is a need to determine the acute toxicity of the extract to ensure its safety for human use. Overall, the problem of this research is to evaluate the phytochemical composition and anti-inflammatory activities of the methanolic leaf extract of *Cnidicollus anconitifolius* and determine its acute toxicity to develop new drugs for the treatment of inflammation-related diseases with fewer side effects.

**1.3 Aim and Objectives of the Study**

The aim of the research is to evaluate the phytochemical composition and anti-inflammatory activities of the methanolic leaf extract of *Cnidicollus anconitifolius* on albino rats. The specific objectives of this study are:

1. To determine the phytochemical composition of the methanolic leaf extract of *Cnidicollus anconitifolius.*
2. To evaluate the anti-inflammatory activities of the methanolic leaf extract of *Cnidicollus anconitifolius* on albino rats by inducing edema.
3. To determine the acute toxicity of the methanolic leaf extract of *Cnidicollus anconitifolius.*

**1.4 Significance of the Study**

The phytochemical analysis of the methanolic leaf extract of *Cnidicollus anconitifolius* will provide valuable information regarding the presence of bioactive compounds, helping to establish its chemical composition.

The evaluation of anti-inflammatory activities will contribute to the understanding of the potential therapeutic effects of the plant extract, particularly in the context of inflammation-related diseases.

The findings may provide scientific evidence to support the traditional use of *Cnidicollus anconitifolius* in folk medicine as an anti-inflammatory agent.

The identification of mechanisms of action underlying the anti-inflammatory effects will deepen our understanding of the plant extract's pharmacological properties.

The safety assessment of the extract will ensure that it does not pose any significant risks or toxic effects, providing important preliminary data for future studies and potential clinical applications

**1.5 Scope of the Study**

The present study will focus on the phytochemical analysis and anti-inflammatory activities of the methanolic leaf extract of *Cnidicollus anconitifolius* on albino rats. The study will be conducted in the Department of Chemical Science and Technology, Federal Polytechnic, Mubi.

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**Chapter TWO**

**Literature Review**

**2.1 Introduction**

This chapter provides a comprehensive review of the relevant literature on the phytochemical composition and anti-inflammatory activities of the methanolic leaf extract of *Cnidicollus anconitifolius* on albino rats. It aims to establish the current knowledge gap and the rationale for conducting the present research study. This literature review will also highlight the previous research findings, methodologies, and limitations, as well as identify potential areas for further investigation.

**2.2 Inflammation**

Inflammation (from [Latin](https://en.wikipedia.org/wiki/Latin_language): [*inflammatio*](https://en.wiktionary.org/wiki/en:inflammatio#Latin)) is part of the complex biological response of body tissues to harmful stimuli, such as [pathogens](https://en.wikipedia.org/wiki/Pathogen), damaged cells, or [irritants](https://en.wikipedia.org/wiki/Irritation), and is a protective response involving [immune cells](https://en.wikipedia.org/wiki/Immune_cells), [blood vessels](https://en.wikipedia.org/wiki/Blood_vessel), and molecular mediators. The function of inflammation is to eliminate the initial cause of cell injury, clear out [necrotic](https://en.wikipedia.org/wiki/Necrotic) cells and tissues damaged from the original insult and the inflammatory process, and initiate tissue repair (Ferrero-Miliani *et al*., 2017).

The five [cardinal signs](https://en.wikipedia.org/wiki/Cardinal_signs) are heat, pain, redness, swelling, and [loss of function](https://en.wikipedia.org/wiki/Functio_laesa) (Latin *calor*, *dolor*, *rubor*, *tumor*, and *functio laesa*). Inflammation is a generic response, and therefore it is considered as a mechanism of [innate immunity](https://en.wikipedia.org/wiki/Innate_immune_system), as compared to [adaptive immunity](https://en.wikipedia.org/wiki/Adaptive_immune_system), which is specific for each pathogen. Too little inflammation could lead to progressive tissue destruction by the harmful stimulus (e.g. bacteria) and compromise the survival of the organism. In contrast, too much inflammation, in the form of chronic inflammation, is associated with various diseases, such as [hay fever](https://en.wikipedia.org/wiki/Hay_fever), [periodontal disease](https://en.wikipedia.org/wiki/Periodontal_disease), [atherosclerosis](https://en.wikipedia.org/wiki/Atherosclerosis), and [osteoarthritis](https://en.wikipedia.org/wiki/Osteoarthritis) (Hall, 2011).

Inflammation can be classified as either *acute* or *chronic*. Acute inflammation is the initial response of the body to harmful stimuli, and is achieved by the increased movement of [plasma](https://en.wikipedia.org/wiki/Blood_plasma) and [leukocytes](https://en.wikipedia.org/wiki/Leukocyte) (in particular [granulocytes](https://en.wikipedia.org/wiki/Granulocyte)) from the blood into the injured tissues. A series of biochemical events propagates and matures the inflammatory response, involving the local [vascular system](https://en.wikipedia.org/wiki/Vascular_system), the [immune system](https://en.wikipedia.org/wiki/Immune_system), and various cells within the injured tissue. Prolonged inflammation, known as *chronic inflammation*, leads to a progressive shift in the type of cells present at the site of inflammation, such as [mononuclear cells](https://en.wikipedia.org/wiki/Mononuclear_cell_infiltration), and is characterized by simultaneous destruction and [healing](https://en.wikipedia.org/wiki/Healing) of the tissue from the inflammatory process (Piira *et al.*, 2013).

Inflammation has also been classified as Type 1 and Type 2 based on the type of [cytokines](https://en.wikipedia.org/wiki/Cytokines) and [helper T cells](https://en.wikipedia.org/wiki/Helper_T_cells) (Th1 and Th2) involved. Inflammation is not a synonym for [infection](https://en.wikipedia.org/wiki/Infection). Infection describes the interaction between the action of microbial invasion and the reaction of the body's inflammatory response—the two components are considered together when discussing an infection, and the word is used to imply a microbial invasive cause for the observed inflammatory reaction (Doitsh *et al*., 2014).

Inflammation, on the other hand, describes purely the body's immunovascular response—whatever the cause may be. But because of how often the two are [correlated](https://en.wikipedia.org/wiki/Correlation), words ending in the suffix [*-itis*](https://en.wiktionary.org/wiki/-itis) (which refers to inflammation) are sometimes informally described as referring to infection. For example, the word [*urethritis*](https://en.wikipedia.org/wiki/Urethritis) strictly means only "urethral inflammation", but clinical [health care providers](https://en.wikipedia.org/wiki/Health_care_provider) usually discuss urethritis as a urethral infection because urethral microbial invasion is the most common cause of urethritis. Inflammation is part of the body's defense mechanism. It is the process by which the immune system recognizes and removes harmful and foreign stimuli and begins the healing process. Inflammation can be either acute or chronic (Adeneye *et al.,* 2012).

Inflammation is your body's defense against injury and infection. The five cardinal signs of inflammation are pain, heat, redness, swelling, and loss of function. However, some people with inflammation do not have any symptoms.  Inflammation is a fundamental and complex biological response that occurs in the body as a reaction to various harmful stimuli, such as infections, injuries, tissue damage, or irritants. It is a vital component of the body's immune system and serves several essential functions (Olusola *et al.,* 2018):

**Protection**: The primary purpose of inflammation is to protect the body from harm. When the body detects a threat, such as a pathogen (e.g., bacteria, viruses), physical injury (e.g., a cut or burn), or the presence of foreign substances (e.g., toxins), it initiates an inflammatory response. This response is designed to isolate and neutralize the threat.

**Tissue Repair**: Inflammation plays a crucial role in tissue repair and regeneration. It helps remove damaged cells, pathogens, and debris from the site of injury or infection. It also stimulates the production of new tissue and blood vessels to facilitate healing.

**Immune Response**: Inflammation is closely linked to the immune system. Immune cells, such as white blood cells (leukocytes), are mobilized to the site of inflammation to fight off infections and remove foreign invaders. This immune response helps to eliminate the source of the problem.

**2.2.1 Acute Inflammation**

Tissue damage due to trauma, microbial invasion, or noxious compounds can induce acute inflammation. It starts rapidly, becomes severe in a short time and symptoms may last for a few days for example cellulitis or acute pneumonia. Subacute inflammation is the period between acute and chronic inflammation and may last 2 to 6 weeks (Grace *et al.*, 2016).

**2.2.2 Chronic Inflammation**

Chronic inflammation is also referred to as slow, long-term inflammation lasting for prolonged periods of several months to years. Generally, the extent and effects of chronic inflammation vary with the cause of the injury and the ability of the body to repair and overcome the damage (Grace *et al.*, 2016).

**2.3 Phytochemical Composition of *Cnidicollus anconitifolius***

*Cnidicollus anconitifolius*, commonly known as "African spinach," belongs to the family Amaranthaceae. The plant has been traditionally used in various cultures for its medicinal properties. Several studies have reported the presence of various phytochemical constituents in *Cnidicollus anconitifolius*, including alkaloids, flavonoids, phenolic compounds, terpenoids, saponins, and tannins (Adeneye *et al.,* 2012; Aiyegoro *et al.,* 2010; Olusola *et al.,* 2018). These phytochemicals are known to possess diverse biological activities, such as antioxidant, anti-inflammatory, and antimicrobial properties.

**2.3.1 Alkaloids in *Cnidicollus anconitifolius***

Alkaloids are a class of nitrogenous organic compounds known for their diverse biological activities. Several studies have identified the presence of alkaloids in Cnidicollus anconitifolius. For example, Adeneye *et al*. (2012) reported the presence of alkaloids in the methanolic leaf extract of Cnidicollus anconitifolius. These alkaloids contribute to the plant's overall phytochemical composition and may have potential implications for its anti-inflammatory activities.

**2.3.2 Flavonoids in *Cnidicollus anconitifolius***

Flavonoids are a group of plant secondary metabolites known for their antioxidant and anti-inflammatory properties. Aiyegoro *et al*. (2010) identified the presence of flavonoids in *Cnidicollus anconitifolius*. Flavonoids have been extensively studied for their potential anti-inflammatory effects through the inhibition of inflammatory mediators (Anyanwu *et al*., 2019). The flavonoid content in *Cnidicollus anconitifolius* contributes to its overall phytochemical profile and may play a role in its anti-inflammatory activities.

**2.3.3 Phenolic Compounds in *Cnidicollus anconitifolius***

Phenolic compounds are widely distributed in plants and have been recognized for their antioxidant and anti-inflammatory properties. Olusola *et al*. (2018) identified the presence of phenolic compounds in *Cnidicollus anconitifolius*. These compounds are known to exert anti-inflammatory effects by modulating key signaling pathways involved in the inflammatory response (Olusola *et al.,* 2018). The phenolic compounds present in *Cnidicollus anconitifolius* contribute to its phytochemical composition and may contribute to its anti-inflammatory activities.

**2.3.4 Terpenoids in *Cnidicollus anconitifolius***

Terpenoids, also known as isoprenoids, are a diverse class of secondary metabolites found in various plants. Although limited studies have specifically investigated the terpenoid content of *Cnidicollus anconitifolius*, it is suggested that the plant may contain terpenoids based on its phylogenetic classification within the family Amaranthaceae. Further research is needed to identify and characterize the specific terpenoids present in *Cnidicollus anconitifolius* and explore their potential anti-inflammatory activities.

**2.3.5 Saponins in *Cnidicollus anconitifolius***

Saponins are a group of secondary metabolites known for their diverse biological activities, including anti-inflammatory properties. Adeneye *et al.* (2012) reported the presence of saponins in the methanolic leaf extract of Cnidicollus anconitifolius. These saponins may contribute to the overall phytochemical composition of the plant and play a role in its anti-inflammatory activities.

**2.3.6 Tannins in *Cnidicollus anconitifolius***

Tannins are polyphenolic compounds widely distributed in plants and known for their antioxidant and anti-inflammatory properties. Aiyegoro *et al.* (2010) identified the presence of tannins in Cnidicollus anconitifolius. Tannins have been reported to exhibit anti-inflammatory effects by modulating inflammatory mediators and reducing inflammatory responses (Aiyegoro *et al.,* 2010). The presence of tannins in Cnidicollus anconitifolius contributes to its phytochemical composition and may contribute to its anti-inflammatory activities.

**2.4 Anti-inflammatory Activities of *Cnidicollus anconitifolius***

**2.4.1 Carrageenan-Induced Paw Edema Model**

The carrageenan-induced paw edema model is a widely used experimental model to assess the anti-inflammatory effects of plant extracts. Adeneye *et al.* (2012) evaluated the anti-inflammatory activity of the methanolic leaf extract of *Cnidicollus anconitifolius* using this model. The study demonstrated a significant reduction in paw edema at various time points, indicating the potential anti-inflammatory effects of the extract. The observed reduction in edema suggests that *Cnidicollus anconitifolius* may inhibit the release of inflammatory mediators and modulate the inflammatory response.

**2.4.2 Cotton Pellet-Induced Granuloma Model**

The cotton pellet-induced granuloma model is another commonly used method to evaluate the anti-inflammatory properties of plant extracts. Aiyegoro *et al.* (2010) investigated the anti-inflammatory activity of *Cnidicollus anconitifolius* using this model. The study reported a significant reduction in granuloma formation and granuloma dry weight in the group treated with the methanolic leaf extract. These findings suggest that *Cnidicollus anconitifolius* possesses anti-inflammatory properties, possibly through the inhibition of granuloma formation and tissue inflammation.

**2.5 Uses of *Cnidicollus anconitifolius***

**2.5.1 Nutritional uses**

*Cnidicollus anconitifolius*, commonly known as tree spinach or tree cabbage, is widely utilized for its nutritional benefits. The leaves of this plant are rich in essential nutrients, including proteins, vitamins, minerals, and dietary fiber. A study by Afolayan and Jimoh (2009) highlighted the nutritional quality of Cnidicollus anconitifolius, indicating its potential as a valuable dietary resource.

**2.5.2 Culinary uses**

*Cnidicollus anconitifolius* is commonly used as a leafy vegetable in various cuisines. The tender leaves are cooked and consumed as a nutritious and flavorful addition to soups, stews, and stir-fries. The plant's leaves have a slightly bitter taste, which adds a distinct flavor profile to dishes. Chukwuma *et al.* (2015) emphasized the culinary potential of *Cnidicollus anconitifolius* and its contribution to local food culture.

**2.5.3 Medicinal uses**

*Cnidicollus anconitifolius* has a long history of traditional medicinal use in various regions. Different parts of the plant, including the leaves, stems, and roots, are used to prepare herbal remedies for treating various ailments. The plant is believed to possess anti-inflammatory, analgesic, and antimicrobial properties. Onwukaeme *et al.* (2010) conducted a study highlighting the anti-inflammatory activity of the ethanolic leaf extract of *Cnidicollus anconitifolius* in rats.

**2.5.4 Traditional uses**

In addition to its nutritional and medicinal uses, *Cnidicollus anconitifolius* holds cultural and traditional significance in various communities. The plant is often incorporated into traditional ceremonies, rituals, and festive celebrations. Grace *et al.* (2006) documented the cultural importance and management practices of *Cnidicollus anconitifolius* in southern Africa, highlighting its connection to local customs and traditions.

**2.5.5 Antioxidant Activity**

*Cnidicollus anconitifolius* has been found to possess antioxidant properties. Antioxidants help in neutralizing harmful free radicals in the body, which can cause oxidative damage and contribute to various diseases. Anyasor *et al.* (2014) conducted a study on the essential oils and crude extracts of three *Cnidoscolus* species, including *Cnidicollus anconitifolius*, and highlighted their antioxidant potential.

**2.5.6 Anti-Diabetic Properties**

In traditional medicine, *Cnidicollus anconitifolius* has been used for managing diabetes. Adedayo *et al.* (2017) conducted a study on medicinal plants used for the treatment of diabetes in Nigeria and reported the use of *Cnidicollus anconitifolius* as a potential anti-diabetic remedy.

**2.5.7 Toxicological Properties**

While *Cnidicollus anconitifolius* has various beneficial properties, it is important to consider potential toxicological aspects. Ogundare *et al.* (2013) conducted a study to evaluate the toxicological properties of *Cnidicollus aconitifolius* leaves. The study indicated that the plant exhibited low toxicity, supporting its safe use in traditional medicine and culinary applications.

**2.5.8 Industrial Applications**

Beyond its nutritional and medicinal uses, *Cnidicollus anconitifolius* has potential industrial applications. The plant's fibers can be extracted and utilized for the production of textiles, paper, and other fiber-based products. Opabode *et al.* (2015) evaluated *Cnidicollus aconitifolius* for its nutritional and industrial uses, highlighting its potential as a renewable resource.

**2.6 Summary**

In summary, the literature review demonstrates that *Cnidicollus anconitifolius* possesses a rich phytochemical composition, including alkaloids, flavonoids, phenolic compounds, terpenoids, saponins, and tannins. These phytochemicals contribute to its anti-inflammatory activities, as evidenced by studies using animal models. The mechanisms of action underlying the anti-inflammatory effects of *Cnidicollus anconitifolius* involve the inhibition of inflammatory mediators and modulation of key signaling pathways.

Different studies have collectively demonstrated the diverse range of uses associated with *Cnidicollus anconitifolius,* including its nutritional value, medicinal properties, antioxidant activity, potential anti-diabetic effects, cultural significance, and industrial applications. However, further research is necessary to explore its full potential, optimize its usage, and understand any potential contraindications or side effects.

Despite the existing body of literature on the phytochemical composition and anti-inflammatory activities of Cnidicollus anconitifolius, there are still certain gaps and limitations that need to be addressed. First, most of the studies have focused on animal models, particularly albino rats, and there is a need to conduct further research using other animal models and, eventually, clinical trials involving human subjects. Additionally, the specific mechanisms through which the phytochemical constituents of *Cnidicollus anconitifolius* exert their anti-inflammatory effects require further elucidation. Understanding these mechanisms would provide insights into the molecular targets involved and facilitate the development of targeted therapies.

**Chapter Three**

**Materials and Methods**

**3.1 MATERIALS**

**3.1.1 Equipment/Apparatus**

Beaker, conical flask, test tubes, wash glass, weighing balance, measuring cylinder, syringe (1ml and 5ml), grinder, water bath, Soxhlet extraction, centrifuge, desiccator, Whatman filter paper, micropipette, maxing tape.

**3.1.2 Chemicals, Solvents and Reagents**

Absolute methano, ibuprofen, ferric chloride, concentrated ammonium, concentrated hydrochloric acid, distilled water, potassium mercury iodide (mayers solution) sulphuric acid (H2SO4) ferric chloride (FeCl3) olive oil, indomethacin.

**3.1.3 Plant Material Collection and Identification**

The leaves of *Cnidicollus anconitifolius* will be collected from a designated location in a botanical garden in Federal Polytechnic, Mubi, during June of 2023. The plant will be identified and authenticated by a qualified botanist from the Chemical Science Department. Voucher specimen number will be assigned to the plant material, which will be stored for future reference.

**3.1.4 Experimental Animals**

Healthy adult albino rats (Rattus norvegicus) of both sexes, weighing between 180-200g, will be obtained from the Animal and Health Production Department of Federal Polytechnic, Mubi. The animals will be acclimatized for two weeks under standard laboratory conditions (temperature: 22±2°C, relative humidity: 55±5%, 12-hour light/dark cycle) with free access to standard laboratory diet and water ad libitum. The animals will be handled according to the guidelines set by the Institutional Animal Ethics Committee.

**3.2 METHODOLOGY**

**3.2.1 Preparation of Methanolic Leaf Extract**

The collected leaves of *Cnidicollus anconitifolius* will be thoroughly washed under running tap water to remove any adhering dirt and foreign particles. After air-drying, the leaves were finely ground to a powder using an electric grinder. The powdered leaves (100 g) were macerated in 500 mL of methanol in a clean and sterile container for 72 hours with intermittent shaking. The macerate was filtered through Whatman filter paper, and the filtrate was concentrated under reduced pressure using a rotary evaporator. The obtained methanolic extract was stored in airtight containers at 4°C until further use.

**3.2.2 Phytochemical Screening**

**3.2.2.1 Qualitative Analysis**

The methanolic leaf extract of *Cnidicollus anconitifolius* will be subjected to preliminary phytochemical screening to identify the presence of various secondary metabolites. Standard qualitative tests were performed to detect the following classes of phytochemicals: alkaloids, flavonoids, tannins, saponins, phenols, terpenoids, glycosides, and steroids. The tests were carried out according to established procedures as described by Harborne (1998) and Trease and Evans (2002).

**3.2.2.1.1 Test for Alkaloids**

0.4g of *Cnidicollus anconitifolius* extract will be stirred with 8ml of 1% HCl and the mixture was warmed and filtered. 2ml of filtrate was treated separately with a few drops of potassium mercuric iodide (mayers reagent). Turbidity or precipitation with either of these reagents will be taken as evidence for existence of alkaloids.

**3.2.2.1.2 Test for Flavoniods**

20mg of extract will be suspended in 20ml of distilled water to get the filtrate. 5ml of dilute ammonia solution will be added to 5ml of filtrate followed by few drops of concentrated H2SO4. Presence of flavonoids will be confirmed by followed coloration.

**3.2.2.1.3 Test for Tannins**

50mg of extract will be boiled in 20ml of distilled H2O and filtered. A few drops of 0.1% FeCl3 will be added in filtrate and observed for colour change brownish green or a blue-black coloration will be taken as evidence for the presence of tannins.

**3.2.2.1.4 Test for Saponins**

The ability of saponins to produce emulsion with oil will be used for the screening test. 10mg of the plant extract will be boiled in 20ml of distilled water in a water with, for 5min and filtered. 10ml of the filtrate will be mixed with 5ml of distilled water and shaken vigorously for froth formation. 3 drops of olive, oil will be mixed with froth, shaken vigorously. The emulsion development will be observed.

**3.2.2.1.5 Test for Anthraquinones**

20mg of plant extract will be boiled with 6ml of 1% HCl and filtered. The filtrate will be shaken with 5ml of benzene filtered and 2ml of 10% ammonia solution will be added to the filtrate. The mixture will be shaken and the presence of a pink, violet or red colour in the ammonical phase indicated the presence of free hydroxyl anthraquinones.

**3.2.2.1.6 Test for Cardiac Glycosides**

5ml of glacial acetic acid having one drop of FeCl3 (Ferric Chloride solution). To the mixture obtained 1ml of concentrated, H2SO4 will be added to form a layer. The presence of browning of the interface indicated deoxy sugar-characteristics of cardiac glycosides.

**3.2.3 Quantitative Analysis**

The quantitative analysis of selected phytochemical constituents in the methanolic leaf extract was performed using appropriate spectrophotometric methods. The concentrations of total phenolic content, total flavonoid content, and total alkaloid content were determined following established protocols described in the literature.

**3.2.4 Acute Toxicity Study**

An acute toxicity study will be conducted to determine the safe dose range of the methanolic leaf extract of *Cnidicollus anconitifolius*. The study will follow the guidelines provided by the Organization for Economic Co-operation and Development (OECD) Test Guideline 423. A total of 20 albino rats will be randomly divided into four groups (n=5). The animals fasted overnight prior to the experiment. The extract will be administered orally at doses of 100, 500, 1000, and 2000 mg/kg body weight. The rats will be observed continuously for the first 4 hours and then at regular intervals for 24 hours to record any signs of toxicity, morbidity, or mortality.

**3.3 Anti-Inflammatory Activity Evaluation**

**3.3.1 Induced Paw-Edema Method**

The anti-inflammatory activity of the methanolic leaf extract will be evaluated using the carrageenan-induced paw edema method in rats. Thirty-six (36) albino rats will be randomly divided into Six (6) groups. Inflammation will be induced in all groups of albino rats with 5% formaldehyde except one group which will serve as the normal control group. The seven groups will be labelled as follows;

**Normal control group:** will be fed with normal diet and it will serve as normal control.

**Inflamed control group:** will be fed with formalin and will be fed with normal diet.

**Standard control group:** will be administered with 2mg/kg body weight paraceutamol orally for 7 days in addition to the normal diet and it will serve as the treatment or treated control or reference.

**Inflamed and will be treated with 100mg/kg:** The animals in this group will be administered with 100mg/kg aqueous extract of methanolic leaf extract of *Cnidicollus anconitifolius* for 7 days in addition to normal diet.

**Inflamed and will be treated with 300mg/kg:** The animals in this group will be administered with 300mg/kg aqueous extract of methanolic leaf extract of *Cnidicollus anconitifolius* for 7 days in addition to normal diet.

**Inflamed and will be treated with 400mg/kg:** The animals in this group will be administered with 400mg/kg aqueous extract of methanolic leaf extract of *Cnidicollus anconitifolius* for 7 days in addition to normal diet.

**3.3.2 Method**

The measurement of paw volume will be done by means of volume displacement technique one hour after formalin injection and after the treatment with the methanolic leaf extract of *Cnidicollus anconitifolius* by the displacement of water (De Miranda *et al.,* 2000). Briefly, an empty 250ml beaker will first be dipped into a 1000ml beaker which will contain 500ml of water volume and the volume of water that will be displaced by the empty 250ml beak will be recorded. An albino rat will be then placed in the empty beaker 250ml and dipped in the bigger beaker.

The volume that will be displaced by the empty beaker and the albino rat will also be recorded. The volume of the empty beaker will then be subtracted from the volume of albino rat in the beaker. This procedure will be repeated for all of the albino rats in various groups.

Reduction in paw volume compared to the treated control albino rat will be considered as the anti-inflammatory response.

The percentage inhibition will be obtained using the following ratio:

x 100

Where;

V0 = will represent the average paw volume of the albino rats for each group after inducing with formaline and,

Vt = will represent the average paw volume of each group after treatment with methanolic leaf extract of *Cnidicollus anconitifolius* (De Miranda *et al.,* 2000).

**3.3.3 Biochemical Analysis**

At the end of the experiment, the animals will be sacrificed, and blood samples will be collected by cardiac puncture. The blood samples will be centrifuged to obtain serum, which will be used for the estimation of biochemical markers.

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