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(RESEARCH ARTICLE)

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Formulation and evaluation of antimicrobial properties of ointment of *Chromolaena odorata* ethanolic extract

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Abstract

Herbal medicines are complex mixtures of organic chemicals that may come from any raw or processed part of a plant which are used to make about a quarter of the medications prescribed around the world today. *Chromolaena odorata L.* is an important perennial herb globally. Several parts of this plant are widely used to treat skin infections, burns and wound as well as possess anticancer, antidiabetic, anti-hepatotoxic, anti-inflammatory, antimicrobial and antioxidant properties. The medicinal values of *C. odorata L.* abides in their phytochemicals which produce defined physiological action in the human body. Extraction of the dried leave part of *C. odorata L.* was carried out using absolute ethanol. Simple ointment BP was formulated using different concentration of the following excipients (Hard paraffin, Soft paraffin, Wool fat and Cetostearyl alcohol). Organoleptic evaluation of both Simple ointment BP and *C. odorata* ointment were conducted. Antimicrobial evaluation of the samples was conducted on *Candida albican, Staphylococcus aureus* and *Streptococcus pyogenes. C. odorata* ointment showed activity against *S. aureus* with inhibition diameter of 10 mm while zero activity was observed against *S. pyogenes,* and *C. albican*. *C. odorata* ethanolic extract incorporated in commercial product (Nixoderm®) showed activity against the three pathogens, *S. aureus, S. pyogenes,* and *C. albican* with inhibitory zone of 7 mm, 2 mm and 10 mm respectively. *S. aureus, S. pyogenes* and *C. albican* were significantly inhibited with inhibition zones of 20 mm, 10 mm and 15 mm respectively by the Commercial antimicrobial product (Nixoderm®) which served as the positive control. Formulation of *C. odorata* ethanolic extract as a pharmaceutical dosage form, ointment was a success which showed a significant activity against *S. aureus* used in the study.

Keywords: *Chromolaena odorata*; Ethanolic extract; Ointments; *Staphylococcus aureus*; *Streptococcus pyogenes*; *Candida albican*

1. Introduction

Herbal medicines are naturally occurring, plant-derived substances that are used to treat illnesses within local or regional healing practices [1]. These products are complex mixtures of organic chemicals that may come from any raw or processed part of a plant [1]. Traditional Chinese Medicine and Ayurvedic Medicine are two well-known herbal medicine systems that believe that the focus should be on health rather than disease. Plants are used to make about a quarter of the medications prescribed around the world [1]. A lot of researchers have explored the potentials of natural products for medicinal purposes [2-8].

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The benefits of using herbal extracts over isolated chemicals include the treatment of complicated pathophysiological disorders as Skin and Soft Tissue Infections (SSTIs). The same herbal remedy used to treat SSTIs contains components that have the ability to operate primarily as antimicrobials, antioxidants, anti-inflammatories, or even wound healers. Thus, by focusing on the crucial processes involved in the pathophysiology of various disorders, herbal medicines' multi-target activity can aid treatment of infections [9]. Synergism between herbal medicine's constituents can enhance the potency of its pharmacological effects, particularly in the treatment of SSTIs. As an illustration, it has been noted that combinations of natural chemicals exhibit higher antibacterial action than isolated substances [9].

About 28 herbal species from various genera have been documented in studies published in the last ten years to simultaneously have the four biological activities crucial to the therapy of SSTIs (antimicrobial, antioxidant, anti-inflammatory, and healing properties). The families Asteraceae, Apocynaceae, and Bignoniaceae can be highlighted among these species because they account for around 21 % of the reported species collectively [9]. The Asteraceae family, which has about 23000 species and 1600 genera worldwide and contains significant phytochemicals such polyphenols, flavonoids, and diterpenoids, is one of the largest groups of flowering plants [10].

Chromolaena odorata (Siam weed) a specie of Chromolaena (L.) from the Family, Asteraceae is a perennial scandent or semi-woody shrub [11]. It is a widespread weed that grows in a wide range of soil types, abundant in open wastelands and along roadside edges, and prevents the growth of other vegetation [12].

Chromolaena odorata L., has been reported to be used frequently to heal wounds, burns and certain skin infections in addition to having anticancer, anti-diabetic, anti-hepatotoxic, anti-inflammatory, antimicrobial, and antioxidant qualities [13].

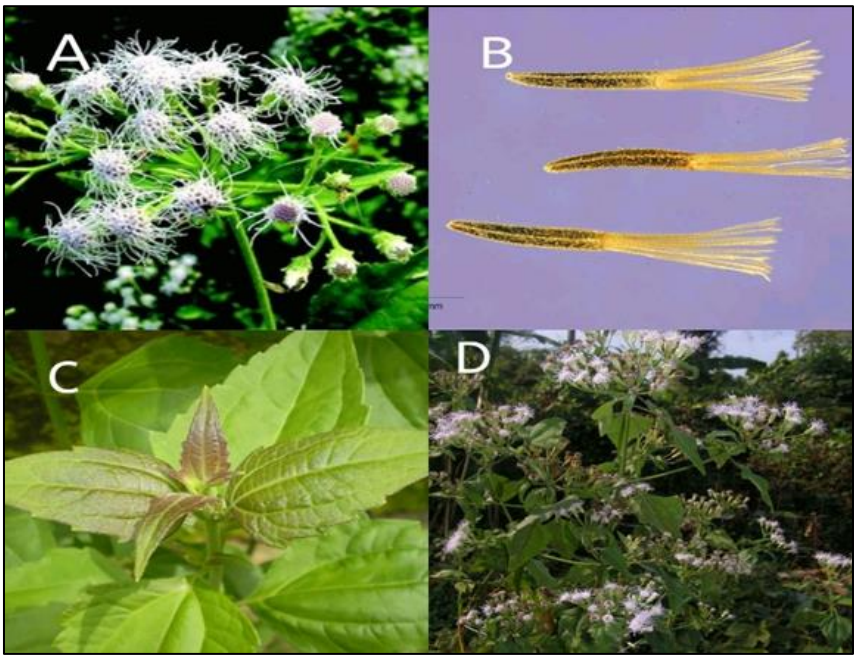

Figure 1 (a) flowers (b) seeds (c) leaves (d) whole plant of *Chromolaena odorata* L. [14]

1.1. Pharmacological uses *Chromolaena odorata*:

Due to its widespread ethno-medical use, particularly in Africa and Asia, the plant has garnered more attention during the past 60 years. Additionally, there is growing biological and pharmaceutical support for some of the purported ethno medicinal advantages of *C. odorata*, particularly in relation to oxidative damage and wound healing [15]. The phenolic is the main group reported in the species with biological activities important to the SSTIs treatment published in 2009–2019 [9]. Phenolic compounds are multi-target bio-actives because they have a wide range of structural variety and can specifically alter the activity of proteins, nucleic acids, and bio-membrane [16]. Table 1 below show some of the reported medicinal properties of the plant *C. odorata*.

Table 1 Some of the Medicinal Properties of *C. odorata*

S/N	Medicinal Property	References
1	Anticancer activities	[12, 17-20]
2	Anti-inflammatory activities	[21-23]
3	Antidiabetic properties	[24]
4	Wound healing properties	[25]
5	Antibacterial properties	[26]
6	Antifungal properties	[27]

2. Materials and methods

2.1. Materials

C. odorata (L), Pure cultures of pathogenic *Staphylococcus aureus, Streptococcus pyogenes* and *Candida albican*, Wool fat, White Soft paraffin, Hard paraffin, Cetostearyl alcohol, Absolute ethanol, Salbrose dextrose agar, Mueller hinton agar, Nutrient Broth, Macconkey agar, Blood agar sourced from Pharmaceutical Microbiology and Biotechnology Laboratory, Faculty of Pharmaceutical Sciences COOU, Igbariam Campus, Anambra State, Nigeria.

2.2. Methods

2.2.1. Collection of pyogenic pathogens

The pyogenic microorganisms; *Staphylococcus aureus, Streptococcus pyogenes* and *Candida albican* were procured from Microbial Type Culture Center in the Pharmaceutical Microbiology and Biotechnology COOU Laboratory. These organisms where sub-cultured on their selective media.

2.2.2. Collection and Storage of plant materials

The leaves of *C. odorata* were harvested during the early hours of the morning (around 6AM WAT) within the premises of Entrepreneur Department COOU and identified in the Department of Pharmacognosy and Traditional Medicine, COOU. The leaves were washed in running tap water to remove dust and sand particles and were airdried at room temperature (28^{o}C) for about 14 days. Dried leaves were pulverized with an electric blender and were stored in an airtight container [28].

2.2.3. Extraction

About 30 g quantity of *C. odorata* powdered leaves were emptied into 150 ml volume of absolute ethanol and shaken occasionally for 24 hours. The supernatant was filtered using Whatman's No 1 filter paper. The residue was added to another 150ml of volume of absolute ethanol and was shaken occasionally for another 24 hours, then filtered. The procedure was repeated again for another 24 hours. The filtrate was concentrated by evaporation using a water bath at 40^{o} C for 4 days [28]. The concentrated filtrate was stored in a wide mouthed and tightly closed bottle at 4^{o} C until used.

2.2.4. Percentage yield of extract

Percentage yield was calculated as a function of total weight of the extract divided by the weight of the dried leaves used for the study, all multiplied by 100.

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Total weight of dried pulverized leaves}} \text{ X } 100. \text{ ---- } \textit{Eqn. 1}$$

2.2.5. Preparation of ointment

Simple ointment BP was formulated using formula as shown in Table 2. below**.** Using fusion method, hard paraffin, soft paraffin, cetostearyl alcohol and wool fat were melted successively in decreasing order of melting point and the fluid mixture stirred continuously until cooled, avoiding aeration [29].

Ointment of *C. odorata* ethanolic extract was prepared by melting 100 mg of simple ointment BP in a beaker over a hot water bath maintained at 80 ^{o}C, then 40 ml of molten ointment was taken and emptied into a beaker and 80 mg of *C. odorata* ethanolic extract was added and mixed vigorously. The mixture was transferred into an ointment jar and stored.

Table 2 Formula for the preparation of Simple ointment BP

Ingredients	BP Formulae (g)	Amount used (g)	Melting point (ºC)
Wool fat	50.0	12.5	36-44
Hard paraffin	50.0	12.5	48-66
Cetostearyl alcohol	50.0	12.5	50
White soft paraffin	850.0	212.5	38-56
Total	1000.0	250.0	

2.2.6. Antimicrobial study

To carry out the antimicrobial (antibacterial and anti-fungal) susceptibility test, Agar well diffusion method was used. Mueller Hinton agar, Blood agar and Salbrose dextrose agar were freshly prepared and poured in different sterile plates which stood for 1 hour to solidify. A loopful of each test isolate was prepared to McFarland standard. Using sterile swab sticks each test isolates in suspension of nutrient broth were taken. *S. pyogenes, S. aureus and C. albican* respectively were aseptically swabbed on the surface of already cooled agar. Four wells were bored into the various agars using a cork borer and 1ml of different test samples were placed into the wells and the plates were incubated at 37^{o}C for 24hours. The antimicrobial activities of the test samples were evaluated by measuring the diameter of circular inhibition zones around the well [30].

3. Results

3.1. Percentage Yield

The total weight of the concentrated extract was 3.64g while the total weight of the pulverized leaves was 30g. Putting the values in Eqn. 1 above gave the percentage yield of 12.13%.

3.2. Organoleptic and physical properties

The organoleptic and physical properties such as color, texture, odor, phase separation, appearance, homogeneity and skin feel of the ointment formulations, are shown in Table 3. Both ointments (Simple ointment BP and *C. odorata* ointment) had no phase separation, were both homogenous, and had smooth texture and good aesthetic appeal. *C. odorata* ointment was emerald green in color with aromatic odor while Simple ointment BP was cream in color and odorless.

Table 3 Result of organoleptic evaluation

Organoleptic features	Simple ointment BP	*C. odorata* ointment
Color	Cream	Emerald Green
Odor	Nil	Aromatic
Texture	Smooth	Smooth
Physical appearance	Opaque	Opaque
Skin feels	No grittiness	No grittiness
Phase separation	Nil	Nil
Homogeneity	Homogenous	Homogenous

Figure 2A *C. odorata* ointment

Figure 2B Simple ointment BP

3.3. Antimicrobial study

The results showed that the Simple ointment, *C. odorata* ointment, Commercial antimicrobial product (Nixoderm®) and *C. odorata* extract incorporated in commercial product showed different degrees of antimicrobial activities against *C. albican*, *S. pyogenes* and *S. aureus.*

The Commercial antimicrobial product (Nixoderm®) showed higher activity against the test organisms while the simple ointment showed no activity against the test organisms. *C. odorata* extract incorporated in commercial product showed a reduced activity to the test organisms compared to the activity of the commercial product only. However, *C. odorata* ointment had an activity against *S. aureus* and no effect on *C. albican* and *S. pyogenes.*

Figure 3 Antifungal activity of Commercial product (Nixoderm®) (positive control C1), Simple ointment BP (Negative control C2), Extract of *Chromolaena odorata* incorporated in commercial product (C3), Ointment of *Chromolaena odorata* (C4)) against pathogenic *C. albican*

Figure 4 Antibacterial activity of commercial product (Nixoderm®) (positive control S1), Simple ointment BP (Negative control S2), Ointment of *Chromolaena odorata* (S3), Extract of *Chromolaena odorata* incorporated in commercial product (S4) against pathogenic *Staphylococcus aureus*

Figure 5 Antibacterial activity of commercial product (Nixoderm®) (positive control S1), Simple ointment BP (negative control S2), Extract of C. odorata incorporated in commercial product (S3), Ointment of *Chromolaena odorata* (S4) against pathogenic *Streptococcus pyogenes*

Table 4 Zones of inhibition (mm) of the different samples

Microorganisms	Simple ointment (mm)	*Chromolaena odorata* ointment (mm)	Extract in commercial product (mm)	Commercial product (mm)
Staphylococcus aureus	0	10	7	20
Streptococcus pyogenes	0	0	2	10
Candida albican	0	0	10	15

4. Discussion

Staphylococcus aureus, Streptococcus pyogenes and *Candida albican* were not susceptible to Simple ointment BP which served as a negative control. This was expected as there are no active pharmaceutical ingredients present in the formulation. *Chromolaena odorata* ointment showed activity against *Staphylococcus aureus* with inhibition diameter of 10 mm while zero activity was observed against *Streptococcus pyogenes,* and *Candida albican*. *C. odorata* ethanolic extract incorporated in commercial product (Nixoderm®) showed activity against the three pathogens, *Staphylococcus aureus, Streptococcus pyogenes,* and *Candida albican* with inhibitory zone of 7 mm, 2 mm and 10 mm respectively. *S. aureus, S. pyogenes* and *C. albican* were significantly inhibited with inhibition zones of 20 mm, 10 mm and 15 mm respectively by the Commercial antimicrobial product (Nixoderm®) which served as the positive control. *C. odorata* ointment at the 0.8mg/ml concentration showed significant activity against *S. aureus* as much as half of the activity shown by the commercial product. This implies that if the concentration of the extract is increased in the formulation, greater activity may have resulted. Also, it is important to note the reduction in activity when the *C. odorata* ethanolic extract was incorporated into the commercial product which contained chemically synthesized antimicrobial agent, this shows an antagonistic herbal-drug interaction. Further studies to evaluate the dose-dependent antimicrobial activity of the *C. odorata* ointment is recommended from this study.

5. Statistical analysis

The values of the zones of inhibition of the different samples were obtained in triplicates and the mean determined.

6. Conclusion

Formulation of *C. odorata* ethanolic extract as an ointment dosage form was a success. The *C. odorata* extract showed a significant activity against *S. aureus* used in the study but no activity was observed against the fungus *C. albican.* Further studies may be designed to validate the findings from this current study.

Compliance with ethical standards

Acknowledgement

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Disclosure of conflict of interest

The authors declare the complete absence of any conflict of interests while carrying out this research work.

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