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Formulation and Evaluation of Polyherbal Antioxidant Face Cream Containing Ethanol Extracts of *Psidium Guajava* and *Ocimum Gratissimum*

***M. O. ILOMUANYA[A-F], T. AJAYI[B,C,F], I. CARDOSO-DAODU[C-F], T. AKHIMIEN[B,F], O. ADEYINKA[B.F], C. AGHAIZU[B,F]**

Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy. University of Lagos, PMB 12003, Surulere, Lagos, Nigeria.

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: Aging is a natural progressive process that leads to aesthetic and functional changes in the skin. The aim of this research work is to formulate and evaluate a polyherbal antioxidant face cream using the ethanol extracts of *psidium guajava* and *ocimum gratissimum*.

Method: 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.

Results: All the formulations showed good spread ability, good consistency, homogeneity, appearance, pH without phase separation occurring. Rheological tests showed that the all formulations exhibited non-Newtonian pseudo plastic flow. All six formulations also showed concentration dependent antioxidant activity. Ascorbic acid a potent antioxidant served as the standard for these tests. Formulation AFCR6 showed the highest antioxidant activity with IC50 value of 80.1 µg/mL.

Conclusion: The polyherbal antioxidant cream containing extracts of *Psidium guajava* and *Ocimum gratissimum* have been shown to have excellent antioxidants 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.

Keywords: Anti-oxidant, Face cream, *Psidium guajava*, *Ocimum gratissimum*

INTRODUCTION

Aging is a natural progressive process that leads to aesthetic and functional changes in the skin (Mardani-Nejada *et al.,* 2016) 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 (Nardostachy *et al.,* 2014). In normal conditions there is a balanced equilibrium existing between these radicals and the skin's natural antioxidants such as vitamin E, co-enzyme Q 10, ascorbates, and carotenoids (Matangi *et al.,* 2014). The excess generation of free radicals overwhelms the skins natural cellular antioxidants creating a 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 antioxidants (Matangi *et al.,* 2014). Antioxidants are substances that are capable of counteracting the damaging, but normal effects of the physiological process of oxidation in normal tissues. Antioxidants minimize the cellular damage due to oxygen and other free radicals; although age related macular degeneration is not a reversible process, antioxidants have the ability to clearly slow down the progression of aging (Saidulu *et al.,* 2014). The production of squalene peroxide due to oxidation of squalene cells in the epidermis by free radicals has

been identified as one of the most powerful acne causing substances in the world.

Plant extracts with antioxidant properties raise great interest in the phytocosmetic field as they present molecules that could inactivate reactive oxygen species restoring skin homeostasis and preventing erythema and premature aging of the skin. (Saewan and Jimtaisong 2013). Plants containing flavonoids have been reported to possess strong antioxidant properties. Studies have demonstrated that herbal extracts in formulations offer better efficacies than equivalent doses of individual herbs when used alone. (Zhang *et al.,* 2014). There are different types of herbal drugs which can be formulated in various forms such as gel, powders and creams.

Ocimum gratissimum is a herbaceous plant which belongs to the family Lamiaceae. It is commonly known as *scent leaf.* The plant is indigenous to tropical areas, especially India, and it is also found in West Africa. It is used in traditional medicine for treatment of several ailments such as urinary tract and gastro intestinal infections (Leal *et al.,* 2006). The leaves are used as general tonic and possess antidiarrheal properties. The essential oils contain eugenol, thymol and p-Cymene which show some evidence of antioxidant, anti-bacterial, anthelminthic and insecticidal properties (Hakkim *et al.,* 2008).

Psidium guajava is a small tree in the Myrtaceae family which is native to Mexico, Central America and Nigeria. It is a typical Myrtoideae, with tough dark leaves that are opposite, simple, elliptic to ovate and 5-15 cm long. Ethanolic extracts of *Psidium guajava* leaves contain carotenoids and polyphenols like (+)-gallocatechin and leucocyadin and flavonoids i.e. quercetin. The main constituent of the leaf oil is α-pinene, 1,8-cineole and β-caryophylene. The leaves are noted to have antidiarrheal, antidiabetic and antioxidant properties. (Cragg, and Newman 2013).

Psidium guajava and *Ocimum gratissimum ethanolic* extracts have been shown to a vast complex armory of active ingredients also known as phytochemicals which have the ability to calm soothe heal and protect the skin and they also possess UV absorbing activity attributed to the presence of flavonoids which is the major active ingredient common to both plants (Sahu *et al.,* 2012; Roy *et al.,* 2012). A combination of the two herbs as opposed to individual herbs may offer a synergistic activity. This study is therefore designed to formulate a polyherbal face cream that would serve as an antioxidant that will not only prevent the skin from the effects of reactive oxygen and free radicals but promote an overall healthy skin condition regardless of age.

MATERIALS AND METHODS

Materials

The materials used include Stearic acid (BDH Chemicals, England), cetylstearyl alcohol (BDH Chemicals, England), soft paraffin, liquid paraffin, hard paraffin, methyl paraben, Propyl paraben (Sigma Aldrich St. Louis, USA) and Triethanolamine (Merck, German). All other chemical and reagents were of analytical grade. The leaves of *Ocimum gratissimum* and *Psidium guajava* were obtained from Igbogila farm in Ogun state. The two plants were authenticated by taxonomists in the botany department of the University of Lagos, Nigeria with herbarium number LUH6981 and LUH 6980, respectively.

Methods

Extraction

The shade dried and coarsely powdered leaves of *Ocimum gratissum* and *Psidium guajava* were extracted using 100% v/v ethanol (Xu *et al.,* 2014). The extracts were then filtered and concentrated in the vacuum at 40-50℃ using a rotary evaporator (Retch USA). Evaporation of solvent in the rotary evaporator produces a crude extract. These extracts were dried in an oven at 40°C to obtain the dried ethanol extract and then the powdered ethanol extract of *Ocimum gratissum* (EEOG) and *Psidium guajava* EEPG were transferred appropriately into labeled sample bottles and stored in a refrigerator at 4^{o}C for subsequent use.

Formulation of the creams

Oil in water (o/w) emulsion- based cream (semisolid formulation) was formulated. The emulsifier (stearic acid) and other oil soluble components (cetylstearyl alcohol, soft paraffin, liquid paraffin and hard paraffin) were dissolved as the oil phase and heated to 75℃. The preservatives (methyl paraben and propyl paraben) and other aqueous components (triethanolamine, water and ethanol extracts of *Ocimum gratissmum* and *Psidium gratissimum*) were also heated to 75℃. The aqueous phase was added in portions to the oil phase with continuous stirring. The perfume (orange oil) was added after the temperature dropped to (45 ± 0.5)℃ (Table 1).

Table 1. Formula for preparing antioxidant cream formulations utilizing extracts of *Ocimum gratissum* and *Psidium guajava*

Ingredients	AFCR1	AFCR2	AFCR3	AFCR4	AFCR5	AFCR6
EEOG (g)	0.025	0.025	0.04	0.04	0.025	0.04
EEPG (g)	0.04	0.04	0.025	0.025	0.04	0.04
Stearic acid (g)	2.5	2.5	5.0	5.0	2.5	5.0
Cetyl stearyl alcohol (ml)	3.75	3.75	5.0	5.0	5.0	3.75
Liquid paraffin (ml)	4.0	3.0	4.0	3.0	4.0	3.0
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
Orange oil (ml)	0.5	0.5	0.5	0.5	0.5	0.5
Purified water (mls)to	100	100	100	100	100	100

Evaluation of cream formulations

Determination of emulsion type

Amaranth dye was utilized to determine the phase of the emulsion type via microscopy (Eclipse E100 Nikon TX, USA). A drop of the cream was placed on a microscopic slide, covered with a cover slip and examined under a microscope. If the dispersed globules appear red and the dispersion medium is colorless, the cream is oil-in-water type. The reverse condition occurs in water-in-oil type cream i.e. the disperse globules appear colorless in the red background.

pH of the formulation

The pH meter was calibrated using a standard buffer solution. About 0.5 g of the cream was weighed and dissolved in 50 mL of distilled water and its pH was measured (780 Metrohm pH meter CA, USA). Measurements were taken in triplicate

Viscosity

The viscosity of the formulation was determined using the spindle No. 4 of a Brookfield (Brookfield Engineering Laboratories NW, USA). viscometer at 10, 20, 30 40 and 60 revolutions per minute.

Organoleptic tests

The formulations were tested for homogeneity by judging their visual appearance and touch affinity. The appearance of the cream was judged and graded by its color, pearl essence and roughness. Emolliency, slipperiness and amount of residue left after the application of fixed amount of cream also was checked. A rubout was carried out to evaluate spread ability and wetness. A fixed amount of cream was applied on the dorsal skin surface of human volunteer, the type of film or smear formed on the skin and the ease of removal of the cream applied was examined by washing the applied part with water. The Lagos University Teaching Hospital Research Ethics Committee of the College of Medicine University of Lagos provided ethical approval for the study.

Skin sensitivity test (Draize test)

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

Microbial limit test

Microbial analysis was carried out for all the cream formulations according to the world health organization (WHO) guidelines for product evaluation (WHO 2016). It included total bacterial count, total fungal count, presence of *Escherichia coli, Staphylococcus aureus* and Bacillus subtilis.

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* 2016. The radical scavenging assay of the formulations against 1,1 diphenyl1-1-picryl 1 hydrazyl (DPPH) radical via UV absorbance at 517nm was done, utilizing ascorbic acid as standard and ethanol as control. Cream (100 mg) was extracted using absolute ethanol in a separating funnel. To an ethanolic solution of DPPH (100mmol/L, 2ml), 2ml of the test sample dissolved in ethanol was added at different concentrations (5-25mg/ml). Absorbance was recorded at 517 nm at 30 min. (Muthukumarasamy *et al.,* 2016). The scavenging activity was calculated.

$$\% \text{ Scavenging activity} = [(\text{Absorbance}_{517\text{control}} - \text{Absorbance}_{517\text{sample}}) / \text{Absorbance}_{517\text{control}}] \times 100.$$

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 40ºC and parameters were recorded on day 0, 10, 15, 30, 90. The formulations were re-evaluated for pH, homogeneity, appearance, ease of removal, after feel and spreadability and anti-oxidant activity.

Statistical analysis

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

RESULTS AND DISCUSSION

All creams gave a pleasant citrus fragrance due to the presence of orange peel oil extracts. Orange peel oil which is derived from the plant *citrus siensis* primarily provides fragrance for the topical formulation. It also doubles as an antiseptic, antinflammatory and antimicrobial. It also possesses antioxidant properties due to the presence of ascorbic acid (Oladipupo *et al.,* 2015).Orange peel oil extract can also serve as a source of nourishment for dry, irritated and acne prone skin (Oladipupo *et al.,* 2015). All six creams had a slightly green appearance which can be attributed to the presence of the herbal extracts. Cetyl stearyl alcohol was present in the cream as a viscosity and consistency enhancer, liquid paraffin as an emollient, methyl and propyl paraben were included as preservatives.

The viscosity and rheological properties of each of the formulations were evaluated. All formulations where found to exhibit non-Newtonian, pseudo-plastic flow when subjected to shear stress. An increase in the shear rate led to a decrease in the viscosity of each of the formulations, this type of flow is expected and usually exhibited by emulsions. Figure 1 shows that time taken during the reading of viscosity affected the viscosity readings due to increased shearing.

Shearing is a phenomenon which occurs whenever the fluid is physically moved or distributed, as in spreading or rubbing will determines how the cream will behave when applied to the skin surface (Chang *et al.,* 2013). During topical application the cream is automatically be subjected to shear stress by rubbing hence it is therefore important to study how the substance will behave when subjected to these conditions (Chang *et al.,* 2013). Pseudo plastic behavior of the creams imply that when shear stress is applied, flow thinning occurs (Figure 1), thus the formulation flows easily due to breaking of its organized structures thus allowing easy application all over the body (Mastropietro *et al.,* 2013). It is expected that a good suspension or emulsion base should have a relatively high degree of pseudo plastic behavior (Chang *et al.,* 2013). All the formulations showed a good flow thinning which was showed in Figure 1 where an increase in the rpm resulted in a decrease in the viscosity of the creams. AFCR 3, 4, 5 and 6 exhibited high viscosity on initial formulation, however when rpm was progressively increased all formulations AFCR 1-6 had viscosity 1146 – 1459 mPas at 40 rpm with the viscosity being uniform for all formulations at 50 rpm as 1046 mPas ± 5.02 mPas.

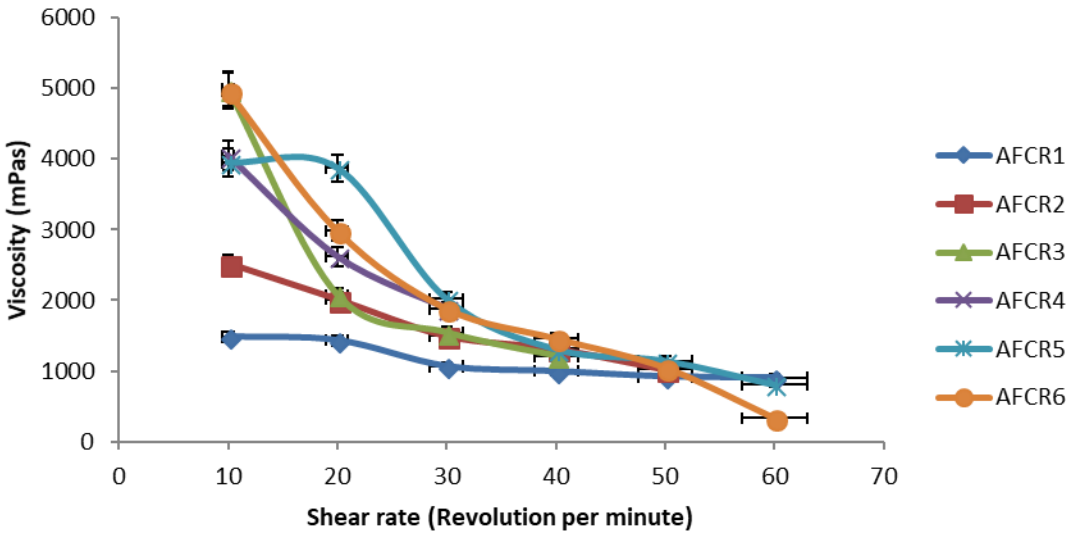

Fig. 1: The effect of shear rate on the viscosity of formulations (n=3±S.D)

The prepared poly herbal face cream was oil in water type of emulsion possibly enabled improvement in the dispersion of additives during formulation, it may also improve spreadability and permeation properties of the formulation.

The pH of the formulations ranged between 6.86 to 7.39, close enough to the pH of the epidermal layer (Table 2). All six formulations did not show any skin irritation or allergy when applied on the skin for the period of 24 h. After feel test showed that the formulated creams were emollient and the type of smears formed were non greasy. All six creams remained stable over this period of time during the accelerated stability test (Table 2). The results of the Draize test for sensitivity demonstrated that the formulations AFCR1-6 were safe and skin irritation and allergic sensitization were scarce or absent. All formulations showed no redness, edema, inflammation and irritation during application as shown in Table 2.

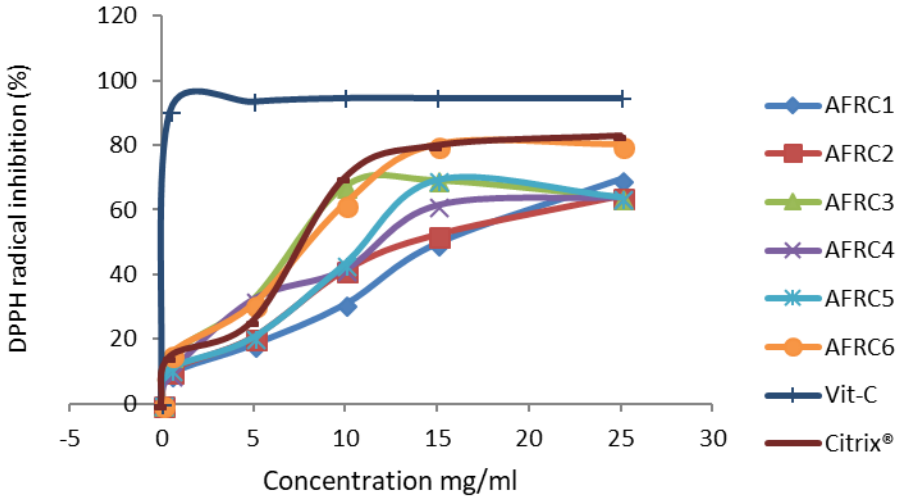

Figure 2: Scavenging of DPPH activity by the polyherbal cream formulations utilizing ascorbic acid as the antioxidant standard (n=3±S.D)

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

Time (Duration)	Formulation	pH	Dynamic Viscosity mPas at 40rpm
DAY 0	AFCR 1	7.09 ±0.04	1459 ±2.64
	AFCR 2	7.05 ±0.03	1309 ±1.98
	AFCR 3	6.86 ±0.01	1109 ±2.33
	AFCR 4	6.95 ±0.11	1212±1.73
	AFCR 5	7.03 ±0.07	1314±2.09
	AFCR 6	7.06 ±0.03	1009±2.34
DAY 10	AFCR 1	7.02 ±0.10	1461 ±1.64
	AFCR 2	7.05 ±0.02	1312 ±1.98
	AFCR 3	6.86±0.03	1119 ±2.53
	AFCR 4	6.95±0.10	1211±2.03
	AFCR 5	7.03±0.31	1319±3.11
	AFCR 6	7.00±0.21	998±2.34
DAY 15	AFCR 1	7.03±0.03	1459 ±1.71
	AFCR 2	7.05±0.02	1306 ±2.08
	AFCR 3	6.86±0.05	1111 ±4.50
	AFCR 4	6.95±0.21	1203±3.00
	AFCR 5	7.03±0.32	1314±2.71
	AFCR 6	7.01±0.03	997±3.01
DAY 30	AFCR 1	7.04±0.02	1458 ±1.41
	AFCR 2	7.01±0.01	1310 ±2.11
	AFCR 3	6.86±0.03	1114 ±4.72
	AFCR 4	6.95±0.11	1200±3.70
	AFCR 5	7.03±0.03	1315±2.71
	AFCR 6	7.03±0.21	1000±1.01
DAY 90	AFCR 1	7.04±0.22	1458 ±1.21
	AFCR 2	7.00±0.24	1310 ±2.31
	AFCR 3	6.76±0.21	1114 ±4.52
	AFCR 4	7.01±0.21	1200±3.50
	AFCR 5	7.03±0.17	1315±2.51
	AFCR 6	7.00±0.18	1000±1.11

The DPPH radical scavenging activities of the cream formulations containing ethanol extract of *psidium guajava* and *ocimum gratissimum* were assessed. DPPH radicals react with suitable reducing agents which lose color stoichiometrically and the number of electrons consumed was measured spectrophotometrically at 517 nm. Radical scavenging activity assay, on all six formulations exhibited concentration dependent antioxidant activity. A graph of concentration against antioxidant activity was plotted with ascorbic acid as a standard Figure 2. It was deduced that formulation AFCR6 had the highest radical scavenging activity which was comparable to commercially available antioxidant cream Citrix® which was also evaluated as shown in Figure 2. AFRC 6 showed a significant antioxidant activity i.e. 25 mg/mL (IC50) having activity of 80.4% in comparison to standard ascorbic acid having 95.1% activity i.e. AFRC6 > AFRC1 > AFRC5 > AFRC3 > AFRC2 > AFRC4); All the formulations showed radical scavenging activity with AFCR 4 showing an activity of 63.84% as the lowest activity recorded.

All the formulations passed the microbial limit test which included some parameters like total bacterial count and total fungal count. AFRC 1 was the only formulation that showed a total bacterial count of 43 CFU/g and this was well within the acceptable limit. Pathogenic bacteria like *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* were absent in all formulations studies. There was also absence of fungal growth in all the formulations studied.

The total number of microorganisms present in a formulation did not exceed the minimal stipulated standard for in the British Pharmaceutical Codex (Chang *et al.,* 2013). According to the pharmaceutical codex formulations for topical applications are not expected to contain any pathogens whilst a total viable count of ≤ 300 cfu/g is expected (Hitchins 2017). It is important that the microbial load be kept be within specifications as a higher microbial load would potentially impair the skin and mucous membrane defense mechanisms.

Most of the constituents used in the herbal formulation are viable nutrients for microbial growth it is thus imperative that the herbal formulations prepared should not be contaminated with pathogenic organisms in order to reduce susceptibility to biodegradation of the formulation and risk of topical infection if used.

The cream formulation has been shown to have high antioxidant activity which will have beneficial effects on the process of skin aging, skin sun protection or skin cancer. Acute exposure of human skin to UV radiation in vivo leads to oxidation of cellular biomolecules that could be prevented by a prior antioxidant treatment via utilization of this polyherbal face cream. The inclusion of *citrus sinensis* essential oil in the formulation increased the antioxidant effect of the polyherbal creams. *Citrus sinensis* oils are reported to be one of the rich sources of bioactive compounds namely coumarins, flavonoids, carotenes, terpenes and linalool etc. (Mondello *et al.,* 2005). Recently, Citrus peel essential oils have also been searched for their natural antioxidant and antimicrobial properties (Tepe *et al.,* 2005; Jayaprakasha *et al.,* 2007; Viuda-Martos *et al.,* 2008).

CONCLUSION

Poly-herbal face cream formulation containing *psidium guajava* and *ocimum gratissimum* were successfully prepared. The herbal antioxidant formulation was light green in colour, with pH within the limits compatible with stratum corneum, with non-Newtonian rheological profile. It was seen to be stable over a 90 day period during accelerated stability testing. Formulation AFRC 6 had the highest antioxidant activity comparable with a marketed antioxidant formulation. A combination of the *Psidium guajava* and *Ocimum gratissimum* extracts confers a synergistic activity that potentially creates a strong antioxidant activity useful for regression of age related macular degeneration. This herbal cream formulation can be further developed and translated to a marketed formulation for commercial use.

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*Address for correspondence
Margaret O. Ilomuanya
Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy. University of Lagos, PMB 12003, Surulere, Lagos, Nigeria.
E-mail: milomuanya@live.com; milomuanya@unilag.edu.ng
Tel: +234- 8033295077

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