Pharmaceutical Application of Thermally Modified Arrinracho Clay as Sunscreen Agent

Edah A.O\(^1\), Wetpin R.A\(^2\), Kolawole J.A\(^2\)

\(^1\)Department of Chemistry University of Jos, Jos Nigeria, 
\(^2\)Department of Pharmaceutical Chemistry University of Jos, Jos Nigeria. PMB 2084 Jos, 930001, Nigeria

Abstract: The overexposure of animal skin to natural sun rays often display a debilitating effect that may manifest as skin cancers and ulcers. Topical sunscreens creams are applications that are readily used to address the issue of prolonged exposure to UVA and UVB rays. Materials, such as clay has been used around the world for centuries by indigenous people as an important medicinal and cosmetic tool. The objective of the study is to evaluate the sunscreen potentials of locally obtained Arrinracho clay deposit in Barkin Ladi, Plateau State, Central Nigeria. The sampling points were located by GPS corresponding to latitude (09° 51' 56.4˝ to 09° 28´´ 55.5˝), longitude (008° 51'05.6' to 08° 51'11.4") and an elevation between (4305 to 4590)ft. The beneficiated and pulverized thermally modified (BPTM) and the non-modified clay samples were tested for microbial contamination. Four sunscreen creams were formulated using standard zinc oxide cream formula and replacing the zinc oxide with the clay sample as the active ingredient. The formulated sunscreen creams were evaluated for topical Sun Protective Factor (SPF) on albino rat's skin, physical appearance and pH. The formulated creams were compared with commercially used Aloe sunscreen cream with SPF 30. The results indicated that the thermally modified clay sample was free from microbial contamination unlike the non-modified clay sample which was found to be contaminated with Staphylococcus aureus and Bacillus specie. The new formulation provided a similar efficacy against sunburn as the commercially used Aloe sunscreen cream with SPF 30. The clay of the present research work is defined as Arrinracho clay as sunscreen agent. The BPTM Arrinracho clays presented a high potential as sunscreen agent.

Keywords: Sun Protective Factor, Cosmetic and Exfoliating Agents

I. INTRODUCTION

The aim of this research is to evaluate the sunscreen potentials of locally obtained Arrinracho clay deposit in Barkin Ladi, Plateau State in Central Nigeria. Clay has been used around the world for centuries by indigenous people as an important medicinal and cosmetic tool. Recently, medical research has emerged which supports the efficacy of clay and it is now becoming increasingly popular as a rediscovered treatment for many health and skin conditions. Externally clay is used to absorb excess oils, dirt and toxins from the skin while simultaneously exfoliating and improving skin circulation. Some clay, such as bentonite is primarily ingested for medicinal purposes such as detoxification or to meet mineral deficiency requirements. Other clay, such as French Green clay and Rhassoul clay are used externally for skin conditions and for cosmetic processes. Clays comes in a variety of colors such as red, green, white, gray and can range in texture from coarse and heavy to fine and fluffy condition. The different colors of clays occur because of their natural mineral content (Holly Xing and Timothy Garland 2012). Cosmetic clay is made up of different mineral content and each clay type has a different effect on the skin. The high mineral content of clay rejuvenates the skin while the clay exfoliates and stimulates blood circulation to the skin. Regular use of clay facials will remove dead skin cells, improve circulation to the skin, remove debris from the skin pores and brings about smooth healthy glow. Each clay has the ability to absorb toxins from the skin but they differ in their levels of absorption. Clays contain massive amount of trace minerals, necessary for good health. This may explain many of the healing properties of clay. Specific trace minerals, which various clays possess, vary widely. Also the amount of any particular trace minerals in any specific clay varies a lot among clay in different locations. For example, the amount of iron in several bentonite clay can vary from well below 1% and up to 10% (Holly Xing and Timothy Garland 2012). The clay of the present research work is defined as Arrinracho pulverized clay samples (ACS) with the following physical properties: the colour ranged from white to slightly off white; its texture is smooth, displaying a free flowing pattern on discharge. The particle size passed 30μm mesh, indicating a fine nature. These are comparable to the acceptable properties of clays characterized by United States Geological Agency (Foley, 1999). The chemical properties mainly contain Silica oxide (49.01%), Aluminum oxide (34.20%), Iron Oxides (3.96%) and minor components of Copper oxide (0.10%), Nickel oxide (0.09%), Zinc oxide (0.21%), Chromium oxide (0.14%), Titanium oxide (0.01%), Calcium oxide (0.27%), and Manganese Oxides (0.01%) (Edah et al 2012). The silica act to provide a physical barrier to the sun. The silica also assists in the dispersion the iron oxide particles within the clay when applied on the human skin (Holly and Timothy 2012). The physicochemical properties of the clay include Parahopeite, Narcite, Kaolinite, Anthop (composite 1), Halloysite, Chrysotile, talc, Riebeckite, Antigorite (composite 2), Gruinit, Cristobalite, Riebeckite, Antigorite (composite 3) and Halloysite, Kaolinite, Antigorite, Anthop (composite 4). (Edah et al 2012).
II. MATERIALS AND METHOD

Thermal Modification of the Clay Materials:
Arrinrasho clay sample from Barkin Ladi, Plateau, Central Nigeria Mortar and pestle; 180µm mesh (sieve), Hot air oven weighing balance and crucible.

Procedure: Fifty grams (50g) of the clay sample was weighed accurately and transferred into a crucible. The crucible containing the weighed clay sample was placed in a calibrated oven at about 300°C and allowed for about 4 hours prior to its formulation into sunscreen cream and microbial contamination testing.

Microbial Contamination Test Materials:

Nutrient Agar (Media). Glass Wares: Petri dish, bijou bottles, conical flask, beaker, measuring cylinder, universal bottles, pipette, and spirit lamp. Others: Hot plate (Gallen Kamp), Autoclave (ALL AMERICAN), Heating incubator (Hospibrand USA), Hot air oven and weighing balance.

Preparation of Nutrient Agar:
Mulleur Hinton Agar was prepared according to manufacturer's instruction. It was mixed in distilled water and boiled, dispensed into bottles and sterilized at 121°C for 15 minutes. Each bottle of Mulleur Hinton was poured in a sterile Petri dish and allowed to set. The plates were dried in hot air oven.

Standardization of Clay Sample:

One gram (1g) of the modified clay sample and the non modified clay sample were diluted into 10mls of distilled water separately. The resulting solutions of the two samples were further standardized by serial dilutions of 1 in 10 dilutions into six different concentrations for each of the samples (modified and non modified clay samples).

Procedure:
The set Agar was flooded with 0.5mls of the diluted concentrations of the two samples on separate sets of Petri dish each and swirled evenly to properly distribute the samples. The inoculated culture media were incubated at 37°C for 24 hours. After which presence or absence of microbial growth was observed.

Microbial Identification Test (Staining):
Materials: Electron light Microscope, slides, spirit lamp, wire loop, immersion oil.
Reagents used: Crystal violet, Lugol Iodine, Ethanol, Safanine.

Procedure:
A colony on the culture grown was picked and transferred unto a slide, fixed and air dried, after which it was flooded with Crystal violet, allowed to stand for 30 seconds then washed with water. The slide was again flooded with Lugol Iodine for 1 minute and then washed off with water. After which the slide was flooded again with Safarine, allowed to stand for 30 seconds and then flooded with water. The resulting slide was placed under the electron light microscope, viewed and snapped with the aid of a phone camera.

Formulation of the sunscreen cream

Materials:
Clay samples, calcium hydroxide, oleic acid, wool fat, Arachis oil and purified water, measuring cylinder, 1ml pipette, test tubes, spatula, dispensing bottles, mortar and pestle, weighing balance, stirring rod.

Procedure:
The clay samples were mixed with calcium hydroxide, triturated to a smooth paste with a mixture of oleic acid and arachis oil. Wool fat was then incorporated after been melted and added gradually with continuous stirring. Sufficient water was then added to produce 50g. The resulting formulations were optically observed for appearance, texture and spreadability. After which their various pH were measured at 25°C using a pH meter.

Table I: Formulation of the investigated sunscreen cream

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>F2 cream (modified clay)</th>
<th>F3 cream (non modified clay)</th>
<th>F4 cream, placebo (bentonite clay)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clay</td>
<td>16g</td>
<td>16g</td>
<td>16g</td>
</tr>
<tr>
<td>CaOH</td>
<td>0.0225g</td>
<td>0.0225g</td>
<td>0.0225g</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>0.25g</td>
<td>0.25g</td>
<td>0.25g</td>
</tr>
<tr>
<td>Arachis oil</td>
<td>16g</td>
<td>16g</td>
<td>16g</td>
</tr>
<tr>
<td>Wool fat</td>
<td>4g</td>
<td>4g</td>
<td>4g</td>
</tr>
<tr>
<td>Purified water</td>
<td>50g</td>
<td>50g</td>
<td>50g</td>
</tr>
</tbody>
</table>

Ingredients in Aloe Sunscreen:
Homosalate 10.0%, Octisalate 5.0%, Avobenzene 3.0%, Enulsilizole 3.0%, Octocrylene 2.79%, Aloe barbadensis leaf juice (stabilized aloe vera gel), water (aqua), butyloctyl salicylate, caprylic/capric triglyceride, glycerin, cetearyl olivate, sorbitan olivate, sodium chloride, cetyl alcohol, glyceryl stearate, polyglyceryl-4 isostearate, cetyl peg/ppg-10/1 dimethicone, hexyl laurate, cetyl dimethicone, phenoxyethanol, peg-75 stearate, triethanolamine, xanthan gum, ceteth-20, steareth-20, tocopheryl acetate, fragrance (parfum), methylisothiazolinone.

In Vivo Testing Of the Creams

Materials:
Albino Wistar Rats (15), Veet hair removal cream, a piece of hand towel.
Creams: F1-Aloe sunscreen cream (standard), F2-modified clay sunscreen cream, F3-non modified clay sunscreen cream, F4-bentonite clay sunscreen cream (control)

Procedure:

The rats were group into three groups with each group having five rats and labeled according to each formulation. The hair of the rats at the back side was shaved using the Veet hair removal cream, wash-dried with a hand towel soaked in water mixed with soap to remove the remaining hair removal cream on the body of the rats. The rats were allowed for 24 hours to rest after the hair removal. The creams were applied dropwise using a 1ml syringe at a surface area of 2 mg/cm\(^2\) of skin according to the method of (Haywood et al., 2003) with each animal specified for each formulation and labeled accordingly. The cream was allowed to dry for 15 minutes and exposed to direct sunlight for at least three hours consecutively for 21 days, with repeated application of the creams prior to exposure each day. The rats were observed for skin erythematous response (skin pigmentation darkening).

Calculation of the Sun Protective Factor of the Sunscreen Creams

In general definition, SPF is the ratio of the time duration of UV radiation required to produce erythema in the protected skin (with sunscreen) to that required to produce erythema in the unprotected skin (without sunscreen). Express mathematically as

\[
SPF = \frac{\text{Time duration of UV radiation required to produce erythema in the protected skin (with sunscreen)}}{\text{Time duration of UV radiation required to produce erythema in the unprotected skin (without sunscreen)}}
\]

III. RESULTS

Microbial contamination test results

The result of the microbiological contamination test of the clay samples (modified and non modified clay) are presented in Table II as follows;

<table>
<thead>
<tr>
<th>Concentration</th>
<th>1x10(^{-1})</th>
<th>1x10(^{-2})</th>
<th>1x10(^{-3})</th>
<th>1x10(^{-4})</th>
<th>1x10(^{-5})</th>
<th>1x10(^{-6})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of colony (Non modified clay sample)</td>
<td>uncountable</td>
<td>uncountable</td>
<td>72</td>
<td>6</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Number of colony (modified clay sample)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Stability and Physical Appearance Evaluation Results: The results of the stability and physical characteristics evaluation of the prepared sunscreen cream formulation is presented in Table III.
Table: III. Stability & physical characteristics evaluation of the prepared sunscreen cream formulation

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Modified clay sunscreen cream</th>
<th>Non modified clay sunscreen cream</th>
<th>Bentonite clay sunscreen cream</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creaming</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Coalescence</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Elegance</td>
<td>Maintained</td>
<td>Maintained</td>
<td>Maintained</td>
</tr>
<tr>
<td>pH</td>
<td>8.27</td>
<td>8.53</td>
<td>7.07</td>
</tr>
<tr>
<td>Remark</td>
<td>Satisfactory</td>
<td>Satisfactory</td>
<td>Satisfactory</td>
</tr>
</tbody>
</table>

The evaluation of the stability of the formulated sunscreen cream revealed that the formulations were stable as characterized by the absence of coalescence of the internal phase, absence of creaming, and maintained elegance and stable appearance over a period of one month of storage at 25°C and the readings obtained with the pH values.

Response Observed On The Rats after Exposure to Sun Radiation

After 72hrs of exposure to sun radiation the observed response on the rats are presented in figure 2.

Figure: 2. Group (1) Rats after 3days of exposure to sun radiation.

With F1 = Sunscreen cream as the standard showing no skin irritation (well protected by sunscreen), no notable pigmentation darkening of the skin. F2 = Sunscreen cream preparation with modified clay, sunscreen cream presented no skin irritation, pigmentation darkening of the skin, which is well shielded from sun radiation). F3 = Sunscreen cream prepared from the non modified clay, the sunscreen cream showed mild skin irritation, pigmentation darkening of the skin. The F4 = Null-sunscreen cream as the control showed excessive skin irritation, pigmentation darkening of the skin. F1, F2, F3, F4 are formulations 1 to 4 respectively.

In vivo Evaluation Test Results

In an in vivo evaluation of the sunscreen cream formulations the following results were obtained as presented in the Table IV.

Table: IV in vivo evaluations of the investigated sunscreen formulations

<table>
<thead>
<tr>
<th>Sunscreen creams</th>
<th>Duration of exposure</th>
<th>Duration of time of observed response (skin irritation, pigmentation and darkening)</th>
<th>Sun protective factor values (SPF) Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1 sunscreen cream</td>
<td>21 days</td>
<td>17 days</td>
<td>5.67 ± 2.57</td>
</tr>
<tr>
<td>F2 sunscreen cream</td>
<td>21 days</td>
<td>15 days</td>
<td>5.00 ± 2.24</td>
</tr>
<tr>
<td>F3 sunscreen cream</td>
<td>21 days</td>
<td>15 days</td>
<td>5.00 ± 2.24</td>
</tr>
<tr>
<td>F4 sunscreen cream</td>
<td>21 days</td>
<td>3 days</td>
<td>1.00 ± 0.58</td>
</tr>
</tbody>
</table>
IV. DISCUSSION

Thermally Modified Clay Sample

We observed that the thermal exposure of the BPTM clay samples generated a modification yielding a brilliant looking clay sample with dry and smooth texture characteristics on touch. The modification of the clay prior to its incorporation into sunscreen cream enhanced its potency. The particles were duly activated to physically serve as barriers that readily absorb UVA and UVB natural rays from the sun.

Microbiological Testing

The microbiological test of the BPTM clays, revealed that the modified clays sample were free from pathogenic bacteria, while the non modified clays sample revealed growth of both Staphylococcus aureus and Bacillus subtilis as shown in figure 1a and 1b respectively. Staphylococcus aureus which is a common cause of skin infections such as abscesses, pimples, impetigo, boils, cellulitis, folliculitis, carbuncles and scalded skin syndrome should not be found in APIs or excipients of formulation. This therefore makes the non-modified clay inappropriate for sunscreen cream formulation.

Stability and Physical Appearance

The stability evaluation of the formulated investigated sunscreen cream revealed the formulations were stable. The creams characterized by the absence of coalescence of the internal phase, absence of creaming, and maintenance of elegance stable appearance over a period of one month of at 25°C and the readings obtained with the pH values revealed that the formulated investigated sunscreen creams have pH of 8.27, 8.43 and 8.07 for F2 modified clay sunscreen cream, F3 non modified clay sunscreen cream and F4 Bentonite clay creams respectively. The observed pH values were normal for topical cream.

In Vivo Evaluation of the Investigated Sunscreen Creams

The in vivo evaluation of the investigated sunscreen creams revealed calculated sun protective values (mean ± SD) for the aloe sunscreen formulation (standard), modified clay sunscreen formulation and the non modified sunscreen formulation were obtained as 5.67 ± 2.57, 5.0 ± 2.22 and 5.0 ± 2.22 respectively as shown in Table IV above. This reveals no statistical difference between the sun protective factors (SPF) of the modified clay sunscreen formulation and that of the non modified clay sunscreen formulation. This implies that modification of the clay prior formulation has no influence on the sun protective factor (SPF). Hence, thermal modification is not a requirement for improved sun protection except for its bacterial free advantage. This could be attributed to the presence of more than one organic sunscreen actives in the formulation (Homosalate 10.0%, Octisalate 5.0%, avobenzone 3.0%, ensulizole 3.0% and Octorylene 2.79%). It also reveals that the skin was protected 5 times better with a sunscreen cream applied than without a sunscreen cream applied, as suggested by the calculated Sun Protective Factor (SPF) values of the investigated sunscreen creams. This can be attributed to the presence of inorganic UV filters/physical barriers which are ZnO, TiO₂ and Fe₂O₃ (Hoang-Minh et al., 2010; Ngole et al., 2010) in the clay sample of the investigated sunscreen formulation which serves as actives against UV radiation. As regards the safety of the inorganic filters included in the formulation on the skin, Inorganic pigments, especially titanium dioxide, were particularly viewed as safe as they do not penetrate into the skin. This has been shown in skin penetration studies (Lademann et al., 1999, Nohyne, et al., 2007 ).

V. CONCLUSION

The sunscreen cream formulations generated from the beneficiated, pulverized and thermally modified Arrinrasho clay deposit in Barkin Ladi, Plateau State, Central Nigeria, displayed good potentials as Sunscreen agent. It effectively protected the skin of the white Albino Rats against sunburn. An equivalent sun protection factor (SPF) was obtained with the BPTM Arrinrasho clay with value of 5.00 as obtained with the standard Aloe sunscreen cream prepared for effective shielding against UVA and UVB rays, thus giving a wide spectrum of activity with well expressed characteristic properties. The preliminary application of this preparation on volunteers has not yielded any adverse effect. An expanded preliminary application of this preparation on volunteers in order to establish a wider data base with respect to issues on the adverse effect(s) would add value to this work.

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CONFLICT OF INTEREST DECLARATION

The authors of this work hereby declare that there is no area of competing interests as at the time of this submission.

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