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AS MEN OF HONOUR WE JOIN HANDS

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Formulation and Evaluation of Antioxidant Topical Cream Using Ethanol Extract of *Markhamia Tomentosa* Leaves

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ABSTRACT

Background: Global warming which results from the thinning of the ozone layer has led to an increase in the level of exposure to ultraviolet rays from the sun. Long term exposure to these rays can lead to photo-aging. The aim of this study is to develop an antioxidant cream using ethanol extract of *Markhamia tomentosa* leaves as an active ingredient and to evaluate its physico-chemical properties as well as free radical scavenging and ferric reducing antioxidant activities.

Methods: The leaves of *Markhamia tomentosa* was obtained from Oke-Igbo, Ondo state, Nigeria in August 2019. The leaves of *Markhamia tomentosa* were dried, pulverized and macerated to obtain *Markhamia tomentosa* ethanol extract. Four water-in-oil emulsion creams containing *Markhamia tomentosa* ethanol leaf extract were formulated using fusion technique. Physico-chemical properties such as the pH, viscosity, skin irritancy, spreadability and organoleptic tests were evaluated. Subsequently the (1, 1-diphenyl1-2-picrylhydrazyl) DPPH radical scavenging and Ferric reducing antioxidant power assays were also carried out.

Results: All formulations were brown, with a smooth and non-greasy feel on dermal application. They had no characteristic smell and were easily washed off the skin. Formulations had a pH that fell within the range of 5.5-7.5 which is safe for dermal application. All formulations had a spreadability above 100 mm^2 g^{-1} and exhibited non-Newtonian properties with a decrease in viscosity at the increase of shear stress emphasizing the ease of spreadability. FMT1 (*Markhamia tomentosa* ethanol leaf extract cream) had the highest antioxidant activity which was concentration dependent, with a percentage radical scavenging activity of 76.48% and an absorbance of 0.276 in the ferric reducing antioxidant power assay, at wavelengths of 517 and 700nm respectively.

Conclusion: This *Markhamia tomentosa* ethanol leaf extract cream formulation exhibited strong antioxidant properties and can serve as a valuable archetype that can be developed and translated to a pharmaceutical antioxidant formulation for clinical use.

1. Introduction

Global warming can be explained as the overall increase in the temperature of the atmosphere on earth which is as a result of the depletion of the ozone layer.[1] The thinning of the ozone layer due to pollution allows an increase in the amount of ultraviolet rays from the sun that reaches the earth.[1] Ultraviolet radiation due to sun exposure interacts with oxygen present in the skin to give reactive oxygen species also known as free radicals.[2] In an ideal situation, dermally there is a balance between the skins natural antioxidants and reactive oxygen species. Excessive presence of the reactive oxygen species leads to a decline in skin elasticity and premature wrinkling.[3]

Photo aging which is described as photo induced aging of the skin due to sun exposure has become a common problem. The use of herbal antioxidants have proven to be a safe and viable approach to blocking the damaging effects of ultraviolet rays. The most advantageous reason for use of herbal antioxidants is that they are directly sourced from plants and have negligible side effects compared to their synthetic counterparts. Plants have a vast variety of active

phyto-constituents some of which have the ability to sooth, heal and protect the skin.[4]

Markhamia tomentosa is a plant 5-30 feet high, it is from the family Bignoniaceae. The plant possess large yellow flowers. Phyto-chemical screening of *Markhamia tomentosa* has led to the confirmation of sitosterol, flavonoids, steroids, saponins and terpenes as phytoconstituents. The leaves of *Markhamia tomentosa* are known to be used to treat edema, headache, chest pain and gout traditionally.[5, 6] A couple of phyto-constituents of *Markhamia tomentosa,* such as phenol and sitosterol are particularly known have antioxidant properties. However its medicinal application as an antioxidant is only a recent concept.

The aim of this study is to develop an antioxidant cream using ethanol extract of *Markhamia tomentosa* leaves as an active ingredient and to evaluate its physico-chemical properties as well as free radical scavenging properties and ferric reducing antioxidant power. The *Markhamia tomentosa* antioxidant cream is expected to counteract the damage cause by oxidation of skin tissues due to exposure to free radicals, minimizing cellular damage through scavenging of free radicals. This formulation is a safe and viable option for preventing dermatological damage due to photo-aging whilst enhancing a healthy micro-environment for skin tissues.[6]

2. Methodology and Materials

2.1 Materials

The following materials were used for this study, Amaranth dye (Sigma Aldrich, St. Louis USA), stearic acid (Surfachem, UK), cetostearyl alcohol (Pure Nature, NZ), soft paraffin (Unicorn petroleum, India), hard paraffin (Okchem, China), triethanolamine (Merck, Germany), methyl paraben and propyl paraben (Sigma Aldrich, St. Louis USA).

2.2 Plant collection authentication and extraction

The leaves of *Markhamia tomentosa* was obtained from Oke-Igbo, Ondo state, Nigeria in August 2019. They were identified taxonomically and authenticated in the Herbarium of Department of Botany and Microbiology, University of Lagos. A herbarium specimen was deposited with a voucher number LUH 8056.[5]

The leaves of *Markhamia tomentosa* were air dried and then dried in an oven at 60°C. Approximately 350g of dried leaves were grinded using a laboratory mill. Extraction was conducted by macerating the grounded powder in ethanol for 4 days (cold maceration). The resultant mixture was then filtered and concentrated using an oven at 40°C to obtain the *Markhamia tomentosa* ethanol leaf extract.[7]

2.3 Formulation development

A water-in-oil emulsion cream was formulated using fusion technique. The surfactant (stearic acid) and oil phase (Cetostearyl alcohol, soft paraffin, and hard paraffin) and the aqueous phase (methylparaben, propyl parabene, triethanolamine, water and ethanol extract of *Markhamia tomentosa* leaves) were individually heated in a water bath to 80°C. The emulsifier, stearic acid and the aqueous phase was gradually added into the oil phase with constant stirring. The mixture was stirred and allowed to cool until the cream congealed at room temperature (Table 1).[8]

Table 1 Formula for preparing antioxidant cream formulations utilizing extracts of *Markhamia tomentosa*.

Ingredients	FMT1	FMT2	FMT3	FMT4
Markhamia tomentosa leaf extract (g)	5.0	2.5	2.5	0.5
Stearic acid (g)	3.5	5.0	3.5	5.0
Cetostearyl alcohol (mL)	4.5	5.0	4.5	5.0
Triethanolamine (mL)	0.75	0.75	0.75	0.75
Hard paraffin (g)	2	2	2	2
Soft paraffin (g)	4	4	4	4
Methyl parabene (mL)	0.3	0.3	0.3	0.3
Propyl parabene (mL)	0.025	0.025	0.025	0.025
Water to (mL)	100	100	100	100

2.3.1 Determination of Emulsion Type

The emulsion type was determined by adding drops of Amaranth dye to the formulations (FMT1-FMT4) (1g). The stained formulation was viewed for phase type under a microscope (Eclipse E100 Nikon TX, USA). A drop of the emulsion was placed on as microscopic slide and observed. [8]

2.3.2 pH of the formulations

The pH of formulations FMT1-FMT4 was determined using a pH meter. All readings were recorded in triplicates. [9]

2.3.3 Organoleptic test

Formulations FMT1-FMT4 were evaluated for homogeneity by assessing their visual appearance and texture. The appearance of the cream was judged and graded by its color, pearl essence and smoothness. The emolliency and slipperiness were also evaluated.[10]

2.3.4 Spreadability

The formulations (FMT1-4) were evaluated for spreadability. Exactly 0.4g of each formulation was placed in between two glass slides and a 100g mass was placed on both sides for 60 seconds to compress the cream formulation and obtain uniformed thickness, excess cream at the edges of the slide was cleaned off. A 50g mass M was tied to the upper slide. The time required to move the slides across a distance of 10cm was taken as the measure of spreadability. The Equation 1 was used for calculating spreadability.[11]

$$S = M\,\frac{L}{T} \quad \text{..... Equation 1}$$

M = Weight tied to the upper slide, L = Length of glass slide, T = Time taken to separate the slides.

2.3.5 Skin Irritancy

Ethical approval for the study was covered by approval number (CMUL/HREC/07/22/1071). An area of the human skin ($1cm^2$) was marked and about 0.3g amount of cream formulation (FMT1-4) was applied topically and the time lapse was noted. The specified area was checked for signs of irritancy such as a rash, erythema or edema at regular intervals for 24h.[10]

2.3.6 Viscosity

The viscosity of the formulations FMT1-FMT4 was determined utilizing a Brookfeild viscometer (Spindle 4.0) at a shear rate of 10, 20, 30, 40, 50 rpm. Results were recorded in triplicates.[9]

2.3.7 *In-vitro* antioxidant activity of the formulation FMT1- FMT4 using DPPH assay

The antioxidant activity of the formulations (FMT1 – FMT4) against 1, 1-diphenyl1-2-picrylhydrazyl (DPPH) radical through UV absorbance at 517 nm, using ascorbic acid as standard and ethanol as control was carried out. The formulations were assayed spectrophotometrically using a modification of the method described by Ilomuanya *et al* 2018.[8] Exactly 100mg of the ethanol extract of *Markhamia tomentosa* leaves, each formulation and ascorbic acid were extracted utilizing absolute ethanol via a separating funnel. About 2mLs of each of the test samples were dissolved in ethanol and introduced at different concentrations (5-25mg/mL) to the ethanol solution of DPPH (100mmol/L, 2mL). Absorbance was noted at 517nm at 30 min. [8] The percentage antioxidant activity was calculated using Equation 2

$$\%\,\text{Antioxitant Activity} = \frac{\text{Absorbance}_{\,517\ control} - \text{Absorbance}_{\,517\ sample}}{\text{Absorbance}_{\,517\ control}}\ x\ 100$$

..... Equation 2

2.3.8 Ferric reducing antioxidant power assay

The ferric reducing antioxidant power of the different samples was assessed according to the method described by Vijayalakshmi and Ruckmani. [12] Exactly 100mg of the ethanol extract of *Markhamia tomentosa* leaves, each of the formulations (FMT1-4) and ascorbic acid was extracted utilizing absolute ethanol via a separating funnel. To the extracted sample 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium hexacyanoferrate solution was added to 1mL of the extracted samples. The reaction was then incubated at 50°C for 20 min. After incubation, 2.5 mL of 10% trichloroacetic acid was added to the mixture and centrifuged at 3,000 rpm for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL of deionised water and 0.5 mL of 0.1% ferric chloride. The mixture was left to stand for 10 minutes and the absorbance read at 700nm. A mixture of the buffer served as the control. Increased absorbance of the sample mixture means higher reducing power of the *Markhamia tomentosa* ethanol leaf extract, formulations (FMT1-4) and ascorbic acid.[12]

2.3.9 Centrifugation test

The stability of the formulations FMT1-FMT4 were determined with centrifugation test at 4,000 rpm for 10minutes. The appearance and stability were assessed by

macroscopic observation for creaming or phase seperation.[13]

2.4 Statistical Analysis

To determine the level of significance ($p < 0.05$), statistical analyses were performed using a one-way ANOVA test followed by Bonferroni's multiple comparisons test (if applicable) performed on GraphPad Prism version 7.00 for Windows (GraphPad Software, La Jolla, CA, USA).[14]

1. Results

3.1 Organoleptic properties, skin irritancy, and emulsion type tests for the formulations FMT1-FMT4.

All formulations were homogenous on macroscopic observation. Formulations FMT1 was dark brown due to the presence of a higher concentration of *M.tomentosa* ethanol leaf extract, FMT2 and 3 were brown in appearance, while FMT4 had a lighter shade of brown as it had the lowest concentration of *M.tomentosa* ethanol leaf extract. The emulsion type test gave a water in oil emulsion. No erythema or edema was observed during the skin irritancy test indicating the dermal safety of the formulations.

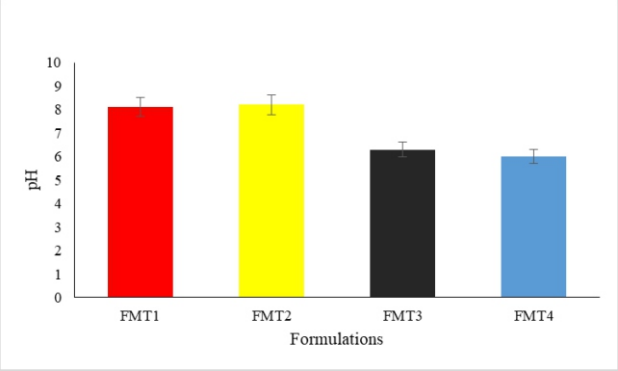

Figure 1 shows the pH of the various formulations FMT1-FMT4. Values are expressed as mean ± SD (n=3).

3.2 pH of formulations FMT1-FMT4

In Figure 1, the pH of the formulation FMT1 was the highest followed by FMT2 and FMT3 the lowest was FMT4 which was 6.0. The pH expresses the level of basicity or acidity of the formulations. The pH of the skin is between 5.0- 6.0. All the formulations had a pH ranged within 5.5-7.5, which is safe for dermal application.

3.3 Spreadability of formulations FMT1-FMT4.

The spreadability of the formulations is shown in Figure 2, with FMT2 having the lowest spreadability (104 $mm^2 g^{-1}$), followed by FMT1 (135 $mm^2 g^{-1}$), then FMT3 (157.5 $mm^2 g^{-1}$). The formulation with the highest spreadability was FMT4 with a value of 189 $mm^2 g^{-1}$. The spreadability of a formulation is an important parameter as the formulation must be evenly distributed around the site of action on the skin.

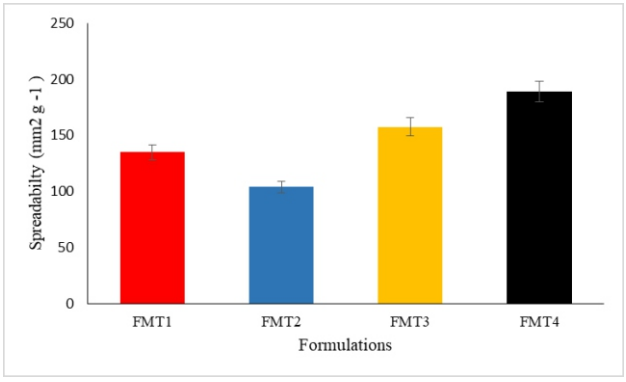

Figure 2 Spreadability of formulations FMT1-FMT4. Values are expressed as mean ± SD (n=3).

3.3 Viscosity of the formulations FMT1-FMT4

The viscosity of the formulations was evaluated. Figure 3 shows that the formulation with the overall highest viscosity was FMT2 while the formulation with the least viscosity at 50 RPM was FMT4. There was an overall trend of decrease in viscosity when shear rate was increased.

3.4 Stability of the formulations FMT1-FMT4

After centrifugation test formulations were visually examined for phase separation, creaming or cracking. None of the formulations showed any evidence of physical instability.

3.5 Antioxidant properties of formulations FMT1-FMT4

The antioxidant properties of the four creams were evaluated using DPPH radical scavenging assay and Ferric reducing antioxidant power (FRAP). The DPPH radical scavenging assay can be described as an established method for ascertaining the antioxidant power of herbal extracts. It measures the degree of scavenging activity against free radicals while ferric reducing antioxidant power assay utilizes antioxidants as reducing agents in a redox linked colorimetric reaction where Fe^{3+} is reduced to Fe^{2+} accompanied by a color change from yellow to intense blue.[18,19,20]

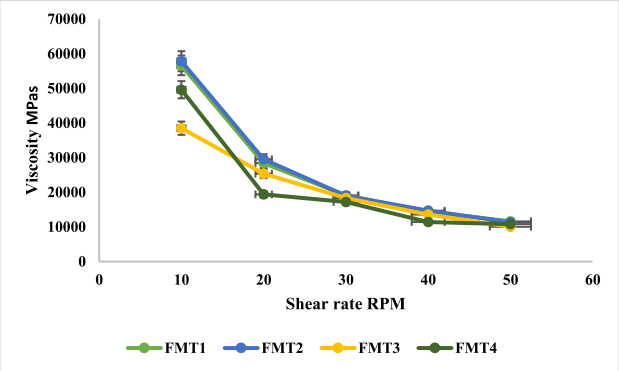

Figure 3 Effect of increase in shear rate on the viscosity of the formulations FMT1-FMT4. Values are expressed as mean ± SD (n=3).

The formulation with the lowest antioxidant property in the DPPH scavenging assay at 100 µg/ml was FMT4 while the highest was the standard (ascorbic acid), then *Markhamia tomentosa* ethanol leaf extract, followed by FMT1.

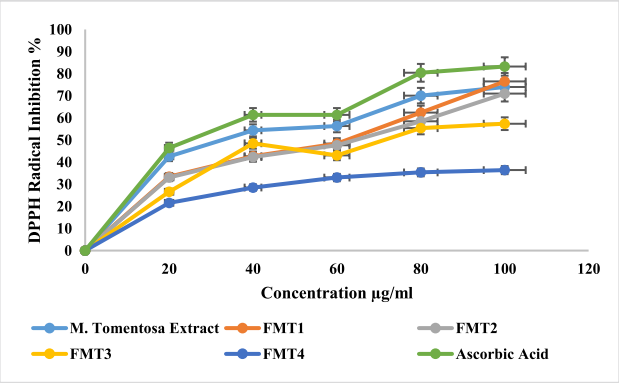

Figure 4 Scavenging of DPPH activity by the herbal cream formulations FMT1-4 and the *M.tomentosa* ethanol leaf extract, with ascorbic acid representing the antioxidant standard (n=3±S.D).

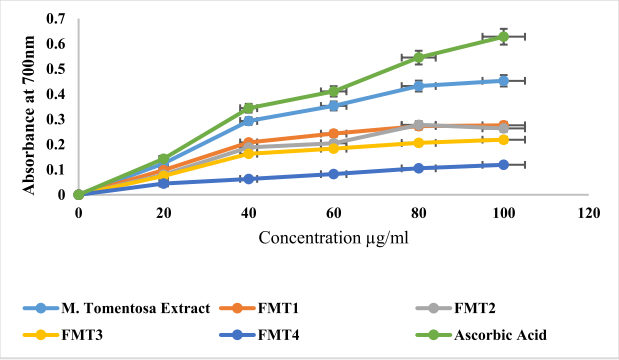

Figure 5 Ferric reducing antioxidant power (FRAP) of the herbal cream formulations FMT1-4 and the *M.tomentosa* ethanolic leaf extract, with ascorbic acid representing the antioxidant standard (n=3±S.D).

This is due to the higher concentration of the extract in formulation FMT1. The high antioxidant activity of FMT1 means that the formulation will be the most efficient in mopping up free radicals on dermal application preventing effects of oxidative stress due to sun exposure. The formulation with the highest and lowest ferric reducing antioxidant power are FMT1 and FMT4, respectively.

FMT1 had the overall highest antioxidant activity with a percentage radical scavenging activity of 76.48% and an absorbance of 0.276 in the ferric reducing antioxidant power assay, at a wavelength 517 and 700nm respectively. [20]

4. Discussion

One of the effects of global warming which is often overlooked is photo-aging. *Markhamia tomentosa* is a plant popularly known for its anticancer properties. Its antioxidant properties are yet to be fully explored. There is a need for the development of an herbal topical formulation with potent antioxidant properties and minimal side effects. In this study a water in oil emulsion was formulated with *Markhamia tomentosa* ethanol leaf extract as the active pharmaceutical ingredient for antioxidant therapeutic activity. The importance of antioxidants in defending against free radical damage cannot be over emphasized hence its use in regressing the process of aging. This topical antioxidant formulation not only mops up free radicals but also aids in the protection of the skin from photo-aging and its effects. The formulations were developed utilizing fusion method to ensure a high degree of stability.

Organoleptic tests, are tests based on human senses. The color, feel, and consistency of the creams were assessed. The brown tint observed in the formulations was due to the presence of the *Markhamia tomentosa* ethanol extract. The smooth feel and consistency of the creams was as a result of the presence of the strong emollient, liquid paraffin. All formulations were smooth and non-greasy on dermal application they had no characteristic smell and were easily washed off the skin. Emulsion type test indicated that all formulations were water-in-oil type of emulsion. No erythema or oedema was observed in the skin irritancy test during and after 24 hours showing that the formulations are safe for topical use. [15]

Spreadability of a topical formulation is the degree to which a formulation can distribute throughout a given area on the

skin on application. This is an important parameter in topical administration as it affects the uniformity of dose of active pharmaceutical ingredient (API) that is absorbed by the skin at the site of action. It also influences the overall therapeutic efficacy of the topical formulation. The consistency of the cream affects the level of thickness of the emulsion, this affects the viscosity of the formulation and the spreadability.[11] The spreadability was highest in the formulation with the lowest viscosity. The formulation with the lowest viscosity was FMT4 therefor it had the highest spreadability with a value of 189 $mm^2 g^{-1}$. FMT2 had the highest viscosity so it's spreadability was the lowest, 104 $mm^2 g^{-1}$. [16]

The viscosity of a cream can be described as the level of resistance experienced when the cream is subjected to deformation under shear stress. Based on the viscosity evaluation, the formulations were found to be non-newtonian. The decrease in viscosity observed after shear rate was increased means that the formulations easily spreads without resistance during dermal application. [17] FMT2 had the highest viscosity this can be due to the presence of the high concentration of the viscosity increasing agent Cetostearyl alcohol while FMT3 had the overall lowest viscosity which is due to the lower concentration of Cetostearyl alcohol present in the formulation. Centrifugation is the process of subjecting a system (two phase system such as an emulsion) to centripetal force provided by a centrifuge imitating an in-situ stress storage condition. A centrifuge equipment can reproduce stress conditions by up surging the gravitational acceleration within its field subjecting the formulation to such conditions. All formulations FMT1-FMT4 remained stable after the centrifuge test with no cracking, creaming or phase separation observed. [18]

The DPPH radical scavenging activities of the formulations FMT1-FMT4 were evaluated. DPPH radicals react with suitable reducing agents (antioxidant molecules) which change color and the number of electrons usurped is measured spectrophotometrically at 517nm. The percentage antioxidant activity of *Markhamia tomentosa* leaf extract was closest to that of the standard Ascorbic acid at all concentrations.[19] FMT1 had the highest antioxidant activity due to the high concentration of *Markhamia tomentosa* leaf extract in it, whilst FMT4 showed the lowest antioxidant properties probably due to the low concentration of *Markhamia tomentosa* leaf extract in the formulation. Ferric reducing antioxidant power (FRAP) is a test that depends on electron transfer. It assesses the ability of an antioxidant to form a complex with the metal atom, Iron, thereby reducing Fe^{3+} to Fe^{2+}. The standard (ascorbic acid) reflected the highest Ferric reducing antioxidant power followed closely by the *Markhamia tomentosa* leaf extract and then formulation FMT1, FMT2, FMT3 and lastly FMT4. In both antioxidant tests FMT1 showed the highest antioxidant power, indicating its potency and ability to act as a skin protectant from the gruesome effects of excessive exposure to the sun.[20]

5. Conclusion

Antioxidant herbal formulations FMT1-FMT4 were successfully prepared with *Markhamia tomentosa* ethanol leaf extract as active ingredient. Formulation FMT1 was found to be optimal because it had an ideal pH, safe for dermal use. It also had good spreadability and viscosity properties. FMT1 had the highest antioxidant activity in both DPPH radical scavenging and Ferric reducing antioxidant power (FRAP) assays. *Markhamia tomentosa* ethanol leaf extract topical cream formulation exhibits antioxidant properties which can be applied to block or regress photoaging. *Markhamia tomentosa* ethanol leaf extract antioxidant cream formulation can serve as an archetype that can be developed and translated to a pharmaceutical formulation for clinical use.

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