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The Use of Selected Nigerian Natural Products in Management of Environmentally Induced Free Radical Skin Damage

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Abstract: This study investigated the effectiveness of natural extracts from honey, (*Apis mellifera* L. Apidae), cam wood (*Pterocarpus osun* Craib (Fabaceae) and Shea butter (*Butyrospermum parkii* G. Don, kotschy (Sapotaceae) incorporated for topical application in traditional cosmetic formulations in Nigeria to protect free radical induced skin damage. The effect of these samples on oxidative damage was assessed using the method of inhibition in lineolic acid peroxidation and vitamin E the predominant antioxidant in 'human skin as reference. Total antioxidants was measured as TAE (Total Antioxidant Equivalent). All the samples have antioxidant activity. The antioxidant activity (expressed as percent inhibition of oxidation) ranged from as high as 38.51-81.23% in honey depending on source since honey is produced from many different floral sources and subject to adulteration with syrup. 73.65% in shea butter and 43.26% in cam wood. Antioxidant activity had a direct relationship with TAE (Total Antioxidant Equivalent). It is concluded that natural antioxidants could induce depigmenting effect and replace use of synthetics.

Key words: Skin, lipid peroxidation, antioxidants, natural extracts, oxidative damage

INTRODUCTION

Skin reflects our heritage, emotional and physical well-being and gives away our age. Skin disorders are often visible to others and can result in psychological effects, which include depression, a negative self-image, increased emotional distress and quality of life deterioration. Skin is the largest organ of the body. It is responsible for many physical and biochemical processes such as protection, sensation, temperature regulation and vitamin D production. Although the skin is virtually waterproof, it does allow absorption of vitamins, hormones, drugs and chemicals[1].

Skin provides an essentially waterproof barrier and moderate to severe moisture loss is usually replaced internally, not externally. Without protective lipids, skin moisture is lost to evaporation[2]. Young skin and connective tissue contain mostly elastic or soluble collagen and as a result, it can absorb moisture and plump up. This ongoing process of moisturization and swelling keeps young, elastic skin looking sleek and smooth. Skin, which has a highly differentiated and certainly complex organizational structure, is particularly vulnerable to free radical damage because of its contact with oxygen. Other environmental stimuli and atmospheric pollutants like sun exposure, cigarette smoking and normal aging also cause oxidative damage to the skin. Smoking has an even greater effect on premature wrinkling than extensive sun exposure. Smoking depletes L-ascorbic acid, resulting in lowered serum levels[3-6]. This is an important source of oxidative and nitrosative stress both to terrestrial plants and animals. This damage is the same sort of thing that happens to collagen, which is inelastic, unable to absorb water well and does not plump up. With loss of elasticity and moisture, lines and wrinkles form, especially in areas exposed to sunlight, the face, the neck and the backs of the hands[7].

Free radicals are believed to have an important role in skin aging and in development of many inflammatory skin disorders and skin cancers. Free radicals have also been shown to be involved in UV-induced and ionizing irradiation damage to epidermal cells[8]. The epidermis, which is the first line of defense against free radicals, contains a variety of antioxidants than the dermis since it is directly exposed to solar radiation. Antioxidants are substances that mop up free radicals, the highly reactive oxygen molecules that are responsible for oxidative damage.

Antioxidant defenses include the lipid-soluble antioxidants vitamin E and carotenoids, the water-soluble antioxidants vitamin C and glutathione and the enzymes superoxide dismutase, catalase, glutathione reductase and

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glutathione peroxidase[8]. These antioxidants remove free radicals and thus counteract their potentially damaging effects. It therefore seems reasonable to try to increase levels of protective low molecular weight antioxidants through a diet rich in fruits and vegetables or by direct topical application. Indeed, various in vitro and animal studies have proved that low molecular weight antioxidants, especially vitamins C and E, ascorbate and tocopherol, as well as lipoic acid, exert protective effects against oxidative stress. Research has also demonstrated protective effects of French maritime pine bark and rosemary extracts against UV induced skin damage[9,10].

The purpose of this study was to investigate the effectiveness of natural extracts from honey, cam wood and shea butter incorporated for topical application in cosmetic formulations to protect free radical-induced skin damage.

Shea tree is the only sapotacea on the dry soils of the African Savannah. The fruit; similar to a small avocado, with a tasteful pulp contains a nut which kernel yields the precious butter by crushing[11]. Our shea butter is obtained exclusively by traditional crushing of shea nuts. The impurities are removed by treatment with hot water. It is then neutralized and refined by a physical process.

Three families of social bees produce honey, the Bombidae, Meliponidae and Apidae and all collect nectar, from different flowers. It is traditionally applied on sores and it is used against pile, as a therapy for earaches and gastric ulcer in Nigeria, infected leg ulcer in Ghana. Traditionally, the eating of honey will help to increase charm, beauty and success of a person. It is also used to increase fertility[12].

The genus *Pterocarpus* consists of 20 species distributed throughout the tropics, much confusion exists as to the botanical identification of various specimens of red woods known as bar wood and cam wood as the native names are not distinctive. Both bar wood and cam wood are of the class of insoluble red wood, the dyeing principle being insoluble or sparingly soluble, even in boiling water. Not all red extracts from insoluble red woods have emollient properties. The entire plant of the red and white variety is used in Nigeria for amenorrhea[13].

The effect of these samples on oxidative damage was assessed using lipid peroxidation. A simple method to quantify overall oxidative assay, typically utilized to quantify total antioxidant levels was also modified and measured directly as Total Antioxidant Equivalent (TAE). There are reports of the antioxidant activity of honey from other countries[14], but none on cam wood neither shea butter. This is the first report of the antioxidant activity of honey, cam wood and shea butter from Nigeria.

MATERIALS AND METHODS

Sample collection: All the samples used were purchased on separate occasions from different open markets in Lagos Nigeria.

Antioxidant activity: The antioxidant activity was determined using the ferric thiocyanate method as described by Kikuzaki and Nakatani[15]. Two milliliters of 200 mg L^{-1} ethanolic extract of sample, 2 mL of 2.5% (w/v) linolenic acid in ethanol 95% (v/v) 4 mL of 0.05 M of phosphate buffer (pH 7.0) and 2 mL of distilled water were mixed in a 10 mL test tubes covered with aluminum foil and fastened with rubber band. A blank sample was prepared using 4 mL of ethanol, 2 mL of 2.5% (w/v) linolenic acid in ethanol (95%) 4 mL of 0.05 M of phosphate buffer (pH7.0). The test tubes were placed in a water bath at 37°C and kept in the dark cupboard to accelerate oxidation.

0.1 mL of mixture above was added to 9.7 mL of 75% ethanol and 0.1 mL of 30% (w/v) ammonium thiocyanate. After 5 min, 0.1 mL of 0.02 M ferrous chloride solution in 3.5% (v/v) HCl was added to the mixture and stirred. The amount of peroxide formed was determined by reading absorbance at 500 nm at intervals for 24 h during incubation. α-Tocopherol (Sigma) was used as standard antioxidant while a blank of ethanol was ran with each assay. All determinations were carried out in triplicate. The inhibition of lipid peroxidation as a percentage was calculated by following equation:

$$\% \text{ Inhibition} = \frac{A_1 - A_2}{A_1} \times 100\%$$

Where, A_1 was the absorbance of the control reaction and A_2 was the absorbance in the presence of the extract sample.

Total antioxidants: Five gram of each sample was percolated with 50 mL of petroleum ether for 16 h at room temperature in 50 mL beakers covered with aluminum foil and fastened with rubber band. Filtrate was extracted with 3x20 mL of methanol. The methanol layer was concentrated to dryness and residue dissolved in ethyl acetate. The ethyl acetate extracts of the samples were kept in test tubes, covered with aluminum foil and diluted to strength of 1:200 to facilitate UV analysis. A standard curve was plotted with different concentrations of water-soluble vitamin E. Each measurement was expressed as percentage vitamin E equivalent per gram of sample. (Total Antioxidant Equivalent) All determinations were carried out in triplicate.

RESULTS AND DISCUSSION

The effects of these samples on oxidative damage were assessed using lipid peroxidation. A standard curve was plotted with different concentrations of vitamin E and a linear correlation was observed between absorbance and concentration of vitamin E (Fig. 1). All the samples have antioxidant activity. Activity increased in the order of cam wood, shea butter and honey. Inhibition of lipid oxidation by honey showed a great variation depending on source (Table 1).

Honey is produced from many different floral sources and subject to adulteration with syrup. The antioxidant activity of honey has also been demonstrated as inhibition of chemiluminescence in a xanthine-xanthine oxidase-luminol system that works via generation of superoxide radicals. This antioxidant activity may be at least partly what is responsible for the anti-inflammatory action of honey, as oxygen free radicals are involved in various aspects of inflammation, such as further recruitment of leucocytes that initiate further inflammation[14,16]. Even if the antioxidants in honey do not directly suppress the inflammatory process they can be expected, by scavenging free radicals, to reduce the amount of damage that would otherwise have resulted from these. There were no records on the activity of shea butter and cam wood in literature though shea butter is universally used as am emollient.

The values obtained by the TAE assay are designed to provide information on the full complement of antioxidants present. The TAE measurements showed a great variation in concentration of antioxidants, with the highest being 20.3 times that of the lowest. TAE positively correlated (R^2 = 0.9994) with antioxidant activity. Floral source is an important factor in the evaluation of honey as supplemental providing antioxidants[14].

The substances most commonly used as depigmenting agents are synthetic hydroquinone and its compounds, in particular its ethers such as hydroquinone monomethyl ether and monoethyl ether. Although they have a certain level of efficacy, these compounds are unfortunately not free of side effects on account of their toxicity, which can make them difficult or even hazardous to use hence its control by the National Agency for Food Drug Administration and Control (NAFDAC) in Nigeria. This toxicity arises from the fact that they interfere with fundamental mechanisms of melanogenesis, by killing cells, which then risk disrupting their biological environment and consequently force the skin to eliminate them by producing toxins. Thus, hydroquinone is a compound which is particularly irritant and cytotoxic to melanocytes and whose total or partial replacement has been envisaged by many researchers[17-20].

Table 1: Antioxidant Activity (AA) and Total Antioxidant Equivalent (TAE) of samples

Samples	(%) AA	(%) TAE
Honey 1	38.51±1.21	40.08±0.02
Honey 2	72.59±1.53	79.86±0.14
Honey 3	56.98±0.32	60.09±0.07
Honey 4	81.23±0.21	90.01±0.38
Cam wood	43.26±1.48	43.42±0.02
Shea butter	73.65±0.86	80.36±0.17

All values in this table represent the mean±SD (n = 3)

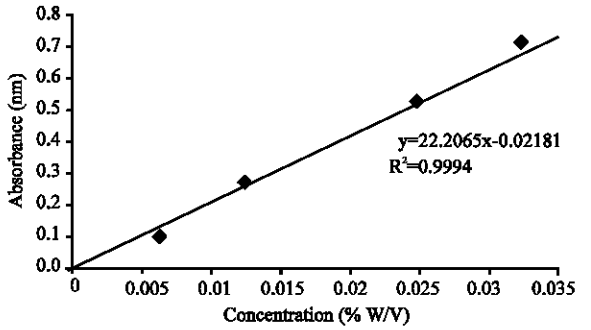

Fig. 1: Vitamin E calibration curve showing a linear correlation between absorbance and concentration

Substances have thus been sought which are not involved in the mechanism of melanogenesis, but which act upstream on tyrosinase by preventing its activation and are consequently much less toxic. Kojic acid is commonly used as tyrosinase-activation inhibitor, this acid complexing the copper present in the active site of this enzyme. Unfortunately, this compound is unstable in solution, which somewhat complicates the manufacture of the composition[21].

Oxidation has been found to have a strong relationship with melanin formation. But even if the antioxidants in these samples do not directly suppress the photo aging process they can be expected, by scavenging free radicals, to reduce the amount of damage that would otherwise have resulted from these to induce a depigmenting effect and replace use of synthetics. Thus highlighting the effectiveness of a natural antioxidant biotechnology in the management of skin.

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