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Protective effect of *Erythrina senegalensis* sequential extracts against oxidative stress in SC-1 fibroblasts and THP-1 macrophages

[Efecto protector de los extractos secuenciales de *Erythrina senegalensis* contra el estrés oxidativo en fibroblastos SC-1 y macrófagos THP-1]

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Abstract

Context: *Erythrina senegalensis* (ES) DC. is used traditionally in the management of various free radical-related diseases, including wound healing.

Aims: To evaluate sequentially prepared leaf extracts of ES for the ability to protect against oxidative stress in fibroblasts and macrophages.

Methods: Sequential extracts were prepared using hexane, ethyl acetate, methanol and water in increasing polarity. Chemical profiling was done with ultra-performance liquid chromatography coupled with mass spectrometry (UPLC-MS). Cytotoxic potential was determined using the sulforhodamine B staining assay, phase contrast, PlasDIC, and live/dead staining microscopy. The ability to scavenge the ABTS and DPPH free radicals was assessed, while the effect on AAPH-induced oxidative stress was evaluated by measuring reactive oxygen species (ROS) release.

Results: A previously reported compound (neobavaisoflavone), and three others (kaempferol, rutin, and rotenone) were tentatively detected. With regards to cytotoxicity, no IC_{50} could be determined up to the highest concentration tested (100 µg/mL) in both THP-1 and SC-1 cells. The methanol extract displayed the greatest antioxidant activity against the free radicals (IC_{50} = 44.86 µg/mL [ABTS]; 291.1 µg/mL [DPPH]) and AAPH-induced intracellular ROS in macrophages (1.0-fold reduction at 100 µg/mL). The protective effect of the extracts (1 – 100 µg/mL) was comparable to the positive control (Trolox), which decreased ROS by 1.30-fold at 5 µg/mL.

Conclusions: A significant ability to protect against oxidative stress was exhibited by extracts from ES, with no observed cytotoxicity. Secondary metabolites from the plant may have potential for use as alternative medicines in ROS-induced diseases such as chronic wounds.

Keywords: antioxidant activity; cytotoxicity; oxidative stress; wounds.

Resumen

Contexto: *Erythrina senegalensis* (ES) DC. se usa tradicionalmente en el tratamiento de diversas enfermedades relacionadas con los radicales libres, incluida la curación de heridas.

Objetivos: Evaluar extractos de hojas de ES preparados secuencialmente para la capacidad de proteger contra el estrés oxidativo en fibroblastos y macrófagos.

Métodos: Los extractos se prepararon usando hexano, acetato de etilo, metanol y agua en polaridad creciente. La elaboración de perfiles químicos se realizó con cromatografía líquida de ultra alto rendimiento junto con espectrometría de masas (UPLC-MS). El potencial citotóxico se determinó usando el ensayo de tinción con sulforodamina B, contraste de fase, PlasDIC y microscopía de tinción viva/muerta. Se evaluó la capacidad de eliminar los radicales libres ABTS y DPPH, mientras que el efecto sobre el estrés oxidativo inducido por AAPH midiendo la liberación de especies reactivas de oxígeno (ROS).

Resultados: Un compuesto previamente informado (neobavaisoflavona) y otros tres (kaempferol, rutina y rotenona) fueron detectados tentativamente. Con respecto a la citotoxicidad, no se pudo determinar la CI_{50} hasta la concentración más alta probada (100 µg/mL) en las células THP-1 y SC-1. El extracto de metanol mostró la mayor actividad antioxidante contra los radicales libres [IC_{50} = 44,86 µg/mL (ABTS); 291,1 µg/mL (DPPH)] y ROS intracelular inducida por AAPH en macrófagos (reducción de 1,0 vez a 100 µg/mL). El efecto protector de los extractos (1 - 100 µg/mL) fue comparable al control positivo (Trolox), que disminuyó las ROS en 1,30 veces a 5 µg/mL.

Conclusiones: Los extractos de ES exhibieron una capacidad significativa para proteger contra el estrés oxidativo, sin citotoxicidad observada. Los metabolitos secundarios de la planta pueden tener potencial para su uso como medicamentos alternativos en enfermedades inducidas por ROS tales como heridas crónicas.

Palabras Clave: actividad antioxidante; citotoxicidad; estrés oxidativo; heridas.

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INTRODUCTION

Physiological levels of reactive oxygen species (ROS) are crucial for the normal functioning of cells (Brieger et al., 2012; Chen et al., 2018). An imbalance between ROS production and the organism's endogenous antioxidant protection systems could result in oxidative stress (Brieger et al., 2012). This causes cellular damage, leading to many chronic diseases such as cancer and delayed wounds (Dunnill et al., 2017; Chen et al., 2018). Antioxidant supplementation aids with the management and prevention of these conditions (Hosseinzadeh and Nassiri-Asl, 2014; Pisoschi and Pop, 2015).

Medicinal plants remain a major source of antioxidants (Pisoschi and Pop, 2015). One of the common medicinal plants employed for healing chronic diseases in Western Africa is *Erythrina senegalensis* DC (*Fabaceae*). In Ghana, aerial parts of the plant are used to manage gastrointestinal disorders like diarrhea and stomach-ache, as emmenagogues, and to induce abortion (Christensen et al., 2015; Larsen et al., 2016). Extracts prepared from the plant are also used in Ivory Coast and other countries for bacterial and parasitic infections, and for the treatment of wounds (Kone et al., 2012; Ilodigwe et al., 2014). Although used, there is a paucity of information in the scientific literature regarding its phytoconstituents and ability to protect against oxidative stress.

Previously the authors reported the presence of appreciable antioxidant activity in water extracts from *E. senegalensis* (Yahaya et al., 2018a). However, water extracts mostly contain a complex mixture of hydrophilic compounds (Sasidharan et al., 2011). The sequential extraction technique was employed in the current study to extract compounds of various polarities from the plant into different solvents for easy identification and isolation. This study was aimed at determining the protective ability of sequentially prepared extracts from *E. senegalensis* against oxidative stress, and to tentatively identify the phytoconstituents present.

MATERIAL AND METHODS

Chemicals

Organic solvents and trichloroacetic acid (TCA) were purchased from Merck Chemicals (South Africa), trypsin/versene from The Scientific Group (South Africa), and hemagglutination buffer [phosphate-buffered saline (PBS)] from BD Scientific (Paris, France). 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH), 2,7-dichlorofluorescein diacetate (H_2-DCF-DA), 2,2'-azino-bis(3-ethyl-benzothiazoline-6-sulphonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, fluorescein diacetate, kaempferol, quercetin, neobavaisoflavone, phorbol-12-myristate 13-acetate, propidium iodide, rotenone, rutin, sulforhodamine-B (SRB) powder and culture media were obtained from Sigma-Aldrich (St. Louis, USA).

Plant material

Leaves of *E. senegalensis* were collected from the botanical garden of the University of Cape Coast, Ghana (GPS coordinates; 5°7′4.251″ N 1°17′36.347″ W). The identity of the specimen was authenticated at the University's School of Biological Sciences herbarium, where a voucher specimen (UCCH 0091215) was deposited. Samples were washed thoroughly, air-dried at room temperature, finely powdered with a grinder (Glen Creston, UK) and stored in sterile airtight containers prior to use. Ethical approval for conduct of the research was obtained from the Research Ethics Committee of the University of Pretoria, Faculty of Health Sciences, with reference number 194/2017.

Sequential extraction of plant material

Extraction was performed sequentially using four different solvents in increasing polarity as described previously (Yahaya et al., 2018b). Powdered plant material (10 g) was sonicated (Bransonic 52, Branson Ultrasonics, Danbury, USA; 120 W) in 100 mL hexane for 15 min, shaken for 30 min on an electronic shaker and incubated at 4°C for 24 h. The solvent was decanted, and the marc air-

dried. The marc was re-extracted with ethyl acetate, methanol and water following the same procedure, although no further sonication took place. Filtrates were dried *in vacuo* with a rotary evaporator (Buchi Rotavapor R-200) at 40°C, with exception of the water extract which was freeze-dried (Freezone 6 Freeze Dry System, Labconco 31). Gravimetric yields were determined, and extracts stored at -20°C until needed.

Chemical profiling of extracts

Chemical profiling of extracts was tentatively done using ultra-performance liquid chromatography in tandem with mass spectrometry (UPLC-MS) as described previously (Yahaya et al., 2018a). A Waters UPLC system (Waters Corporation, Manchester, UK) coupled with a Waters photodiode array (PDA) detector (Waters Corporation, Manchester, UK) and a SYNAPT G1 HDMS QTOF mass spectrometer was used. The mass spectrometer was operated in both negative and positive modes of electrospray ionization to enable greater coverage of the metabolome. Phytochemical standards (Fig. 1) were dissolved in methanol to a concentration of 1 mg/mL, whilst plant extracts were dissolved to 10 mg/mL.

Culture of cell lines

Culture and maintenance of SC-1 fibroblasts

SC-1 cells (CRL-1404) were purchased from the American Type Culture Collection and were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat inactivated fetal calf serum (FCS), 100 µg/mL streptomycin and 100 mg/L penicillin at 37°C in a humidified incubator under an ambient pressure air atmosphere containing 5% CO_2.

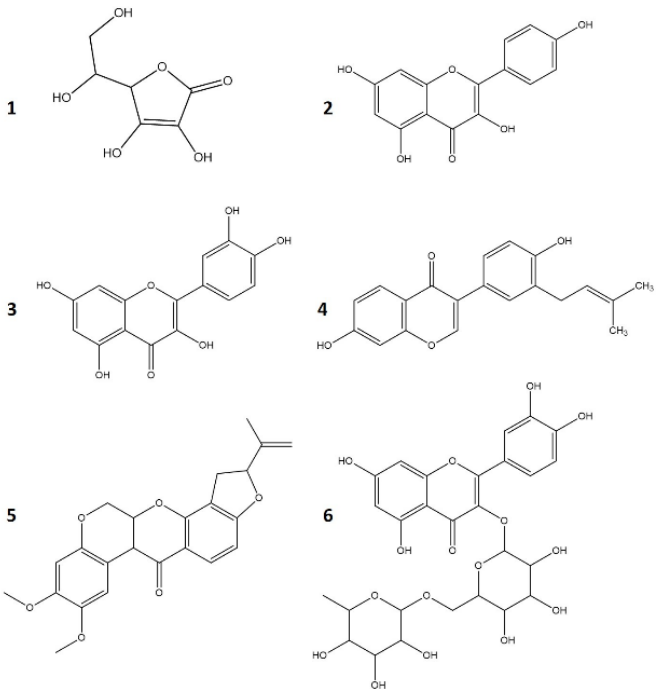

Figure 1. Compounds assessed in UPLC-MS fingerprint analysis.
(1) ascorbic acid; **(2)** kaempferol; **(3)** quercetin; **(4)** neobavaisoflavone; **(5)** rotenone; **(6)** rutin.

Culture, maintenance, and differentiation of THP-1 monocytes

THP-1 cells (TIB-202) were purchased from the American Type Culture Collection and were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium supplemented with L-glutamine (2 mM), HEPES (10 mM), sodium pyruvate (1 mM), glucose (2.5 g/L), 2-mercaptoethanol (50 µM), 10% FCS, and 1% penicillin/streptomycin at 37°C in a humidified incubator under an ambient pressure air atmosphere containing 5% CO_2. THP-1 cells were differentiated into macrophages using an established protocol (Lund et al., 2016). Briefly, cells were treated with 0.1 µM phorbol 12-myristate 13-acetate in 10% FCS-supplemented RPMI and incubated for 48 h to allow for differentiation. Differentiated THP-1 cells are characterized by attachment to the vessel surface, which was confirmed microscopically. Following differentiation, the media was replaced with fresh 10% FCS-supplemented RPMI (400 µL), and the macrophages used immediately.

Evaluation of the cytotoxic potential of extracts

Sulforhodamine-B staining assay

Extracts were assessed for possible cytotoxicity using a modified sulforhodamine B (SRB) assay as previously described (Yahaya et al., 2018b). Cells (100 µL, 1×10^4 cells/well) were seeded into 96-well plates in 10% FCS-supplemented medium and incubated overnight to allow for attachment. To determine the cytotoxicity profiles of each sample, 100 µL of the extract in culture medium was added to the plates at a final working concentration range of 1-100 µg/mL and incubated for 24 h or 48 h. A 1% saponin (in-reaction) served as positive control, whilst 100 µL of DMEM (RPMI for THP-1) was added as negative control. Dimethyl sulfoxide (1% v/v) in culture medium was used as vehicle control, whilst culture medium only served as the blank control.

Cells were fixed with 50 µL of cold TCA solution (50% w/v), and the plate incubated at 4°C overnight to allow for fixation. After fixation, the plate was gently washed with water (four times) to remove excess TCA and dried in a low-temperature oven. The fixed cells were stained with 100 µL of 0.057% (w/v in 1% acetic acid) SRB solution and the plate incubated for 30 min at room temperature. Dried plates were washed twice with 200 µL of 1% acetic acid solution (v/v) to remove excess unbound dye, and the plate dried again. The bound dye was dissociated using 200 µL of a 10 mM Tris-based solution (pH 10.5) amidst shaking for 30 min on an electronic shaking device. Absorbance was then measured at 540 nm with a reference wavelength of 630 nm, (Nascimento et al., 2013) using a plate reader (BioTek ELx800) and cell density calculated as follows [1]:

$$Cell\ density\ (\%\ of\ the\ negative\ control) = \frac{As}{Ac} \times 100\% \quad [1]$$

Where As and Ac are the blank-corrected absorbance of the sample and average negative control, respectively.

Light microscopy

Cells were seeded in 24-well plates at a density of 2.5×10^4 cells/well in 400 µL DMEM supplemented with 10% FCS, and incubated for 24 h to allow for attachment. Cells were treated with 10 and 100 µg/mL extract and incubated for 24 h and 48 h under the conditions described above. Following incubation, cells were analyzed using a phase contrast microscope (Axiovert 40 CFL) equipped with an inverted camera (Zeiss AxioCam MRm, Oberkochen, Germany) at 10× magnification for signs of apoptosis and necrosis. Apoptotic cells are characterized by cytoplasmic shrinkage, nuclear condensation, membrane blebbing and apoptotic body formation, while necrosis is characterized by swelling and cell lysis (Nanji and Hiller-Sturmhofel, 1997). PlasDIC was conducted at 40× magnification, and pictures taken and edited using AxioVision 4.

Live-dead staining

Cells were treated as described above. Following incubation, cells were washed twice with PBS (200 µL) and stained for 5 min with 50 µL of the staining solution containing 2 µg/mL fluorescein diacetate (FDA) and 10 µg/mL propidium iodide

(PI) in PBS. Cells were washed twice with 200 µL PBS, and covered with FCS-free DMEM (200 µL) for evaluation using a fluorescence microscope (Axiovert 40 CFL) fitted with an inverted camera (Zeiss AxioCam MRm, Oberkochen, Germany). All images were captured at 10× magnification.

Free radical scavenging activity of extracts

ABTS radical scavenging effect

The ABTS radical scavenging effect of extracts was determined as described previously (Re et al., 1999). A stock solution of ABTS containing 7 mM ABTS salt and 2.4 mM potassium persulfate, was prepared in distilled water and incubated in the dark for 16 h at 4°C. The resultant ABTS radical ($ABTS^{+}$) solution was diluted with distilled water to an absorbance of 0.70 ± 0.02 at 734 nm (PerkinElmer Lambda 25 UV/VIS spectrometer). An aliquot of 180 µL $ABTS^{+}$ solution was mixed with 20 µL of varying concentrations of the extracts (20 – 100 µg/mL in-reaction), and 0.6 – 5.0 µg/mL of the positive control (Trolox) in a 96-well plate. Equivalent volumes of distilled water and 1% DMSO in methanol served as negative controls for the water and organic extracts, respectively. The absorbance was read after 30 min incubation in the dark using the Synergy 2 microplate reader (BioTek Instruments, Inc.) at 734 nm. The $ABTS^{+}$ scavenging capacity was calculated as follows [2]:

$$ABTS\ radical\ scavenging\ activity\ (\%\ relative\ to\ negative\ control) = \frac{Ac - As}{As} \times 100 \quad [2]$$

Where *Ac* and *As* represent the absorbance of the average negative control and sample, respectively. The half-maximal inhibitory concentration (IC_{50}) values were calculated using nonlinear regression analysis and used to indicate antioxidant activity.

DPPH radical scavenging effect

The DPPH radical scavenging effect of extracts was estimated using a previously described method (Manzocco et al., 1998). A solution of 0.135 mM DPPH in methanol was prepared prior to experimentation with an initial absorbance of 0.48 ± 0.02. An aliquot of 180 µL DPPH solution was mixed with 20 µL of varying concentrations of the extracts (20 – 320 µg/mL in-reaction), and 0.6 – 5.0 µg/mL Trolox in a 96-well plate. Equivalent volumes of distilled water and 1% DMSO in methanol served as negative controls for the water and organic extracts, respectively. The reaction mixture was incubated in the dark for 30 min at room temperature and absorbance read spectrophotometrically at 515 nm using a microplate reader (Synergy-2, BioTek Instruments, Inc.). The ability of extracts to scavenge the DPPH radical was calculated using the following equation [3]:

$$DPPH\ radical\ scavenging\ activity\ (\%\ relative\ to\ negative\ control) = \frac{Ac - As}{As} \times 100 \quad [3]$$

Where *Ac* and *As* represent the absorbance of the average negative control and sample, respectively. The IC_{50} values were calculated as described earlier.

Activity against oxidative stress

The ability of extracts to alter intracellular ROS was assessed in an AAPH-induced oxidative stress model as previously described (Lopachev et al., 2016). Cells were seeded into a 96-well plate at a density of 1×10^4 cells/well and allowed to attach overnight. The culture medium was replaced with 100 µL fresh medium containing 10 µM H_2-DCF-DA and incubated for 30 min in the dark. Excess H2-DCF-DA was removed by washing twice with 100 µL PBS, followed by exposure to 1, 10 and 100 µg/mL extract (in 100 µL of 10% FCS-supplemented medium) for 4 h. All control wells were treated with equivalent volumes of the respective medium during this period, except antioxidant controls to which 5 µg/mL Trolox was added. Cells were washed twice with 100 µL PBS and 50 µL PBS containing 100 µM AAPH added, except for the negative controls to which 50 µL PBS was added. The relative fluorescence intensity (RFI) was measured every minute for 2 h at an excitation and emission wavelength of 485 nm and 530 nm respectively using a Synergy 2 microplate reader (BioTek Instruments, Inc.). Intracellular ROS was estimated as follows [4]:

$$Intracellular\ ROS\ (fold - change\ relative\ to\ control) = \frac{RFIs}{RFIc} \quad [4]$$

Where *RFIs* and *RFIc* represents the RFI of each sample and the average negative control, respectively.

Statistical analysis

Data represents results of at least three independent experiments conducted in technical triplicates. Statistical analysis was performed using GraphPad Prism 5.00 data analysis software. Data was expressed as the mean ± standard error of mean (SEM). The difference between groups was determined by ANOVA followed by Bonferroni post-tests. *P* values less than 0.05 were considered significant.

RESULTS

Chemical profiling of extracts

Extracts were profiled to determine the presence of some phytochemical compounds using UPLC-MS in order to provide fingerprints. Four compounds were tentatively identified in the extracts (Fig. 2). Neobavaisoflavone (*m/z* = 321.11), a previously identified compound (Kuete et al., 2014; Yahaya et al., 2018a), was detected in the hexane and methanol extracts of the plant. Three other compounds, kaempferol (*m/z* = 285.04), rotenone (*m/z* = 393.13), and rutin (*m/z* = 609.14), were detected in the methanol extract of the plant.

Determination of cytotoxic potential

The IC_{50} for all tested extracts was greater than 100 µg/mL in both fibroblasts and macrophages (Fig. 3). This was corroborated by findings of the microscopic examination of treated cells. Both phase contrast (Fig. 4A and 5A) and PlasDIC microscopy (Fig. 4B and 5B) indicated that extract-treated cells had similar morphology as the untreated controls. Moreover, live/dead staining of the cells with FDA and PI indicated similarity between treated and untreated cells (Fig. 4C and 5C). Living cells are stained green by the cell-permeable esterase substrate (FDA), whilst the cell-impermeable acid stain (PI) stains dead or damaged cells red (Diaz et al., 2004).

Effect on oxidation

Acellular antioxidant potential

The ABTS and DPPH radical scavenging effects of the extracts are as shown on Table 1. The methanol and hexane extracts showed the strongest (IC_{50} = 44.86 µg/mL) and weakest (IC_{50} > 100 µg/mL) ability to quench the free radicals, respectively. None of the extracts had a notable effect on the DPPH free radical. With an IC_{50} of 291.10 µg/mL, the methanol extract produced the strongest scavenging activity against DPPH.

Amelioration of cellular oxidative stress

The exposure of fibroblasts and macrophages to AAPH resulted in an increase of 1.70- and 1.57-fold in intracellular ROS, respectively (Fig. 6). Pre-treatment with the extracts at concentrations up to 100 µg/mL before AAPH exposure yielded diverse effects on cellular oxidation. Intracellular ROS in pre-treated fibroblasts was lower when compared to cells that were not pre-treated with extracts. The most prominent effect was observed upon pre-treatment with the ethyl acetate and methanol extracts at 100 µg/mL. These two extracts caused a 0.76 and 0.74-fold decrease in oxidative stress, respectively, when compared to untreated cells.

The effect of the extracts on AAPH-induced intracellular ROS generation in the differentiated macrophages was even more pronounced. Extract pre-treatment of macrophages resulted in a 0.5 – 1.1-fold reduction in intracellular ROS level. The most prominent effect was observed upon pre-treatment with the methanol and water extracts. Whilst the methanol extract, at 100 µg/mL, caused a fold decrease of 1.0 in intracellular ROS, the water extract exhibited a possible pro-oxidant effect. ROS levels increased to 1.1-fold upon treatment with 100 µg/mL of the extract, compared to 0.9-fold change at 1 µg/mL. These effects were comparable to that observed upon pre-treatment of cells with Trolox, the antioxidant positive control. Trolox, at 5 µg/mL, caused a 1.30-fold decrease in intracellular ROS, compared to cells that were not pre-treated.

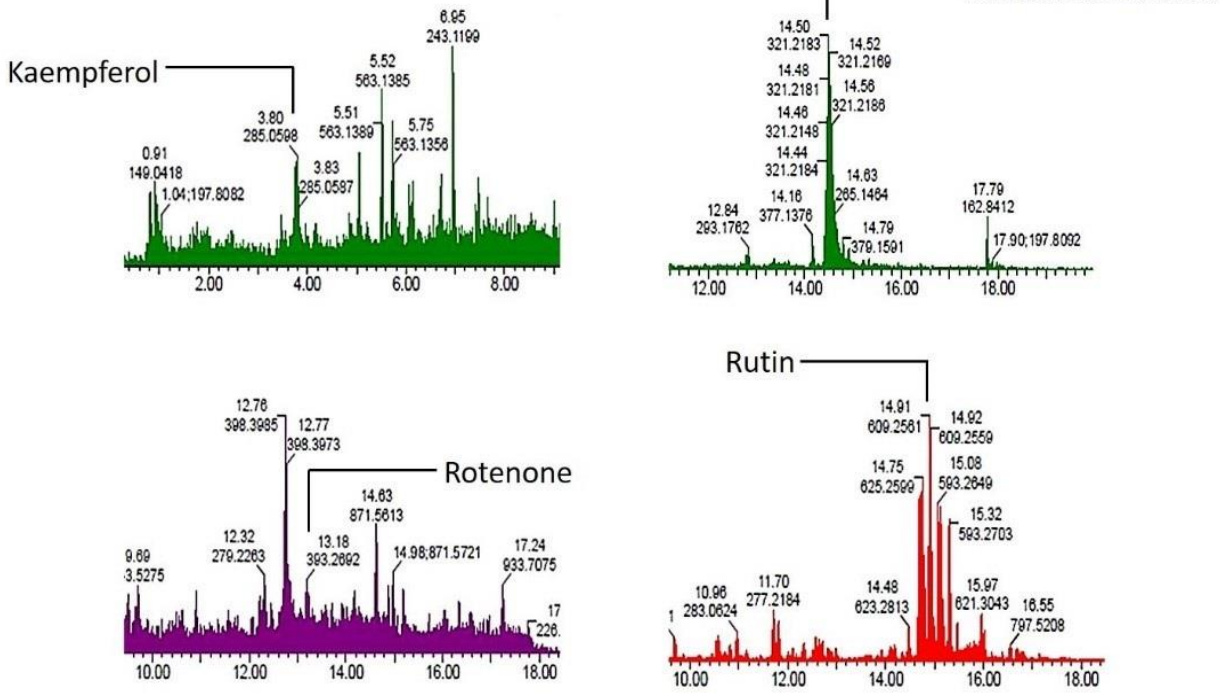

Figure 2. UPLC-MS chromatograms of compounds detected in the sequential extracts of *E. senegalensis*.

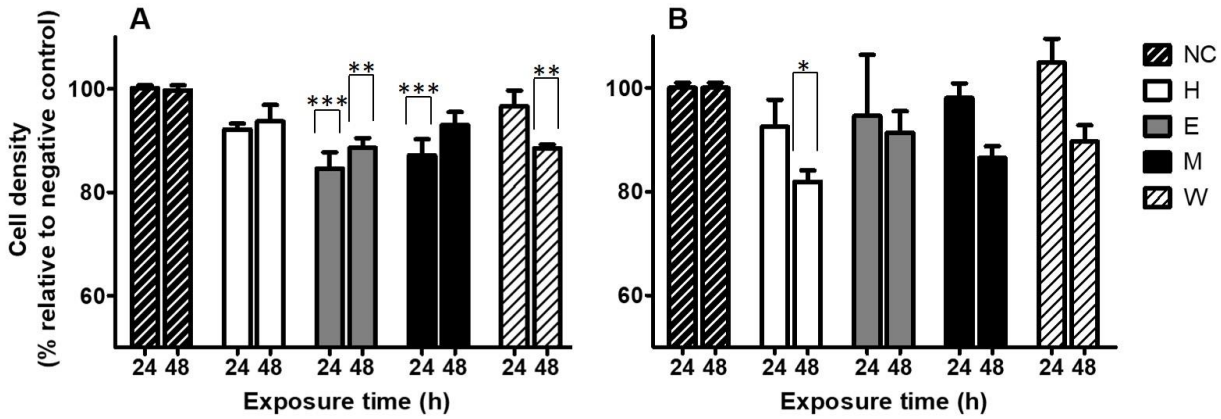

Figure 3. Cytotoxic evaluation of sequential extracts of *E. senegalensis* in **(A)** SC-1 fibroblasts and **(B)** THP-1 differentiated macrophages using the SRB assay.

H: hexane; E: ethyl acetate; M: methanol; W: water; NC: negative control. Data expressed as mean ± SEM (n = 9). Significant difference from NC by ANOVA followed by Bonferroni post-tests denoted by *($p < 0.05$), **($p < 0.01$) and ***($p<0.001$).

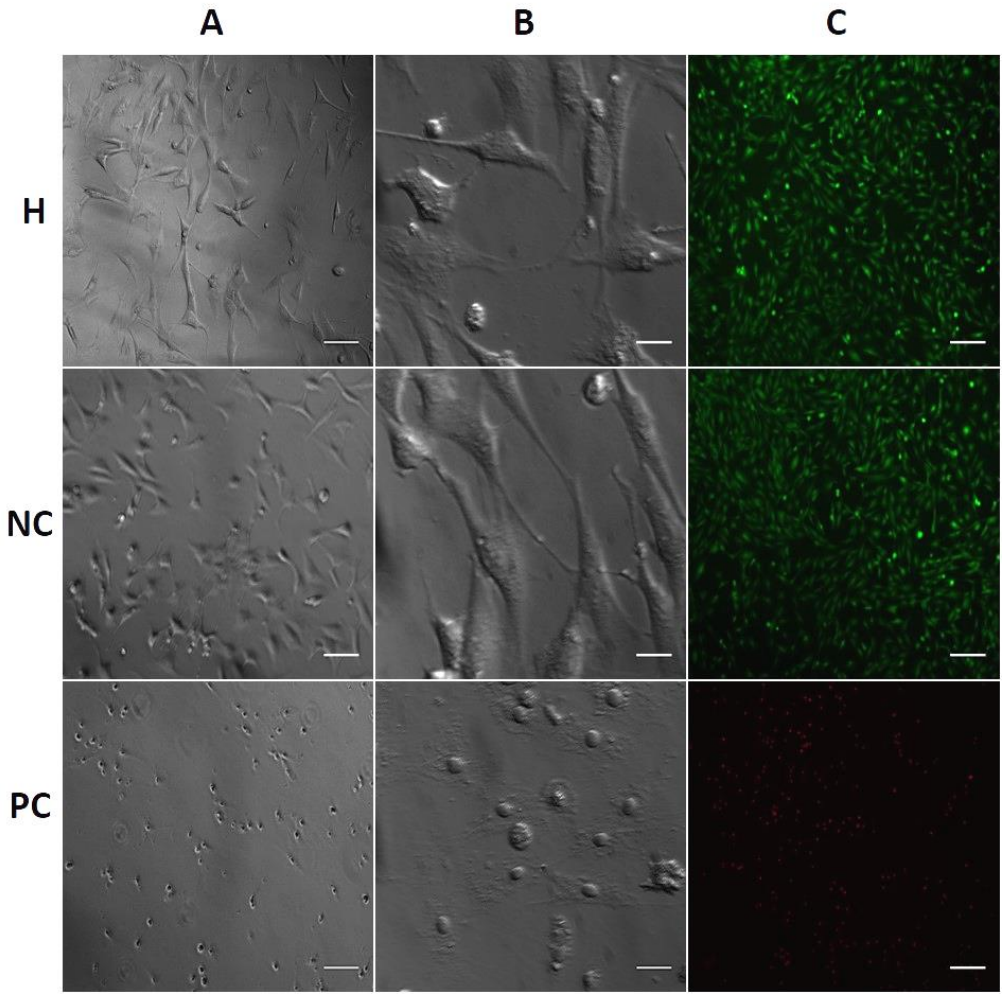

Figure 4. Cytotoxic potential of sequential extracts prepared from *E. senegalensis* in SC-1 fibroblasts.

Hexane (H) extracts were used as a representative image as all extracts displayed the same effect. **(A)** phase contrast; **(B)** PlasDIC; **(C)** composite images of live/dead staining; NC: negative control; PC: positive control; fluorescein diacetate staining, green; propidium iodide staining, red. Images were captured at 10× (phase contrast and live/dead staining) and 40× (B) magnifications. Scale bar; 100 µm (A, C), 25 µm (B).

Table 1. The scavenging effects of sequential extracts from *E. senegalensis* on ABTS and DPPH radicals.

Extract	IC_{50} ± SEM (µg/mL)	
	ABTS	**DPPH**
Hexane	> 100	> 320
Ethyl acetate	53.29 ± 1.07	> 320
Methanol	44.86 ± 1.03	291.10 ± 2.46
Water	49.05 ± 1.04	> 320
Positive control (Trolox)	2.92 ± 1.02	6.27 ± 1.07

Data expressed as mean ± SEM (n = 9). IC_{50}: the half maximal concentration or concentration required to decrease the initial absorbance of ABTS and DPPH by half.

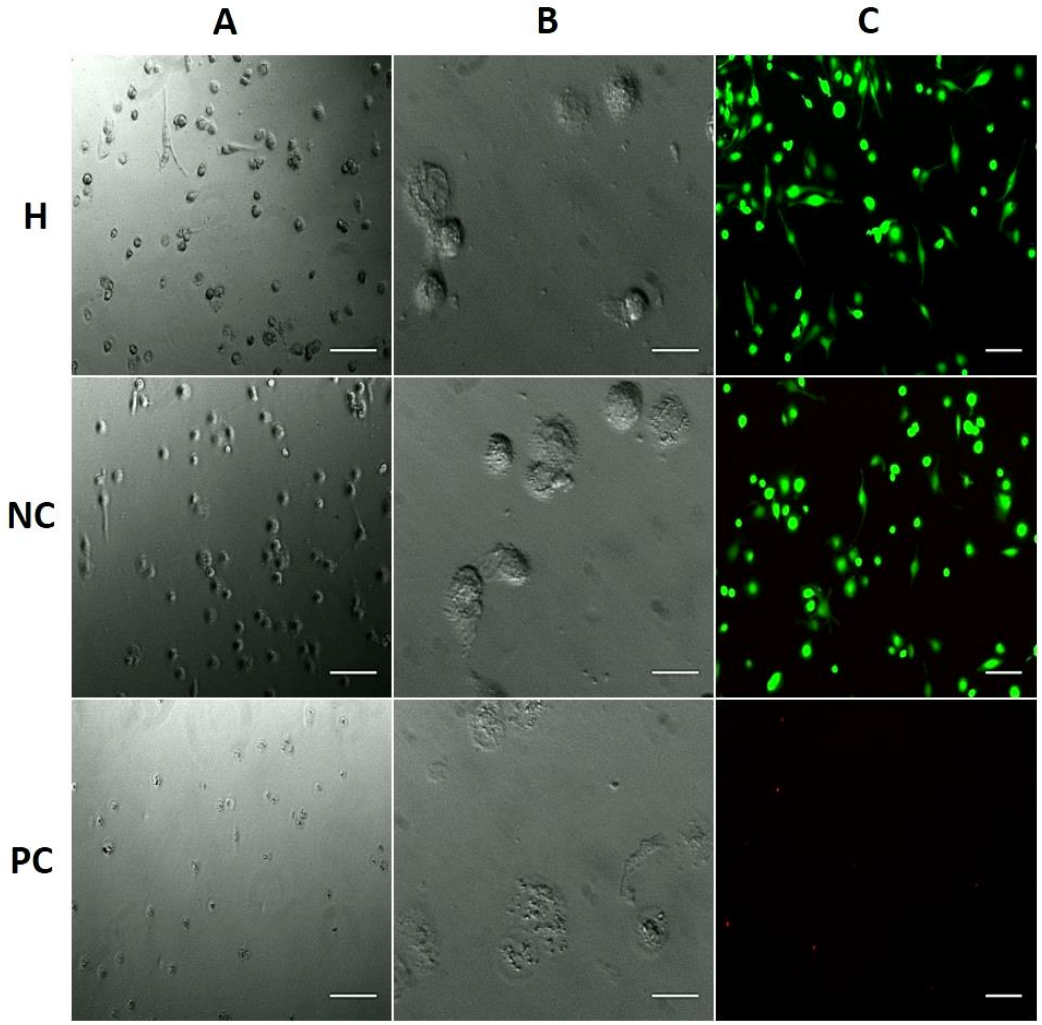

Figure 5. Cytotoxic potential of sequential extracts prepared from *E. senegalensis* in THP-1 differentiated macrophages.

Hexane (H) extracts were used as a representative image as all extracts displayed the same effect. **(A)** phase contrast; **(B)** PlasDIC; **(C)** composite images of live/dead staining; NC: negative control; PC: positive control; fluorescein diacetate staining = green; propidium iodide staining = red. Images were captured at 10× (phase contrast and live/dead staining) and 40× (B) magnifications. Scale bar; 100 µm (A, C), 25 µm (B).

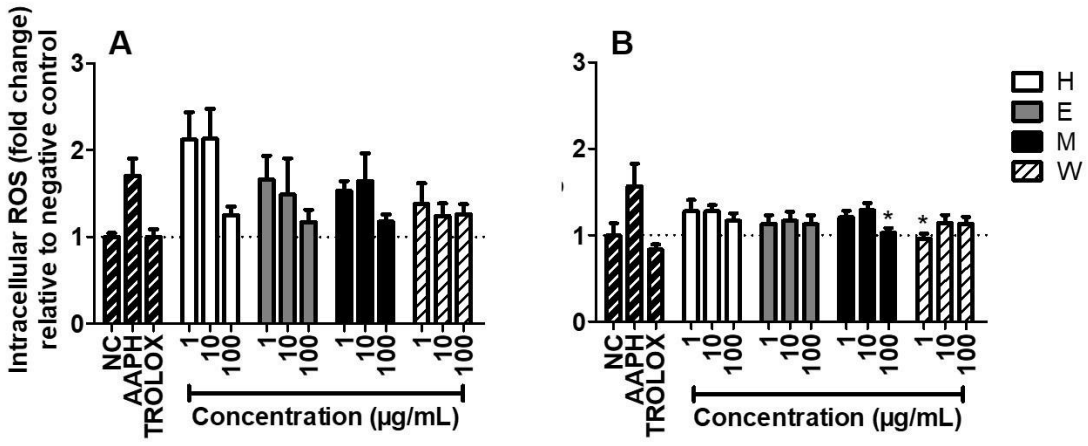

Figure 6. Effect of *E. senegalensis* sequential extracts on AAPH-induced oxidative stress in **(A)** SC-1 fibroblasts and **(B)** THP-1 differentiated macrophages.

H: hexane; E: ethyl acetate; M: methanol; W1. water; AAPH: 2,2'-azobis(2-amidinopropane) dihydrochloride; NC: negative control. Data expressed as mean ± SEM (n = 9). *Significant difference ($p<0.05$) from the AAPH-only cells by ANOVA followed by Bonferroni post-tests.

DISCUSSION

The study aimed at evaluating the ability of extracts of *E. senegalensis*, prepared sequentially from four solvents with incremental polarity, to protect cells from oxidative damage and to determine the phytoconstituents present. Fingerprint analysis of the extracts using UPLC-MS indicated the presence of multiple compounds. Of the four tentatively identified compounds, only neobavaisoflavone was previously indicated to be present in the plant (Kuete et al., 2014; Yahaya et al., 2018a). To the best of our knowledge, this is the first study indicating the presence of kaempferol, rotenone and rutin in the plant. Further analysis will be required to confirm this.

Data from the cytotoxic analysis using both spectroscopic and microscopic techniques indicated no sign of extract-induced cellular damage up to the maximum tested concentration of 100 µg/mL. However, because topical application of plant remedies often involves the use of higher concentrations, there is need to evaluate the risk at concentrations above 100 µg/mL. Although cytotoxicity of some compounds isolated from the plant has been shown in cancerous cells (Kuete et al., 2014), no such results have been reported in normal cells. The ethnomedicinal extract of the leaves was previously found to have no effect on the viability of fibroblasts (Yahaya et al., 2018a). Moreover, the safety of the plant in animal models has been well documented (Donfack et al., 2008a;b). Therefore, our finding is further proof of the safety of *E. senegalensis* extracts.

Since the ability to attenuate oxidative stress is directly related to a compound's antioxidant potential (Dunnill et al., 2017; Yahaya et al., 2018b), the extracts were assessed for antioxidant effects using ABTS and DPPH radical scavenging as markers of activity. All treatments had superior radical scavenging activity against the ABTS, compared to the DPPH radical (Table 1). This is indicative of the appreciable antioxidant activity of the extracts. The ABTS radical scavenging effect of the ethnomedicinal extract of the plant's leaves was previously demonstrated in a study which indicated strong antioxidant potential (Yahaya et al., 2018a). The antioxidant potential exhibited in the current study could be attributed to the presence of phytochemicals such as polyphenols and steroids (Souleymane et al., 2016). Two of such compounds (kaempferol and rutin) were tentatively found to be present in the extracts (Fig. 2), compounds that are known to exhibit antioxidant potential albeit weak in the case of rutin (Hosseinzadeh and Nassiri-Asl, 2014; Badmus et al., 2016).

Kaempferol's ability to protect against oxidative damage has been established (Liao et al., 2016). Hence, the presence of kaempferol in the methanol extract could be responsible for the good antioxidant effect observed. Although the water extract exhibited appreciable antioxidant activity, it did not test positive for any of the standard antioxidants tested, suggesting that other phytochemicals could be responsible for the activity noted.

Several factors may account for the varied scavenging ability of the extracts against the two free radicals. Some antioxidant components may selectively quench one free radical, and not the other. Hydrophilic antioxidants, for instance, are poorly detected with DPPH (Niki, 2010). Therefore, the poor DPPH scavenging ability of the tested extracts could be due, in part, to the presence of these water-loving antioxidants. Also, the radicals have different stereoselectivity which results in different electron and hydrogen atom transfer potentials (Yu et al., 2002).

Assessment of the ability of extracts to protect fibroblasts and macrophages against AAPH-induced oxidative stress revealed promising outcome. Except for the hexane extract, all others exhibited some ability to protect the cells against oxidative stress. Generally, the extracts demonstrated a greater ability to suppress ROS generation in differentiated macrophages, compared to fibroblasts. This could be ascribed to the inherent ability of phagocytic cells to secrete ROS through the activities of reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, mitochondrial electron transport chain, and xanthine oxidase (Droge, 2002). It has been documented that a correlation exists between compounds with the ability to donate electrons, such as antioxi-

dants, and oxidative stress suppression (Dunnill et al., 2017; Yahaya et al., 2018b). The protective effect of the extracts against oxidative stress could therefore be a result of their antioxidant potential. Previous reports indicate that three of the identified compounds, neobavaisoflavone, kaempferol, and rutin, have antioxidant potential (Xiao et al., 2010; Hosseinzadeh and Nassiri-Asl, 2014; Badmus et al., 2016), while the fourth (rotenone) is capable of inducing oxidative stress by reducing activity of intracellular antioxidants such as glutathione, amongst others (Swarnkar et al., 2009).

Although the water extract showed good antioxidant activity (ABTS IC_{50} = 49.05 µg/mL), it was observed to exhibit the opposite effect on intracellular ROS in macrophages at higher concentrations. This could be ascribed to the increased stress exhibited on the cells at higher concentrations of the extract. Whereas cell growth was unaffected after 24 h, exposure of macrophages to the water extract reduced cell density by 10% after 48 h (Fig. 3). This is an indication of possible long-term cytotoxicity upon exposure to higher concentrations of the extract, and therefore could explain the prooxidant effect observed.

A previous study indicated the cytoprotective potential of the ethnomedicinal extract of the plant in isolated fibroblasts (Yahaya et al., 2018a). Though similar cellular studies on the plant were hard to come by, some accounts of the ability of its extracts and isolated compounds to protect against toxicity in animal experiments were noted (Donfack et al., 2008a;b). The current study findings therefore add to the available information on the protective ability of the plant against oxidative damage.

CONCLUSIONS

Extracts of *E. senegalensis* have exhibited notable potential to protect against oxidative stress in cells. The findings demonstrate that extracts from the plant have potential for use in the protection against oxidative damage, and in the management of oxidative stress-related conditions such as chronic wounds. Further experimentation will be required to identify and confirm the phytochemicals responsible for the activity in the extracts.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

Badmus JA, Ekpo OE, Rautenbach F, Marnewick JL, Hussein AA, Hiss DC (2016) Isolation and antioxidant activity of flavonoids from *Holarrhena floribunda* (G.don) leaves. Acta Biochim Pol 63(2): 353–358.

Brieger K, Schiavone S, Miller Jr FJ, Krause K-H (2012) Reactive oxygen species: from health to disease. Swiss Med Wkly 142: w13659.

Chen Q, Wang Q, Zhu J, Xiao Q, Zhang L (2018) Reactive oxygen species: key regulators in vascular health and diseases. Brit J Pharmacol 175(8): 1279–1292.

Christensen CB, Soelberg J, Stensvold CR, Jager AK (2015) Activity of medicinal plants from Ghana against the parasitic gut protist *Blastocystis*. J Ethnopharmacol 174: 569–575.

Diaz TM, Pertega S, Ortega D, Lopez E, Centeno A, Manez R, Domenech N (2004) FDA/PI flow cytometry assay of complement-mediated cytotoxicity of antibodies generated during xenotransplantation. Cytom Part A 62a(1): 54–60.

Donfack J, Njayou F, Rodrigue T, Chuisseu D, Tchana N, Finzi V, Tchounguep M, Ngadjui T, Moundipa F (2008a) Study of a hepatoprotective and antioxidant fraction from *Erythrina senegalensis* stem bark extract: *In vitro* and *in vivo*. Pharmacologyonline 1: 120–130.

Donfack JH, Nico FN, Ngameni B, Tchana A, Chuisseu PD, Finzi PV, Ngadjui BT, Moundipa PF (2008b) *In vitro* hepatoprotective and antioxidant activities of diprenylated isoflavonoids from *Erythrina senegalensis* (Fabaceae). Asian J Trad Med 3(5): 72–178.

Droge W (2002) Free radicals in the physiological control of cell function. Physiol Rev 82(1): 47–95.

Dunnill C, Patton T, Brennan J, Barrett J, Dryden M, Cooke J, Leaper D, Georgopoulos NT (2017) Reactive oxygen species (ROS) and wound healing: the functional role of ROS and emerging ROS-modulating technologies for augmentation of the healing process. Int Wound J 14(1): 89–96.

Hosseinzadeh H, Nassiri-Asl M (2014) Review of the protective effects of rutin on the metabolic function as an important dietary flavonoid. J Endocrinol Invest 37(9): 783–788.

Ilodigwe E, Okonkwo B, Agbata C, Ajaghaku D, Eze P (2014) Wound healing activity of ethanol leaf extract of *Erythrina senegalensis*. Brit J Pharm Res 4(4): 531.

Kone WM, Vargas M, Keiser J (2012) Anthelmintic activity of medicinal plants used in Cte d'Ivoire for treating parasitic diseases. Parasitol Res 110(6): 2351–2362.

Kuete V, Sandjo LP, Kwamou GM, Wiench B, Nkengfack AE, Efferth T (2014) Activity of three cytotoxic isoflavonoids from *Erythrina excelsa* and *Erythrina senegalensis* (neobavaisoflavone, sigmoidin H and isoneorautenol) toward multi-factorial drug resistant cancer cells. Phytomedicine 21(5): 682–688.

Larsen BHV, Soelberg J, Kristiansen U, Jager AK (2016) Uterine contraction induced by Ghanaian plants used to induce abortion. S Afr J Bot 106: 137–139.

Liao W, Chen L, Ma X, Jiao R, Li X, Wang Y (2016) Protective effects of kaempferol against reactive oxygen species-induced hemolysis and its antiproliferative activity on human cancer cells. Eur J Med Chem 114: 24–32.

Lopachev AV, Lopacheva OM, Abaimov DA, Koroleva OV, Vladychenskaya EA, Erukhimovich AA, Fedorova TN (2016) Neuroprotective effect of carnosine on primary culture of rat cerebellar cells under oxidative stress. Biochemistry (Mosc) 81(5): 511–520.

Lund ME, To J, O'Brien BA, Donnelly S (2016) The choice of phorbol 12-myristate 13-acetate differentiation protocol influences the response of THP-1 macrophages to a pro-inflammatory stimulus. J Immunol Methods 430: 64–70.

Manzocco L, Anese M, Nicoli M (1998) Antioxidant properties of tea extracts as affected by processing. LWT-Food Sci Technol 31(7): 694–698.

Nanji AA, Hiller-Sturmhofel S (1997) Apoptosis and necrosis: two types of cell death in alcoholic liver disease. Alc Health Res World 21(4): 325–330.

Nascimento AK, Melo-Silveira RF, Dantas-Santos N, Fernandes JM, Zucolotto SM, Rocha HA, Scortecci KC (2013) Antioxidant and antiproliferative activities of leaf extracts from *Plukenetia volubilis* Linneo (Euphorbiaceae). Evid Based Complement Alternat Med 2013: 950272.

Niki E (2010) Assessment of antioxidant capacity *in vitro* and *in vivo*. Free Rad Biol Med 49(4): 503–515.

Pisoschi AM, Pop A (2015) The role of antioxidants in the chemistry of oxidative stress: a review. Eur J Med Chem 97: 55–74.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Rad Biol Med 26(9): 1231–1237.

Sasidharan S, Chen Y, Saravanan D, Sundram K, Latha LY (2011) Extraction, isolation and characterization of bioactive compounds from plants' extracts. Afr J Tradit Complement Altern Med 8(1): 1–10.

Souleymane F, Charlemagne G, Moussa O, edraogo Eloi, P, Baptiste NJ, Pierre GI, Jacques S (2016) DPPH radical scavenging and lipoxygenase inhibitory effects in extracts from *Erythrina senegalensis* (Fabaceae) DC. Afr J Pharm Pharmacol 10(11): 185–191.

Swarnkar S, Tyagi E, Agrawal R, Singh MP, Nath C (2009) A comparative study on oxidative stress induced by LPS and rotenone in homogenates of rat brain regions. Environ Toxicol Pharmacol 27(2): 219–224.

Xiao G, Li G, Chen L, Zhang Z, Yin J-J, Wu T, Cheng Z, Wei X, Wang Z (2010) Isolation of antioxidants from *Psoralea corylifolia* fruits using high-speed counter-current chromatography guided by thin layer chromatography-antioxidant autographic assay. J Chromatogr A 1217(34): 5470–5476.

Yahaya ES, Cordier W, Steenkamp P, Steenkamp V (2018a) Effect of ethnomedicinal extracts used for wound healing on cellular migration and intracellular reactive oxygen species release in SC-1 fibroblasts. S Afr J Bot 118: 11–17.

Yahaya ES, Cordier W, Steenkamp PA, Steenkamp V (2018b) Attenuation of oxidative stress and artificial wound closure in C2C12 myoblasts induced by sequential extracts of *Boerhavia diffusa*. J Pharm Pharmacol 70(1): 111–123.

Yu L, Haley S, Perret J, Harris M, Wilson J, Qian M (2002) Free radical scavenging properties of wheat extracts. J Agric Food Chem 50(6): 1619–1624.

AUTHOR CONTRIBUTION:

Contribution	Yahaya ES	Cordier W	Steenkamp PA	Steenkamp V
Concepts or ideas	x			x
Design	x	x	x	x
Definition of intellectual content	x	x	x	x
Literature search	x			
Experimental studies	x	x		
Data acquisition	x	x		
Data analysis	x	x	x	x
Statistical analysis	x			
Manuscript preparation	x			
Manuscript editing		x	x	x
Manuscript review	x	x	x	x

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