South African Journal of Botany 167 (2024) 209–216

Contents lists available at ScienceDirect

South African Journal of Botany

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

The antithrombotic potential of selected South African plants for venous thromboembolism

Lilitha Lwando Denga[a], Bianca Diedericks[a], Anna-Mari Kok[a,b], Namrita Lall[a,c,d,e,*]

[a] Department of Plant and Soil Sciences, University of Pretoria, Pretoria 0002, South Africa
[b] South African International Maritime Institute (SAIMI), Nelson Mandela University, Gqeberha 6019, South Africa
[c] School of Natural Resources, University of Missouri, Columbia, MO, United States
[d] College of Pharmacy, JSS Academy of Higher Education and Research, Mysuru, Karnataka, India
[e] Bio-Tech R&D Institute, University of the West Indies, Kingston, Jamaica

ARTICLE INFO

Article History:
Received 29 September 2023
Revised 22 January 2024
Accepted 16 February 2024
Available online xxx

Edited by: Dr S.C. Pendota

Keywords:
Anti-FXa
Cyclooxygenase-1
Medicinal plants
Antithrombotic
Venous thromboembolism

ABSTRACT

Venous thromboembolism (VTE) is a collective term for the cardiovascular diseases namely, deep vein thrombosis and pulmonary embolism. Thrombosis causes the progression of VTE. Many of those who live in developing countries rely on herbal medicinal products as a primary source of healthcare. The study investigated the antithrombotic properties of the selected South African plant extracts. Eleven (11) plant species traditionally used for pain management and wound healing including plants reported in literature with anti-inflammatory and antioxidant activity were selected for this study. Extracts were prepared for each plant using ethanol, dichloromethane, and water. The extracts were tested for their antiproliferative effects on human liver carcinoma (HepG2) cells as well as antioxidant, activated coagulation factor X (anti-FXa) and cyclooxygenase-1 (COX-1) inhibitory activity. The results were interpreted using published information on the phytochemistry of the relevant active extracts. The most effective antioxidant activity was observed for the aqueous extract of *Heteropyxis natalensis* (*H. natalensis*) (IC_{50} = 2.71 μg/mL), which also displayed potent anti-FXa activity with an IC_{50} value of 2.64 μg/mL. The same extract inhibited COX-1 at an IC_{50} value of 25.32 μg/mL. Due to the potent antiproliferative, antioxidant, and anti-FXa activity, as well as favourable COX-1 inhibition, the aqueous extract of *H. natalensis* was identified as the best candidate for future antithrombotic studies.

1. Introduction

Venous thromboembolism (VTE) is a collective term that consists of two cardiovascular diseases namely, deep vein thrombosis (DVT) and pulmonary embolism (PE) (Anderson and Spencer, 2003). Patients with DVT develop a blood clot (thrombus) in the large veins of the leg, which can break away and travel through the circulatory system as an embolus (Tarbox and Swaroop, 2013). When an embolus reaches the pulmonary circulation in the lungs, it results in PE which occurs in up to one third of DVT cases and is the main contributor to mortality (Mackman, 2008). Cardiovascular diseases cause the most deaths globally (WHR, 2023), therefore, the development of novel treatments is relevant. Medicinal plants have the potential to advance drug discovery in the global market, therefore, the medicinal properties of plants may be explored for the possible treatment of VTE (Frenzel and Teschke, 2016). Various secondary metabolites from plants have displayed a myriad of medicinal properties and have been used extensively in the pharmaceutical industry (Jain et al., 2019).

The key biological mechanism that causes VTE is thrombosis, which is regulated by coagulation within the coagulation cascade summarized in Fig. 1 (Raskob et al., 2014). Warfarin, known for the treatment and prevention of blood clots, rarely causes liver injury when used in antithrombotic therapy, however, there has been several cases reported of clinical acute liver injury attributable to it. Coumarin derivatives such as acenocoumarol and phenprocoumon has more commonly attributed to liver injury, for this reason they have

Abbreviations: ANOVA, Analysis of variance; COX, Cyclooxygenase; COX-1, Cyclooxygenase-1; DCM, Dichloromethane; DMEM, Dulbecco's Modified Eagles Medium; DMSO, dimethyl sulfoxide; DPPH, 2,2-diphenyl-1-picrylhydrazyl; DVT, Deep vein thrombosis; EDTA, Ethylenediaminetetraacetic acid; EI, Electron ionization; FXa, Activated coagulation factor X; GGACK, 1,5-Dansyl-Glu-Gly-Arg Chloromethyl Ketone; GPP8, GraphPad Prism 8; HepG2, Hepatocellular carcinoma; IC_{50}, 50% inhibitory concentration; K_m, Michaelis constant; NIST, National Institute of Standards and Technology; PE, Pulmonary embolism; PEG-6000, Polyethylene glycol 6000; Tris, tris (hydroxymethyl)aminomethane; TXA_2, Thromboxane A_2; V_{max}, maximum rate of reaction; VTE, Venous thromboembolism

* Corresponding author.
E-mail address: namrita.lall@up.ac.za (N. Lall).

https://doi.org/10.1016/j.sajb.2024.02.028

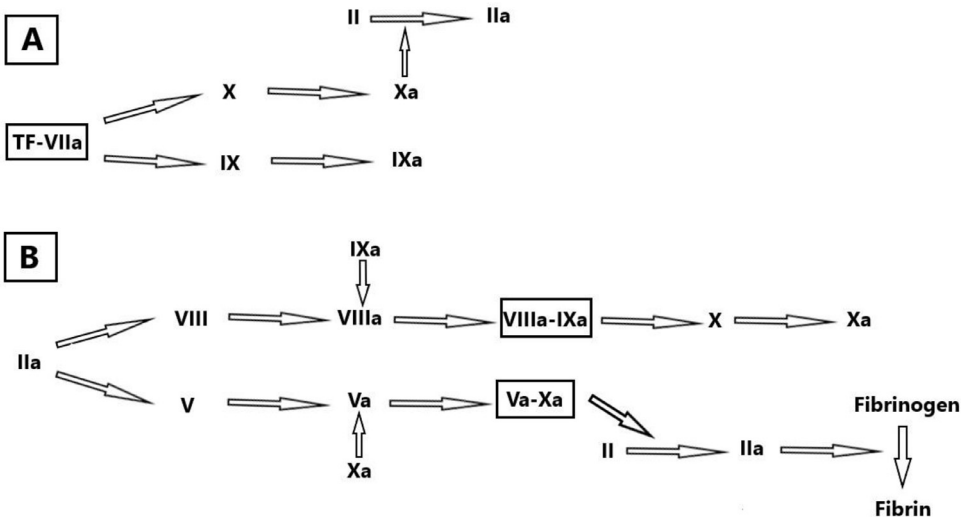

Fig. 1. The coagulation cascade (Paint 3D) based on Kamal et al. (2007)**. (A) Initiation of the coagulation cascade:** Tissue factor (TF) forms complex with activated coagulation factor VII (VIIa). Complex activates coagulation factors X (X) and IX (IX) resulting in activated coagulation factors X (Xa) and IX (IXa). Xa converts prothrombin (II) to thrombin (IIa). **(B) Propagation of the coagulation cascade:** Thrombin activates coagulation factors VIII (VIII) and V (V) resulting in activated coagulation factors VIII (VIIIa) and V (Va). VIIIa forms tensase complex with IXa resulting in additional Xa, which forms prothrombinase complex with Va. Prothrombinase complex converts II to IIa, which converts fibrinogen to fibrin.

been banned to be used as an antithrombotic treatment in countries such as the United States (Arora and Goldhaber, 2006; Klein, 2009). The liver is mainly involved in metabolism and detoxification of endogenous and exogenous compounds, therefore, any damage to the liver will have negative health implications (Subramaniam et al., 2015). Antithrombotic agents, which do not cause any damage to the liver, are therefore required to treat VTE.

Activated coagulation factor Xa (FXa) is involved in both the initiation and progression of the coagulation cascade (Kubitza et al., 2014). Inhibition of FXa is therefore an effective antithrombotic mechanism. Another is reducing platelet activity as platelets provide virtually all the specificity required for procoagulant activity (Monroe et al., 2002). Seeing that cyclooxygenase-1 (COX-1) synthesizes the platelet aggregatory mediator thromboxane A_2 (TXA_2), inhibition of COX-1 will therefore reduce platelet activity (Gabrielsen et al., 2010). Research conducted in the last decade has provided evidence that plant extracts with significant and well-known antioxidant properties also potentially have antithrombotic effects (Tham et al., 2019). Nutritional antioxidants support the synthesis of prostacyclin by preventing lipid hydroperoxide-mediated inhibition of prostacyclin synthetase. Prostacyclin is one of the body's fundamental cardioprotective hormones and has a powerful antithrombotic role (McCarty, 1986; Mitchell et al., 2019).

In this study, the antithrombotic properties of ethanolic, dichloromethane (DCM), and aqueous extracts of various South African plants were evaluated. A total of 33 plant extracts from 11 plant species (Table 1) were investigated. Plants traditionally used for pain and wound healing were chosen including plants with reported anti-inflammatory and antioxidant activity. The antiproliferative effects of the plant extracts were investigated using the human hepatocellular carcinoma (HepG2) cell line which has consistent, homogenous features and has been used extensively *in vitro* hepatotoxicity testing models (Tham et al., 2019). Furthermore, the antioxidant, anti-FXa and COX-1 inhibitory activity of selected plant extracts were also investigated.

2. Methods

2.1. Materials, chemicals, and reagents

The HepG2 cell line was donated by Prof Lyn-Marie Birkholtz from the University of Pretoria. Dulbecco's modified Eagle's medium (DMEM), penicillin, streptomycin, fungizone, trypsin ethylenediaminetetraacetic acid (EDTA), fetal bovine serum and PrestoBlue™ cell viability reagent were purchased from Thermo Fisher Scientific (Johannesburg, Gauteng, South Africa). Organic solvents, Actinomycin D, Vitamin C, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Dimethyl sulfoxide (DMSO), tris(hydroxymethyl)aminomethane (Tris), NaCl, Polyethylene glycol 6000 (PEG-6000), FXa from bovine plasma and FXa chromogenic substrate were purchased from Sigma Aldrich (Modderfontein, Gauteng, South Africa). Cell culture flasks and plates were purchased from Lasec SA Pty Ltd. (Midrand, South Africa). The COX-1 Inhibitor Screening Assay Kit (ab204698, Abcam) and 1,5-Dansyl-Glu-Gly-Arg-Chloromethyl Ketone (GGACK) dihydrochloride were purchased from Biocom Africa (Pty) Ltd. (Centurion, South Africa).

2.2. Plant collection

The plants identified for the current study were collected from the University of Pretoria's Hatfield and Hillcrest campuses including a select few purchased from Random Harvest Nursery (Krugersdorp, South Africa). The plant voucher specimens were submitted to the HGWJ Schweickerdt Herbarium (PRU), Pretoria, South Africa, for identification.

2.3. Preparation of extracts

Ethanolic, dichloromethane (DCM) and aqueous extracts of the leaves and non-woody stems of each plant were prepared using methods previously described by Mativandlela et al. (2006) with slight modifications (Table 2). Succulent plants were collected and immediately used whereas the non-succulent plants were shade dried and macerated to a powder form.

2.3.1. Ethanolic and DCM extract preparation

Ethanol (99 %) and dichloromethane (DCM) were added to the powdered plant material of the non-succulent plants as well as the fresh plant material of the succulent plants at a specific plant mass to solvent volume ratio (Table 2). The succulent plant material was macerated in a blender in 99 % ethanol, whereas the succulent plant material in DCM was macerated using a mortar and pestle. This was then left for 3 days with continuous shaking and filtered through a Whatmann® filter system (11 μm pore size). The filtrate was concentrated in a rotary evaporator (Buchi, RII) under reduced pressure at

Table 1
Traditional use and pharmacological properties of selected plant species.

Plant Species	Traditional use	Pharmacological Properties	References
***Barleria obtusa* Nees**	Burns	N/A*	(Afolayan et al., 2014)
***Carpobrotus dimidiatus* (Haw.) L. Bolus**	Wound healing, burns, and toothache	Anti-inflammatory and antioxidant	(Van Wyk and Gericke, 2018; Mulaudzi et al., 2019)
***Cotyledon orbiculata* L.**	Boils, corns, warts, earache, and toothache	Anti-inflammatory	(Moteetee and Van Wyk, 2011; Maroyi, 2019)
***Cussonia spicata* Thunb.**	Abdominal pain	N/A*	(County et al., 2021)
***Heteropyxis natalensis* Harv.**	Toothaches, blood purifier, wounds, bleeding gums, and nose-bleeding	Anti-inflammatory and antioxidant	(Henley-Smith et al., 2018; Van Wyk et al., 2009; Long, 2005; Muchuweti et al., 2006; Frum and Viljoen, 2006)
***Hypoestes aristata* (Vahl) Sol. ex Roem & Schult.**	Sore eyes and sore throats	N/A*	(Hulme, 1954)
***Hypoestes forskaolii* (Vahl) R.Br.**	Wound healing and headaches	N/A*	(Beyi, 2019; Al Haidari, 2018)
***Pelargonium citronellum* J.J.A.van der Walt**	N/A*	Antioxidant	(Lalli et al., 2008)
***Pelargonium graveolens* L'Hér.**	Wound healing and sore throats	Antioxidant	(Lalli et al., 2008; Hutchings and Scott, 1996; Ali et al., 2020)
***Portulacaria afra* Jacq.**	Chronic sores and earaches	Antioxidant	(Nciki et al., 2016; Hulley and Van Wyk, 2019; (Khanyile et al., 2021)
***Sideroxylon inerme* L.**	N/A*	Antioxidant	(Shelembe et al., 2016)

* **N/A:** Not applicable.

36 °C and then frozen at −80 °C for 24 h where upon the frozen extracts were then freeze dried (Alpha 1–2 LDplus) until complete solvent evaporation has occurred.

2.3.2. Aqueous extract preparation

Hot distilled water was added to the powdered plant material of the non-succulent plants as well as the fresh plant material of the succulent plants at a specific plant mass to solvent volume ratio (Table 2). The succulent plant material was macerated with the hot water in a blender, refrigerated at 8 °C for 24 h and then left to shake continuously for a further 24 h. This was then filtered through a Whatmann® filter system (11 μm pore size) and the filtrate frozen at −80 °C. The frozen extracts were then freeze dried (Christ Alpha 1–2 LDplus) until complete solvent evaporation occurred.

2.3.3. Extract percentage yield calculation

The extract percentage yield was calculated using the following equations:

Table 2
Plant collection and extraction data.

Plant species	Succulent/ Non-succulent	Voucher specimen (PRU) number	Extraction Type	Plant mass (g): Solvent (ml)	Percentage Yield (%)
***Barleria obtusa* Nees** [a]	Non-succulent	PRU 125926	Ethanolic	1:20	2.39
			DCM	1:20	6.26
			Aqueous	1:20	16.00
***Carpobrotus dimidiatus* (Haw.) L. Bolus** [a]	Succulent	PRU 125927	Ethanolic	1:5	2.27
			DCM	1:5	0.92
			Aqueous	1:5	3.84
***Cotyledon orbiculata* L.** [b]	Succulent	PRU 128848	Ethanolic	1:10	0.85
			DCM	1:5	0.23
			Aqueous	1:5	1.23
***Cussonia spicata* Thunb.** [a]	Non-succulent	PRU 128851	Ethanolic	1:20	1.71
			DCM	1:20	6.36
			Aqueous	1:20	17.85
***Heteropyxis natalensis* Harv.** [c]	Non-succulent	PRU 096405	Ethanolic	1:20	14.22
			DCM	1:20	7.41
			Aqueous	1:20	2.45
***Hypoestes aristata* (Vahl) Sol. ex Roem & Schult.** [a]	Non-succulent	PRU 125925	Ethanolic	1:20	6.42
			DCM	1:20	19.06
			Aqueous	1:20	16.87
***Hypoestes forsakaolii* (Vahl) R.Br.** [a]	Non-succulent	PRU 127863	Ethanolic	1:20	13.48
			DCM	1:20	28.86
			Aqueous	1:20	8.25
***Pelargonium citronellum* J.J.A.van der Walt** [a]	Non-succulent	PRU 127869	Ethanolic	1:20	10.43
			DCM	1:20	7.40
			Aqueous	1:20	12.07
***Pelargonium graveolens* L'Hér** [a]	Non-succulent	PRU 128847	Ethanolic	1:20	7.89
			DCM	1:20	3.66
			Aqueous	1:40	25.23
***Portulacaria afra* Jacq.** [a]	Succulent	PRU 128849	Ethanolic	1:5	3.57
			DCM	1:5	0.53
			Aqueous	1:5	1.70
***Sideroxylon inerme* L.** [b]	Non-succulent	PRU 128850	Ethanolic	1:20	9.43
			DCM	1:20	3.20
			Aqueous	1:20	23.84

[a] Collected from University of Pretoria Hatfield Campus.
[b] Purchased from Random Harvest Nursery (Krugersdorp, South Africa).
[c] Collected from University of Pretoria, Hillcrest Campus.

(i) Non-succulent plants

$$\text{Extract percentage yield } (\%) = \frac{\text{Final extract mass (g)}}{\text{Dried plant material mass(g)}}$$

(ii) Succulent plants

$$\text{Extract percentage yield } (\%) = \frac{\text{Final extract mass (g)}}{\text{Fresh plant material mass(g)}}$$

2.4. Antiproliferative activity assay

The antiproliferative activity of all plant extracts was tested against the HepG2 cell line using a previously described cell culture based antiproliferative assay with slight modifications (Lall et al., 2016). The cells were grown in a flat-sided tissue culture flask in DMEM supplemented with 10 % foetal bovine serum and 1 % antibiotics (100 U/mL penicillin, 100 μg/mL streptomycin, 250 μg/L fungizone) at 37 °C and 5% CO_2. After the formation of a monolayer of cells, they were detached from the surface of the flask with Trypsin-EDTA (0.25 % Trypsin, 0.01 % EDTA) solution.

The cells were plated in 96-well plates to a final concentration of 1000 cells/well and incubated for 24 h at 37 °C and 5 % CO_2 to allow for adherence. The plant extracts and Actinomycin D were added to the plates in triplicate at concentrations ranging from 1.56 – 400 μg/mL and 3.91×10^{-4} – 0.05 μg/mL respectively. The plates were incubated for a further 72 h, after which PrestoBlue™ cell viability reagent was added to all the wells of the plates. The plates were further incubated for 2 h followed by the measurement of fluorescence (Ex/Em = 560/590 nm) using the Victor® Nivo™ multimode plate reader (PerkinElmer, Waltham, Massachusetts).

Actinomycin D served as the positive control, 2 % DMSO as the solvent control, DMEM as the negative control and PrestoBlue™ with DMEM and no cells served as the PrestoBlue™ control. The cell viability was determined using the following equation and the 50 % inhibitory concentration (IC_{50}) was determined using GraphPad Prism© 8 software (GraphPad Software, San Diego, California, USA).

$$\text{Percentage viability } (\%) = \frac{\text{Fluorescence Extract} - \text{Fluorescence PrestoBlue}^{TM}\text{control}}{\text{Fluorescencesolventcontrol} - \text{FluorescencePrestoBlue}^{TM}\text{control}} \times 100$$

2.5. DPPH antioxidant assay

The plant extracts that displayed an IC_{50} higher than 400 μg/mL in the antiproliferative assay were tested for their antioxidant activity using the DPPH antioxidant assay. The plant extracts were tested using a previously described method with modifications (Du Toit et al., 2001). Aqueous extracts were dissolved in distilled water, DCM extracts were dissolved in dichloromethane and ethanolic extracts were dissolved in 99 % ethanol. Vitamin C was dissolved in 99 % ethanol and served as the positive control. Ethanol (99 %) served as the blank and diluted plant extract only as the negative control. The plant extracts were tested in triplicate at concentrations ranging from 0.41 – 100 μg/mL for the *H. natalensis* aqueous extract and Vitamin C and 3.91 – 500 μg/mL for all other extracts tested. The reaction was initiated by the addition of DPPH followed by incubation for 30 min at room temperature in the dark. The DPPH antioxidant was omitted from the negative control reactions. Absorbance was read at 515 nm using a BIO-TEK®PowerWave™ XS multi-well plate reader (A.D.P., Weltevreden Park, South Africa) and KC Junior software (BIO-TEK®, Winooski, Vermount, USA). The percentage antioxidant activity was determined using the following formula below and the IC_{50} values determined using GraphPad Prism© 8 software (GraphPad Software, San Diego, California, USA).

$$\textit{Percentage antioxidant activity } (\%) = \frac{\textit{Absorbance Blank} - (\textit{Absorbance Extract} - \textit{Absorbance Negative Control})}{\textit{Absorbance Blank}} \times 100$$

2.6. Anti- Activated coagulation factor X (Anti-FXa) assay

The plant extracts that displayed an IC_{50} value lower than 20 μg/mL in the DPPH antioxidant assay, were tested for their anti-FXa activity using a previously described method with slight modifications (Chu et al., 2000). The enzyme assay was conducted in buffer (0.15 M NaCl, 0.05 M Tris, 0.1 % PEG-6000, pH 7.5) with GGACK dihydrochloride dissolved and serially diluted in buffer. Plant extracts were dissolved and serially diluted in 10 % DMSO and the FXa chromogenic substrate dissolved in buffer. Serially diluted samples were pre-incubated with FXa from bovine plasma with a final concentration of 0.425 μM, for 5 min at 37 °C in a 96-well plate. The plant extracts were tested in triplicate at final concentrations of 1.56 – 100 μg/mL for the *H. natalensis* aqueous extract and 7.8 – 500 μg/mL for all other extracts tested. 1,5-Dansyl-Glu-Gly-Arg-Chloromethyl Ketone (GGACK) dihydrochloride was tested in triplicate at final concentrations of 0.008 – 0.5 μg/mL. The buffer and 0.5 % DMSO served as the solvent controls and GGACK dihydrochloride as the positive control. The reaction was initiated by adding FXa chromogenic substrate to each well at a final concentration of 161 μM and the absorbance of the reaction read at 405 nm for 15 min at 37 °C using a BIO-TEK® PowerWave™ XS multi-well plate reader and KC Junior software. The IC_{50} values were determined using GraphPad Prism© 8 software (GraphPad Software, San Diego, California, USA).

2.7. Anti-FXa enzyme kinetics

The aqueous extract of *H. natalensis* displayed significant anti-FXa activity and was therefore subjected to further enzyme kinetic studies to determine the type of inhibition. The aqueous extract of *H. natalensis* and GGACK dihydrochloride were tested in triplicate at the previously determined IC_{50} values. The plant extract was dissolved in 10 % DMSO while GGACK dihydrochloride was dissolved in buffer solution. The buffer and 0.5 % DMSO served as solvent controls. The FXa chromogenic substrate was dissolved and serially diluted in buffer. The diluted samples were preincubated with FXa from bovine plasma for 5 min at 37 °C in a 96-well plate. The final concentration of FXa from bovine plasma in each well was 0.425 μM. The reaction was initiated by adding FXa chromogenic substrate to each well at a final concentration range of 1.26 – 161 μM. The absorbance of the reaction was read at 405 nm for 15 min at 37 °C using a BIO-TEK®PowerWave™ XS multi-well plate reader and KC Junior software. The Michaelis constant (K_m) and maximum rate of reaction (V_{max}) were determined using Michaelis-Menten analysis in GraphPad Prism© 8 software (GraphPad Software, San Diego, California, USA) including development of the kinetic graphs.

2.8. Cyclooxygenase-1 (COX-1) inhibition assay

The COX-1 inhibitory potential of the aqueous extract of *H. natalensis* which displayed pronounced anti-FXa activity was tested using the COX-1 Inhibitor Screening Assay Kit (ab204698, Abcam). The assay was conducted according to the kit instructions provided. The COX assay buffer served as the enzyme control, SC560 as the inhibitor control and 0.5 % DMSO as the solvent control. The COX reaction mixture consisting of the COX assay buffer, COX-1, COX cofactor and the COX probe was prepared and added to a 96-well plate at room temperature. The plant extract was dissolved and serially diluted in 10 %

Table 3
Antiproliferative, DPPH antioxidant and anti-FXa activity of selected plant extracts.

Sample	Extract	Antiproliferative IC_{50}[f] (μg/mL) ± SD[k]	DPPH Antioxidant IC_{50} (μg/mL) ± SD	Anti-FXa IC_{50} (μg/mL) ± SD
***Barleria obtusa* Nees**	Ethanolic	146.3 ± 0.12 [d]	N/T[l]	N/T
	DCM	297.2 ± 0.06 [d]	N/T	N/T
	Aqueous	NIA[h]	14.93 ± 0.04 [d]	NIC[j]
***Carpobrotus dimidiatus* (Haw.) L. Bolus**	Ethanolic	NIA	11.92 ± 0.02 [d]	NIC
	DCM	205.1 ± 0.04 [d]	N/T	N/T
	Aqueous	396.7 ± 0.04 [d]	N/T	N/T
***Cotyledon orbiculata* L.**	Ethanolic	NIA	41.04 ± 0.04 [d]	N/T
	DCM	61.96 ± 0.18 [d]	N/T	N/T
	Aqueous	NIA	64.86 ± 0.03 [d]	N/T
***Cussonia spicata* Thunb.**	Ethanolic	99.40 ± 0.04 [d]	N/T	N/T
	DCM	232.4 ± 0.04 [d]	N/T	N/T
	Aqueous	NIA	27.94 ± 0.03 [d]	N/T
***Heteropyxis natalensis* Harv.**	Ethanolic	72.76 ± 0.11 [d]	N/T	N/T
	DCM	132.7 ± 0.53 [d]	N/T	N/T
	Aqueous	NIA	2.71 ± 0.03 [d]	2.64 ± 1.71[e]
***Hypoestes aristata* (Vahl) Sol. ex Roem & Schult.**	Ethanolic	26.10 ± 0.02 [d]	N/T	N/T
	DCM	14.76 ± 0.03 [d]	N/T	N/T
	Aqueous	294.60 ± 0.24 [d]	N/T	N/T
***Hypoestes forsakaolii* (Vahl) R.Br.**	Ethanolic	IL[g]	N/T	N/T
	DCM	38.61 ± 0.04 [d]	N/T	N/T
	Aqueous	7.66 ± 0.18 [d]	N/T	N/T
***Pelargonium citronellum* J.J.A. Van der Walt**	Ethanolic	26.77 ± 0.05 [d]	N/T	N/T
	DCM	34.14 ± 0.04 [d]	N/T	N/T
	Aqueous	212.6 ± 0.11 [d]	N/T	N/T
***Pelargonium graveolens* L'Hér.**	Ethanolic	94.05 ± 0.12 [d]	N/T	N/T
	DCM	306.5 ± 0.62 [d]	N/T	N/T
	Aqueous	NIA	15.67 ± 0.03 [d]	NIC
***Portulacaria afra* Jacq.**	Ethanolic	NIA	28.92 ± 0.03 [d]	N/T
	DCM	327.3 ± 0.14 [d]	N/T	N/T
	Aqueous	NIA	35.51 ± 0.04 [d]	N/T
***Sideroxylon inerme* L.**	Ethanolic	151.4 ± 0.06 [d]	N/T	N/T
	DCM	NIA	NIB[i]	N/T
	Aqueous	342.2 ± 0.65 [d]	N/T	N/T
Controls				
Actinomycin D [a]	N/T	0.0006 ± 0.07	N/T	N/T
GGACK dihydrochloride [b]	N/T	N/T	N/T	0.23 ± 0.07[e]
Vitamin C[c]	N/T	N/T	2.50 ± 0.04	N/T

[a] positive control - antiproliferative assay.
[b] positive control – anti-FXa assay.
[c] positive control- DPPH antioxidant assay.
[d] p-value < 0.05 when compared to positive control (one-way ANOVA $p < 0.05$, Tukey's multiple comparison test, $n = 3$).
[e] p-value = 0.0940 when compared to positive control (Unpaired t-test with Welch's correction, $n = 3$).
[f] **IC50:** 50 % inhibitory concentration.
[g] **IL:** Inhibition lower than lowest tested concentration of 3.13 μg/mL.
[h] **NIA:** No inhibition at highest tested concentration of 400 μg/mL.
[i] **NIB:** No inhibition at highest tested concentration of 500 μg/mL.
[j] **NIC:** No inhibition at highest tested concentration of 500 μg/mL.
[k] **SD:** Standard deviation.
[l] **N/T:** Not tested.

DMSO and added to the 96-well plate. The plant extract was tested in triplicate at concentrations ranging from 1.56 – 100 μg/mL. The inhibitor control was prepared by adding 2 μL of SC560 to the COX assay buffer. The reaction was initiated by adding the arachidonic acid and NaOH (50:50) solution. The fluorescence of the reaction was read in kinetic mode for 15 min at 25 °C using the Victor® Nivo™ multimode plate reader (Ex/Em = 535/587 nm). The maximum slopes and the IC_{50} values were determined using GraphPad Prism© 8 software (GraphPad Software, San Diego, California, USA).

2.9. Statistical analysis

The IC_{50} values were determined using a 4-parameter logistic equation in GraphPad Prism© 8 software (GraphPad Software, San Diego, California, USA) with constraints on the top (100) and bottom (0) parameters. A one-way ANOVA followed by a Tukey's Multiple Comparison Test was used to determine whether the differences between the positive controls and the treatments were significant in both the antiproliferative activity and DPPH antioxidant assays. The unpaired parametric T-test with Welch's correction was used to determine whether the difference between the positive control and the treatment in the anti-FXa and COX-1 inhibition assays were significant.

3. Results and discussion

3.1. Antiproliferative activity

The antiproliferative activity of the selected plants extracts against HepG2 cells is summarized in Table 3. In general, the ethanolic and DCM extracts of all the plants had higher antiproliferative activity than their respective aqueous extracts. This trend could also be seen in a study done by Fan et al. (2020), where the ethanolic and DCM extracts of *Hagenia abyssinica* had higher antiproliferative activities with a higher inhibition rate against HepG2 cells than the aqueous extract tested. The higher inhibition values could potentially be due to alcoholic solvents such as ethanol being a better solvent when compared to water when extracting compounds from medicinal

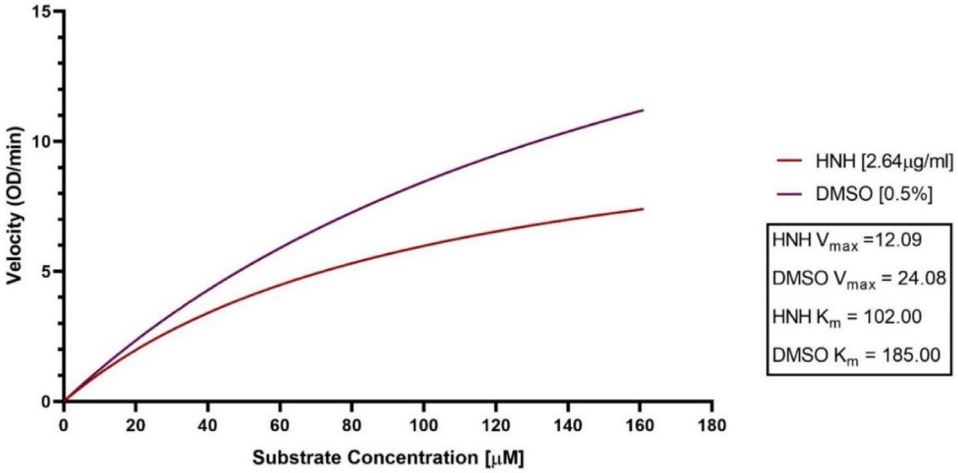

Fig. 2. Competitive inhibition of FXa by *H. natalensis*' aqueous extract (HNH). DMSO [0.5 %] curve represents enzyme at 100 % activity.

plants, this could be due to ethanol being more polar than water and extracting compounds with higher polarity than water (Gberikon et al., 2015). Whereas DCM is slightly polar, however not as polar as water and edges on being non-polar, therefor DCM can extract compounds that are below the polarity of water (Wang and Zenobi, 2010). Therefore, ethanol and DCM has the ability to include a wider variety of compounds within their extracts. The ethanolic, DCM and aqueous extracts of *Hypoestes forskaolii* displayed IC_{50} values lower than 30 μg/mL with their antiproliferative effect potentially due to the alkaloids which have previously been isolated from the plant (Abdel-sattar et al., 2020). In this same study a phenanthrol [9,10-b] quinolizidine-type alkaloid isolated from a methanolic extract of *H. forskaolii* exhibited an IC_{50} value of 10 ng/mL (24.45 nM) against MRC5 (human diploid embryonic lung) cells. For *H. forskaolii, H. aristata* and *P. citronellum*, all the solvents tested (ethanolic, DCM and aqueous) exhibited antiproliferative effects with IC_{50} values lower than 300 μg/mL (Table 3). Due to the liver being responsible for the metabolism of many important drugs currently found on the market, any cytotoxic effects as a result of the ingestion of a plant extract, on the hepatic cells may cause adverse hepatic effects when administered concurrently with these drugs (David and Hamilton, 2010). The information obtained from the current study can be of added advantage when evaluating the medicinal properties of these plants in relation to the treatment of any disease.

3.2. DPPH antioxidant activity

The antioxidant activity of the selected plant extracts is summarized in Table 3. The aqueous extract of *H. natalensis* showed the highest antioxidant activity with an IC_{50} of 2.71 ± 0.03 μg/mL which is comparable to the positive control, Vitamin C, which exhibited an IC_{50} of 2.50 ± 0.04. In a previous study, the ethanolic leaf extract of *H. natalensis* scavenged 29.7 % of the DPPH free radicals at a concentration of 1 mg/L (Muchuweti et al., 2006). Therefore, the aqueous extract in the current study appears to be more potent when compared to the ethanolic extract. Studies have determined that *H. natalensis* is rich in polyphenolic compounds with galangin (3,5,7-trihydroxyflavone) and quercetin previously isolated from the plant (Muchuweti et al., 2006; Henley-Smith et al., 2018). Both galangin and quercetin are known to have strong antioxidant effects (Bacanlı et al., 2018; Xu et al., 2019). Quercetin has also previously been isolated from *P. graveolens*, which displayed strong antioxidant activity in the current study (15.67 ± 0.03 μg/mL) for its aqueous extract (Boukhris et al., 2015). The aqueous extracts of *B. obtusa* also displayed strong antioxidant activity with an IC_{50} value of 14.93 ± 0.04 μg/mL, which may be due to the presence of carotenoids and flavonoids in this plant, compounds previously shown to exert high antioxidant activity (Musil et al., 2002; Fiedor and Květoslava, 2014). Flavonoids are also present in *C. dimidiatus* (Mulaudzi et al., 2019) which may account for the high antioxidant activity (11.92 ± 0.02 μg/mL) demonstrated by the ethanolic extract in the current study.

3.3. Anti-FXa activity and enzyme kinetics

The anti-FXa activity of the selected plant extracts is summarized in Table 3. The only plant extract that displayed noteworthy anti-FXa activity was the aqueous extract of *H. natalensis* with an IC_{50} value of 2.64 ± 1.71 μg/mL when compared to the positive control (0.23 ± 0.07 μg/mL). Further enzyme kinetic studies revealed that the V_{max} and K_m values of *H. natalensis* were lower than the V_{max} and K_m values of the 0.5 % DMSO control, indicating competitive inhibition (Fig. 2). Also, the V_{max} and K_m values of GGACK dihydrochloride were lower than the V_{max} and K_m values of the buffer, also indicating competitive inhibition (Fig. 3).

3.4. COX-1 inhibition

The COX-1 inhibitory activity for the aqueous extract of *H. natalensis* was determined to be 25.32 μg/mL. According to the product protocol, SC560 has an IC_{50} of 6.45 nM which translates to 0.002 μg/mL. Previously chalcone (E)−1-(2′,4′-dihyroxy,5′-methoxy,3′- methylphenyl)−3-phenylprop-2-en-1-one has been isolated from *H. natalensis* (Adesanwo et al., 2009). During the study conducted by Bandgar et al. (2012), chalcone derivatives from *H. natalensis* inhibited 68.47 − 79.95 % of the COX-1 activity. Therefore, the presence of chalcones present in the aqueous extract of *H, natalenisis* may account for the COX-1 inhibitory activity observed in the current study. The inhibition of COX-1 exhibited by the extract could potentially reduce TXA_2 production and prevent platelet aggregation (Crescente et al., 2019).

4. Conclusion

From all the extracts tested in the current study the aqueous extract of *H. natalensis* competitively inhibited FXa at a low concentration, indicating that the extract will prevent progression of initiation and propagation of the coagulation cascade as previously described. The extract similarly inhibited COX-1 and will therefore be able to reduce TXA_2 production and prevent platelet aggregation.

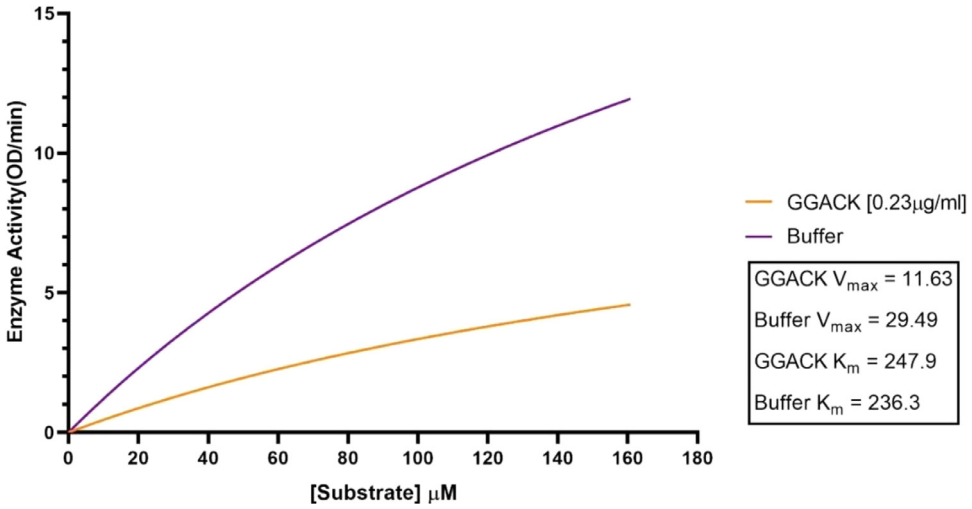

Fig. 3. Competitive inhibition of FXa by GGACK dihydrochloride (GGACK). Buffer curve represents enzyme at 100 % activity.

Furthermore, due to the low antiproliferative activity exhibited by the aqueous extract of *H. natalensis*, it can be extrapolated that the extract is not likely to cause any cytotoxic effects. However, further investigation is needed to conclude this observation, including testing the aqueous extract on several other cancerous and non-cancerous cell lines of importance. The results of the study revealed that amongst all the extracts tested, the aqueous extract of *H. natalensis* was the best candidate for further antithrombotic studies in relation to VTE. Future studies should focus on the mechanisms of inhibitory action of the aqueous extract of *H. natalensis* on both FXa and COX-1, including the further identification and isolation of compounds that may be responsible for the activity seen during the current study.

Declaration of competing interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This research was funded by the National Research Foundation of South Africa, grant number 98334. We acknowledge Mr. Jason Sampson for his assistance with plant identification.

References

Abdel-sattar, E., Abdallah, H.M., Kiyohara, H., Yamada, H., El-mekkawy, S., Ichino, C., 2020. Experimental parasitology antimalarial alkaloid from *Hypoestes forskaolii*. Exp. Parasitol. 211, 107851. https://doi.org/10.1016/j.exppara.2020.107851.

Adesanwo, J.K., Shode, F., Aiyelaagbe, O., Oyede, R.T., Baijnath, H., 2009. Isolation and characterization of a new chalcone from the leaves of *Heteropyxis natalensis*. Int. J. Med. Sci. 1, 28–32. https://www.researchgate.net/publication/242689038_Isolation_and_characterization_of_a_new_chalcone_from_the_leaves_of_Heteropyxis_ natalensis.

Afolayan, A.J., Grierson, D.S., Mbeng, W.O., 2014. Ethnobotanical survey of medicinal plants used in the management of skin disorders among the Xhosa communities of the Amathole District, Eastern Cape, South Africa. J. Ethnopharmacol. 153, 220–232. https://doi.org/10.1016/j.jep.2014.02.023.

Al Haidari, R.A., 2018. A review of traditional uses, phytochemicals and bioactivities of the genus *Hypoestes*. Afr. J. Tradit. Complement. Altern. Med. 15, 1–17. https://doi.org/10.21010/ajtcamv15i3.1.

Ali, I.B.E., Tajini, F., Boulila, A., Jebri, M.A., Boussaid, M., Messaoud, C., Sebaï, H., 2020. Bioactive compounds from Tunisian *Pelargonium graveolens* (L'Hér.) essential oils and extracts: α-amylase and acethylcholinesterase inhibitory and antioxidant, antibacterial and phytotoxic activities. Ind. Crops. Prod. 158, 112951. https://doi.org/10.1016/j.indcrop.2020.112951.

Anderson, F.A., Spencer, F.A., 2003. Risk factors for venous thromboembolism. Circ 107, 9–16. https://doi.org/10.1161/01.CIR.0000078469.07362.E6.

Arora, N., Goldhaber, S.Z., 2006. Anticoagulants and transaminase elevation. Circ 113, 698–702. https://doi.org/10.1161/CIRCULATIONAHA.105.603100.

Bacanlı, M., Başaran, A., Başaran, N., 2018. Chapter 34 - Galangin as a plant phenolic and usage in health and disease. Polyphenols: Prevention and Treatment of Human Disease, Second Ed. Academic Press, pp. 433–438. https://doi.org/10.1016/B978-0-12-813008-7.00034-5.

Bandgar, B.P., Hote, B.S., Dhole, N.A., Gacche, R.N., 2012. Synthesis and biological evaluation of novel series of chalcone derivatives as inhibitors of cyclooxygenase and LPS-induced TNF-α with potent antioxidant properties. Med. Chem. Res. 21, 2292–2299. https://doi.org/10.1007/s00044-011-9746-6.

Beyi, M.W., 2019. Ethnobotanical investigation of traditional medicinal plants in Dugda District, Oromia Region. SM J. Med. Plant. Stud. 2, 1007. https://doi.org/10.36876/smjmps.1007.

Boukhris, M., Hadrich, F., Chtourou, H., Dhouib, A., Bouaziz, M., Sayadi, S., 2015. Chemical composition, biological activities and DNA damage protective effect of *Pelargonium graveolens* L'Hér. essential oils at different phenological stages. Ind. Crops Prod. 74, 600–606. https://doi.org/10.1016/j.indcrop.2015.05.051.

Chu, V., Brown, K., Colussi, D., Choi, Y.M., Green, D., Pauls, H.W., Spada, A.P., Perrone, M.H., Leadley, R.J., Dunwiddie, C.T., 2000. *In vitro* characterization of a novel factor Xa inhibitor, RPR 130737. Thromb. Res. 99, 71–82. https://doi.org/10.1016/s0049-3848(00)00227-9.

County, M., Kigen, G., Kipkore, W., Wanjohi, B., Haruki, B., Kemboi, J., 2021. Medicinal plants used by traditional healers in Sangurur. Elgeyo. Pharmacognosy Res. 9, 333–347. https://doi.org/10.4103/pr.pr.

Crescente, M., Menke, L., Chan, M.V., Armstrong, P.C., Warner, T.D., 2019. Eicosanoids in platelets and the effect of their modulation by aspirin in the cardiovascular system (and beyond). Br. J. Pharmacol. 176, 988–999. https://doi.org/10.1111/bph.14196.

David, S., Hamilton, J.P., 2010. Drug-induced liver injury. US. Gastroenterol. Hepatol. Rev. 73–80.

du Toit, R., Volsteedt, Y., Apostolides, Z., 2001. Comparison of the antioxidant content of fruits, vegetables and teas measured as vitamin C equivalents. J. Toxicol. 166, 63–69. https://doi.org/10.1016/S0300-483X(01)00446-2.

Fan, M., Chen, G., Zhang, Y., Nahar, L., Sarker, S.D., Hu, G., Guo, M., 2020. Antioxidant and anti-proliferative properties of *Hagenia abyssinica* roots and their potentially active components. Antioxidants 9, 143. https://doi.org/10.3390/antiox9020143.

Fiedor, J., Květoslava, B., 2014. Potential role of carotenoids as antioxidants in human health and disease. Nutrients 6, 466–488. https://doi.org/10.3390/nu6020466.

Frenzel, C., Teschke, R., 2016. Herbal hepatotoxicity: clinical characteristics and listing compilation. Int. J. Mol. Sci. 17, 1–38. https://doi.org/10.3390/ijms17050588.

Frum, Y., Viljoen, A.M., 2006. *In vitro* 5-lipoxygenase activity of three indigenous South African aromatic plants used in traditional healing and the stereospecific activity of limonene in the 5-lipoxygenase assay. J. Essent. Oil Res. 18, 85–88. https://doi.org/10.1080/10412905.2006.12067127.

Gabrielsen, A., Qiu, H., Bäck, M., Hamberg, M., Hemdahl, A.L., Agardh, H., Folkersen, L., Swedenborg, J., Hedin, U., Paulsson-Berne, G., Haeggström, J.Z., Hansson, G.K., 2010. Thromboxane synthase expression and thromboxane A2 production in the atherosclerotic lesion. J. Mol. Med. 88, 795–806. https://doi.org/10.1007/s00109-010-0621-6.

Gberikon, G.M., Adeoti, I., Aondoaclaa, A.D., 2015. Effect of ethanol and aqueous solutions as extraction solvents on phytochemical screening and antibacterial activity of fruit and stem bark extracts of *Tetrapleura tetrapteraon, Streptococcus salivarus*, and *Streptococcus mutans*. Int. J. Curr. Microbiol. Appl. Sci. 4, 404–410.

Henley-Smith, C.J., Botha, F.S., Hussein, A.A., Nkomo, M., Meyer, D., Lall, N., 2018. Biological activities of *Heteropyxis natalensis* against micro-organisms involved in oral infections. Front. Pharmacol. 9, 1–9. https://doi.org/10.3389/fphar.2018.00291.

Hulley, I.M., Van Wyk, B.-E., Quantitative medicinal ethnobotany of Kannaland (Western Little Karoo, South Africa): non-homogeneity amongst villages. S. Afr. J. Bot. 122, 225–265. https://doi.org/10.1016/j.sajb.2018.03.014.

Hulme, M.M., 1954. Wildflowers of Natal. Shuter and Shooter, Pietermaritzburg.

Hutchings, A., Scott, A.H., 1996. Zulu Medicinal Plants: an Inventory. University of Natal Press, Pietermaritzburg, South Africa.

Jain, C., Khatana, S., Vijayvergia, R., 2019. Bioactivity of secondary metabolites of various plants: a review. Int. J. Pharm. Sci. Res. 10, 494–504. https://doi.org/10.13040/IJPSR.0975-8232.10(2).494-04.

Kamal, A.H., Tefferi, A., Pruthi, R.K., 2007. How to interpret and pursue an abnormal prothrombin time, activated partial thromboplastin time, and bleeding time in adults. Mayo Clin. Proc. 82 (7), 864–873. https://doi.org/10.4065/82.7.864.

Khanyile, A., Maliehe, T., Shandu, J.S., Khan, R., 2021. *In vitro* antibacterial, antioxidant, anti-quorum sensing and cytotoxic properties of *Portulacaria afra* leave extract. Biosci. Res. 18, 455–463. https://www.researchgate.net/publication/345671969_In_vitro_antibacterial_antioxidant_anti-quorum_sensing_and_cytotoxic_properties_of_Portulacaria_afra_leave_extract.

Klein, R., 2009. Evidence for immunological (allergic) mechanisms in a subgroup of patients with phenprocoumon-induced liver disease. Eur. J. Clin. Pharmacol. 65, 1195–1201. https://doi.org/10.1007/s00228-009-0705-9.

Kubitza, D., Becka, M., Mück, W., Krätzschmar, J., 2014. Pharmacodynamics and pharmacokinetics during the transition from warfarin to rivaroxaban: a randomized study in healthy subjects. Br. J. Clin. Pharmacol. 78, 353–363. https://doi.org/10.1111/bcp.12349.

Lall, N., Kumar, V., Meyer, D., Gasa, N., Hamilton, C., Matsabisa, M., Oosthuizen, C., 2016. *In vitro* and *in vivo* antimycobacterial, hepatoprotective and immunomodulatory activity of *Euclea natalensis* and its mode of action. J. Ethnopharmacol. 194, 740–748. https://doi.org/10.1016/j.jep.2016.10.060.

Lalli, J.Y.Y., Van Zyl, R.L., Van Vuuren, S.F., Viljoen, A.M., 2008. *In vitro* biological activities of South African *Pelargonium* (Geraniaceae) species. S. Afr. J. Bot. 74, 153–157. https://doi.org/10.1016/j.sajb.2007.08.011.

Long, C., 2005. Eswatini's Flora - siSwati names and uses, Eswatini National Trust Commission. http://eswatininaturereserves.com/flora/clbotalpha.asp?l=h&pg=2 (accessed February 22, 2021).

Mackman, N., 2008. Triggers, targets and treatments for thrombosis. Nature 451, 914–918. https://doi.org/10.1038/nature06797.Triggers.

Maroyi, A., 2019. Phytochemical and ethnopharmacological review of *Heteropyxis natalensis*. Asian J. Pharm. Clin. Res. 12, 8–15. https://doi.org/10.22159/ajpcr.2019.v12i3.29375.

Mativandlela, S.P.N., Lall, N., Meyer, J.J.M., 2006. Antibacterial, antifungal and antitubercular activity of (the roots of) *Pelargonium reniforme* (CURT) and *Pelargonium sidoides* (DC) (Geraniaceae) root extracts. S. Afr. J. Bot. 72, 232–237.

McCarty, M., 1986. An antithrombotic role for nutritional antioxidants: implications for tumor metastasis and other pathologies. Med. Hypotheses. 19, 345–357. https://doi.org/10.1016/0306-9877(86)90109-x.

Mitchell, J.A., Shala, F., Elghazouli, Y., Warner, T.D., Gaston-Massuet, C., Crescente, M., Armstrong, P.C., Herschman, H.R., Kirkby, N.S., 2019. Cell-specific gene deletion reveals the antithrombotic function of COX1 and explains the vascular COX1/prostacyclin paradox. Circ. Res. 125, 847–854. https://doi.org/10.1161/CIRCRESAHA.119.314927.

Monroe, D.M., Hoffman, M., Roberts, H.R., 2002. Platelets and thrombin generation. Arterioscler. Thromb. Vasc. Biol. 22, 1381–1389. https://doi.org/10.1161/01.ATV.0000031340.68494.34.

Moteetee, A., Van Wyk, B.-E., 2011. The medical ethnobotany of Lesotho: a review. Afr. Biodivers. Conserv. 41, 209–228. https://doi.org/10.4102/abc.v41i1.52.

Muchuweti, M., Nyamukonda, L., Chagonda, L.S., Ndhlala, A.R., Mupure, C., Benhura, M., 2006. Total phenolic content and antioxidant activity in selected medicinal plants of Zimbabwe. Int. J. Food Sci. Tech. 41, 33–38. https://doi.org/10.1111/j.1365-2621.2006.01258.x.

Mulaudzi, R.B., Aremu, A.O., Rengasamy, K.R.R., Adebayo, S.A., McGaw, L.J., Amoo, S.O., Van Staden, J., Du Plooy, C.P., 2019. Antidiabetic, anti-inflammatory, anticholinesterase and cytotoxicity determination of two *Carpobrotus* species. S. Afr. J. Bot. 125, 142–148. https://doi.org/10.1016/j.sajb.2019.07.007.

Musil, C.F., Chimphango, S.B.M., Dakora, F.D., 2002. Effects of elevated ultraviolet-B radiation on native and cultivated plants of southern Africa. Ann. Bot. 90, 127–137. https://doi.org/10.1093/aob/mcf156.

Nciki, S., Vuuren, S., Van Eyk, A., De Wet, H., 2016. Plants used to treat skin diseases in northern Maputaland, South Africa: antimicrobial activity and *in vitro* permeability studies. Pharm. Biol. 54, 2420–2436. https://doi.org/10.3109/13880209.2016.1158287.

Tarbox, A.K., Swaroop, M., 2013. Pulmonary embolism. Int. J. Crit. Illn. Inj. Sci. 3, 69–72. https://doi.org/10.4103/2229-5151.109427.

Raskob, G.E., Angchaisuksiri, P., Blanco, A.N., Buller, H., Gallus, A., Hunt, B.J., Hylek, E.M., Kakkar, A., Konstantinides, S.V., McCumber, M., Ozaki, Y., Wendelboe, A., Weitz, J.I., 2014. Thrombosis: a major contributor to global disease burden. Arterioscler. Thromb. Vasc. Biol. 34, 2363–2371. https://doi.org/10.1111/jth.12698.

Shelembe, B., Moodley, R., Jonnalagadda, S.B., 2016. Secondary metabolites isolated from two medicinal plant species, *Bridelia micrantha* and *Sideroxylon inerme* and their antioxidant activities. Acta Pol. Pharm. 73, 1249–1257. https://www.researchgate.net/publication/309403257_Secondary_metabolites_isolated_from_two_medicinal_plant_species_Bridelia_micrantha_and_Sideroxylon_inerme_and_their_antioxidant_activities.

Subramaniam, S., Khan, H.B.H., Elumalai, N., Lakshmi, S.Y.S., 2015. Hepatoprotective effect of ethanolic extract of whole plant of *Andrographis paniculata* against CCl_4-induced hepatotoxicity in rats. Comp. Clin. Path. 24, 1245–1251. https://doi.org/10.1007/s00580-015-2067-2.

Tham, T.T., Hwang, S., Bang, J., Yi, H., Park, Y., Kang, S., Kang, H., Kim, Y., Ku, H., 2019. High-content analysis of *in vitro* hepatocyte injury induced by various hepatotoxicants. J. Vet. Sci. 20, 34–42. https://doi.org/10.4142/2Fjvs.2019.20.1.34.

Van Wyk, B.-E., Gericke, N., 2018. People's plants: A guide to Useful Plants of Southern Africa. Briza Publications, Pretoria.

Van Wyk, B.-E., van Oudtshoorn, B., Gericke, N., 2009. Medicinal Plants of South Africa. Briza Publications, Pretoria. second ed.

Wang, R., Zenobi, R., 2010. Evolution of the solvent polarity in an electrospray plume. J. Am. Soc. Mass Spectrom. 21, 378–385. https://doi.org/10.1016/j.jasms.2009.10.022.

World Heart Report (WHR), 2023. World Heart Report 2023: Confronting the World's Number One Killer. World Heart Federation, Geneva, Switzerland.

Xu, D., Hu, M.J., Wang, Y.Q., Cui, Y.L., 2019. Antioxidant activities of quercetin and its complexes for medicinal application. Molecules 24, 1123. https://doi.org/10.3390/molecules24061123.