LyophilisedAloe excelsafractions, photo-protection and actinic damage retardation claim substantiation: Novel natural sunscreens from tropical plants

ABSTRACT

Introduction: Ultraviolet radiation is potentially harmful to plants'physiological structures and theirphotosynthetic apparatus throughinduction of photo-oxidative damage and photosynthetic inhibition. *Aloe excelsa*a resilient (sub)tropical plant hasevolvedvariousphoto-protective mechanisms to proliferate in these harsh environments. With the current FDA, over-the-Counter Monograph M020 castigating 14 of the 16 approved sunscreens as "unsafe" to the ecology and human health, the hunt for safer sunscreens is on and phytoconstituents from photo-resilient plants may just be the next generation, safe and efficacioussunscreens.

Aims: This study investigated the photo-protectiveactivity of lyophilizedhydro-ethanolic*Aloe excelsa*extracts through phytoscreening and i*n-vitro* estimations of their SPF, UVAPF, UVA/UVB ratio, critical wavelength, anti-oxidancy as well as anti-inflammatory potential.

Methods: The *Aloe excelsa*gel matrix was physically extracted from transversely cut fresh rossetes,macerated in 70% ethanol, lyophilized and then subjected to both quantitative and qualitative phytochemical screening techniques. The antioxidant activity was measured using theDPPH scavenging assay, the photoprotection investigation was performed *in-vitro*using directives fromCOLIPA 2011/FDA Final Rule 2011 as guidelines and the anti-inflammatory capacity was evaluated using the protein denaturing test.

Results: Qualitative screening confirmed the presence of numerous primary and secondary metabolites of pharmacological interest, Quantitative phyto-analysis revealed that *Aloeexcelsa*has higher levels of total phenols, tannins and flavonoids than *Aloe vera*. Anti-inflammatory capacity was closely related to the standard Diclofenacand the anti oxidancy was almost equivalentto ascorbic acid. A prepared 50% *Aloe excelsa* gel had an SPF of 7.6 and a UVAPF of 4 and a critical wavelength of 375.The calculated photostabilities: $\%SPF_{eff}$ and $\%UVAPF_{eff}$ were both above 97%.

Conclusion:The resultsconfirm that*Aloe excelsa* is a multifunctional photoprotective material with confirmed anti-oxidancy, anti-inflammatory and UVR protection attributes. The 50% lyophilised *Aloe excelsa* gel can be technically classified as a low SPF sunscreen. The abundant primary and secondary metabolites correlate directly with the observed and calculated photoprotective parameters obtained in this study.

Key words: *Aloe excelsa*, natural sunscreen, photoprotection, anti-inflamattory, anti-oxidant.

1 INTRODUCTION

1.1 Ultraviolet radiation and natural photoprotection

The potentially harmful Ultra Violet Radiation (UVR) energies dissipated by the solar system display a linear reduction patternin relation to theirincidence on the globe,as the angle widens from the

perpendicular position of the sun characteristic of areas along or close to the equator[1].The solar zenith angle, spectral irradianceand AVA/B intensity, which is also depended on the time of day and season of the year is therefore high all year roundfor most Southern African Tropical and subtropicalplant habitats[2]. This geographic consequence as well as the relatively high altitudes in Southern African climes are believed to contribute considerably to the adverse UVR induced damage of resident plant species, coupled with photo inhibition of their various metabolic processes[3]. Photons from UVRhave been reported as damaging tomost plant species' photosynthetic apparatus due to their capacity to induce photo-oxidative damage while photoinhibiting the efficiency of the photosynthetic processes. UVR Intensity has been shown to have a geographical bias; maximum UVR intensity is received between the Equator and 30 degrees latitude North and South. UVA Intensity remains almost constant from the equator to 60 degrees North and South latitude[4]. During the winter months, very little UVB is detectable above 50 degrees latitude, the winter UVB levels in tropical countries is higher than the summer UVB levels in temperate areas[3,]. UVR is also affected by altitude, an increase in altitude of 1000m results in a 15% increase in UVB while UVA remains practically constant. Organisms, both Flora and fauna living in tropical and subtropical areas are therefore generally exposed to higher levels of actinic damaging UVR than any other geographical location[4].For the organisms to adapt to these conditions they should have optimizedvarious photo protective mechanisms so as to counteract free radical species generation and thus block both photo inhibition and photo damage. Thephotoprotective mechanisms in plant species which are believed to be based on the ultimate quenching of known singlet and triplet reactive states of the abundant chlorophyll as well as the reparative and proliferative process for repairing the damaged photosystemsare still very much under scientific debate and review[5].

1.2 Current sunscreens and the need for newer safer products

According to the FDA and COLLIPA guidelines, there are only 16 materials approved as sunscreens: 14 organic UV blockers and two inorganic (mineral) UV blockers[6]. The approved materials restrict UVA and UVB radiation onslaughts via two (not fully) agreed upon primary mechanisms. Organic UVR sunscreens are assumed to capture UVR and dissipate it in other metabolically lesser harmful energy forms, thereby protecting underlying skin structures from absorbing the damaging rays. The mineral Inorganic UV blockers are also capable of using the prior mechanism but also do have an added capacityto reflect UVR, depending on the formulation rheology and excipients employed[7]. However, even though there is abundant evidence relating to their immense benefits in retarding actinic damage and malignancy in consumers, the approved materials have been confirmed tocontribute to otherpotential health and environmental hazards[7]. Continuous confirmation of these hazards in scientific reports hasincited the need for newer safer alternatives and has tightened regulations on commercial sunscreens. Unfortunately, these stiffer current regulations provide an unassailable hurdle to the registration of new sunblock's. Globally, thebarriers to the adoption of any new material as a sunscreen are exceptionally high such that under the FDA, there has never been any new sunscreen registered in almost 30years[8]. It should also be noted that the current approved 16 sunscreens actually did not pass through the stringent regulatory frameworkbut were just incorporated into the system when the monographs were originally drafted. It is very doubtful if any of them could have passed the current regulatory requirements[9].The regulatory challenges invokethe need to think in other terms and develop non-traditional health and ecologically conscious sunscreen platforms.

Over the past few years, wild plant species rich in photo protective phytochemicals such as phenolics, flavonoids, tannins and other secondary metabolites have been investigated with interest as next generation non-traditional sunscreens[9].These phytoactives are fast gaining much attention specially after the gazzetting of the most recent FDA Over-the-Counter Monograph M020 which essentially passed a vote of no confidence in all sunscreens being used officially in the USA and much of the world[10].

The demand for natural actives assun protecting agents does not only stimulatea promising market of consumers who believe in their advantages but perhaps presages the future of natural sunscreens which are free from the controversies surrounding the current 16 approved sunscreen materials by the FDA[11].Since the majority of human phenotypes need sunscreens for survival from UVR, the quest for alternativenon-traditional sunscreens for photo protection has never been more urgent.Phenolic compounds abundant in highly resilient subtropical plant species including the Zimbabwe Tree aloe *(Aloeexcelsa)*are well known fortheir free radical scavenging and antioxidant potential and this work

seeks tocorrelatethese potential photo protective benefits with possible retardation of the biological end points for photodamage in humans[11,12].According to ourliterature search, systematic studies on the SPF, UVAPF as well as the antioxidant capacity of *Aloe excelsa* have not yet been reported. The aim of this studytherefore was to investigatethephoto protection potential of lyophilized*Aloe excelsa*extractsthrough*in vitro*determination of SPF,UVAPF, anti-oxidancy, anti-inflammatory capacity as well as to phyto screen and understand the general photo protective secondary metabolites of various lyophilized Zimbabwe tree aloe extracts.The leaf gelfrom thisresilient plant thriving in tropical and subtropical ecosystems is widely used in African traditional medicine as a photo protection balm.

1.3 Aloe excelsa

Figure 1: (a) *Aloe excelsa* plants amongst piles of stone and walls, near the Great Enclosureof the ancient Great Zimbabwe city ruins (b) Aloe excelsa rosettes in full bloom: Photos by Bart Wursten (Flora of Zimbabwe Home page-https://www.zimbabweflora.co.zw/)

Aloe excelsa iscommonly known as the Zimbabwe Tree aloe due to the prevalence of large populations of specimens found growing around the ancient ruins of Great Zimbabwe and various ancient settlementsin the region (Figure1)[13]. The cultural medical importance of this plant is supported by this abundance at ancient civilizations monuments scattered in the region which point to perhaps, an assertion that the ancient civilizations artificially propagated this plant close to their settlements due to its medicinal and photo protective importance to them.This unique drought resistant “tree” is non-branching and can stem up to 6m in height. The unusual aloe is usually wrapped with the remains of dead leaves up the stem[14].At the apex, the fleshy leaves form a compact arching and sometimes simply spreading rosette. The dull green leaves, distinguished from all other aloe species by an array of spines at the back can grow up to a meter long with a width of up to 150mm at the stem base. The leaf margins have unusual and sharp 3-angular reddish-brown teeth[14,15]. The flowers of the Zimbabwe tree aloe are distinguishable from other related aloes due to their classically flattened array downward on racemes. The *Aloe excelsa* flowers usually bloom in the late tropical winter with an astonishing variability of colors that include red, orange, yellow and even white variations[10]. Various studies on the plant show that it has widely been used traditionally by the native people as a treatment for various skin ailments most of which are consequent of the harsh UVR onslaught in the environments. Apart from the oral traditional use in photo protectiveskin treatment, the plant was chosen for this study primarily because of scientific reports that it contains various polyphenols which have spectrophotometric peaks in the UVR ranges of photo protectiveand pharmaceutical interest and should therefore potentially act as viable sunscreens[12,16]. Despite the fact that the exact mechanisms and active secondary metabolites involved or generated from the observed protection of the various chloroplasts are relatively unknown for *Aloe excelsa*,Indigenous knowledge systems and traditional medical practices in Zimbabwe and other subtropical regions have long recognized this sun damage protective capacity of *Aloe excelsa* and have positively correlated it to potential application in photo protectionof human skin types.

In the current study, we report the results of an investigation into the sun-screening and photo protective capacity of high-veld, wild tropical *Aloeexcelsa.*

2 MATERIALS AND METHODS

2.1 Chemicals and plant material

2.1.1 Plant materials

Fresh rosettes of *Aloe excelsa* leaves cut off from the racemesstems were collected from the Domboshava area of Zimbabwe (17^0 36′28.24″S 31^0 10′07.31″E) on 27 June 2023. The plant material was authenticated taxonomically as *Aloe Excelsa*by the national herbarium in Harare, Zimbabwe.

2.1.2 Chemicals and equipment

All chemicals, including 2,2-Diphenyl-2-picrylhydrazyl (DPPH) stable radical,thionyl chloride,Mayer's reagent,Millon's reagent, gallic acid, polysorbate 80, citric acid, Sodium benzoate, Ascorbic acid,chloroform, H_2SO_4, butylated hydroxy anisole (BHA), butylated hydroxytoluene (BHT), α-naphthol solution, sodium nitrite, Folin-Ciocalteu reagent, phenol,methanol,ethanol, acetone, hydrochloric acid, acetic acid, sodium carbonate, hexane, Carbopol 940®, trietanolamine, propylene glycol,glycerinewere jointly obtained at various stages of the studies from laboratories of either the University of Zimbabwe, School of pharmacy,Harare, Zimbabwe or the department of Chemistry and biochemistry at the University of California, Los AngelesCA90095, USA.All the spectrophotometric data were obtained either using aThermo Scientific Evolution 60S UV-Visible spectrophotometer (Shanghai, China) ora UV Transmittance Analyzer UV-2000S, Labsphere (North Sutton, NH, USA or Lambda 35 UV/Vis-Spectrometer, Perkin Elmer Instruments from either of the 2 institutionsabove where the studies were jointly conducted.

2.2 Extract preparation

The fleshy leaves were cut off transversely from the base of the rosette. The skin was scrapped off by a scalpel and*Aloe excelsa*juice was obtained by extracting the gel matrix sap from the leaves. The crude gel matrix was instantly preserved by adding 0.1% citric acid, 0.1% BHT and 0.1% Sodium benzoate.The plant gel matrix extracts were optimized by incubating 200g of the plant extract in 70% (v/v) ethanol for 1 L of the preparation for 72 hours in the dark. The extracts were filtered and evaporatedunder low pressure (Rotavapor® R-300, Buchi, Switzerland), followed by lyophilization (Lyovapor l-200, Buchi, Switzerland)under 120Pa pressure and -20 °C. To perform the photoprotection assay, the lyophilized extract of the *Aloeexcelsa* wasincorporated into a water based gel at a concentration of 50% (w/w).

2.3 Phytochemical screening

In a 200ml round bottomed flask, 10g of the lyophilized hydro-ethanolic extracts of *Aloe excelsa*were dissolved in 90ml of distilled water and subjected to various phyto-screening techniques to confirm the presence or absence of relevant phytoconstituentsof pharmacological interest to this study. The following qualitative tests were conducted on the extract liquor:

2.3.1 Qualitative Screening for primary metabolites

2.3.1.1 Detection of proteins and amino acids

The detection of proteins and amino acids was done using Millon's test. In a test tube,2 ml of Millon's reagent was addedto 5ml of the extract liquor, and the test tube contents were heated in a water bath at 70 ^oC for 10 minutes. The test tube was then cooled and 2 to 3 drops of sodium nitrite were added. The presence of proteins and amino acids was observed by the formation of a white thermo chromic precipitate which turned red when heated above 50 ^oC[17].

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2.3.1.2 Detection of carbohydrates

The Molisch's test was used to determine the presence of carbohydrates in the lyophilized extract. 2 to 3 drops of α-naphthol solution was added to a test tube,with 5 ml of the extract liquor. The presence of carbohydrates was detected by the development of a violet ring at the mixture phase junction[18].

2.3.2 Qualitative Detection of secondary metabolites

2.3.2.1 Detection of Anthraquinones andglycosides

The modified Borntrager's assay was used to determine the presence of anthraquinone glycosides. 5ml of the extract liquor was mixed with 5ml of dilute hydrochloric acid. The mixture was subsequently treated with 3ml ferric chloride solution and immersed in a water bath at 80^{o}C for 10 minutes. After cooling, extraction was done with 10ml of benzene. The resultant benzene layer was decanted and treated with 5ml ammonia solution. The mixture was observed for the development of a pink color which signals the presence of anthranol glycosides[19].

2.3.2.2 Tests for alkaloids

The presence of alkaloids was determined through the Mayer's test. Two drops of Mayer's reagent were added to 5 ml of the lyophilized extract liquor in a test tube. The presence of alkaloids was determined by the development of a white creamy precipitate[20].

2.3.2.3 Tests for tannins and phenolics

The presence of tannins in the extract was determined by the ferric chloride test. To a test tube,2-3 drops of ferric chloride was added to 5 ml of the prepared extract liquor. The test sample was observed for the presence of catechic tannins signaled by the development of a green-blue color or a blue-black color development which indicates the presence of Gallic tannins[21].

2.3.2.4 Test for flavonoids

The presence of flavonoids was determined by means of the alkaline reagent test. 2 to 3 drops of a 50 % NaOHlye were added to 5ml of the lyophilized liquor in a test tube. The development of a deep yellow colorwhich gradually paled to a colorlesshue after the further addition of 3 to 4 drops of dilute HCL, confirmed the presence of flavonoids[22].

2.3.2.5 Test for terpenoids

To confirm the presence of terpenoids. 5ml of the extract liquor was added to 2 or 3 granules of tin metal in 2 ml thionyl chloride solution in a test tube. The formation of a pink color indicated the presence of terpenoids[23].

2.3.2.6 Tests for steroids

The presence of steroids in the hydro-ethanolic extract of *Aloe Excelsa*was confirmed by adding 5 ml of chloroform to 5 ml of the extract liquor in a test tube,followed by the addition of 1 ml of concentrated Sulphuric acid. The development of a reddish browncolorindicated the presence of sterols in the extract[24].

2.3.2.7 Test for saponins

The simplified foam test was used to confirm the presence of saponins in the test sample. 5ml of the extract was added to 30ml distilled water in a 100ml measuring cylinder, the mixture was shaken for 3 minutes and the development of at least 1 cm head of foam in the test tube confirmed the presence of saponins[25].

2.3.3 Quantitative phytochemical analysis

The quantitative analysis compared the abundance of the secondary metabolites: phenolic, tannins and flavonoids in the aqueous, versus hydro-ethanolic extractions of *Aloe excelsa* versus a hydro-ethanolic

extract of *Aloe Vera.*The Quantitative phytochemical tests were done with the widely studied *Aloe Vera* as a standard reference.

2.3.3.1 Quantification of Total Phenolic and total tannins content

The total phenolic and total tannins content of the extracts were determined according to the Folin-Ciocalteu spectrophotometric method with some modifications. To prepare a calibration curve, phenol (Gallic acid) stock solution (5 mg/mL) was added into 100 mL volumetric flasks, and then diluted to volume with water. From each calibration solution, 0.25 mL was mixed with 1.25 mL of 10-fold diluted Folin-Ciocalteu's phenol (1 mL Folin reagent and 9 mL deionized water) reagent and allowed to react for 5 min. Then, 1 mL of 7.5% Na_2CO_3 solution was added, and the final volume was made up to 5 mL with deionized water. After 1 h of reaction at room temperature, the absorbance at 760 nm was determined by spectrophotometer (Lambda 35 UV/Vis-Spectrometer, Perkin Elmer Instruments). The test was done in triplicate. Calibration curves were plotted to determine the level of phenolics and tannins in the samples. The same procedure was done for different parts of *Aloe excelsa and Aloe Veraextracts* in concentrations. The test was done in triplicate. The results were expressed as Gallic acid equivalents (GAE, mg/g) of either of the *Aloe* extracts[26].

2.3.3.2 Quantification of total flavonoids

The total flavonoid content was estimated spectrophotometrically at 510 nm. In the test, 1mg of lyophilized extract was dissolved in 2mL of distilled water. To this solution, 0.5mL of IM sodium nitrite was addedtogether with 2ml of a 1M, NaOH solution, distilled water was then added to make the volume to 10ml. The solution was shaken and allowed to stand at room temperature for 15 min and the absorbance was subsequently measured. The total flavonoid content was estimated as mg of quercetin equivalent (mg QE/g extract) on a dry weight basis using the standard curve[27].

2.4 Antioxidant evaluation of *Aloe excelsa*

The antioxidant activity ofthe *Aloeexcelsa* was determined using the DPPH free radical scavenging assay[28]. To universal bottles, 50 µL of the lyophilized *Aloeexcelsa*extract in concentrations from 5 to 150 mg/ml were added followed by 5 ml of 0.004% (w/v) solution of DPPH. The resultant mixture was vortexed and incubated for 30 minutes at room temperature in a dark cupboard and then read using a UV spectrophotometer (Lambda 35 UV/Vis-Spectrometer, Perkin Elmer Instruments) at 517 nm. The blank was 70% (v/v) methanol. Ascorbic acid (Vitamin C) was used for comparison. Measurements were taken in triplicate[28].

DPPH scavenging effect was calculated using the following equation:

$$:\text{DPPH scavenging effect}(\%) = \{\frac{A^\circ - A}{A^\circ}\}\, x\, 100$$

Equation 1

Where A^0 is the absorbance of negative control (0.004% DPPH solution) and A is the absorbance in presence of extract. The results were reported as IC_{50} values and ascorbic acid equivalents (AAE, mg/g) of Aloe extracts.

2.5 In-Vitro SPF determination and photo stability evaluation of the lyophilized*Aloe excelsa*extract

2.5.1 Determination of SPF, UVAPF and the critical wavelength

Directives on Sunscreen testing and labelling of products prescribed by the FDA and COLIPA were used as guides in this determination of the SPF[29,30].The *in vitro*SPF determinations (from 290-400nm) were carried out using aSpectrophotometer equipped with two photodiode array spectrographs and coupled to an integrating sphere,Ultraviolet Transmittance Analyzer (UV-2000S, Labsphere, USA). The spectrophotometer had a xenon flash lamp, which permitted emission of the required continuouspeaklessspectrum ofradiation. The lamp supplied energies in the spectral range between 290–450 nm. The incremental step was 1nm and the irradiance was conveniently kept low so as not to

introduce potential photo stability to the *Aloe excelsa*lyophilized extract.For the *invitro* SPF determination the lyophilized extract was incorporateddirectly at 50% into a gel base composed of 0.25%Carbopol 940 ® and 0, 6 % Trietanolamineand water to make up the volume to 100%. The incorporation was done at 40^{o}C and the emulsion was left to cool to ambient room temperature before mounting onto the plates. The 50% *Aloe excelsa*gel was applied at a rate of 2mg/cm^{2} to square Polymethylmethacrylate (PMMA) plates which were roughed on one side (Helioplate™ HD6,HelioScreen, France) and was spread evenly over the plates with a fingertip covered in a vinyl glove. The natural sunscreens,50mg in total per each plate were directly weighed and applied in droplets onto the plates. Care was taken to prevent any material losses. Three plates were prepared for each sample and the filmed plates were kept in the dark to equilibrate for 15 minutes at 28 degrees Celsius. The equilibrated plates were then subsequently mounted ontothe light-path of the Ultraviolet Transmittance Analyzer (UV -2000S). The UV radiation transmittance patterns through the mounted samples was measured using the equipment settings above at 6 different sites of the plates. The blank was prepared by mounting onto the PMMA plates, the base emulsion without the 50% Aloe gel as per recommended guidelines. The built in equipment software used the recorded transmission patterns to calculate and determine I*n-vitro* UVA/B photo protection efficacy accordingly: The UVRphoto protectionefficacy of the *Aloe excelsa* gel was determined through the calculation of the UVB protection efficacy (SPF) and the UVA protection efficacy (UVAPF),the UVA/UVB ratio and the critical wavelength λ_c.
The *invitro* SPF as evaluated as per the following equation 2.

$$\mathrm{SPF}_{\text{in vitro}} = \frac{\int_{\lambda=290\,\mathrm{nm}}^{\lambda=400\,\mathrm{nm}} \mathrm{E}\lambda \,\mathrm{x}\mathrm{I}(\lambda)\mathrm{x}\,\mathrm{d}(\lambda)}{\int_{\lambda=290\,\mathrm{nm}}^{\lambda=400\,\mathrm{nm}} \mathrm{E}(\lambda)\mathrm{x}\,\mathrm{I}(\lambda)\,\mathrm{x}\,10^{\mathrm{A}_0\,(\lambda)}\mathrm{x}\,\mathrm{d}\lambda}$$

Equation 2
Where
E (λ); erythema action spectrum,
I (λ): spectral irradiance,
A0 (λ):mean monochromatic absorbancebefore UVexposure,
Dλ: wavelength step (1 nm).

The UVAPF value was generated after a coefficient of *in-vitro*adjustment'c' was calculated as shown in Equation 3.The value generated by the equipment softwareis regarded as the SPF label value.

$$\mathrm{SPF}_{\text{in vitro adjustment}} = \mathrm{SPF\ label} = \frac{\int_{\lambda=290\,\mathrm{nm}}^{\lambda=400\,\mathrm{nm}} \mathrm{E}(\lambda)\mathrm{x}\mathrm{I}\,(\lambda)\mathrm{x}\,\mathrm{d}(\lambda)}{\int_{\lambda=290\,\mathrm{nm}}^{\lambda=400\,\mathrm{nm}} \mathrm{E}(\lambda)\mathrm{x}\,\mathrm{I}(\lambda)\mathrm{x}10^{\mathrm{A}_0\,(\lambda)\mathrm{x}\,\mathrm{C}}\,\mathrm{x}\,\mathrm{d}\lambda}$$

Equation 3

Using the coefficient of Adjustment "c" the UVA protection factor wascalculatedusing Equation 4 after determining the dose of irradiation (D) by Equation 5.

$$\mathrm{UVAPF}_0 = \frac{\int_{\lambda=320\,\mathrm{nm}}^{\lambda=400\,\mathrm{nm}} \mathrm{P}(\lambda)\mathrm{x}\,\mathrm{I}(\lambda)\mathrm{x}\,\mathrm{d}(\lambda)}{\int_{\lambda=320\,\mathrm{nm}}^{\lambda=400\,\mathrm{nm}} \mathrm{P}(\lambda)\mathrm{x}\,\mathrm{I}(\lambda)\mathrm{x}\,10^{\mathrm{A}_0(\lambda)\mathrm{x}\,\mathrm{C}}\mathrm{x}\,\mathrm{d}(\lambda)}$$

Equation 4

$$\mathrm{D} = \mathrm{UVAPF}_0\mathrm{x}\mathrm{D}_0$$

Equation 5

Where
P (λ) is the PPD action spectrum

D_0=1.2

The finalUVAPF was calculated according to Equation 6

$$\mathrm{UVAPF} = \frac{\int_{\lambda=320\,\mathrm{nm}}^{\lambda=400\,\mathrm{nm}} \mathrm{P}(\lambda)\mathrm{x\ I}\ (\lambda)\mathrm{x}\ \mathrm{d}\lambda}{\int_{\lambda=320\,\mathrm{nm}}^{\lambda=400\,\mathrm{nm}} \mathrm{P}(\lambda)\mathrm{x\ I}\ (\lambda)\ \mathrm{x}\ 10^{-\mathrm{A}(\lambda)\mathrm{x\ C}}\ \mathrm{x}\ \mathrm{d}\lambda}$$

Equation 6

Where
A (λ) is the mean monochromatic absorbance after UV exposure.
At each wavelength increment a (λ) was calculated using Equation 7 and the (λc),

$$\mathrm{A}(\lambda) = \log\frac{\mathrm{C}_\lambda}{\mathrm{P}_\lambda}$$

Equation 7

The UVA/UVB ratio was therefore consequently calculated as the ratio between thefinal UVAPF and the SPF label.

2.5.2 Evaluation of the lyophilised extract gel photostability
The observations and calculations above for SPF, UVAPF, UVA/UVB ratio and the critical wavelength were noted at 0, 30, 60, 90 and 120 minutes so as to assess the photostability of the gel as a function of time.

2.6 Evaluation of the Anti-inflammatory capacity of *Aloe excelsa*

The protein denaturation assay as described by Madhuranga HDT (2023)was the test of choice to assess theanti-inflammatory capacity of the lyophilised *Aloe excelsa* extract[31]. The test was used to determine whether the extract could hinder egg albumin from becoming denatured in PBS. In this assay 0.5 ml fresh egg albumin from a free range domesticated hen (Gallus domesticus)was added to 10 ml of PBS (pH 7.2) and mixed with 5ml solutions of the lyophilised extract. The lyophilised *Aloe excelsa*extract was used in varying concentrations. The test concentrations used in the assays in µg/ml were 125,250,500,1000, 2000, 4000, 6000 and 8000. The resultant mixtures were then incubated (Shel lab SRI3 Low Temperature BOD Incubator) at 36.8°C for 15 minutes and then heated at 65°C for 10 min. After cooling, their absorbance was determined at 660 nm (UV spectrophotometer, Lambda 35 UV/Vis-Spectrometer, Perkin Elmer Instruments)usingthe vehicleas blank. Diclofenac sodium was used as a comparative reference standard at related concentrations of (µg/ml) 125, 250, 500, 1000, and 2000 respectively. The vehicle was used as the control. The percentageinhibition of protein denaturation was calculated by usingthe following formula in equation 8:

$$\mathrm{Inhibition}(\%) = \frac{\mathrm{A}_{\mathrm{control}} - \mathrm{A}_{\mathrm{sample}}\ \mathrm{x}\ 100}{\mathrm{A}_{\mathrm{sample}}}$$

Equation 8
Where
$A_{s\ ample}$ is the absorbance of samples and $A_{control}$ is the Absorbance of the blank.

The Half-maximal inhibitory concentration (IC50) valuewas determined to be the anti-inflammatory inhibition Percentage of 50 % concentration

3 RESULTS AND DISCUSSION

3.1 Qualitative phytochemical screening

The Aloe family contains vast phytochemical classes. The widely studied*Aloe Vera* has been found to have phytochemical groups including anthraquinones, chromones, anthrones, phenolic compounds, flavonoids, tannins, steroids, and alkaloids and many others which contribute to its reputed pharmacological activities[32]. The most popular compound found across all studied Aloe species is Aloin[33], an anthraquinone with well-knownpharmacological activity. Another anthraquinone, aloe emodin present in almost all studied aloe specie has reported toxicity issues which limits the quantities of Aloe species that can be taken orally[32]. In the present assay the strong presence of anthraquinones was also noted alongside presence of all expected secondary metabolites reported in literature from other studied aloe species shown in Table 1.

3.1.1 Primary and secondary metabolites screening

Table 1: *Aloe Excelsa* qualitative phytochemicals screening for primary and secondary metabolites

Test for	Presence in hydro-ethanolic extract	Presence in distilled water extract
Alkaloids	++	+
Phytosterols	+	-
Flavonoids	+++	+
Saponins	-	-
Proteins and Amino Acids	+	+
Fixed oils and fats	-	-
Phenolic compounds	+++	++
Tannins	++	++
Carbohydrates	++	+
Glycosides	+	+
Terpenoids	+	-
Anthraquinones	+++	++

3.1.2 Quantitative phytochemical screening

In quantitative phytochemical estimation, the total phenol content of the hydroethanolicand aqueous extracts of *Aloe Excelsa* revealed 78 mg GAE/gHydroethanolic extract and 48 mg GAE/g aqueous extract respectively. The tannin content showed 15 mg GAE/g extract and 612mg GAE/g extract respectively while the total flavonoid content revealed 0.088 mg QE/g hydroethanolic extract and 0.062mgQE/g aqueous extract respectively. The GAE calibration curve was done to help determine the total phenolic content of thelyophilized *Aloe excelsa* extracts. Similar estimates done for *Aloe Vera* as a comparative standard revealed lower figures for the secondary metabolites under review compared tothe *Aloeexcelsa*(Table 2)

Table 2: Total Phenolic, tannins and flavonoids Content Aloe excels vs *Aloe Vera*

Extract	Total phenol	Total tannins	Total flavonoids
	mgGAE/g extract	mgGAE/g extract	mgQE/g extract
A. excelsa- Hydroethanolic	78	15	0.088
A. excelsa- Aqueous	48	12	0.062
A. Vera-Hydroethanolic	22	9	0.052
A. Vera -Aqueous	9	7	0.078

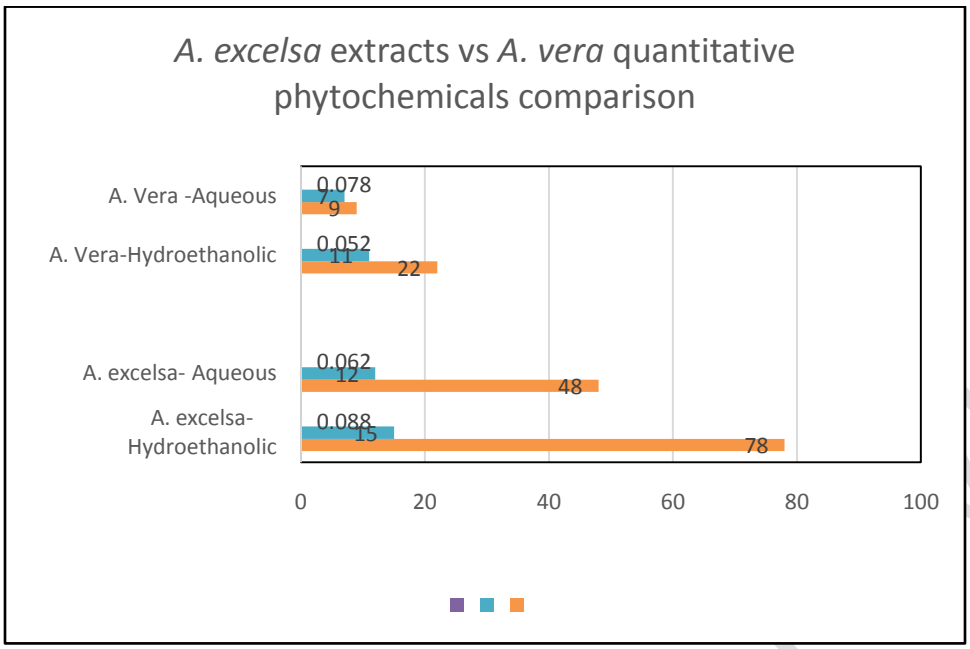

Figure 2: *Aloe excelsa* versus *Aloe Vera* Quantitative phytoscreeninganalysis

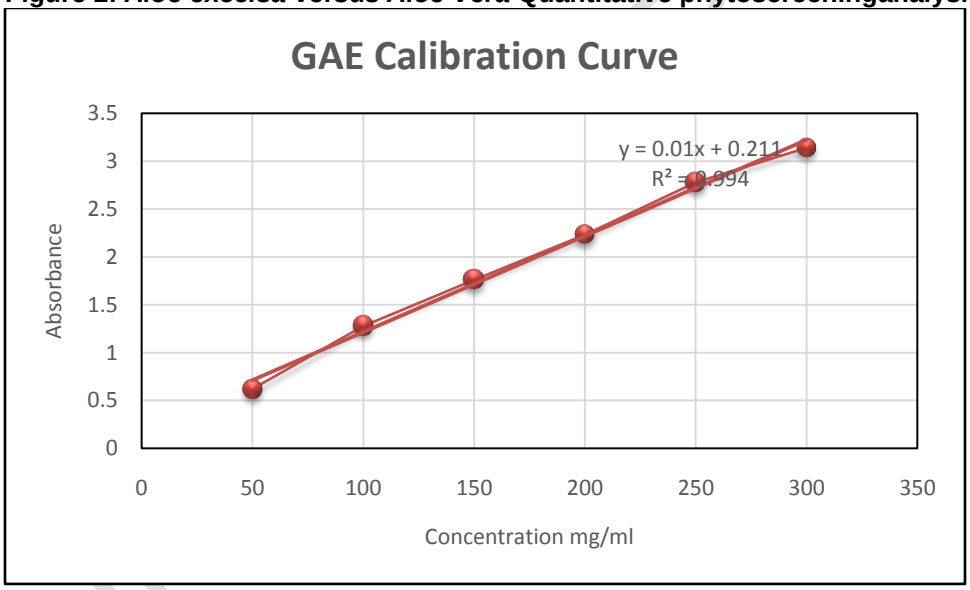

Figure 3: *Aloe excelsa* Gallic acid standard curve for Folin-Ciocalteu assay

The comparative tests with *Aloe Vera*(table 2, figure 2 and figure 3) show that the *Aloe excelsa* studied here has more than 4 times the total phenolics found in *Aloe Vera* and almost double the total tannins and considerably more flavonoids. This could be attributed to the fact that our *Aloe excelsa* sample was from a wild variety whereas the *Aloe Vera* was artificially propagated. It is scientifically proven that plants synthesise secondary metabolites as defence mechanisms to cope with environmental challenges[4,8]. It is therefore sensible to assume that the wild *Aloe excelsa* sample thrived in a more stressful environment than the artificially nurtured *Aloe Vera* and hence developedhigher volumes of secondary metabolites to deal with pathogens, climatic stresses and resource competition with other species.

3.2 Antioxidant assay

DPPH scavenging activity was 93.56% for ascorbic acid (the standard used) at 150 µg/ml. While *the Aloe excelsa*hydro-ethanolic extract was 87.8% and 77.9% for the aqueous extract (figure 4). The IC_{50} values (figure 5) were 26.02µg/ml, 46.9µg/ml and 63.63µg/ml for ascorbic acid, *Aloe excelsa*hydro-ethanolic extract and the aqueous extract respectively.

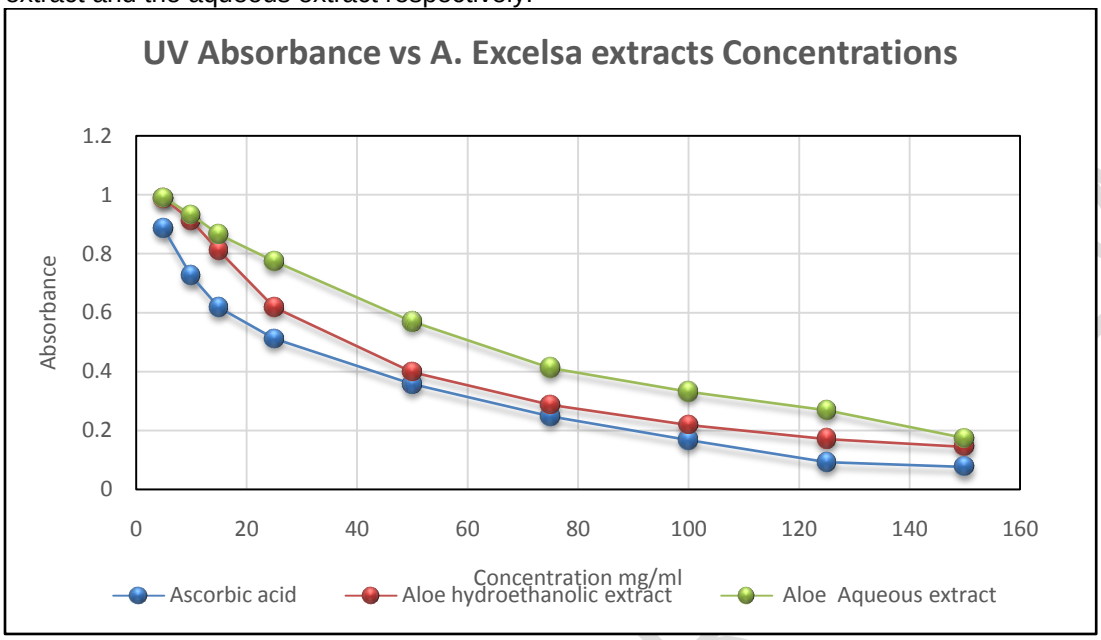

Figure 4: DPPH scavenging assay of *Aloeexcelsa*extract compared to the ascorbic acid

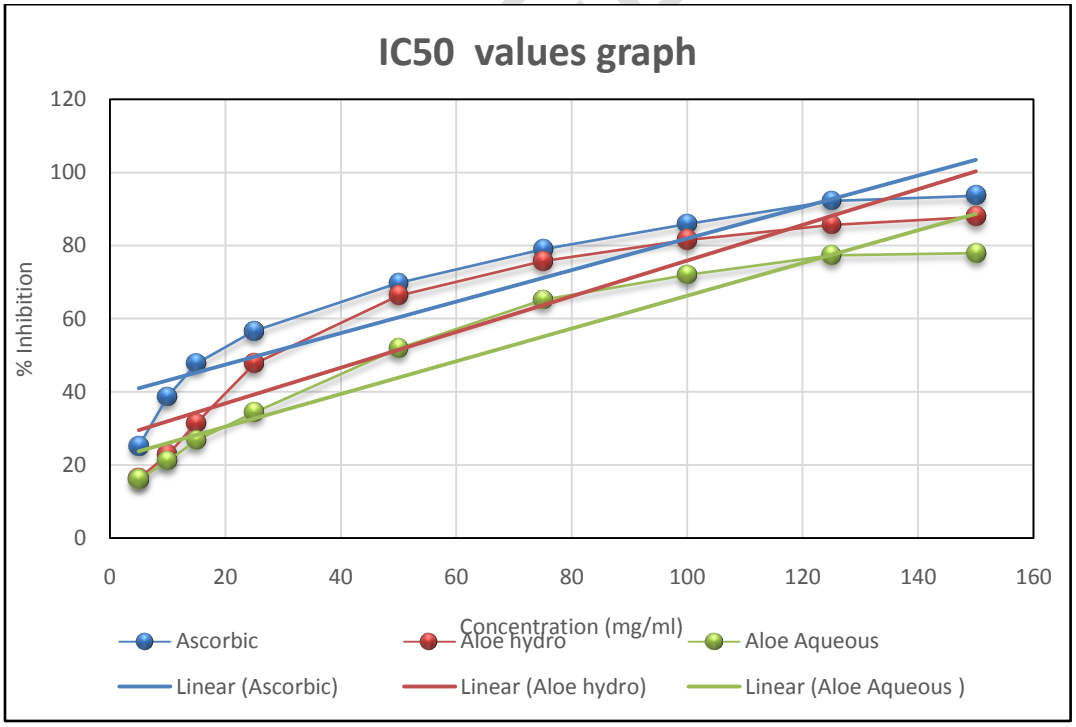

Figure 5: Evaluation of IC50 of *Aloe excelsa*extracts and ascorbic acid

UVR has the capacity to generate reactive oxygen species (ROS) or free radicals in living systems. These have the potential to cause oxidative stress, DNA mutations, lipid peroxidation, and protein oxidation which all have deleterious effects on skin structure, barrier properties and reparative functions[2]. ROS contribute tophoto aging, Immunosuppression, and photo carcinogenesis which are all aspects of actinic damage. Antioxidants have the capacity to scavenge for the ROS from living systems thereby preventing or reducing actinic damage and maintaining redox states through quenching of these harmful ROS[2,4,8]. In plant metabolism processes, antioxidant activity depends on the presence of specific bio-active secondary metabolites, especially polyphenols, tannins and carotenoids, which are all present in abundance in *Aloe excelsa* according to our phytoscreening results above. DPPH free radical scavenging is perhaps the mostaccepted test protocol for screening for the antioxidant activity of plant extracts. Our resultsrevealed that the hydro-ethanolic extract of *Aloe excelsa* has almost similar free radical scavenging activity as compared with standard ascorbic acid (Fig.4 and figure 5). The high total polyphenols content shown in table 2 correlate very well with the observed free radical scavenging antioxidant activity of *Aloe excelsa*shown in Figure 4.

3.3 Anti-inflammatory capacity of *Aloe excelsa*

Table 3: Effects of *Aloe excelsa* and Diclofenac against protein denaturation

Concentration	% Inhibition	% Inhibition
µg/ml	**Aloe excelsa**	**Diclofenac**
250	10± 0.07	31±0.24
500	14±0.09	56±0.72
1000	24±0.14	204±3.82
2000	76±1.16	640.20± 6.24
4000	180±1.64	Not tested
6000	390±2.86	Not tested
8000	584±4.76	Not tested

The crudeHydroethanolic Aloe*excelsa* extract exhibited anti-inflammatory effects that are approximately 25% of the anti-inflammatory attributes of Diclofenac. The anti-inflammatory activity of the aloe became comparable to the least dose of the positive control Diclofenac(250 µg/ml) used in this study at around 1000µg/ml Theanti-inflammatory effect of 2000 µg/ml of the positive control Diclofenac was only 8.75% greater than that for 8000 µg/ml lyophilised crude extracts of *Aloe excelsa*. These results are interesting for they are notably high for a crude extract.

Plant secondary metabolites are known to possess anti-inflammatory activities[34]. Various phytochemicals responsible for the anti-inflammatory activities include polyphenols, terpenoids, flavonoids, saponins, and tannins all of which are present in *Aloe excelsa* from the preliminary phytochemical screening done in this study. Denaturation of tissue proteins is believed to be one of the underlying factors in the production of auto antigens and as a marker for inflammatory conditions. It makes technical sense to conclude that agents that prevent protein denaturation also have anti-inflammatory attributes. The tests carried out above are therefore valid tests as preliminary screens to confirm the presence of anti-inflammatory factors in samples. In this present study the protein denaturation bioassay confirmed anti-inflammatory properties of lyophilised*Aloe excelsa* extracts at levels almost comparable to Diclofenac.

3.4 In-Vitro SPF determination andphoto stabilityof lyophilized *Aloe excelsa*extract

The *in-vitro* photo protectiontesting for SPF and UVAPF was done following the “Sunscreen Testing According to COLIPA 2011/FDA Final Rule 2011 Using UV/Vis LAMBDA Spectrophotometers” guidelines. After testing materials for photoprotective activity using the protocols, sunscreen SPFs are labelled as either low, moderate, high or very high as shown in table 4[29,30]. According to these guidelines, marketed consumer products with SPFs below 6 cannot be classified as sunscreens because the

protective effect is low and the objectives of sun protection cannot be achieved. The guidelines also nullify the use of the word “sun-block” in efficacy claims and labelling so as to prevent misinterpretations by consumers.

Table 4: The four protection classes for SPF labelling by COLIPA 2011/FDA Final Rule 2011

Label SPF	Protection class
6	low
10	low
15	moderate
20	moderate
30	high
50	high
50+	very high

For ease of commercial application and to ensure that products compliance can be achieved from 1 testing guideline, the FDA Final Rule 2011 test protocol parameters are aligned to COLIPA 2011 sunscreen guidelines[29,30]. Apart from the SPF, both directives require proof of UVA protection factor (UVAPF) from products which should be equal to or more than 1/3 of the SPF, The directives also specify an *in-vitro* Critical Wavelength value greater than 370 nm, in order for a product to comply with requirements for broad spectrum sunscreen protection.

Table 5: *In vitro* photo protection and photo stability of 50% *Aloe Excelsa* gel

Parameter	Time, minutes				
	0	**30**	**60**	**90**	**120**
SPF	7.60±0.40	7.5±0.54	7.45±0.76	7.41±0.32	7.40±0.24
UVAPF	4	4	4	4	4
λ_C	375	375	375	374	375
UVA/UVB ratio	0.78	0.77	0.77	0.77	0.76
$\%SPF_{eff}$	-	98.6	98	97.5	97.4
$\%UVAPF_{eff}$	-	100	100	100	100

According to the above results, the *in-vitro* photoprotectiveefficacy of *Aloe excelsa* as depicted by SPF and the UVAPF and the photostability of the formulated 50% *Aloe excelsa*gel was successfully evaluated. We used a gel formulation ahead of emulsions because dermatologists often recommend the use of gel based formulations ahead of emulsions for acne prone skin types because they are less greasier than emulsions. Acne prone skin types also stand to benefit more from the other anti-inflammatory attributes of *Aloe excelsa*also investigated in this study. Our previous studies also confirmed that Gel based formulations are absorbed faster in ordinary use conditions due to their lightweight[35,36].Since perceptible sunburn is only caused by UVB, the sunburn protection factor(SPF) therefore only evaluates the efficacy of protection from UVB, it becomes imperative to evaluate other parameters which contribute to all the solar induced potential damages referred to as actinic damage. Therefore, in this study in order to relate our findings to the concept of broad spectrum photo protection, we also measured and reported the UVAPF, the UVB/UVA ratio as well as the critical wavelength ƛc as shown in Table 4. From the same table we also report the results from the photostability of the extracts evaluated over 2 hours. The results confirm that the photo screening extracts retain theirbroad spectrumphoto protectioncapacity over a 2 hour post irradiation period. Our results show that, their efficacy remained stable throughout the

observation period without any significant aberrations in the tested parameters. According to our reported results here, the gel formulation of *Aloe excelsa*has an SPF of 7.6 and a UVAPF of 4 and a critical wavelength of 375. These interesting results infer that, even at 50% concentration,the hydroethanolic lyophilisedcrude extracts of *Aloe excelsa* meet the COLIPA 2011definition of a "sunscreen"[29,30]. The calculated *in-vitro* efficacy percentages: $\%SPF_{eff}$ and $\%UVAPF_{eff}$which are above 97%for the lyophilized crude extracts point to interesting potential for uses of *Aloe excelsa*gel in general actinic damage retardation cosmeceuticals or for the addition of crude *Aloe excelsa* to organic and inorganic sunscreens for added synergistic effects which will in turn lead to a reduction of the used percentages of potentially harmful organic sunscreens in consumer products to obtain high SPFs.

4 Conclusions

The current investigation substantiates the potential for use of *Aloe excelsa* as a multifunctional photoprotective material. According to the COLIPA 2011/FDA Final Rule 2011 sunscreen testing protocols and labelling guidelines, the results for the 50%*Aloe excelsa*gel reported here, qualifies the extract under the category of low SPF sunscreens.The SPF and UVA PF results confirm the lyophilised extract capacity to directly absorb and dissipate UV light. Other important parameters confirmed include, antioxidant capacity, photostability and anti-inflammatory attributes which are key to actinic damage photo protection. The abundant primary and secondary metabolites identified in the extract correlate directly with theobserved and calculated phyto active photoprotective efficacy. To the best of our knowledge, the technical comparisons with *Aloe Vera* reported here for the first time establish *Aloe excelsa* as one of the most pharmacologically active Aloe species in nature.

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