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(RESEARCH ARTICLE)

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Evaluation of the anti-oxidation and wound healing activities of *Chromolaena odorata* methanol leaf extract using rat model

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Abstract

Since decades ago, humans have been using natural products such as plants in medicines. Currently, traditional medicine has become popular. Plants contain numerous phytochemicals that may act synergistically through different mechanisms for prophylaxis and therapy of diseases. This study evaluated the anti-oxidative and wound healing activities of *Chromolaena odorata* methanol leaf extract using mature male rat models. Phytochemical analysis, acute toxicity study, anti-oxidative potentials as well as wound healing activities of the leaf extract were assessed. In anti-oxidative study, the effects of *Chromolaena odorata* methanol leaf extract on malondialdehyde (MDA) and catalase were tested. In the wound healing, excision wounds were created on the back of the rats and the wounds were treated with three different doses of *Chromolaena odorata* methanol leaf extract embedded in petroleum jelly. Cikatrin powder and petroleum jelly served as positive and negative controls respectively. The phytochemicals present were alkaloids, flavonoids, proteins, tannins, and steroids. LD_{50} > 5,000 mg/kg body weight was noted. In anti-oxidative assay, the leaf extract had better lipid peroxidation inhibition. The 500 mg/kg body weight of the extract reduced MDA to 5.63 ± 0.33 x 10^{-5} µmol/ml compared to 14.16 ± 0.59 x 10^{-5} µmol/ml obtained with distilled water. The 5% and 10% extracts had better percentage wound healing of 95.52% and 97.14 % respectively than cikatrin which had 87.95% after 12 weeks of treatment. *Chromolaena odorata* methanol leaf extract had good safety profile and a dose dependent increase in wound healing that is significantly ($p < 0.05$) more potent than cikatrin.

Keywords: Anti-oxidation; *Chromolaena odorata*; Malondialdehyde; Wound healing activities

1. Introduction

From time immemorial, humans have used natural products, such as plants, animals, microorganisms, and marine organisms, in medicines to relieve and treat diseases (Yuan *et al*., 2016). According to the researchers, fossil records show that the human use of plants as medicines may have begun as far back as at least 60,000 years. Traditional medicine is a treatment method that makes use of natural and spiritual resources either separately or jointly for treatment, diagnoses and prevention of illnesses or maintenance of healthy living. The popularity of traditional medicine has increased due to high cost of orthodox drugs and proliferation of drug resistant disease causing microorganisms (Fokunang *et al*., 2011). According to the World Health Organization (WHO), 80% of the emerging world's population depend on traditional medicine for cure of their ailments (Gajender *et al*., 2023). During the past decades, the developed world has also observed an ascending trend in the application of complementary and alternative medicines (CAM), particularly herbal remedies (Mahomoodally *et al*., 2013). African traditional medicine is a form of

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complete health care system that comprises three areas notably divination, spiritualism, and herbalism, although there may be overlap in some circumstances (Ezekwesili-ofili *et al*., 2019). The increased rate of traditional medicine use in Africa has been attributed to cultural and economic factors. Moreover, plants contain numerous phytochemical compounds that may act synergistically through different mechanisms to effect cure or prevent diseases. Herbal medicine is a distinct and noticeable form of traditional medicine, in which the traditional healer specializes in the use of herbs to treat various ailments. In Nigeria, there has been high rate of traditional medicine usage. This is attributed to its accessibility, affordability and anticipated low risk of adverse reaction when compared to orthodox medicines. A certain study in Ibadan, Nigeria found that people were more likely to choose traditional medicine because of supposed higher effectiveness, accessibility, and affordability (Li *et al*., 2020). Traditional medicines are used to treat a varieties of diseases in Nigeria. These diseases include: malaria, ulcers, sexually transmitted diseases, infertility, and wounds among others. Injuries and wounds are ignored but widespread illness in developing countries and unexpectedly they cause greater than five million deaths annually. This approximates to the number of deaths associated with HIV/AIDS, malaria and tuberculosis combined together (Rechard *et al*., 2009). This is worsened in low-income countries due to poorly developed trauma care and rehabilitation systems and little or no social welfare infrastructure. Research to proffer solution to this menace has not been given the full attention it deserves and resources have been insufficient. Wound healing orthodox medicines are not only expensive but also not readily available and are associated to adverse reactions. Consequently, more researches are now focusing on traditional medicines which are cheaper, accessible and have less toxicity. The fact that wounds and injuries are becoming more prevalent in developing countries should not be overemphasized. This is because, there are a lot of predisposing factors such as trauma, accidents which can be as a result of bad road networks, falls especially among children and elderly ones, as well as paucity of wound healing medications among other factors. On the other hand, there are little availability of orthodox medicines that are indicated specifically for wound healing; most of the known drugs used for this purpose are antimicrobials, antioxidants and anti-inflammatory medications. *Chromolaena odorata* has been reported to possess antioxidant activities. This prompeted the desire to test it for wound healing potentials with the intention of proffering solution to the scarcity of wound healing drugs. In this study therefore, we investigated the wound healing activities of *Chromolaena odorata* which stems from the fact that *Chromolaena odorata* has been shown to exhibit remarkable antioxidant effects; and having been found to be very active, it will contribute in reducing the prevalence of different types of wounds in Nigeria and beyond.

1.1. Process of wound healing

Wound is a distortion of the skin as a result of an injury, burn, or deficiency of enzymes such as glucose-6-phosphate dehydrogenase. Wound healing is a complex process of tissue repair. Historically, plants and plant-based constituents have been widely used for the treatment and management of different types of wounds. Herbal drugs have played a significant role in curing diseases including healing wounds throughout the history of mankind. Herbal medicines used in wound treatment or care include those with disinfectant, debridement, and moisturizing capacities as to provide an appropriate natural healing environment. Folklore cultures employ a significant number of plants to treat cuts, wounds, and burns (Sharma *et al*., 2021). The process of wound healing following an injury begins when platelets come into contact with exposed collagen. This causes platelet aggregation as well as the release of coagulation factors which in turn result in the formation of fibrin clot at the injury site. Pro-inflammatory cells such as cytokines as well as growth factors also arrive at the injury site. The fibrin clot functions as a temporary matrix while the fibroblast is the connective tissue responsible for collagen deposition that is necessary for tissue repair. Collagen is also essential because it repair the defect and restore anatomical structure and function of the damaged tissue. There are different types of wounds which include: acute wounds, closed wounds, open wounds, incised wounds, tear or laceration wounds, puncture wounds, abrasive wounds, chronic wounds among others (Chhabra *et al*., 2017). Chronic wounds are wounds that have not gone through the usual healing stages and hence reach a state of pathologic inflammation. They need extended healing time.

1.2. Some wound healing plants

For more than 5000 years, Egyptians, indigenous peoples of Africa, Asia, Romans, and the Americas have used medicinal plants as first-line therapy for inflammation, burns, ulcers, and surgical wounds (Shedoeva *et al*., 2019). Medicinal plants contain many natural phytochemical compounds that makes the process of wound healing faster and regenerate tissue at the wound site. Some examples of medicinal plants and their wound healing effects include: Centella (*Centella asiatica*) which is also known as Asian pennywort, used to facilitate healing of wounds. Extracts from the *Centella asiatica* are reported to facilitate the healing of the chronic ulcers irrespective of their distance, depth, and scale. Some of these extracts are Asiaticoside, triterpenes, madecassoside which promotes collagen synthesis and angiogenesis at the wound site (Diniz *et al*., 2023). Curcumin which is a component of Curmeric (*Curcuma longa*) has been shown to interact at transcription, translation, and post-translation levels with key cellular processes and enzymes such as pro-inflammatory cytokines, apoptosis, nuclear factor kappa B (NF-kb), cyclooxygenase 2, 5-lipoxygenase, prostaglandin E2 among others. Researches show that curcumin plays vital role in wound healing by altering the pericellular and

extracellular matrix, stimulating fibroblast proliferation, development of granulation tissue and the deposition of collagen in the cutaneous wounds (Kumari *et al*., 2022). Alcohol extracted from *Wedelia trilobata* leaves have been used to treat rheumatism, persistent wounds and sore arthritic joints (Shedoeva *et al*., 2019). The researchers also reported that luteolin, a flavonoid in the leaves of *Wedelia trilobata* has been shown to confer neuroprotective, anti-cancer, antioxidant, and immunomodulatory activities to the plant. Traditional healers treat skin wounds using the *Wedelia trilobata* leaves. Luteolin inhibits the expression of nuclear factor kapper B (NF-Kb)-regulated pro-inflammatory cytokines, a characteristic feature of skin infection and psoriasis (Weng *et al*., 2014). Acetone extracts from *Aloe vera* leaves show great antimicrobial activity. *Aloe vera* tends to be more susceptible to gram-positive bacterial species than gram-negative species. Saponins, acemannan, and anthraquinone derivatives are compounds with a proven antimicrobial activity. Acemannan is an effective stimulator for the operation of macrophages and T cells and induces the transcription of pro-inflammatory messenger RNAs (mRNAs), tissue necrosis factor α (TNF-α), prostaglandin E2 (PGE2), and nitrous oxide. Topically applied acemannan, has been documented to significantly reduce the time for wound closure (Sharma *et al*., 2021). In a clinical trial, the antioxidant, antimicrobial, anti-inflammatory, anti-diabetic, antiviral, anti-cancer, and hepatoprotective effects of *Arctium lappa* were detected (Nermeen *et al*., 2022). *Arctium lappa* has also been reported to control cell adhesion and gene expression in canine dermal fibroblasts, influencing the Wnt/β-catenin signaling pathway, known to be a key wound cure regulator. In a pilot study of *Arctium lappa,* healing of human first and second-degree burns were found to be handled more efficiently than the control procedure (Shedoeva *et al*., 2019). It has been shown that *Panax ginseng* decreases inflammation, and confers antioxidant, anti-cancer, antibacterial, and immunomodulatory capacity (Riaz *et al*., 2019). According to the research, *Panax ginseng* comprises many bioactive compounds, of which the most potent is a saponin called ginsenosides. Panax ginseng root extracts have been shown to protect the skin from acute ultraviolet beam (UVB) irradiation and significantly improved healing following laser burning and excisional wound injury.

1.3. *Chromolaena odorata*

The proximate and quantitative phytochemical analyses of both aqueous and methanolic extracts of the leaves of *Chromolaena adorata* indicated that the leaves contained carbohydrate (1.10±1.14%), Protein (24.08±0.08%), Lipid (14.00±0.01%), fiber (50.26±0.01%), Ash (10.98± 2.00%) and moisture content of 5.65±0.02%. An energy content of 220.20 kcal was recorded. The leaves also contained mineral elements such as calcium, sodium, potassium, iron, manganese, zinc, copper, phosphorus, and magnesium. The leaves also yielded 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%) (Nwinuka *et al*., 2009). The researchers also reported the anti-microbial effects of the methanolic extracts to be positive for *Bacillus subtilis*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Candida albicans, Aspergillus saccharomyces cerevisiae, Rhizopus species, Penicillium species* and negative for *Pseudomonas pyrogenes* and *Escherichia coli*. Several biochemicals are involved in the healing process of the body, including antioxidants and cytokines. Phytochemicals from numerous plants suggested that they have positive effects on different stages of the wound healing process via various mechanisms. *Chromolaena odorata* exhibits anti-inflammatory, antipyretic, analgesic, antimicrobial, cytotoxic and numerous other relevant medicinal properties on an appreciable scale. A review of this plant summarized its role in the wound healing activities of biological systems, which are crucial to its potential future drug design, development and application for the treatment of wounds (Vijayaraghavan, *et al*., 2017a).

1.4. Antioxidant property of *Chromolaena odorata*

Polyphenols from the ethanol extract of *Chromolaena odorata* are rich in natural antioxidants such as p-hydroxyl benzoic, p-coumaric, protocatechuic, ferulic and vanillic acids (Bhargava *et al*., 2013). They noted that the antioxidant potentials of 25-400 µg/ml *Chromolaena odorata* ethanol extract was similar to 10-80 µg/ml of standard ascorbic acid in vitro. As preventive antioxidants, they may enhance the oxidative capacity of hepatocytes to prevent glutathione depletion. Which will mitigate lipid peroxidation and protect liver from damage. In another study, *Chromolaena odorata* was shown to contain polyphenols and antioxidant enzymes that activate biological defense mechanisms and stress-sensing transcription factors to prevent oxidative damage and heat stress in chicken (Lartey *et al*., 2020). The study further indicated that Chromomoric acid C-1 from *Chromolaena odorata* methanol extract, at 10 µg, demonstrated anti-inflammatory potential by activating Nrf2 and suppressing NF-κB at an inhibition capacity (IC_{50}) of 6.9 µM. These biological defense properties of this plant have the potential to enhance anti-oxidative physiology for cellular oxidative balance, and mitigate oxidative damage in the presence of heat stress. Another research recently done on *Chromolaena odorata* leaves reported its potent antioxidant activity. This study evaluated the antioxidant activity with various doses of ethanolic extract of *Chromolaena odorata* leaves against male Wister rats induced by paracetamol. The researchers concluded that ethanol extract of *Chromolaena odorata* at a dose of 500 mg/Kg body weight exhibited 58.974% reduction of malondialdehyde serum level and improve the histological structure of hepatocytes (Solihah *et al*., 2020). A study done to characterize soluble and cell wall polysaccharides isolated from the *Chromolaena odorata* leaves and to evaluate their antioxidant and immunomodulatory properties indicated significant radical scavenging and

immunostimulatory activities. The study support the ethnomedicinal use of the leaves of *Chrmolaena odorata* (Boudjeko *et al*., 2015). *Chromolaena odorata* is widely used as traditional medicines for diabetes and soft tissue wounds treatment in some regions in East Indonesia. A new flavanone isolated from the methanol extract and elucidated as odoratenin together with two known compounds: isosakuranetin and subscandenin exhibited very potent 2,2-diphenyl-1-picryl-hydrazyl (DPPH) and 2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radicals inhibitory activity (Putri *et al*., 2019). *Chromolaena odorata* is one of the plants used by the community as traditional medicine. Some of community in Ambon, Indonesia used *Chromolaena odorata* leaves as wound medicine due to the free radical scavenging activity from fraction of methanol extract of the plant (Maulida *et al*., 2019). In an earlier study, the free radical scavenging and antioxidant activity of the leaves of *Chromolaena odorata* was evaluated and the researchers reported that the ethanol and methanol extracts reveal significant antioxidant properties (Bhargava *et al*., 2013).

1.5. Wound healing activities of *Chromolaena odorata*

Researches have shown that several phytochemicals are involve in the healing process of wounds. Among these compounds are antioxidants and cytokines. *Chromolaena odorata* was also reviewed and found to exhibits anti-inflammatory, antipyretic, analgesic, antimicrobial, antioxidant among other medicinal properties. These properties confer to it the wound healing activities of biological systems, which are crucial to its potential future drug design, development and application for the treatment of wounds (Vijayaraghavan *et al*., 2017b). In a similar study, it was pointed out that *Chromolaena odorata* extract has been used to stop bleeding and in wound healing in many tropical countries. The researchers examined the molecular mechanisms by which the plant extract affected hemostatic and wound healing activities. They discovered that *Chromolaena odorata* promoted Balb/c 3T3 fibroblast cell migration and proliferation; Subsequently, they found that heme oxygenase-1 (HO-1), the accelerating wound healing enzyme, was increased at the transcriptional and translational levels by *Chromolaena odorata* treatments (Hataichanok *et al*., 2013). Furthermore, a study which aimed at exploring the wound healing potential of aqueous and ethanol extracts of *Chromolaena odorata* in a rat excision wound model was documented. The researchers noted that *Chromolaena odorata* exhibited a faster reduction in wound area compared to control and Betadine-treated groups (Vijayaraghavan1 *et al.*, 2017b). In addition, the topical application of the extract increased collagen synthesis and its stabilization at the wound site, as evidenced by the increase in hydroxyproline and hexosamine levels and expression of collagen. A review of *Chromolaena odorata* wound healing property showed its importance in that aspect (Sirinthipaporn *et al*., 2017). The researchers summarized the role of *Chromolaena odorata* and its biomarkers in the wound healing activities of biological systems, which are crucial to its potential future use for the treatment of wounds (Bhuyan *et al*., 2019). In this present study therefore, we investigated the antioxidant activities of *Chromolaena odorata* and then evaluated its wound healing potentials in male Wister rat models using cikatrin powder and petroleum jelly as positive and negative controls respectively.

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^{o}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 1.56×10^5 $M^{-1}cm^{-1}$.

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

4. Discussion

The results of the phytochemical analysis showed that alkaloids, flavonoids, proteins, tannins, and steroids are present in the methanol leaf extract of *Chromolaena odorata.* However, reducing sugar, saponins, amino acids, triterpenoids and glycosides are absent. These present phytochemicals might have contributed to the wound healing potency of the extract due to their potentials in anti-oxidation, anti-inflammation, anti-diabetic, antimicrobial, analgesic among other pharmacological activities. A certain review aimed at summarizing the antioxidant effects of alkaloids by both classical in vitro scavenging assay and at the cellular level. The researchers considered studies that used the DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay and the effect of alkaloids on NADPH-oxidase, the key enzyme for reactive oxygen species production, at the cellular level. More than 130 alkaloids were tested by DPPH assay and the researchers concluded that some of the alkaloids were either similar to or even more active than standard antioxidants and the number of aromatic hydroxyl groups seems to be the major determinant for their activities. The data on inhibition of NADPH-oxidase activity by alkaloids demonstrated that there is little relationship to the DPPH assay. The mechanism seems to be based on inhibition of synthesis, activation or translocation of NADPH-oxidase subunits. (Macakova *et al*., 2019). In another study, flavonoids were considered as indispensable component in a variety of nutraceutical, pharmaceutical, medicinal and cosmetic applications. This was attributed to their anti-oxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties coupled with their capacity to modulate key cellular enzyme function (Panche *et al*., 2016). A recent study described oxidative stress and chronic inflammation as the common pathological bases of chronic diseases such as atherosclerosis, cancer, and cardiovascular diseases. There has been an increasing interest to identify food-derived bioactive compounds that can mitigate the pathological pathways associated with oxidative stress and chronic inflammation. Egg white contain a variety of biologically active proteins, many of which have antioxidant and anti-inflammatory activities and usually show better activity after enzymatic hydrolysis. The researchers concluded that egg white proteins exhibited anti-oxidative stress and anti-inflammatory activities and clarifies their mechanism of action in vivo and in vitro (Zhou *et al*., 2022). In another study, the classification and extraction sources of plant tannins were reviewed, as well as the biological functions of plant tannins in animals. It was shown that tannins have antioxidant, antibacterial, anti-parasitic, anti-inflammatory, antidiarrheal actions (Zhenkai *et al*., 2022). In another study, three steroids and one fatty acid and two fatty acid esters in *Cyperus sexangularis* (CS) leaf were reported to show considerable antioxidant, anti-inflammatory and anti-elastase properties. The n-hexane and dichloromethane leaf extracts were chromatographed on a silica gel column to yield the compounds. The inhibitory effect of each compound against 2, 2-diphenyl-1-picrylhydrazyl (DPPH), nitric oxide (NO) and ferric ion radicals were determined by standard in vitro antioxidant methods. The in vitro anti-inflammatory response was measured using egg albumin denaturation (EAD) assay, while the anti-elastase activity of each compound in human keratinocyte (HaCaT) cells was also monitored. The compounds were characterized as three steroidal derivatives, stigmasterol (1), 17-(1-

methyl-allyl)-hexadecahydro-cyclopenta[a]phenanthrene (2) and β-sitosterol (3), dodecanoic acid (4) and two fatty acid esters, ethyl nonadecanoate (5) and ethyl stearate (6). Stigmasterol (1) exhibited the best biological properties, with IC_{50} 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 6.25 µg/mL, stigmasterol inhibited EAD by 50%. Compounds 1, 3, 4 and 5 showed comparable anti-elastase activity with an $IC_{50} \geq 50$ µg/mL, whereas the activity of ursolic acid (standard) was double fold with an IC_{50} of 24.80 ± 2.60 µg/mL when compared to each of the compounds. In conclusion, this study has identified three steroids (1–3), one fatty acid (4), and two fatty acid esters (5 and 6) in C. sexangularis leaf for the first time. The compounds showed considerable antioxidant, anti-inflammatory and anti-elastase properties (Gugulethu *et al*., 2023). Results of the acute toxicity study showed that the methanol leaf extract of *Chromolaena odorata* did not record any mortality up to the high dose of 5,000 mg/kg body weight. It is therefore considered to be safe for human consumption. In accordance to an earlier study, herbal medicines are generally considered to be safe and effective agents. Therefore, people more and more turn to herbal medicine because they believe that plant remedies are free from undesirable side effects. However, medicinal plants can be toxic intrinsically or when taken in combination with other preparations which may be due to purity, consistency of active compounds, and contaminants. The researchers therefore recommended that health care professionals should remain vigilant for potential interactions between herbals and prescription medications (Mohammad-Reza *et al*., 2013). According to the results of antioxidant assays of malondialdehyde (MDA) and catalase (CAT) activities, it was shown that the methanol leaf extract of *Chromolaena odorata* exhibited dose dependent reduction in lipid peroxidation which at all tested doses were significant ($p < 0.05$) when compared with the normal control group which recorded MDA concentration of 14.16 ± 0.59 ($x10^{-5}$ µmol/ml) as against 5.63 ± 0.33 ($x10^{-5}$ µmol/ml) recorded by group 5 that received 500 mg/kg body weight of the extract. In an earlier study, free radicals were noted to generate the lipid peroxidation process in an organism. Malondialdehyde (MDA) is one of the final products of polyunsaturated fatty acids peroxidation in the cells. An increase in free radicals causes overproduction of MDA and MDA level is commonly known as a marker of oxidative stress and the antioxidant status (Gawel *et al*., 2004). The results also recorded a dose dependent increase in serum levels of catalase enzyme which were significant ($p < 0.05$) at the medium and high tested doses. In another study, reactive species produced in the cell during normal cellular metabolism was implicated in chemically reacting with cellular biomolecules such as nucleic acids, proteins, and lipids, thereby causing their oxidative modifications leading to alterations in their compositions and potential damage to their cellular activities. Fortunately, cells have evolved several antioxidant defense mechanisms (as metabolites, vitamins, and enzymes) to neutralize or mitigate the harmful effect of reactive species and/or their byproducts. Any perturbation in the balance in the level of antioxidants and the reactive species results in a physiological condition called "oxidative stress." According to the researchers, catalase is one of the crucial antioxidant enzymes that mitigates oxidative stress to a considerable extent by destroying cellular hydrogen peroxide to produce water and oxygen (Nandi *et al*., 2019). This implied that *Chromolaena odorata* methanol leaf extract protected the animals from oxidative stress in a manner comparable to the protection by aspartic acid which is a standard anti-oxidative stress drug. The results also showed that the methanol leaf extract of *Chromolaena odorata* was more potent than cikatrin in wound healing activities. The group 1 rats treated with petroleum jelly did not show any significant change in the wound area. This group had a mean wound area of 0.77 ± 0.07 cm from the onset which became 0.67 ± 0.02 cm after day 15 of treatment which is just equivalent to 12.99% reduction of the wound areas. Both the cikatrin powder, 5% crude extract ointment and 10% crude extract ointment prepared by mixing *Chromolaena odorata* leaf extract with petroleum jelly were able to heal the wound completely by the end of day 15. However, the extracts were faster in healing the wounds and the 10% concentration was the fastest. On day 6 of treatment, the wound areas of the cikatrin treated group reduced from 0.83 ± 0.03 cm to 0.60 ± 0.05 cm, equivalent to 27.71% which was insignificant ($p > 0.05$); while groups 3 and 4 treated with 5 and 10% crude extract ointments respectively had significant ($p < 0.05$) reduction in wound area both having 0.47 ±0.03 (29.85%) and 0.47 ± 0.07 (32.86%) respectively. From day 9, the reduction in wound area became more significant in group 4 with 0.25 ± 0.05 cm (64.29%) as against 0.33 ± 0.03 (50.74%) in group 3. On day 12 of treatment, cikatrin powder, 5% and 10% crude extract ointment had 87.95, 95.52, and 97.14% reduction in wound areas respectively. Despite the three treatments achieving 100% healing on day 15, *Chromolaena odorata* methanol leaf extract had dose dependent increase in wound healing activities and showed more potency than cikatrin. This was attributed to *Chromolaena odorata*'s profound anti-oxidative, anti-inflammatory, anti-microbial among other characteristics. A certain study enumerated the mechanisms of wound healing activities of medicinal plants to include: *Cinnamomum verum* which has some properties such as antioxidant, antiulcer, antimicrobial, antidiabetic, hypoglycemic, hypolipidemic and anti-inflammatory activities which can be beneficial in types of wound such as diabetic and infected wounds; *Aloe vera* extract has some beneficial properties which can decrease inflammation; enhance mature granulation tissue and resulting in help to accelerate wound healing. It also decreases the blood glucose which can be beneficial in diabetic wounds. Another herb *Anethum graveolens* L. (dill) (Apiaceae) was known to have some properties such as antimicrobial, antidiabetic and anti-inflammatory that can improve wound healing. Some compounds including cis-carvone, limonene, α-phellandrene, and anethofuran are major compounds in dill essential oil. Alpha-phellandrene is other major compounds in dill essential oil which may decrease bacterial growth and

colonization and is to be beneficial in infected wounds (Reza *et al*., 2019). *Chromoleana odorata* exhibited all of these properties and this was evident in the rapid mode of wound healing that it demonstrated.

5. 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

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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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