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Research Article

Development and Evaluation of Herbal Nail Polish Using the Ethanolic Extract of *Chromolaena odorata*

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ABSTRACT

Plant extracts have been incorporated in the formulation of nail polish. *Chromolaena odorata* has been reported to exhibit antifungal and antimicrobial activities. In this study, the ethanolic extract of *C. odorata* has been incorporated into nail polish. Even though different extracts have been incorporated into nail polish formulations *C. odorata* has never been used in nail polish formulations. The study design involved the incorporation of *C. odorata* extract into nail polish formulations and comparing their performance with a known brand of nail polish sold on the market. The antimicrobial activity of the extract and the formulated nail polish were first determined by agar diffusion. Five (5) wells were created in each plate using a cork borer (No.3, 4 mm). 20 μ L of 50% concentration of the ethanolic extract of *C. odorata* was dispensed into three wells representing triplicate repeat, 20 μ L of tetracycline and Nystatin (positive control) were dispensed in the middle well (bacteria and fungus respectively) and 20 μ L of dilute ethanol (2 mL of ethanol: 2 mL of distilled water) was dispensed in the last well (negative control). The set up was incubated at 37 °C for 24/ 48 h and the zones of inhibitions recorded. The plates were then checked for the presence or absence of growth in the Nutrient agar or SDA. The extract showed good antimicrobial activity when tested against *Staphylococcus aureus* (NCTC 12493) (8.60 ± 2.85 mm), (nail paronychia), *Candida albicans* (ATCC 90028) (Onycholysis and Onychomycosis) (18.33 ± 0.63 mm), and *Streptococcus pyogenes* (Clinical). (14.60 ± 0.39 mm). The formulated products exhibited good antimicrobial activity, were stable over the testing period, and showed good functionality when subjected to different stability tests.

INTRODUCTION

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Nail cosmetics have gained popularity due to advertisements, online presence and social media networks leading to increased consumer appetite for these products (Koch et al, 2019). Nail cosmetics are mainly used for adorning and enhancing the appearance of nails but sometimes this comes with some negative side effects, including dermatitis; nail discoloration, and other nail infections [1]. Nail polish, hardeners, moisturizers, and prostheses are the four broad categories of nail cosmetics. Nail polish are mostly applied as basecoats or topcoats of both toenails and fingernails [2]. These basic components include plasticizers and resins, solvents, film-forming agents and pigments [3]. Nitrocellulose is added to nail polish formulations to enhance their hardness, water resistance, viscosity and because they dry rapidly to create a hardened thin film [4]. Plasticizers and resins are used to enhance adhesion, toughness, brightness, flow quality and flexibility of a nail polish. Examples of plasticizers include alkyl resins, acrylates, vinyl, or polyesters [5]. The chemical composition of most additives in cosmetic products affect the properties of the final cosmetic product developed [6].

C. odorata is a perennial shrub that grows abundantly in Asia and sub-Saharan Africa, it has been reported to be used in the treatment of many ailments and disease conditions such as diabetes, malaria, wounds, inflammation and fever. It has antidiabetic, anticancer, anti-inflammatory, antimicrobial, antiparasitic, antinociceptive, antipyretic and wound healing activities [7] *C. odorata* have antibacterial, antispasmodic, antiprotozoal, antifungal, antihypertensive, anti-inflammatory, astringent, diuretic, and hepatotropic effects, which makes it suitable for use in cosmetic products [8]. The presence of saponins, phenols, and tannins in the aqueous and ethanolic leaf extracts of *C. odorata* have been reported. The extracts have been found to exhibit

hemolytic, anti-inflammatory, antioxidative, immunostimulant, and antibacterial properties [9]. The ethanolic extract of *C. odorata* has been reported to improve cardiac conditions by lowering blood pressure, boosting circulation, and preventing the buildup of arteriosclerosis plaque and blood clots [10]. A bioprocess for the synthesis of ethanol using mixed invasive species (including *C. odorata*) has been reported. A composite biomass of the weeds upon acid hydrolysis, alkaline delignification and enzymatic hydrolysis gave pentose-rich and hexose-rich hydrolyzates, which were each fermented under sonication. The phenolic extract of *C. odorata* has been found to inhibit protein fibrillation and oxidation *in vitro* because of the presence of phenols, flavonoids and terpenoids [11].

In this study, we present the formulation of herbal nail polish incorporated with the ethanolic extract of *C. odorata*, evaluation of their properties and as well as their antimicrobial activity. This work reports the first incorporation of *C. odorata* extracts into nail polish formulations.

MATERIAL AND METHOD

Extraction of plant samples

Dried leaves of *C. odorata* were obtained from the Ho municipality, Volta Region, Ghana. The botanical identity of the sample was confirmed by experts from the School of Basic and Biomedical Sciences of the University of Health and Allied Sciences before being transferred to the laboratory. The extraction procedure followed previously published methods with slight modifications [12]. The dried sample was pulverized and weighed. About 700 g of the sample was macerated in 2 L of ethanol (95 % v/v) for a week. It was then filtered and the solvent removed by rotary evaporation, after which the extract obtained was allowed to sit for one week to



evaporate all the residual solvent to yield a constant weight of extract. The yield of the extract was calculated and screened for its phytochemical constituents.

Phytochemical Screening

The phytochemical tests of the ethanolic extract of *C. odorata* was determined according to the standard methods [13-15].

Standard Reagents

The selected chemicals used in this study include: nitrocellulose (10%) (film-forming agent) was obtained from Abro chemicals, ethyl acetate (solvent), (Merck Chemicals), camphor (plasticizer), castor oil (resin), oil-based colorant (blue, yellow, and red), polyethylene glycol (thickener) were purchased from Unique Fragrances (Kasoa, Ghana). Ethanol, sodium hydroxide, hydrochloric acid, sodium chloride and Potassium Mercuric Iodide were obtained from Merck Sigma, Germany. The plant sample (dried *C. odorata*) was obtained from the Ho municipality, Volta Region, Ghana. Two antibiotic agents; Tetracycline and Nystatin were used as positive controls in the microbial analysis whilst ethanol and ethyl acetate were used as negative controls.

Test organisms

The test organisms used in this study included methicillin-resistant *Staphylococcus aureus* (NCTC 12493), (nail paronychia), *Candida albicans* (ATCC 90028) (Onycholysis and Onychomycosis), and *Streptococcus pyogenes* (Clinical) (nail psoriasis) are all known to cause nail infections. These microorganisms were obtained from the Microbiology unit of the School of Basic and Biomedical Sciences, University of Health, and Allied Science (UHAS) and were

activated by sub-culturing them on the nutrient agar (Oxoid, United Kingdom) for 24 hours at 37 °C in an incubator after which they were made ready for the microbial analysis.

Preparation of plant and nail polish samples

A stock solution of the extract was prepared by weighing 1 g of the extract into two clean sterile 15 mL falcon tubes containing 5 mL of ethanol. From this stock solution, an aliquot of 2 mL was transferred into two clean and sterile 15 mL falcon tubes, 2 mL of ethanol was added and used for further antimicrobial analysis. An aliquot of 1 mL of the stock solution (100%) were used to prepare 100 mg/mL of each sample.

Antimicrobial determination of plant extract

The antimicrobial activity of the ethanolic extract of *C. odorata* was determined using both the Kirby-Bauer agar well diffusion and the broth micro-dilution methods. From the Kirby-Bauer agar well diffusion, the zones of inhibition were measured. The Kirby-Bauer agar well diffusion involved a test agar plate with a standardized concentration of test organisms and either antibiotic disc or wells with antibiotic agents placed on the lawn of microorganisms. After overnight incubation, the diameter of the zone of inhibited growth were measured. The method used was based on reported work with slight modifications [16-19]

Broth microdilution method of plant extract

The minimum inhibitory concentrations (MICs) of the extract were determined by the micro broth dilution method using the 96 well microtiter plates per the method according to the Clinical and Laboratory Standards Institute [20]. The method used was consistent with reported work with slight modification [21-24]. A 50% concentration of the



stock solution of the extract was prepared as earlier described. Ten different concentrations were obtained by serial dilution, an aliquot of 100 μL of double-strength Mueller Hinton broth (for bacterial strains) and Brain-Herat Infusion broth (for fungal strain) (Oxoid Limited, United Kingdom) were distributed into each 96-well plate (Cito test Labware Manufacturing Co. Ltd, Jiangsu, China) and mixed with 100 μL of the plant extract to prepare well concentrations ranging from 0.1–50.0 mg/mL. Wells 11 and 12 were designated positive control (Broth + organism only) and negative control (Broth with no organism) respectively for each microbial strain on each column. This was followed by the addition of 100 μL of each of the 0.5 McFarland standardized test organisms at a concentration of 10^5 CFU after which the plates were subjected to incubation at 37 °C for 24–48 hours for bacterial and fungal strains respectively. The MIC values were then evaluated by adding 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye and kept in the incubator for 30 min after which the MIC was observed as the least concentration which does not change color from yellow to red. The experiment was performed in triplicate and their averages were recorded as the MICs.

Determination of Minimum Bactericidal Concentrations (MBC) and Minimum bactericidal Concentrations/ Minimum Inhibitory Concentration (MBC/MIC)

The MBC/MIC was determined to establish whether the ethanolic extract of *C. odorata* could destroy the microbial cells. Aliquots from each well from the susceptibility tests were transferred to plates with Nutrient agar and then incubated for 24–48 hours at 37 °C. The plates were then examined for growth on Nutrient agar [25].

Nail polish formulation

The preparation of the different nail polish formulations in this study was according to the method reported with slight modifications [26].

Formulation of antimicrobial nail polish with *C. odorata* leaves

a) First Formula

Two different formulas were used in preparing the herbal nail polish with *C. odorata* leaves. Table 1 below shows the first formula for the preparation of herbal nail polish with *C. odorata* leaves and ones without the plant extract. The formula below was used for the first nail polish formulations with the *C. odorata* extract (CO1, CO2, CO3, CO4, and CO5) and the ones without *C. odorata* extract (WCWCO and CWCO), 10 g of camphor was grounded into powder and dissolved in 140 mL of ethyl acetate with stirring. 20 g of nitrocellulose, 30 g of castor oil and 10 g polyethylene glycol were added and stirred until all the components were dissolved.

The formulated stock nail polish in the beaker was then dispensed into 20 mL nail polish containers. Different amounts (0.1 g to 0.5 g) of the *C. odorata* extract were weighed and incorporated into five different formulations. A red colourant was then added to six of the formulations including the five formulations containing the plant extract. The nail polish formulation with colourant (red) but without *C. odorata* extract (CWCO) and a nail polish formulation with no colourant nor *C. odorata* extract (WCWCO) were used as controls for the study.

Table 1: Formula for antimicrobial nail polish with *C. odorata* leaves extract (CO1, CO2, CO3, CO4, and CO5) and without extract (CWCO and WCWCO)

FORMULA	QUANTITY
Nitrocellulose (10% nitrocellulose)	20 g
Ethyl acetate	140 mL



Camphor	10 g
Castor oil	30 g
Colorant (Red)	q. s
Polyethylene glycol	10 g
Acheampong leaves	Varying amounts
CO1	0.1 g
CO2	0.2 g
CO3	0.3 g
CO4	0.4 g
CO5	0.5 g
Controls	Varying amounts
WCWCO	No plant extract
CWCO	No plant extract

b) Second Formula

The second formula used in formulating the herbal nail polish with *C. odorata* leaves and ones without the plant extract are indicated in Table 2. The formula below was used for the formulation of COb1, COb2, COb3, COb4, and COb5 with varying amounts of *C. odorata* extract incorporated. The formulations without the extract were (CWCOb and WCWCO₂), 19 g of camphor was grounded into powder and dissolved in 70 mL of ethyl acetate with stirring, and 60 g of nitrocellulose was added and stirred to obtain a clear solution.

The formulation was dispersed into 10 mL containers and the different amounts of extract of *C. odorata* incorporated. The formulated nail polish was then dispensed into 10 mL containers, different amounts of the *C. odorata* extract was incorporated into the relevant sample. The blue colour was incorporated and mixed thoroughly to obtain COb1, COb2, COb3, COb4, and COb5. Two control formulations were prepared one contained only the colourant (blue) without *C. odorata* extract (CWCOb), the other nail polish had no colourant nor the *C. odorata* extract (WCWCO₂).

Table 2 Formula for antimicrobial with *C. odorata* leaves extract (COb1, COb2, COb3, COb4, and

COb5) and without extract (CWCOb and WCWCO₂)

FORMULA	QUANTITY
Nitrocellulose (10% nitrocellulose)	60 g
Ethyl acetate	70 mL
Camphor	19 g
colorant (blue)	q.s.
<i>C. odorata</i> leaves	Varying amount
COb1	0.1 g
COb2	0.2 g
COb3	0.3 g
COb4	0.4 g
COb5	0.5 g
Controls	Varying amount
CWCOb	No plant extract
WCWCO ₂	No plant extract

Evaluation of Herbal Nail Polish

The nail polish formulations were evaluated for their functionality and stability using the Bureau of Indian standards. They were also tested against some microorganisms that cause nail infections (methicillin-resistant *Staphylococcus aureus* (NCTC 12493), (nail paronychia), *Candida albicans* (ATCC 90028) (Onycholysis and Onychomycosis), and *Streptococcus pyogenes* (Clinical) (nail psoriasis) These evaluations were conducted as a quality control measure on the nail polish to understand their antimicrobial properties.

Functionality tests

Stability tests

This is the measure of the durability of the nail polish. All the nail polish formulations were taken through accelerated stability tests where they were stored at a temperature of 25 ° ± 2 °C and 37 °C ±2 °C, after 1 month, the formulations were inspected for their organoleptic characteristics (color, smell, texture, and consistency).

Bureau of Indian Standards

Smoothness to flow



About 1 mL of each nail polish formulation and marketed nail polish were pipetted on a glass slide of area 137.81 cm² and raised vertically. The smoothness of flow was observed visually and determined by comparing it with a marketed nail polish used as a standard [27].

Gloss

The gloss of a nail polish refers to how shiny and smooth it looks when observed visually. The gloss of formulated nail polish samples and the marketed nail polish were determined by applying a film each at the same consistency with the nail polish brush on a glass slide of area 137.81 cm² and observed visually using the marketed nail polish as a standard of comparison [27]

Drying time

The optimal drying time of an ideal nail polish is between 1 to 2 minutes without developing bloom. A film of the same consistency for each nail polish sample and the marketed nail polish was applied on a glass slide of area 137.81cm² with the help of the nail polish brush. The time required to form a dry touch film at room temperature was recorded using a stopwatch. It was then compared with the marketed nail polish [28]. Figure 1 gives the

pictorial representation of sample CO3 undergoing drying.



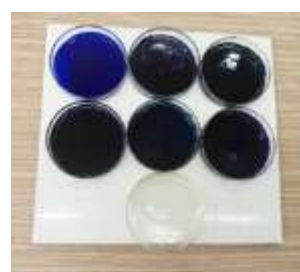
Figure 1 Drying of CO3 nail polish sample

Non-volatile content

The empty clean sterile petri dish plate for the nail polish samples were weighed first and denoted as W1. About 3 mL of each nail polish sample including the marketed nail polish were pipetted into the weighed clean sterile petri dish plates and their weight were noted as W2. The difference (W2-W1) was the actual weight of the nail polish samples. The petri dishes plates with the nail polish samples were placed in a hot air oven at 105 ± 2 °C for 1 hour. The plates were then removed, allowed to cool and weighed, W3. The difference in weight (W3-W1) for each nail polish sample was calculated and the non-volatile content was expressed in percentages (Rasheed, et al, 2012). Figure 2 (a, b) shows pictorial presentations of some stages in the non-volatile content test.



(a)



(b)

Figure 2 Non-volatile tests for (a) *C. odorata* (red), and (b) *C. odorata* (dark blue color)

In-vitro adhesion test

An area of 3.6 cm by 2.4 cm was marked with a marker and a rule on a glass slide of area 11.45 cm

by 12.45 cm. A few drops of each nail polish sample as well as the marketed nail polish weighing 0.0645 g were applied on the marked area on the glass slide and spread evenly with the

Table 3: Phytochemical screening of *C. odorata* leaves extract

Plant Constituent	Test/ Reagent	Ethanollic AL extract
Alkaloids	Mayer's test	-
Flavonoids	Alkaline Reagent Test	+
Saponins	Foam test	+
Tannins	Gelatin test	-
Reducing Sugars	Benedict's test	-

Key, (+) = presence of the phytochemical; (-) = undetected

Determination of Minimum Bacteriostatic Concentration (MBC) and Minimum Bacteriostatic Concentration/Minimum Inhibitory Concentration (MBC/MIC)

The antimicrobial studies of the ethanolic *C. odorata* extract was evaluated through the broth micro-dilution method to obtain the MICs and MBCs. Table 4 gives the MIC, MBC and MBC/MIC concentrations of the plant extract.

Table 4: Antimicrobial activity (MIC analysis) of the extract of *C. odorata* against strains that cause nail infections

Organisms	MIC	MBC	MBC/ MIC	Comment
<i>C. albicans</i>	0.49	1.563	3.19	Fungicidal
<i>S. aureus</i>	12.50	25.00	2.00	Bactericidal
<i>S. pyogenes</i>	6.25	25.00	4.00	Bactericidal

Kirby-Bauer agar well diffusion method of plant extract *C. odorata* Leaves

The ethanolic extract of *C. odorata* leaves was tested against selected microorganisms. The extract of *C. odorata* showed the highest activity against *Candida albicans* followed by *Streptococcus pyogenes* and the least microbial activity against *Staphylococcus aureus*. The positive control used for the bacterial stains (tetracycline) showed good antimicrobial activity

against *Staphylococcus aureus* (22 ± 2.08 mm) but *Streptococcus pyogenes* showed resistance to it as well as resistance to the negative control (ethanol). *Candida albicans* was resistant to the negative control and the positive control (Nystatin). Table 5 shows the zones of inhibitions of agar disc diffusion for the ethanolic extract of *C. odorata* leaves against the selected organisms. The zones of inhibition of *C. odorata* leaves extract against the selected organisms (Figure 6)

Table 5 Zones of inhibitions of agar well diffusion for the ethanolic extract of *C. odorata* leaves against test organisms

Zones of inhibitions/ mm Mean/ Standard dev.			
Plant Extract	<i>Candida albicans</i>	<i>Streptococcus pyogenes</i>	<i>Staphylococcus aureus</i>
	18.33± 0.63	14.60± 0.39	8.60 ± 2.85



(a)



(b)



(c)

Figure 6 Zone of inhibition for the ethanolic extract of *C. odorata* against (a) *C. albicans* (b) *S. pyrogenes* (c) *S. aureus* (PC: Nystatin, PC: Tetracycline, NC: Ethanol)

Formulated antimicrobial nail polish with *C. odorata* The pictures of the formulated nail polish samples tested in this work. (Figure 7)

a) First formulation



Figure 7 Formulated nail polish (red) with *C. odorata* and the controls without the extract

(b) Second formulation

The formulated nail polish (blue) with the *C. odorata* leaves and ones without the plant extract

together with the marketed nail polish (Figure 8a and 8b)

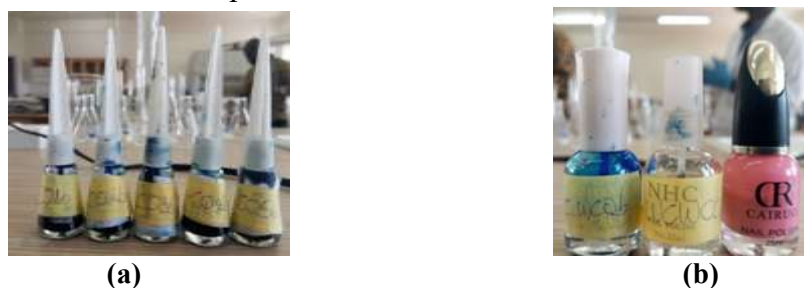


Figure 8 (a, b) Formulated nail polish (blue) with *C. odorata*, with color, colorless and standard nail polish.

Evaluation of Herbal Nail Polish

The formulated nail polish were subjected to preliminary evaluation tests which included functionality tests, the Bureau of Indian standards and antimicrobial studies against microorganisms that cause nail infections

Functionality Tests

Stability tests

The formulated nail polish showed good stability when stored at 37 ± 2 °C. After 1 month, the formulated nail polish samples were inspected for their organoleptic characteristics (color, smell,

texture, and consistency) and there was no significant change in these characteristics.

Bureau of Indian Standards

Smoothness to flow

The smoothness of flow for all the nail polish samples showed a satisfactory flow property when compared to the marketed nail polish, however, the flow of the formulated nail polish samples was faster than the marketed nail polish with formulations CO1b, CO2b, CO3b, CO4b, CO5b, WCWCO₂ and CWCO_b giving a rough film texture when felt with the fingers.

Gloss

The gloss of the nail polish samples and the marketed nail polish were determined by applying a film at the same consistency with the nail polish

brush on a glass slide of area 137.81cm² and observing them visually. Table 6 gives a summary of the results for the gloss property of the nail polish samples.

Table 6 Results of the gloss property of the nail polish samples.

Nail polish samples	Gloss property	Comment
Standard nail polish	Pass	Shiny and wet-looking
CO1	Pass	Shiny and wet-looking
CO2	Pass	Shiny and wet-looking
CO3	Pass	Shiny and wet-looking
CO4	Fail	Dark, rough with particles of plant extract
CO5	Fail	Dark, rough with particles of plant extract
CO1b	Satisfactory	Losses gloss upon drying
CO2b	Satisfactory	Losses gloss upon drying
CO3b	Satisfactory	Losses gloss upon drying
CO4b	Satisfactory	Losses gloss upon drying
CO5b	Satisfactory	Losses gloss upon drying
WCWCO	Pass	Shiny and wet-looking
WCWCO ₂	Satisfactory	Losses gloss upon drying
CWCO	Pass	Shiny and wet-looking
CWCO _b	Satisfactory	Losses gloss upon drying

Drying time

The drying times for the nail polish formulations were found to be between 131.01 ± 6.25 seconds and 2606.00 ± 1.12 seconds on average at room temperature. The marketed nail polish showed an average drying time of 82.36 ± 6.30 seconds Table 7 gives the average drying time of the nail polish samples.

Table 7: Drying time of the formulated nail polish samples

Nail polish samples	Drying time(s) Mean/Standard dev.
Standard nail polish	82.36 ± 6.30
CWCO _b	131.01 ± 6.25
CO5b	181.11 ± 6.10
CO4b	214.51 ± 6.00
CO3b	222.90 ± 5.90
CO1b	391.00 ± 5.48
WCWCO ₂	522.19 ± 5.09
CO2b	634.00 ± 4.74
CO5	1024.00 ± 3.59
CO4	1160.00 ± 3.19
CO3	1164.00 ± 3.17

CO2	1284.00 ± 2.82
CO1	1962.00 ± 0.70
WCWCO	2361.00 ± 0.39
CWCO	2606.00 ± 1.12

Non-volatile content

This test was done to check the quantity of the non-volatile content in the nail polish formulations since the solvent used was volatile. The non-volatile content in percentage terms for the formulated nail polish formulations were found to be in the range of 29.52 ± 1.40% and 52.24 ± 9.11% with the least non-volatile content observed in CO1b and the highest observed in CO5b. Table 8 illustrates the non-volatile content of the nail polish samples expressed in percentages Mean/Standard dev.

Table 8 Non-volatile content of the nail polish samples expressed in percentages Mean/Standard dev.

Nail polish Samples	Percentage non-volatile content
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SNP	18.90±6.31%
CO1	39.06±3.01%
CO2	36.54±1.85%
CO3	37.76±2.40%
CO4	39.55±3.23%
CO5	43.91±5.25%
CO1b	29.52±1.40%
CO2b	39.71±3.31%
CO3b	36.96±2.04%
CO4b	33.98±0.66%
CO5b	2.24±9.11%
WCWCO	21.18±5.30%
WCWCO ₂	27.40±2.38%
CWCO	20.73± 5.47%
CWCOb	28.60±1.83 %

In vitro adhesion

In vitro adhesive strength of nail polish samples was determined by the film peel-off test with the nail polish spread evenly on a constant area of 8.64cm². The percentage of film peel-off in the nail polish samples was found to be in the range of 2.90± 3.32% to 93.17± 4.48%. Table 9 gives the results of the *in vitro* adhesion (peel-off test) of the nail polish samples expressed in percentages. Percentage peel off = (area of peel off /area of spread) × 100%. Figure 9 gives the pictures of the *in vitro* adhesion results of some nail polish samples.

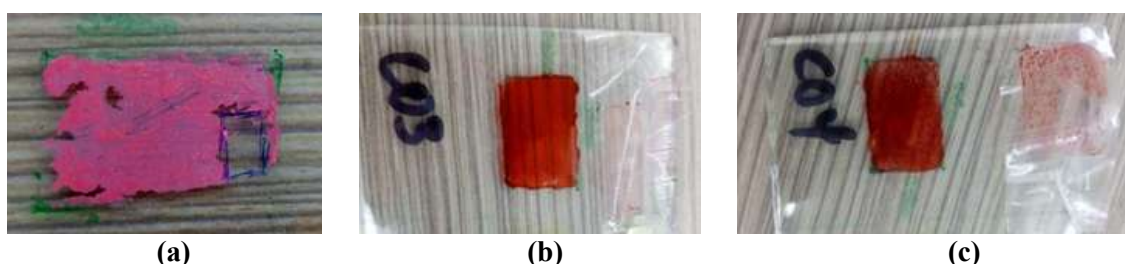


Figure 9 *In-vitro* adhesion tests results of (a) Standard nail polish (SNP) (b) CO3 (c) CO4

Water resistance

Water resistance tests were conducted to evaluate the water resistance of prepared nail polish samples. All the nail polish samples showed a decrease in weight after the water resistance test indicating a satisfactory water resistance and lower water permeability except for CO3 and CO1 that showed poor water resistance resulting in increased weight. CO4b showed excellent water resistance due to a decreased weight after the water resistance tests while the other nail polish samples showed good water resistance due to moderate decrease in weight after the water resistance tests. The results of the water resistance tests are given in Table 10.

Table 10 Results of water resistance for the nail polish samples Mean/Standard dev.

Nail polish samples	Difference in weight (g)	Water resistance
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CO4b	0.0347 ±0.016	Excellent
CO1b	0.0321 ±0.014	Good
CO2	0.0297 ±0.011	Good
WCWCO ₂	0.0291 ±0.011	Good
CWCOb	0.0285 ±0.010	Good
CO4	0.0275 ±0.009	Good
CO3b	0.0265 ±0.008	Good
CO5b	0.0244 ±0.006	Good
CO2b	0.0235 ±0.005	Good
CO5	0.0220 ±0.003	Good
SNP	0.0201 ±0.002	Good
CO1	0.0232 ±0.088	Poor
CO3	0.0704 ±0.09	Poor

Antimicrobial property determination of the formulated nail polish

Kirby-Bauer agar well diffusion method

The nail polish samples (CWCO and WCWCO) formulated using the first formula and without the plant extract were tested against the test organisms as described previously and they showed variable

antimicrobial activities. With WCWCO showing no activity against *S. pyrogenes*. The nail polish sample showed the highest antimicrobial activity against *C. albicans* (23 ± 1.09 mm) and the lowest activity against *S. aureus* (7.33 ± 2.00 mm) the results showed good antimicrobial activity against most of the selected strains. *C. albicans* and *S. pyrogenes* were resistant to both the positive and negative controls. *S. aureus* however, was susceptible to the positive control (22 ± 1.09 mm), Figures 10 and Figure 11 give the results of the antimicrobial activity of WCWCO against the test

organisms. Figures S1, S2 and S3 give the zones of inhibition of the ethanolic *C. odorata* extract, WCWCO and CWCO when tested against *S. aureus*, *C. albicans*, and *S. pyrogenes*.

CWCO gave the highest anti-microbial activity against *S. aureus* (8.60 ± 0.40 mm) and followed by *C. albicans* (8.33 ± 0.47 mm) and the lowest activity was observed for *S. pyrogenes* (2.33 ± 1.92 mm) with all organisms resistant to the negative control as shown in Figure 11 below.

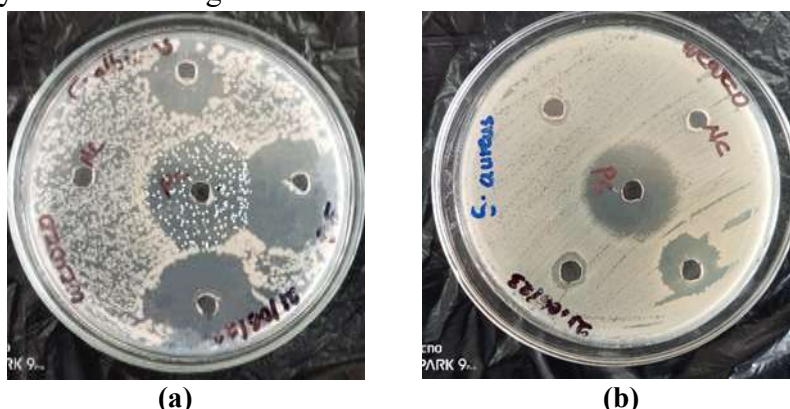


Figure 10: Zone of inhibition of WCWCO nail polish sample when tested against *C. albicans* and *S. aureus* select set of organisms (NC: negative control (ethyl acetate) PC: Positive control (Tetracycline))

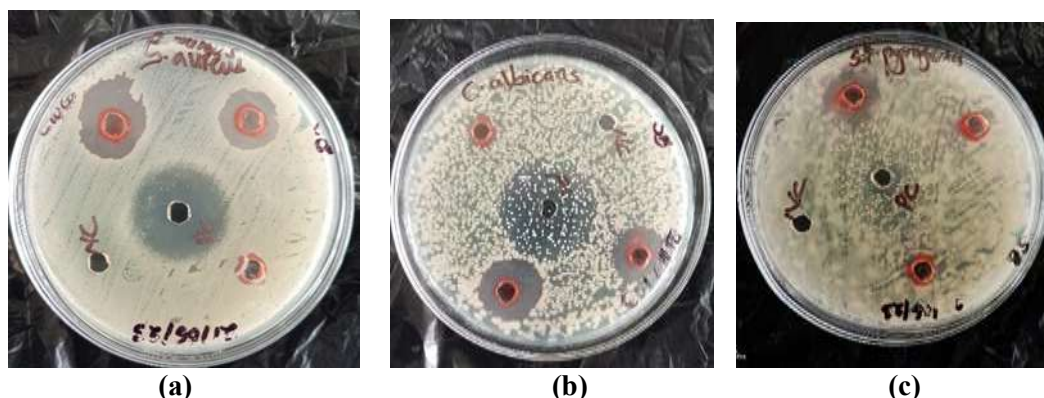


Figure 11: Zone of inhibition of CWCO nail polish sample when tested against (a) *S. aureus* (b) *C. albicans* and (c) *S. pyrogenes*.

Determination of MBC and MBC/MIC Concentration

The antimicrobial studies of nail polish samples (CO1b, CO2 CO3b, CO4b, CO5b, CWCOb and WCWCO₂) were evaluated using the broth micro-dilution methods and the MICs and MBCs results

were obtained. The nail polish samples formulated with *C. odorata* and a red colorant interfered with the red dye used in the MICs analysis hence the blue coloured nail polish was used.

Table 11 gives the MIC, and MBC results for all the nail polish formulations and controls against *C. albicans*, *S. aureus* and *S. pyrogenes*.

Table 11 MIC and MBC results for all the nail polish formulations and controls against *C. albicans*, *S. aureus* and *S. pyrogenes*.

Organisms		MIC	MBC	MBC/MIC	Comment MBC/MIC
<i>C. albicans</i>	CO1b	25	25	1.00	Fungicidal
	CO2b	25	50	2.00	Fungicidal
	CO3b	50	50	1.00	Fungicidal
	CO4b	50	50	1.00	Fungicidal
	CO5b	50	>50	>1.00	Fungicidal
	CWCOb	50.00	50.00	1.00	Fungicidal
	WCWCO ₂	50.00	>50.00	>1.00	Fungicidal
<i>S. aureus</i>	CO1b	25	50	2.00	Bactericidal
	CO2b	50	50	1.00	Bactericidal
	CO3b	50	50	1.00	Bactericidal
	CO4b	50	50	1.00	Bactericidal
	CO5b	50	>50	>1.00	Fungicidal
	CWCOb	25.00	50.00	2.00	Bactericidal
	WCWCO ₂	25.00	50.00	2.00	Bactericidal
<i>S. pyrogenes</i>	CO1b	25	50	2.00	Bactericidal
	CO2b	25	50	2.00	Bactericidal
	CO3b	50	>50	>1.00	Bactericidal
	CO4b	50	>50	>1.00	Bactericidal
	CO5b	50	>50	>1.00	Bactericidal
	CWCOb	50.00	50.00	1.00	Bactericidal
	WCWCO ₂	50.00	>50.00	>1.00	Bactericidal

Key: Values are in $\mu\text{L}/\text{mL}$, MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration. Experiment was carried out in triplicate.

Reference range for MBC/MIC: The antimicrobial activity of the nail polish samples were classified as bactericidal/fungicidal if the ratio of MBC/MIC was less than or equal to 4, and as bacteriostatic/fungistatic if it was more than 4 [35].

DISCUSSION

Herbal cosmetics are natural and devoid of any potentially dangerous synthetic compounds that could endanger human health. These herbal

cosmetics are used substantially in various permissible range of cosmetic ingredients to provide specific cosmetic health benefits and are termed cosmeceuticals [36]. This has created an increasing demand for herbal cosmetics since they comprise a remarkably diverse array of bioactive substances with a variety of unique pharmacological and technical applications, including natural antioxidants, natural preservatives and natural colorants [37]. These herbal cosmetics have also been tested and proven to have less allergic reactions on the skin, hair and nails compared with synthetic ones with profound allergic reactions and irritations to the body and being environmentally unfriendly [38]. We therefore decided to formulate herbal nail polish with antimicrobial properties against some nail



infection causing organisms using the ethanolic extract of *C. odorata*. The plant extract was tested for its antimicrobial activity against the selected organisms that cause nail infections to justify its inclusion in the nail polish formulations. The antimicrobial activity of the *C. odorata* leaf extract using agar well diffusion showed a significant activity with the highest activity observed against *C. albicans*, followed by *S. aureus* and *S. progenies*. The results corresponds to a study that assessed the antimicrobial activity of *C. odorata* leaves against organisms that cause wound infections, this showed substantial antimicrobial activity against *S. aureus* [39] and *C. albicans* when the effect on the plant extract against some human pathogens were assessed [40]. The results of the antimicrobial susceptibility tests of the same ethanolic plant extract also showed higher activity against *C. albicans* and *S. pyogenes* with moderate activity observed for the benzene, ethyl acetate, chloroform, and water extracts against the same microorganisms [41]. Above all *S. aureus* was susceptible to the Tetracycline but *S. pyrogenes* was resistant this was also observed for *C. albicans* to Nystatin. All the organisms were resistant to the negative control except *S. aureus*. Comparing the MICs and MBC/MIC of *C. odorata* leaf extract [100 mg/mL] against the organisms in the study, the extract was fungicidal against *C. albicans* with the extract being the best at the lowest concentrations. All the nail polish formulations were subjected to stability, functionality, and bureau of Indian standard tests. The stability tests were used to determine the shelf life and storage condition of the nail polish samples and the results indicated that the nail polish formulations with the herbal extract or without, showed good stability when it was stored at a temperature of 37 ± 2 °C. After 1 month, the formulated nail polish samples were inspected for their organoleptic characteristics (color, smell, texture, and consistency and there was no

significant change in the organoleptic characteristics which also correlates with a study of formulated medicated nail polish samples with tolnaftate [42], and were followed up with the bureau of Indian Standards tests. Smoothness of flow for all the nail polish samples showed satisfactory flow compared to the marketed nail polish however the rate of flow of the nail polish samples were faster than the marketed nail polish due to low viscosity of the nail polish. Formulations with *C. odorata* leaves (CO1, CO2 and CO3) passed the gloss test with a shiny look when compared with the marketed nail polish but two formulations (CO4 and CO5) failed the test with dark particles of the plant extracts on the surface when applied on the glass slide, this may be due to the higher amounts of the extract used in the formulation which did not dissolve completely. Formulations from the second formula (CO1b, CO2b, CO3b, CO4 and CO5b) showed satisfactory gloss properties when compared to the marketed nail polish. The controls of the first formulation (CWCO and WCWCO) had similar gloss in comparison to the marketed nail polish whilst the samples of the second formula (CWCO_b and WCWCO₂) showed satisfactory gloss property. Drying time for all the nail polish formulations were found to be between 131.01 ± 6.25 seconds and 93.98 ± 20.47 seconds on average under room temperature as compared to the marketed nail polish 82.36 ± 6.30 seconds, however there was a decrease in drying time as the amount of *C. odorata* extract increased showing that the plant extract increased the viscosity of the formulations making them dry faster. The nail polish formulations were subjected to non-volatile content test and it was observed that for the formulation with *C. odorata* (CO1, CO2, CO3, CO4 and CO5) the non-volatile content was between $36.54 \pm 1.85\%$ and $43.91 \pm 5.25\%$ with CO2 being the lowest and CO5 the highest. For the second formulation with *C. odorata* leaf extract,



(CO1b, CO2b, CO3b, CO4b and CO5b) the non-volatile content was between $29.52 \pm 1.40\%$ and $52.24 \pm 9.11\%$. The controls were also between $21.18 \pm 5.30\%$ and $28.60 \pm 1.83\%$ with the marketed nail polish sample containing between $18.90 \pm 6.31\%$ non-volatile content. It can therefore be observed that the non-volatile content decreases as the amount of the plant extract increases with a few inconsistencies (Table 11), which is contrary to a reported study where the polymer concentration increases as the non-volatile content increases [43].

In vitro adhesive strength of nail polish samples was determined by film peel off test. The percentage of film peel-off in the nail polish samples was found to be in the range of $2.90 \pm 3.32\%$ to $93.17 \pm 4.48\%$. Comparing the nail polish formulations that showed the best adhesions, CO3, CO4, and CO5 showed excellent adhesion to the glass slide with no significant peel-off, also CO2 and CO1 showed very good adhesion with an area of peel-off being 0.25 cm^2 and 0.5 cm^2 which was slightly better than standard nail polish peel off with peel off area of 0.6 cm^2 . The least adhesion with the highest peel off % was seen in CO3b and the highest adhesion but the lowest peel off % was seen in CO2. It was also observed that the higher the area of peel off the lower the adhesion of the nail polish samples. Water resistance of prepared nail polish formulations were evaluated using the water resistance test. The amount of water absorbed by the nail polish samples after keeping in water bath at 37°C for 24 hours was found to be generally low among the nail polish samples except for CO3 and CO1 which showed a higher increase in weight after the water resistance test with CO3 having higher increase in weight ($0.1309 \pm 0.18 \text{ g}$) than CO1 ($0.0837 \pm 0.09 \text{ g}$). Thus, CO3 had a poorer water resistance than CO1. Amongst the nail polish samples with a decreased weight after

the water resistance test, the marketed nail polish sample (SNP) showed the highest decrease in weight ($0.0404 \pm 0.007 \text{ g}$) and therefore gave a good water resistance. CO4b showed the smallest decrease in weight ($0.0258 \pm 0.028 \text{ g}$) amongst the formulated nail polish samples hence had excellent water resistance. In all, CO3 showed the highest increase in weight and hence the lowest water resistance whilst CO4b showed the lowest increase in weight and hence the highest water resistance. The antimicrobial activity of the nail polish samples without the plant extract using the first formula (CWCO and WCWCO) showed substantial antimicrobial activity against *C. albicans*, *S. aureus* and *S. pyrogenes*. CWCO nail polish sample [100 mg/mL] showed the highest zone of inhibition in mm from the agar diffusion assay against *S. aureus*. All organisms were resistant to ethyl acetate the negative control throughout the study. The WCWCO nail polish sample showed a higher activity against *C. albicans* followed by *S. aureus* but showed no activity against *S. pyrogenes*. The MIC analysis showed CO1b exhibit a similar antimicrobial activity against *C. albicans*, *S. aureus* and *S. pyrogenes* with an MIC of $25 \mu\text{L}/\text{mL}$. CO2b gave an MIC of $25 \mu\text{L}/\text{mL}$ for *C. albicans* and *S. pyrogenes* and an MIC of $50 \mu\text{L}/\text{mL}$ for *S. aureus*. CO3b, CO4b, CO5b gave MICs of $50 \mu\text{L}/\text{mL}$ against *C. albicans*, *S. aureus* and *S. pyrogenes*. Comparing these results to the MIC of the *C. odorata* extract, it was observed that *C. odorata* leaves give MIC as low as $0.49 \mu\text{L}/\text{mL}$, $12.50 \mu\text{L}/\text{mL}$ and $6.25 \mu\text{L}/\text{mL}$ to kill 50% of the organisms whilst the nail polish samples gave MICs of 25 and $50 \mu\text{L}/\text{mL}$ for activity against the same organisms. The nail polish samples without the plant extract (CWCO_b and WCWCO₂) gave MICs of $>50 \mu\text{L}/\text{mL}$ whilst CWCO_b gave an MIC of $25 \mu\text{L}/\text{mL}$ against *S. aureus*. It can be observed that generally the higher the amount of the plant extract the lower the antimicrobial activity which



is contrary to a reported study [44], where a mixture of herbal plants (lemon grass, garlic etc) were used in very small amounts in nail polish formulations against *C. albicans* and discovered that as the concentration of the herbal extracts increases the antimicrobial activity also increased. This suggest that there is a threshold concentration for optimum activity beyond which the extract in the presence of other constituents of the formulation lead a lower activity. Using the extract alone the activity increases with increasing concentration but in the presence of nitrocellulose, ethyl acetate, camphor, castor oil, the colourant and polyethylene glycol the activity of the *C. odorata* leaf extract is suppressed.

CONCLUSION

C. odorata leaf extract is widely used as topical treatment for wounds, nail infections and in other cosmetics in wound- healing balms with promising antimicrobial activity. From the results obtained it can be concluded that the formulations showed satisfactory stability and functionality. They showed good antimicrobial activity against most of the organisms that cause nail infections but the extract showed a higher activity as well as the controls for both formulation except *S. pyrogenes* in the first formulation. The findings from this work has the potential to change how nail polish products are marketed. With these products they can be marketed for their wound healing properties as well. The incorporation of the extracts into the nail polish reduced the antimicrobial activity in some cases. It is suggested that for future work the compounds in *C. Odorata* should be isolated, characterized and tested for their suitability as additives in the formulated nail polish.

Authorship Contributions

Concept Design: F.O., Literature search: F.O., S.B., Data Collection: S.B., D.N., C.K., Analysis

or Interpretation of results: F.O., S.B., D.N., Writing: Review and editing: F.O., S.B., D.N., J.W.A.J., E.Q., C.K.,

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