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Phytochemical, Wound Healing and Toxicological Profiles of the Methanol Extract of *Chromolaena odorata* (L.) King & Rob. (Asteraceae) Leaves

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Abstract

Chromolaena odorata (Asteraceae), commonly called ''siamweed, is a medicinal shrub traditionally used to stop bleeding and treat wounds, burns, and skin infections. The extracts of this plant have been reported to contain alkaloids, flavonoids, essential oils, saponins, tannins, and terpenoids. *The aim of the current study was to evaluate the wound healing and sub-chronic toxicity profiles of the aqueous methanol extract of C. odorata.* Sub-chronic toxicity studies were conducted by assessing the hematological parameters, liver, and kidney functions in experimental animals after oral administration of the extract for 14 days. In *vivo* wound healing activity was assessed in rats using an incision wound model. Topical application of a 50% extract in shea butter significantly ($P < 0.05$) increased the contraction rate of the incision wound relative to the control. However, oral administration of the extract for 14 days had no positive effect on wound healing but showed marked adverse effects on liver function and hematological parameters in the animals. Therefore, prolonged oral administration of *C. odorata is not advisable.*

Keywords: *Chromolaena odorata,* Phytochemistry, Wound healing, Toxicology, Haematology

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Introduction

Over the last few decades, there has not been significant success in the development of pharmaceutically active substances with appreciable wound healing activity to combat endemic cases of foot ulcers associated with peripheral neuropathy. Wound healing has become increasingly important in therapeutics due to the burden of chronic wounds and limb amputations. Locally sourced remedies, primarily from the field of phytochemistry, have become increasingly relevant due to their availability, low cost, and safety. Studies have suggested that the development of resistance to herbal portions is relatively more difficult than that to synthetic or orthodox pure compounds because of the inability of microorganisms to decode simultaneously the multiple mechanisms of action and activity profiles of the several bioactive compounds present (Ruddaraju *et al.*, 2019). However, several studies are required to validate these claims. The rising cases of multi-drug resistance have prompted more research, especially as phytochemicals found in nature could present a robust source of active compounds with enormous potential. With these challenges in mind, the combination of standardized antibiotics with plant extracts could present a paradigm shift for medicinal chemists and drug discovery scientists (Imaga, 2010). This novel combination could lead to an expansion of the antimicrobial spectrum, prevention of the emergence of drug-resistant mutants, and minimization of toxicity levels (Dudhatra *et al.*, 2012; Tatiraju *et al.*, 2013).

Chromolaena odorata (Asteraceae) (Figure 1), commonly known as Siam weed, is a medicinal shrub traditionally used in tropical Africa to stop bleeding and treat wounds, burns, dysentery, and skin infections. In Nigeria, it is locally called *Abani di egwu* or *Nsiibilibe* (Igbo language), *ewé Akíntọ́lá* (Yorùbá), or *Akamtoro* in the Nsukka dialect (Lalith, 2009), and is traditionally used for a variety of ailments, including malaria, toothache, diarrhea, diabetes, skin disease, and fever (Phan *et al.*, 2001; Akinmoladun and Akinloye, 2007; Zachariades *et al.*, 2009). Previous reports have portrayed *C. odorata* as a rich source of bioactive compounds. Flavonoids such as querceta-getin-6,4'-dimethyl ether (Wollenweber *et al.*, 1995), aromadendrin-7,4'-dimethyl ether, luteolin, and acetin, isolated from the plant, have been reported to be effective against lung and breast cancer cells (Suksamrarn *et al.*, 2004). The compound 2-hydroxy-4',5',6,4-tetramethoxy chalcone, also known as odoratin, has been identified from the plant and has been reported to exhibit cytotoxic and anticlonogenic activity against several cancer cell lines (Kouame *et al.*, 2013). Eriodictyol-7-4'-dimethyl ether, naringenin-4'-methyl ether and 2',4-dihydroxy-4',5',6-trimethoxy chalcone isolated from the plant have been reported to exert strong inhibitory effects against methicillin-resistant *Staphylococcus aureus* (Johari *et al.*, 2012). Tannins isolated from *C. odorata* have been reported to possess antiviral (Lu *et al.*, 2014), antibacterial (Akiyami *et al.*, 2001), and antiparasitic (Kolodziej and Kiderlen, 2005) activities, justifying the use of *C. odorata* for the treatment of skin diseases and for accelerating blood clotting (Buck, 2003; Cox and Cox, 2009). The juice from the leaves is also applied as a topical ointment to stop bleeding and serves as an antidote to the sting of the spine of the common sea catfish.

Studies (Frykberg and Banks, 2015) have shown that approximately 6 million people suffer from chronic wounds worldwide, while in the United States, traumatic wounds lead to many hospitalizations every year. In Nigeria, the prevalence of diabetic foot ulcers among hospitalized patients was 11% to 32 % %in 2024, accompanied by a high rate of amputation (Ekwebene, 2024). While some data are available on the wound healing properties of *C. odorata* extracts (Debashisha *et al*., 2010), information on the topical wound healing properties of its blend with shea butter is lacking. Furthermore, the sub-chronic toxic effects of the extract on haematological parameters and liver and kidney functions after oral administration have not been adequately evaluated. Therefore, we report the phytochemical, wound healing, and toxicological profiles of the methanolic extract of *Chromolaena odorata* leaves obtained from the University town of Nsukka, Nigeria.

Materials and Methods

Chemicals and Equipment

Analytical grades of the following solvents and chemicals were used: methanol, ethanol, chloroform (JHD, China), sodium hydroxide, potassium hydroxide, ammonia solution, ferric chloride hydrochloric acid, sulphuric acid (Sigma Aldrich), Mayer's reagent, Dragendoff's reagent, Wagner's reagent, Tween-80, Shea butter, Leishman Stain (Bemac Sci. & Chem. Co. Nigeria), amoxicillin tablets, penicillin ointment, and ketamine.

The following equipment were also used: microcapillary tube (Marienfeld, Germany), microhematocrit centrifuge (Hawksley, England), microhematocrit reader (Hawksley, England), hemocytometer set containing an improved Neubauer counting chamber and diluting pipettes (Hawksley, England), Laboratory Tally counter (Clay Adams, New Jersey), Light Microscope (Leica Inc. U.S.A), red blood cell diluting fluid, test tubes, and automatic pipette (Superfit Equip, Ames).

The Plant Materials

The leaves of *C. odorata* were collected from Ihe-Owerre, Nsukka, Enugu State, Nigeria, in May 2023. The plant was identified and authenticated by a botanist, Mr. Alfred Ozioko, of the International Center for Ethnomedicine and Drugs Development (InterCEDD), Nsukka, Enugu State, Nigeria, where a voucher specimen (voucher no. InterCEDD) was deposited. The leaves were air-dried in the shade and pulverized into tiny particles before extraction.

Experimental animals and sample collection

Adult Wistar rats of both sexes (120 –250 g) were obtained from the Animal House of the Department of Veterinary Medicine, University of Nigeria Nsukka. The animals were housed in clean steel cages with good ventilation and maintained at ambient temperature. They were fed mixed pelleted feed (Top Feeds) and given access to clean portable water at all times.

All protocols were approved by the University of Nigeria Ethics Committee on the use of experimental animals (approval no. NHREC/05/01/2008B) and Institute of Health, Mexico City, Mexico accepted principles for laboratory animals' use and care in the National Committee for Scientific Research, Mexico City, Mexico (protocol number.

Solvent Extraction

A 300 g sample of the dried plant material was extracted using 1500 ml of 95% aqueous methanol within 72 h using cold maceration. The mixture was agitated vigorously at intervals. The crude extract was concentrated *in vacuo* using a rotary evaporator set at 40 $^{\circ}C$. The percentage yield of the extract was then determined.

Qualitative Phytochemical Analysis

Qualitative phytochemical analysis was performed on the crude extract using standard protocols (Trease and Evans, 1983).

Preparation of the Extract and Formulation of the Crude Extract with Shea Butter Ointment

A 1 g sample of the crude extract was dissolved in 5 ml of tween-80 and the mixture was stirred until a very thick gel was obtained. A 2 g amount of fresh methanolic crude extract was appropriately weighed and transferred to a clean Petri dish. In addition, 2 g of shea butter was transferred into the same Petri dish. The two were mixed to form an ointment. The ointment was transferred into a sample bottle and labeled.

Wound Healing Activity Study

In *vivo* wound healing activity was assessed in mice using an incision wound model (Masson-Meyers et al., 2020). The animals were divided into six groups (n=4). Each animal was anesthetized using 0.5 ml of ketamine to keep them calm while inflicting wounds. To all the anaesthesized animals, the middle dorsal posterior area was properly shaven to get rid of the hairs and expose the skin of the animal and using a clean blade, a wound about 4 cm in length and 2 cm deep was created. After bleeding stopped, the drugs were administered to the animal groups. Group 1 was topically administered the extract alone, formulated as a sol-gel. Group 2 was topically administered the crude extract/sheabutter ointment. Group 3 served as the positive control group and was topically administered penicillin ointment. Group 4 received only 0.2 ml of Tween 80 orally and served as the negative control. Group 5 was administered 400 mg/kg orally and topical application of the crude extract gel. Group 6 served as the positive control and received 20 mg/kg ampiclox orally and penicillin ointment topically. Treatments and measurements were performed every alternate day for 14days.

Haematological Tests

The test sample was evaluated for its effect on haematological parameters (packed cell volume, Hb level, red blood cell count, total white blood cell count, and differential white blood cell count) in adult Wistar rats after 14 days of oral administration of 400 mg/kg to the animal group (n=4), using selected standard protocols. The control group received 1 mL of Tween-80. Packed cell volume (PCV) was determined using the microhematocrit method (Thrall and Weisere, 2002). The hemoglobin concentrations of the blood samples were determined using the cyanomethaemoglobin method (Higgins *et al*., 2008)).

The red blood cell counts and the total white blood cell count were determined using the Haemocytometer method (Thrall and Weisere, 2002), while the Leishman technique (Thrall and Weisere, 2002) was followed the determination of differential white blood cell count (differential leukocyte count)

Serum Biochemistry

The extract (400 mg/kg) was administered orally to the animal group (n=4) daily for 14 days. The control group received 1 mL of Tween-80. After 14 days, blood was collected from the tail vein of each animal and centrifuged to obtain serum for the assay.

Effect on the Liver Enzymes Activity

Determination of serum Aspartate Amino Transferase (AST) activity and serum Alanine Amino Transferase (ALT) Activity Level were by the Reitman-Frankel colorimetric method (Reitman and Frankel, 1957) using the Quimica Clinica Aplicada AST test kit (QCA, Spain)

The TECO ALP Test Kit (TECO Diagnostics, Anaheim, California, USA) was used for the determination of serum Alkaline Phosphatase (ALP) Activity following the Thymolpthalein monophosphate method (Colville, 2002; Roy, 1970)

Effect on Kidney Function

Serum protein levels were measured using Randox total protein and albumin test kits (Randox Laboratories Ltd., County Antrim, United Kingdom) following the Direct

Biuret method (Lubran, 1978). The Bromocresol Green Method (Johnson, 2008; Doumas and Peters, 1997) was used to assay serum albumin. Serum total bilirubin levels were determined using a Randox Bilirubin test kit (Randox Laboratories Ltd., Crumlin, County Antrim, UK) (Doumas et al., 1973).Serum creatinine levels were measured using the method described by Lamb and Price (2008) with a QuimicaClinicaAplicada (QCA) test kit (QCA, Spain).The modified Berthelot reaction method (Lamb and Price, 2008) was used to assay plasma/serum urea using a Dialab Urea test kit (Dialab, Neudorf, Austria).

Statistical Analysis

All data obtained were analyzed using one-way analysis of variance (ANOVA) followed by Student's t-test (GraphPad Prism version 7.0 (GraphPad software, San Diego, USA). Values were expressed as mean ± Standard deviation (SD), and were considered significant relative to the control at $P < 0.05$

Results

Phytochemical Constituents

Qualitative phytochemical analysis revealed relatively high quantities of saponins, alkaloids, flavonoids, terpenoids, and traces of tannins. Steroids were not detected in the extracts (Table 1).

Effect on wound healing

Topical application of a 50 % extract in shea butter significantly increased the contraction rate of the incision wound relative to the control (Table 2, Figure 2). After 14 days of assay, the best wound healing profile was exhibited by the crude extract delivered in shea butter and applied topically to the skin. The percentage of wound contraction as a function of wound healing activity was relatively high (Figure 3). The group that received the oral preparation of the extract recorded the least effective wound healing activity and showed signs of acute toxicity after the third day (Table 2).

Effect on the Biochemical Parameters (Liver and Kidney Functions)

The treatment group showed elevated levels of liver function markers (AST, ALT, and ALP), indicating hepatotoxicity.

Effect of Haematological Parameters

There was also a significant decrease ($P < 0.05$) in the PCV, hemoglobin concentration, and total RBC count in the treated group compared to the control group. The was also significant elevation ($P < 0.05$) in the total white blood cell count, neutrophil, lymphocyte count, monocyte and eosinophil level in the treated group compared to the control (Table 5)

Figure 1. *Chromolaena odorata* plant

Table 1: Phytochemical constituents

Phytochemicals	Relative abundance
Saponins	+++
Tannins	+
Flavonoids	++
Steroids	_
Terpenoids	++
Alkaloids	++

KEY: - (not detected); + Trace; ++ Moderately present; +++ Highly abundant

Table 2: Daily wound contraction length

Treatment	Mean length of contraction (cm)							
	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14
No treatment (negative control)	4.00 ± 0.00	5.10 ± 0.06	4.80 ± 0.10	4.40 ± 0.10	3.70 ± 0.00	3.20 ± 0.20	3.00 ± 0.20	2.90 ± 0.20
Pure extract (topical)	4.00 ± 0.00	4.30 ± 0.10***	4.13 ± 0.21***	4.00 ± 0.10***	3.70 ± 0.10	3.20 ± 0.10	3.00 ± 0.10	2.90 ± 0.10
Extract with shea butter (topical)	4.00 ± 0.00	4.10 ± 0.00***	4.00 ± 0.10***	3.80 ± 0.00***	3.30 ± 0.10***	2.97 ± 0.06	2.77 ± 0.06	2.47± 0.06**
Penicillin ointment (topical)	4.00 ± 0.00	4.37 ± 0.06***	4.00 ± 0.10***	3.77 ± 0.06***	3.23 ± 0.06***	2.97 0.06	2.90 ± 0.10	2.77 ± 0.06
Extract (oral 400 mg/kg and topical)	4.00 ± 0.00	4.40 ± 0.10***	4.20 ± 0.10**	4.20 ± 0.10	4.10 ± 0.00	3.70 ± 0.10	3.60 ± 0.10	3.40 ± 0.00
Oral amoxicillin (20 mg/kg) and penicillin ointment	4.00 ± 0.00	4.60 ± 0.10***	4.50 0.00	4.20 ± 0.00	3.70 ± 0.06	3.60 ± 0.10	3.00 ± 0.00	2.60 ± 0.10*

Values are presented as mean ± SD; n = 4; * P < 0.05. ** P < 0.01, *** P < 0.001 significant difference relative to negative control

Table 3: Percentage wound contraction after 14 days treatment

Animal treatment group	Percentage wound contraction (%)
No treatment (Control)	27.5
Extract with Shea butter (Topical)	37.5
Penicillin ointment (Topical)	30.0
Pure extract (oral 400mg/kg and topical)	15.0
Oral amoxicillin (20mg/kg) and topical penicillin ointment	35.0

Table 4: Effect of the extract on serum biochemical parameters

Parameters	Treated	Control
AST (IU/L)	84.96 ± 20.17	60.15± 9.36
ALT(IU/L)	24.10 ± 5.04	21.25 ± 1.46
ALP (IU/L	50.58 ± 4.55	35.28 ± 4.71
Total bilirubin (mg/dL)	0.36 ± 0.06	0.45 ± 0.06
Total protein (g/L)	66.26 ± 2.81	70.62 ±2.82
Albumin (g/L)	34.02 ± 0.99*	38.14 ± 0.85
Globulin (g/L)	28.24 ± 1.82	32.48 ± 3.67
Urea (mg/dL)	41.31 ± 1.67	37.58 ± 3.68
Creatinine (mg/dL)	0.53 ± 0.18	0.65 ± 0.35

Values are presented as mean ± SD; n = 4; *P < 0.05 significant difference relative to control using unpaired Student's t-test

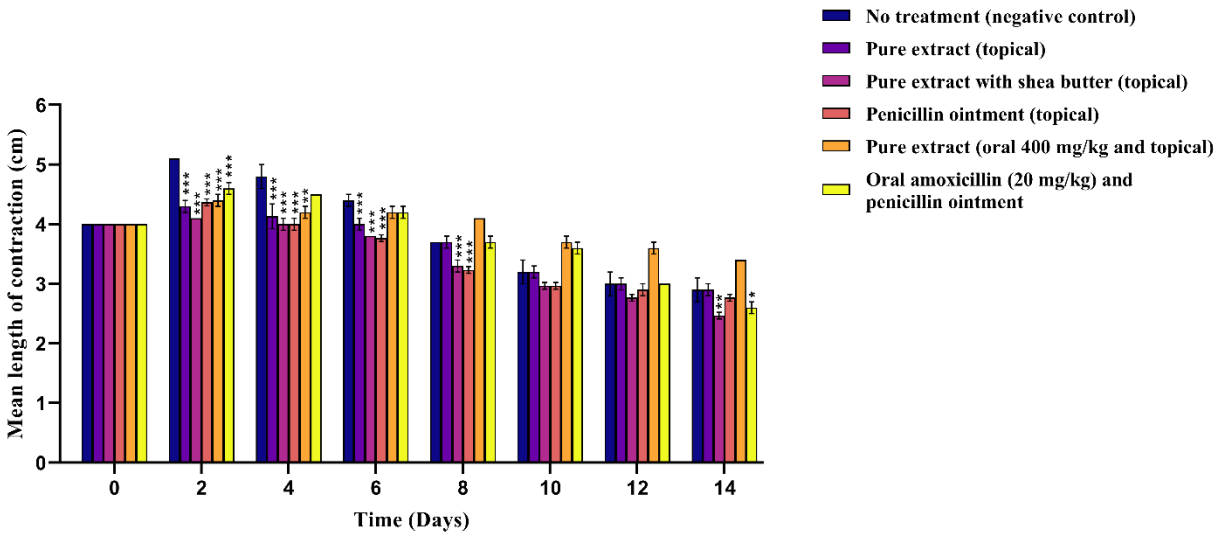

Figure 2: Effect of the extract on wound healing

Values are presented as mean ± SD; n = 3; * P < 0.05. ** P < 0.01, *** P < 0.001 significant difference relative to negative control

Table 5: Effect of the extract on haematological parameters

Parameters	Treated	Control
PCV (%)	38.25 ± 1.77*	43.25 ± 1.77
Haemoglobin conc. (g/dL)	14.68 ± 0.67*	10.88 ± 7.01
RBC Count (10^6/µL)	9.56 ± 1.80*	12.35 ± 0.35
Total WBC count (10^3/µL)	23.20 ± 6.51*	12.55 ± 0.64
Lymphocyte count (10^3/µL)	12.58 ± 2.27*	8.00 ± 1.01
Neutrophils count (10^3/µL)	9.19 ± 3.52*	3.40 ± 0.52
Eosinophils count (10^3/µL)	0.96 ± 0.57	0.58 ± 0.47
Monocytes count (10^3/µL)	0.47 ± 0.13	0.58 ± 0.65

Values are presented as mean ± SD; n = 4; * P > 0.05 significant difference relative to control using unpaired Student's t-test

Discussion

The results of the qualitative phytochemical analysis showed that saponins were abundant in *C. odorata leaves.* This may be responsible for the foaming properties of this plant as well as the wound healing effect. In ethnomedicine, the leaves of the plant are squeezed, and the foaming juice is applied to cuts or fresh wounds to stop bleeding (Sirinthipaporn and Jiraungkoorskul 2017). Saponins have been previously reported to have wound-healing effects attributed to their perceived antimicrobial and anti-inflammatory properties (Hassan *et al*., 2011). Similar plants, such as *Aspilia Africana*, known for their high saponin content, are used to treat wounds and stop bleeding. Some studies have reported a possible potentiation of the wound healing activity of this plant due to its inherent antimicrobial activity (Nwinuka *et al.,* 2009). *Chromolaena odorata* showed significant antibacterial effects on some microorganisms, such as *Bacillus subtilis*, *Klebsiella pneumoniae*, *and Staphylococcus aureus*, and *antifungal activities* against *Candida albicans*, *Aspergilus, Saccharomyces*, *cerevisiae*, and *Rhizopus spp.* (Nwinuka *et al.,* 2009). The long-term use *of Chromolaena odorata* can be harmful. It has been speculated that the presence of phytochemicals such as sesquiterpenes and pyrrolizidine alkaloids present in the plant is responsible for the acute toxicity of the plant (Paulose *et al.,* 2016). This suggests that oral administration is not advised, as this could evoke life-threatening effects, end-organ damage/failure, and eventually, death. This is further explained by the effect of the plant extract on the liver and kidneys, as shown in the biochemical assay results. The marked decrease in the PCV, hemoglobin concentration, and total RBC count by the extract suggests destruction of the erythrocytes and reduction in the oxygen-carrying capacity of the blood, while the elevation in the total white blood cell count, neutrophil, lymphocyte count, monocyte, and eosinophil levels signify a response to injury, stress, and inflammation. Local and systemic factors can affect wound healing, such as oxygen, infections, stress, diabetes, and age. Fasuyi *et al.* (2005) reported that *Chromolaena odorata* decreased the oxygen-carrying capacity of the blood. The serum biochemical results suggested impairment of hepatic and renal functions. Acute toxicity or injury of the hepatocytes can lead to an abnormal elevation of these enzymes, often in quantities proportional to the degree of injury (Ashan *et al*, 2009). The serum AST concentration was markedly increased in the treated animals compared to that in the control group, suggesting necrosis and trauma of the hepatocytes. The ALT and ALP levels also increased significantly, suggesting extreme stress in the hepatic. muscles due to infection or bruising (Ashan *et al*, 2009). In clinical settings, serum urea and creatinine levels are used as indicators to assess kidney function. Elevated levels of these substances indicate abnormal renal function, which could suggest acute renal injury.

Conclusion

In conclusion*, Chromolena odorata* demonstrated wound-healing properties when applied topically. The formulation of the extract with shea butter improved wound healing activity. Oral administration of the extract had no significant effect on wound healing but adversely affected liver function and hematological parameters. Therefore, prolonged oral use of *Chromolaena odorata* is not advised.

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Availability of data and materials

The data that support the results of this study are available from the Department of Pharmaceutical and Medicinal Chemistry, University of Nigeria, Nsukka. These data can be obtained from the authors upon request.

Conflict of Interest

The authors declare that there are no conflicts of interest associated with this work.

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