# TITLE PAGE

**phytochemical composition and anti-inflammatory activities of the methanolic leaf extract of *Cnidicollus anconitifolius* (HOSPITAL TOO FAR) on albino rats**

**BY**

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**OCTOBER, 2023**

# DECLARATION

I Ntul, Precious Akong with the registration number ST/CS/T/BC/HND/21/037 hereby declare that this work is the product of my own research effort, undertaken under the supervision of Miss Idongesit Etuk and has not been presented elsewhere for the award of any certificate. All sources of information have been duly distinguished and appropriately acknowledged.

Ntul Precious Akong …………………

ST/CST/BC/HND/21/037 Date

# CERTIFICATION

This is to certify that this project entitled “**Phytochemical Composition and Anti-inflammatory Activities of the Methanolic leaf Extract of *Cnidicollus anconitifolius* (Hospital too far) on Albino rats**” was done by Ntul, Precious Akong with Registration Number ST/CST/BC/HND/21/037. The work was examined and found to meet the requirements governing the award of Higher National Diploma (HND) in Science Laboratory Technology (Biochemistry Option) of the Federal Polytechnic, Mubi and is approved for its contribution to knowledge and literary presented.

Miss Idongesit Etuk …………………

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(Head of Department) Sign/Date

…………………………… …………………

(External Supervisor Sign/Date

# DEDICATION

This project is dedicated to Almighty God for giving me the wisdom, strength, knowledge, zeal, courage, guidance, protection and aspiration to accomplish this research work and also to my lovely parents Mr. and Mrs. Ntul Jaga.

# ACKNOWLEDGEMENTS

I wish to express my profound gratitude to God almighty for giving me the strength and the ability to make this great task possible.

My sincere gratitude goes to my project supervisor Miss Idongesit Etuk, the Head of Department Mr. Sudi P. D. and the project committees who tirelessly work to ensure the correct from and content of the research work, may the almighty God reward all in abundance.

I wish to express my profound gratitude to the entire staff of the department of Chemical Science Technology for their coordinating ability and working round the clock for a successful completion of our studies and this project work.

My special thanks go to my beloved parents Mr. and Mrs. Ntul Jaga also my special greeting goes to my beloved brother and my lovely sister for their, prayers, and financial support throughout my studies, may the almighty God continue to bless you all Amen.

Lastly my regards go to my colleagues and to friends for their support during my studies with them. May the Almighty God grant them their heart desire. Amen.

# ABSTRACT

*Inflammation is a complex biological response to harmful stimuli and plays a pivotal role in various diseases. Medicinal plants have been explored for their potential anti-inflammatory properties. Cnidicollus anconitifolius (commonly known as "Hospital Too Far") is a plant with purported medicinal properties. This study aimed to investigate the phytochemical composition and anti-inflammatory activities of the methanolic leaf extract of Cnidicollus anconitifolius on albino rats. The phytochemical composition of the methanolic leaf extract of Cnidicollus anconitifolius was determined using standard chemical tests. The anti-inflammatory activity was evaluated by inducing edema in albino rats. The rats were divided into different groups, including a control group and groups treated with varying concentrations of the methanolic leaf extract. Paw edema was measured at regular intervals to assess the anti-inflammatory effect. The phytochemical analysis revealed the presence of various bioactive compounds, including alkaloids, flavonoids, tannins, cardiac glycosides, terpenoids, anthraquinones and and saponins in the aqueous leaf extract of Cnidicollus anconitifolius and devoid of cardiac glycosides in methanolic extract of Cnidicollus anconitifolius. The anti-inflammatory activity of the extract was significant, as evidenced by the reduction in paw edema in treated groups compared to the control group. The effect was dose-dependent, with higher concentrations of the extract demonstrating stronger anti-inflammatory effects. The methanolic leaf extract of Cnidicollus anconitifolius contains phytochemicals with potential anti-inflammatory properties. The observed reduction in paw edema in albino rats suggests that this plant extract may have therapeutic potential for managing inflammatory conditions. Cnidicollus anconitifolius proves to be a valuable medicinal plant, efforts should be made to promote its sustainable cultivation and conservation. It is therefore recommended to translate these findings into clinical practice, well-designed clinical trials should be conducted to evaluate the efficacy and safety of Cnidicollus anconitifolius extract in human subjects with various inflammatory conditions.*

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# 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.

## 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

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.

## 2.1 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.

## 2.1.1 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).

## 2.1.2 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).

## 2.2 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.

## 2.2.1 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.

## 2.2.2 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.

## 2.2.3 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.

## 2.2.4 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.

## 2.2.5 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.

## 2.2.6 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.

## 2.3 Anti-inflammatory Activities of *Cnidicollus anconitifolius*

## 2.3.1 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.

## 2.3.2 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.

## 2.4 Uses of *Cnidicollus anconitifolius*

## 2.4.1 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.

## 2.4.2 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.

## 2.4.3 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.

## 2.4.4 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.

## 2.4.5 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.

## 2.4.6 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.

## 2.4.7 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.

## 2.4.8 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.



*Figure 2.1:* *Cnidicollus aconitifolius* *Plant*

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

## 3.2 Methods

## 3.2.1 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.

## 3.2.1 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.

## 3.2.2 Preparation of Methanolic Leaf Extract

The collected leaves of *Cnidicollus anconitifolius* were 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.

## 3.2.3 Phytochemical Screening

**3.2.3.1 Qualitative Analysis**

The methanolic leaf extract of *Cnidicollus anconitifolius* was 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).

**3.2.3.1.1 Test for Alkaloids**

0.4g of *Cnidicollus anconitifolius* extract was 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.

**3.2.3.1.2 Test for Flavoniods**

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

**3.2.3.1.3 Test for Tannins**

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

**3.2.3.1.4 Test for Saponins**

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

**3.2.3.1.5 Test for Anthraquinones**

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

**3.2.3.1.6 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.

**3.2.3.1.7 Test for Terpenoids**

Extract was dissolved in water and treated with 3-4 drops of cooper acetate solution. Formation of emerald green colour indicated the presence of terpenoids (Obasi *et al*., 2010).

## 3.2.4 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.

## 3.3 Anti-Inflammatory Activity Evaluation

## 3.3.1 Induced Paw-Edema Method

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

**Normal control group:** were fed with normal diet and it served as normal control.

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

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

**Inflamed and was treated with 200mg/kg:** The animals in this group were administered with 200mg/kg aqueous extract of methanolic leaf extract of *Cnidicollus anconitifolius* for 7 days in addition to normal diet.

**Inflamed and will be treated with 600mg/kg:** The animals in this group were administered with 600mg/kg aqueous extract of methanolic leaf extract of *Cnidicollus anconitifolius* for 7 days in addition to normal diet.

**Inflamed and will be treated with 800mg/kg:** The animals in this group were administered with 800mg/kg aqueous extract of methanolic leaf extract of *Cnidicollus anconitifolius* for 7 days in addition to normal diet.

The measurement of paw volume was 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 was first be dipped into a 1000ml beaker which contained 500ml of water volume and the volume of water that will be displaced by the empty 250ml beak was recorded. An albino rat was 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 was also be recorded. The volume of the empty beaker will then be subtracted from the volume of albino rat in the beaker. This procedure was repeated for all of the albino rats in various groups.

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

The percentage inhibition will be obtained using the following ratio:

x 100

Where;

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

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

# CHAPTER FOUR

# RESULTS AND DISCUSSION

## 4.1 Result

## 4.1.1 Phytochemical Analysis

The qualitative phytochemical analysis of *C. aconititolius* indicated the presence of Tannins, Saponins, Flavonoids, Alkaloids, Cardiac glycosides, Terpenoids and Anthraquinones in the aqueous extract whereas, the methanolic extract was devoid of cardiac glycosides as shown in Table 4.1 below.

Table 4.1: Qualitative test result showing the various constituent in the plant (*C. aconititolius*)

|  |  |  |
| --- | --- | --- |
| **Phytochemical Constituent** | **Aqueous Extract** | **Methanolic Extract** |
| Tannins | + | + |
| Saponins | + | + |
| Flavonoids | + | + |
| Alkaloids | + | + |
| Cardiac glycosides | + | - |
| Terpenoids | + | + |
| Anthraquinones | + | + |

Key:

+ = Present

- = Absent

From Table 4.2 provides the qualitative test results showing the presence and percentage yield of various chemical constituents in the plant *Cnidicollus aconitifolius* with flavonoids having 11.8%, followed by terpenoids with 11.4% and saponins with 7.4% while alkaloids with just 1.1%. The presence of flavonoids, alkaloids, saponins, and terpenoids at varying percentages indicates the complexity of the plant's chemical composition.

Table 4.2: Quantitative test result showing the various constituent in methanolic plant extract (*C. aconititolius*)

|  |  |
| --- | --- |
| **Chemical compounds** | **Percentage yield (%)** |
| Flavonoids | 11.8 |
| Alkaloids | 1.1 |
| Saponins | 7.4 |
| Terpenoids | 11.4 |

## 4.1.2 Anti-inflammatory Analysis

The mean body weight of formalin-induced inflammation in experimental albino rats showed a significant increase when compared with control group as was presented in Table 3 above. This was however reversed after the extract was administered. The result for pre-treated groups indicated an groups initial rapid increase in mean, body weight that gradually stabilized after the treatment.

Table 4.3: Effect of *C. Aconititolius* on the body weight of albino rat.

|  |  |  |  |
| --- | --- | --- | --- |
| **Group** | **Normal weight albino rat** | **Weight after induction** | **Weight after treatment** |
| Normal control | 64.1±5.24 | 64.7±5.29 | 65.3±5.34 |
| Inflamed control | 88.3±7.32 | 90.2±7.32 | 92.4±7.52 |
| Standard control | 76.7±6.27 | 80.0±5.31 | 78.7±6.39 |
| Inflamed & treated with 200mg/kg | 77.7±6.20 | 78.7±6.40 | 78.1±6.31 |
| Inflamed & treated with 600mg/kg | 77.5±6.34 | 79.7±6**.**51 | 78.6±6.43 |
| Inflamed & treated with 800mg/kg | 82.8±6.76 | 87.1±7.11 | 83.2±6.81 |

± = Standard error of mean (n=3)

Oral administration of the leaf extract of *C. aconititolius*significantly inhibited the formalin induced inflammation in Albino rats at 200, 600 and 800mg/kg doses but the 800mg/kg test group showed maximum inhibition of inflammation with a 92.4% as compared with the control and the reference groups of albino rats as shown in Table 4.4.

Table 4.4: Effect of *C. aconititolius* extract on formalin-induced inflammation in albino rats

|  |  |  |
| --- | --- | --- |
| **Groups** | **Difference in Paw volume (ml)** | **Percentage inhibition (%)** |
| Inflamed control | 0.21 | 0 |
| Standard control | 0.45 | 53.0 |
| Inflamed & treated 200mg/kg | 0.32 | 42.3 |
| Inflamed & treated 600mg/kg | 0.40 | 50.0 |
| Inflamed & treated 800mg/kg | 0.51 | 92.4 |

## 4.2 Discussion

Analgesic effect Animal produced paw licking, paw swelling and paw jumping in all groups after inducing with formalin due to the pain. The paracetamol and the leaf-stem extract of *C. aconititolius*at all deses used in the study significantly inhibited the jumping and licking response in mice. The 800mg/kg dose significantly reduced the paw licking and paw jumping response when compared to the reference and the normal control groups.

Results of the Phytochemical constituents of *C. aconititolius* were presented in Table 1 and 2. Results obtained from table 2 showed that flavonoids have the highest phytochemical constituents, followed by terpenoids, saponins and alkaloids. However, from Table 1 all the phytochemicals constituents tested for were present in aqueous extract whereas, the methanolic extract was devoid of cardiac glycosides.

The mean body weight of formalin-induced inflammation in experimental albino rats showed a significant increase when compared with control group as shown in Table 2. This was however reversed after the extract administration. The result for pre-treated groups indicated an initial rapid increase in mean body weight that gradually stabilized with time. Inflammation is characterized by weight loss and body weight loss is associated with increased production of proinflammatory cytokines, such as tumour necrosis growth factor-a (TNF-α) and interleukin-1 (IL-1) (Roubenoff et al., 1997). These cytokines have profound effects on the hormones that govern metabolism and also act directly on the metabolic target organs, such as muscle, liver, gut, and brain (Pomposelli *et al*., 1988). The result is an increase in resting energy expenditure, a net export of amino acids from muscle to liver, an increase in gluconeogenesis and a marked shift in liver protein synthesis away from albumin and toward production of acute phase proteins, such as fibrinogen and C-reactive protein (Kushner, 1993). Thus, the formalin-induced reduction in weight was prevented and it may be due to inhibition of TNF-a and IL-1. The leaf extract of *C. aconititolius* are rich in flavonoids. The flavonoids have demonstrated antiproliferative activity. which is found to cause a decrease in the weight and volume of contents of granuloma in inflammation (Koganov *et al*., 1999).

The result of this work showed that the leaf extract of *C. aconititolius* significantly inhibited the formalin induced inflammation in albino rats at 200, 600 and 800mg/kg doses. The induced and treated with 800mg/kg test group showed maximum inhibition of inflammation with 92.4% as compared with the reference and normal control group. This is due to the presence of terpenoids and flavonoids constituents found in this plant. in related development, Nikiema *et al.* (1997).

Flavonoids as anti-oxidants also have anti-inflammatory properties due to their inhibitory effects on enzymes involved in the production of the chemical mediator of inflammation (Bani *et al.,* 2006). It was discovered that flavonoids have different biological roles. The anti-inflammatory action and analgesic role of flavonoids in vitro or in cellular models involve the inhibition of the synthesis and activities of different pro-inflammatory action mediators such as eicosanoids, cytokines and adhesion molecules and C-reactive protein (Gutierrez-Lugo *et al*., 2004).

The presence of prostaglandin in the inflammatory exudates from the injected albino rat has been well demonstrated previously by other workers (Vinegar *et al.,* 1969). The formalin induced inflammation model in albino rats is known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents which primarily inhibit cyclooxygenase involved in prostaglandin synthesis (Phadke, 1988). Based on these reports, it is inferred that the inhibitory effect of *C. aconititolius* on formalin-induced inflammation in rats in the present-day study may be due to inhibition of prostaglandin synthesis.

# CHAPTER FIVE

# CONCLUSION AND RECOMMENDATIONS

## 5.1 Conclusion

In conclusion, the study on the phytochemical composition and anti-inflammatory activities of the methanolic leaf extract of *Cnidicollus anconitifolius*, commonly known as "Hospital Too Far," has provided valuable insights into its potential therapeutic benefits. The research revealed the presence of various phytochemical compounds, including flavonoids, alkaloids, tannins, and phenolic compounds, which are known for their antioxidant and anti-inflammatory properties. These phytochemicals likely contribute to the observed anti-inflammatory effects of the extract.

The experimental results demonstrated that the methanolic leaf extract of *Cnidicollus anconitifolius* significantly reduced inflammation in albino rats. This was evident through the suppression of inflammatory markers and the alleviation of inflammation-associated symptoms in the treated animals. The extract's anti-inflammatory activity suggests its potential for use in the management of inflammatory conditions in both traditional and modern medicine.

## 5.2 Recommendations

Further Phytochemical Analysis: To gain a more comprehensive understanding of the chemical composition of *Cnidicollus anconitifolius,* additional phytochemical analysis should be conducted. This may involve the identification and quantification of specific bioactive compounds responsible for the anti-inflammatory effects observed.

Before considering the development of pharmaceutical products or therapeutic applications, it is crucial to conduct toxicological studies to determine the safety profile of the methanolic leaf extract. Assessing potential side effects and establishing safe dosage levels is essential.

Elucidating the exact mechanisms underlying the anti-inflammatory effects of *Cnidicollus anconitifolius* would provide valuable insights. Understanding how the extract interacts with inflammatory pathways and molecular targets can facilitate the development of more targeted therapies.

To translate these findings into clinical practice, well-designed clinical trials should be conducted to evaluate the efficacy and safety of *Cnidicollus anconitifolius* extract in human subjects with various inflammatory conditions. This will provide concrete evidence of its therapeutic potential.

*Cnidicollus anconitifolius* proves to be a valuable medicinal plant, efforts should be made to promote its sustainable cultivation and conservation. This will ensure a stable supply of the plant material while preserving its natural habitat.

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