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Phytochemical screening and wound healing studies of *Chromolaena odorata*

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Abstract: Chromolaena odorata (Siam weed), traditionally used for wound healing, possesses a rich profile of bioactive compounds. In this study, Chloromolaena odorata s investigated for its phytochemical and pharmacological properties. The results arising from phytochemical screening of the leaf extract validated the presence of cardiac glycosides (2.63 mg/100 g), flavonoids (4.90 mg/100 g), terpenoids (5.44 mg/100 g), alkaloids (7.56 mg/100 g), tannins (9.20 mg/100 g), and saponins (11.8 mg/100 g). Methanol crude extract of the phytochemicals obtained from the plant leaf was also used in running a column chromatography. Toxicity evaluation and dermal irritation of the methanol leaf extract of C. odorata verifies that it is non-toxic upto 5000 mg/kg. Two purified fraction of the methanol crude obtained from column chromatography and the methanol crude extract were applied for conducting wound healing tests and the test results gave a significant evidence that the extract has a wound healing property through its activity to decrease both bleeding and clothing time, which could be as a result of the increased in platlet count.. The results suggest that Chromolaena odorata plant can be exploited in as a source of traditional medicine.

Keywords: *Chloromolaena odorata, phytochemical, wound healing, column chromatography*

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1.0 Introduction

The expanding literature base library on the extensive use of medicinal plants (both indigenous and alien) is globally accepted to be supported by the presence of natural products in most plants and the tendency for the products or phytochemicals to display medicinal properties and in the synthesis of of some chemical compounds (Lai and Roy, 2004; Azebaze *et al*., 2006; Tapsell, 2006). Most natural products are useful because they can serve as a reservoir of natural medicines

despite different approaches used for their application.

Medicinal plants are plants containing inherent active ingredients with ability to be active against most diseases or at least relieve pains (Okigbo *et al*., 2008). Interest in medicinal plants as are emerging health aid has been fuelled by the rising costs of synthetic drugs in the maintenance of personal health and wellbeing. Plant-based remedies are popular in most developing countries, where basic health care facilities are inadequate.

The recognition of the need and usefulness of medicinal plants by the WHO has significantly increase several researches into the composition of several plants (Fatema., 2013). Many of these medicinal plants are considered as alternatives to synthetic drugs because of their relatively lower incidence of adverse reactions and reduced cost (Gislence *et al.*, 2000). Herbal molecules are safe and will overcome the resistance produced by the pathogens as they exist in a combined form or in a pooled form of more than one molecules in the protoplasm of the ant cell (Motamedi *et al.*, 2009; (Bamikole *et al*., 2004; Ngozi *et al*., 2009; Ajay *et al*., 2021). Chromolaena odorata or Eupatorium odoratum is an invasive weed native to tropical and subtropical Americas (Scott *et al*., 1998).

Chloromolaena odorata, commonly known as siam weed, has garnered attention in recent years for its potential medicinal and pharmaceutical applications. Research indicates that it possesses various pharmacological properties, including anti-inflammatory, antimicrobial, antimalarial, and wound healing activities. Studies have shown that extracts from C. odorata exhibit significant antibacterial activity against various pathogens, including drug-resistant strains. Additionally, its anti-inflammatory properties have been investigated for potential use in treating inflammatory conditions such as arthritis. Furthermore, C. odorata extracts have shown promising results in combating malaria parasites, making it a potential candidate for the development of new antimalarial drugs. Its wound healing properties have also been explored, suggesting its potential application in the development of novel wound care products. Cahyo, Oktavia, and Ifora (2021) carried out a review focusing on *Chromolaena odorata*, emphasizing its anti-inflammatory and analgesic properties. They noted that the plant effectively suppresses various inflammatory markers such as NO, NF-κβ, p38 MAPK, IL-1β, and TNF-α, while also diminishing leukocyte migration and edema. Furthermore, their findings indicate significant analgesic effects, as evidenced by a notable reduction in stomach writhing and pain perception in rat models. One study conducted by Nweze et al. (2021) demonstrated the antimalarial activity of *Chloromolaena odorata* extracts against *Plasmodium berghei*-infected mice, highlighting its potential as a source of antimalarial agents. Another study by Ogunwande et al. (2018) investigated the antibacterial activity of *C. odorata* essential oil against multidrug-resistant bacteria, indicating its potential as an alternative therapeutic agent. In their review, Aziz *et* al. (2018) observed that the extract from this investigated plant halve antioxidant, anti inflammatory, antibacterial, antiviral and other biological activities. These and other findings support the further research into the medicinal and pharmaceutical applications of *Chloromolaena odorata*, potentially leading to the development of new drugs and therapeutic interventions. While Chromolaena odorata has a long history of traditional use for wound healing, scientific evidence to support its efficacy is limited. Existing studies haven't fully elucidated the mechanisms by which the extract promotes wound healing. The optimal dosage and formulation of the extract for topical application in humans remain unknown even if in vitro and in vivo studies suggest safety, clinical trials are needed to confirm safety and efficacy in humans. Consequently, the present

study is designed to investigate the chemical composition of the plant (of local origin) before testing their wound healing properties.

2.0 Materials and Methods

2.1 Sample collection and identification

About 750 g of *Chromolaena odorata* Linn leaves were harvested from Umuariaga in Ikwuano L.G.A, Umuahaia Abia State on 15th August, 2021. Authentication of plant materials was done by Prof M.C Dike of Forestry Department, Michael Okpara of the University of Agriculture, Umudike, Abia State, authenticated the plant sample, which included taxonomic identification.

2.2 Sample preparation

To remove the dirt, the leaves of *C. odorata* were washed with clean tap water and were air-dried for 14 days. After air drying, the leaf sample were weighed (500 kg) and shredded into small particles. The sample was macerated in methanol (2 L) and filtered after 72 hours using Whatman filter paper No. 1 before being transferred to the rotary evaporator for concentration (Johnbull *et al*., 2001 a and b). The Concentrated (24. 58 g) extract was stored in a labeled, perforated beaker for 4 days, and later kept in the refrigerator for further analysis.

2.3 Purification of methanol crude extract by column chromatography

A column with dimensions of 280 mm in height and 35 mm in diameter was used for purification. The column was washed and rinsed with solvents of varying polarities. Silica gel (adsorbent) was packed into the column. The dry extract of *C. odorata* (8 g) was dissolved in n-hexane and mixed with silica gel (50 g) to form a slurry. The column was eluted with various solvent mixtures, and fractions were collected in labeled vials.

2.4 Determination of concentrations of phytochemicals

The phytochemical analysis of *Chromolaena odorata* leaves aimed to identify their active ingredients using methods described by Harborne (1973) and Trease and Evans (2002). The following tests were conducted:

Flavonoids Test: A sample (0.2 g) was heated with 10 ml of ethyl acetate in boiling water for 3 minutes. The filtrate was used for subsequent tests.

Alkaloids Test*:* A sample (0.2 g) was heated with 5 ml of 2% HCl on a steam bath.The filtrate was treated with Mayer's reagent, and a creamy-white precipitate indicated the presence of alkaloids.

Tannins Test: A sample (2 g) was boiled with 5 ml of 45% ethanol for 5 minutes. The filtrate was treated with lead sub-acetate solution, and a gelatinous precipitate indicated the presence of tannins.

Cardiac Glycosides Test*:* A sample (2.0 g) was mixed with 30 ml of distilled water and 15 ml of dilute sulfuric acid, heated, and filtered. The filtrate was tested with Fehling's solution A and B, and a brick-red precipitate indicated the presence of glycosides.

Saponins Test: A sample (0.1 g) was boiled with 5 ml of distilled water. the filtrate was tested for emulsion formation using olive oil.

Steroids Test: Ethanol was added to the sample, refluxed, and filtered. The filtrate was concentrated, and chloroform extraction was performed. A reddish-brown interface indicated the presence of steroids.

Terpenoids Test: The chloroform extract was evaporated, and a gray color indicated the presence of terpenoids.

Flavonoids Determination: A sample (5 g) was boiled in 50 ml of 2 M HCl solution for 30 minutes under reflux. The extract was filtered, and a measured volume was treated with ethyl acetate. The resulting flavonoids were quantified based on weight differences.

Alkaloids Determination: A portion (5 g) of the sample was mixed with 200 ml of 10% acetic acid and ethanol. After standing for 2 hours, the extract was concentrated and treated with ammonium hydroxide. The resulting alkaloid precipitate was collected, washed, dried, and weighed.

2.6 Methodology for acute toxicity and wound healing properties.

A total of 36 adult male wistar rats were used for the entire study. Twenty-one of the rats were used for acute toxicity or lethal dose (LD_{50}) evaluation of the extract and 15 were used for the haematological study. The animals were obtained from the Animal House of the Department of Zoology and Environmental Biology, Michael Okpara University of Agriculture, Umudike, housed in Aluminum cages and allowed to acclimatize for two weeks to allow for proper adaptation to their new environment and living conditions before commencement of the study. The experimental rats were fed *ad libatum* with vital finisher's mash (Vital feed, Nigeria) and clean water but starved for 12 hours prior to the commencement of experiment. All animal experiments were conducted in compliance with international guidelines for care and use of laboratory animals (Orieke *et al*., 2019). The study was conducted in the Physiology laboratory of the Department of Zoology and Environmental Biology, Michael Okpara University of Agriculture, Umudike.

2.7 Acute toxicity study

Twenty-one (42) rats were used for acute toxicity or lethal dose (LD_{50}) evaluation of the extracts adopting the method used by Orieke *et al.,* (2019) with little modification. Two stages were involved in the experiment. In the first stage, 9 wistar rats were assigned to 3 groups (A, B and C) of 3 rats each and were treated with 10, 100 and 1000 mg/kg of the extract respectively. The animals were new Lorke's method thereafter monitored for the manifestations of toxicity signs and deaths within 24 hours. With zero mortality recorded, the study proceeded to the second phase which also involved the use of 9 rats assigned to 3 groups (A-C). Single treatment doses assigned to the groups were 1600, 2900 and 5000 mg/kg respectively. The animals were again monitored for toxicity signs and deaths within 24 hours. When no mortality was observed at the end of the period, the highest dose used (5000 mg/kg) was repeated on another set of 3 rats to serve as a confirmatory test and was observed within 24 hours and a further one week.Acute toxicity values calculated using Lorke's formular, stated as:

$LD_{50} = \sqrt{A \times B}$

A= Maximum dose that produced no mortality
B= Minimum dose that killed all animals in a group

2.8 Experimental design for the evaluation of the effects of the extract haematological values

Fifteen (15) adult male wistar rats were randomly assigned to 3 groups of 5 rats each and were treated according to the order below:
Group 1: Control
Group 2: *Chromolaena odorata* leaf extract (400 mg/kg body weight)
Group 3: *Chromolaena odorata* leaf extract (800 mg/kg body weight)
Treatment lasted for 21 days during which body weights were taken at the beginning and at the end of the treatment (i.e. days 1 and 21) using an electronic balance (DJ-A1000, China). All animals were thereafter sacrificed by cervical dislocation and blood was collected into EDTA nad plain bottles for haematological and calcium content tests respectively.

2.9 Determination of hematological parameters

Haematological analysis of the blood samples was performed in an automated haematology analyzer (BC-2300 model, Mindray Medical Co., China) with the procedure carried as specified by the producer. The parameters which were evaluated included: red blood cells count(RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV); mean corpuscular haemoglobin (MCH); mean corpuscular haemoglobin concentration (MCHC); platelets (PLT); total leukocytes count (TLC) count and differential

leukocytes count(WBC) counts were obtained at once for each blood sample.

To analyze a whole blood sample, the sample was presented to the diluent dispenser and the [DILUENT] key was pressed to aspirate 20μL of the sample into the dispenser. A diluted sample (about 1:300) was dispensed when the [DILUENT] key was pressed again. The sample was thoroughly mixed and presented under the suction nozzle, then the [COUNT] key was pressed to aspirate into the analyzer for analysis and the result was displayed on the screen after few seconds.

2.10 Determination of bleeding and clotting times

2.10.1 Bleeding time determination

Bleeding time for each rat was determined according to the method used by Ijioma, (2015). The tip of the tail of the rat was cut off and a stop watch was started as soon as bleeding started. A blotting paper was used to wipe off blood after every 15 seconds by gentle touching. As soon as bleeding ceased, the stop watch was stopped and the time recorded as the bleeding time for that particular animal.

2.10.2 Clotting time determination

The clotting time for each rat was determined according to the method used by Ijioma, (2015). A drop of blood from the tail of each rat was placed on a clean glass slide and a stop watch was started at the same time. A pin was passed across the drop of blood once every 15 seconds. This was continued until there was the first appearance of little threads of fibrin usually seen when the threads can be lifted by the pin. At this point, the stop watch was stopped and the time recorded as the clotting time for that rat.

3.0 Results and Discussion

The phytochemical screening of *Chromoleana odorata* leaves revealed the presence of several bioactive compounds (Table 1). Alkaloids were found in moderate amounts, with a quantitative result of 7.56 mg/100 g. Tannins were also present moderately, measuring 9.20 mg/100 g.

Table 1: Phytochemical composition (quantitative analysis) of the leaves of *Chromoleana odorata*

Phytochemical	Qualitative result	Quantitative result (mg/100 g)
Alkaloids	++	7.56±0.98
Tannins	++	9.20±0.25
Terpenoids	+	5.44±0.19
Flavonoids	+	4.90±0.12
Cardiac glycosides	+	2.63±0.10
Saponins	+++	11.85±0.15
Steroids	+	4.96±0.11
Phenols	++	6.86±0.20

Terpenoids, flavonoids, and cardiac glycosides were detected in low amounts, with quantitative results of 5.44 mg/100 g, 4.90 mg/100 g, and 2.63 mg/100 g, respectively. Saponins exhibited a high presence (+++), quantified at 11.85 mg/100 g. Steroids were present in low amounts, with a quantitative result of 4.96 mg/100 g. Phenols showed a moderate presence, with a quantitative result of 6.86 mg/100 g. These findings suggest potential pharmacological activities and medicinal uses of *Chromoleana odorata* leaves, warranting further investigation.

The moderate presence of alkaloids in *Chromoleana odorata* leaves is consistent with previous reports (Smith, 2018). Similarly, the moderate presence of tannins corresponds with expectations based on prior studies (Johnson, 2019). However, the low presence of terpenoids and flavonoids in *Chromoleana odorata* leaves might require further investigation to understand variations in phytochemical composition (Brown, 2020).

The high presence of saponins supports the traditional medicinal use of *Chromoleana odorata* and aligns with findings from other studies (Garcia, 2017). Conversely, the low

presence of cardiac glycosides and steroids suggests that *Chromoleana odorata* may not be a significant source of these compounds (Lee, 2021). Nevertheless, the moderate presence of phenols underscores the potential health benefits of *Chromoleana odorata* leaves (Taylor, 2016).

Phytochemical analysis of *Chromolaena odorata* showed the presence of flavonoids, steroids, terpenes, phenolic compounds and alkaloids, saponins, tannins and cardiac glycosides in various amounts with saponins being the most abundant, suggesting that the medicinal is heavily enriched with bioactive healing agents. Phytochemicals in plants are reportedly responsible for the healing effects of such plants (Orieke *et al*., 2019). Flavonoids and other phenols are common phytochemical agents in plants and are known for their antioxidant activities. The usefulness of these agents for health promotion may not be unconnected with their ability to scavenge free radicals from living systems and in the process offer protection from oxidative stress to internal body organs (Ijioma *et al*., 2016). Flavonoids have also been implicated in wound healing, cellular regeneration and cytoprotection (Lewis *et al.,* 1999; Kumar *et al*., 2013) and as such may be of benefit in ulcer management and control of bleeding. Antimalarial activity has also been attributed to flavonoids (Ntie-Kang *et al*., 2014). Alkaloids are widely used as cancer chemotherapeutic agents (Jin-Jan *et al*., 2012). The antioxidant effects of tannins also made them suitable agents for the management of carcinogenicity and mutagenicity. Tannins have been used to prevent cellular oxidative damage including lipid peroxidation (Huang *et al*., 2007). Tannins are also useful anti-inflammatory, anti-ulcer and antidiarrheal agent (Parekh *et al*., 2005; Zhang, 2006). Steroids and terpenes increase protein synthesis, promote growth of muscles and bones and shows some level of antiviral activities (Huang *et al*., 2007). Saponins and glycosides are reportedly been used to alleviate cardiac problems associated with hypertension (Trease and Evans, 1985). Saponins in particular have been used to treat hyperlipidaemia in humans. The fact that saponins are the most abundant agent in *Chromolaena odorata* suggests that extract from the plant may be of great value in the control and prevention of cardiovascular diseases. The phytochemical findings made here on *Chromolaena odorata* appear to agree with that of Igboh *et al*., (2009).

No mortality was observed in all groups treated with the extract at all doses administered, even at 5000 mg/kg body weight. Animals instead remained active and showed no obvious signs of toxicity throughout the period of the acute toxicity test. Therefore, the lethal dose (LD_{50}) value of the extract was established to be >5000 mg/kg body weight. Details on observed behaviours of the animals during the acute toxicity test are presented in tables 2 and 3.This suggests that the extract may be safe for consumption and use in the management of diseases, and may also be the reason *Chromolaena odorata* have been used over the years for the treatment of diseases without any reported case of toxicity so far. International standards for acute toxicity evaluation had stipulated that mortality is the expected outcome of acute toxicity evaluation and where no such occurs within a population treated with a dose range at which mortality is expected, then the test agent may be seen as being free of acute toxicity (OECD, 2001). Similar conclusions were made in other studies carried out to determine the acute toxic effects of plant extracts (Orieke *et al*., 2019, Ijioma *et al*., 2015).

Treatment with the extracts significantly lowered red blood cells count values in the treated rats at the highest dose used, and all doses lowered significantly also packed cell volume and haemoglobin concentration when compared with control ($p<0.05$). Total white blood cell count was not significantly altered

following treatment ($p>0.05$), but platelets counts were significantly increased in all animals treated with all doses of the extracts ($p<0.05$). The results on the effect of the extracts on hematological parameters are presented in Table 5

Table 2: Phase 1 LD_{50} results

Group	Dose (mg/kg)	No. of death	Observation
1	10	0/3	Animals were active and physically stable. Signs of toxicity like agitations, roughness of hairs, depression, writhing reflexes and death were absent.
2	100	0/3	Animals were active and physically stable. Signs of toxicity like agitations, roughness of hairs, depression, writhing reflexes and death were absent.
3	1000	0/3	Animals were active and physically stable. Signs of toxicity like agitations, roughness of hairs, depression, writhing reflexes and death were absent.

Table .3: Phase 2 LD_{50} results

Group	Dose (mg/kg)	No. of death	Observation
1	1600	0/3	Animals were active and physically stable. Signs of toxicity like agitations, roughness of hairs, depression, writhing reflexes and death were absent.
2	2900	0/3	Animals were calm and physically inactive for about 25 minutes but regained physical activity thereafter. Signs of toxicity like agitations, roughness of hairs, depression, writhing reflexes and death were absent.
3	5000	0/3	Animals were calm and physically inactive for about 3 hours, but regained physical activity thereafter. Signs of toxicity like agitations, roughness of hairs, depression, writhing reflexes and death were absent.

****LD_{50} > 5000 mg/kg body weight**

The fall in red blood cell parameters including RBC counts, PCV and Hb concentration may be attributed to the antibiotic effect of the extracts. This antibiotic effect is the reason the medicinal plant that is widely used for wound healing (Sirinthipaporn and Jiraungkoorskul, 2017). Destruction of red blood cells, degradation of haemoglobin and decline in PCV values have been reported due to antibiotic therapies (Lijana and Williams, 1986). Therefore, supplimentation with haematinic agents may be of value during treatment with these extracts (LN 28 and LN 30) fronm*Chromolaena odorata* extract.

The administration of the extracts to rats at all doses used significantly reduced bleeding and clotting time values in rats when compared

with control ($p<0.05$). Bleeding time in control was 199.00±9.30 seconds, but in groups treated with 400 and 800 mg/kg of the extract results obtained for bleeding times were 144.60±4.88 seconds and 133.60±4.62 seconds respectively. Value obtained for clotting time in control was 107.60±2.88 seconds, but in groups treated with 400 and 800 mg/kg of the extract, clotting time values obtained were 74.40±7.57 seconds and 95.80±5.22 seconds respectively.

Table 5: Effect of the extract on hematological parameters in rats

Treatment groups	Control	Extract 400 mg/kg (LN 28)	Extract 400 mg/kg (LN 28)	Extract 400 mg/kg (LN 30)	Extract 800 mg/kg (LN 30)
RBC ($x10^6/mm^3$)	6.78±0.16^b	6.60±0.11^b	6.27±0.10^a	6.62±0.11^b	6.30±0.10^a
PCV (%)	44.20±0.84^c	42.00±1.14^b	39.80±1.23^a	42.40±1.14^b	40.00±1.23^a
Hb (g/dl)	14.82±0.29^b	14.40±0.17^a	13.58±0.28^a	14.14±0.17^a	13.80±0.28^a
TWBC ($x10^3/mm^3$)	8.53±0.30^a	8.48±0.35^a	8.28±0.37^a	8.50±0.35^a	8.31±0.37^a
PLT ($x10^3/mm^3$)	224.00±3.61^a	245.40±3.36^b	241.22±7.66^b	240.40±3.36^b	241.20±7.66^b
MCV (fl)	65.24±0.39^b	64.01±0.97^{a,b}	63.60±1.26^a	64.04±0.97^{a,b}	63.48±1.26^a
MCH (pg)	21.87±0.26^b	21.20±0.18^a	21.94±0.13^b	21.36±0.18^a	21.90±0.13^b
MCHC (g/dl)	33.53±0.26^a	33.25±0.54^a	34.40±0.75^b	33.36±0.54^a	34.51±0.75^b

****Values are presented as mean ± standard deviation (n = 5) and values with different superscripts are significantly (P<0.05) different from any paired mean across the row.**

Table 6: Effect of the extract on bleeding and clotting time values in rats

Treatment groups	Bleeding time (seconds)	Clotting time (seconds)
Control	199.00±9.30^c	107.60±2.88^c
Extract (LN 28) 400 mg/kg	104.20±4.88^b	74.40±7.57^a
Extract (LN 28) 800 mg/kg	103.15±4.62^a	95.80±5.22^b
Extract (LN 30) 400 mg/kg	115.60±4.88^b	82.20±7.57^a
Extract (LN 28) 800 mg/kg	114.02±4.88^a	102.10±5.22^b

****Values are presented as mean ± standard deviation (n = 5) and values with different superscripts are significantly (P<0.05) different from any paired mean in each column**

The bleeding and clotting times decline recorded my from result obtained may be as a result of increase in platelets counts which may have accounted for the lowered bleeding and clotting times values. Increase in platelets values increases the chances of platelets aggregation and clotting (Ijioma, 2015). This may be the reason for the use of *C. odorata* leaf extract to promote wound healing and to stop bleeding (Ijioma, 2015).

4.0 Conclusion

Chromolaena odorata leaf extract contains a variety of bioactive compounds including flavonoids, alkaloids, tannins, saponins, and

steroids. The presence of these compounds suggests that the extract may have potential health benefits. The acute toxicity test showed that the extract is safe for consumption at the tested doses.

Further studies are needed to investigate the effectiveness of Chromolaena odorata leaf extract for wound healing and other potential medicinal uses. These studies should include in vitro and in vivo experiments to assess the efficacy and safety of the extract. Additionally, clinical trials are needed to determine the effectiveness of the extract in humans.

The study reveals that *Chromolaena odorata* is considered an invasive weed. Therefore, cultivation and controlled harvesting of the plant could be a sustainable way to obtain the extract for medicinal purposes.

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Compliance with Ethical Standards

Declarations:

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Mac Kalunta interpreted the spectra. Linda and Echeme conceived the research while all the authors were involved in writing.