European Journal of Integrative Medicine 29 (2019) 100925

Contents lists available at ScienceDirect

European Journal of Integrative Medicine

journal homepage: www.elsevier.com/locate/eujim

Research paper

The anti-aging potential of medicinal plants in Cameroon - *Harungana madagascariensis* Lam. and *Psorospermum aurantiacum* Engl. prevent in vitro ultraviolet B light-induced skin damage

Jacqueline Manjia N.[a,b], Frederic Njayou N.[a,*], Apeksha Joshi[b], Kapil Upadhyay[b], Kavita Shirsath[b], Ranjitsinh Devkar V.[b], Paul Moundipa F.[a]

[a] Laboratory of Pharmacology and Toxicology, Department of Biochemistry, Faculty of science, University of Yaoundé I, PO. Box 812 Yaoundé, Cameroon
[b] Division of Phytotherapeutics and Metabolic Endocrinology, Department of Zoology, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, 390002, India

ARTICLE INFO

Keywords:
Medicinal plants
Cameroon
Anti-Aging
Antioxidant
Anti-Elastase
Anti-Tyrosinase

ABSTRACT

Introduction: Plants are used by the populations of Foumban (West Region) and Nkol Anga'a (Center Region) for skin care in Cameroon, Central Africa. In this study, the anti-aging potential of the medicinal plants were investigated by evaluating their *in vitro* antioxidant, anti-elastase and anti-tyrosinase activities followed by the analysis of their protective effect on ultraviolet B light-induced oxidative stress.

Methods: Antioxidant potential to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydrogen-hydroxyl (OH°), reduce potassium ferrocyanate and phosphomolybdenum and inhibit lipid peroxidation (LP) was assessed while the anti-elastase and anti-tyrosinase activities were determined spectrophotometrically. The protective potential of the most active extracts were determined on ultraviolet B light-induced oxidative stress in fibroblasts and melanoma cells by measuring the mRNA levels of Nrf-2 and HO-1

Results: Twenty-six medicinal plants species belonging to sixteen families were stock-listed. The crude extracts of *Harungana madagascariensis* and *Psorospermum aurantiacum* possessed high total polyphenol content. Likewise, the antioxidant activity of both plants was comparable to that of ascorbic acid in all the studied oxidative models. Tested at 100 µg/mL, these extracts were found to inhibit the activity of elastase at 100 and 77.58% respectively. Both extracts exhibited low inhibitory activity on tyrosinase as compared to ascorbic acid while the isolated hexane and methanol fractions demonstrated strong antioxidative activities. The hexane fraction of *P. aurantiacum* induced significant ($p < 0.01$) up-regulation of antioxidant genes (Nrf-2 and HO-1).

Conclusion: The above cited plants exhibited high antioxidative, anti-tyrosinase and anti-elastase activities and, their active compounds may be promising for skin care.

1. Introduction

Human skin is constantly exposed to by microorganisms, chronic light exposure, pollution, ionizing radiation, chemicals, and toxins that produce reactive oxygen species (ROS) and that inevitably result in detrimental outcomes which can speed up skin aging [1,2]. Skin aging is a complex mechanism characterize by a loss of skin elasticity and strength as well as development of pigmentation disorders, acnes and pimples [3,4]. Moreover, hyperpigmentation, deterioration of collagen and elastin fibers resulting in wrinkles, laxity, skin dryness, poor wound healing [5] has been attributed to up-regulation of tyrosinase, collagenase and elastase, respectively [6,7]. Such skin appearances are the result of oxidative stress due to skin exposure to sun UV-B (280–320 nm) [4]. To efficiently counteract reactive oxygen species (ROS), skin cells are equipped with special elements such as nuclear factor erythroid 2-related factor 2 (Nrf-2). Nrf-2 that is involved in the regulation of antioxidant genes (heme oxygenase- 1 (HO-1), superoxide dismutase, catalase…). However overproduction of ROS impairs the

Abbreviations: PA, psorospermum.aurantiacum; HM, harungana madagascariensis; Nrf-2, nuclear factor Erythroid 2-related factor 2; HO-1, heme oxygenase-1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; DMSO, dimethyl sulfoxide

* Corresponding author.
E-mail addresses: jmanjia@gmail.com (J. Manjia N.), njayou@yahoo.com (F. Njayou N.), apekshajoshi134@gmail.com (A. Joshi), kplupadhyay192@gmail.com (K. Upadhyay), kavitashirsath04@gmail.com (K. Shirsath), rv.devkar-zoo@msubaroda.ac.in (R. Devkar V.), pmoundipa@hotmail.com (P. Moundipa F.).

https://doi.org/10.1016/j.eujim.2019.05.011
Received 30 March 2019; Received in revised form 24 May 2019; Accepted 24 May 2019

good functioning of these antioxidant genes [3].

Topical bleaching agents such as hydroquinone, kojic acid, arbutin and azelaic acid can reduce signs of skin aging but have also been reported for their inefficiency and side effects [8,10]. Therefore, the need for discovery and development of new strategies to halt skin aging has become highly evident. Natural compounds are known as activators of antioxidant genes offering protection against UV-induced cytotoxicity [3,4].. Thus, the skin anti-aging abilities of the extracts has been reported by several research groups. In this regard, green tea polyphenols and fruit of *P. strobilacea* were shown to prevent oxidative damage, anti-elastase property in several experimental models [8,9]. Caffeic acid and quercetin have also been reported to reduce oxidative stress and inhibit tyrosinase [11]. Ellagic acid has been found to suppress oxidative stress and up regulate Nrf-2 and HO-1 in human skin cells [12]. Effective compounds with minimal side effects remain scarce and hence screening of other natural products for potential anti-skin aging activity is much needed.

In this perspective, an ethnopharmacological survey was carried out amongst local communities and traditional healers in Cameroon to collect medicinal plants used for skin care. These plants were screened *in vitro* for their anti-skin aging activities including antioxidant, anti-tyrosinase and anti-elastase activities. Then, the most active extracts were selected, fractionated and, fractions assessed for their effects on antioxidant genes and experimentally induced intracellular oxidative stress in fibroblast and melanoma cells.

2. Materials and methods

2.1. Chemicals

Elastase (type VI), N-Suc-(Ala)3-P-nitroanilide (sucAla3-PNA), Tyrosinase, L-tyrosine, 2,2-diphenyl-1-picrylhydrazyl (DPPH), potassium persulfate, ferrozine, ferrous sulfate, potassium ferricyanide, trichloroacetic acid, ferric chloride, sodium nitroprusside, L-ascorbic acid, Folin-Ciocalteu reagent, were purchased from Prolabo (Paris, France) and Sigma–Aldrich chemical company (Hamburg, Germany). All chemicals of molecular biology grade were purchased commercially. Dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Sisco Research Laboratory Pvt. Ltd. (Mumbai, India). Fetal bovine serum (FBS), Dulbecco's Modified Eagle's Medium (DMEM), trypsin phosphate versene glucose (TPVG) and antibiotic-antimycotic solution were purchased from Hi-media Laboratories (Mumbai, India). TRIzol reagent, DreamTaq and Green master mix were procured from Invitrogen (CA, USA). iScript cDNA synthesis kit was purchased from Bio-Rad (CA, USA). Skin mouse embryonic fibroblast (NIH/3T3) and skin mouse melanoma (B16F10) were procured from National Centre for Cell Science (NCCS, Pune, India).

2.2. Ethnopharmacological survey, plant collection and extraction

The symphony between the indigenous populations and their traditional practice such as herbal therapy led us to identify the localities in Cameroon namely Foumban and Nkol Anga'a as our study areas. A total of 32 healers were surveyed in both of the study areas (20 in Foumban in the West region and 12 in Nkol'Anga in the Centre region). A survey was conducted in August 2011 after prior consent of interviewers and with a clause that information gathered was purely for scientific studies and not for any commercial use. The current study was approved in accordance to the regulations of the Graduate School from the University of Yaoundé I (Cameroon). Participants who freely accepted to share their traditional knowledge on herbal medicine with our research team were selected and interviewed. Each individual was administered a questionnaire, and the answers to questions were completed by the participant or by our team member in accordance with his statement based on their degree of literacy. The participants were either traditional healers or elderly people who had the experience of using medicinal plants for skin care. Information on local names and traditional description of the medicinal plant species, parts of plants used, preparation methods, administration methods and their therapeutic efficacies were obtained through the survey. Plants identified by the person interviewed were harvested in the same area, photographed and labeled specimens for the herbarium were prepared. At the end of the investigation, the specimens were transported to the National Herbarium of Cameroon in Yaoundé (HNC) for identification.

Aerial plant parts (stem, barks and leaves) were harvested between 6 and 11 a.m. in Foumban and Nkol Anga'a washed with clean water, shade dried at room temperature, grinded, packed, stored at −20 °C and then analyzed separately. Due to the fact that participants used various solvents and methods, our team adopted to use mixture of methylene chloride-methanol (1: 1 v / v) as the common extraction solvent. Thus, 50 g of powder of each plant part was macerated in this solvent for 24 h while stirring at regular intervals and filtered (Whatman No. 1 filter paper). The filtrates obtained were concentrated under vacuum by a rotary evaporator (Heidolph WB 2000). Based on their good biological activities potential, two plant extracts were selected and fractionated by liquid-liquid partitioning using hexane, methylene chloride, ethyl acetate and methanol. After calculating the yields, the evaporated extracts and fractions were then stored at −20 °C for the further experiments.

2.3. Antioxidant assays

All the plant extracts were tested at final concentrations of 0.1, 1, 10 and 100 µg/mL.

2.3.1. Ferrous reducing power assay

The reducing power of plant samples was assessed as described by (Patil et al., 2009). A volume of 50 µL of each plant extract was added to 1.l mL of phosphate buffer (0.6 M, pH 6.6) and 1 mL of potassium ferrocyanate [K3Fe $(CN)_6$] (0.25%). After incubation for 20 min at 50 °C, 1 mL of trichloroacetic acid (TCA) (10%) was added and the mixture was centrifuged (3000 rpm, 10 min, 4 °C). The supernatant collected was mixed with 1 mL of distilled water and 0.2 mL of ferrichloride (1%). The mixture was well homogenized and allowed to stand for 10 min at room temperature. The absorbance was measured at 700 nm against a blank solution. Ascorbic acid was used as positive control.

2.3.2. Total antioxidant capacity assay

Determination of total antioxidant capacity was done by the phosphomolybdenum method as reported by (Kumar and Hemalatha, 2011). The tubes containing the plant extracts and reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) were incubated at 95 °C for 90 min. After the mixture has cooled to room temperature, the absorbance of each solution was measured at 695 nm against a blank. The antioxidant capacity was expressed as ascorbic acid equivalent (AAE/g of extract).

2.3.3. Diphenyl-1-picrylhydrazyl ($DPPH^{\circ}$) radical scavenging assay

The free radical-scavenging ($DPPH^{\circ}$) activity of the plant extracts was measured according to the method of [13]. A volume of 3.1 mL of freshly prepared DPPH (40 µg/mL) in pure methanol was mixed with 50 µL of the test sample to achieve the desired concentration. The mixture was shaken vigorously and incubated for 30 min in the dark at room temperature. The absorbance was recorded at 517 nm against a blank. Control samples contained the same volume of solvent without the plant extract (or ascorbic acid as positive control). The percentage of DPPH scavenging activity was calculated according to the Eq. (1) below and used for half scavenging concentration (SC_{50}).

$$\text{Inhibition } \% = [A_0 - (A2-A1)]/A_0 \times 100\% \quad (1)$$

Where A_0 is the absorbance of the incubated DPPH°· solution without addition of the sample or positive controls, A1 is the absorbance of the sample without DPPH°· Solution and A2 is the absorbance of the incubation mixture containing both the test sample and DPPH°· solutions.

2.3.4. Hydroxyl (OH°) radical scavenging assay

Hydroxyl radical scavenging assay was performed according to the method described by [14]. The reaction mixture consisted of 50 µL of each plant extract, 0.7 mL of Fe_2SO_4 (3 mM), 1 mL of H_2O_2 (1 mM), 1 mL of distilled water and 0.4 mL of sodium salicylate (10 mM). The mixture was then incubated at 37 °C for 1 h, and the absorbance measured at 562 nm. The scavenging activity was calculated using the formula described in the Eq. (1) above.

2.3.5. Lipid peroxidation assay

The inhibition of lipid peroxidation by plant extracts and ascorbic acid was determined according to the thiobarbituric acid method. $FeCl_2$–H_2O was used to induce lipid peroxidation in liver homogenates [14]. Each plant extract was mixed with 1.0 mL of a 10% liver homogenate, and then, an appropriate volume of Fe_2Cl_2 (0.5 mM) and H_2O (0.5 mM) was added. The mixture was incubated at 37 °C for 60 min, and then, 1.0 mL each of trichloroacetic acid (15%) and thiobarbituric acid (0.67%) were added, and the mixture was heated to 100 °C for 15 min. After centrifugation (3500 rpm, 5 min), the absorbance of the supernatant was recorded at 532 nm. The inhibition percentage was calculated according to the Eq. (1) mentioned above.

2.4. Elastase inhibitory assay

A preliminary test was carried out to determine the linear zone where the initial velocities could be calculated and this was found to be in the interval of 60–180 seconds (data not shown). The enzyme activity was measured in the presence or absence of inhibitor (vitamin C) or the test extracts. The activity of porcine pancreatic elastase was examined using N-Suc-(Ala) 3-P-nitroanilide (sucAla3-PNA) as substrate. The release of P-nitroaniline was measured spectrophotometrically at 410 nm as described by [15]. Briefly, the reaction was carried out in a volume of 780 µL of Tris − HCl buffer (pH 8.0, 0.2 M), 50 µL of plant extract and 50 µL of elastase solution (1 µg/mL), the blanks tubes contained the buffer instead of enzyme. The reaction mixture was pre-incubated for 15 min at 25 °C before adding 20 µL of sucAla3-PNA (0.8 mM) and then homogenized by inversion. The change in absorbance of the mixture was monitored at 410 nm and the absorbance was measured every 15 s for 5 min. The initial rates of hydrolysis of the substrate by the enzyme were calculated in the linear zone and the inhibition percentages were determined according to the formula of the Eq. (2) below.

$$\text{Inhibition (\%)} = [(A–B)/A] \times 100 \quad (2)$$

Where A is absorbance without plant extract, and B is the change in absorbance in presence of plant extract.

2.5. Tyrosinase inhibitory assay

Each plant extract was assayed for tyrosinase inhibitory activity by using the method described by [16] with some slight modifications. The reaction was carried out in 500 µL of phosphate buffer (pH 6.8, 0.1 M), 250 µL of l-tyrosine (1.5 mM). After preincubation for 15 min at 37 °C, 50 µL of plant extract and 100 µL of mushroom tyrosinase (31 units/mL) maintained at 37 °C were added. The blank contained the buffer instead of the enzyme. The mixture was incubated for 45 min at 25 °C. The absorbance of the mixture was read at 490 nm against blank. The inhibition percentage was calculated according to the formula above (Eq. (2)).

2.6. Determination of total phenolic content (TPC) and phytochemical screening

The total phenolic content was analyzed according to the Folin–Ciocalteu method as described by [17]. Briefly, 50 µL of tested samples was mixed with 2.4 mL of distilled water, and 200 µL of Folin–Ciocalteu's reagent (1/10) were added to 500 µL of Na_2CO_2 20%. The reaction mixture was incubated at 25 °C for 40 min, and the absorbance read at 725 nm. The results were compared to a chlorogenic acid calibration curve, and the total phenolic content was expressed as milligrams of chlorogenic acid equivalents (CAEs) per gram of extract.

Phytochemical screening of the different fractions was performed according to a procedure described by [18,19]

2.7. Determination of half inhibitory concentration (IC_{50}) of plant extracts and correlations between phenolic content with antioxidant potency

IC_{50} values were automatically calculated by using the software STATGRAPHICS 5.0 plus. Correlation between total phenolic content of fractions and their antioxidant activity were determined using the Microsoft Excel 2007, and the coefficient of correlation (r^2) value was deducted from the graph.

2.8. Cell culture and treatment

Fibroblast (NIH/3T3) and Melanoma (B16F10) cells were cultured in DMEM (10% FBS and 1% antibiotic antimycotic solution) at 37 °C in 5% CO_2 in air atmosphere. Cells were trypsinized using 1 ml of trypsin-EDTA (0.25%W/V and 0.53 mM) and sub-cultured every two or three days. The study was grouped as Control (untreated cells), UV−B control (UV−B irradiated cells), positive control (UV−B irradiated cells + ascorbic acid) and UV-B-irradiated cell + plant fraction. A stock solution of plant fraction was prepared in [DMSO: phosphate-buff ;er saline (PBS)] (0.5–1%) at a concentration of 1000 µg/mL, and the solution was filtered through 0.22 µm filter membrane. The final concentrations (100, 50, 25, 12.5 µg/mL) were achieved by dilution with culture medium.

2.9. Cell viability assay

The cytotoxicity of plant extracts toward NIH/3T3 Fibroblast and Melanoma B16F10 cells was determined using 2-(2, 5-dimethyl-2thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay according to [20] with slight modifications. A cell suspension containing 10^5cells were plated in 96-well plates in 100 µL growth media and left overnight. The cells were exposed to various concentrations of plant fractions for 24 h. 100 µl MTT of 5 mg/mL concentration was added to each well and after 4 h of incubation at 37°c the supernatant was removed. The remaining purple formazan crystals were dissolved in 150 µl of dimethyl sulfoxide. Plates were gently shaken for 15 min at room temperature. Absorbance was measured at 540 nm using ELX800 universal Microplate Readers (Bio-Tek instruments, Inc., Winooski, VT). Cell viability at each concentration of plant extracts was determined by comparison with the negative control without plant extract. After cell viability calculation, the concentration of plant extract which showed at least 80–100% of viability was selected to perform further experiments.

2.10. Intracellular oxidative stress assay

The effect of crude extract and fractions on ROS production in B16F10 and NIH/3T3 induced by UVB was measured using 2′,7′-dichlorofluorescein diacetate (DCFDA) and following previously described procedures of [4]. Cells were grown at 5×10^5cells per well in a 6-well plate with 2000 µL of supplemented medium overnight. The medium was removed, and cells were treated with crude extract and

fractions for 2 h in FBS-free medium. Then, the cells were irradiated at a dose of 39.6 and 29.7 mJ/cm2 for B16F10 and NIH/3T3 respectively, and they were incubated at 37 °C for 15 min. DCFDA was added (25μM) in FBS-free medium and cells were incubated for additional 15 min at 37 °C in a CO_2 incubator. Finally, the cells were washed twice with PBS and images were taken on floid cell imaging station (Life Technologies, USA). The images were analyzed using image J software.

2.11. Anti-oxidant gene mRNA level modulation studies

Cells (5×10^5 per well) were seeded in 6 well plates with fresh complete culture medium for 24 h. The cells were pre-treated with various concentrations of plant fraction for 2 h followed by UVB exposure and incubated for further 24 h. After treatment, total RNA was extracted from cells using TRIzol Reagent according to the manufacturer's instructions. RNA concentration and purity were determined by reading the absorbance at 260 and 280 nm by using spectrophotometer (Thermo Scientific). The 260/280 ratio of our RNA preparation ranged from 1.8 to 2.1. Total RNA (1 μg) was reverse transcribed using iScript cDNA Synthesis kit as described by the manufacturer. Semi-quantitative PCR was used for analysis the mRNA levels of target genes. GAPDH was used as an internal control. The sequences of all primers used in this study are presented in the Table 1. All primers of interest genes were synthetized by TSINGKE Biological Technology Company (Beijing, China).

2.12. Statistical analysis

Data analysis was carried out using the GraphPad Instat 3.0 software. The Kolmogorov test was used to verify the normality of the values. For normal distribution, the one-way analysis of variance (ANOVA) test was used. The results were expressed as mean ± SEM of three independent experiments run in duplicate. The difference was significant when $p < 0.01$. For gene expression, data were presented as mean ± SEM and analyzed by one-way ANOVA using Graph Pad Prism 6.0 (CA, USA) followed by Dunnett's multiple comparisons test. Results were considered to be significant when $p < 0.05$.

3. Results

3.1. Plant survey and identification

From the survey, twenty-six plants with medicinal potential used against skin affections were collected, identified belonging to sixteen different plant families. These plants are listed in alphabetical order and per family in Table 2. Each plant has its available National Herbarium reference number. In addition, other information regarding the part of the plant used, method of preparation, dosage and yield of the preparation of its extract is enlisted.

3.2. Antioxidant activities

The antioxidant activity was evaluated by testing the reducing power, the antioxidant power of extracts, and also by evaluating their ability to scavenge the DPPH°, OH° radicals and to inhibit lipid peroxidation. A qualitative screening of phytochemical composition of extracts was done and the quantification of the total phenolic content was also determined.

3.2.1. Reducing power of plant extracts

It resulted that the reducing ability of all plant extracts increased in a concentration-dependent manner (data not shown). The extracts of *H. madagascarinesis* (1.48 ± 0.02), *B. micrantha* (1.49 ± 0.02) and *P. aurantiacum* (1.43 ± 0.02) at a concentration 100 μg/mL showed greater reduction ability than ascorbic acid (1.41 ± 0.04) as shown in Table 3.

3.2.2. Total antioxidant capacity of plant extracts

The total antioxidant capacity of the plant extracts, expressed as equivalents of ascorbic acid per gram of extract (AAE /g of extract) is ranged from 33.09 ± 0.35 to 218.25 ± 2.16 mg of AAE / g of extract and shown in Table 3. All the extracts showed an increase in antioxidant capacity with increasing concentrations. At 100 μg/mL, it was found that the total antioxidant capacity was higher with the extracts of *H. madagascariensis* (212.17 ± 4.58 AAE /g extract) and *B. micrantha* (218. 25 ± 2.16 AAE /g extract).

3.2.3. Antiradical activity of extracts

The concentration required to scavenge radical at 50% (IC_{50}) of plant extracts is presented in Table 3. It was found that the extract of *H. madagascariensis* exhibited the highest DPPH radical scavenging activity with SC50 of 3.20 ± 0.01 μg/mL compared to ascorbic acid 3.40 ± 0.03 μg/mL. On the other hand, the extracts of *C. odorota* (26.30 ± 6.08 μg/mL), *P.* aurantiacum (29.70 ± 5.37 μg/mL), *A. vera* (29.90 ± 1.13 μg/mL) ; *A. africana* (30.60 ± 0.01 μg/mL), *H. madagascariensis* (31.30 ± 0.01 μg/mL) and *S. alata* (32.85 ± 6.01 μg/mL) showed good hydroxyl radical scavenging activities similar to that of ascorbic acid(30.80 ± 0.01 μg/mL).

3.3. Inhibitory effect of crude extracts on lipid peroxidation

All the plant extracts effectively inhibited the lipid peroxidation in a concentration-dependent manner (data not shown). Extracts of *P. umbellatum* (8.15 ± 0.21 μg/mL), *H. madagascariensis* (9.35 ± 0.21 μg/mL), *A. africana* (9.90 ± 0.01 μg/mL) and *P. aurantiacum* (10.80 ± 0.01 μg/mL) showed low IC_{50} compared to ascorbic acid (19.50 ± 2.12 μg/mL) (Table 3).

3.4. Inhibitory effect of crude extract on elastase and tyrosinase

The inhibitory effect of 26 plant extracts on tyrosinase and elastase is shown in Table 4.

Highest elastase inhibition percentages were found with *P. aurantiacum* (77.58 ± 0.23%) and *H. madagascariensis* (100 ± 0.01%) extracts compared to others plant extracts. Similarly, *A. vera* (IC_{50} = 50.07μg/mL) strongly inhibited the activity of tyrosinase less than ascorbic acid (IC_{50} = 6.20 μg/mL)

3.5. Phytochemical compounds and total phenolic content of plant extracts

Several classes of phytochemicals constituents have been detected in twenty-seven extracts as: flavonoids, triterpenes, tannins, alkaloids,

Table 1
Primers for semi-quantitative PCR.

Gene Name	Accession number	Forward Primer (5'-3')	ReversePrimer (5'-3')	Product length
Nrf-2	NM_010902.4	AAGAATAAAGTCGCCGCCCA	AGATACAAGGTGCTGAGCCG	170
HO-1	NM_010442.2	GAATCGAGCAGAACCAGCCT	CTCAGCATTCTCGGCTTGGA	135
GAPDH	NM_017008.4	GTCGGTGTGAACGGATTTGG	AGATGCCTGCTTCCCATTCT	192

Nrf-2: Nuclear factor Erythroid 2-related factor 2; HO-1: Heme oxygenase-1; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

Table 2
Medicinal plants used for skin care by populations of Foumban (West Region Cameroon) and Nkol Anga'a (Center Region, Cameroon).

Family	Scientific name Voucher Specimen Number	Vernacular names		Part(s) used	Mode of preparation and dose	Skin affections treated	Yield (%)
		Bamoun	Beti				
Acanthaceae	*Eremomastax speciosa* (hochst) Cufod 24 182 / SFR/ cam	Pâ - nkwen		Leaves	Leaves are soaked in water and is taken internally two or three times a day	Skin allergy and Pimples	5,72
	Acanthus montanus 26 004/SFR/cam	fonzap	Ndoue	Leaves	The juice of crushed leaves is applied topically	Pimples and Itching	5,98
Asteraceae	*Ageratum conyzoides* Lin 6575 SFRK	Mejô têfû	Nyat ilouak	Leaves and Stems	The juice of leaves and stems is applied topically and decoction is taken orally	Cuts to stop bleeding and Pimples	7,48
	Aspilia africana (per) CD Adams 16 478/ SFR/ cam	wa'wa'	Lyalgue dzié	Leaves and Stems	The juice of crushed leaves and stems is applied topically	Wounds and cut to get quick relief	5,24
	Dichrocephala integrifolia (Lin.) O.Ktze16641/ SFR/cam	Yière	koukoua	Leaves	The juice of crushed leaves is applied topically	Wounds and Sores	0,66
	Emilia coccinia (sims.) G.Don 29441/NHC	Shin mû	Aloa mvou	Leaves	The leaf of juice of is applied topically	Healing of wounds	6,60
	Sonchus oleraceus Lin 44928/NHC	Melile ntam-ntam	Ta'a bekon	Leaves	The juice of crushed leaves is applied topically	pimples	3,34
	Chromolena Odorata	Be jamais	Ndogmou Nkondengui	Leaves	The paste of leaves is applied topically	Cuts and wounds to stop bleeding	8,12
Crasulaceae	*Kalonchoe crenata* (Andr) Haw 35196/NHC	Mâ-tut	Adog	Leaves	Decoction of leaves is taken orally twice a day	Pimples	3,00
Cesalpilaceae	*Senna alata* (Lin.) Link 29494/NHC	Sùe'nâsa	Ngome	Leaves	Leaves is crushed with a little quantity of salt and applied topically	Scurf	12,68
Commelinaceae	*Commelina SP.*	Gou gouete	Nkarnin	Sap	The sap is applied topically	Ringworm and on scalp to avoid baldness	2,16
Euphoboraceae	*Bridelia micrantha* 38578/NHC		Iwolode	Bark	Decoction of bark is used to bath and is also taken internally twice a day	Pimples	1,44
Fabaceae	*Mucuna prurieus* (Linn) DC 42582/NHC		Akon	Leaves	The infusion of leaves is taken orally	Pimples and skin allergy	8,22
	Phaseolus lunatus Linn 42652/NHC	Joubiere		Whole plant	The whole plant paste is applied on scalp	Dandruff	7,08
Hypericaceae	*Harungana madagascarinsis* Lam 22866/SFR/ cam	Tûne	Atondo	Bark and Stem	The bark and Stem are burnt and ash is applied topically	Skin irritation	28,32 (Barks)
	*Psorospermum aurantiacum*16855/SFR/cam	Mâ bane		Leaves and bark	Decoction of bark and leaves is taken orally	Pimples	6,12
Liliaceae	*Aloe vera*			Gel	The gel is applied topically	Skin dryness	4,88
Malvaceae	*Sida rhombifolia* linn41749/NHC	Sisem	Zizin	Leaves	The paste of leaves is applied topically	wounds	10,98
Mimosaceae	*Albizia gummifera* 13,224 /SFR/cam	Pâ		Leaves	The crushed of leaves is used like soap for the bath	Skin dryness and skin allergy	5,20
Moraceae	*Fiscus exasperata* Vahl.	Ghùghu	Akole	Leaves	Decoction of leaves is taken orally	Pimples and eczema	1,08
Piperaceae	*Piper umbellatum* 20 934/SFR/cam	Boupouete	Aboam zana	Leaves	The crushed of leaves is applied topically	Pimples	8,82
Portulaceae	*Portulaca oleracea* (Linn.)17542/SFR/cam	Kepigup		Leaves	The crushed of leaves is applied topically	wound	9,28
Rubiaceae	*Spermacoce latifolia* (Aubl) K.Schum 28331/ SFR/cam			Leaves	The paste of leave is applied topically	Disorders pigmentation	4,84
	Mitracarpus scaber 21049/SFR/cam		Oyen Ze'e	Leaves	The paste of leave is applied topically	Disorders pigmentation	2,42
Solanaceae	*Solanum acaleastrum* dunal 25878/SFR/cam	kûkùe	Ozezan komba	Fruits	The fruit is burnt and ash is mixed with oil and applied topically	Disorders pigmentation	10,84
	Capsicum fruitescens L.10737/SFR/cam	Yuwuo'metu	Ndodo'o	Leaves	The Leaves and fruit are ground and its paste is applied topically	Wounds for quick relief	14,86

Table 3
Antioxidant activities of some Cameroonian medicinal plants used traditionally for skin care.

Scientific name	DPPH°SC50 (µg/mL)	HO^- SC50 (µg/mL)	RPA (OD at 700 nm)	TAC (mg AAE/g)	LP IC50 (µg/mL)
Eremomastax speciosa	84.85 ± 1.06[b]	> 100	0.39 ± 0.00	85.41 ± 1.47	> 100
Acanthus montanas	> 100	> 100	0.11 ± 0.02	52.81 ± 0.09	> 100
Ageratum conyzoides	> 100	95.35 ± 2.47[b]	0.23 ± 0.00	91.58 ± 1.55	98.6 ± 0.00[b]
Aspilia africana	> 100	30.60 ± 0.00[a]	0.34 ± 0.05	77.66 ± 1.38	9.90 ± 0.00[b]
Dichrocephala integrifolia	> 100	> 100	0.12 ± 0.00	84.25 ± 3.8	> 100
Emilia coccinia	> 100	> 100	0.23 ± 0.02	119.54 ± 2.24	> 100
Sonchus oleraceus	> 100	> 100	0.15 ± 0.02	33.09 ± 0.35	> 100
Chromolena Odorata	> 100	26.30 ± 6.08[a]	0.57 ± 0.01	90.6 ± 3.97	39.10 ± 9.48[b]
Kalonchoe crenata	> 100	> 100	0.40 ± 0.02	99.45 ± 4.75	> 100
Senna alata	42.20 ± 2.40[b]	32.85 ± 6.01[a]	1.39 ± 0.00	115.57 ± 5.97	44.85 ± 1.77[b]
Commelina SP.	> 100	> 100	0.15 ± 0.01	46.76 ± 0.86	> 100
Bridelia micrantha	33.85 ± 1.77[b]	48.25 ± 1.06[b]	1.49 ± 0.02	218.25 ± 2.16	87.75 ± 0.64[b]
Mucuna prurieus	> 100	87.90 ± 3.82[b]	0.39 ± 0.01	75.46 ± 1.21	> 100
Phaseolus lunatus	> 100	> 100	0.10 ± 0.01	68.13 ± 1.73	> 100
Harungana madagascariensis	3.20 ± 0.00[a]	31.30 ± 0.00[a]	1.48 ± 0.02	212.27 ± 4.58	9.35 ± 0.21[b]
Psorospermum aurantiacum	23.25 ± 1.77[b]	29.70 ± 5.37[a]	1.43 ± 0.02	152.81 ± 4.06	10.80 ± 0.00[b]
Aloe vera	> 100	29.90 ± 1.13[a]	0.34 ± 0.00	106.29 ± 3.54	> 100
Sida rhombifolia	> 100	> 100	0.26 ± 0.02	73.26 ± 0.66	> 100
Albizia gummifera	> 100	> 100	0.27 ± 0.00	126.01 ± 0.17	> 100
Fiscus exasperate	> 100	77.30 ± 0.00[b]	0.25 ± 0.00	73.20 ± 2.85	> 100
Piper umbellatum	98.30 ± 1.27[b]	47.30 ± 3.11[b]	0.26 ± 0.02	84.04 ± 0.95	8.15 ± 0.21[b]
Portulaca oleracea	> 100	> 100	0.08 ± 0.00	48.29 ± 6.3	81.60 ± 0.57[b]
Spermacoce latifolia	> 100	> 100	0.06 ± 0.00	52.14 ± 7.03	> 100
Mitracarpus scaber	> 100	95.00 ± 6.65	0.31 ± 0.01	99.21 ± 2.16	> 100
Solanum acaleastrum	> 100	> 100	0.18 ± 0.02	101.53 ± 2.33	> 100
Capsicum fruitescens	> 100	93.30 ± 0.00[b]	0.29 ± 0.04	89.99 ± 0.58	> 100
Ascorbic acid	3.40 ± 0.00[a]	30.80 ± 0.00[a]	1.41 ± 0.05	ND	19,50 ± 2,12[b]

Results are represented as mean ± standard deviation, n = 3. ND: not determined because of the low inhibition percentages obtained with the highest dose of extract during the test. Data with the same letter are not significantly different while data having different letters are significantly different at $p < 0.01$. SC_{50}: half scavenging concentration; HO^-: Hydroxyl radical; RPA: Reducing power ability; TAC: Total antioxidant capacity; DPPH°: 2, 2-diphenyl-1-1-picrylhydrazyl; AAE: Ascorbic acid equivalent; OD: Optical density; LP: Lipid Peroxidation.

Table 4
Tyrosinase and elastase inhibitory potential of some Cameroonian medicinal plants used traditionally for skin care.

Scientific name	% Inhibition of Tyrosinase (at 100 µg/ml)	% Inhibition of Elastase (at 100 µg/ml)
Eremomastax speciosa	6.73 ± 2.77	10.97 ± 0.78
Acanthus montanus	NA	NA
Ageratum conyzoides	17.02 ± 2.47	37.72 ± 0.31
Aspilia africana	2.299 ± 2.88	34.16 ± 0.33
Dichrocephala integrifolia	2.29 ± 2.91	NA
Emilia coccinia	26.84 ± 2.18	32.72 ± 0.59
Sonchus oleraceus	12.11 ± 2.61	5.79 ± 0.83
Chromolena Odorata	25.43 ± 2.22	22.3 ± 0.39
Kalonchoe crenata	33.38 ± 1.98	26.5 ± 0.65
Senna alata	43.43 ± 1.68	33.57 ± 0.33
Commelina SP.	15.85 ± 2.50	37.72 ± 0.31
Bridelia micrantha	11.88 ± 2.62	NA
Mucuna prurieus	9.31 ± 2.70	24.43 ± 0.66
Phaseolus lunatus	NA	NA
Harungana madagascariensis	NA	100 ± 0.01
Psorospermum aurantiacum	10.94 ± 2.65	77.58 ± 0.23
Aloe vera	64 ± 1.07	41.21 ± 0.30
Sida rhombifolia	NA	NA
Albizia gummifera	41.1 ± 1.75	23.49 ± 0.39
Fiscus exasperata	20.99 ± 2.35	12 ± 0.77
Piper umbellatum	1.36 ± 2.93	28.82 ± 0.36
Portulaca oleracea	4.23 ± 5.99	34.76 ± 0.33
Spermacoce latifolia	3.93 ± 2.86	3.72 ± 0.85
Mitracarpus scaber	13.05 ± 2.59	26.45 ± 0.37
Solanum acaleastrum	NA	NA
Capsicum fruitescens	3.23 ± 2.88	NA
Ascorbic acid	100 ± 0.01	ND
Epigallocatechingallate	ND	100 ± 0.01

Results are represented as mean ± standard deviation, n = 3. ND: not determined because of the low inhibition percentages obtained with the highest dose of extract during the test, NA: Not active.

sugars, saponins, sterols and leucoanthocyans as shown in Table 5. All plant extracts revealed the presence of polyphenols. The total phenolic content ranged from 6.031 to 35.67 mg AAE/g extract. The extract of *H. madagascariensis* was found to contain 35.67 ± 0.37 AAE/g extract.

3.6. Correlations between total polyphenol content and antioxidant activity

The correlation coefficient for the selected plant extracts was found to be ranged from -1.43 to 0.995 (Table 6) in at least three out of five oxidation models used. This is indicative of negative or positive correlation between the total phenolic content and antioxidant end points studied.

3.7. Antioxidant, antityrosinase and antielastase activities of isolated fractions

The extracts of *P. aurantiacum* (PA) and *H. madagascariensis* (HM) were selected for further investigation due to their good biological activities. Fractions isolated from theses extracts were tested for antioxidant, anti-tyrosinase and anti-elastase activities. The results indicate that all the fractions exhibited considerable elastase inhibitory activity (greater than 50% inhibition) as shown in Table 7. Hexane fraction of PA produced highest anti-elastase activity at 10 µg/mL compared to others plant fractions (data not shown). In order to determine the IC_{50} value for hexane fraction we conducted experiments to assess the dose-response relationships. The IC_{50} value for hexane fraction was 15.40 µg/mL. Fractions of HM and PA were also investigated for tyrosinase inhibition. In this assay, all the fractions were tested at a single concentration of 100 µg/mL compared to ascorbic acid (positive control). Fractions inhibited tyrosinase lower than ascorbic acid. For antioxidant activities, hexane fraction (0.350 ± 0.024) of HM at a concentration of 100 µg/mL showed greater reduction ability than ascorbic acid (0.290 ± 0.009). On the other hand, the methanolic fraction of PA exhibited the highest DPPH°· radical scavenging activity with IC_{50} of

Table 5
Total phenolic content and phytochemical composition of some Cameroonian medicinal plants used traditionally for skin care.

Scientific names	TPC (mg CAE/g of extract)	Phytochemicals classes of compounds							
		Polyphenols	Flavonoids	Alkaloid	Terpens	Tannins	Saponin	Sterols	Sugar
Eremomastax speciosa	2.16 ± 0.69	–	–	–	–	+	–	+	+
Acanthus montanus	10.38 ± 0.35	–	–	+	+	–	–	–	+
Ageratum conyzoides	17.30 ± 0.81	–	–	+	–	–	+	+	–
Aspilia africana	16.10 ± 0.55	+	+	+	+	–	–	–	+
Dichrocephala integrifolia	10.28 ± 2.04	–	–	+	+	–	–	–	+
Emilia coccinia	23.67 ± 1.35	–	–	+	–	–	+	+	–
Sonchus oleraceus	9.67 ± 0.20	–	–	+	+	+	–	–	+
Chromolena Odorata	28.29 ± 0.92	+	+	+	–	+	–	–	+
Kalonchoe crenata	14.47 ± 0.89	–	+	–	+	+	+	–	+
Senna alata	31.46 ± 0.69	+	+	–	+	+	–	–	–
Commelina SP.	10.03 ± 0.14	+	–	–	–	–	+	–	+
Bridelia micrantha	31.48 ± 1.30	+	+	–	–	–	+	+	+
Mucuna prurieus	18.48 ± 0.23	+	+	+	+	+	+	–	+
Phaseolus lunatus	8.12 ± 0.09	–	–	+	+	–	–	–	+
Harungana madagascariensis	35.67 ± 0.37	+	+	–	+	+	+	–	+
Psorospermum aurantiacum	30.18 ± 0.95	+	+	–	–	+	–	+	–
Aloe vera	12.35 ± 0.60	–	–	+	–	–	+	–	–
Sida rhombifolia	19.01 ± 0.58	–	–	+	+	+	–	–	+
Albizia gummifera	17.54 ± 0.06	–	–	+	–	–	–	–	+
Fiscus exasperata	12.33 ± 0.12	–	–	+	–	–	–	+	+
Piper umbellatum	14.99 ± 0.09	+	+	+	–	+	–	+	+
Portulaca oleracea	7.06 ± 0.14	–	–	+	–	–	–	–	+
Spermacoce latifolia	10.44 ± 0.32	–	–	+	+	+	+	–	+
Mitracarpus scaber	17.74 ± 0.37	–	–	+	+	+	–	–	+
Solanum acaleastrum	8.87 ± 0.00	–	–	–	–	–	–	–	+
Capsicum fruitescens	17.30 ± 0.06	–	+	+	+	+	+	–	+

Results are represented as mean ± standard deviation, n = 3. TPC: Total phenolic contain; CAE: Chlorogenic acid equivalent; +: Presence of compounds; -: Absence of compounds.

Table 6
The r^2 (correlation coefficient) values between antioxidant activities and total polyphenolic content.

Scientific name	DPPH°· radical scavenging activity	HO° radical scavenging activity	Lipid Peroxidation
Ageratum conizoides	0,934	ND	ND
Psorospermum aurantiacum	−0,41	−0,23	−1,43
Ficus. Exasperata	−7,22	ND	ND
Chromolena odorata	0,792	ND	0,994
Bridelia micrantha	0,61	0, 981	0,929
Sena alata	−1,23	0,932	0,885
Mitracarpus scaber	0,629	ND	ND
Eremonatax speciosa	ND	0,754	ND
Capsicum frutescens	−0,66	ND	ND
Mucuna pruriens	−0,6	ND	ND
Aspilia africana	−2,14	ND	0,958
Piper umbellatum	−6,22	0,995	0,794
Aloe vera	0,857	ND	ND
Portulaca eracea	ND	ND	0,946
Harungana madagascariensis (bark)	0,964	0,043	0,335

Results are represented as mean ± standard deviation, n = 3. ND: not determined because of the low inhibition percentages obtained with the highest dose of extract during the test; HO°: Hydroxyl radical; DPPH°·: 2, 2-diphenyl-1-1-picrylhydrazyl.

4.55 ± 0.05 µg/mL compared to ascorbic acid 7.45 ± 0.25 µg/mL. Hexane and methanol fractions of both plants where selected because of their good activities for further studies.

3.8. Effect of crude extract and fractions on cell viability

Cell viability was determined on B16F10 and NIH/3T3cell lines using MTT assay. It was found that crude extract, hexane and methanol fractions of HM and PA were non-toxic at 12.5, 25, 50 µg/ml on NIH 3T3 cells (Fig. 1). In contrast, crude and hexane extracts of HM significantly decreased cell viability at the concentration of 100 µg/mL. It is interesting to note that after fractionation of crude extract of HM, the hexane and methanol fractions obtained were less toxic at the concentration of 100 µg/ml, and it therefore suggested that the fractionation might contribute to eliminate some toxic substances within the crude extract. On the other hand, crude extract of PA and its hexane fraction exhibited a concentration-dependent decrease of cell viability with the highest concentration having between 50–60% of cell viability. On the contrary, the methanol fraction was found to be non-toxic at all tested concentrations as shown in Fig. 2. In addition, we found that the crude extract of HM and PA with their hexane fractions were highly toxic on B16F10 cells at100 µg/mL with cell viability of about 30–35% (Fig. 2). Methanol fractions of HM and PA did not have any significant reduction in cell viability even at the highest concentration used.

Table 7
Antioxidant, tyrosinase and elastase inhibitory activities of plant fractions.

Medicinal plant	Fractions	DPPH°· % Inhibition (at 100 µg/mL)	DPPH°· radical scavenging activity SC_{50} (µg/mL)	RPA (Absorbance at 700 nm)	Tyrosinase % Inhibition (at 100 µg/ml)	Elastase % Inhibition (at 100 µg/ml)
Harungana madagascariensis	Hexane	94.82 ± 0.17	25.85 ± 0.55*	0.350 ± 0.024	47.00 ± 2.12	51.50 ± 0.04
	Methylene chloride	87.28 ± 0.17	41.00 ± 0.60*	0.280 ± 0.002	28.00 ± 1.6	88.73 ± 0.05
	Ethyl acetate	91.48 ± 0.1	7.55 ± 0.35ns	0.320 ± 0.007	39 .00 ± 2.61	82.79 ± 0.08
	Methanol	93.33 ± 0.35	7.65 ± 0.05ns	0.260 ± 0.014	45.00 ± 0.5	90.5 ± 0.04
	Crude extract	95.43 ± 0.17	23.85 ± 2.35*	0.305 ± 0.014	48.82 ± 1.75	91.10 ± 0.04
Psorospermum aurantiacum	Hexane	80.99 ± 1.75	41.55 ± 3.25*	0.0.270 ± 0.002	42.00 ± 6.11	95.25 ± 0.02
	Methylene chloride	84.20 ± 5.25	25.30 ± 2.50*	0.220 ± 0.002	15.00 ± 0.6	85.17 ± 0.07
	Ethyl acetate	92.84 ± 4.54	8.65 ± 0.15ns	0.260 ± 0.037	23.00 ± 0.87	91.20 ± 0.04
	Methanol	89.64 ± 0.35	4.55 ± 0.05ns	0.290 ± 0.009	26.5 ± 0.05	92.30 ± 0.03
	Crude extract	95.43 ± 0.17	9.80 ± 0.10ns	0.280 ± 0.015	35.00 ± 1.74	88.61 ± 0.10
Ascorbic acid		91.85 ± 0.35	7.45 ± 0.25	0.290 ± 0.009	96.64 ± 0.97	ND
Epigallocatechin gallate		ND	ND	ND	ND	98.96 ± 0.01

Results are represented as mean ± standard deviation, n = 3. ND: not determined because of the low inhibition percentages obtained with the highest dose of extract during the test. RPA: Reducing power ability; DPPH°: 2, 2-diphenyl-1-1-picrylhydrazyl; SC_{50}: Half scavenving concentration.

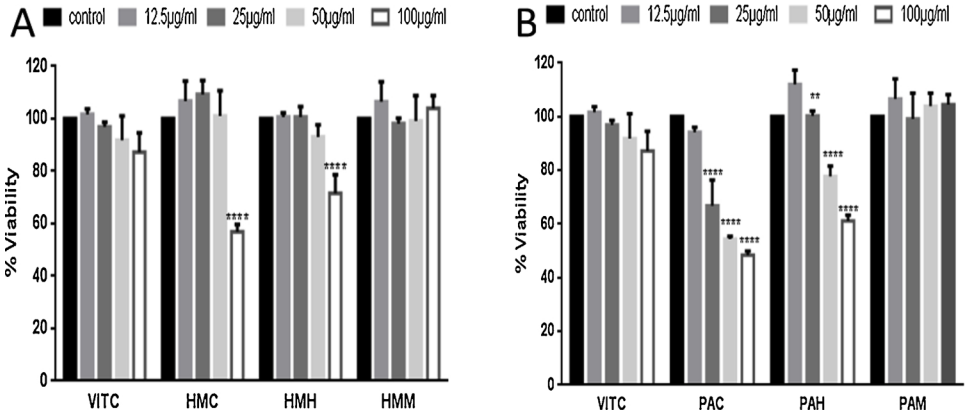

Fig. 1. Effect of crude extract and fractions on the viability of NIH3T3 cells. NIH3T3 cells were treated with various concentrations of plant extracts and then incubated for 24 h. Cell viability was determined using the MTT assay. Data shown are mean values ± S.D. (n = 3), Data are analyzed using one-way ANOVA followed by Dunnett's test:*p < 0.05 compared to the control group. HMC: H. madagascariensis crude extract; HMH: H. madagascariensis Hexane fraction; HMM: H. madagascariensis methanol fraction; PAC: P. aurantiacum crude extract; PAH: P. aurantiacum Hexane fraction; PAM: P. aurantiacum methanol fraction; VIT C: vitamin C.

3.9. Protective effect of plant extract on UVB irradiation induced intracellular oxidative stress in B16F10 and NIH/3T3 cells

Intracellular oxidative stress was qualitatively assessed in B16F10 and NIH/3T3 cells by staining cells with DCFDA. HM extract, PA extract and their fractions recorded weaker fluorescence suggesting fractions mediated decrement in intracellular oxidative stress as shown in Figs.3 and 4.

3.10. Effect of crude extract and fractions on expression of Nuclear factor Erythroid 2-related factor 2 (Nrf-2) and Heme oxygenase-1 (HO-1) in NIH/3T3 and B16F10 cells

In our study, HO-1 mRNA level was down regulated following UV−B treatment but Nrf-2 was not significantly up regulated as compared to control in NIH/3T3. Hexane fraction of HM accounted for HO-1 upregulation that was significantly higher than ascorbic acid treated

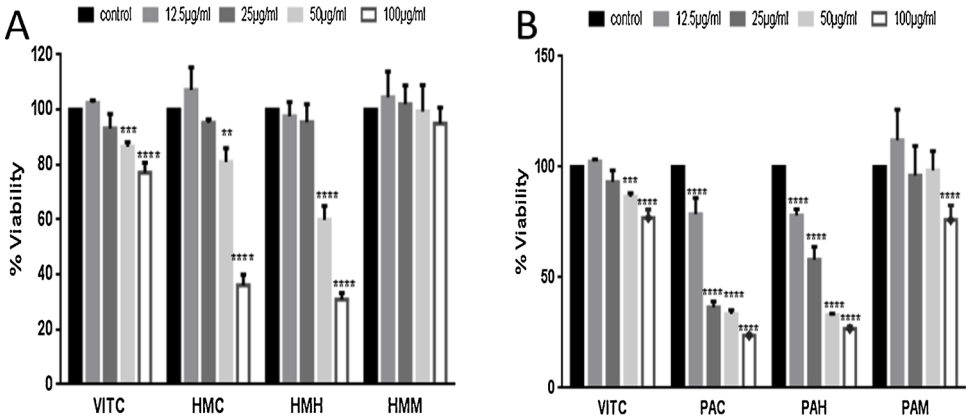

Fig. 2. Effect of crude extract and fractions on the viability of B16F10 cells. B16F10 cells were treated with various concentrations of plant extracts and then incubated for 24 h. Cell viability was determined using the MTT assay. HMC: H. madagascariensis crude extract; HMH: H. madagascariensis Hexane fraction; HMM: H. madagascariensis methanol fraction; PAC: P. aurantiacum crude extract; PAH: P. aurantiacum Hexane fraction; PAM: P. aurantiacum methanol fraction. Data shown are mean values ± S.D. (n = 3), Data are analyzed using one-way ANOVA followed by Dunnett's test: group*p < 0.05 compared to the control group; VIT C, vitamin C.

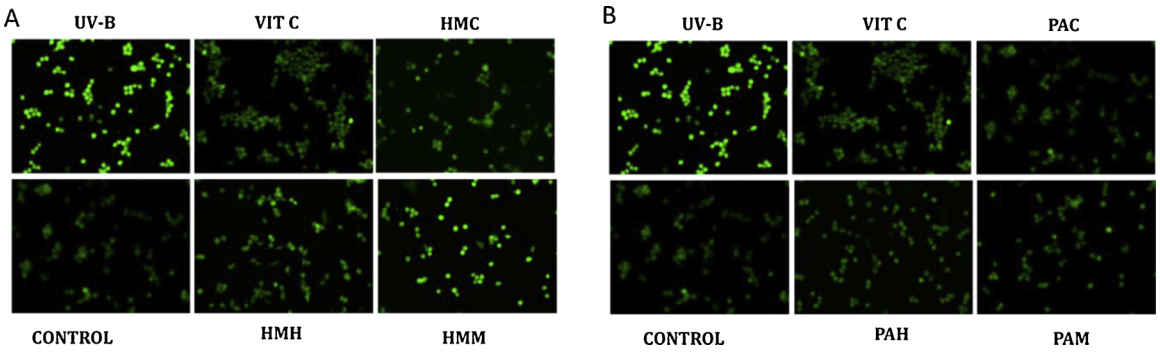

Fig. 3. Effect of plant extract on UVB-induced intracellular oxidative stress in NIH/3T3. The cells were pre-treated with different amount of selected plant extracts for 1 h, followed by UVB exposure (29.7 mJ/cm2) for 30 min. Inhibition of intracellular levels of (A, B) ROS is shown. HMC: H. madagascariensis crude extract; HMH: H. madagascariensis Hexane fraction; HMM: H. madagascariensis methanol fraction; PAC: P. aurantiacum crude extract; PAH: P. aurantiacum Hexane fraction; PAM: P. aurantiacum methanol fraction; VITC: vitamin C.

group. Similar responses were recorded following PA (crude and hexane) extracts treated groups, but methanol extract of PA failed to record upregulation of HO-1 as shown in Fig.5. Alterations in mRNA levels of Nrf-2 indicate a confusing pattern where in the expected up-regulation in ascorbic acid treated group is not recorded. But crude and hexane extracts of PA recorded significant increment in mRNA levels of Nrf-2 that were in agreement with HO-1 levels. The Nrf-2 and HO-1 levels were found to be upregulated in B16F10 following UV exposure as shown in Fig.6. A similar trend was observed in ascorbic acid, Hexane fraction (HM and PA) and methanol fraction of PA, but methanol fraction of HM showed significant decrement in Nrf-2 and HO-1 levels.

4. Discussion

In the present study, plants with medicinal properties used by the populations of Foumban and Nkol Anga'a were investigated for their skin anti-aging potential. The data obtained during the ethnopharmacological survey revealed a sizable number (26 plants) used by the resident population for treatment of skin and related ailments. Plants belonging to Fam. Asteraceae were the most commonly used (6 species). Decoction, maceration in water or application of raw shoot were the main methods of preparing potions for traditional usage. Except for the reports on *H. madagascariensis* bark (anti-inflammatory) and *A. vera* (anti-tyrosinase) other plants lacked scientific information and hence were studied for the first time for their anti-skin aging properties [21,22]. Oxidative stress and free radicals are strongly implicated in the mechanism of skin aging and during which there is an increase in the activities of the enzymes (tyrosinase, elastase and collagenase) which degrade the skin components [10].

The *in vitro* antioxidant activity of the plants showed their potential to protect skin-cell constituents such as lipids, DNA and proteins from oxidative damage induced by oxidative stress which may occur *in vivo* upon generation of deleterious radicals such as hydrogen peroxide, hypochlorous acid, hydroxyl radicals, nitric oxide radicals and singlet oxygen [23]. Oxidative stress is a complex process and the experimental model cannot be described by a single method [23]. It is therefore essential to use several methods to study the antioxidant activity of a plant extract [17]. In this study, this activity was evaluated *in vitro* by measuring the ability of our extracts to reduce ferric ion (Fe^{3+}) and phosphomolybdic complex, scavenge DPPH°· and HO° radicals and inhibit lipid peroxidation.

DPPH°· free radical method is a sensitive way to determine the antioxidant activity of plant extracts [24]. DPPH°· is a free radical and can accept an electron or hydrogen radical from a donor compound to become stable molecule. Hydroxyl radicals (HO°) formed in the biological systems have been recognized to be the most reactive species and extremely damaging [25]. Therefore, the scavenging activity of HO° can be considered as one of the best indicators of the antioxidant potential of a compound. All the plant extracts tested scavenged the DPPH°· and quenched hydroxyl radicals. Although their respective actions were different, this result proved the capability of these extracts to donate an electron or hydrogen to stabilize DPPH°· and HO° radicals. The variation observed between the scavenging or quenching activities of the extracts could be attributed to their unequal content in total polyphenols content shown in Table 5. Among of the extracts tested, the extract from *H. madagascariensis* exhibiting a positive correlation between its DPPH°· and HO° scavenging activity and polyphenols content (Table 6) coupled

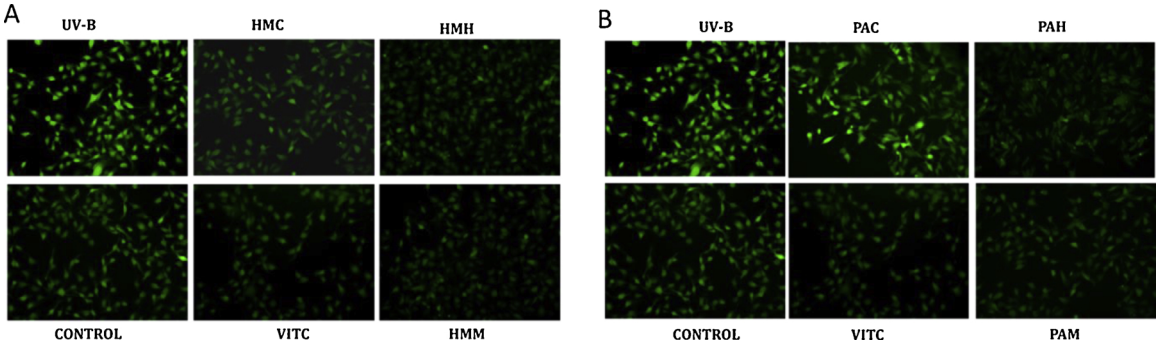

Fig. 4. Crude extracts and fractions reduce UVB-induced intracellular oxidative stress in B16F10. To test the anti-oxidant potential of plant extracts, generation of ROS was measured in a cell-free system using fluorescent probes DCFDA. The cells were pretreated with different amount of plant extracts for 2 h, followed by UVB exposure (39.6 mJ/cm2) for 30 min. Inhibition of intracellular levels of ROS (A,B) is shown. HMC: H. madagascariensis crude extract; HMH: H. madagascariensis Hexane fraction; HMM: H. madagascariensis methanol fraction; PAC: P. aurantiacum crude extract; PAH: P. aurantiacum Hexane fraction; PAM: P. aurantiacum methanol fraction; VIT C: vitamin C.

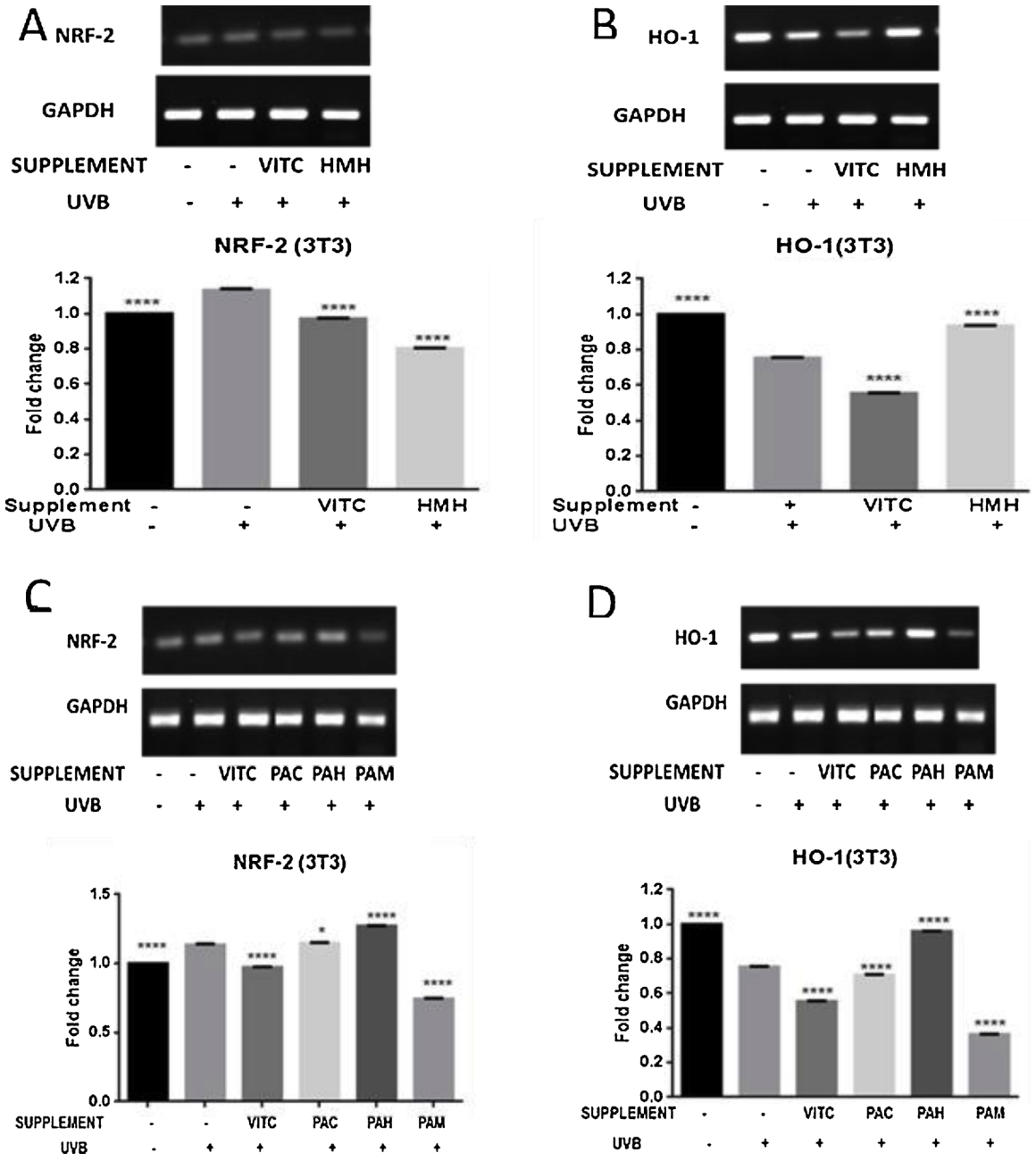

Fig. 5. Effect of crude extract and fractions on expression of Nuclear factor Erythroid 2-related factor -2 (Nrf-2)) and Heme oxygenase-1 (HO-1) in NIH/3T3 cells. Cells were pre-treated with various amounts of plant, HMH and VITC (A,B) then PAC, PAH,PAM (C,D) for 2 h followed by UVB exposure (29.7 mJ/cm^2) and incubated for further 24 h. The mRNA levels of NRF-2 and HO-1 were then determined by reverse transcription-polymerase chain reaction and quantified by image J software. GAPDH mRNA was used as internal control. Data shown are mean values ± S.D. (n = 3), Data are analyzed using one-way ANOVA followed by Dunnett's test:*p < 0.05 compared to UVB-exposed group. Nrf-2, Nuclear factor Erythroid 2-related factor 2; HO-1: Heme oxygenase-1; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; HMH: H. madagascariensis Hexane fraction; PAC: P. aurantiacum crude extract; PAH: P. aurantiacum Hexane fraction; PAM: P. aurantiacum methanol fraction; VITC: vitamin C.

to its lower SC_{50} values presented in Table 3, was identified as the highest antiradical. Lipid peroxidation is also involved in skin aging since epidermis layer is rich in polyunsaturated fatty acids [26]. The formation of HO° radical by the Fenton reaction in iron-rich tissues contributes to the initiation of lipid peroxidation [27]. Extracts of plants under study were tested for their lipid peroxidation inhibitory activity and have been found to inhibit the phenomenon. Extracts from *H. madagascariensis* and *P. aurantiacum* exhibited higher activity. This observation suggests these plant extracts are able to scavenge hydroxyl radical or chelate iron. This inhibitory activity may be due to phytochemical compounds other than polyphenols. In fact, there is a lower or no correlation (Table 6) between this activity and the total polyphenol content. The reducing power of the compounds could serve as a significant indicator of the antioxidant potential [28]. So, to evaluate this property of the plant extracts, we measured their ability to reduce Fe^{3+} to Fe^{2+}. The respective extract of *H. madagascariensis*, and *P. aurantiacum* showed the highest reducing capacity as shown by their respective optical density = 1.48 and 1.43 which remained higher than the standard optical density = 1.41 (Table 3). The extract of *H. madagascariensis* showed the highest antioxidant capacity.

Elastin is the main component of the elastic fibers and is a part of extracellular matrix protein that provides elasticity to the connective

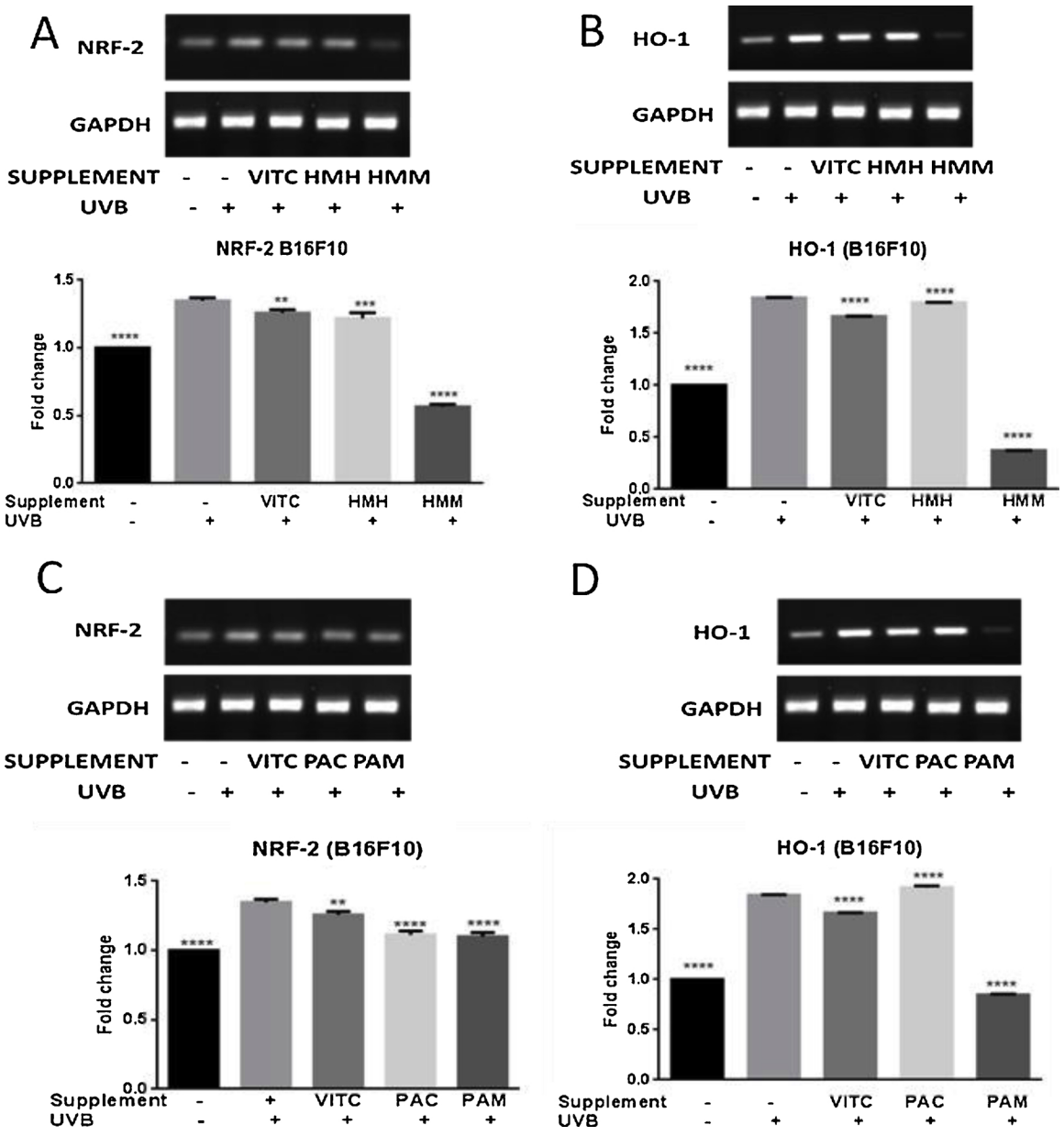

Fig. 6. Effect of crude extract and fractions on expression of Nuclear factor Erythroid 2-related factor 2 (Nrf-2) and Heme oxygenase-1 (HO-1) in B16F10 cells. Cells were pre-treated with various amounts of plant, HMH: HMM and VITC (A,B) then PAC, PAH, PAM (C,D) for 2 h followed by UVB exposure (39.6 mJ/cm^2) and incubated for further 24 h. The mRNA levels of NRF-2 and HO-1 were then determined by reverse transcription-polymerase chain reaction and quantified by image J software. GAPDH mRNA was used as internal control. Data shown are mean values ± S.D. (n = 3), Data are analyzed using one-way ANOVA followed by Dunnett's test:*p < 0.05 compared to UVB-exposed group. Nrf-2: Nuclear factor Erythroid 2-related factor 2; (HO-1) 1: Heme oxygenase-1; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; HMH: H. madagascariensis Hexane fraction; HMM: H madagascariensis methanol fraction; PAC: P. aurantiacum crude extract; PAH: P. aurantiacum Hexane fraction; PAM: P. aurantiacum methanol fraction; VITC: vitamin C.

tissues. Elastase is the proteinase enzyme able to attack all major connective tissue matrix proteins, including elastin, collagen, proteoglycans, and keratins, and this leads to the decreased of skin elasticity and triggers several diseases in the body such as chronic inflammatory diseases, rheumatoid and lung disease [10]. Similarly, tyrosinase is an enzyme which catalyzes two rate-limiting steps in melanin synthesis; however, an activation of tyrosinase caused by exposure to UV light or ROS (reactive oxygen species) leads to uncontrolled melanogenesis and pigmentation disorders. Thus, inhibition of these enzymes is important to prevent all these disorders [8] The extracts were tested for their inhibitory effect on elastase and tyrosinase. From all the extracts surveyed, extract of *H. madagascariensis*, and *P. aurantiacum* were found to strongly inhibit both enzymes as testified by their higher inhibition percentages (Table 4). Triterpenoids and flavonoids were evidenced (Table 4) in the extracts. Since these phytoconstituents are recognized to be inhibitors of elastase and tyrosinase inhibitory activities [16,29] it may be suggested these phytochemicals contribute in part to the enzymes inhibitory action of these extracts.

In this study, an extract was considered to have good anti-aging potential if it inhibited both enzymes and exhibited high activity in all the five oxidative models tested. Therefore, based on EC_{50}/IC_{50} values, quantity of ascorbic acid equivalent/g extract values and absorbance as presented in Table 3, inhibition percentage of elastase and tyrosinase activities (Table 4), crude extract of *P. aurantiacum* and *H. madagascariensis* were considered to be most antioxidative and inhibitors of elastase and tyrosinase. These extracts were fractionated and fractions

tested for antioxidant activities, elastase and tyrosinase inhibitory activities. Hexane and methanolic fractions of both plants showed higher antioxidant and enzymes inhibitory activities (Table 7) and were selected for further studies.

Ultraviolet radiations are one of the prime causes of skin ailments including an increased risk of skin cancer [20]. Oxidative stress and cytotoxicity is routinely induced *in vitro* by treating cultured cells with UV-B [4]. To further study the antioxidant activity of the both plant extracts and fractions, we assessed their ability to protect skin mouse cells lines from UVB-induced oxidative stress. The 2´, 7´-dichlorofluorescein diacetate (DCFDA) stain is commonly used to assess intracellular oxidative stress depicted in the form of prominent fluorescence [30]. It was observed that UV-B treated cells showed prominent fluorescence whereas cells treated with tested extracts or ascorbic acid showed varying levels of decreased fluorescence. These results provide evidence that the extracts are able to efficiently protect skin cells from UV-B induced oxidative stress.

Depletion of intracellular antioxidants is one of the key reasons of cell damage and mortality while its prevention by exogenous treatment is an effective strategy in variety of somatic cells including fibroblast and melanoma. Nuclear factor Erythroid 2-related factor 2 (Nrf-2) and Heme oxygenase (HO-1) have been identified as key players that protect depletion of intracellular glutathione by a prompt response following an experimentally induced cellular damage [31]. The results showed that hexane fractions of HM and PA modulated the mRNA levels of these genes suggesting these fractions as well as the crude extract of PA having potent antioxidant capacity that are involved in the protection of skin cells against UV-B.

A drawback of the study is that only methylene chloride-methanol (1: 1 v / v) extract was used to screening the efficacy of the plant extracts. In fact, it was shown in a studw that the phytochemicals present in the plant extract were present in different concentrations in different solvents [32]. Hence, it is possible that more potent forms of phytochemicals present in the plants tested may have been obtained in different solvents used during the extraction process.

5. Conclusion

Use of medicinal plants for treating skin ailments were surveyed in local populations but, only few traditional healers provided detailed information. A total of twenty six medicinal plants could be surveyed belonging to sixteen families with maximum representation from Family of Asteraceae. The extracts of *H. madagascariensis* and *P.aurantiacum* were non-toxic at lower levels and exhibited strong antioxidative, anti-elastase and anti-tyrosinase activities. Hexane fractions from these extracts showed varying degrees of upregulation of mRNA levels in intracellular antioxidant genes and prevented melanoma and fibroblast cells lines from UVB- induced intracellular oxidative stress. These results provide compelling evidence on skin anti-aging potential of *H. madagascariensis* and *P.aurantiacum* and warrants detailed *in vivo* studies in relevant experimental models. It is therefore useful for further studies to charaterize compounds present in these extracts that are responsible of these properties.

Conflict of interest

The authors declare no conflicts of interest

Author contributions

FNN, RVD and PMF defined the research subject and its aims, conceived and designed the experiments. JNM, AJ and KU performed the experiments. JNM, KS, RDV, FNN and PMF analyzed the data and wrote the paper. All the authors read and approved the final version of this manuscript

Acknowledgements

We are grateful to the traditional healers for volunteering and sharing the information about their rich heritage and the Cameroon National Herbarium for identification of plants. Authors are thankful to The Maharaja Sayajirao University of Baroda, India for technical support.

Funding

Part of this work was carried out at The Maharaja Sayajirao University of Baroda, India, when Mrs. Jacqueline Njikam Manjia was a visiting scientist, under the CV Raman International Fellowship for African Researchers.

References

[1] C. Mathen, R. Thergaonkar, M. Teredesai, G. Soman, S. Peter, Evaluation of anti-elastase and antioxidant activity in antiaging formulations containing terminalia extracts, Int. J. Herb. Med. (2014) 95–99.
[2] R. Negi, D. Pande, A. Kumar, R.S. Khanna, H.D. Khanna, Evaluation of biomarkers of oxidative stress and antioxidant capacity in the cord blood of preterm low birth weight neonates, J. Matern. Fetal. Neonatal. Med. 25 (2012) 1338–1341, https://doi.org/10.3109/14767058.2011.633672.
[3] M. Ben-Yehuda Greenwald, S. Ben-Sasson, H. Bianco-Peled, R. Kohen, Skin redox balance maintenance: the need for an Nrf2-Activator delivery system, Cosmetics. 3 (2016) 1, https://doi.org/10.3390/cosmetics3010001.
[4] K. Bravo, L. Duque, F. Ferreres, D.A. Moreno, E. Osorio, Passiflora tarminiana fruits reduce UVB-induced photoaging in human skin fibroblasts, J. Photochem. Photobiol. B 168 (2017) 78–88, https://doi.org/10.1016/j.jphotobiol.2017.01.023.
[5] H. Swalwell, J. Latimer, R.M. Haywood, M.A. Birch-Machin, Investigating the role of melanin in UVA/UVB- and hydrogen peroxide-induced cellular and mitochondrial ROS production and mitochondrial DNA damage in human melanoma cells, Free Radic. Biol. Med. 52 (2012) 626–634, https://doi.org/10.1016/j.freeradbiomed.2011.11.019.
[6] J.-H. Koo, H.T. Kim, H.-Y. Yoon, K.-B. Kwon, I.-W. Choi, S.H. Jung, H.-U. Kim, B.-H. Park, J.-W. Park, Effect of xanthohumol on melanogenesis in B16 melanoma cells, Exp. Mol. Med. 40 (2008) 313, https://doi.org/10.3858/emm.2008.40.3.313.
[7] C. Wiegand, C. Raschke, P. Elsner, Skin aging: a brief summary of characteristic changes, in: M.A. Farage, K.W. Miller, H.I. Maibach (Eds.), Textb. Aging Skin, Springer Berlin Heidelberg, Berlin, Heidelberg, 2017, pp. 55–65, , https://doi.org/10.1007/978-3-662-47398-6_5.
[8] T. Kim, H.J. Kim, S.K. Cho, W.Y. Kang, H. Baek, H.Y. Jeon, B. Kim, D. Kim, Nelumbo nucifera extracts as whitening and anti-wrinkle cosmetic agent, Korean J. Chem. Eng. 28 (2011) 424–427, https://doi.org/10.1007/s11814-010-0357-6.
[9] T.S. Thring, P. Hili, D.P. Naughton, Anti-collagenase, anti-elastase and anti-oxidant activities of extracts from 21 plants, BMC Complement, Altern. Med. 9 (2009), https://doi.org/10.1186/1472-6882-9-27.
[10] Hulya Celik Onar, Elastase, tyrosinase and lipoxygenase inhibition and antioxidant activity of an aqueous extract from Epilobium angustifolium L. Leaves, J. Med. Plants Res. 6 (2012), https://doi.org/10.5897/JMPR11.1127.
[11] S. Jadoon, S. Karim, M.H.H.B. Asad, M.R. Akram, A. Kalsoom Khan, A. Malik, C. Chen, G. Murtaza, Anti-aging potential of phytoextract loaded-pharmaceutical creams for human skin cell longetivity, Oxid. Med. Cell. Longev. 2015 (2015) 1–17, https://doi.org/10.1155/2015/709628.
[12] Y.-C. Hseu, C.-W. Chou, K.J. Senthil Kumar, K.-T. Fu, H.-M. Wang, L.-S. Hsu, Y.-H. Kuo, C.-R. Wu, S.-C. Chen, H.-L. Yang, Ellagic acid protects human keratinocyte (HaCaT) cells against UVA-induced oxidative stress and apoptosis through the up-regulation of the HO-1 and Nrf-2 antioxidant genes, Food Chem. Toxicol. 50 (2012) 1245–1255, https://doi.org/10.1016/j.fct.2012.02.020.
[13] G. Zengin, Y.S. Cakmak, G.O. Guler, A. Aktumsek, In vitro antioxidant capacities and fatty acid compositions of three Centaurea species collected from Central Anatolia region of Turkey, Food Chem. Toxicol. 48 (2010) 2638–2641, https://doi.org/10.1016/j.fct.2010.06.033.
[14] X.-Y. Su, Z.-Y. Wang, J.-R. Liu, In vitro and in vivo antioxidant activity of Pinus koraiensis seed extract containing phenolic compounds, Food Chem. 117 (2009) 681–686, https://doi.org/10.1016/j.foodchem.2009.04.076.
[15] K.K. Lee, J.-D. Choi, The effects of Areca catechu l extract on anti-aging, Int. J. Cosmet. Sci. 21 (1999) 285–295, https://doi.org/10.1046/j.1467-2494.1999.196563.x.
[16] A. Manosroi, P. Jantrawut, T. Akihisa, W. Manosroi, J. Manosroi, *In vitro* anti-aging activities of *Terminalia chebula* gall extract, Pharm. Biol. 48 (2010) 469–481, https://doi.org/10.3109/13880200903586286.
[17] B. Blažeković, S. Vladimir-Knežević, A. Brantner, M. Štefan, Evaluation of Antioxidant Potential of Lavandula x intermedia Emeric ex Loisel. “Budrovka”: A Comparative Study with L. angustifolia Mill, Molecules 15 (2010) 5971–5987, https://doi.org/10.3390/molecules15095971.
[18] J.B. Harborne, Phytochemical Methods: a Guide to Modern Techniques of Plant Analysis, Chapman and Hall, London ; New York, 1980.
[19] O. Odebiyi, E. Sofowora, Antimicrobial alkaloids from a Nigerian chewing stick

(fagara zanthoxyloides), Planta Med. 36 (1979) 204–207, https://doi.org/10.1055/s-0028-1097271.
[20] M.A.A. Mohamed, M. Jung, S.M. Lee, T.H. Lee, J. Kim, Protective effect of Disporum sessile D.DOn extract against UVB-induced photoaging via suppressing MMP-1 expression and collagen degradation in human skin cells, J. Photochem. Photobiol. B 133 (2014) 73–79, https://doi.org/10.1016/j.jphotobiol.2014.03.002.
[21] E.O. Iwalewa, V. Naidoo, V. Bagla, J.N. Eloff, Nitric Oxide Producing Effect of Six Extracts From Harungana Madagascariensis Lam, ex Poiret (Guttiferae) stem bark, 2009, pp. 14–21.
[22] P.K. Mukherjee, N. Maity, N.K. Nema, B.K. Sarkar, Bioactive compounds from natural resources against skin aging, Phytomedicine. 19 (2011) 64–73, https://doi.org/10.1016/j.phymed.2011.10.003.
[23] L. Fu, B.-T. Xu, R.-Y. Gan, Y. Zhang, X.-R. Xu, E.-Q. Xia, H.-B. Li, Total phenolic contents and antioxidant capacities of herbal and tea infusions, Int. J. Mol. Sci. 12 (2011) 2112–2124, https://doi.org/10.3390/ijms12042112.
[24] P.S. Kumar, S. Sucheta, V. Sudarshana Deepa, P. Selvamani, S. Latha, Antioxidant activity in some selected Indian medicinal plants, Afr. J. Biotechnol. 7 (2008) 1826–1828, https://doi.org/10.5897/AJB2008.000-5030.
[25] M. Maizura, A. Aminah, W.M. Wan Aida, Total phenolic content and antioxidant activity of kesum (Polygonum minus), ginger (Zingiber officinale) and turmeric (Curcuma longa) extract, Int. Food Res. J. 18 (2011) 529–534.
[26] T.-K. Lin, L. Zhong, J. Santiago, Anti-inflammatory and skin barrier repair effects of topical application of some plant oils, Int. J. Mol. Sci. 19 (2017) 70, https://doi.org/10.3390/ijms19010070.
[27] P. Costa, S. Gonçalves, P.B. Andrade, P. Valentão, A. Romano, Inhibitory effect of Lavandula viridis on Fe2+-induced lipid peroxidation, antioxidant and anti-cholinesterase properties, Food Chem. 126 (2011) 1779–1786, https://doi.org/10.1016/j.foodchem.2010.12.085.
[28] A.S. Rao, S.G. Reddy, P.P. Babu, A.R. Reddy, The antioxidant and antiproliferative activities of methanolic extracts from Njavara rice bran, BMC Complement, Altern. Med. 10 (2010), https://doi.org/10.1186/1472-6882-10-4.
[29] N. Sultana, N.H. Lee, Antielastase and free radical scavenging activities of compounds from the stems ofCornus kousa, Phytother. Res. 21 (2007) 1171–1176, https://doi.org/10.1002/ptr.2230.
[30] R.R. Sonani, R.P. Rastogi, N.K. Singh, J. Thadani, P.J. Patel, J. Kumar, A.K. Tiwari, R.V. Devkar, D. Madamwar, Phycoerythrin averts intracellular ROS generation and physiological functional decline in eukaryotes under oxidative stress, Protoplasma. 254 (2017) 849–862, https://doi.org/10.1007/s00709-016-0996-5.
[31] R.N. Jadeja, N.H. Urrunaga, S. Dash, S. Khurana, N.K. Saxena, Withaferin-a reduces acetaminophen-induced liver injury in mice, Biochem. Pharmacol. 97 (2015) 122–132, https://doi.org/10.1016/j.bcp.2015.07.024.
[32] S.C. Ugochukwu, I. Arukwe Uche, Onuoha Ifeanyi, Preliminary phytochemical screening of different solvent extracts of stem bark and roots of Dennetia tripetala G, Baker 3 (2013) 10–13.