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FORMULATION AND EVALUATION OF CREAMS CONTAINING THE ETHANOLIC EXTRACTS OF *OCIMUM GRATISSIMUM L. (LAMIACEAE) AND CHROMOLAENA ODORATA L. (ASTERACEAE)* FOR THEIR ANTIMICROBIAL ACTIVITY

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ABSTRACT

Some medicinal plants have found usefulness as natural antibiotics. Formulating such into suitable dosage forms would help to enhance their administration and effectiveness. The aim of this research was to formulate creams containing ethanolic leaf extracts of *Ocimum gratissimum* and *Chromolaena odorata* for their antimicrobial activity and to evaluate their physicochemical properties. The leaves of the two medicinal plants were extracted using ethanol and dried thereafter. The extracts were tested against bacteria (*Staph aureus, Pseudomonas aeruginosa and Bacillus subtilis)* and fungi (*Candida albicans, Aspergillus niger).* The combined extracts was formulated into creams *using* Aqueous Cream and Buffered Cream BP as cream bases and were evaluated for their physicochemical properties and also tested against the test organisms. The results show that the extraction yield was 9.2 and 26.71% for *Ocimum gratissimum* and *Chromonaela odorata* respectively. The extracts and the formulated creams were active against the test organisms. *Staph. aureus* was the most susceptible bacterium with

an average inhibition zone of 28.75±6.09 mm for the concentrations tested, and while both fungi were sensitive to the extracts, *Candida albicans* was more sensitive than *Aspergillus niger*. Both cream bases showed good release of the active ingredient against the test organisms with an average inhibition zone of 22 ±3.52 and 23±3.16 mm for Aqueous cream BPC and Buffered cream BP bases respectively. The creams could be effectively used in the treatment of microbial skin infections.

KEYWORDS: medicinal plants; test organism; antimicrobial; creams.

INTRODUCTION

Medicinal plants have been the cornerstone of traditional medicine systems for thousands of years, offering a rich repository of therapeutic agents for various ailments. In many parts of the world, especially within communities with limited access to modern healthcare, these plants continue to play a vital role in health maintenance and disease treatment.[1] Among the vast diversity of medicinal flora, *Ocimum gratissimum* (clove basil, scent leaf) and *Chromolaena odorata* (Siam weed) have emerged as plants of interest due to their potent antimicrobial properties.[2,3]

Interest in medicinal plants has been on the increase in recent times because of their medicinal properties as antimicrobial, antidiabetic, antihypertensive, antipeptic ulcer, hepatoprotective, anti-inflammatory, antinoceptive, etc as a result of phytochemical constituents they contain such as alkaloids, flavonoids, tannins, saponins, terpenes, anthocyanins, etc.[4] Also, there is growing increase in antimicrobial resistance against conventional medicines which when left unattended to by way of natural remedy may lead to health catastrophe.[5,6] Bioactive compounds from plants have been serving as lead agents for discovering new antimicrobials, antimalarials, anti-inflammatory agents, etc. To enhance the administration and effectiveness of these medicinal plants, they have to be formulated into suitable dosage forms such as tea, mixtures, lotions, creams, ointments, capsules, tablets, etc.[7,8] Creams are semi-solid dosage forms which may be oil-in-water or water-in oil preparation being stabilized by an emulsifier or combination of emulsifiers.[7,8] They come as oily, aqueous or buffered creams. These can serve as cream bases for incorporation of medicament. The plant *Chromolaena odorata* (Siam weed) is from the family asteraceae and is known for its wound healing properties as a result of its phytochemical constituents such as flavonoids, alkaloids, saponins, tannins, coumarins, steroids, etc[9] in the same vein, the plant *Ocimum gratissimum* (Scent leaf) from the family lamiaceae has been known for its

antimicrobial, antidiabetic, anti-inflammatory, analgesic, etc properties.[10] This work was aimed at exploiting the additive or synergistic antimicrobial properties of both plants by combining the ethanolic extracts of the two plants and formulating them into creams, and to evaluate the physicochemical and antimicrobial properties of the formulations.

MATERIALS AND METHODS

MATERIALS

Collection, drying and processing of Plant material

Fresh leaves of *Chromolena odorata* and *Ocimum gratissimum* were collected from Jos metropolis and were authenticated at the Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, University of Jos by Mr Thomas Yakubu with voucher number UJ/PCG/HSP/24A01, and was confirmed by Mr Joseph Azila, a Taxonomist at the Federal College of Forestry, Jos. The plant material was air dried in the laboratory and thereafter pulverized using pestle and mortar, packaged and stored for extraction. Isolates of *Staph aureus*, *Pseudomonas aeruginosa, Bacillus subtilis, Candida albicans,* and *Aspergillus niger* were gotten from the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, University of Jos, Jos, Nigeria.

METHODS

Extraction of plant constituents

A 350 g of the plant leaf powder was subjected to maceration for seven days using ethanol (95%). The extract was filtered using muslin bag first and thereafter Whatman filter paper. The filtrate was exposed to air in the laboratory for drying. After drying, the extract was stored in a well closed glass bottle and stored for subsequent use.

Microbiological evaluation

Overnight culture of these organisms was carried out using flame sterilized loop for aseptic transfer of the organisms into nutrient broth in universal bottles and incubated at 37 ^{o} C for 24 h. *Standardization of test organisms* Universal bottles were sterilized in the autoclave (B. Bran, Y x 400B 2010, England) for 1 hour at 121 ^{0}C. 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 solution of bacteria isolates and aseptically transferred into the test bottles containing nutrient broth. The preparation was incubated for 24 h at 37^{0}C.

Preparation of Muellen Hilton Agar

The Muellen Hilton agar was prepared by dissolving 9.5 g of Muellen Hilton agar powder into 250 ml of distilled water and mixed properly. The mixture was placed on the hot plate and allowed to boil. A 20 ml of the Muellen Hilton agar was dispensed into test bottles and then sterilized in an autoclave for 1 hour at 121^0C.

Qualitative test

The sterile Muellen Hilton agar was poured into sterilized Petri dishes and allowed to solidify. After solidifying, six (6) holes were bored on the media using the cork borer number 5. The holes were labeled according to each concentration of 150, 75, 37.5, and 18.75 mg/ml. The Petri dishes were labeled according to the test organisms. Each plant was tested against the five (5) organisms - *Staph aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Candida albicans*, and *Aspergillus niger*. Each of the holes were filled with 0.1 ml of plant extract according to the labeled concentrations respectively. And the fifth hole was filled with a 0.1 ml of standard, which were fluconazole (for fungal organisms) and Gentamicin (for bacterial organisms) and the sixth hole was filled with 0.1ml of DMSO. The plates were incubated for 24 h at 37^0C. The zone of inhibitions was then measured and recorded.

Quantitative test

Minimum inhibitory concentration (MIC) determination

The Muellen Hilton broth was prepared and sterilized for the preparation of the seven (7) concentrations and allowed to cool. A 2 ml of the ethanolic plant extract was aseptically and serially diluted to obtain 150, 75, 37.5, 18.75, 9.375, 4.6875, and 2.34375 mg/ml concentrations using the sterile 2 ml double strength broth in test tubes A 0.1ml of the standardized suspension of the organisms was added to each of the test tube and properly shaken. The tubes were incubated for 24 h at 37^0C and results recorded.

Minimum bactericidal concentration (MBC)

For the determination of MBC, the tube that showed no growth were then streaked on Petri dishes containing nutrient agar and incubated for 24 h at 37^0C. The lowest concentration that showed no visible bacteria growth after sub-culturing was recorded as the MBC.

Preparation of cream

Aqueous Cream BPC contains 30 % Emulsifying ointment, chlorocresol 0.1% and purified water to 100 % while **Buffered Cream BP contains 30 %** Emulsifying Ointment, 0.25%

sodium phosphate, 0.05% citric acid monohydrate, 0.1% chlorocresol and purified water to 100%. The creams as bases were prepared according to BPC and BP methods. They were prepared by melting the ingredients together and stirring until set.

Physical evaluation of formulated creams

The physicochemical properties of the prepared creams such as color, odor, texture, pH, viscosity, extrudability were evaluated. **Colour, Odour, Texture**: The creams were examined visually for colour and appearance, smelled to assess odor and rubbed on the hand to evaluate texture, and recorded.

pH: The pH of the creams was determined using Digital pH meter (Model PHS, China.

Viscosity: The viscosity of the creams 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 reported.

Extrudability: A small quantity of each cream 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 0.5 kg was placed on the plunger of the syringe for 10 seconds and the weight of the content extruded was taken. The results were the average of the replicate experiments.[11]

Viscosity measurement

The viscosity of the herbal creams was determined using a digital rotational viscometer (Model BDV 1 S, China). The viscosity reading was taken at 28 ± 2 ^{o}C using spindle number four (4) at 30 revolutions per minute (rpm). The viscosity (mPa.s) was read off and recorded.[11]

Table 1: Formula for the Herbal creams.

Ingredients	Creams	
	Aqueous	Buffered
Emulsifying Ointment	30 g	30 g
Sodium phosphate	-	2.5 g
Citric Acid Monohydrate	-	0.5 g
Chorocresol	0.1 g	0.1 g
Purified Water	69.9 g	66.9 g
Plant Extract	15 g	15 g

RESULTS AND DISCUSSION

The extract yield for the various plant was 9.2 and 26.1% for *Ocimum gratissimum* and *Chromolaena odorata* respectively. The extract from *Ocimum gratissimum* was dark green, hard and sticky with its characteristic smell, while that of *Chromolaena odorata* was greenish brown, and sticky with its characteristic odour. The sensitivity of the test organisms to the extracts varied with individual organism and extract concentration. The qualitative test (Tables 2,3 and 4) revealed that 18.75 mg/ml concentration of both plant extracts inhibited the growth of all the test organisms. These results support the work of Sagar et al and Bunkaew et al[2,3] *Staph. aureus* was more sensitive to *Ocimum gratissimum* than *Chromolaena odorata* at all the concentrations tested, while *Bacillus subtilis* was sensitive to both extracts. *Pseudomonas aeruginosa* was more sensitive to *Chromolaena odorata* than *Ocimum gratissimum* at various tested concentrations. For the fungi isolates, *Candida albicans* was more sensitive to *Chromolaena Odorata* than *Ocimum gratissimum* while *Aspergillus niger* was more sensitive to *Ocimum gratissimum* than *Chromolaena odorata*. The combined concentration of 75 mg/ml each of the extract (150 mg/ml), revealed increased sensitivity of *Staph. aureus* to the extracts in all the tested concentrations. In the same vein, *Basillus subtilis* sensitivity was also improved by the extracts combination. There was also an improvement in the sensitivity of *Pseudomonas aeruginosa*. For the fungi isolates, *Candida albicans* was more sensitive than *Aspergillus niger* to the combined extracts at all the concentrations.

Table 2: Inhibition Zone of *Chromonaela odorata* Leaf extract against Test Organisms.

Inhibition Zone Diameter (IZD) in mm at various concentrations (mg/ml)							
Test Organism	150	75	37.5	18.75	DMSO	*IZDm average	Std
Staph. Aureus	28	26	23.5	21	0	24.63±2.63	25
Bacillus subtilis	27	25	22	20.5	0	23.63±2.53	26
P. aeruginosa	24	23	20	17	0	21.00±2.74	21
Candida albicans	20.5	18	15	13	0	16.63±2.85	20
Aspergillus niger	20	17	16	12	0	16.30±2.86	17
*IZD average	23.9±3.2	21.8±3.6	19.3±3.3	16.7±3.7			21.8±3.3

*IZDc average –inhibition zone diameter average with respect to a particular microorganism concentration; *IZDm average inhibition zone diameter average with respect to -; STDs: gentamycin (20 µg/ml) for bacteria Fluconazole (50 µg/ml) for fungi.

Table 3: Inhibition Zone of *Ocimum gratissimum* Leaf extract against Test Organisms.

Inhibition Zone Diameter (IZD) in mm at various concentrations (mg/ml)							
Test Organism	150	75	37.5	18.75	DMSO	IZDm average	Std
Staph. Aureus	30	28	26	23	0	26.75±2.58	25.5
Bacillus subtilis	26	24	23	21	0	23.50±1.80	26
P. aeruginosa	20	17	15	13.5	0	16.38±2.85	20
Candida albicans	18	16	14	12	0	15.00±2.23	20
Aspergillus niger	20	18	17	15	0	17.5±1.80	18
IZDc average	22.8±4.5	20.6±4.6	19.0±4.7	16.9±4.3			21.9±3.2

*IZDc average –inhibition zone diameter average with respect to a particular microorganism concentration; *IZDm average inhibition zone diameter average with respect to -; STDs: gentamycin (20 µg/ml) for bacteria; Fluconazole (50 µg/ml) for fungi.

Table 4: Inhibition Zone of *Ocimum gratissimum and Chromolaena odorata* Leaf extracts against Test Organisms.

Inhibition Zone Diameter (IZD) in mm at various concentrations (mg/ml)							
Test Organism	150	75	37.5	18.75	DMSO	IZDm	Std
Staph. aureus	32	29	28	26	0	28.75±6.09	25.5
Bacillus subtilis	30	28	25	22.5	0	26.38±2.85	26
P. aeruginosa	25	23	20	18	0	21.50±2.69	21
Candida albicans	21	20	17	15	0	18.25±2.38	19
Aspergillus niger	17	15.5	14	12.5	0	14.75±1.67	17
IZDc average	25.0±5.5	23.0±5.0	20.8±5.1	18.8±4.9			21.4±3.2

*IZDc average –inhibition zone diameter average with respect to a particular microorganism concentration; *IZDm average inhibition zone diameter average with respect to -; STDs: gentamycin (20 µg/ml) for bacteria; Fluconazole (50 µg/ml) for fungi.

For the quantitative evaluation, Tables 5, 6, and 7 show the result of minimum inhibitory concentration (MIC). *Bacillus subtilis* had the least MIC of 4.6875 mg/ml for *Ocimum gratissimum*, followed by *Staph aureus* (9.375 mg/ml) for both extracts, and *Pseudomonas aeruginosa* (9.375 mg) for only *Ocimum gratissimum*. Among the bacteria, *Pseudomonas aeruginosa* had the highest MIC (18.75 mg/ml) with *Chromolaena odorata* extract while Staph and Bacillus had the least (9.375 mg/ml), and for *Ocimum gratissimum*, Pseudomonas and Staph had the highest MIC of 9.375 mg/ml while Bacillus had the least (4.6875 mg/ml). The combined extracts improved the sensitivity of Staph and Bacillus pushing their MIC down to 4.6875 mg/ml but that of Pseudomonas remained at 18.75 mg/ml. For the fungal isolates, Candida had MICs of 9.375 and 18.75 mg/ml for Chromolaena and Ocimum extracts

respectively, while that of Aspergillus was 18.75 mg/ml for both extracts. When the extracts were combined, Candida MIC was pushed down to 4.6875 mg/ml while that of Aspergillus remained at 18.75 mg/ml.

Table 5: Minimum Inhibitory Concentration (mg/ml) of *Chromolaena odorata* Leaf extract.

Organisms	150	75	37.5	18.75	9.375	4.687	2.34
Staph. aureus	-	-	-	-	-	+	+
B. subtilis	-	-	-	-	-	+	+
P.aeruginosa	-	-	-	-	+	+	+
C. albican	-	-	-	-	-	+	+
A. niger	-	-	-	-	+	+	+

Key: growth (+); no growth (-)

Table 6: Minimum Inhibitory Concentration (mg/ml) of *Ocimum gratissimum* Leaf extract.

Organisms	150	75	37.5	18.75	9.375	4.687	2.34
Staph. aureus	-	-	-	-	-	+	+
B. subtilis	-	-	-	-	-	-	+
P.aeruginosa	-	-	-	-	-	+	+
C. albican	-	-	-	-	+	+	+
A. niger	-	-	-	-	+	+	+

Key: growth (+); no growth (-)

Table 7: Minimum Inhibitory Concentration of combined *C.odorata* and *O. gratissimum* Leaf extracts.

(mg/ml) Organisms	150	mg/ml 75	37.5	18.75	9.375	4.687	2.34
Staph. aureus	-	-	-	-	-	-	+
B. subtilis	-	-	-	-	-	-	+
P.aeruginosa	-	-	-	-	+	+	+
C. albican	-	-	-	-	-	+	+
A. niger	-	-	-	-	+	+	+

Key: growth (+); no growth (-)

For the minimum bactericidal concentration (MBC), as shown in Tables 8, 9 and 10, Bacillus, Staph and Pseudomonas had 18.75, 37.5 and 75 mg/ml respectively for *Chromolaena odorata*. Both Staph and Bacillus had 18.75 mg/ml and Pseudomonas had 75 mg/ml for Ocimum. For the fungal isolates, candida had MBC of 37.5 and 75 mg/ml for Chromolaena and Ocimum respectively while Aspergillus had 75 mg/ml for both extracts.

Table 8: Minimum Bactericidal Concentration of *Chromolaena odorata* Leaf extract.

(mg/ml) Organisms	150	mg/ml 75	37.5	18.75	9.375	4.687	2.34
Staph. aureus	-	-	-	+	+	+	+
B. subtilis	-	-	-	-	+	+	+
P.aeruginosa	-	-	+	+	+	+	+
C. albican	-	-	-	+	-	+	+
A. niger	-	-	+	+	+	+	+

Key: growth (+); no growth (-)

Table 9: Minimum Bactericidal Concentration of *Ocimum gratissimum* Leaf extract.

(mg/ml) Organisms	150	mg/ml 75	37.5	18.75	9.375	4.687	2.34
Staph. aureus	-	-	-	-	+	+	+
B. subtilis	-	-	-	-	+	+	+
P.aeruginosa	-	-	+	+	+	+	+
C. albican	-	-	+	+	+	+	+
A. niger	-	-	+	+	+	+	+

Key: growth (+); no growth (-)

Table 10: Minimum Bactericidal Concentration of combined *Chromolaena odorata* and *Ocimum gratissimum* Leaf extracts.

(mg/ml) Organisms	150	mg/ml 75	37.5	18.75	9.375	4.687	2.34
Staph. aureus	-	-	-	+	+	+	+
B. subtilis	-	-	-	+	+	+	+
P.aeruginosa	-	-	+	+	+	+	+
C. albican	-	-	-	+	+	+	+
A. niger	-	-	+	+	+	+	+

Key: growth (+); no growth (-)

The formulated herbal creams (Tables 11 and 12) showed similar inhibition zones to the extracts. The aqueous creams show inhibition zones of 25, 25, 16, 24,and 20 mm for Staph, Bacillus, Pseudomonas, Candida, and Aspergillus respectively, while the inhibition zones of 26, 24, 17, 25, and 23 mm were for Staph, Bacillus, Pseudomonas, Candida, and Aspergillus respectively for buffered cream. In a similar manner, the standard antibacterial cream, clindamycin, had inhibition zone of 20, 31and 17 mm for Staph, Bacillus and Pseudomonas respectively, while the standard anti-fungal cream ketoconazole had inhibition zone diameter of 16 mm for both Candida, and Aspergillus. The results from the qualitative, quantitative, and formulated herbal creams show that all the test organisms were sensitive to the herbal extracts. Physicochemical evaluation of creans as shown in Table 12 reveals that **b**oth creams had a dark green colour with a characteristic odour. The aqueous cream had smooth texture

while that of buffered cream was slightly rough. Their pH values 4.70 and 6.27 for herbal aqueous cream and herbal buffered cream respectively were within the safe range (4.5 - 6.5) for skin application.[7,8] The viscosity values of 15,546±9.57 and 15,547±2.5 mPa.s and extrudability values of 31.25±0.5 and 29.41 ±0.5 g/sec for aqueous and buffered creams respectively, reveals that herbal aqueous cream was less viscuous than herbal buffered cream, and also contributed to qualitative and quantitative microbial evaluation performances by way of ease of diffusion through the agar. Both creams showed good extrudability and, consequently, the applicability of the herbal creams would be easy.

Extracts

Table 11: Antimicrobial Activity of Formulated Cream of *Ocimum gratissimum and Chromolaena odorata* Leaf.

Inhibition Zone Diameter (IZD) in mm Organisms						
Cream	*Staph.aureus*	*B.subtilis*	*P. aeruginosa*	*C. albican*	*A.niger*	IZD average
Aqueous	**25**	**25**	**16**	**24**	**20**	**22±3.52**
Buffered	**26**	**24**	**17**	**25**	**23**	**23±3.16**
Clindamycin	**20**	**31**	**17.5**	**-**	**-**	
Ketoconazole				**16**	**16**	

Stds: clindamycin cream (bacteria); ketoconazole cream (fungi)

Table 12: Evaluation of Physical properties of formulated Creams.

Physical properties	**Aqueous Cream**	**Buffered Cream**
Colour	Dark green	Dark green
Odour	Characteristic	Characteristic
Texture	Smooth	Rough
pH	4.79	6.27
Viscosity (mPa.s)	15,546±9.57	15,547±2.5
Extrudability (mg/sec)	31.25±0.5	29.41 ±0.5

These results substanciate the reported work of some researchers[2,12,13] on the antimicrobial activity of *Ocimum gratissimum* on some microbes such as *Staph. aureus, P.aeruginosa* and *E. coli*. In a similar manner, other workers[3,14,15] reported on the anti-microbial activity of *C. odorata* against some bacteria such as *Strept. Pyogenes, B. subtilis, Staph. aureus, P. aeruginosa and E. coli*. Among the bacteria tested, Bacillus was the most susceptible while Pseudomonas was the least susceptible. The combined extracts showed additive effect on the sensitivity of the test organisms as shown in the qualitative test. Also among the fungal isolates, Candida was more sensitive to the extracts than Aspergillus. Both cream bases showed good release of the plants extracts against the test organisms. The herbal creams

could be effectively used in the treatment of bacterial and fungal infections of the skin. Clindamycin is one of the antibacterials used in the treatment of Staph. aureus infection, therefore, the creams could be used to effectively treat Staph skin infection.

CONCLUSION

The ethanolic extracts of *Chromolaena odorata* and *Ocimum gratissimum* demonstrated good activity against the tested pathogenic fungi and bacteria similar to the standard drugs. The antimicrobial activity improved with the combined extracts of both plants. The formulated herbal creams with the combined extracts using Aqueous Cream BPC and Buffered Cream BP as herbal cream bases also showed antimicrobial effect similar to the standard antimicrobial creams used for the treatment of fungal and bacterial infections. The herbal creams could be effectively used in the treatment of susceptible fungal and bacterial infections.

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