Polyherbal Antioxidant Topical Preparation Comprising Ethanol Extract of *Tetracarpidium conophorum* and *Ocimum gratissimum*: Formulation and Evaluation

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ABSTRACT: The use of antioxidants is an effective approach to prevent symptoms related to photo-induced aging of the skin. The aim of this research work was to formulate and evaluate a polyherbal antioxidant face cream using the ethanol extracts of *Tetracarpidium conophorum* and *Ocimum gratissimum*. The ethanol extract of the herbs was incorporated at varying concentrations into six different emulsion bases. Antioxidant activity of the formulations was assessed using 2,2-diphenyl-1-picrylhydrazyl method. The formulations were evaluated for pH, viscosity, spreadability and microbial content. Accelerated stability tests were performed on all the formulations to assess stability at varying storage conditions. All the formulations showed good spreadability, good consistency, homogeneity, appearance, pH and no phase separation occurred. Non-Newtonian pseudo-plastic flow influenced by increased shear was experienced by all the formulations. Concentration dependent antioxidant activity was observed with FC2 and FC4 showing the highest antioxidant activity with IC_{50} value of 80.1 and 83.2 µg/ml, respectively. The polyherbal antioxidant preparation containing extracts of *T. conophorum* and *O. gratissimum* shown to exhibit excellent antioxidant properties. It can serve to protect the skin from reactive oxygen species created by UV radiation and environmental toxin, thus protecting the skin from photo aging.

Key words: Anti-oxidant; Polyherbal; *Tetracarpidium conophorum*; *Ocimum gratissimum.*

INTRODUCTION

Key traditional therapeutic herbal strategy exploits the combination of several medicinal herbs to achieve extra therapeutic effectiveness. This is known as polyherbalism. Polyherbal formulations are mixture of herbs, prepared in a number of formulations such as decoctions, elixirs, infusions, creams, gels, ointments and paste etc, in order to achieve maximum therapeutic efficacy.[1] Polyherbal formulations have plant-based pharmacological agents which may exert synergistic, potentiate, agonistic or antagonistic actions by virtue of its diverse active principles within themselves.[1,2] These pharmacological principles work together in a dynamic way to produce maximum therapeutic efficacy with minimum side effects.[1] The use of polyherbal formulations have been prominenet in management of the effects of aging and improvement of skin tone. Aging is a natural progressive process that leads to aesthetic and functional changes in the skin[2] promoted by a group of molecules known as radicals. These radicals also known as reactive oxygen species can be created by combustion of by-products and UV radiation interacting with the oxygen present in the skin.[3] In normal conditions a balanced equilibrium existing between these radicals and the skin's natural antioxidants such as vitamin E, co-enzyme 10Q, ascorbates, and carotenoids.[4] The excess generation of free radicals overwhelms the skins natural cellular antioxidants creating a

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condition which is known as oxidative stress. Oxidative stress leads to oxidative damage which manifest physically as aging, a process which can be effectively retarded by the use of externally applied antioxidants.[4]

Plant extracts possessing antioxidant properties have been explored in phytocosmetic field as they present molecules that could inactivate reactive oxygen species restoring skin homeostasis and preventing erythema and premature aging of the skin.[5] African walnut *Tetracarpidium conophorum* has a long history as food plant and is grown by farmers across West African rain forest. *T. conophorum* is widely distributed and consumed by the inhabitants of the Guinea Zone of West and Central Africa.[6] Studies have shown that the African walnut possess some beneficial properties like antibacterial, antioxidant.[6,7] and immune-stimulating activities. It is commonly used in Nigerian folkloric medicine for the treatment of bacterial infections and ailments caused by oxidative stress.[6] Photochemical screening of ethanol extracts of *T. conophorum* showed presence of alkaloids, saponins, glycosides, flavonoids and tannins. Akomolafe *et al*.[8] evaluated the anti-peroxidative activity of the leaves of *T. conophorum* by determining their capacity to reduce malondialdehyde levels. The results suggest that the extract from *T. conophorum* leaves had greater capacity to reduce lipid peroxidation and thus, this plant may be useful in the treatment/management of cellular damage involving reactive oxygen species. Amaeze *et al*.[9] evaluated the antioxidant activity of *T. conophorum* extracts of fresh and dried leaves. The result revealed that ethanol extract of the dried leaves had high antioxidant as well nitric oxide radical inhibition activity comparable to that of rutin and ferric reducing power.

Ocimum gratissimum is a herbaceous which belongs to the family Lamiaceae. It is commonly known as *scent leaf*. The plant is indigenous to India and West Africa. The essential oils contain eugenol, thymol and p-cymene which show some evidence of antioxidant, anti-bacterial, anthelminthic and insecticidal properties.[10] The antioxidant capacity of essential oils *Ocimum* spp. were evaluated using a HPLC-based hypoxanthine/xanthine oxidase and DPPH assays with strong antioxidant capacity being evident in all the oils.[11] Extracts from the leaves of *O. gratissimum* were investigated for their phytochemical constituents and antioxidant activity suggesting the rich phytochemical content of *O. gratissimum* and its good antioxidant activity.[11] The aim of this research work was to formulate and evaluate a polyherbal antioxidant face cream using the ethanol extracts of *T. conophorum* and *O. gratissimum* having known antioxidant activity that will protect the skin from the effects of reactive oxygen and free radicals as well as act as an emollient.

MATERIALS AND METHODS

Materials. Stearic acid (BDH Chemicals, England), cetylstearyl alcohol (BDH Chemicals, England), soft paraffin, liquid paraffin, hard paraffin, methyl and propyl paraben (Sigma Aldrich, St. Louis, USA), triethanolamine (Merck, Germany). All other chemicals and reagents were of analytical grade.The leaves of *Ocimum gratissimum* were obtained from Igbogila farm in Ogun state at latitude of 7.0539°N and longitude of 2.9751°E while the leaves of *Tetracarpidium conophorum* were collected from farms in Nkwere Local Government Area, Imo state, Nigeria at latitude 5° 45' 33.01" N and longitude 7° 06' 13.82" E. The two plants were authenticated by a taxonomist in the Department of Botany, University of Lagos, Nigeria with herbarium specimen number LUH6981 and LUH 6972, respectively.

Extraction. The shade dried and coarsely powdered leaves of *O. gratissum* and *T. conophorum* were extracted using absolute ethanol.[12] The extracts were then filtered and concentrated in the vacuum at 40-50ºC using a rotary evaporator. Evaporation of solvent in the rotary evaporator produced a crude extract. These extracts were then dried in an oven at 40°C to obtain for the dried ethanol extract. These powdered ethanolic extract of *O. gratissum* (EEOG) and *T. conophorum* (EETC) were transferred

appropriately into labeled sample bottles and stored in a refrigerator at 4°C for subsequent use.

Formulation of the creams. Oil in water (o/w) emulsion-based cream (semisolid formulation) has been formulated. The emulsifier (stearic acid) and other oil soluble components (cetylstearyl alcohol, soft paraffin, liquid paraffin, hard paraffin) were dissolved in the oil phase and heated to 75°C. The preservatives (methyl paraben and propyl paraben) and the aqueous components (triethanolamine, water, EEOG and EETC) were heated to 75°C. The aqueous phase was added in portions to the oil phase with continuous stirring. *Citrus cinensis* oil was added after the temperature dropped to (45 ± 0.5°C) (Table 1).

Table 1. Formula for preparing antioxidant cream formulations utilizing extracts of *O. gratissum* (EEOG) and *T. conophorum* (EETC).

Ingredients	FC1	FC2	FC3	FC4	FC5	FC6
EEOG (g)	0.25	0.5	0.4	0.8	0.25	0.4
EETC (g)	0.4	0.8	0.25	0.5	0.4	0.4
Stearic acid (g)	2.5	2.5	5.0	5.0	7.5	7.5
Cetylstearyl alcohol (ml)	3.75	3.75	5.0	5.0	5.0	3.75
Liquid paraffin (ml)	4.0	4.0	4.0	4.0	4.0	4.0
Triethanolamine (ml)	1.0	1.0	1.0	1.0	1.0	1.0
Hard paraffin (g)	2.5	2.5	2.5	2.5	2.5	2.5
Soft paraffin (g	5.0	5.0	5.0	5.0	5.0	5.0
Methyl paraben (ml)	0.05	0.05	0.05	0.05	0.05	0.05
Propyl paraben (ml)	0.025	0.025	0.025	0.025	0.025	0.025
Citrus sinensis oil (ml)	0.5	0.5	0.5	0.5	0.5	0.5
Purified water (g) to	100	100	100	100	100	100

Evaluation of cream formulations. Type of emulsion under dye test to determine the phase of the emulsion type *via* microscopy was carried out. pH, viscosity and organoleptic tests of the cream formulation was carried out.

Skin sensitivity test. A fixed amount of cream was applied on intact skin of three human volunteers and left for 24 hrs. The applied part of the skin was observed for any adverse reactions. Physical indications such as redness, inflammation, swelling, or rash were noted.

Microbial limit test. Microbial analysis was carried out for all the cream formulations according to the world health organization (WHO) guidelines.[13] All formulations were then autoclaved, and the biological load was computed using equation shown below.

$$\text{No. of bacteria} = \frac{\text{No. of colonies x Dilution factor}}{\text{Volume of inoculumn}} \quad \text{Eq. 1}$$

Antioxidant activity of the cream using DPPH assay. The free radical scavenging activity of the six formulations were evaluated and assayed spectrophotometricaly using a modification of the method described by Muthukumarasamy *et al.*[14] The radical scavenging activity of the formulations against 1,1 diphenyl1-1-picryl-1-hydrazyl (DPPH) radical *via* UV-Vis absorbance at 517 nm, was evaluated utilizing ascorbic acid as standard and ethanol as control. 100 mg of the cream was extracted using absolute ethanol in a separating funnel. To a methanolicsolution of DPPH (100 mmol/l, 2 ml), 2 ml of the test sample dissolved in ethanol was added at different concentrations (5-25 mg/ml). Absorbance was recorded at 517nm at 30 minutes. The scavenging activity was calculated as shown in Equation 2

$$\% \text{ Scavenging activity} = \frac{\text{Absorbance}_{517\text{control}} - \text{Absorbance}_{517\text{sample}}}{\text{Absorbance}_{517\text{control}}} \times 100 \quad \text{Eq. 2}$$

Here, ascorbic acid was used as a standard. A commercial antioxidant (Citrix® antioxidant sunscreen cream Topix Pharmaceuticals, Inc. NY, USA) was also evaluated and compared with the formulated antioxidant creams.

Accelerated stability testing. ICH guidelines (40 ºC/75 %RH) were followed in the accelerated stability testing of the polyherbal cream formulation. The creams were packed in amber colored jars and kept in a stability chamber with set temperature and relative humidity. The formulations were subjected to accelerated stability testing at both room temperature and at 40°C and parameters were recorded on day 0, 10, 15, 30, 90. The formulations were evaluated for pH, homogeneity, appearance, ease of removal, and spreadability, and anti-oxidant activity.

Statistical analysis. The data were expressed as mean ± standard deviation and was evaluated using one-way analysis of variance (ANOVA) (±SD). Significant differences ($p < 0.05$) of mean values were determined by Tukey test.

RESULTS AND DISCUSSION

All the formulated cream was found to be an oil in water (O/W) type emulsion, hence can be easily washed with water thus making it aesthetically appealing.

The formulated antioxidant cream was evaluated for several physicochemical tests, all creams had a pleasant citrus odor and were pale green in color. The formulated creams were not greasy after application to the skin. This process was confirmed by visual examination. There was no change in colour of formulated cream upon storage for long time. After feel test showed that the formulated cream were emollient.

All formulations have shown no sign of skin irritancy *i.e.* there were no signs of erythema, edema and irritancy within 24 hrs.

Rheological analysis of creams is essential to access the optimum stability as well as the changes produced with aging, stress and temperature. It also acts as a preliminary tool for the imminent failure of the product during storage.[15] Viscosities of all the formulations decreased gradually with increased shear rate, which indicated shear thinning or pseudoplastic behavior of all samples. This is a satisfactory rheological parameter due to formation of coherent film which covers skin surface upon application (Figure 1). This type of flow is mostly exhibited by emulsions, and it implies that an increase in the force as the cream is applied to the body will lead to easy spreading of the cream on the body. Formulation 6 was found to have the best flow and rheological property.

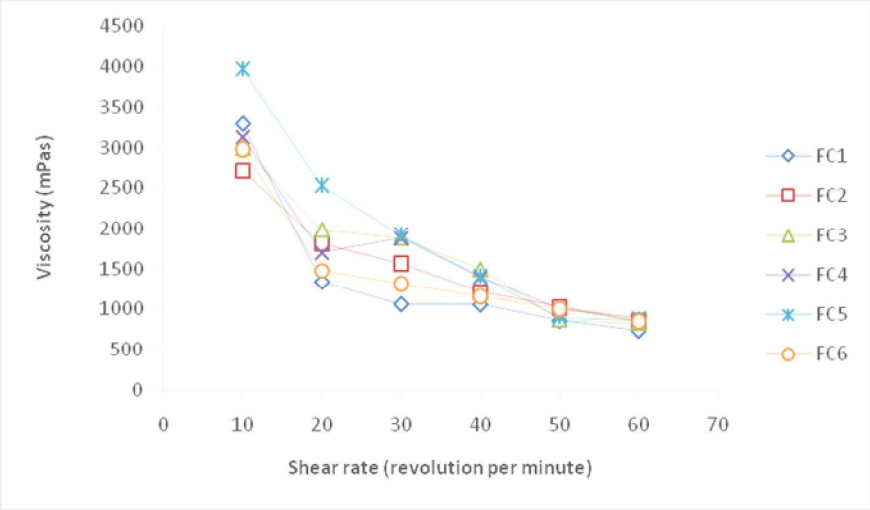

Figure 1. The effect of shear rate on the viscosity of formulations (n=3±S.D).

Suspension or emulsions are expected to have a relatively high degree of pseudoplastic behaviour.[15] The study of rheology is essential in cream formulation as it is expected that the formulations should have a low viscosity at high shear rates and also must be able to recover quickly and return to a high viscosity upon standing. This is due to the fact that the cream should be able to flow when applied to the skin and still retain its sufficient viscosity so as to remain on the skin and not flow off after application.[15,16]

Table 2. Accelerated stability testing on the polyherbal face creams ($p \leq 0.05$).

Time (Duration)	Formulation	pH	Dynamic Viscosity mPas at 40 rpm
DAY 0	FC 1	7.01±0.14	1060 ±1.56
	FC 2	7.03±0.03	1212 ±1.01
	FC 3	6.99±0.01	1490 ±2.97
	FC 4	6.99±0.01	1394±2.93
	FC 5	7.03 ±0.05	1405±1.04
	FC 6	7.01±0.06	1158±1.11
DAY 10	FC 1	7.03±0.14	1061 ±1.96
	FC 2	7.02±0.13	1210 ±1.71
	FC 3	7.00±0.21	1495 ±1.88
	FC 4	7.00±0.11	1395±2.20
	FC 5	7.03 ±0.05	1412±1.84
	FC 6	7.01±0.06	1158±2.91
DAY 15	FC 1	7.01±0.14	1061 ±1.96
	FC 2	7.03±0.03	1210 ±1.71
	FC 3	7.00±0.08	1495 ±1.88
	FC 4	7.00±0.09	1395±2.20
	FC 5	7.03 ±0.10	1412±1.84
	FC 6	7.01±0.10	1158±2.91
DAY 30	FC 1	7.01±0.14	1061 ±1.96
	FC 2	7.03±0.03	1210 ±1.71
	FC 3	7.00±0.11	1495 ±1.88
	FC 4	7.01±0.21	1395±2.20
	FC 5	7.03 ±0.15	1412±1.84
	FC 6	7.01±0.11	1158±2.91
DAY 90	FC 1	7.01±0.35	1061 ±1.96
	FC 2	7.03±0.26	1213 ±2.21
	FC 3	7.01±0.31	1495 ±1.99
	FC 4	7.01±0.11	1395±2.30
	FC 5	7.03 ±0.07	1412±1.74
	FC 6	7.01±0.08	1158±1.81

The antioxidant activity of the polyherbal formulation was assessed using DPPH radical scavenging activity. The highest radical scavenging activity (80%) was recorded in the leave extract FC6 at a concentration of 25mg/mL (IC_{50}). This was comparable with the marketed antioxidant cream Citrix® which showed comparable DPPH radical scavenging activity. There was a progressive increase in DPPH radical scavenging activity with an increase in concentration of the polyherbal cream formulation suggesting a concentration based release. FC1 and FC5 showed high DPPH radical scavenging activity (68.32 and 70%, respectively), at 25 ml/ml. A graph of concentration against antioxidant activity was plotted with ascorbic acid as a standard figure 2. Ascorbic acid which was used as the standard showed the highest activity followed by FC6 > FC5 > FC1 > FC3 > FC4 > FC2). All the polyherbal creams formulated showed radical scavenging activity with FC 2 showing the least activity (61.73%) at 25 mg/ml.

The microbial limit test included parameters like total bacterial count and total fungal count and eveidence of pathogenic bacteria like *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*. There was an absence of bacterial and fungal growth in all the polyherbal creams formulated. Absence of microorganisms in the formulation is imperative to prevent contamination of the skin which can impair the defense mechanism of the stratum corneum and mucous membranes.

Antioxidant extracted from natural herbal source have wide applications in preparation of cosmetic because of their easy availability and nontoxicity.[6,9] Antioxidants neutralize free radicals, unstable oxygen molecules that break down skin cells and cause wrinkles, thus preventing impairment at the cellular level. They inhibit inflammation leading to collagen depletion and offer protection against photo damage and skin cancer. The blend of EEOG and EETC have shown to be non irritating and the polyherbal cream has been shown to have excellent rheological characteristics that make it desirable as a cream. The inclusion of *Citrus sinensis* essential oil in the formulation increased the antioxidant effect of the polyherbal creams due to its rich coumarins, flavonoids, carotenes, terpenes and linalool content.[6,8]

Antioxidant creams are widely used today as it appears to be an interesting way to safeguard the skin against oxidative stress caused by various extrinsic sources. As part of a synergistic approach, most formulations have begun to thread the path of using multiple known antioxidant containing plants to achieve the required goals. The research work suggests that, to ensure the quality and purity of the cream it must have the consistency and uniformity in the ingredients of the herbal antioxidant cream. Topical application of anti-oxidant cream will be effective against UV radiation and protect the skin from major consequence of UV damage.

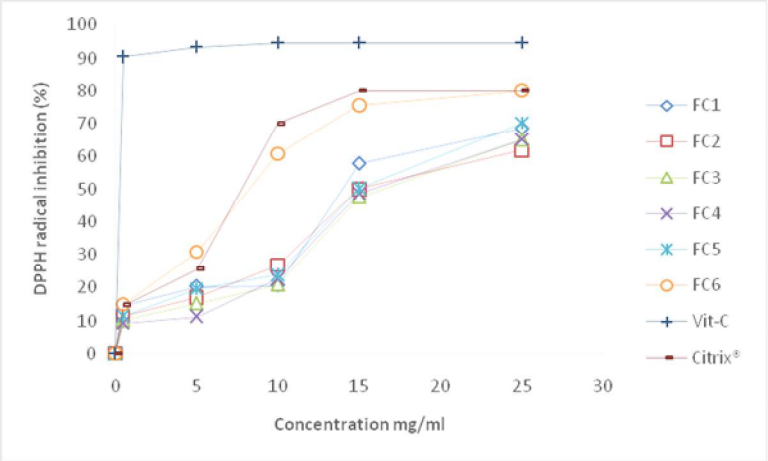

Figure 2. Scavenging of DPPH activity by the polyherbal cream formulations utilizing ascorbic acid as the antioxidant standard (n=3±S.D).

CONCLUSION

Polyherbal topical formulation containing *T. conophorum* and *O. gratissimum* was successfully prepared with pH within the limits compatible with stratum corneum with evident stability over 90 days. Antioxidant activity measured *via* DPPH scavenging activity was comparable to marketed formulations in FC1 and FC5. A combination of *T. conophorum and O. gratissimum* extracts confers a synergistic activity that potentially be useful for regression macular degeneration due to age and harsh environmental conditions. This herbal cream formulation can be further developed and translated to a marketed formulation for commercial use.

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