Evaluation of physicochemical and antimicrobial properties of creams formulated using *Pterocarpus santalinoides* seeds methanol extract

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**ABSTRACT**

To formulate *Pterocarpus santalinoides* seed methanol extract creams and evaluate them for antimicrobial activity. About 200 g of powdered dried seeds was macerated in 400 ml of methanol for 48 hours, and its collected filtrate was concentrated at 60°C using a rotary evaporator for 6 hours. The antimicrobial activities of the methanol extract against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus niger*, and *Candida albicans* were evaluated. Six cream formulations, P1–P6, were formulated with or without varying the methanol extract concentrations of *P. santalinoides*. The creams were oil-in-water in nature, with good homogeneity, extrudability, spreadability, and ease of removal. The creams’ pH was between 5.0 ± 0.29 and 5.5 ± 0.17. The viscosities of the formulations ranged from 3,600 to 48,950 mPas at 6 rpm and 1,240 to 6,390 mPas at 60 rpm using spindle 4. The microorganisms used were susceptible to the effect of the methanol extract at 100 mg/ml. All bacteria tested were also susceptible to the creams, but the fungi tested were susceptible only to formulations P4, P5, and P6. Creams with good physicochemical and antimicrobial activities against test organisms were successfully prepared using the methanol extract of *P. santalinoides*.

**INTRODUCTION**

Creams are semisolid dosage forms prepared for external use in microbes, plants, animals, and humans. They are usually applied to the skin and also mucous membranes such as the rectum or vagina. They are usually semisolid emulsions or viscous liquids with opaque appearances. Their rheological character and consistency depend on the emulsion type, oil-in-water (o/w) or water-in-oil (w/o) type, and on the type of the solids in the dispersed phase (Idson and Lazarus, 2009; Rai et al., 2019) and work at critical micellar concentrations. They are topical preparations that are usually used for cosmetic purposes or therapeutic use. Topical delivery is the skin application of formulations that contain active pharmaceutical ingredients to directly treat disorders of the skin or the skin manifestations of a general disease (e.g., psoriasis) (Chauhan and Gupta, 2020). Various medicaments have been added to creams to achieve therapeutic or prophylactic effects, and they include antimicrobials (Deepika and Singh, 2017; Okafo et al., 2021; Pal et al., 2013), antibacterials/anti-inflammatories (Sekar and Jalil, 2017), analgesics/anti-inflammatory (Bolla et al., 2020), antiaging medicaments (Moldovan et al., 2017), and antioxidants (Hartiadi and Sahamasutri, 2020). The incorporated medicaments may be of synthetic or herbal origin.

Medicinal plants are of great use in traditional or herbal medicine and as a precursor of many modern medicines (Emencheta et al., 2019; Odeh and Tor-Anyiin, 2014). This
is because of the presence of many secondary metabolites in medicinal plants. Plant parts such as leaves, fruits, seeds, stems, roots, and barks or whole plants are used for healing purposes to cure different human or other animal diseases or as prophylactics (Mba-Omeje et al., 2017). Recently, there has been a rise in the usage of herbal medicines. This is because of the belief that herbal medicines have little or no side effects, unlike orthodox medicines. They are readily available and usually affordable. Different plant species have been screened to substantiate the traditional claim of their effectiveness as therapeutic agents.

Pterocarpus santalinoides L’Herit. ex DC, family Leguminosae, is a tree that can grow up to a height of 9–12 m and a diameter of 1 m with low, straggling branches (Emencheta et al., 2019). It provides a shade tree that grows along the riverine forests of Africa and tropical South America (Emencheta et al., 2019; Odeh and Tor-Anyiin, 2014). In Nigeria, the plant is used for its medicinal and food values. Soups are prepared from tender and fresh leaves (Ogbonna and Idumah, 2018). The leaves, seeds, fruits, and stem bark are used in treating different ailments. The plant is used in treating gastrointestinal diseases, skin diseases, malaria, diabetes, etc. (Emencheta et al., 2019; Nworu et al., 2009; Odeh and Tor-Anyiin, 2014). The antimicrobial (Emencheta et al., 2019; Odeh and Tor-Anyiin, 2014) and antioxidant (Akanire-Ejim et al., 2018) activities of extracts from several plant parts have been studied. Escherichia coli, Salmonella typhi, Staphylococcus aureus, Proteus mirabilis, and Candida albicans were susceptible to the antibacterial properties of butanol, ethanol, and the aqueous extracts of P. santalinoides, but Streptococcus pyogenes and Pseudomonas aeruginosa were resistant (Odeh and Tor-Anyiin, 2014). Emencheta et al. (2019) reported the antimicrobial potentials of the different plant parts against S. typhi and E. coli but negligible antifungal activities. Most of the research works on the antimicrobial potentials of P. santalinoides were conducted using the leaves, barks, and stems, but very little has been done using the seeds.

This study was carried out to formulate the methanol extract of P. santalinoides seeds into creams and to determine the physicochemical and antimicrobial potentials of the prepared creams.

MATERIALS AND METHODS

Materials

The materials used were as follows: methanol (JHD, Guangdong Guanghua Chemical Factory Co. Ltd., China), liquid paraffin (Niram Chemicals, India), nutrient agar, Sabouraud dextrose agar (Titan Biotech, India), Mueller-Hinton agar (MHA) (Titan Biotech, India), nutrient broth, propylparaben (Kernal, India), methylparaben (Central Drug House, India), olive oil (Goya), Tween 80 (Guangdong Guanghua Sci-Tech Co. Ltd., Guanghua, China), Span 80 (Guangdong Guanghua Sci-Tech Co. Ltd., Guanghua, China), cetostearyl alcohol, glycerin (Merck Schuchardt OHG, Hohenbrunn, Germany), ketoconazole, and gentamicin.

Organisms

Staphylococcus aureus, P. aeruginosa, Bacillus subtilis, E. coli, Aspergillus niger, and C. albicans were collected from the

Department of Pharmaceutical Microbiology and Biotechnology’s laboratory, Delta State University, Abraka.

Collection and identification of plant material

Pterocarpus santalinoides seeds were obtained from a farm in Enugu, Nigeria. The plant was identified by Mr. Felix Nwafor, a taxonomist in the Department of Pharmacognosy and Environmental Medicine, University of Nigeria, Nsukka, Nigeria. It was issued a voucher number, PCG/UNN/0036.

Extraction of P. santalinoides seeds

Pterocarpus santalinoides seeds were dried under shade and ground to a powder. A 200 g powder was macerated for 48 hours in 400 ml of methanol. It was filtered using a Whatman filter paper, and the filtrate was concentrated to semisolid form at 60°C for 6 hours using a rotary evaporator (Rotavapor RII, Büchi Labortechnik AG, Flawil, Switzerland). It was concentrated until a constant weight to ensure complete evaporation of methanol.

Physicochemical properties of the extract

The extract’s organoleptic properties such as color and odor were observed. The extract’s pH was also measured.

Phytochemical screening

The extract was examined for the absence or presence of phytochemicals such as alkaloids, saponins, flavonoids, terpenoids, and phenols using standard methods as stated in the literature (Biswas et al., 2013; Mba-Omeje et al., 2017; Onyekere et al., 2019; Trease and Evans, 2002). Phytochemicals were also determined quantitatively using the methods of Ezewu and Ejikeme (2016) and Khalifa et al. (2017).

Antimicrobial assay of the methanol extract of P. santalinoides seeds

This was conducted using the agar well diffusion technique. MHA was prepared following the manufacturer’s specifications. It was autoclaved, and 20 ml of it was aseptically transferred into each Petri dish. These were then allowed to solidify. The extract was two-fold serially diluted to obtain the following concentrations: 100, 50, 25, 12.5, 6.25, and 3.125 mg/ml. The bacteria were spread on the agar in the Petri dishes to inoculate them. Wells were bored into each agar plate using a sterile cork borer. The different concentrations of the extract were transferred to the respective wells and labeled appropriately. Gentamicin was used as the positive control (40 mg/ml) and was transferred to the well at the center. The plates were incubated for 24 hours at a temperature of 37°C. The inhibition zone diameters (IZDs) were measured in millimeters. The process was repeated using SDA and fungal isolates. The incubation was done for 48 hours at room temperature. Ketoconazole (20 mg/ml) was used as the positive control. The IZDs were measured in millimeters.

Determination of minimum inhibition concentration (MIC)

The method of Okafo et al. (2020) was used. The two-fold serial dilutions (1 ml) of the extract were transferred into respective labeled Petri dishes. A 19 ml quantity each of molten MHA for test bacteria and molten SDA for test fungi were transferred into the labeled Petri dishes. The agars in the Petri
dishes were allowed to solidify, and the respective test organisms were streaked on them. The Petri dishes containing the bacteria were incubated for 24 hours at 37°C, and those containing the fungi were incubated for 48 hours at room temperature. The MIC of the extract for the test organisms was determined.

**Preparation of creams**

*Pterocarpus santalinoides* creams were prepared using the method of Hartiadi and Sahamastuti (2020) according to the formula shown in Table 1. About 7.15 g of Tween 80 was mixed with 10 g of glycerin in a beaker using a magnetic stirrer with a hot plate. Then, 0.54 g of methylparaben and 0.06 g of propylparaben were added to the Tween 80/glycerin mixture and stirred properly at 70°C using the magnetic stirrer with a hot plate. A 2.5 g quantity of the *P. santalinoides* methanol extract was dissolved in 58.9 g of distilled water and was added to the Tween 80/glycerin mixture. The mixture was stirred using the magnetic stirrer with a hot plate at 70°C to form the aqueous phase. The oil phase was produced by melting 8 g of cetostearyl alcohol in a water bath at 70°C. A 10 g quantity of olive oil or liquid paraffin and a 2.85 g quantity of Span 80 were added and stirred properly with a glass rod. The oil phase was added gradually to the aqueous phase and stirred using a magnetic stirrer at a temperature of 70°C for 5 minutes. The stirring continued until the mixture cooled. The cream was transferred into a container and was stored in a cool place. This was repeated for all the formulations using the formula in Table 1.

**Evaluation of creams**

**Physical appearance**

The organoleptic properties like color, odor, and phase separation were noted.

**pH**

The pH of the creams was determined using a pH meter (Hanna Instruments, India).

**Spreadability**

The method of Kaushik *et al.* (2020) was used with slight modifications. Spreadability was evaluated by placing 0.5 g of the cream within a premarked circle of a 1 cm diameter on a glass plate. A second glass plate was placed over the first plate, and a 300 g weight was kept on the upper glass plate for 5 minutes. The weight caused the cream to spread, and this resulted in an increase in the diameter of the circle. The increase was measured in centimeters, and the results were taken as comparative values for spreadability.

**Homogeneity**

The creams were viewed under a microscope to examine their homogeneity (Hartiadi and Sahamastuti, 2020). Homogeneity was also examined by visual inspection (Okafo *et al.*, 2019; Ubaid and Murtaza, 2016) and by pressing the cream between the thumb and index finger (Chen *et al.*, 2016).

**Ease of removal**

This was evaluated by washing off the cream applied to a specific part of the body with flowing tap water (Ashish *et al.*, 2013; Sekar and Jalil, 2017).

**Viscosity**

The viscosity of the creams was evaluated with a Brookfield viscometer at a temperature of 28°C. Spindle 4 was inserted in the creams and was rotated at 6, 12, 30, and 60 rpm, respectively. The displayed readings were recorded.

**Centrifugation test**

A 2 g quantity of the sample was put in the centrifuge (Remi Elektrotechnik Ltd., Vasai, India) and was rotated for 30 minutes at 3,000 rpm at room temperature. The creams were evaluated for phase separation at the end of the centrifugation (Chandrasekar *et al.*, 2018).

**Extrudability**

Extrudability was based upon the quantity in the percentage of cream extruded from the tube on the application of a certain load. Collapsible lacquered aluminum tubes with 10 mm openings were filled with the respective formulations. A 1 kg load was placed on each tube, and the quantity of the formulation that was extruded 60 seconds after each of the tubes was opened was recorded (Fauzee and Walker, 2020; Ilievksa *et al.*, 2016; Khan *et al.*, 2020; Ugandar and Deivi, 2013). The extrudability of the formulations was calculated using the following equation (Asija *et al.*, 2015):

<table>
<thead>
<tr>
<th>Formulations</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
<th>P5</th>
<th>P6</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pterocarpus santalinoides</em> methanol extract (g)</td>
<td>—</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>10</td>
</tr>
<tr>
<td>Olive oil (g)</td>
<td>—</td>
<td>10</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Liquid paraffin (g)</td>
<td>10</td>
<td>—</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Tween 80 (g)</td>
<td>7.15</td>
<td>7.15</td>
<td>6.674</td>
<td>7.15</td>
<td>7.15</td>
<td>7.15</td>
</tr>
<tr>
<td>Span 80 (g)</td>
<td>2.85</td>
<td>2.85</td>
<td>5.326</td>
<td>2.85</td>
<td>2.85</td>
<td>2.85</td>
</tr>
<tr>
<td>Cetostearyl alcohol (g)</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Methylparaben (g)</td>
<td>0.54</td>
<td>0.54</td>
<td>0.54</td>
<td>0.54</td>
<td>—</td>
<td>0.54</td>
</tr>
<tr>
<td>Propylparaben (g)</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>—</td>
<td>0.06</td>
</tr>
<tr>
<td>Glycerin (g)</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Distilled water (g)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
Swabrick et al., 2005 and dilution tests (Swabrick et al., 2005). A scarlet red dye was mixed with the creams. A drop of the cream was placed on a microscope slide, covered with a cover slip, and then examined under a microscope. Observations of the color of the dispersed globules and the background were made. The dilution test was conducted by adding 10 ml of distilled water to 1 g of the cream in a beaker. It was stirred very well and was observed for separation.

**Antimicrobial susceptibility test of the creams**

The antimicrobial activity of the creams was evaluated using the disc diffusion method. MHA and SDA were prepared, sterilized, and transferred into Petri dishes, where they were allowed to solidify. They were inoculated with the test bacteria and fungi, respectively, using sterile cotton swabs. Sterile blank discs were impregnated individually with the respective cream formulations and were placed onto the inoculated Petri dishes. The Petri dishes containing bacteria were inverted and incubated for 24 hours at 37°C while those that contained fungi were incubated at 25°C for 48 hours. The antimicrobial activity was evaluated by measuring the IZDs to the nearest millimeter (Sekar and Jalil, 2017).

**Skin irritation test**

The Research and Ethical Committee of the Faculty of Basic Medical Sciences of Delta State University, Abraka, Nigeria, issued an ethical clearance (Resolution No. REC/FBMS/DELSU/21/98) for this study before it was carried out. Male Wistar rats were used for this study. The Wistar rats were divided into four groups, namely, control, standard, test group 1, and test group 2. The hairs in the dorsal region of the rats were removed a day prior to the start of the study. Formalin (0.8%), a standard irritant, was applied to the standard group after 24 hours of shaving the rat’s skin. Formulations P3 and P6 were applied to test groups 1 and 2, respectively. Distilled water was applied to the control group. The rats were observed for any irritation such as erythema or edema at the end of 24 hours (Khullar et al., 2012; Shaik et al., 2019).

**Accelerated stability testing**

The method of Hartiadi and Sahamastuti (2020) was used. The creams were subjected to three cycles of the freezing and thawing test. The freezing was carried out in a refrigerator at 4°C for 24 hours, and the thawing was carried out in an oven set at 40°C for 24 hours to complete a cycle. This was done for three cycles, after which the creams were reexamined for organoleptic properties, pH, homogeneity, centrifugation, and viscosity.

**Statistical analysis**

All experiments were carried out in triplicate for the validity of the statistical analysis and the results were expressed as mean ± SD. The statistical analysis was done using Microsoft Excel and IBM SPSS 23 software. Differences between means were determined with a one-way analysis of variance (ANOVA) at a significance level of $p < 0.05$.

**RESULTS**

**Extraction and phytochemical screening**

The percentage yield of the methanol extract was 10.1% w/w. The extract was dark brown in color with a honey-like odor. The pH of the extract was 5.1. Phytochemical screening of the plant extract was done to ascertain the presence of alkaloids, saponins, terpenoids, flavonoids, and phenols (Table 2). This was evident by the color change when the reagents were added. The quantitative analysis results (Table 2) also indicated the concentrations of the different phytochemical constituents contained in the extract.

**Antimicrobial screening of methanol extract of *P. santalinoides***

The result of the antimicrobial susceptibility test (Table 3) revealed that the methanol extract of *P. santalinoides* at a 100 mg/ml concentration and above had antimicrobial activity against all the microorganisms studied.

**Physicochemical evaluation of methanol extract of *P. santalinoides* seeds creams**

The creams’ physicochemical properties are shown in Table 4. Creams were of varying colors: white (P1), light brown (P2), cream (P3, P4, and P5), and brown (P6). Their pH was between 5.0 ± 0.29 and 5.5 ± 0.17. All the creams had good homogeneity on visual and microscopic inspection. Palpable masses were absent when the creams were felt between the index finger and the thumb. All the creams were easily washed off from the applied skin surface by flowing tap water. The creams were of the o/w type. Upon centrifugation, formulations P1 and P3 were noted to be stable but other formulated creams separated. Formulation P2 cracked and could not reform to a normal cream upon stirring, indicating permanent instability. Formulations P4, P5, and P6 when stirred returned to normal creams; therefore, their instability was temporary (creaming). The formulated creams had good spreadability, though formulations P1 and P4 were less spreadable than the rest. The formulated creams were easily extruded from collapsible tubes (% extrudability of 66.33 ± 0.70 to 90.95 ± 1.46%). All the creams showed pseudoplastic flow (Fig. 1). They displayed shear-thinning behavior; the viscosity decreased as shear stress (spindle speed) increased. The creams’ viscosity was between 1,240 and 48,950 mPas. There was skin irritation among the rats in the control group but none among those rats in the standard and test groups (Fig. 2).

### Table 2. Phytochemical constituents of *P. santalinoides* methanol extract.

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Concentration (% w/w)</th>
<th>Occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>1.5</td>
<td>Present</td>
</tr>
<tr>
<td>Saponins</td>
<td>0.7</td>
<td>Present</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>2.1</td>
<td>Present</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>1.4</td>
<td>Present</td>
</tr>
<tr>
<td>Phenols</td>
<td>1.2</td>
<td>Present</td>
</tr>
</tbody>
</table>
Table 3. Antimicrobial susceptibility of test organisms to methanol extract of *P. santalinoides*.

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Zone of inhibition (mm) produced by different extract concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>200 mg/ml</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>11</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>12</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>10</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>8</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>13</td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>9</td>
</tr>
</tbody>
</table>

Table 4. Physicochemical parameters of formulated creams.

<table>
<thead>
<tr>
<th>Physicochemical property</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
<th>P5</th>
<th>P6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>White</td>
<td>Light brown</td>
<td>Cream</td>
<td>Cream</td>
<td>Cream</td>
<td>Brown</td>
</tr>
<tr>
<td>Type of cream</td>
<td>o/w</td>
<td>o/w</td>
<td>o/w</td>
<td>o/w</td>
<td>o/w</td>
<td>o/w</td>
</tr>
<tr>
<td>pH</td>
<td>5.5 ± 0.17</td>
<td>5.3 ± 0.25</td>
<td>5.4 ± 0.23</td>
<td>5.4 ± 0.46</td>
<td>5.4 ± 0.15</td>
<td>5.0 ± 0.29</td>
</tr>
<tr>
<td>Homogeneity</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
</tr>
<tr>
<td>Ease of removal</td>
<td>Easy</td>
<td>Easy</td>
<td>Easy</td>
<td>Easy</td>
<td>Easy</td>
<td>Easy</td>
</tr>
<tr>
<td>Centrifugation</td>
<td>Stable</td>
<td>Cracked</td>
<td>Stable</td>
<td>Separated (creamed)</td>
<td>Separated (creamed)</td>
<td>Slightly separated</td>
</tr>
<tr>
<td>Skin irritation</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Spreadability* (cm)</td>
<td>3.66 ± 0.54</td>
<td>4.30 ± 0.43</td>
<td>4.56 ± 0.52</td>
<td>3.63 ± 0.75</td>
<td>5.25 ± 0.87</td>
<td>4.50 ± 0.58</td>
</tr>
<tr>
<td>Extrudability* (%)</td>
<td>66.33 ± 0.70</td>
<td>90.95 ± 1.46</td>
<td>82.20 ± 1.38</td>
<td>82.77 ± 0.67</td>
<td>88.78 ± 0.38</td>
<td>84.33 ± 0.74</td>
</tr>
</tbody>
</table>

*Data were expressed as mean ± SD (n = 3).*

Figure 1. Viscosity curves of cream formulations P1–P6.
Antimicrobial susceptibility of the microorganisms to cream formulations P1–P6

The antimicrobial property of the extract was retained by the creams on all the bacteria studied (Table 5). Formulation P1 which contained no extract showed some activity against the bacteria, probably due to the presence of a preservative (methyl- and propylparaben) in its formula. Formulations P1, P2, and P3 had no antifungal effect on the fungi used. The fungi were susceptible to formulations P4, P5, and P6.

Accelerated stability testing

After three freeze-thaw cycles, all the creams maintained good homogeneity and ease of removal. There was a minor decrease in cream pH values (Fig. 3a), but they were not appreciably different (p < 0.05) except for formulation P5. There was an increase in spreadability for formulations P1, P3, P4, and P5 but a decrease in P2 and P6 (Fig. 3b); however, the change was not significant for formulations P2, P3, and P5 (p < 0.05). The shear-thinning property of the creams was retained after the freeze-thaw cycles (Fig. 4a and b), but there was a decrease in viscosity for P1, P2, P4, and P6 and a rise in viscosity for P5. The viscosity of formulation P3 was relatively stable throughout the freeze-thaw cycles. The change in viscosity was significant (p < 0.05) for all the creams except formulation P3.

DISCUSSION

Extraction and phytochemical screening

The yield of the methanol extract of *P. santalinoides* (10.1% w/w) in this work was higher than the 1.97% reported by Emencheta et al. (2019). This could be because of environmental or geographical differences in the plant sources, which may result in different quantities and types of phytochemicals present. The process and efficiency of the extraction method could be because of the causes of the difference in percentage yield. The existence of saponins, flavonoids, alkaloids, and phenols in the extract was confirmed, as reported by Ogbonna and Idumah (2018). Odeh and Tor-Anyiin (2014) reported the presence of terpenoids and saponins-glycosides, which were also observed in this study.

Antimicrobial screening of methanol extract of *P. santalinoides*

*Pseudomonas aeruginosa,* a Gram negative bacterium, was susceptible even at an extract concentration of 6.25 mg/ml. It had a 4–12 mm IZD for the concentrations of 6.25–200 mg/ml used in the research work. The IZDs of the extract at the 25–200 mg/ml concentrations for *E. coli* were 5–11 mm and 7–10 mm for *S. aureus.* The fungi were susceptible to higher concentrations (≥100 mg/ml) of the extract; *C. albicans* had IZD of 10–13 mm while *A. niger* had IZD of 5–9 mm. This study agrees with the work of Odeh and Tor-Anyiin (2014) that reported sensitivity of *S. aureus, E. coli,* and *C. albicans* to ethanol, butanol, and the aqueous extract of the *P. santalinoides* leaf; however, unlike this study, they reported that *P. aeruginosa* was resistant. This study was in disagreement with Emencheta et al. (2019), who reported that *C. albicans* and *A. niger* were resistant to 100 mg/ml of the *P. santalinoides* seeds crude methanol extract, butanol, ethyl acetate, and n-hexane fractions. It is however in agreement with their report on the sensitivity of *E. coli* to the crude methanol extract.

Physicochemical evaluation of methanol extract of *P. santalinoides* seeds creams

The creams possessed various colors due to their varied content of ingredients. Formulation P1 which did not contain the methanol extract remained white in color. Formulation P2 which contained 6.25 mg of the extract and olive oil was light brown while formulations P3, P4, and P5 which contained 6.25 mg of the extract and liquid paraffin were milk or cream colored. Formulation P6 which contained 10 g of the extract and liquid paraffin was brown in color. The brown color was because of the large quantity of extract (dark brown) in the formulation. The color of the creams did not change after the freeze-thaw cycles. Color changes in a cream on storage may signify instability.

The creams’ pH (5.0 ± 0.29–5.5 ± 0.17) was in the normal skin pH range (4–6) (Chen et al., 2016); therefore, there would be a very minute risk of skin irritation (Bolla et al., 2020), and this was corroborated by the results of the skin irritation test, which showed that there was no skin irritation by any of the formulations used. After the three freeze-thaw cycles, the pH values of the creams decreased slightly, though they were still within the normal skin pH range. Marked change in pH for creams is an indication of instability of the formulation. This may arise due to microbial degradation or incongruity between the ingredients in the formula. All the creams possessed good homogeneity on visual and microscopic inspection before and after the accelerated stability study. When the creams were felt between the index finger and the thumb, there were no palpable masses. The creams were washed off easily from the applied skin parts before and after the freeze-thaw cycles, and this showed that there

Figure 2. Skin irritation studies 24 hours after application of (A) formalin, (B) distilled water, (C) formulation P3, and (D) formulation P6.
Figure 3. (a) pH and (b) spreadability of the formulated creams before and after three freeze-thaw cycles. Values are expressed as the mean ± SD ($n = 3$).

Figure 4. Viscosity curves of formulated creams (a) P1–P3 and (b) P4–P6 before (P) and after (P’) three sets of freeze-thaw cycles. Values are expressed as the mean ± SD ($n = 3$).
The determination of tube extrudability is very important in the evaluation of topical preparations such as creams, emulgels, and ointments. Extrudability is the force that is applied to remove the formulation from the tube. The larger the quantity that is extruded, the better the extrudability (Fauzee and Walker, 2020; Ilievksa et al., 2016). Easy extrusion of the preparation from the tube enhances patient compliance. Preparations that are highly viscous may be difficult to extrude, while low viscous ones may be extruded quickly (Obanewa and Oyeniran, 2019).

Viscosity as a parameter is salient in the evaluation of topical preparations since extrudability as well as drug release depends on it (Obanewa and Oyeniran, 2019). The viscosity of all the creams was 3,600–48,950 mPas before and 1,240–6,390 mPas after the accelerated stability study. The creams exhibited pseudoplastic flow. They displayed shear-thinning behavior because the viscosity decreases as shear stress (spindle speed) increases. Formulation P3 maintained stable viscosity before (35,800 mPas) and after (35,950 mPas) the freeze-thaw cycle. Formulations such as P1, P2, and P4 recorded a significant decrease in viscosity, P6 recorded a slight decrease, while P5 recorded an increase. Marked change in viscosity of a cream on storage is an indication of instability.

### Antimicrobial susceptibility of the microorganisms to the creams

The creams retained the activity of the methanol extract against the bacteria used in this study (Table 5). The IZDs recorded by formulation P1 (2–5.5 mm) were low compared to the other formulations (P2–P6) that contained the methanol extract (3–15 mm). The IZDs displayed by the creams against the microorganisms were very much less than those recorded for the control (gentamicin). This may be because the extract was still in the crude form and required further purification and fractionation to isolate the pure active constituent. Formulation P6 (formulated with 100 mg/ml of the extract) exhibited IZD of 6 mm for *C. albicans* and 4.5 mm for *A. niger*, which was comparable to those produced by the methanol extract alone (IZD of 10 mm for *C. albicans* and 5 mm for *A. niger*). However, formulations P4 and P5 that contained 25 mg/ml of the methanol extract showed IZD of 4.5–8.5 mm against *C. albicans* and IZD of 5–6 mm against *A. niger*, respectively. This activity against the fungi by formulations P4 and P5 could not be easily explained because they were formulated with the 25 mg/ml extract concentration and the extract alone at that concentration did not show any inhibitory activity against the fungi. Formulation P2, which had no inhibitory activity against the fungi used in the study, was formulated with the same ingredients as formulation P4 except that olive oil instead of liquid paraffin was used in its formulation. Formulations P1, P2, and P3 were inactive against the test fungi.

### CONCLUSION

This study showed that the methanol extract of *P. santalinoides* has antibacterial activity at a concentration of 25 mg/ml and antifungal activity at 100 mg/ml. The creams had antibacterial activity against the test bacteria. The cream formulated with 100 mg/ml of the extract (P6) exhibited antifungal activity against *C. albicans* and *A. niger*. Some of the creams containing 25 mg/ml (P4 and P5) displayed antifungal activity against the test fungi while the rest did not. The creams all exhibited good physicochemical properties: good homogeneity, ease of removal, type of cream, and spreadability. The pH of the creams was within the normal pH range for the skin. The creams had a good shear-thinning property. Formulations P1 and P3 were stable, formulations P4 and P6 were slightly unstable but reformed to good creams when agitated, while formulations P2 and P5 were completely unstable.

Formulation P3 is chosen as the optimized antibacterial formulation because it exhibited good physicochemical properties and was stable after accelerated stability testing, though it lacked antifungal activity against the test fungi. Formulation P6 displayed good antimicrobial activity against all test bacteria and fungi but was slightly unstable after accelerated stability testing. The other

### Table 5. Antimicrobial susceptibility of the test organisms to formulated creams.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
<th>P5</th>
<th>P6</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>2.0 ± 0.00</td>
<td>8.0 ± 1.41</td>
<td>9.0 ± 1.41</td>
<td>6.5 ± 0.71</td>
<td>4.0 ± 0.00</td>
<td>7.0 ± 1.41</td>
<td>34.0 ± 2.83</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>2.5 ± 0.71</td>
<td>3.0 ± 1.41</td>
<td>4.5 ± 0.71</td>
<td>3.5 ± 0.71</td>
<td>4.5 ± 0.71</td>
<td>4.0 ± 0.00</td>
<td>28.5 ± 0.71</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>4.0 ± 0.00</td>
<td>5.0 ± 1.41</td>
<td>13.0 ± 1.41</td>
<td>15.0 ± 1.41</td>
<td>12.0 ± 1.41</td>
<td>12.0 ± 1.41</td>
<td>27.5 ± 2.12</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>5.5 ± 0.71</td>
<td>9.5 ± 0.71</td>
<td>5.5 ± 0.71</td>
<td>5.0 ± 1.41</td>
<td>6.0 ± 1.41</td>
<td>7.5 ± 0.71</td>
<td>43.0 ± 1.41</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>4.5 ± 0.71</td>
<td>8.5 ± 0.71</td>
<td>6.0 ± 0.00</td>
<td>20 ± 0.12</td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>5.0 ± 1.41</td>
<td>6.0 ± 0.00</td>
<td>4.5 ± 0.71</td>
<td>17 ± 0.32</td>
</tr>
</tbody>
</table>

Data were expressed as mean ± SD.
formulations were either completely unstable or lacked good antimicrobial activity.

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CONFLICTS OF INTEREST

The authors declared no conflicts of interest.

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AUTHORS’ CONTRIBUTIONS

Concept/design and supervision were done by S. E. Okafo, C. O. Anie, and C. A. Alalor. Data acquisition, data analysis, and interpretation were carried out by S. E. Okafo, C. O. Anie, C. A. Alalor, and L. U. Nwankwo. Drafting of the manuscript was done by S. E. Okafo. Critical revision of the manuscript, funding, and final approval were done by S. E. Okafo, C. O. Anie, C. A. Alalor, and L. U. Nwankwo. Statistical analysis, admin, technical, and material support were provided by S. E. Okafo, C. O. Anie, and L. U. Nwankwo.

ETHICAL APPROVALS

The Research and Ethical Committee of the Faculty of Basic Medical Sciences of Delta State University, Abraka, Nigeria, issued an ethical clearance (Resolution No. REC/FBMS/Delsu/21/98).

DATA AVAILABILITY

All data generated and analyzed are included within this research article.

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