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Formulation and Evaluation of Coat Button (*Tridax Procumbens; Asteraceae*) Leaf Extract Simple Syrup for Pain Management

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A B S T R A C T

People suffer distressing and unpleasant sensations induced by pain signals, which considerably impair an individual's quality of life. As a result, many turn to analgesics, which are specifically developed to relieve pain. This study aims to examine the analgesic properties of coat button (Tridax procumbens; Asteraceae) leaf extract produced as a syrup as a natural alternative to standard analgesic medicines. The goal of this study is to determine the least hazardous concentration of Coat Button Leaf Extract and the most effective concentration of the formulated Coat Button extract (50%, 60%, and 70%) as an analgesic. Identify any significant differences in analgesic activity between Coat Button concentrations with the strongest pain inhibition and the standard medicine (Paracetamol syrup). Determine whether the formulated syrup fits the standard standards. The researchers used experimental analysis on the plant sample to determine its analgesic qualities, which resulted in the following data. To determine the dangerous and effective concentrations, the plant sample was examined utilizing an acute oral toxicity test as well as many animal pain models. Additionally, organoleptic studies are used to analyze the physicochemical qualities of the prepared syrup. The collected data were systematically entered into tables for statistical analysis and interpretation. This study determined that the minimal harmful concentration is 75%. The researchers determined that all three concentrations were effective, albeit using different techniques. When comparing the analgesic activity of Coat Button and Paracetamol syrups, both syrups were effective. Notably, two out of three methods showed no significant difference, and are deemed equally effective. Finally, the researchers determined that the created syrup matched the standard parameters for a syrup.

Keywords: Pain, Pain management, *Tridax procumbens*, Syrup

1. Introduction

Health and wellness refer to all aspects of physical, mental, and emotional well-being, including the presence or absence of disease and discomfort. It entails comprehending how the body works, the elements that contribute to disease, and the numerous ways in which pain can affect an individual's overall health and quality of life. Exploring health and wellbeing enables a thorough examination of disease and pain in the context of human well-being and healthcare (Felman, 2023).

Health is described as a state of complete well-being in which a person is free of illness and pain. When a person is disease-free, it signifies that their body is operating properly, with no illness or medical condition that could jeopardize their health (National Academies Press (US), 2023). Similarly, being pain-free refers to a condition of bodily comfort and the lack of anguish or suffering. As a result, being disease- and pain-free is frequently seen as the pinnacle of health due to the enormous impact it has on one's total well-being. It enables people to participate in daily activities without constraints, allowing them to completely enjoy life and pursue their goals and dreams (Oleribe et al., 2018).

A disease is a condition that interferes with an organism's normal functions. Infections, genetic mutations, environmental pollutants, and autoimmune disorders are all potential causes of disease (National Institutes of Health, 2007). Pain is a common symptom of many disorders and can be caused by inflammation, tissue damage, nerve pressure, or alterations in brain chemistry. When the body's immune system reacts to injury or infection, it produces inflammation. This can cause pain, swelling, and redness (Chen et al. 2017). Disease-related tissue damage can also cause discomfort. A heart attack, for example, might induce chest pain by damaging the heart muscle. When disease puts pressure on nerves, it can produce discomfort in the place where the nerve passes. Furthermore, sickness can modify how the brain receives pain signals, resulting in persistent discomfort without continued tissue damage (American Heart Association, 2021).

According to the International Association for the Study of Pain (IASP) 1979, pain is an unpleasant sensation induced by real or potential tissue damage that is accompanied by sensory and emotional responses. It can emerge in varying degrees and involve a wide variety of emotions. These sensations differ considerably, even among people who have similar diseases or scars. Pain ranges from slight discomfort to profound agony, and everyone’s perception is unique. This definition of pain is accepted worldwide and supported by healthcare experts and organizations such as the World Health Organization

(WHO), emphasizing the importance and validity of understanding and treating pain in human life. Pain is an important function that protects the body from injury. It is triggered by unpleasant stimuli, which is subsequently transmitted to the central nervous system (CNS) via specialized neuronal networks for processing. In this sophisticated process, pain serves as a key warning system, alerting people to potential hazards and pushing them to take the required precautions to safeguard their health. Pain, in its different forms and intensities, is a widespread health issue that affects people of all ages, making it a major clinical, social, and economic problem in communities around the world (Robertson, 2023).

Although there are five main categories for pain, some can be classified into more than one, which is where complications arise. The five most prevalent forms of pain are radicular, neuropathic, nociceptive, and acute. For example, acute pain is a type of temporary pain that is brought on by an injury or sickness and often goes away in a few weeks or months. Contrarily, chronic pain is a persistent form of pain that lasts longer than three months and is frequently brought on by illnesses like fibromyalgia, cancer, or arthritis. Persistent pain can hinder everyday tasks and be extremely debilitating. Another form of pain brought on by injury to the nerves is neuropathic pain.

It can be caused by a few things, such as an accident, an infection, or specific diseases. The most prevalent kind of pain is nociceptive pain, which is brought on by activation of nociceptors, pain receptors for tissue damage. Sharp or painful pain is a common description of nociceptive pain. Another form of pain brought on by inflammation or compression of a nerve root is referred to as radiating pain. A common description is that it feels like a searing or shooting pain that travels down the afflicted nerve. Spinal stenosis and herniated discs are frequently linked to radicular pain (Beaumont, 2021).

Untreated pain has a significant and negative impact on a person's overall quality of life. This effect has been shown for diverse pain kinds and causes as well as across age groups. However, by reducing pain and the suffering it causes, efficient pain management with analgesic therapy has raised people's quality of life. The main objective of pain treatment is analgesia, which is defined as the absence of pain in response to stimuli that would normally cause pain (Cleveland Clinic, 2021).

Standard painkillers are generally accessible and effective, but they have several drawbacks, including the possibility of toxicities and other adverse effects. These include conditions that can cause increased bleeding, such as gastrointestinal irritation, gastric ulcers, changes in renal function, blood pressure effects, hepatic damage, and platelet inhibition (Tadesse et al., 2020). Given these challenges, there is a growing imperative to intensify research into the potential analgesic properties of medicinal plants as alternatives for pain management.

For centuries, traditional medicinal plants have been utilized to alleviate pain. Recent research indicates that many of these plants possess potent analgesic properties. One such plant is Coat Button (*Tridax procumbens*; Asteraceae), a common weed considered a pest in many parts of the world. Despite being considered a weed in many regions; Coat Button has a rich history of traditional use in folk medicine. Its conventional use for medicinal purposes has been well documented by Jayasundera et al. (2021) noting that "these weeds are traditionally known to have medicinal properties and have been used for treatments." In addition to this study, indigenous communities have long recognized its therapeutic potential, employing various parts of the plant to treat various ailments, including pain. These traditional uses have piqued the interest of modern scientific researchers, who seek to validate and harness the potential of this plant in contemporary medicine.

Tridax procumbens, commonly known as "Coat Button," is a medicinal herb found in the Asteraceae family, also known as the Daisy family, due to its flower's appearance. It originates from Central America and is a semi-prostrate, creeping weed categorized as an annual or perennial plant. The leaves are simple, lanceolate, or ovate, with coarsely toothed margins and a hairy texture, growing oppositely without stipules. Its tubular and yellow flowers with hairs appear at the tips of long, occasionally twisted stems measuring 25 to 40 cm. The stems are typically green and hairless along most of their length but can be reddish and heavily haired at the base. Coat Button blooms year-round, bearing bisexual flowers with basal placentation. This resilient herb can endure various environmental conditions, including drought, heat, humidity, pollution, seashore locations, slopes, and windy areas. It commonly grows in tropical and subtropical regions like the Philippines, pastures, fallow lands, cultivated fields, roadsides, and disturbed areas (StuartXchange, 2018).

Coat Button (*Tridax procumbens*) contains various phytochemical compounds, some of which have demonstrated anti-inflammatory and analgesic properties in preliminary studies. These compounds include flavonoids, alkaloids, tannins, carotenoids, and saponins. Such bioactive constituents have the potential to interact with pain pathways in the human body, offering a natural alternative to conventional analgesic drugs. However, while these findings are encouraging, a considerable gap exists in understanding the precise mechanisms underlying Coat Button analgesic actions and the optimal formulations for its use (Pawar et al., 2021). This study aims to explore the analgesic potential of *Tridax procumbens* formulated as a syrup. The formulation of this botanical specimen as a syrup presents a potentially convenient and palatable method of administration, particularly for individuals who may have difficulty swallowing pills or capsules. Syrups are also known to provide rapid absorption of active compounds, which can be advantageous for achieving quick pain relief (Le, 2022). Through a systematic investigation, the researchers seek to determine the analgesic potential of *Tridax procumbens* extract, identify the plant constituents in the acquired extract behind its analgesic activity, and study the plant constituents by which *Tridax procumbens* extract exerts its analgesic effects (Ingole et al., 2022).

By pursuing these objectives, the researchers aim to contribute valuable scientific insights into the potential role of Coat Button (*Tridax procumbens*) as a natural analgesic agent. The researchers also aim to develop a safe and effective natural analgesic agent from *Tridax procumbens* extract, as an alternative to conventional pain management therapies.This research has the potential to increase the knowledge of the researchers, future researchers, other healthcare professionals, and the public regarding medicinal plants, as well as to provide novel approaches to addressing pain and enhancing the quality of life for individuals with a variety of painful conditions.

Furthermore, in delineating the scope and delimitations of our study, we aim to ensure a focused and rigorous investigation that remains well-aligned within manageable and feasible parameters. Firstly, the study will specifically utilize only the leaves of Coat Button, excluding the consideration of the entire plant. Additionally, the research will be limited to the examination of Coat Button and will not involve the testing of other medicinal plants, with a geographical restriction to those within the Philippines. Furthermore, the evaluation of analgesic efficacy will be exclusively conducted on female Wistar Albino rats, eliminating the inclusion of other species or variations. The assessment will be restricted to the Writhing, Plantar, and Formalin-induced paw-licking test, providing a defined framework for the experimental procedures. The extraction process will solely involve obtaining constituents from the plant sample, without isolating these constituents. Lastly, the research paper will concentrate solely on the analgesic properties of Coat Button, omitting discussions on its potential anti-inflammatory property. These delineations serve to maintain the study's coherence and precision, ensuring that the research remains within the confines of available resources and time constraints.

1.1 Analgesic potential of Coat Button (Tridax procumbens; Asteraceae) based on traditional medicinal use.

Coat Button (*Tridax procumbens*) has been used in folk remedies and practices for its various health benefits. The listed studies highlight the widespread use of Coat Button in traditional medicine which suggests that it may hold promise as a potential analgesic agent. The plant is prevalent throughout India and is utilized in indigenous medicine for a range of ailments. In Ayurvedic medicine, it is widely used due to its medicinal properties, including reducing blood pressure, bronchial catarrh, malaria, dysentery, diarrhea, stomachache, wound healing, headaches, and preventing hair loss and hemorrhage from cuts and bruises (Chaudhari, 2022). *Tridax procumbens* was recognized and employed in aquaculture as a fish growth enhancer (Adeshina I, et al., 2021). This versatile herb also has been associated with treating various diseases, including malaria, leishmaniasis, and dysentery (Ebiloma et al., 2017).

Up-to-date recent research studies have noted that lactating pregnant women in Chiquimula, Guatemala, suffering from anemia could alleviate their symptoms by using *Tridax procumbens* (Beck et al., 2018). Aqueous extracts of Coat Button exhibit significant activity against Trypanosoma brucei, along with antibacterial and wound-healing properties (Agyare et al., 2016). The flowers and leaves possess antiseptic, insecticidal, and parasiticidal properties, and the plant exhibits various pharmacological activities such as immunomodulatory, antidiabetic, antihepatotoxic, antioxidant, anti-inflammatory, analgesic, anticancer, and marked depressant action on respiration (Pardeshi and Bhiungade, 2016). Coat Button is utilized to treat gastrointestinal and respiratory infections, high blood pressure, and diabetes (Giovannini et al., 2016). It plays a crucial role in defluoridation of water, providing an inexpensive method in regions where the natural level of fluoride mineral is high in groundwater (Ingle NA, et al., 2014).

In 2011, Appiah-Opong et al. have documented that extracts of Coat Button (*Tridax procumbens*) exhibit anti-plasmodial activity against chloroquine-resistant P. falciparum parasites. In a study conducted by Agrawal S, et al., in 2010, aqueous leaves of Coat Button (*Tridax procumbens*) possessed cardiovascular effects, reducing heart rate and blood pressure. The leaf juice serves as a potent antiseptic, insecticidal, and parasiticidal agent, also used to control hemorrhages from cuts, bruises, and wounds. It has additional applications for bronchial catarrh, dysentery, diarrhea, prevention of hair fall, and promotion of hair growth. The plant demonstrates insect-repellent activity, a hypotensive effect, and potent immune-modulating properties (Mundada & Shivare, 2010). Furthermore, Coat Button is employed as a bio-adsorbent for chromium (VI), a highly toxic ion released into the environment through leather processing and chrome plating industries (Mundada S and Shivhare R, 2010). Other studies conducted in the early 2000s have indicated that the herb acts as a wound-healing agent and exhibits hepatoprotective activity (Ravikumar V., et al., 2005).

In certain parts of the world, traditional medical practitioners and native peoples use the plant's leaves as a remedy against conjunctivitis (Nia R, et al., 2003). The Coat Button (*Tridax procumbens*) is found worldwide and has a history of use in Central America for treating anemia, colds, inflammation, and hepatopathies (Taddei and Rosas Romero, 2000). In Guatemala, it is employed to address vaginitis, stomach pain, diarrhea, mucosal inflammations, and skin infections (Taddei and Rosas-Romero, 2000).

To supplement the limited recent studies on the medicinal use of Coat Button, additional information is drawn from earlier studies. Plant extracts were utilized for human well-being long before medical facilities existed, aiming to prevent various chronic and infectious diseases (Diallo D, et al., 1999). This medicinal herb was found to have paradoxical use against malaria, hemorrhage, diarrhea, stomachache, parasitic infection, liver disorder, and inflammation. The tribal inhabitants of Udaipur district in Rajasthan (India) orally used the leaf powder, along with other herbs, to treat diabetes. In 1998, Caceres et al. reported that in Guatemala, Coat Button is utilized as an antibacterial, antifungal, and antiviral treatment. The same study found that the leaf juice of Coat Button is effective in treating wounds and stopping bleeding.

1.2 Constituent of Coat Button (Tridax procumbens)

A plant constituent is a chemical compound that is found in plants and has medicinal properties. *Tridax procumbens* demonstrates significant potential as a plant species renowned for producing metabolites. Various cultures have long depended on the ancestral wisdom of the advantageous properties of this plant (Beck et al., 2018). Identifying phytochemicals in this herb involved the investigation of several substances, such as alkaloids, carotenoids, flavonoids, (particularly procumbenetins and flavones), saponins, and tannins. Phytoconstituents are known to exert a substantial influence on the biological aspects of plants.

Alkaloids belong to naturally occurring substances found in many plants, including *Tridax procumbens*. Alkaloids have analgesic properties, according to Chinese medical herbs, with the anti-inflammatory effect being the most apparent and often utilized in the treatment of rheumatoid arthritis, ankylosing spondylitis, and other rheumatic immunological illnesses (Li et al., 2020). Alkaloids are categorized according to structural categories based on Chinese medical herbs, and the plant sources, applicable conditions, and anti-inflammatory mechanisms of 16 types of alkaloids routinely used in clinical treatment, such as berberine, tetrandrine, and stephanine, are reviewed. As a result, the analgesic activity of crude alkaloids is attributable to the inhibition of cyclooxygenase or 5-lipoxygenase pathways, which may be associated with the inhibition of inflammatory cytokines and interleukins (Li et al., 2020).

Another metabolite produced in Coat Button is carotenoids which are responsible for characteristic colors found in nature. Carotenoids are classified into two main groups, depending on their chemical structure and functions: xanthophylls (lutein, zeaxanthin, and astaxanthin) and carotenes (α-carotene, β-carotene, and lycopene). Carotenoids have been shown to have a wide range of biological activities due to their antioxidant, anti-inflammatory, and anti-tumor properties. They can also block angiogenesis, induce apoptosis, halt the cell cycle, and improve the immunological response (Zasowska-Nowak et al., 2022).

In carotenoids, the capacity of antioxidants, carotenoids protect cells and tissues from harmful radical oxygen species (ROS) is a well-established fact that free radicals play an important role in pain. Pain is "an unpleasant sensory and emotional experience associated with actual or potential tissue damage" and carotenoids have potential therapeutic significance in pain and inflammation management (Hernández-Ortega et al., 2012). Carotenoids with both anti-inflammatory and antioxidant properties seem to be an excellent example of naturally occurring active substances capable of alleviating neuropathic pain. Additional carotenoid supplementation in parallel with standard analgesic treatment may not only contribute to better pain control and alleviation of side effects of treatment, but also due to additional properties may improve other inflammation-related symptoms, such as cancer-related cachexia, anorexia, or asthenia (Zasowska-Nowak et al., 2022).

Flavonoids, naturally occurring compounds, are abundant in various plants, including the *Tridax procumbens*. These flavonoids encompass diverse classes, each characterized by a distinctive chemical structure, albeit sharing a common foundation composed of three interconnected rings called the flavan nucleus. The specific arrangement of substituents in one of these rings, notably the hydroxyl group (-OH), plays a pivotal role in determining the mechanism through which flavonoids exert their effects. This structural variation underscores the multifaceted nature of flavonoid activity (Sylvia, 2022). Flavonoids have gained widespread recognition for their manifold benefits, including antioxidant properties and their capacity to alleviate pain and reduce inflammation. Importantly, these compounds have demonstrated a favorable safety profile in preclinical and clinical studies (Schiller, 2021).

1.3 Analgesic Properties of Coat Button (Tridax procumbens) Formulated as a Syrup

Oral route administration is one of the most convenient ways to administer medication. Taking medication or substances orally is typically straightforward and requires no special equipment or medical expertise. It can be done by the patients themselves, reducing the need for healthcare professionals to administer the treatment (Pharmapproach, 2023).

Syrup is a liquid medication that is readily broken down and absorbed in the stomach. The liquid nature of syrups facilitates rapid absorption, leading to a faster onset of action compared to solid dosage forms. This attribute is particularly relevant in instances where quick therapeutic effects are imperative. Hence, the enhanced bioavailability associated with analgesic properties can contribute to improved patient outcomes and management of acute conditions (Le, 2022). When *Tridax procumbens* extract is incorporated into a syrup that can be reconstituted, it offers several advantages. Firstly, it ensures the chemical stability of the active ingredient throughout its shelf life, a crucial consideration for any medication. Secondly, the resulting syrup significantly reduces the overall product weight, making it a more practical and convenient option for patients (Adepu & Ramakrishna, 2021). Moreover, the bioavailability of this plant in a syrup form will be enhanced compared to traditional tablets and capsules.

The formulation of *Tridax procumbens* as a syrup provides an innovative solution that combines the analgesic properties of this medicinal plant with the advantages of an easily administered, stable, and patient-friendly oral dosage form. This approach aligns with modern pharmaceutical advancements and addresses the challenges associated with traditional solid dosage forms, ultimately enhancing the therapeutic effectiveness of Coat Button as an analgesic agent.

Alkaloids, carotenoids, and flavonoids are present in the plant and possibly display a potential anti-inflammatory and analgesic effect. Additionally, Coat Button (*Tridax procumbens*) exhibits significant positive impacts on central, peripheral, and inflammatory pain models, possibly due to the presence of flavonoids and sterols, which contribute to its protective effects. The extract of Coat Button (*Tridax procumbens*) which possibly contains these constituents will be incorporated into the syrup so that the syrup will exhibit analgesic properties (Prabhu V. V. et al. (n.d.).

The analgesic activity of Coat Button (*Tridax procumbens*) in syrup form has various advantages, including the potential for rapid effects. Unlike solid dosage forms such as pills or capsules, liquid dosage forms such as syrups or solutions allow for faster absorption and commencement of action (Jaiswal et al., 2020). Liquid analgesic medications are swiftly absorbed into the bloodstream through the gastrointestinal tract when taken orally, resulting in a speedier start of pain relief. This is because liquid dosage forms, unlike solid dosage forms, do not require disintegration or dissolution, which can contribute to a faster onset of action. Furthermore, liquid dose forms might help individuals with difficulties swallowing or needing pain relief quickly (Cleveland Clinic,2021).

1.4 Paracetamol as an Effective Synthetic Pain Reliever

Paracetamol, or acetaminophen, is considered effective as a synthetic pain reliever, with its primary mechanism of action thought to be related to its influence on the central nervous system. While the precise details are not fully elucidated, paracetamol is believed to inhibit the activity of the enzyme cyclooxygenase (COX), particularly in the brain. The reduction of COX activity leads to decreased synthesis of prostaglandins, which are signaling molecules involved in the perception of pain and regulation of body temperature (Anderson, B. J. 2019). Paracetamol is widely used to treat postoperative pain and is well known for its morphine-sparing effect. Therefore, the effect of morphine–paracetamol combination can be synergistic, additive, or infra-additive. The primary aim of our study is to define the median effective analgesic doses (ED50s) of paracetamol, morphine, and the combination of both. Also, the nature of the interaction for postoperative pain after moderately painful surgery using an up-and-down method and is bolographic analysis was determined. Paracetamol (acetaminophen) is often used alone or in combination with traditional analgesics to treat mild and moderate postoperative pain. Its primary site of action remains unclear. Several studies postulated that paracetamol acts centrally in the brain rather than peripherally (at the nerve endings). Paracetamol is an effective analgesic, especially when administered intravenously, useful in a broad range of clinical conditions. Its mechanism of action is yet to be fully determined and is likely to involve several pain pathways (Baker, 2021). Paracetamol demonstrates efficacy comparable with that of standard equivalent doses of many NSAIDs (including ibuprofen, diclofenac, ketorolac, and parecoxib), tramadol, and 10 mg IV morphine, with fewer side-effects. This applies across a variety of surgical procedures, as well as for other sources of acute and chronic pain such as musculoskeletal pain and headaches, including tension-type headache and migraine. As a component of a multimodal analgesic regime, it is generally considered to have useful opioid-sparing effects; a reduction in opioid consumption is mostly if not universally borne out with the statistical significance in clinical studies, but the addition of regular paracetamol invariably reduced pain scores and the incidence of nausea and vomiting, and improved patient satisfaction (Baker, 2021). Paracetamol is effective in the management of tension-type headache and migraine, though not for cluster headaches. In a meta-analysis of six studies, paracetamol was equianalgesic to low-dose NSAIDs in the treatment of tension-type headache. A combination of paracetamol and caffeine has also been shown to be equivalent to sumatriptan in the acute treatment of migraine (Baker, 2021).

2. Methodology

The experimental method was used for the study. The experimental method in educational research is the application and adaptation of the classical method of experimentation. It is a scientific method of conducting research in which one or more independent variables are altered and applied to one or more dependent variables to determine their influence on the latter. It is an attempt by the researchers to maintain control over all factors that may affect the result of an experiment. In doing this, the researcher attempts to determine or predict what may occur (Zubair, 2023). The researchers subjected Coat Button extract in an experimental analysis to assess its analgesic properties. Moreover, the analgesic potential of Coat Button was tested in various animal pain models, including the writhing test, plantar test, and formalin-induced paw-licking test. Before the conduct of experimentation, an animal certificate was obtained. The Coat Button plat sample was authenticated at Don Mariano Marcos Memorial State University Northern Campus, Sapilang, Bacnotan, La Union. Treatment of data undermines the authority and trustworthiness of researchers. It introduces information into scientific records that could cause harm to broader society, as when the dangers of medical treatment are understated. Treatment of Data 1: To answer Statement of the Problem Number 1, the researchers performed an Acute Oral Toxicity Test based on OECD Guidelines. Treatment of Data 2: To answer Statement of the Problem Number 2, the researchers used the Mean to measure the concentration that exhibits the most effective Analgesic Activity according to the:

2.1 Writhing test

The mean average of the number of writhing movements of each group was calculated for each concentration of the analgesic. This allowed the researchers to determine which concentration exhibits the most effective analgesic activity in reducing pain-induced writhing in the rats. The extract concentration that exhibited the least number of stretches was considered the most effective concentration.

2.2 Plantar test

The mean average of the withdrawal latency time was calculated for each group. This enabled the researchers to identify the concentration that showed the highest analgesic activity in alleviating pain in the rats during the plantar test. The extract concentration that exhibited a more prolonged time was considered the most effective concentration.

2.3 Formalin-Induced Paw-Licking test

The formalin-induced paw-licking test's mean average obtained determined the extract concentration that exhibited the least amount of paw-licking and was considered the most effective concentration. Treatment of Data 3: To answer Statement of the Problem Number 3, the researchers used One way ANOVA with Tukey's Test for Post-Hoc Analysis to determine if there is a significant difference between the formulated syrup and the Paracetamol syrup commercially available drugs as to their Analgesic activity, as well as to determine the relationship between dependent and independent variables. Treatment of Data 4: To answer Statement of Problem Number 4, the researchers performed organoleptic tests, and a pH meter was used to determine the formulated syrup.

3. Results and Discussions

This chapter demonstrates and discusses the study's findings to investigate the analgesic property of Coat Button (*Tridax procumbens*) leaf extract formulated as syrup. Results are presented systematically in tables for interpretation. Results of the Acute Oral Toxicity Test in the minimum toxic concentration of the extract are displayed. Likewise, the findings of the Writhing test, Plantar test, and Paw-licking test to evaluate the effectiveness of the various concentrations of the extracts (50%, 60%, and 70%) are also demonstrated. A comparative analysis juxtaposing the analgesic activity of the most effective concentration of the Coat Button extract with that of a standard drug, Paracetamol syrup, is also presented. Finally, the physical and chemical characteristics of the formulated syrups, including specific gravity, pH, color, taste, odor, and clarity, are described.

3.1 Determination of the Minimum Toxic Concentration according to Acute Oral Toxicity Test

The ethanolic extract of Coat Button (*Tridax procumbens*) was utilized in the acute oral toxicity test. Following the protocol of the Organization for Economic Cooperation and Development (OECD) Test Guideline 423 (2001), the extract was administered to the rats employing 2 mL extract per 100 g body weight.As per the OECD Guidelines for Acute Toxic Class method, the concentration is considered toxic as the death of two mice exceeded the safe concentration. Two out of three mice exhibited complications such as gagging and difficulty in breathing, followed by death within the 2-hour observation period. Therefore, a higher concentration of the extract does not need to be conducted as a follow-up test to identify the toxic dose. The data of the 75% concentration shows evident mortality. A study conducted by Emmanuel et al. (2021) on *Tridax procumbens* corroborates the results of this study with their findings that the nutritional composition of this plant must have led to its therapeutic and conventional use as a vegetable. Still, the presence of some heavy metals could be a significant health concern, as bioaccumulation might lead to the impairment of some vital organs in the body. Similarly, the study conducted by Abubakar A. et al. (2012) sought to assess the effect of ethanolic extract of *Tridax procumbens* on potassium bromate-induced hepatotoxicity in adult Wistar Albino rats. They observed signs of toxicity, which include salivation, rubbing at the site of application, on the nose and mouth, and restlessness. Contrastingly, a study by Burgos-Pino et al. (2023) found no clinical signs of toxicity were observed in any of the animals dosed with *Tridax procumbens* and Allium sativum extracts. Furthermore, another study conducted by Ferrer-Lino et al. (2022) also reported no evident mortality in their study on the oral Effect of *Tridax procumbens*, Allium sativum, and (3S)-16,17- Didehydrofalcarinol in a Murine Model of Cutaneous Leishmaniasis.

Therefore, the researchers of this study consider that the observed discrepancies in the results may be attributed to the differing amounts of substances administered or the methods employed to extract the substances from their source material. Suggesting that variations in substance concentration and extraction methodologies significantly influence the observed outcomes. Another factor could be the environmental conditions under which the *Tridax procumbens* plants were grown. Factors such as soil quality, temperature, sunlight exposure, and water availability can all influence the growth and development of the plant, thus affecting the plant's concentration and composition of bioactive compounds (Haruna & Yahaya, 2021).

3.2 Determination of the most Effective concentration of Tridax procumbens extract as Analgesic.

The effectiveness of the extracts' various concentrations (50%, 60%, and 70%) as an analgesic was evaluated by conducting the Writhing, Plantar, and Paw-licking tests. Following the pain induction, each group was treated with the appropriate concentration (50%, 60%, 70%) based on their respective groups.

3.3 Writhing Test

During the experiment, female albino rats were subjected to pain induction using Acetic Acid. The researchers then administered different concentrations of the Coat Button extract, extracted from *Tridax procumbens*, to the rats. The concentrations used were 50%, 60%, and 70%. The researchers observed and recorded each rat's writhing response for a period of 10 minutes following the administration of the extract. The writhing response was used to assess the extract's effectiveness in reducing pain perception, with fewer writhes indicating a higher analgesic effect. The decreased number of writhes indicates which concentration is more effective. Therefore, the {70%} concentration, having the lowest mean of 2.67 among the three concentrations, is the most effective for a writhing test. This aligns with a similar study by Patel et al. (2011), which concluded that *Tridax procumbens* leaves possess analgesic and antipyretic activity, as evidenced by the reduction in the number of writhes produced by acetic acid. Alam et al. (2009) further affirmed this claim, stating that any agent that lowers the writhing number demonstrates analgesia by inhibiting prostaglandin synthesis, a peripheral mechanism of pain inhibition. Thus, the studies collectively suggest the potential of *Tridax procumbens* as an effective natural analgesic.

3.4 Plantar Test

Pain was induced in female albino rats using a Plantar Apparatus. Following the induction of pain, the rats were administered varying concentrations of Coat Button extract, specifically 50%, 60%, and 70% by volume. The female albino rats were exposed to infrared radiation from the light source. The time started running, and when the rat removed its paw from the thermal stimulus, the time was stopped and timed. An increase in the duration of the test is indicative of the concentration's effectiveness. Among the three concentrations tested, 60% concentration demonstrated the highest effectiveness, with a mean duration of 7.9 seconds, making it the most effective in the Plantar test. A similar study by Tadesse et al. (2020) on the analgesic properties of Echinops kebericho M. aligns with this research. They found that the paws of mice, which are sensitive to heat without skin damage, revealed the central antinociceptive mechanisms of the extract when exposed to a consistently heated plate. Their results showed that the duration of paw withdrawal extended after administering centrally acting analgesics. They concluded that all test doses demonstrated significant antinociceptive activity in both chemically induced peripheral and thermally induced central pain in a dose dependent manner ($p < 0.01$ and $p < 0.001$). The researchers hypothesized that the 60% concentration had the highest mean effect, potentially due to the optimal solubility of the active compounds at this concentration. Solubility can affect a compound's bioavailability, which is the proportion of the compound that enters circulation when introduced into the body and can have an active effect. This is supported by a study by Rushika Jaiswal et al. (2020), which concluded that solubility could influence a compound's bioavailability. In line with the researchers' interpretation, a study by Patil and Pawar (2018) on *Tridax procumbens* suggested that the solubility of different extracts could influence their bioavailability, and consequently, the plant's analgesic effect.

3.5 Formalin-induced Paw-licking Test

Upon the induction of pain using Formalin, the female albino rats were treated using the different concentrations of Coat Button, which were 50%, 60%, and 70%. The effectiveness of the Coat Button treatment was assessed by observing the time each rat spent licking the paw that was injected with Formalin. A decrease in licking duration signifies increased effectiveness of concentration. On average, the 50% and 70% concentrations demonstrated the highest effectiveness, with a mean duration of 1.33 seconds. However, when broken down into specific time frames, the 50% concentration showed the highest effectiveness at the 5-minute mark, while the 70% concentration was most effective at the 30-minute mark.

Central sensitization by Formalin is the most crucial aspect of the test when evaluating nocifensive behavior. Historical experimental data indicate that the behavioral response observed after the injection is solely due to the direct stimulation and activity of the C-fiber nociceptor (López-Cano et al., 2017). The test was initially described in the late seventies (Dubuisson & Dennis, 1977). Initially, it consisted of the injection of 50 µl of 5% Formalin in the dorsal surface of one forepaw of a rat or a cat. Since then, the Formalin test has been extensively used to assess nociception and inflammation-related responses, thus being adapted according to each study's aim (Tjolsen et al., 1992) A study that corroborates the results gathered was conducted by Sen et al. (2018), where they evaluated the analgesic activity of the methanolic extract of Typha elephantina Roxb using the Formalin-induced hind paw licking test and they confirmed that the leaf extract was found to have significant ($p < 0.001$) analgesia. The researchers interpret one possible explanation for the 50% concentration exhibiting the highest mean in the first 5 minutes as due to the concept of the initial absorption rate. The initial rate is the rate at which a substance is absorbed within the first few minutes of exposure. At a 50% concentration, the substance might be at an optimal level for rapid absorption by the cells, leading to a high initial effect. Meanwhile, the 70% concentration exhibiting the highest mean after 30 minutes may be due to the principle of sustained release. Sustained release refers to a gradual release of the substance over a more extended period, allowing for a prolonged effect. A higher concentration, such as 70%, might be necessary to maintain the effect over 30 minutes as some of the substance may be metabolized or excreted over time. In line with the researchers' interpretation, a study by Patel et al., 2011 on evaluating the of Analgesic and antipyretic activity of *Tridax procumbens* leaf extract found that *Tridax procumbens* extract at a specific concentration showed better analgesic activity. This finding implies that the effectiveness of the plant's pain-relieving properties is dose dependent. In other words, the amount of the extract used can influence the level of pain relief it provides. Dose dependence is a common characteristic of many medicines and therapeutic substances.

3.5 The difference in the analgesic activity between the extract with the highest inhibition of pain and the standard drug.

The efficacy of the Coat Button leaf extract, which exhibits the highest pain inhibition, is compared to that of the standard drug. This comparison is conducted by analyzing the variables using a One-way ANOVA, followed by a Tukey's Test for Post-Hoc Analysis to determine any significant differences. The results will provide valuable insights into the relative efficacy of the Coat Button leaf extract and the standard drug in alleviating pain. Through the One-Way ANOVA, any variations in pain inhibition among the different treatments can be assessed.

In Writhing Test, The P-value is greater than the significance level of 0.05. Therefore, the researchers fail to reject the null hypothesis, indicating that there is no significant difference between the extract with the highest inhibition of pain and the standard drug in the writhing test. In contrast, the P-value of the Plantar Test is less than the significance level of 0.05. Therefore, the researchers reject the null hypothesis. This indicates that there is a significant difference between the extract with the highest inhibition of pain and the standard drug in the plantar test. Similarly, in the Paw-Licking Test, the P-value is greater than the significance level of 0.05. Thus, the researchers fail to reject the null hypothesis, suggesting that there is no significant difference between the extract with the highest inhibition of pain and the standard drug in the paw-licking test. In summary, based on the t-tests conducted at a ± 0.05 significance level reveal a significant difference between the extract and the standard drug in the Plantar Test. However, no significant difference was found in the Writhing and Paw-Licking Tests.

3.6 Writhing Test

In this test, the effectivity of the *Tridax procumbens* syrup is evaluated by comparing it with paracetamol syrup, a standard analgesic drug. The test involved administering the syrups to respective groups of the rats and then observing their reactions to pain, which is measured by how much they writhe. By comparing the average number of writhes per second in each group, the researchers can see if *Tridax procumbens* syrup is as effective as paracetamol syrup in relieving pain. The group with a 60% concentration exhibits an average of 3.0 writhes per second, whereas the Positive Control Group shows a slightly lower average of 2.7 writhes per second. The p-value derived from the ANOVA table is 0.7953, which is relatively high. Typically, a p-value exceeding the default alpha value of 0.05 indicates a failure to reject the null hypothesis. In this context, it suggests that there is insufficient evidence to assert a statistically significant difference in the mean writhing frequency between the two groups.

3.7 Plantar Test

In the Plantar test, the effectivity of the *Tridax procumbens* syrup is evaluated by comparing it with paracetamol syrup, a standard analgesic drug. The test involved administering the syrups to respective groups of rats and then observing their reactions to pain, which is measured by the withdrawal time of the rat from the heat stimulus. By comparing the average time (seconds) in each group, the researchers can see if *Tridax procumbens* syrup is as effective as paracetamol syrup in relieving pain. The group with a 60% concentration exhibited a mean withdrawal time of 7.93 seconds, while the Positive Control group showed a mean time of 3.90 seconds.

The effect of the treatment was found to be significant, with a p-value of 0.0185. As this value is below the significance threshold of 0.05, the null hypothesis was rejected. This suggests a significant disparity in the analgesic activity between the extract demonstrating the highest pain inhibition and the standard drug. This conclusion is supported by the results obtained from the plantar test apparatus. It's crucial to note that the information provided in this discussion is based solely on the observed behavior of the rats and is presented without any guarantees. While the formulation appears to exhibit superior analgesic effects, it does not necessarily undermine the efficacy of the commercially available syrup. Instead, it suggests that the extract's concentration triggered a significant response in the rats' behavior.

The researchers hypothesized that the significant difference observed in favor of the formulated syrup may relate to dose response. This phenomenon is often referred to as hormesis, where a substance at low doses can have a different effect compared to higher doses. In a study conducted by Calabrese, E.J. and Mattson, M.P. (2011), in the case of syrup, a lower concentration might trigger a more pronounced response because it interacts differently with the body's receptors or metabolic pathways. This can be due to various factors such as receptor saturation, different metabolic pathways being activated, or even potential toxicity at higher concentrations The term adaptive response refers to a plastic response, that occurs after exposure to mild doses of a toxic agent, aimed at the restoration of homeostasis (Samson and Cairns 1977). Similarly, adaptive homeostasis is used to explain the increased or decreased seen in the homeostatic range of an animal exposed to sub-lethal conditions (Davies 2016). While the term cross tolerance refers to the animal's ability to defend against damage caused by stressor B after a brief exposure to stressor A (Gruber and Keyser 1945).

3.8 Formalin-induced Paw-licking Test

In the paw-licking test, the effectiveness of the *Tridax procumbens* syrup was evaluated by comparing it with paracetamol syrup, a standard analgesic drug. The test involved administering the syrups to respective groups of the rats and then observing their reactions to pain, which was measured by the number of licks to the paw that the rat made. By comparing the average mean licking time in seconds for each group, the researchers can see if *Tridax procumbens* syrup was as effective as paracetamol syrup in relieving pain.

There is no significant difference in the analgesic activity between the extract with the highest pain inhibition and the standard drug when using the Formalin-induced Paw-licking Test. The mean duration of licking the hind paw for the standard drug is 1.3, with a standard deviation of 0.58, indicating some variability. Group 1's value is 1.7, with a standard deviation of 1.15, indicating more variability. Group 2's value is 1.3, with a standard deviation of 0.58, indicating some variability. For Group 3, it is 2.7, with a standard deviation of 0.58, indicating some variability. For Group 4, it is 1.8, with a standard deviation of 0.87, indicating more variability than Group 1, 2, and 3, but less than Group 1. The p-value is 0.1858, which is greater than the standard level of 0.05. A higher p-value suggests that the differences between the groups' duration of licking the hind paw are not statistically significant.

3.9 Characteristics of the Coat Button (Tridax procumbens) Syrup

The physicochemical properties of the formulated *Tridax procumbens* syrup, including specific gravity, pH, color, taste, odor, and clarity, were meticulously assessed through organoleptic observation and precise pharmaceutical computation. The physicochemical properties of the formulated *Tridax procumbens* syrup are compared to those of a simple syrup, which serves as a control due to its lack of additional flavoring or medicinal agents that could confound the results. The comparison was based on recognized pharmacopeial standards to ensure compliance with established quality benchmarks. This evaluation enables the researchers to identify deviations or deficiencies that may necessitate reformulation or process optimization to achieve desired quality attributes. The comparative analysis of the physicochemical properties, as presented in Table 3.4, provides a clear and quantifiable measure of the formulated syrup's conformance to quality standards. This systematic assessment reinforces confidence in the formulation process and underscores the commitment to delivering pharmaceutical products of high quality and reliability.

Both syrups have a specific gravity of 1.3, which suggests that the syrup is a concentrated solution, indicating that the syrups are denser than water (1.0). In addition to this, both syrups have a neutral pH level. However, for the color, taste, and odor, the formulated syrup appeared green, tasted sweet with a bitter taste, and exuded a tobacco like scent. In contrast, the simple syrup appeared colorless, tasted sweet with no aftertaste, and gave a sweet odor. Lastly, both syrups are clear, indicating no visible particulate matter or cloudiness. The differences observed between the color, taste, and odor may be due to the properties of the Coat Button plant itself, imparting its pigment, scent, and herbal taste.

4. Conclusions and Recommendations

This chapter summarizes the findings from this study's data collection and analysis. The conclusions are outlined to understand the outcomes, clearly addressing the research problems. A set of recommendations is also presented based on the insights gained from this research. These suggestions serve as a roadmap for future improvements and potential areas of exploration.

Summary of Findings

The study outcomes are elaborated in the text below after extensive data collection and detailed analysis. The findings are presented comprehensively, providing insightful details and valuable information.

Based on the Acute Oral Toxicity Test, the 75% concentration of the Coat Button extract exhibited evident mortality, as noted by the complications observed.

The most effective concentration among the formulated Coat Button (*Tridax procumbens*) extract (50%, 60%, and 70%) exhibits analgesic effects that vary for each pain model. The 70% concentration is the most effective for the Writhing Test, with a mean of 2.67. Meanwhile, the Plantar Test indicated that 60% concentration was the most effective, with a mean of 7.9. Subsequently, the most effective concentration(s) for the Paw-Licking Test is 50% and 70%, with a mean of 1.33. Considering the findings, the 50% concentration shows effectiveness immediately. However, it does not have a long-term effect, with a mean of 1.7 for the first 5 minutes, while the 70% concentration has a more prolonged effect despite taking a more extended period before taking effect with a mean of 0.3.

The comparison between the formulated syrup and the commercial syrup reveals both significant and non-significant differences. The results of the Writing Test suggest that the 70% concentration with a p-value of 0.7953 is not considered significant at 0.05. The Plantar Test indicates that the 60% concentration, with a p-value of 0.0185, is considered significant at 0.05, showing a significant difference between the 60% concentration and the positive control. In the case of the Formalin-induced Paw-licking Test, the 50% concentration is considered significant at 0.05 during the initial phase (0-5 minutes), with a p-value of 0.0474. However, during the combined/cumulative intervals from 0-30 minutes, the 50% concentration resulted in a p value of 0.1858, which is not significant at 0.05.

The syrup made from Coat Button has a specific gravity of 1.3, suggesting that it is a concentrated solution and denser than water. It has a neutral pH of 7, meaning it is neither acidic nor basic. The syrup's green color and tobacco-like odor can be attributed to the properties of the Coat Button (*Tridax procumbens*) plant itself.

Conclusion

Certain conclusions have been carefully drawn based on the information examined and analyzed. The findings arrived at after considering the relevant data from this study. Based on the Acute Oral Toxicity Test, the minimum toxic concentration of the Coat Button leaf extract is 75%. The most effective concentration is not clearly identified, as the results show no consistency. All three concentrations exhibited efficacy, albeit through different tests. Based on the three methods used in comparing the analgesic activity of the Coat Button and the Paracetamol syrup, two out of three methods showed no significant difference, and are deemed equally effective. The formulated Coat Button syrup has a specific gravity of 1.3 and a pH level of 7. The syrup is green in color with a sweet taste and tobacco-like odor.

Recommendations

The researchers acknowledge that, although the findings appear promising, considerable gaps may have existed in their understanding of the analgesic property of the Coat Butto. Therefore, to further enhance the study, here are several recommendations to be considered:

1. Future researchers are advised to explore other extraction methods, such as the Soxhlet extraction method or other methods that are more advanced.

2. Future researchers are encouraged to isolate the constituents of the Coat Button to identify a pure active constituent with a potentially more substantial analgesic effect.

3. Future researchers are urged to utilize other formulations compatible with the active ingredients and appropriate for administration.

4. Future researchers should consider evaluating the analgesic properties of the Coat Button using parts of the plant other than the leaves.

5. Future researchers are encouraged to conduct the study with other test methods to enhance the credibility of the results.

6. Future researchers are also encouraged to conduct the study with different animal species to enhance the credibility of the results.

7. Future researchers are advised to compare the results with other methods published to further enhance the clarity and validity of the results, especially on the use of the Plantar test.

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