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Formulation and evaluation of ointments containing methanolic extracts of *Chromolaena odorata* Linn (Asteraceae) and *Borreria verticillata* Linn (Rubiaceae) leaves for antimicrobial activity

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Abstract

Medicated ointments are used topically for the treatment of skin infections such as viral, bacterial, and fungal infections using synthetic or natural compounds. The aim of this study was to formulate the methanolic leaf extract of *Borreria verticillata* and *Chromolaena odorata* as herbal ointments for antimicrobial activity, and to evaluate the product. The leaves were extracted using methanol, and extracts were screened for their antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Aspergillus niger*. The extracts were formulated into ointments with Emulsifying and Simple ointments BP, and evaluated for their antimicrobial activity and physicochemical properties. The results showed that the extraction yield was 14.75 and 26.00% for *C. odorata* and *B. verticillata* respectively. All the test organisms were sensitive to both formulated herbal ointments but ointment prepared with Emulsifying Ointment BP gave higher inhibition zone diameter than that formulated with Simple Ointment BP. On the basis of the results, the extracts could be formulated in both ointments for antimicrobial activity.

Keywords: Antimicrobial; Herbal Ointments; Formulation; Evaluation

1. Introduction

Microbial skin infections are very rife globally especially among resource limited nations of the world including Africa. These infections present as viral, bacterial, and fungal skin infections. Lately, these microbes have started developing resistance to the existing antimicrobials especially synthetic ones [1, 2]. Aside development of resistance, the accessibility and affordability of this antimicrobial in low-income settings of most African countries pose a serious challenge. Also, some of these antimicrobials are formulated as single class treatment, e.g., antibacterial, anti-fungal, anti-viral, and when treating fungal and bacterial infections, the formulation would contain antifungal, antibacterial, and anti-inflammatory agents. The quality, affordability and side effects of these combined formulations especially those with steroids may not be as it supposed to be. Consequently, medicinal plants with antimicrobial and anti-inflammatory properties without the side effects of steroids are the best alternative. Some medicinal plants such as *Ocimum gratissimum*, *Momordica balsamina*, *Chromolaena odorata*, and *Borreria verticillata* have demonstrated antimicrobial and anti-inflammatory properties [3,4,5]. The leaves, stems, roots and flowers of *Chromolaena odorata* possess various constituents of medicinal importance. These compounds give the plant strong antibacterial, antifungal, anti-inflammatory, and antioxidant properties making it effective in treating wounds, infections, and skin diseases [6]. It has also been shown to accelerate wound healing by reducing inflammation and promoting tissue regeneration [7]. It has been reported that extracts of the plant possess anticancer, antidiabetic, antipyretic and antimicrobial properties [5].

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In the same vein, *Borreria verticillata* leaves, fruits and stems possess various bioactive constituents of medicinal importance. The leaf and stem extracts possess anti-inflammatory and analgesic effects which support its use in pain management [8]. It is reported to possess hepatoprotective properties, indicating potential benefits for liver health. It has been reported that extracts of this plant possess hypoglycemic, anti-larvicidal, anti-nociceptive and antimicrobial properties [4].

The aim of this work was to formulate *Chromolaena odorata* and *Borreria verticillata* ointments for their antimicrobial activity.

2. Materials and Methods

Materials used in the work include methanol, Liquid Paraffin, Hard Paraffin, Yellow Soft Paraffin, Cetostearyl Alcohol, Wool fat, Nutrient agar, Muller Hilton Agar, Muller Hilton broth, Sterile water, *Chromolaena odorata* and Borreria verticilata plants, Test Organisms (*Staphylococcus aureus, Bacillus Subtilis, Pseudomonas aeruginosa, Escherichia coli, and Aspergillus niger*).

2.1. Collection and identification

The samples used consist of fresh leaves of *Chromolaena odorata* and Borreria verticilata. The plants were collected in March 2025 in Jos North Area of Plateau State. The leaves were authenticated in the Pharmacognosy laboratory, Faculty of Pharmaceutical Sciences, University of Jos, by Mr. Thomas, and voucher specimens were deposited with the following voucher numbers: UJ/PCG/HSP/24A01 and UJ/PCG/HSP/25B04 for *Chromolaena odorata* and *Borreria verticilata* respectively. The test organisms were obtained from the Pharmaceutical Microbiology Laboratory, University of Jos, Plateau State.

2.2. Preparation of *Chromolaena odorata* extract

The fresh plant leaves *Chromolaena odorata* and Borreria verticilata were washed, shade dried for seven days, ground into powder using a pestle and mortar. A 650 g of *Chromolaena odorata* powder was weighed using the digital weighing balance (Mettler P165, Switzerland) and was macerated in 5 L of methanol for seven days with frequent shaking. It was then filtered through muslin cloth and Whatman No. 3 filter paper, and the filtrate was concentrated to dryness using rotary evaporator (Model RE 100, England). The same procedure was carried out on 730 g of *Borreria verticilata*. The extracts were stored in airtight containers until required for use.

2.3. Preparation of extracts stock

A 2 g of each of the extract was dissolve in 10 ml of Dimethyl sulphoxide (DMSO) to give a concentration of 200 mg/ml, it was further diluted to give concentrations of 100, 50, 25, and 12.5 mg/ml.

2.4. Standardization of test organisms

Universal bottles were sterilized in the autoclave (B. Bran, Y x 400B 2010, England) for 1 hour at 121 ℃. The single strength nutrient broth was sterilized, and 5 ml of the nutrient broth was transferred into the sterile bottles. Flame sterilized loop was dipped into the stock suspension of bacteria isolates and aseptically transferred into the test bottles containing nutrient broth. The preparation was incubated for 24 h at 37 ℃.

2.5. Preparation of Mueller-Hilton Agar

The Mueller-Hilton Agar was prepared for five (5) test organisms. It was prepared by dispersing 19 g of Mueller-Hilton agar powder into 500 ml of distilled water and mixed properly. The mixture was placed on a hot plate and allowed to boil. A 20 ml of the Mueller-Hilton agar was dispensed into test bottles and then sterilized in an autoclave for 1 hour at 121 ℃.

3. Microbiological sensitivity test

The sterile Mueller-Hilton Agar was poured into sterilized Petri dishes and allowed to solidify. After solidifying, holes were made using the cork borer number 6. The holes were labelled according to each concentration of 200, 100, 50 and 25 mg/ml. The petri dishes were labelled according to the plant extracts and the test organisms. Both plants were tested alone and in combination against the five (5) organisms. Each of the holes were filled with 0.1 ml of the plant extract according to the labelled concentrations respectively. The last hole was filled with 0.1 ml of the standard (fluconazole,

50 µg) for the fungus and gentamicin (20 µg) for bacteria. The plates were incubated for 24 h at 37 °C. The zones of inhibition were measured and recorded.

3.1. Determination of minimum inhibitory concentration (MIC)

The Mueller-Hilton broth was prepared and sterilized for the different concentrations 200, 100, 50, 25, 12.5, 6.25, 3.13 and 1.56 mg/ml and allowed to cool. A 2 ml of the methanolic plant extract was transferred aseptically into a test tube containing sterile 2 ml of the double strength broth. A 2 ml was drawn from the first tube into the second test tube containing 2 ml of the broth, 2 ml was also taken from the second test tube and transferred into the third test tube containing 2 ml of broth. This process was repeated until the final tube. A 0.1 ml of each the test organisms was added to the appropriate test tubes as labelled, and then properly shaken. The tubes were incubated for 24 h at 37 °C and the results recorded.

3.2. Minimum bactericidal concentration (MBC) determination

For the determination of MBC, the tubes that showed no growth were streaked on Petri dishes containing nutrient agar and incubated for 24 h at 37 °C. The lowest concentration that showed no visible growth after sub-culturing was recorded as the MBC.

3.3. Preparation of ointments

A 10% w/w ointment was prepared in two bases, simple ointment and emulsifying ointment. Methanolic extracts of *Chromolaena odorata* and *Borreria verticillata* were incorporated in a 1:1 ratio by the fusion method. A 15 g of each extract (30 g) was incorporated into 300 g of each base after melting on a water bath at 70 °C with continuous stirring. The mixtures were allowed to cool with gentle stirring and the finished ointments were transferred into clean, well labelled containers for storage.

Table 1 Formula for the Herbal Ointments

Ingredients	Simple Ointment	Emulsifying Ointment
Hard paraffin	15 g	60 g
Yellow Soft paraffin	255 g	150 g
Wool fat	15 g	-
Emulsifying wax	-	90 g
Ceto stearyl alcohol	15 g	-
Extracts	30 g	30 g
Total	330 g	330 g

3.4. Evaluation of ointment

3.4.1. Antimicrobial Efficacy of Formulated Ointments

The well diffusion method was used to assess the antimicrobial efficacy of the formulated herbal ointments prepared with the plant extracts of *Chromolaena odorata* and *Borreria verticilata*. A sterile Mueller-Hilton agar seeded with 0.1 ml of a 24 h broth culture of test organisms was used. Wells were created using the cork borer number 6 and filled with topical products of the plant extracts. The plates were pre-incubated for 24 h at 37°C.. A commercial brand of gentamicin ointment (Antibacterial) and ketoconazole cream (Antifungal) were used as standard. The zones of inhibition were determined and recorded.

3.4.2. Physical evaluation of formulated ointments

The prepared ointments were evaluated for the organoleptic and physicochemical properties including colour, odour, texture, pH, viscosity, extrudability and spreadability.

- **Physical examination**:The ointments were examined visually for colour and appearance, smelled to assess odour and rubbed on the palms to evaluate texture, and the results recorded.

- **pH**: The pH of the ointments was determined by using Digital pH meter (Model PHS, China). A 10 g of the formulations were weighed and the pH tested.
- **Viscosity**: The viscosity of the ointments was carried out with W & J Viscometer (Model NDJ-8S, Spindle No. 4 at 6 rpm). Readings were taken in triplicate and the average values recorded.
- **Extrudability**: A small quantity of each ointment was loaded into a 5 ml syringe with the aid of a spatula after removing the plunger. The plunger was replaced and a small quantity was extruded through the orifice to ensure proper packing and smooth extrusion. The loaded syringe was clamped on a retort stand using a cotton wool as a cushion. A load of 1.3 kg was placed on the plunger of the syringe until the whole content was extruded. The result was the average of replicate experiments.
- **Spread ability**: A 1 g of each ointment was weighed and placed between two glass slides. A weight of 500 g was applied for 5 minutes. After removal of the weight, the diameter of the spread ointment was measured directly in centimeter and the spread area was calculated using the equation $A=\pi r^2$ were A = Area, r = radius and π = 22/7.

4. Results and Discussion

The extract yield of *Borreria verticillata* and *Chromolaena odorata* was 26.00 and 14.75% respectively. The extracts were dark green in color and sticky with their characteristic odor (Table 2).

Table 2 Yield and Physical Properties of Extracts

Properties	**Chromolaena odorata**	***Borreria verticillata***
Yield	14.75%	26.00%
Consistency	Smooth and sticky	Gritty and sticky
Colour	Dark green	Dark green
Odour	Characteristic	Characteristic

The antimicrobial screening of the plant extracts showed that both extracts exhibited concentration dependent antibacterial activity, and the test organisms had unique sensitivity to the extracts individually, and according to extracts concentration (Table 3 and 4). The qualitative test (Table 3 and 4) revealed that *Staph aureus* and *E. coli* were more sensitive to the *Chromolaena odorata* extract than *P. aeruginosa* and *B. subtilis* [9,10]. It was reported that *C. odorata* extract was sensitive against *Staph. aureus*, *E. coli*, *P. aeruginosa* and *B. subtilis*. *Aspergillus nige*r was also sensitive to the extract at various tested concentrations. The absence of inhibition in the negative controls confirmed that the observed activity was solely due to the incorporated plant extracts. In a similar manner, *E. coli* was the most sensitive to *B. verticilata* extract, and was followed by *Staph. aureus* and *P. aeruginosa*. The result agrees with the review of Izuogu et aL [11] on the antimicrobial activity of *B. verticilata*. The sensitivity of *A. niger* to *B. verticilata* extract was similar to that of *C. odorata* at the least concentration (25 mg/ml) tested. The combined extract at 25 mg/ml concentration (Table 6) shows improved activity against the test organisms. . For the quantitative evaluation, *Staph aureus* and *B. subtilis* had the least MIC of 3.13 mg/ml for both *C. odorata* and *B. verticilata* extracts. *E. coli* and *A. niger* had the same MIC of 6.25 mg/ml.

Table 3 Zone of Inhibition (mm) for *Chromolaena odorata*

Test organism	**Zone of Inhibition (mm)**				
	200 mg/ml	**100 mg/ml**	**50 mg/ml**	**25 mg/ml**	**Standard**
S. A	21.67 ± 1.53	17.00 ± 1.00	15.00 ± 1.00	13.00 ± 1.00	30.33 ± 0.58
P. A	18.00 ± 1.00	16.00 ± 1.00	15.00 ± 1.00	12.00 ± 1.00	28.33 ± 0.58
E.C	21.00 ± 1.00	18.17 ± 0.76	15.00 ± 1.00	12.50 ± 0.50	31.50 ± 0.50
B. S	18.50 ± 0.50	16.50 ± 0.50	13.17 ± 0.76	11.33 ± 0.58	25.33 ± 0.58
A. N	18.00 ± 1.00	17.00 ± 1.00	14.50 ± 0.50	13.50 ± 0.50	27.67 ± 0.58

S.A: *Staphylococcus aureus;* P.A: *Pseudomonas aeruginosa*, E.C: *Escherichia coli;* B.S: *Bacillus Subtilis*; A.N: *Aspergillus Niger*

Table 4 Zones of inhibition of *Borreria verticillata*

Test organism	Zone of Inhibition (mm)				
	200 mg/ml	**100 mg/ml**	**50 mg/ml**	**25 mg/ml**	**Standard**
S. A	19.33 ± 0.58	18.50 ± 0.50	16.17 ± 0.76	15.33 ± 0.58	32.33 ± 0.58
P. A	20.17 ± 0.76	17.67 ± 0.58	16.00 ± 0.50	13.00 ± 0.50	33.00 ± 1.00
E.C	22.00 ± 0.50	19.67 ± 0.58	16.83 ± 1.26	16.00 ± 0.50	32.67 ± 0.58
B. S	18.00 ± 1.00	15.50 ± 0.50	14.00 ± 0.50	12.17 ± 0.76	29.17 ± 0.76
A. N	18.00 ± 1.50	15.83 ± 0.76	15.00 ± 0.50	13.50 ± 0.50	28.17 ± 0.76

S.A*: Staphylococcus aureus*, P.A: *Pseudomonas aeruginosa*, E.C: *Escherichia coli*, B.S: *Bacillus Subtilis*, A.N: *Aspergillus Niger*

The highest MIC (12.50 mg/ml) came from *P. aeruginosa* for both plant extracts. In a similar fashion (Table 6), *Staph aureus*, *E. coli*, *A. niger* had the least MBC of 12.50 mg/ml for *C. odorata* extract, while *Staph. aureus*, *E. coli*, and *B. subtilis* had 12.50 mg/ml for *B. verticilata* extract. This finding supports the report of Rufai et al. [12] concerning the antimicrobial activity of methanolic *B. verticilata* flower bud extract against *Staph. aureus* and *E. coli*. The highest MBC of 50.00 mg/ml) came from *P. aeruginosa* for both plant extracts. The formulated herbal ointments (Table 7) show varying inhibition zones to the extracts. The herbal emulsifying ointment shows inhibition zones of 16.00, 12.00, 15.67, 11.00, and 14.33 mm for Staph, Pseudomonas, Escherichia, Bacillus, and Aspergillus respectively, while the inhibition zones of 17.50, 14.17, 16.00, 13.70, and 15.00 mm were for Staph, Pseudomonas, Escherichia, Bacillus, and Aspergillus respectively for herbal simple ointment. In a similar manner, the standard ointment, gentamycin, and ketoconazole cream for bacteria and fungi respectively gave 23.00, 20.30, 23.20, 19.00, and 16.00 mm for Staph, Pseudomonas, Escherichia, Bacillus, and Aspergillus respectively. The results from the qualitative, quantitative and formulated herbal ointments show the sensitivity of all the test organisms to the herbal extracts. These results support the review of Izuogu et al. [11] on the antimicrobial activity of *B. verticilata* extracts against *P. aeruginosa*, *Staph aureus*, *E. coli*, Candida, and dermatophytes. Among the bacteria tested, *E. coli* was the most sensitive while Pseudomonas was the least sensitive. The combined extracts showed improved effect of the extract against the test organisms. The fungal isolate, *A. niger*, was sensitive to both extracts, almost equally.

Table 5 Minimum Inhibitory and Bactericidal concentrations of *C. odorata* and *B. verticilata* Extracts

Extract	**Concentration (mg/ml)**	***Staph. aureus***	***P. aeruginosa***	***E. coli***	***B. subtilis***	***A. niger***
C. odorata	MIC	3.13	12.50	6.25	6.25	6.25
	MBC	12.50	50.00	12.50	25.00	12.50
B. verticilata	MIC	6.25	12.50	6.25	3.13	6.25
	MBC	12.50	50.00	12.50	12.50	25.00

Table 6 Zones of inhibition (mm) of individual and combined leaf extracts at 25 mg/ml

Test organism	**Cromulent odorata**	**Borreria verticillate**	**Combination of extracts**	**Standard**
S. A	13.00 ± 1.00	15.33 ± 0.58	15.50 ± 0.50	23.17 ± 1.25
P. A	12.00 ± 1.00	13.00 ± 0.50	13.17 ± 0.76	20.00 ± 1.00
E.C	12.50 ± 1.00	16.00 ± 0.50	15.17 ± 0.76	23.33 ± 0.76
B. S	11.33 ± 0.58	12.17 ± 0.76	12.50 ± 0.50	21.17 ± 1.00
A. N	13.50 ± 0.50	13.50 ± 0.50	14.17 ± 0.76	19.50 ± 0.50

S.A*: Staphylococcus aureus*, P.A: *Pseudomonas aeruginosa*, E.C*: Escherichia coli*, B.S: *Bacillus Subtilis*, A.N: *Aspergillus niger*

These results suggest that both plants possess broad spectrum antibacterial potential, and their combination may enhance efficacy of the formulated ointments. Both ointment bases showed good release of the plants extracts against the test organisms but herbal ointment made with Emulsifying Ointment BP performed more than the Simple ointment BP evident by the zones of inhibition (Table 7). This enhanced effect could be attributed to the hydrophilic-lipophilic balance of the emulsifying bases as a result of the presence of sodium laurylsulphate, a surfactant, in the emulsifying ointment base, which promotes better release and diffusion in vitro. The herbal ointments could be effectively used in the treatment of bacterial and fungal skin infections caused by susceptible microorganisms.

Table 7 Antibacterial screening of Formulated Ointments

Ointments	**Zone of Inhibition (mm)**				
	S. A	**P. A**	**E. C**	**B. S**	**A. N**
S/O	16.00 ± 1.00	12.00 ± 0.50	15.67 ± 0.58	11.00 ± 1.00	14.33 ± 0.76
E/O	17.50 ± 1.00	14.17 ± 0.76	16.00 ± 1.00	13.70 ± 1.15	15.00 ± 1.00
P/C	23.00 ± 0.58	20.30 ± 1.00	23.20 ± 1.00	19.00 ± 0.50	16.00 ± 1.00
N/C	-	-	-	-	-

S/O: Simple Ointment; E/O: Emulsifying Ointment; P/C: Positive Control (Gentamycin Ointment, ketoconazole cream); N/C: Negative Control (Blank ointment base); S.A: *Staphylococcus aureus; P.A: Pseudomonas aeruginosa; E.C: Escherichia coli; B.S: Bacillus Subtilis; A.N: Aspergillus Niger*

Physicochemical evaluation of ointments as shown in Table 8 reveals that both ointments had a dark green colour with a characteristic odour and slightly smooth texture. Their pH values 6.16 and 5.77 for herbal simple ointment and herbal emulsifying ointment respectively were within the safe range (4.5 - 6.5) for skin application [13,14]. The viscosity values of 97709±1842 and 96276±1540 reveals that herbal simple ointment was more viscuous than herbal emulsifying ointment, and also contributed to qualitative and quantitative microbial evaluation performances by way of ease of diffusion through the agar. The extrudability for the herbal emulsifying ointment (6.03±0.15) was more than the herbal simple ointment (5.17±0.21), indicating easier dispensing for herbal emulsifying ointment than for herbal simple ointment. Both herbal ointments showed good spreadability but that of herbal emulsifying ointment was slightly higher. Consequently, the applicability of the herbal emulsifying ointment would be easier than the herbal simple ointment.

Table 8 Physicochemical Evaluation of the Herbal Ointments

Properties	**Simple Ointment**	**Emulsifying Ointment**
Colour	Dark green	Dark green
Odour	Characteristic	Characteristic
Texture	Slightly smooth	Slightly smooth
pH	6.16	5.77
Viscosity (mPa.s)	97709 ± 1842	96276 ± 1540
Extrudability (g/s) Spreadabilty (cm^2)	5.17 ± 0.21 11.40±0.26	6.03 ± 0.15 11.90 ±0.36

5. Conclusion

The results of this research showed that the methanolic extracts of *Chromolaena odorata* and *Borreria verticillata* possess activity against the test organisms. The extracts were incorporated into the simple and emulsifying ointment bases successfully. The formulated ointments also showed good physicochemical properties, and anti-microbial activity against the test organisms. The herbal emulsifying ointment released the crude extract better than the herbal simple ointment. The herbal ointments could be effectively used in the treatment of bacterial and fungal skin infections caused by susceptible microorganisms.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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