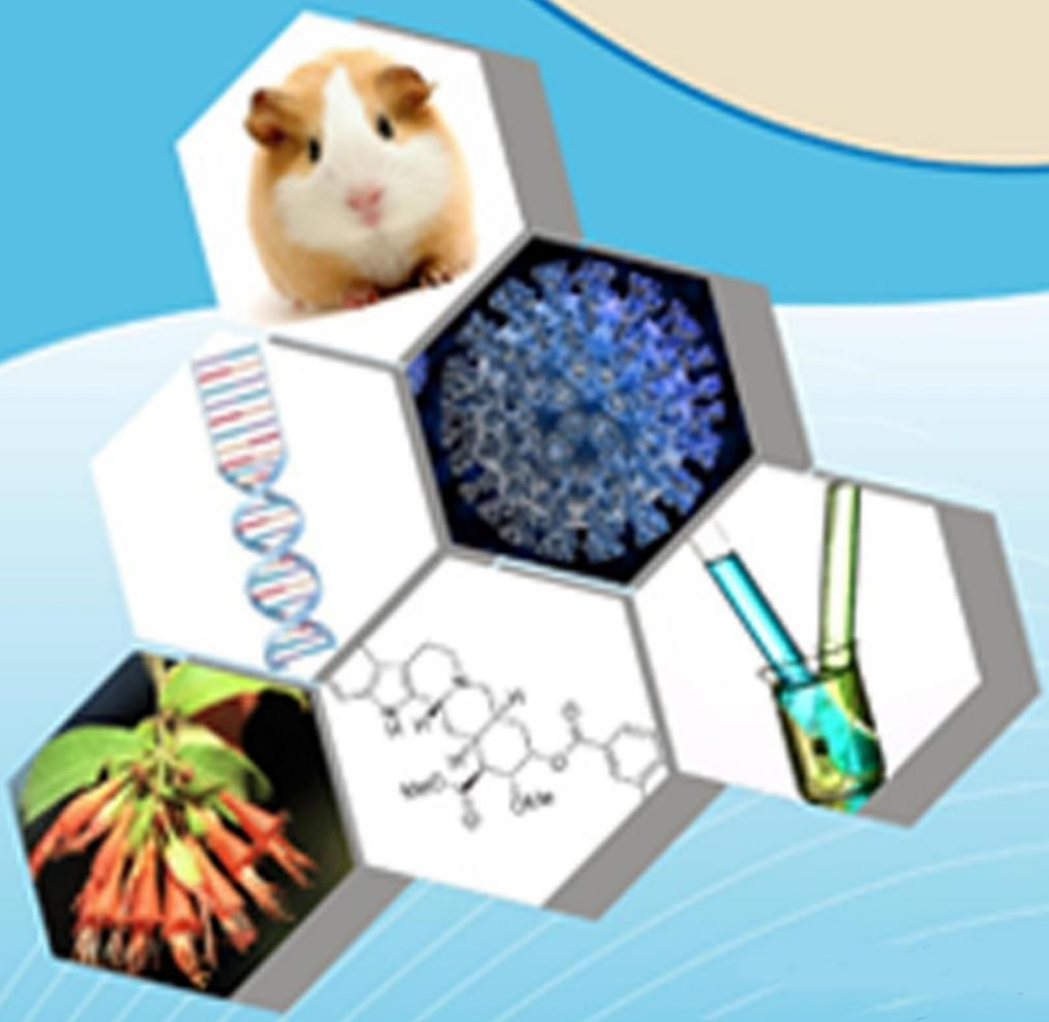
ISSN : 2347-2251

Indo-American Journal of Pharma and Bio Sciences



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Indo-Am. I. Pharm & Bio.Sc.,2025

ISSN 2347-2251 www.iajpb.com

Indo-American Journal of Pharma and Bio Sciences

Vol. 23, Issue 2, 2025

Chromolaena odorata methanol leaf extract as a wound healing agent and antioxidant: a rat model study

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Received on: 03 Apr 2025 Accepted Date: 10 May 2025 Published on: 18 June 2025

Abstract

People have been utilizing plants and other natural things as remedies for a long time. At the moment, conventional medicine is in vogue. In terms of disease prevention and treatment, the many phytochemicals found in plants may work in tandem via a variety of pathways. This research examined the effects of Chromolaena odorata methanol leaf extract on wound healing and antioxidant activity in adult male rats. The leaf extract was evaluated for its phytochemical composition, acute toxicity, anti-oxidative capabilities, and wound healing capabilities. Chromolaena odorata methanol leaf extract was used in an anti-oxidative investigation to see how it affected malondialdehyde (MDA) and catalase. The rats' backs were cut in an excision wound healing experiment, and three different dosages of Chromolaena odorata methanol leaf extract embedded in petroleum jelly were applied to the wounds. The positive control consisted of cikatrin powder, whereas the negative control was petroleum jelly. Among the phytochemicals found were steroids, alkaloids, flavonoids, proteins, and tannins. A half-life of 5,000 mg/kg of body weight was recorded. The anti-oxidative experiment demonstrated that the leaf extract effectively inhibited lipid peroxidation. In contrast to the 14.16 ± 0.59 x 10-5 µmol/ml measured with distilled water, the MDA was lowered to 5.63 ± 0.33 x 10-5 µmol/ml by the 500 mg/kg body weight extract. After 12 weeks of therapy, the wound healing percentage for the 5% and 10% extracts was 95.52% and 97.14% respectively, which was higher than cikatrin's 87.95%. Chromolaena odorata methanol leaf extract outperformed cikatrin in terms of wound healing potency and had a favorable safety profile.

Wound healing activities; malondialdehyde; Chromolaena odorata; Anti-oxidation

1. Introduction

To alleviate and cure illness, people have used natural items as medicine from the beginning of time (Yuan et al., 2016). This includes plants, animals, microbes, and aquatic creatures. Fossil evidence suggests that people may have started using plants for medicinal purposes at least 60,000 years ago, according to the researchers. When it comes to diagnosing and treating diseases, as well as maintaining good health, traditional medicine relies on spiritual and natural resources, either alone or in combination. The rising expense of conventional pharmaceuticals and the spread of bacteria and viruses that are resistant to these medications have contributed to the rise in popularity of alternative health practices (Fokunang et al., 2011). Research conducted by Gajender et al. (2023) indicates that traditional medicine is relied upon by 80% of the people in the developing world, as reported by the World Health Organization (WHO). Additionally, the use of CAM, especially herbal therapies, has been on the rise in the industrialized world over the last few decades (Mahomoodally et al., 2013). Divination, spiritualism, and herbalism are the three main pillars of African traditional medicine, which provides holistic health treatment (Ezekwesili-ofili et al., 2019). A combination of cultural and economic variables has led to a dramatic uptick in the use of traditional African medicine. On top of that, plants have a plethora of phytochemical substances that, via various pathways, may work together to treat or prevent illness. Herbal medicine stands out among traditional medical practices because it relies on the expertise of traditional healers who focus on the use of herbs to treat a wide range of illnesses. A lot of people in Nigeria have been using traditional medicine. This is because, in contrast to conventional treatment, it is easily accessible, inexpensive, and has a very low risk of side effects. Traditional medicine was more popular in Ibadan, Nigeria, according to a research (Li et al., 2020), due to its perceived greater efficacy, accessibility, and price. Many illnesses in Nigeria are treated using traditional remedies. A few examples of these conditions include infertility, wounds, ulcers, and sexually transmitted

infections (STDs). Surprisingly, more than five million people die every year from untreated injuries and wounds, which are common in underdeveloped nations. This is close to the sum of the fatalities caused by TB, HIV/AIDS, and malaria put together (Rechard et al., 2009). This is exacerbated in low-income nations because their social welfare infrastructure is weak and their trauma treatment and rehabilitation systems are underdeveloped. There has been a lack of funding and focus on research that may provide a remedy to this threat. Traditional medical treatments for wounds are not only prohibitively costly, but they are often difficult to get and may have unwanted side effects. Traditional remedies are becoming the subject of increased investigation due to their low toxicity, ease of availability, and low cost. It is important to note that wounds and injuries are on the rise in emerging nations. This is due to the fact that several variables increase the likelihood of injury, including trauma, accidents caused by poorly maintained roads, falls, particularly among the young and the old, and a lack of drugs to speed the healing process. However, conventional wound healing pharmaceuticals are few and far between, with antimicrobials, antioxidants, and anti-inflammatory agents making up the bulk of the available options. It has been shown that Chromolaena odorata contains antioxidant properties. In light of the current shortage of effective wound healing medications, this sparked interest in investigating its possible therapeutic applications. Since Chromolaena odorata has been demonstrated to have outstanding antioxidant effects, and since it is very active, it will help reduce the prevalence of various wound types in Nigeria and beyond, we decided to investigate its wound healing activities in this study.

Section 1.1. Healing procedure for wounds

An accident, burn, or enzyme deficit (such as glucose-6-phosphate dehydrogenase) may distort the skin, causing it to seem like a wound. The process of wound healing involves repairing damaged tissues. Various kinds of wounds have traditionally been treated and managed using plants and plant-based ingredients. Throughout human history, herbal remedies have been an important part in treating a wide variety of illnesses and injuries. Some herbal remedies include antiseptic, debriding, and hydrating properties that make them useful for wound care and therapy. Many plants are used in folk medicine to cure burns, cuts, and other injuries (Sharma et al., 2021). The interaction between platelets and exposed collagen initiates the wound-healing process after an injury. As a consequence, platelets clump together and coagulation factors are released, leading to the development of a fibrin clot at the location of the lesion. At the location of an injury, pro-inflammatory cells such cytokines and growth factors also come. In order to heal damaged tissues, connective tissue cells called fibroblasts deposit collagen, and a fibrin clot acts as a temporary matrix. Collagen is also crucial because it fixes the damage and gets the injured tissue back to how it was anatomically and functionally. Chronic wounds, abrasive wounds, rips or lacerations, puncture wounds, closed wounds, open wounds, incised wounds, and many other kinds of wounds are known (Chhabra et al., 2017). Wounds that have not healed normally and have progressed to the pathologic inflammatory stage are known as chronic wounds. They need more time to recover.

1.2. Different herbs that promote recovery after injury

Shedoeva et al. (2019) notes that medicinal plants have been utilized as a primary treatment for inflammation, burns, ulcers, and surgical wounds by indigenous peoples of Africa, Asia, the Americas, and Egypt for over five thousand years. In order to speed up the healing process and promote tissue regeneration at the wound site, medicinal plants include several natural phytochemical components. One medicinal plant that may speed up the healing process of wounds is centella (Centella asiatica), often known as Asian pennywort. It has been shown that Centella asiatica extracts may help chronic ulcers heal, no matter how big or little they are. Among these extracts, Diniz et al. (2023) identified Asiaticoside, triterpenes, and madecassoside, all of which increase angiogenesis and collagen production at the site of the lesion. Research has demonstrated that curcumin, a component of curcuma longa, interacts with various proteins and processes in the body. These include apoptosis, transcription factors like nuclear factor kappa B (NF-kb), cyclooxygenase 2, 5-lipoxygenase, prostaglandin E2, and many more. Scientific studies have shown that curcumin changes the pericellular and promoting the formation of new connective tissue (granulation tissue), fibroblast proliferation, and collagen deposition in skin wounds via the extracellular matrix (Kumari et al., 2022). According to Shedoeva et al. (2019), the alcohol derived from the leaves of the Wedelia trilobata plant has been used for the treatment of rheumatism, chronic wounds, and painful arthritis joints. A flavonoid found in Wedelia trilobata leaves, luteolin, has antioxidant, anti-cancer, neuroprotective, and immunomodulatory properties, according to the researchers. Wedelia trilobata leaves are used by traditional healers to cure skin wounds. A hallmark of skin infections and psoriasis, luteolin suppresses the production of pro-inflammatory cytokines controlled by nuclear factor kapper B (NF-Kb) (Weng et al., 2014). Aloe vera leaf acetone extracts are very effective against microbes. Some bacterial species, known as gram-positive, may cause greater harm to aloe vera than gram-negative ones. Compounds having shown antibacterial action include saponins, acemannan, and derivatives of anthraquinones. Acemannan triggers the production of nitrous oxide, prostaglandin E2, tissue necrosis factor α (TNF-α), and pro-inflammatory messenger RNAs (mRNAs). It also effectively stimulates the activity of macrophages and T cells. One study found that using acemannan topically shortened the time it took for wounds to heal (Sharma et al., 2021). A clinical investigation found that Arctium lappa has hepatoprotective, anti-inflammatory, anti-viral, anti-cancer, and antioxidant properties

(Nermeen et al., 2022). The Wnt/β-catenin signaling pathway is recognized as an important regulator of wound healing, and Arctium lappa has been shown to regulate gene expression and cell adhesion in canine dermal fibroblasts. Results showed that Arctium lappa was more effective than the control method in facilitating the healing of first- and second-degree burns in humans in a pilot research (Shedoeva et al., 2019). Panax ginseng contains anti-inflammatory, antioxidant, anti-cancer, antibacterial, and immunomodulatory properties, according to research (Riaz et al., 2019). Research indicates that Panax ginseng contains several bioactive components, the most powerful of which are saponins known as ginsenosides. Research has shown that extracts from the roots of the Panax ginseng plant may prevent skin damage from intense UVB radiation and speed up the recovery process after laser burns and excisions.

Section 1.3. Odorata Chromolaena
The carbohydrates (1.10±1.14%), proteins (24.08±0.08%), lipids (14.00±0.01%), fibers (50.26±0.01%), ash (10.98± 2.00%), and moisture content (5.65±0.02%) were determined by the proximate and quantitative phytochemical analyses of the aqueous and methanolic extracts of the Chromolaena adorata leaves. There is an energy level in A total of 220.20 kcal were noted. A number of mineral elements were found in the leaves as well, including magnesium, calcium, sodium, potassium, iron, manganese, zinc, copper, and phosphorus. Nwinuka et al. (2009) found that the leaves contained a variety of compounds, including alkaloids (18.38±0.02%), flavonoids (12.90±0.03%), saponins (14.90±0.05%), cyanogenic glycosides (3.27±0.02%), tannins (0.14±0.01%), and phytic acid (0.05±0.03%). According to the researchers, the methanolic extracts had anti-microbial effects that were negative for Pseudomonas pyrogenes and Escherichia coli, but positive for Bacillus subtilis, Klebsiella pneumoniae, Staphylococcus aureus, Candida albicans, Aspergillus saccharomyces cerevisiae, Rhizopus species, and Penicillium species. Antioxidants and cytokines are two of several biochemicals that play a role in the body's recovery process. There is evidence that phytochemicals found in many plants may promote wound healing at various points in the process via a variety of pathways. Chromolaena odorata has a wide array of useful therapeutic qualities, including anti-inflammatory, antipyretic, analgesic, antibacterial, and cytotoxic effects. Important for its possible future use as a medication in the treatment of wounds, this plant's function in biological systems' wound healing processes was outlined in a review of the literature (Vijayaraghavan, et al., 2017a).

section 1.4. Characteristics of Chromolaena odorata as an antioxidant
According to Bhargava et al. (2013), the ethanol extract of Chromolaena odorata contains polyphenols that are abundant in natural antioxidants such p-hydroxyl benzoic, p-coumaric, protocatechuic, ferulic, and vanillic acids. In vitro, they found that the antioxidant potentials of Chromolaena odorata ethanol extract (10–80 µg/ml) were comparable to those of conventional ascorbic acid (25–400 µg/ml). To avoid glutathione depletion, they may work as preventative antioxidants by increasing hepatocyte oxidative capacity. This will prevent harm to the liver by reducing lipid peroxidation. Another research found that Chromolaena odorata may protect chickens from heat stress and oxidative damage because it contains antioxidant enzymes and polyphenols. These compounds stimulate biological defense systems (Lartey et al., 2020). The research went on to say that at 10 µg, the methanol extract of Chromolaena odorata, which contains Chromomoric acid C-1, has the ability to activate Nrf2 and decrease NF-κB with an inhibitory capacity (IC50) of 6.9 µM, leading to anti-inflammatory effects. Potentially improving anti-oxidative physiology for cellular oxidative equilibrium and mitigating oxidative damage under heat stress are these biological defense features of this plant. Chromolaena odorata leaves have strong antioxidant properties, according to another study. This research aimed to determine the antioxidant activity of an ethanolic extract of Chromolaena odorata leaves against paracetamol-induced male Wister rats using different dosages. According to Solihah et al. (2020), when administered at a dosage of 500 mg/Kg body weight, the ethanol extract of Chromolaena odorata reduced malondialdehyde blood levels by 58.974% and improved the histological structure of hepatocytes. Research into the antioxidant and immunomodulatory effects of chlorolaena odorata leaf polysaccharides found that the plant's soluble and cell wall polysaccharides exhibited strong radical scavenging and immune-enhancing effects. The findings provide credence to the traditional therapeutic use of Chrmolaena odorata leaves (Boudjeko et al., 2015). Some parts of East Indonesia rely on Chromolaena odorata as a traditional medication for treating diabetes and soft tissue lesions. Along with two known compounds, isosakuranetin and subscandenin, a new flavanone called odoratenin was found in the methanol extract. It showed strong inhibitory activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radicals (Putri et al., 2019). One of the plants that the community uses as traditional medicine is Chromolaena odorata. The free radical scavenging activity of the portion of methanol extract of Chromolaena odorata leaves made it a wound medication for certain of the Ambon, Indonesian people (Maulida et al., 2019). Chromolaena odorata leaves were previously tested for their antioxidant and free radical scavenging capabilities; the results showed that both the ethanol and methanol extracts have strong antioxidant capabilities (Bhargava et al., 2013).

Section 1.5. Chromolaena odorata's wound-healing properties

Multiple phytochemicals are involved in wound healing, according to research. Antioxidants and cytokines are

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samples of these chemicals. Chromolaena odorata was also studied and discovered to have a number of useful medical characteristics, including anti-inflammatory, antipyretic, analgesic, antibacterial, and antioxidant. These characteristics make it a biological system with wound-healing capabilities; these features are important for its prospective use as a medication in the future (Vijayaraghavan et al., 2017b). Chromolaena odorata extract has a history of usage in many tropical nations for wound healing and bleeding prevention, according to a related research. Scientists looked into the molecular pathways that the plant extract used to influence hemostatic and wound healing functions. Chromolaena odorata was found to enhance migration and proliferation of Balb/c 3T3 fibroblast cells. The researchers went on to find that Chromolaena odorata treatments increased heme oxygenase-1 (HO-1), an enzyme that speeds up wound healing, at both the transcriptional and translational levels (Hataichanok et al., 2013). Additionally, there was documentation of a research that sought to investigate the wound-healing capacity of Chromolaena odorata aqueous and ethanol extracts using a rat excision wound model. According to Vijayaraghavan1 et al., 2017b, the wound area was reduced more rapidly in the Chromolaena odorata group than in the control and Betadine groups. Additionally, there was a rise in hydroxyproline and hexosamine levels as well as collagen expression with topical administration of the extract, indicating an increase in collagen production and stability at the wound site. According to a study conducted by Sirinthipaporn et al. (2017), the wound healing potential of Chromolaena odorata is significant. Important for its possible future application in wound therapy, the researchers summed up Chromolaena odorata's and its biomarkers' roles in biological systems' wound healing activities (Bhuyan et al., 2019). So, we looked into Chromolaena odorata's antioxidant capabilities and tested its wound-healing capabilities in male Wister rats. We used cikatrin powder and petroleum jelly as controls, respectively, to see how well it worked.

2. Material and methods

2.1. Materials

2.1.1. Animals

Adult Wister rats were procured from the animal house of Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University Awka, Agulu Campus, Anambra State, Nigeria. The rats were acclimatized for one week and were fed with commercially available rat pellets and allowed accesses to drinking water *ad libitum* and were maintained under laboratory conditions of temperature 26 ± 2 °C, humidity of 50 ± 5%, and at a 12 h natural light/dark cycle. All animal experiments were conducted in compliance with NIH guide for care and use of laboratory animals and was approved by the Nnamdi Azikiwe University's Ethical Committee for the use of Laboratory Animals for Research Purposes (Approval number: NAU/AREC/2023/00082).

2.1.2. Chemicals and reagents

The chemicals and drugs used in this research include: cikatrin powder, petroleum jelly, 80% methanol, methylated spirit, Formaldehyde 40% w/v, chloroform, tween-80, 1% thioberbituric acid (TBA) in 20% sodium hydroxide (NaOH), naproxen, distilled water, carbonate buffer, diclofenac, concentrated sulphuric acid, normal saline, trichloroacetic acid (TCA), dilute hydrochloric acid, phosphate buffer (PH 7.0), dichromate acetic acid, ketamine.

2.1.3. Equipment

Glass column, flasks, beakers, test tubes, measuring cylinders, surgical blade, forceps, scissors, graph paper, white transparent paper, rotary evaporator, Analytical Weighing Balance (Metler H30, Switzerland), Electric Oven(Gallenkamp, England), Spectrophotometer (B. Bran Scientific &Instrument Company, England), Water Bath (Techmel & Techmel, Texas, USA), National Blender (Japan), Micropippete (Finnipipette® Labsystems, Finland), Plethysmometer (Biodevices, New Delhi, India) and Intubation tubes, amber colored bottles, refrigerator, centrifuge, cotton wool, gauze bandage.

2.2. Plant materials

2.2.1. Collection and authentication

Fresh leaves *Chromolaena odorata* was collected from school of Pharmacy Agulu. It was authenticated by a Taxonomist at the Department of Botany, Nnmadi Azikiwe University, Awka.

2.2.2. Extraction of plant material

The fresh *Chromolaena odorata* leaves were collected from Agulu, Anaocha Local Government Area, Anambra State. They were then washed and dried away from sunlight at room temperature for 48 hours. The dried leaves were pulverized to powder using an electronic blender and kept in clean airtight amber colored bottle. Then, 750 g of the powdered leaves material was cold macerated in 80% methanol. The mixture was allowed to stand for two days (48 hours) with intermittent agitation. It was filtered and the filtrate concentrated to dryness using water bath at 40 °C for 72 hours. The extract was stored in a refrigerator until used.

2.2.3. Phytochemical analysis of Chromolaena odorata methanol leaf extract

The leaf extract was tested for the presence of various plant constituents like Alkaloids, Flavonoids, Reducing sugars, Saponins, Proteins, Tanins, Amino acids, Steroids, Triterpenoids and glycosides using the methods described by (Kokate, 2001; Harborne, 1998; Khandelwal, 2008).

2.3. Tests for Alkaloids

To small amount of the extract sample was added few drops of dilute hydrochloric acid, mixed and filtered. The following tests for alkaloids were carried out with the filtrates.

- **Mayer's reagent**: A portion of the filtrate was treated with Mayer's reagent and observed. The presence of yellow or creamy precipitate indicates the presence of alkaloids.
- **Dragendoff's reagent:** A portion of the filtrate was treated with Dragendoff's reagent and observed. The presence of a reddish-brown precipitate indicates the presence of alkaloids
- **Wagner's reagent:** A portion of the filtrate was treated with Wagner's reagent and observed. The presence of a reddish-brown precipitate suggests the presence of alkaloids.
- **Hager's reagent:** A portion of the filtrate was treated with Hager's reagent and observed. The presence of yellow precipitate indicates the presence of alkaloids.

2.4. Test for flavonoids

Lead acetate test: The filtrate was treated with a few drops of lead acetate solution. Formation of yellow precipitate indicates the presence of flavonoids.

- **Alkaline reagent test:** The filtrate as treated with a few drops of sodium hydroxide. Formation of intense yellow color, which becomes colorless on addition of few drops of dilute acid, indicates the presence of flavonoids.

2.5. Test for reducing sugar (Carbohydrates)

- **Benedict test**: Small quantities of the test samples in water were treated with Benedict solution and heated to boiling in water bath. Appearance of brick red precipitate indicates the presence of reducing sugar.
- **Fehling's test:** Small quantities of the test samples in water were treated with equal volumes of Fehling's A and Fehling's B solution and heated in a water bath for 10 minutes. Formation of red precipitate indicated the presence of a reducing sugar.

2.6. Test for Saponins

- **Frothing test:** The filtrate was treated with small amount of water and shaken for about 15 minutes in a graduated cylinder. Formation of a stable 1cm foam layer indicates the presence of Saponins

2.7. Test for Proteins

- **Million's test:** A 1 ml of test solutions was treated with sulphuric acid was added to a small amount of million's reagent and boiled. The sample was observed for formation of white precipitate which turns red after warming indicates the presence of protein.
- **Precipitation test:** If the test solutions give white colloidal precipitate with the following reagents: i) 5% $CuSO_4$. ii) 5% Lead acetate indicates the presence of proteins.

2.8. Tests for Tannins

- **Ferric Chloride test**: To 2 ml of the filtrate was added 5% dilute ferric chloride solution, a violet colour formation indicates the presence of tannins.

2.9. Test for Amino acids

- **Ninhydrin test:** To 3 ml of the filtrate, three drops of 5% v/w lead acetate solution were added and heated to boiling in a water bath for 10 min. The change in color of solution to purple or blue indicates the presence of amino acids.

2.10. Test for Steroids and Triterpenoids

- **Salkowski test:** Small amount of chloroform was added to 5 ml of the filtrate and few drops of concentrated sulphuric acid added. The mixture was shaken well and kept aside for some time and observed. Red color appearance indicates the presence of steroids and appearance of yellow color in the lower layer indicates the presence of triterpenoids.

2.11. Test for Glycosides

- **General test:** This was done using the Fehling's method of test for reducing sugar. After the Fehling's method as explained above, a portion of the sample was hydrolyzed with dilute sulphuric acid in separate test tubes. The increase in color intensity indicates the presence of glycosides.

2.12. Acute toxicity studies (LD50) of *Chromolaena odorata*

The median lethal dose (LD_{50}) estimation of the test drug was conducted with the method described by Lorke, (1983). The tests was done in phases; in the first phase three groups of rats (n = 3) were given oral administration of 10 mg/kg body weight, 100 mg/kg body weight and 1,000 mg/kg body weight of test drug. The animals were observed for 24 hours for number of deaths and for any sign of toxicity. In the second stage, new set of four groups of rats (n = 1) were orally administered 2,000, 3,000, 4,000 and 5,000 mg/kg body weight of test drug and were observed for 24 hours for deaths and for sings of toxicity.

The LD_{50} was determined using the formula:

$$LD_{50} = (H \times L)^{1/2}$$

H = Highest dose that resulted to no mortality
L = Lowest dose that resulted to mortality

2.13. Evaluation of antioxidant activities

2.13.1. Analysis of Lipid peroxidation

Malondialdehyde (MDA) an index of Lipid peroxide react with thiobabarturic acid (TBA) to give a complex pink color. This was used to assess lipid peroxidation using the method of Buege and Aust (1978) and also reported by Oraekei *et al*., (2020). An aliquot (1.0 ml) of the diluted serum in normal saline was added to 2.0 ml of (1:1:1 ratio) TCA-TBA- the reagent. (0.37% TBA, 0.24 mM HCl and 15% TCA) Trichloro acetic acid- thiobabarturic acid- hydrochloric acid reagent and boiled at 100°C for 15 min and allowed to cool. Flocculent materials were removed by centrifuging at 3000 rpm for 10 min. The supernatant was removed and the absorbance read at 532 nm against the blank. Malondialdehyde (in µM) was calculated using the molar extinction coefficient MDA – TBA complex of $1.56 \times 10^5\ M^{-1}cm^{-1}$.

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2.13.2. Determination of catalase activity

Catalase activity was determined according to Sinha (1972). It was assayed colorimetrically at 620 nm and expressed as micromoles of H_2O_2 consumed / min / mg protein at 25°C. The reaction mixture contained 0.1 ml of serum, 1.0 ml of 0.01 phosphate, buffer (PH 7.0) and 0.4 ml of 2M H_2O_2. The reaction was stopped by the addition of 2.0 ml of dichromate-acetic acid reagent (5% $K_2Cr_2O_2$ and glacial acetic acid were mixed in the ratio, of 1:3 respectively)

$$\sum = 40\ M^{-1}\ cm^{-1}$$

2.14. Evaluation of wound healing activities of *Chromolaena odorata* methanol leaf extract

The wound healing effect of *Chromolaena odorata* was carried out using excision model as was described by Vijayaraghavan *et al*., (2017b) with slight modifications. A total of 4 groups of 3 rats per group were used. The animals were anesthetized with 10 mg/kg body weight ketamine intraperitoneally (I.P) prior to the wound creation. The furs on the back of the rats was shaved and circular uniform excision wounds of 1.5 cm diameter were created along markings using toothed forceps and pointed scissors. After 24 hours the wounds were cleaned and measured by tracing the wound surface areas using transparent paper after which the paper having the wound dimensions were placed on a graph sheet. The number of squares of the graph sheet that matches the wound dimensions was counted and used to measure the area of the wounds. These were recorded as the basal wound areas. The wounds were left untreated for 18 hours for complete expansion after which the groups then received treatment as follows: group 1 received Petroleum Jelly and served as control; group 2 received cikatrin powder; group 3 received 5% of crude extract ointment; and group 4 received 10% of crude extract ointment. While cikatrin powder served as the positive control, petroleum jelly was the negative control and was used as vehicle control to drench the leaf extracts for the test groups. The drugs were applied once daily until complete epithelization. The wound size were measured at 3 days intervals until the wounds were healed. The degree of the wound was calculated using the formula;

$$W_h = (A - B)/A \text{ multiplied by } 100/1.$$

W_h = percentage reduction in wound area (%)
A = Mean wound size at day 0
B= Mean wound size on corresponding days

2.15. Statistical analysis

Results were presented as mean ± Standard error of mean (S.E.M). Means were analyzed using one way analyses of variance (ANOVA) followed by post hoc Turkey's test for multiple comparisons. $P < 0.05$ was set to be statistically significant. Results analysis was conducted using Statistical Package for Social Science, SPSS- version 20.

3. Results

Table 1 Results of phytochemical analysis

Test		**Occurrence**
Alkaloids	Mayer's	+
	Dragendorf's	-
	Wagner's	+
	Hager's	+
Flavonoidss	Lead acetate test	+
	Alkaline reagent test	+
Reducing sugars	Benedict's test	-
	Fehling's test	-
Saponins	Frothing test	-
Proteins	Millon's test	+

	Precipitation test	+
Tannins	Ferric Chloride test	+
Amino acids	Ninhydrin test	-
Steroids	Salkowski test	+
Triterpenoids	Salkowski test	-
Glycosides	General test	-

+= Present, -= Absent

Table 2 Results of acute toxicity study

Groups	Doses (mg/kg body weight)	Number of rats	Number of deaths
1	10	3	Nil
2	100	3	Nil
3	1,000	3	Nil
4	2,000	1	Nil
5	3,000	1	Nil
6	4,000	1	Nil
7	5,000	1	Nil

According to the results in table 2, no death was recorded up till 5,000 mg/kg body weight; LD50 was > 5,000 mg/kg body weight.

Table 3 Results of lipid peroxidation assay

Groups	Treatments	Mean MDA ± SEM ($x10^{-5}$ µmol/ml)
1	10 ml/kg Distilled Water	14.16 ± 0.59
2	100 mg/kg aspartic acid	5.55 ± 0.38
3	100 mg/kg Crude Extract	9.51 ± 0.95
4	250 mg/kg Crude Extract	7.35 ± 0.35
5	500 mg/kg Crude Extract	5.63 ± 0.33

Table 4 Results of antioxidant study; catalase analysis

Groups	Treatment	Mean CAT ± SEM ($x10^{-4}$µmol/min/mg)
1	10 ml/kg Distilled Water	2.49 ± 0.25
2	100 mg/kg aspartic acid	3.83 ± 0.12
3	100 mg/kg Crude Extract	2.85 ± 0.07
4	250 mg/kg Crude Extract	3.18 ± 0.13
5	500 mg/kg Crude Extract	3.80 ± 0.10

Table 5 Results of wound healing study

Means ± SEM (cm)						
Treatments	Basal	Day 3	Day 6	Day 9	Day 12	Day 15
Petroleum jelly	0.77 ± 0.07	0.77 ± 0.07	0.72 ± 0.07	0.75 ± 0.03	0.70 ± 0.00	0.67 ± 0.02
Cikatrin powder	0.83 ± 0.03	0.70 ± 0.06	0.60 ± 0.05	0.37 ± 0.03	0.10 ± 0.06	0.00 ± 0.00
5% crude extract ointment	0.67 ± 0.03	0.57 ± 0.03	0.47 ± 0.03	0.33 ± 0.03	0.03 ± 0.03	0.00 ± 0.00
10% crude extract ointment	0.70 ± 0.06	0.60 ± 0.06	0.47 ± 0.07	0.25 ± 0.05	0.02 ±0.02	0.00 ± 0.00

Table 6 Percentage reduction in wound areas (%)

Percentage reduction in wound areas (%)						
Groups	**Treatment**	**Day 3**	**Day 6**	**Day 9**	**Day 12**	**Day 15**
1	Petroleum jelly	0	6.49	2.60	9.09	12.99
2	Cikatrin powder	15.66	27.71	55.42	87.95	100
3	5% crude extract ointment	14.93	29.85	50.74	95.52	100
4	10% crude extract ointment	14.29	32.86	64.29	97.14	100

Discussion

Chromolaena odorata's methanol leaf extract contains steroids, alkaloids, flavonoids, proteins, and tannins, according to the phytochemical study. Glycosides, amino acids, triterpenoids, saponins, and reducing sugars are not present, however. Their anti-oxidant, anti-inflammatory, anti-diabetic, antibacterial, analgesic, and other pharmacological activity suggests that these phytochemicals may have had a role in the extract's wound healing effectiveness. There was an effort to compile information on alkaloids' antioxidant properties using cellular methods as well as the traditional in vitro scavenging experiment. Researchers looked at studies that examined the effects of alkaloids on NADPH-oxidase, an enzyme essential for the formation of reactive oxygen species, at the cellular level, as well as those that used the DPPH radical scavenging test. Researchers used the DPPH assay to evaluate over 130 alkaloids; they found that some of the alkaloids were as active as, or even more so than, conventional antioxidants, with the amount of aromatic hydroxyl groups serving as the primary indicator of their effectiveness. There is little correlation between the DPPH test and the results on alkaloids' inhibitory effects on NADPH-oxidase activity. It seems that blocking the production, activation, or translocation of NADPH-oxidase subunits is the underlying mechanism. Source: Macakova et al., 2019. Another research found that flavonoids are great for your health and have many uses in the cosmetic, pharmaceutical, nutraceutical, and therapeutic fields. Their ability to alter essential cellular enzyme activity and their anti-oxidative, anti-inflammatory, anti-mutagenic, and anti-carcinogenic capabilities were the reasons given for this (Panche et al., 2016). Atherosclerosis, cancer, and cardiovascular illnesses all share oxidative stress and chronic inflammation as their pathological foundations, according to a new research. The search for bioactive chemicals in food with the potential to alleviate oxidative stress and chronic inflammation has gained momentum in recent years. The physiologically active proteins found in egg whites are often more effective following enzymatic hydrolysis, and many of these proteins include antioxidant and anti-inflammatory properties. Zhou et al. (2022) found that egg white proteins had anti-oxidative stress and anti-inflammatory effects, and they also explained how these effects worked in both animal and laboratory models. Tannins from plants have many biological uses in animals, and another research looked at how they are classified and where they are extracted. Several studies have shown the anti-inflammatory, anti-parasitic, antibacterial, and antidiarrheal properties of tannins (Zhenkai et al., 2022). Another research found that the Cyperus sexangularis (CS) leaf contained three steroids, one fatty acid, and two fatty acid esters, all of which exhibited strong antioxidant, anti-inflammatory, and anti-elastase characteristics. The chemicals were obtained by chromatographing the leaf extracts with n-hexane and dichloromethane on a silica gel column. Using conventional in

vitro antioxidant techniques, we assessed each compound's inhibitory activity against DPPH, NO, and ferric ion radicals. We used the egg albumin denaturation (EAD) test to assess the anti-inflammatory response in vitro, and we also examined the anti-elastase activity of each drug in human keratinocyte (HaCaT) cells. Among the substances identified as steroidal derivatives were stigmasterol (1), 17-(1- two fatty acid esters, ethyl nonadecanoate (5) and ethyl stearate (6), dodecanoic acid (4), β-sitosterol (3), methyl-allyl)-hexadecahydro-cyclopenta[a]phenanthrene (2), and dodecanoic acid (4). The ideal biological characteristics were shown by stigmasterol (1), which had an IC50 value of 38.18 ± 2.30 µg/mL against DPPH, 68.56 ± 4.03 µg/mL against NO, and 303.58 ± 10.33 µAAE/mg against Fe3+. At a concentration of 6.25 µg/mL, stigmasterol reduced EAD by half. The anti-elastase activity of compounds 1, 3, 4, and 5 was similar, with an IC50 value of 50 µg/mL or higher. In contrast, the reference chemical, ursolic acid, had double the activity, with an IC50 value of 24.80 ± 2.60 µg/mL. To sum up, our work is the first to report the presence of three steroids (1-3), one fatty acid (4), and two fatty acid esters (5 and 6) in the leaves of C. sexangularis. According to Gugulethu et al. (2023), the compounds exhibited strong antioxidant, anti-inflammatory, and anti-elastase capabilities. Acute toxicity testing revealed that Chromolaena odorata methanol leaf extract did not cause death at doses as high as 5,000 mg/kg body weight. As a result, it's thought to be completely harmless for people to eat. An prior research found that herbal medications are typically well-respected for their safety and effectiveness. Because of the widespread belief that plant cures do not have any negative side effects, an increasing number of individuals are turning to herbal therapy. The purity, consistency, and pollutants of therapeutic plants may cause them to be hazardous either on their own or when combined with other preparations. Medical practitioners, according to the study's authors (Mohammad-Reza et al., 2013), should be on the lookout for possible drug interactions between herbal remedies and prescription drugs. The antioxidant assays for malondialdehyde (MDA) and catalase (CAT) activities demonstrated that the methanol leaf extract of Chromolaena odorata reduced lipid peroxidation at doses that were statistically significant ($p < 0.05$). Group 5, which received 500 mg/kg of extract, had an MDA concentration of 5.63 ± 0.33 (x10-5 µmol/ml), in contrast to the normal control group that had an MDA concentration of 14.16 ± 0.59 (x10-5 µmol/ml). Lipid peroxidation was shown to be initiated by free radicals in a previous research. The cellular peroxidation of polyunsaturated fatty acids produces malondialdehyde (MDA) as one of its end products. A typical indicator of oxidative stress and antioxidant status, MDA is overproduced when free radicals rise (Gawel et al., 2004). At the medium and high dosages tested, there was a substantial rise in blood levels of the catalase enzyme, which was dose dependent ($p < 0.05$). Another study found that reactive species, which are naturally occurring in cells as part of normal cellular metabolism, can chemically react with various biomolecules found in cells. This can lead to changes in their composition and even damage to their cellular activities, as these biomolecules undergo oxidative modifications. In order to protect themselves against reactive species and their byproducts, cells have developed a variety of antioxidant defense mechanisms. These include metabolites, vitamins, and enzymes. An imbalance between reactive species and antioxidants causes a physiological state known as "oxidative stress." Scientists have shown that catalase, an essential antioxidant enzyme, greatly reduces oxidative stress by converting hydrogen peroxide into water and oxygen inside cells (Nandi et al., 2019). This suggested that the antioxidant effects of Chromolaena odorata methanol leaf extract were similar to those of the gold standard anti-oxidative stress medication, aspartic acid, in protecting the animals from oxidative stress. Chromolaena odorata methanol leaf extract outperformed cikatrin in wound healing activities, according to the data. No appreciable improvement in wound size was seen in the group 1 rats given petroleum jelly. At the beginning of the therapy, the average wound area in this group was 0.77 ± 0.07 cm; on day 15, it had decreased to 0.67 ± 0.02 cm, a decrease of 12.99%. The lesion was fully healed by the end of day 15 when both the cikatrin powder and the 5% crude extract ointment were used. The ointment was made by combining Chromolaena odorata leaf extract with petroleum jelly. The 10% concentration of the extracts, however, accelerated the wound-healing process the most. The wound areas of the cikatrin group decreased from 0.83 ± 0.03 cm to 0.60 ± 0.05 cm on day 6 of treatment, which is 27.71% but not statistically significant ($p > 0.05$). In contrast, groups 3 and 4, which were treated with 5 and 10% crude extract ointments respectively, showed a significant ($p < 0.05$) decrease in wound area, with 0.47 ± 0.03 cm (32.86%) and 0.47 ± 0.07 cm (32.86%) respectively. Group 4 showed a more substantial decrease in wound area from day 9 onwards, with 0.25 ± 0.05 cm (64.29%) compared to 0.33 ± 0.03 (50.74%) in group 3. Wound areas decreased by 87.95%, 95.52 %, and 97.14% on day 12 of therapy after using 5% crude extract ointment, cikatrin powder, and 10% crude extract ointment, respectively. Despite the fact that all three treatments resulted in complete healing by day 15, the methanol leaf extract of Chromolaena odorata shown more efficacy than cikatrin and a dose-dependent increase in wound healing activities. Reasons for this include the anti-oxidative, anti-inflammatory, and antimicrobial properties of Chromolaena odorata. One study listed the ways medicinal plants aid in wound healing. Cinnamomum verum, for example, has antioxidant, antiulcer, antimicrobial, antidiabetic, hypoglycemic, hypolipidemic, and anti-inflammatory effects; aloe vera extract, on the other hand, can reduce inflammation, improve mature granulation tissue, and speed up the healing process, making it useful for diabetic and infected wounds. In wounds caused by diabetes, it lowers blood glucose levels, which might be helpful. Dill, or Anethum graveolens L., is a member of the Apiaceae family of herbs that has anti-inflammatory, anti-microbial, and antidiabetic characteristics that may speed up the healing process of wounds. Dill essential oil mostly contains cis-carvone, limonene, α-phellandrene, and anethofuran, among other chemicals. One of the other main components of

dill essential oil is alpha-phellandrene, which has antibacterial and antifungal properties.

according to Reza et al. (2019), colonization may be helpful in cases of infected wounds. All of these characteristics were clearly shown by Chromoleana odorata in its lightning-fast wound-healing mechanism.

4. Conclusion

In conclusion, *Chromolaena odorata* methanol leaf extract had a remarkable potency in wound healing activities which is partly attributed to its antioxidant effects. It had a dose dependent increase in wound healing and it is significantly ($p < 0.05$) more potent than cikatrin, a standard wound healing drug.

Compliance with ethical standards

Acknowledgments

I want to appreciate the efforts of Pharm Uche Orji and Mr Chibueze Ikeh; there contributions help in the success of this study. My gratitude also go to Mr Okonkwo Fabian Chukwujekwu and Mr Mbachu Henry Chukwuemeka for their financial support.

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

Maintenance and care of all animals were carried out in accordance with EU Directive 2010/63/EU for animal experiments. Guide for the care and use of Laboratory Animals, DHHS Publ. # (NIH 86-123) were strictly adhered to. Ethical approval was obtained from the Animal Ethical Committee of the Enugu State University of Science and Technology. There was additional approval by the Nnamdi Azikiwe University's Ethical Committee for the use of Laboratory Animals for Research Purposes; (Approval number is NAU/AREC/2023/00082)

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