ISSN 0974 – 5211

Journal of Natural Products
Volume 4 (2011)
www.JournalofNaturalProducts.Com

Research Paper

In Vitro Anti-platelet Aggregation, Antioxidant and Cytotoxic Activity of Extracts of Some Zulu Medicinal Plants

[1]Mosa, R.A., [1]Lazarus, G.G., [1]Gwala, P.E., [2]Oyedeji, A.O., [1]*Opoku, A.R.
[1]Department of Biochemistry and Microbiology, University of Zululand, Private Bag X1001, KwaDlangezwa, 3886;
[2]Department of Chemistry, Walter Sisulu University, Private Bag X1, Mthata 5117, Republic of South Africa
* Corresponding Author
(Received 26 April 2011; Revised 05- 31 May 2011; Accepted 04 June 2011)

ABSTRACT

Three plants (*Protorhus longifolia, Bulbine natalensis, Rapanea melanophloeos*) commonly used by Zulu Traditional Healers to treat blood-clotting related diseases were screened for anti-platelet aggregation, antioxidant and cytotoxic activities. Extracts of air-dried and powdered plant materials showed, to varying degrees of efficacy, antioxidant activity as they scavenged DPPH and ABTS radicals, and exhibited Fe^{2+} chelating activities. The anti-platelet aggregation activity of the extracts was separately investigated on thrombin, ADP and epinephrine induced rat platelet aggregation. The extracts exhibited a concentration dependent anti-platelet aggregation activity. The highest activity, exhibited by the hexane extract of *P. longifolia* (IC_{50} of 0.59mg/ml) and the chloroform extract of *B. natalensis* (IC_{50} of 0.43mg/ml) was observed on the thrombin and epinephrine induced platelet aggregation, respectively. All the extracts showed little cytotoxic effects on brine shrimp. Thus *Protorhus longifolia, Bulbine natalensis, Rapanea melanophloeos* can be used in the management of blood-clotting related diseases.

Keywords: Zulu plants; Antioxidant; Anti-platelet aggregation.

INTRODUCTION

Platelets play a central role in the process of blood clotting and are regarded as key regulators of both haemostasis and pathogenesis of cardiovascular diseases (Bakdash and William, 2008; Xiang, et al., 2008; Fabre and Gurney, 2010). It is therefore important to prevent platelet dysfunctions that could lead to cardiovascular events. Despite the progress made in finding better and effective anti-platelet aggregation agents, cardiovascular diseases are still responsible for a large number of deaths and morbidities. Mode of action and efficacy of the current anti-platelet agents are still clinically doubtful and they are associated with the risk of severe or fatal bleeding (Hsieh, et al., 2007; Fabre and Gurney, 2010). This has fuelled the search for alternative medicine. This trend is a growing interest in finding safer and effective

new natural anti-platelet agents (Amrani, et al., 2009). Besides other traditional medicinal uses, ethnobotanical survey revealed that *P. longifolia, B. natalensis, R. melanophloeos* have been used and recommended by most Zulu traditional healers for management of blood-clotting related diseases (Personal communication and questionnaires, 2008).

Protorhus longifolia (Benrh.) Engl. (Red beech) of *Anacardiaceae* family is an ever green indigenous tall tree. The bark of *P. longifolia* has been traditionally used to cure various diseases such as heartwater and diarrhea in cows (Dold and Cocks, 2001), hemiplegic paralysis, heart burn, and bleeding from the stomach; unspecified parts have been used to strengthen the heart (Hutchings, et al., 1996). The leaves extracts of *P. longifolia* have been reported to possess antimicrobial activity (Suleiman, et al., 2009; 2010). A 10.2 – 18% tanning material and 7% tannin from the bark of *P. longifolia* has been reported (Hutchings, et al., 1996).

Rapanea melanophloeos (L.) Mez (Cape beech) of *Myrsinaceae* family is an evergreen tall tree. The grey bark or sometimes roots are used medicinally for respiratory problems, stomach, muscular and heart complaints. It is used as *iNtelezi* (a charm to protect against evil spirits) by Nguni people (Van Wyk and Van Wyk, 1997) *R. Melanophloeos* has been reported to have molluscicidal and antifungal (Ohtan, et al, 1992) and anthelmintic activity (Githiori, et al, 2002).

Bulbine natalensis Baker (*Asphodelaceae*) is a frost tender evergreen perennial plant. The leaves are used for treating wounds, burns, rashes, itches, ringworm, cracked lips (Pujol, 1990; Rood, 1994; Watt and Breyer-Brandwijk, 1962) and herpes. Extracts of the roots are taken orally to ease vomiting and diarrhea (Pujol, 1990) and also to treat convulsions, venereal disease, diabetes, rheumatism, urinary complaints and blood disorders (Pujol, 1990; Rood, 1994; Watt and Breyer-Brandwijk, 1962).

In this study, we investigate the anti-platelet aggregation, antioxidant and cytotoxic activity of these three plants.

MATERIALS AND METHODS

Chemicals and reagents: Unless otherwise stated, all chemicals and reagents were obtained from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). All the chemicals used including the solvents, were of analytical grade.

***Animals*:** Adult rats (*Sprague-Dawley)* of either sex were collected from the animal house in the Department of Biochemistry and Microbiology, University of Zululand. The animals were maintained under standard conditions (temperature 23 ± 2^{o}C and 12h light dark cycle); had free access to standard pellet feed and enough drinking water. Approval for experimental procedures was obtained from Research Animal Ethics Committee, University of Zululand.

Plant collection: *Bulbine natalensis* Baker was obtained from Twin Stream Nursery, Mtunzini, SA. The other plants (*Protorhus longifolia* (Benrh.) Engl., and *Rapanea melanophloeos* (L.) Mez were collected from Empangeni *muthi* market. The plants were identified and confirmed by L Ntuli, Botany Department, University of Zululand and voucher specimens (RA01UZ, PE01UZ and GGL01 respectively) were prepared and deposited in the University herbarium. The leaves of *B. natalensis*, the bark of *P. longifolia* and the bark of *R. melanophloeos* were thoroughly washed with tap water and then air dried. The air dried plant materials were ground into powder (2mm mesh) and stored in sterile brown bottles at 4°C until used.

Phytochemical screening: The powdered plant materials were screened for the following phytochemicals: saponins, tannins, flavonoids, alkaloids, terpenoids,

cardiac glycosides, steroids, phlobatannins and anthraquinones using standard procedures of Harborne (1973); Odebiyi and Sofowara (1978) and Sofowara (1984).

***Extraction*:** The powdered plant materials were separately extracted (1:5w/v) sequentially with hexane (HE), chloroform (CE), ethyl acetate (EAE), methanol (ME) and water (WE) (24h on platform shaker per solvent, 150 rpm, room temperature). The extracts obtained after filtrations were concentrated under reduced pressure using rotary evaporator (40 ± 2^{o}C); the water extracts were freeze-dried and were stored in sterile bottles at 4^{o}C.

***Determination of total phenolic content*:** Phenolic content of extracts was determined by the Folin-Ciocalteu reagent method (Kähkönen, et al., 1999). Various concentrations of gallic acid (0.01- 0.1mg/ml diethyl ether) and 0.5mg/ml diethyl ether of each extract were prepared. The diethyl ether was evaporated off and to the separate residues 1.5ml of Folin-Ciocalteu reagent (10%) and 1.2ml of sodium carbonate solution (7.5%) were added. The samples were thoroughly mixed and stored in the dark for 30 min. Absorbance of the blue colored mixtures was recorded at 765nm against a blank containing the Folin-Ciocalteu reagent and sodium carbonate solution. The total phenolic content of the extracts was calculated as gallic acid equivalent and expressed as mg/g dry plant material.

***Flavonoid content*:** The method reported by OrdonEz, et al., (2006) was adapted to determine the flavonoid content of the plant extracts. Different concentrations of quercetin (0.01- 0.1mg/ml diethyl ether) and 0.5mg/ml diethyl ether for each extract were prepared. The diethyl ether was evaporated off and to each residue 0.5ml of 2% alcoholic $AlCl_3$ solution (prepared with 80% ethanol) was added. The samples were thoroughly mixed and allowed to stand for 1h at room temperature. Absorbance of yellow colored mixture was read at 420nm against a blank containing 2% $AlCl_3$ solution. The flavonoid content of the extracts was determined as quercetin equivalent and expressed as mg/g dry plant material.

Antioxidant activity

***DPPH radical scavenging assay*:** The DPPH radical scavenging activity of the extracts was investigated using the method described by Brad-Williams (1995). Two milliliters of the crude extract (0-5mg/100ml CH_3OH) were added to 2ml of DPPH (2mg/100ml CH_3OH). The mixture was allowed to stand for 30-60 min with intermittent mixing. Absorbance was measured at 517nm against CH_3OH as blank.

***ABTS radical scavenging assay*:** The method reported by Re, et al., (1999) was used to determine the ABTS radical scavenging activity. ABTS (7mM) was prepared and mixed with 2.45mM potassium persulfate. The mixture was incubated at room temperature in the dark for 16h. The generated ABTS radicals were diluted with methanol (1ml $ABTS^+$: 60ml CH_3OH). One milliliter of the crude extract (0-5mg/100ml CH_3OH) was added to 1ml of $ABTS^+$ and the samples were thoroughly mixed. After 6 min the absorbance at 734nm was read against CH_3OH as blank.

***Reducing power*:** The reducing power of the plant extracts was determined by the method described by Oyaizu (1986). One milliliter of the crude extract (0-5mg/100ml CH_3OH) was mixed with 2.5ml of phosphate buffer (pH 6.6) and 2.5ml of 1% potassium ferricyanide. After 20 min incubation at 50^{o}C, 2.5ml of 10% trichloroacetic acid (TCA) was added. After 5-10 min the mixture was centrifuged (1000 *x g;* 10 min) and 2.5ml supernatant was collected and diluted with 2.5ml distilled water. The sample was mixed with 0.5ml of 0.1% $FeCl_3$ and the absorbance was measured at 700nm. The higher the absorbance reading is the higher the reducing power.

***Chelating activity on Fe^{2+}*:** The metal ion chelating activity of the plant extracts was

tested using the method reported by Decker and Welch (1990). One milliliter of the plant extract (0-5mg/100 ml CH_3OH) was diluted with 3.75ml deionized water. This was mixed with 0.1ml of 2mM $FeCl_2$ and 0.2ml of 5mM 4,4[1]-[3-(2-pyridinyl)-1,2,4-triazine-5,6-dryl] bisbenzene sulphonic acid (ferrozine). After 10 min the absorbance was read at 562nm. Ethylenediaminetetra-acetic acid (EDTA) and citric acid (CA) were used as standards.

Unless otherwise stated commercial antioxidants butylated hydroxytoluene (BHT) and ascorbic acid (AA) were used as standards. The experiment was replicated thrice and the mean ± SEM reported. Percentage inhibition of each parameter measured was calculated from the formula:

$$\% \text{ inhibition} = (1 - A_t/A_0)100$$

- Where A_t is the absorbance of test sample, A_0 is the absorbance of the fully oxidized system sample.
- IC_{50} values were determined using statistical package Origin 6.1.

Brine shrimp lethality bioassay: Cytotoxicity of the extracts was determined following the method described by Meyer et al., (1982) with some modification. Brine shrimp (Artemia salina) eggs were hatched in a 5L bucket half-filled with artificial sea water (3% sea salt). Two grams of brine shrimp eggs were added to 2.5L artificial sea water at 24-28^{o}C. The bucket was covered in black on the sides, light was provided from one source and oxygen was also provided using a million air devices. After 48h shrimp larvae were hatched.

To 50µl of different concentrations of the plant extracts (1- 25mg/ml prepared in 1% DMSO) in different petri dishes set in triplicates, 25ml of artificial sea water was added along with 10 shrimp larvae. Artificial sea water medium containing 1% DMSO was used as control. After 24h the number of surviving and dead shrimps was counted. Percentage mortality rate was determined. In the case of mortality in the control, the corrected percentage mortality was calculated using Abbott's formula (Abbot, 1925). The resulting data was transformed to probit analysis (Probit Program Version 1.5, USA) to determine LC_{50} values of the extracts.

Anti-platelet aggregation activity

Blood platelets: The method of Tomita et al., (1983) was followed to obtain platelets. A rat was killed by a blow to the head and blood was immediately collected from abdominal aorta. The blood was mixed (5:1 v/v) with anticoagulant (acid-dextrose-anticoagulant-0.085M trisodium citrate, 0.065M citric acid, 2% dextrose). The platelets were obtained by a series of centrifugation and washing of the blood. The platelets were finally suspended in a buffer (pH 7.4; containing 0.14M NaCl, 15mM Tris-HCl, 5mM glucose). The platelets were then divided into untreated and enzyme (trypsin, bromelain, and papain) treated platelets.

***Enzyme treated platelets*:** The enzymes-trypsin, bromelain, papain (0.1mg) were separately incubated with 25ml of a 2% suspension of platelets for 60 min at 25ºC. The cells were then washed (3 times) and a 2% suspension prepared.

***Anti-platelet aggregation activity*:** The method of Mekhfi et al., (2004) was followed with some modifications**.** The anti-platelet aggregation activity of the extracts was separately tested on thrombin (5U/ml), ADP (5mM) and epinephrine (10mM) induced platelet aggregation; similar experiments were also carried out on enzyme (trypsin, bromelain and papain) treated platelets. The platelets (100µl) were incubated for 5 min with different concentrations of the crude extracts (1, 3, and 10mg/ml) and an aggregation inducer (20µl) was introduced to the mixtures. Aggregation was determined with the Biotek plate reader using Gen5 software by following change in absorbance at 415nm. DMSO (1%) was used as negative control and heparin was used as positive control.

***Tannin Removal*:** The method described by Toth and Pavia (2001) was adapted to remove tannins from the crude extracts. The extracts were separately mixed (10mg/ml) with polyvinylpolypyrrolidone (PVPP) and the mixtures were incubated for 15 min and centrifuged (3000xg; 10min) several times to reduce tannin to a negligible concentration. The tannin-free extracts were screened for the anti-platelet aggregation activity by the method of Mekhfi, et al., (2004), described above.

Calculation of percentage inhibitory effect of plant extracts on platelet aggregation,

All assays were replicated three times and the mean slope (A) ± SEM reported.

Unless otherwise indicated, the inhibitory effect of the extract on each parameter was calculated as:

$$\% \text{ Inhibition} = \{(A_0 - A_1)/A_0 \times 100\}$$

- Where, A_0 is the mean slope of control and A_1 is the mean slope of the extract.
- IC_{50} values were determined using statistical package Origin 6.1.

RESULTS

***Phytochemical screening*:** The powdered plant materials were qualitatively screened for common phytochemicals. The results are shown in Table-1. The results revealed the presence of most of the phytochemicals that were screened for. The plant materials lacked steroids and the presence of flavonoids, tannins and alkaloids was worth noting.

***Total phenolic and flavonoid content*:** Total amount of phenols and flavonoids in the extracts (determined as gallic acid and quercetin equivalent, respectively) are presented in Table-2. Except *R. melanophloeos* and water extract of *P. longifolia*, the total phenolic and flavonoid content of *P. longifolia* and *B. natalensis* generally seemed to decrease with increase in polarity. The two groups of compounds were extracted more by the non-polar solvents.

***Antioxidant activity*:** The results of the free radical (DPPH and ABTS) scavenging activity and chelating activity on Fe^{2+} are given in Table-3. The reducing powers were also tested. The extracts exhibited to varying degrees of efficacy, a concentration dependent reduction potential, chelating activity on Fe^{2+}, DPPH and ABTS scavenging activities. The highest radical scavenging activity (IC_{50} 0.07mg/ml) was observed in the methanol extract of *P. longifolia* and the ethyl acetate extract of *R. melanophloeos* also showed the highest reduction potential and chelating activity on Fe^{2+} (IC_{50} 0.93mg/ml). The IC_{50} values were even less than or comparable to those of the standards used.

***Brine shrimp lethality bioassay*:** The cytotoxic activity of the plants extracts was determined using brine shrimp lethality test. The cytotoxicity levels (IC_{50} µg/ml) ranged from 2 210 to 54 700µg/ml. The LC_{50} (µg/ml) values of the extracts of *B. natalensis*, *P. longifolia* and *R. melanophloeos* were as low as 2 210, 36 700 and 41 580 respectively.

***Anti-platelet aggregation activity*:** The anti-platelet aggregation activity of the extracts (with tannins and without tannins) is presented in Table-4 and Table-5. Most of the extracts showed a concentration dependent inhibitory activity on the aggregation induced by thrombin, ADP and epinephrine. The activity of the extracts was comparable to that of heparin (IC_{50} 7.4 mg/ml), a commercial anticoagulant. Most extracts showed anti-platelet aggregation activity in the presence of tannins but the activity was lost or reduced when tannins were removed.

DISCUSSION

Despite the availability of the current therapies to prevent platelet dysfunctions, atherothrombotic diseases continue to pose a threat to human health. The search for

new and more effective anti-platelet aggregation agents of natural origin is rather timely and appropriate. Literature reports that plants such as *Urtica dioica* (El Haouari, et al., 2006), *Ocimum basilicum* (Amrani, et al., 2009) and *Nepeta juncea* (Hussain, et al., 2009) have anti-platelet aggregation activity.

The results obtained from this study suggest that the medicinal plants (*P. longifolia, R. melanophloeos* and *B. natalensis* commonly used by Zulu Traditional Healers to manage blood-clotting related diseases) possess the ability to inhibit thrombin, ADP and epinephrine induced aggregation of platelets (Tables- 4).

Free radicals have been reported to stimulate platelet aggregation by interfering with several key steps of platelet functions (Ambrosio, et al., 1997; Bakdash and Williams, 2008). The beneficial effects of antioxidants on the inhibition of platelet activation and aggregation have also been reported (Krotz, et al., 2004; Sobotkvá, et al., 2009). Furthermore, researchers (Mary, et al., 2003; Lin and Hsieh, 2010; Anjana, et al., 2010) have linked the anti-platelet aggregation activity of some plants with their antioxidant activity. However, except the extracts of *R. melanophloeos* that showed some antioxidant activity, the extracts of *P. longifolia and B. natalensis* that exhibited anti-platelet activity were poor antioxidants (Table-3). It is apparent from our results that the anti-platelet aggregation activity of the plants in this study may not necessarily be attributed to their antioxidant activity.

The anticoagulant or anti-platelet aggregation activity of tannins has been demonstrated by various researchers (Dong, et al., 1998; Mekhfi, et al., 2006; Kee, et al., 2008; Tognolini, et al., 2006; Kim and Choi, 2008). The phytochemical composition of the plants, particularly the presence of polyphenolic compounds (Table-1 and Table-2), is therefore worth noting. The anti-platelet aggregation activity was observed mainly in the extracts of the non-polar solvents with the hexane and chloroform extracts of *P. longifolia* and *B. natalensis,* respectively, showing the highest activity (Table-4). Thus the anti-platelet aggregation activity of the extracts could partly be attributed to their relatively high phenolic and/or flavonoid content (Table-2). However, the tannin-free extracts maintained activity. It is apparent that the observed activity of the extracts was due to components other than tannin. The reduced activity of the tannin-free extracts (Table-4) could be an indication of synergistic effect of the plant components.

The proteolytic enzymes (trypsin, bromelain and papain) catalytically hydrolyze proteins (fibrinogen) into smaller fragments (fibrin monomers) with generation of new functional groups. The formation and exposure of the new functional groups stimulates aggregation. The degree to which the extracts inhibited the aggregation of the enzyme-treated platelets (Table-5) does suggest that the extracts may not only be inhibiting thrombin and the other platelet agonists, but may also be preventing aggregation of degraded platelets. However, the platelets' loss of sensitivity to the agonists cannot be ruled out (Vellini, et al., 1986; Metzig, et al., 1999).

Extracts with LC_{50} values less than 250µg/ml are considered significantly active (Rieser, et al., 1996). The extracts of the plants showed relatively weak cytotoxic effects on brine shrimp nauplii. The insignificant toxicity of the plants extracts encourages their use in the concoctions that manage blood-clotting related diseases.

CONCLUSION

In conclusion, the results from this study suggest that *Protorhus longifolia, Rapanea melanophloeos* and *Bulbine natalensis* have compounds that exhibit anti-platelet aggregation activity. The poor antioxidant activity exhibited by some of the most

active extracts suggests that mechanisms other than antioxidant activity could be mediating their anti-platelet aggregation activity. These results support the use of the plants in folk medicine to manage blood-clotting related diseases. Further studies are necessary to isolate and characterize the active components of the extracts and also to elucidate their mechanisms of action in preventing platelet aggregation.

Acknowledgements: The authors are grateful to Medical Research Council (MRC) and the University of Zululand Research Committee for funding this study.

REFERENCES

Abbot, W.S., (1925): A method of computing the effectiveness of an insecticide. *J.Econ. Entomol.,* 18: 265-267.

Ambrosio, G., Tritto, I., Golino, P., (1997): Reactive oxygen metabolites and arterial thrombosis. *Cardiovascular Res.,* 34: 445-452.

Amrani, S., Harnafi, H., Gadi, D., Mekhfi, H., Legssyer, A., Aziz, M., Martin-Nizard, F., Bosca, L. (2009): Vasorelaxant and anti-platelet aggregation effects of aqueous *Ocimum basilicum* extracts. *J. Ethnopharmacol.,* 125: 157-162.

Anjana, G.V., Krishna, K., Joginder, D., Karan, P., Minakshi, B., Chander, S.R., (2010): Inhibitory effect of Seabuckthorn (*Hippophea rhamnoides*) on platelet aggregation and oxidative stress. *J. Complem. Integrative Medi.,* 7: DOI: 10.2202/1553-3840.1295:http://www.bepress.com/jcim/vol7/iss1/7.

Bakdash, N., Williams, M.S., (2008): Spatially distinct production of reactive oxygen species regulates platelet activation. *Free Radical Biology and Medicine,* 45:158-166.

Brad-Williams, W., (1995): Use of a free radical method to evaluate antioxidant activity. *Food Science Technology (London),* 28: 25-30.

Decker, E.A., Welch, B., (1990): Role of ferritin as a lipid oxidation catalyst in muscle food. *J Agric Food Chem*., 38: 674-677.

Dold, A.P., Cocks, M.L., (2001): Traditional veterinary medicine in the Alice district of the Eastern Cape Province, South Africa. *South African Journal of Science,* 97: 375-379.

Dong, H., Chen, S., Kini, R.M., Xu, H., (1998): Effects of tannins from *Geum japonicum* on the catalytic activity of thrombin and factor Xa of blood coagulation cascade. *J. Nat. Prod.,* 61: 1356-1360.

El Haouari, M., Bnouham, M., Bendahou, M., Aziz, M., Ziyyat, A., Legssyer, A., Mekhfi, H., (2006): Inhibition of rat platelet aggregation by *Urtica dioica* leaves extracts. *Phytotherapy Research,* 20: 568-572.

Fabre, J., Gurney, M.E., (2010): Limitations of current therapies to prevent thrombosis: a need for novel strategies. *Mol. bioSyst.,* 6: 305-315.

Githiori, B. J., H□glund, J., Waller, P.J., Baker, L.R., (2002): Anthelmintic activity of preparations derived from *Myrsine Africana* and *Rapanea melanophloeos* against the nematode parasite, *Haemonchus contortus,* of sheep. *J. Ethnopharmacol.,* 80: 187-191.

Harborne, J.B., (1973): Phytochemical methods. Chapman and Hall, Ltd, London, pp. 49-188.

Hsieh, P.W., Huang, T.L., Wu, C.C., Chiang, S.Z., Wu, C.I., Wu, Y.C., (2007): The evaluation and structure-activity relationships of 2-benzoylaminobenzoic esters and their analogues as anti-inflammatory and antiplatelet aggregation agents. *Bioorganic & Medicinal Chemistry Letters,* 17: 1812-1817.

Hussain, J., Jamila, N., Gilani, S.A., Abbas, G., Ahmed, S., (2009): Platelet aggregation, antiglycation, cytotoxic, phytotoxic and antimicrobial activities of extracts of *Nepeta juncea*. *African J. Biotechnol.,* 8: 935-940.

Hutchings, A., Scott, A.H., Lewis, G., Cunningham, A., (1996): Zulu Medicinal Plants: An Inventory. University of Natal Press, Pietermaritzburg.

Kähkönen, M.P., Hopia, A.I., Vuorela, H.J., Rauha, J.P., Pihlaja, K., Kujala, T.S., Heinonen, M., (1999): Antioxidant Activity of Plant Extracts Containing Phenolic Compounds. *J. Agric. Food Chem.,* 47: 3954–3962.

Kee, N.L., Mnonopi, N., Davids, H., Naudé, R.J., Frost, C.L., (2008): Antithrombotic/anticoagulant and anticancer activities of selected medicinal plants of *South Africa. African J. Biotechnol.*, 7:217-223.

Kim, J.M., Choi, H.S.Y., (2008): Antiplatelet effect of flavonoid and flavonoid-glycosides from *Sophora japonica*. *Archives of Pharmacal Research*, 31: 886-890.

Krotz, F., Sohn, H.Y., Pohl, U., (2004): Reactive oxygen species: players in the platelet game, *Arterioscler. Thromb. Vasc. Biol*., 24: 1988–1996.

Lin, T.H., Hsieh, C.L., (2010): Pharmacological effects of *Salvia miltiorrhiza* (*Danshen*) on cerebral infarction. *Chinese Medicine*, 5: http://www.cmjournal.org/content/5/1/22.

Mary, N.K., Achuthan, C.R., Babu, B.H., Padikkala, J., (2003): *In vitro* antioxidant and antithrombotic activity of *Hemidesmus indicus* (L). *J. Ethnopharmacol.*, 87: 187-191.

Mekhfi, M., El Haouari, M., Bnouham, M., Aziz, M., Ziyyat, A., Legssyer, A., (2006): Effects of extracts and tannins from *Arbutus unedo* leaves on rat platelet aggregation. *Phytother. Res.*, 20: 135–139.

Metzig, C., Grabowska, E., Eckert, K., Rehse, K., Maurer, H.R., (1999): Adhesion to bovine endothelial cells and thrombus formation in rat vessels *in vivo. In Vivo*, 13: 7-12.

Meyer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobsen, L.B., Nichols, D.E., McLaughlin, J.L., (1982): A convenient general bioassay for active plant constituents. *Planta Medica*, 45: 31-34.

Odebiyi, A., Sofowora, A.E., (1978): Phytochemical screening of Nigerian medicinal plants. Part III. *Iloydia*, 41: 243-246.

Ohtani, K., Maw, S., Hostettmann, H., (2002): Molluscicidal and antifungal triterpenoid saponins from *Rapanea melanophloeos* leaves. *Phytochemistry*, 33: 83-86.

Ordon Ez, A.A.L., Gomez, J.D., Vattuone, M.A., Isla, M.I., (2006): Antioxidant activities of *Sechium edule* (Jacq.) Swart extracts. *Food Chemistry*, 97: 452-458.

Oyaizu, M., (1986): Studies on products of browning reaction; antioxidant activities of products of browning reactions prepared from glucose amine. *Jap J. Nutrit.*, 44: 307-315.

Pujol, J., (1990): Naturafrica- the Herbalist hand book. Jean Pujol Natural Healers Foundation, Durban.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C.A., (1999): Antioxidant activity applying an approved ABTS radical cation decolourization assay. *Free Radic. Biol. Med*., 26: 123-1237.

Rieser, M.J., Gu, Z.M., Fang, X.P., Zeng, L., Wood, K.V., McLaughlin, J.L., (1996): Five novel mono-tetrahydrofuran ring acetogenins from the seeds of *Annona muricata. J. Natural Products*, 59: 100-108.

Rood, B., (1994): Uit die Veldapteek. Tafelberg, Capetown.

Sobotková, A., Mášová-Chrastinová, L., Suttnar, J., Štikarová, J., Májek, P., Reicheltová, Z., Kotlín, R., Weisel, J.W., Malý, M., Jan, E., Dyr, J.E., (2009): Antioxidant change platelet responses to various stimulating events. *Free Radical Biology and Medicine*, 47: 1707–1714.

Sofowora, A., (1984): Medicinal plants and traditional medicine in Africa, Johnwiley, New York, pp 256-257.

Suleiman, M.M., McGaw, L.J., Naidoo, V., Eloff, J.N., (2009): Evaluation of several tree species for activity against the animal fungal pathogen *Aspergillus fumigatus*. *South African Journal of Botany*, 76: 64-71.

Suleiman, M.M., McGaw, L.J., Naidoo, V., Eloff, J.N., (2010). Detection of antimicrobial compounds by bioautography of different extracts of leaves of selected South African tree species. *African J. Traditional Complementary and Alter. Medicine*, 7: 64-68.

Tognolini, M., Barocelli, E., Ballabeni, V., Bruni, R., Bianchi, A., Chiavarini, M., Impicciatore, M., (2006): Comparative screening of plant essential oils: Phenylpropanoid moety as basic core for antiplatelet activity. *Life Sciences*, 78: 1419-1432.

Tomita, T., Umegaki, K., Hayashi, E., (1983): Basic aggregation properties of washed rat platelets: Correlation between aggregation, phospholipid degradation, malondialdehyde, and thromboxane formation. *J Pharmacology Methods,* 10: 31-44.

Toth, G.B., Pavia, H., (2001): Removal of dissolved brown algal phlorotannins using insoluble polyvinylpolypyrrolidone (PVPP). *J. Chem. Ecol*., 27: 1899-1910.

Van Wyk, B., Van Wyk, P., (1997): Field guide to trees of southern Africa. Struik. Cape Town.

Vellini, M., Desideri, D., Milanese, A., Ominic, C., Daffonchio, L., Hernandez, A., Brunelli, G., (1986): Possible involvement of eicosanoids in the pharmacological action of bromelain. *Arzneimittelforrschung,* 36: 110-112.

Watt, J.M., Breyer-Brendwijk, M.G., (1962): The medicinal and poisonous plants of Southern and Eastern Africa, 2nd edition. Livingstone, London.

Xiang, Y.Z., Kang, L.Y., Kao, X.M., Shang, H.C., Zhang, J.H., Zhang, B.L., (2008): Strategies for antiplatelet targets and agents. *Thrombosis Research,* 123: 35-49.

Table-1: **Results of the phytochemical analysis of the plant material of *P. longifolia, R. melanophloeos and B. natalensis.***

Phytochemical	***P. longifolia***	***R. melanophloeos***	***B. natalensis***
Saponin	+	+	+
Tannins	+	+	+
Steroids	-	-	-
Terpenoids	+	+	-
Alkaloids	+	+	+
Anthraquinones	-	-	+
Flavonoids	+	+	+
Cardiac glycosides	+	+	+
Phlobatannins	-	+	-

- + = Present; - = Absent

Table-2: The total phenolic and flavonoid content (mg/g dry plant material) of the extracts of *P. longifolia, R. melanophloeos* and *B. natalensis* given as gallic acid equivalent.

	P. longifolia		***R. melanophloeos***		***B. natalensis***	
Extract	**Phenolic**	**Flavonoid**	**Phenolic**	**Flavonoid**	**Phenolic**	**Flavonoid**
HE	2.41 ± 0.01	1.71 ± 0.01	0.40 ± 0.06	0.8 ± 0.01	5.03 ± 0.04	3.29 ± 0.05
CE	2.04 ± 0.08	1.24 ± 0.17	1.00 ± 0.05	0.8 ± 0.02	4.55 ± 0.67	2.42 ± 0.07
EAE	0.99 ± 0.13	2.02 ± 0.05	1.00 ± 0.02	0.7 ± 0.00	4.63 ± 0.15	2.84 ± 0.10
ME	0.63 ± 0.09	0.55 ± 0.00	1.20 ± 0.03	1.4 ± 0.01	3.40 ± 0.15	1.78 ± 0.06
WE	2.39 ± 0.00	0.81 ± 0.18	1.00 ± 0.10	0.9 ± 0.08	1.15 ± 0.02	1.89 ± 0.02

- Results are expressed as mean ± SEM, (n=3).

Table-3: The IC_{50} (mg/ml) of chelating activity on Fe^{2+}, DPPH and ABTS scavenging activity of the extracts of *P. longifolia, R. melanophloeos* and *B. natalensis*.

DPPH							
Plant	HE	CE	EAE	ME	WE	BHT	AA
P. longifolia	>5	>5	>5	0.07	>5	1.43	0.49
R. melanophloeos	3.05	4.32	4.83	3.31	3.89	4.06	4.57
B. natalensis	>5	>5	<1	>5	>5	1.29	3.10
ABTS							
Plant	HE	CE	EAE	ME	WE	BHT	AA
P. longifolia	0.27	>5	>5	0.16	2.0	0.42	0.15
R. melanophloeos	3.31	3.22	2.79	1.44	>5	3.73	3.43
B. natalensis	4.72	<5	>5	<5	>5	1.52	>4
Fe^{2+} Chelating							
Plant	HE	CE	EAE	ME	WE	EDTA	CA
P. longifolia	>5	nd	nd	nd	nd	3.68	3.85
R. melanophloeos	4.23	3.55	0.93	3.22	>5	3.31	3.89
B. natalensis	>5	>5	>5	>5	>5	0.73	3.81

- nd- not detected; results are expressed as mean ± SEM, (n=3).

Table-4: Platelet aggregation inhibitory activity (IC_{50} mg/ml) of the extracts of *P. longifolia, R. melanophloeos* and *B. natalensis* with tannins and without tannins in parentheses.

Extract	*P. longifolia*			*R. melanophloeos*			*B. natalensis*		
	a	***b***	***c***	***a***	***b***	***c***	***a***	***b***	***c***
HE	0.59 (9.10)	1.56 (>10)	>10 (>10)	7.10 (nd)	9.25 (9.85)	7.02 (nd)	>10 (>10)	>10 (>10)	5.46 (1.77)
CE	>10 (>10)	4.48 (5.86)	2.38 (4.57)	1.45 (nd)	>10 (9.24)	9.11 (8.13)	>10 (>10)	5.32 (0.37)	0.43 (>10)
EAE	2.13 (nd)	>10 (nd)	2.09 (nd)	1.15 (2.40)	1.59 (2.27)	1.64 (1.86)	>10 (>10)	1.90 (0.18)	2.52 (>10)
ME	nd (>10)	nd (nd)	2.21 (nd)	nd (nd)	nd (nd)	nd (nd)	nd (>10)	1.81 (>10)	>10 (>10)
WE	nd (>10)	nd (nd)	>10 (nd)	2.83 (nd)	nd (9.24)	>10 (nd)	9.71 (0.62)	>10 (0.39)	1.02 (>10)
Heparin	7.40								

- nd- not detected; data were expressed as mean ± SEM, (n=3).
- a-Thrombin-induced platelet aggregation
- b- ADP-induced platelet aggregation
- c- Epinephrine-induced platelet aggregation

Table-5: IC_{50} (mg/ml) values of anti-platelet aggregation activity (enzyme-treated platelets) of the extracts of *P. longifolia, R. melanophloeos* and *B. natalensis.*

Thrombin-induced platelet aggregation									
Extract	***P. longifolia***			***R. melanophloeos***			***B. natalensis***		
	x	***y***	***z***	***x***	***y***	***z***	***x***	***y***	***z***
HE	9.19	5.19	>10	nd	2.63	nd	6.77	0.92	8.53
CE	8.21	6.61	nd	nd	2.21	2.67	9.14	1.90	2.21
EAE	2.17	2.63	2.05	3.38	0.84	1.11	2.30	0.98	2.69
ME	2.42	2.01	nd	41.6	7.22	7.79	1.85	1.51	>10
WE	7.52	8.11	8.46	1.10	1.98	nd	1.07	1.68	9.89
ADP-induced platelet aggregation									
	x	***y***	***z***	***x***	***y***	***z***	***x***	***y***	***z***
HE	1.36	7.77	5.54	nd	7.59	>10	>10	0.28	nd
CE	2.42	2.34	2.46	nd	7.84	nd	2.60	3.92	nd
EAE	>10	8.29	2.66	4.93	3.41	1.12	2.73	7.78	nd
ME	>10	1.61	2.90	5.94	9.49	nd	>10	5.59	nd
WE	>10	>10	>10	4.17	6.22	nd	1.94	>10	nd
Epinephrine-induced platelet aggregation									
	x	***y***	***z***	***x***	***y***	***z***	***x***	***y***	***z***
HE	2.82	9.4		nd	nd	>10	6.47	8.09	nd
CE		nd		nd	>10	8.98	5.46	1.77	nd
EAE	7.12	2.42	1.36	3.67	3.79	3.67	2.38	2.12	nd
ME	>10	>10	2.66	nd	>10	nd	2.52	>10	nd
WE	nd	2.17	nd	7.59	>10	nd	2.47	>10	nd
	>10	>10	nd						

- nd- not detected; data were expressed as mean ± SEM, (n=3).
- x- trypsin treated platelets; y- bromelain treated platelets; z- papain treated platelets.