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Full Length Research Paper

Antithrombotic/anticoagulant and anticancer activities of selected medicinal plants from South Africa

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Nine plants available in the Eastern Cape Province of South Africa were tested for antithrombotic and/or anticoagulant activity. Organic (methanol) and aqueous (distilled water) extractions were performed on the various plant parts. The thrombin assay and clotting time assays (thrombin-induced and $CaCl_2$-induced) were utilised. Several extracts displayed activity, but in most cases this was due to the presence of tannins. Only the aqueous extracts displayed activity after tannin removal. The *Sutherlandia frutescens* leaf extract displayed antithrombotic activity, with an IC_{50} value of 2.17 mg/ml. *Gloriosa superba* and *Zantedeschia aethiopica* leaf extracts displayed anticoagulant properties by inhibiting thrombin-induced clotting, with IC_{50} values of 2.97 and 3.05 mg/ml, respectively. The *Leonotis leonurus* root extract was found to decrease the $CaCl_2$-induced clotting time by 50% at 8.88 mg/ml. A decrease in this value accompanied by a decrease in fibrin formation was preferable for the $CaCl_2$-induced assay, since decreased fibrin formation may have a role in the prevention of cancer metastasis. As tannins were found to contribute minimally to the anticoagulant effect of *L. leonurus*, the cytotoxicity potential of the extracts of this species against five cell lines was determined. Only the organic extract yielded significant cytotoxity.

Key words: Antithrombotic, anticoagulant, anticancer, medicinal plants.

INTRODUCTION

Thromboembolic disorders such as pulmonary emboli, deep vein thrombosis, strokes and heart attacks are the main causes of morbidity and mortality in developed countries (Dickneite et al., 1995). The process of coagulation occurs via a cascade of sequential reactions requiring several enzymes and other molecules known as coagulation (or clotting) factors. Two separate pathways lead to the production of the Stuart-Prower factor (factor Xa where "a" represents an activated factor): the intrinsic pathway and the extrinsic pathway. Factor Xa then participates in the final common pathway that results in the fibrin clot by activating prothrombin (factor II) to thrombin (factor IIa). Thrombin, the final enzyme in the coagulation cascade, in turn converts soluble fibrinogen into insoluble fibrin monomers (Tortora and Grabowski, 2000; Dickneite et al., 1995).

Thrombin, a serine protease, has a central role in haemostasis. Apart from its role in the conversion of fibrinogen to fibrin, it also activates factor XIII, accelerates the formation of factor V, which increases thrombin formation, and activates platelets, thereby enhancing platelet aggregation and the release of phospholipids (Tortora and Grabowski, 2000).

A "hypercoagulable state" is often also associated with

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Abbreviations: S2238, H-D-phenylalanyl-L-pipecolyl-*p*-nitroanilide dihydrochloride; DMSO, dimethyl sulfoxide; PVPP, polyvinylpolypyrrolidone; BSA, bovine serum albumin; DMEM, Dulbecco's Modified Eagles Medium; FBS, fetal bovine serum; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; IC_{50}, 50% inhibition.

cancer. Patients with malignancy are, therefore, at risk of developing thromboembolic disorders. This phenomenon is due to the fact that malignant cells have an increased capacity to initiate the coagulation cascade. Clotting arises from a complex interaction of various mechanisms, including the activation of the coagulation and fibrinolytic systems, disruption of the vascular endothelium, and the generalised activation of the cellular mechanisms resulting in clotting on the surface of monocytes and platelets in circulation (Rickles and Falanga, 2001).

Due to the recognised link between cancer and hypercoagulation, medications able to treat cancer and having antithrombotic/anticoagulant activity would be ideal. Throughout history, plants have been used as a major medicinal source, with interest in herbal formulations increasing globally over the past decade. In addition, extracts of natural products provide a useful source of bioactive compounds which can be developed as drugs directly or provide novel structural templates. Today, a large proportion of drugs in clinical use are produced by the synthesis of natural products and/or their derivatives, and new plant-derived medicines are continually being discovered (Van Wyk et al., 1997; De Medeiros et al., 2000; Rahman et al., 2001).

Primary bioassay screens are important for the initial screening of plants for bioactive chemical constituents and are often the first step in drug development (Rahman et al., 2001). The active compounds in medicinal plants often vary in different parts of the plant, with the whole plant seldom being used in medicine. The leaves and/or twigs, stem, bark and underground parts of plants are most often used for traditional medicines (Van Wyk et al., 1997).

South Africa has a rich diversity of plant species, with about 3000 plant species used as medicines (Van Wyk et al., 1997). A selection of plants, most of which are known to have an anticancer activity, available in the Eastern Cape Province of South Africa was chosen to test for possible antithrombotic and anticoagulant activities. This could identify plants traditionally used in the treatment of cancer that have the additional benefit of an anticoagulant activity. The chosen plants included: *Acokanthera oppositifolia*, *Aloe spesiosa*, *Aloe ferox*, *Catharanthus roseus*, *Gloriosa superba*, *Leonotis leonurus*, *Sutherlandia frutescens*, *Typha capensis* and *Zantedeschia aethiopica*.

MATERIALS AND METHODS

Plant extractions

Plants were identified by Mr C. Thomas of the Botany Department at the Nelson Mandela Metropolitan University.

Plant extractions were performed with either methanol or distilled water (dH_20). The plants were divided into root, stem and leaf sections, dried at 50°C and ground into a powder. Extractions were performed in glass bottles for one hour in a sonicator bath using 10 ml methanol per gram ground plant material, or 20 ml dH_2O per gram ground plant material. The extracts were filtered using Whatman No. 1 filter paper. Methanol extracts were concentrated using a Heidolph rotary evaporator (50°C). All extracts were dried using an Instruvac freeze-dryer and stored at 4°C in the dark until testing.

Three assays were used to test the antithrombotic and anticoagulant properties of the plant extracts.

Thrombin assay

The antithrombotic activity of the plant extracts was determined using a modified method of Rob et al. (1997). The chromogenic thrombin substrate, S2238 (H-D-phenylalanyl-L-pipecolyl-*p*-nitroanilide dihydrochloride, Chromogenix), was used. Plants extracts were screened at concentrations of 1 and 3 mg (dry weight)/ml buffer. It was necessary to solubilise the methanol extracts in dimethyl sulfoxide (DMSO, Merck) before making up the volume with buffer (50 mM Tris-HCl, pH 7.4, 7.5 mM EDTA and 175 mM NaCl), to yield a final DMSO concentration of 1% (v/v). The extract (50 µl) was added to 10 µl thrombin (Sigma) at 30 U/ml in dH_2O. This mixture was left to incubate for 10 min at room temperature and 190 µl 0.76 M S2238 was added. The reaction was monitored at 412 nm for 4 min at 10 s intervals using a microtitre plate reader (Labsystems Multiskan MS). The extracts that inhibited thrombin compared to a control (composed of 1% DMSO (v/v) in buffer without extract) were tested at a range of concentrations to determine an IC_{50} value.

Microtitre plate-based clotting time assays

Clotting time assays were used to determine the anticoagulant effect of the plant extracts on human plasma. The method of Hauptmann et al. (1980) was modified for microtitre plate analysis. Ethical clearance was obtained for the use of plasma that was acquired from the Port Elizabeth blood bank.

Thrombin-induced clotting time assay

The effect of DMSO on clot formation was determined since it is known to inhibit this process. The maximum concentration that could be used without significantly affecting clot formation was 2% (v/v). Plant extracts were tested at 1 and 10 mg/ml in saline containing a DMSO concentration of 2% (v/v). The plant extract (40 µl) was added to human plasma (100 µl) and incubated at room temperature for 5 min. Thrombin (20 µl at 5 U/ml) was added and the rate of clot formation was determined by following the increase of the absorbance at 412 nm using a Labsystems Multiskan MS microtitre plate reader for 20 min at 30 s intervals. Plant extracts that inhibited the rate of clot formation were further tested at a range of concentrations to determine a concentration that inhibited that rate by 50% (IC_{50}). A negative control was performed using 2% (v/v) DMSO in saline, which represented 100% activity. Heparin was used as a positive control.

$CaCl_2$-induced clotting time assay

A $CaCl_2$-induced clotting time assay was developed. This assay allows for the determination of a 50% clotting time and effect on fibrin formation. The extracts were screened at 1 and 10 mg/ml saline, using 2% DMSO (v/v) to solubilise the plant extracts. The assay was performed by adding plant extract (40 µl) to human plasma (100 µl). The reaction was mixed and left to incubate for 5 min at room temperature. Clotting was induced by the addition of 20 µl 0.16 M $CaCl_2$, and the reaction was followed at 412 nm with a Labsystems Multiskan MS microtitre plate reader for 2 h at 3 min intervals.

Tannin quantification and removal

Tannins are present in most plants and have a high affinity for proteins, thereby affecting many biochemical reactions. The quantification and removal of tannins was therefore necessary for the screening of the plant extracts.

The tannin content of the plant extracts was determined using the Hagerman and Butler method for total phenolics as described by Mole and Waterman (1987). The procedure was scaled down for microtitre plate analysis. The plant extracts were analysed at 5 mg dry weight/ml dH_2O. The plant extract (50 µl) was first added to a well, followed by 100 µl SDS/triethanolamine (1% (w/v) SDS / 5% (v/v) triethanolamine in dH_2O) solution and 50 µl $FeCl_3$/HCl (0.81 g $FeCl_3$ / 500 ml 0.001 M HCl) solution. The reaction mixture was allowed to stand at room temperature for 5 min, and the absorbance at 492 nm was measured using a microtitre plate reader (Labsystems Multiskan MS). Tannin concentrations (mg/ml) were determined by extrapolation from a standard curve determined simultaneously using pyrogallol at concentrations of 0 to 1 mg/ml. Tannin removal was performed using either polyvinylpolypyrrolidone (PVPP, Sigma) or bovine serum albumin (BSA, Sigma).

Insoluble PVPP may be used to remove tannins from a plant extract before assaying. PVPP binds tannins by exploiting their strong hydrogen bonding capabilities. This method of tannin removal was modified from that described by Toth and Pavia (2001). Plant extracts were prepared at a concentration of 10 mg/ml in dH_2O. PVPP was added to the extract at 10 mg/ml, shaken for 15 min at 4°C, followed by centrifugation using an Eppendorf centrifuge model 5804 R, at 3645*g* for 8 min at 4°C. The pellet was discarded. Using the supernatant, the procedure was repeated twice, or as many times as required to remove tannins to a negligible concentration. The extract was dried and used for subsequent assays.

Tannins are known to form strong complexes with proteins. This characteristic was exploited in the method of tannin removal using BSA. The plant extract was prepared as for the specific assay being performed. BSA was dissolved in the extract preparation to yield a final BSA concentration of 0.2% (w/v) per well (Harnett, 2005).

Cell culture

A-549 and MCF-7 cells were obtained from the National Cancer Institute (U.S.A.), while all other cell lines were obtained from Highveld Biological (South Africa). HT-29 cells (colon adenocarcinoma) were maintained in Dulbecco's Modified Eagles Medium (DMEM) (Highveld Biological, South Africa) containing 0.2% 100 IU penicillin/100 µg/ml streptomycin (Highveld Biological, South Africa) and 10% fetal bovine serum (FBS) (Gibco, Belgium). A-549 cells (lung epithelial carcinoma) were maintained in Ham's F12 medium (Highveld Biological, South Africa) supplemented with 10% FBS. MCF-7 (breast adenocarcinoma), K562 (chronic myelogenous leukaemia) and HL-60 (acute promyelocytic leukaemia) cells were routinely maintained in RPMI-1640 (Gibco, Belgium) supplemented with 10% FBS.

Cytotoxicity assay

Cells were seeded in 96-well plates (Nunc, Denmark) at a density of 25 000 cells/well in 180 µl culture medium. Adherent cells were allowed to attach to the wells for 24 h prior to treatment. Thereafter, 20 µl of a 10x stock solution of the tannin-free organic and aqueous extracts from *L. leonurus* were added to the cultures, resulting in final concentrations in the well ranging from 1.25 – 500 µg/ml. As a standard, the cytotoxic effects of marrubiin were tested on all cell lines at a final well concentration of 50 µg/ml. The plated cells were maintained in a humidified incubator at 37°C containing 5% CO_2 for 24 h. The effect of the extracts on cell growth (metabolically active cells) was determined by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay as described by Mosmann (1983). Briefly stated, 50 µl 0.5% (v/v) MTT was added to the culture medium at the end of the incubation period. The cells were incubated for a further 2 h at 37°C. The yellow MTT dye is reduced by succinic dehydrogenase in the mitochondria of viable cells to purple formazan crystals, which are then solubilised in 200 µl DMSO. The absorbance was read at 540 nm against a DMSO blank. Cell numbers per well were extrapolated from calibration curves for individual cell lines. The percentage cell viability per well was calculated as follows: % cell viability = [number of viable treated cells]/[number of viable control cells] × 100. The IC_{50} value was determined for extracts reducing cell viability to less than 50% when tested at the initial concentration of 500 µM. All experiments were conducted in triplicate.

RESULTS

The results of the antithrombotic and anticoagulant assays are shown in Table 1. Several plant extracts were found to exhibit antithrombotic and/or anticoagulant activity. However, this activity was found to be predominantly due to the presence of tannins in the extracts, since no significant activity was detected after tannin removal for most of the tested extracts.

Numerous extracts were found to exhibit antithrombotic activity in the presence of tannins (Table 1). All the aqueous and methanol extracts of *T. capensis* exhibited strong thrombin inhibition. The methanol extracts had the lowest IC_{50} values (0.43 and 0.35 mg/ml), indicating that they were potentially the best inhibitors. This value indicates the concentration required to bring about a 50% inhibition of thrombin activity. The aqueous leaf extracts of *G. superba* (5.30 mg/ml), *L. leonurus* (9.69 mg/ml), *S. frutescens* (2.23 mg/ml) and *Z. aethiopica* (4.74 mg/ml) also showed antithrombotic activity. However, after tannin removal, it was found that *S. frutescens* was the only extract to retain its activity. IC_{50} values of 2.23 and 2.17 mg/ml were required before and after tannin removal, respectively, indicating that tannins were not responsible for the antithrombotic activity of the extract (Table 1). The effect of the aqueous *S. frutescens* leaf extract on thrombin activity is shown in Figure 1. The progress curves illustrate the inhibitory effect of increasing extract concentrations on thrombin activity. Since the observed antithrombotic activity of the other plant extracts was lost after tannin removal, their activity was most likely due to the presence of tannins.

The anticoagulant activity of the plant extracts was investigated using the thrombin-induced clotting time assay (Table 1). The extracts that displayed activity in the presence of tannins included: methanol leaf extracts of *G. superba* (5.51 mg/ml), *L. leonurus* (8.26 mg/ml), *S. frutescens* (4.78 mg/ml) and *Z aethiopica* (5.27 mg/ml); stem extract of *L. leonurus* (10.40 mg/ml); rhizome extract of *T. capensis* (8.18 mg/ml); and aqueous leaf extracts of *A. ferox* (7.74 mg/ml), *A. spesiosa* (5.88 mg/ml), *G. superba* (2.50 mg/ml), *S. frutescens* (4.92 mg/ml) and *Z. aethio-*

Table 1. The antithrombotic and anticoagulant effect of plant extracts

Plant extract	Before tannin removal			After tannin removal		
	Thrombin assay (IC_{50}) (mg/ml)	Thrombin-induced (IC_{50}) (mg/ml)	[a]$CaCl_2$-induced (mg/ml)	Thrombin assay (IC_{50}) (mg/ml)	Thrombin-induced (IC_{50}) (mg/ml)	[a]$CaCl_2$-induced (mg/ml)
Methanol extracts						
A. oppositifolia (leaves)	-	-	-	nd	nd	nd
(stems)	-	-	-	nd	nd	nd
(roots)	-	-	-	nd	nd	nd
C. roseus (leaves)	-	-	-	nd	nd	nd
(stems)	-	-	-	nd	nd	nd
(roots)	-	-	-	nd	nd	nd
G. superba (leaves)	-	5.51	-	-	-	-
L. leonurus (leaves)	-	8.26	10.38 (47.9%)	nd	-	-
(stems)	-	10.40	-	nd	-	nd
(roots)	-	-	9.00 (75.9%)	nd	nd	-
S. frutescens (leaves)	-	4.78	-	-	-	-
T. capensis (leaves)	0.43	-	6.92 (38.5%)	-	-	-
(rhizome)	0.35	8.18	7.11 (52.0%)	-	-	-
Z. aethiopica (leaves)	-	5.27	-	-	-	-
dH_2O extracts						
A. oppositifolia (leaves)	-	-	-	nd	nd	nd
(stems)	-	-	10.09 (98.2%)	nd	nd	-
(roots)	-	-	5.41 (60.3%)	nd	nd	-
A .ferox (leaves)	-	7.74	-	nd	nd	nd
A. spesiosa (leaves)	-	5.88	-	nd	nd	nd
C. roseus (leaves)	-	-	-	nd	nd	nd
(stems)	-	-	-	nd	nd	nd
(roots)	-	-	-	nd	nd	nd
G. superba (leaves)	5.30	2.50	-	nd	2.97	-
L. leonurus (leaves)	9.69	-	9.18 (64.9%)	-	nd	-
(stems)	-	-	10.78 (79.5%)	nd	nd	-
(roots)	-	-	5.91 (60.5%)	nd	nd	8.88 (80.94%)
S. frutescens (leaves)	2.23	4.92	-	2.17	nd	-
T. capensis (leaves)	1.87	-	8.50 (54.1%)	-	nd	-
(rhizome)	1.75	25.13	10.85(94.4%)	-	rate increase	-
Z. aethiopica (leaves)	4.74	2.45	-	nd	3.05	-

- = no effect detected.
nd = not determined
[a]Values indicate concentrations required to halve the clotting time compared to a control. Values in brackets indicate percentage of fibrin formation compared to a control.

pica (2.45 mg/ml); rhizome extract of *T. capensis* (25.13 mg/ml). After tannin removal, only the aqueous extracts of *G. superba* and *Z. aethiopica* retained their anti-coagulant activity, as shown by their ability to inhibit the rate of clot formation in the thrombin-induced clotting time assay. The IC_{50} value of *G. superba* increased from 2.50 to 2.97 mg/ml, while that of *Z. aethiopica* increased from 2.45 to 3.05 mg/ml. Since a higher concentration was required after tannin removal, it is proposed that tannins contributed to the initial anticoagulant effects observed. The anticoagulant activity of extracts previously showing activity was, therefore, also possibly due to tannins. Before tannin removal, the aqueous extract of the *T. capensis* rhizome had an IC_{50} of 25.13 mg/ml. After tannin removal, however, the extract was found to have a procoagulant effect at 10 mg/ml. This suggests that *T. capensis* has both an anticoagulant and procoagulant effect, similar to other *Typha* species, namely *T.*

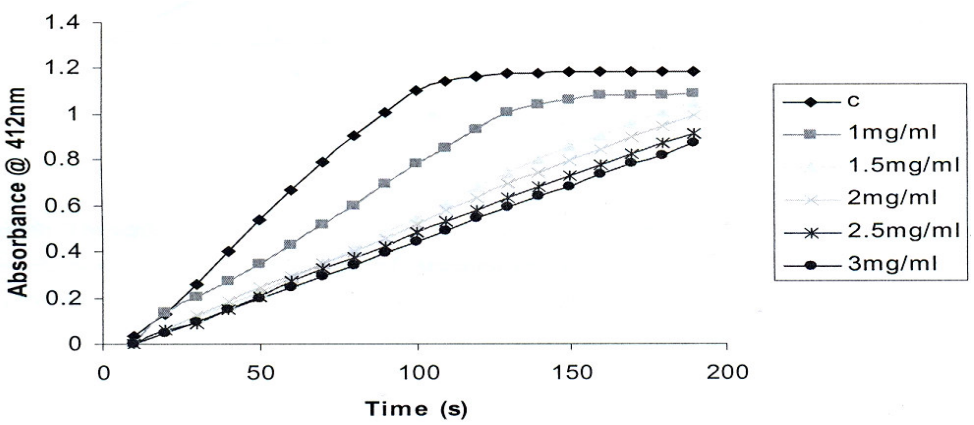

Figure 1. The effect of an aqueous *S. frutescens* leaf extract on the thrombin assay. Increasing concentrations of the extract can be seen to increasingly inhibit thrombin activity. c is a negative control representing 100% thrombin activity.

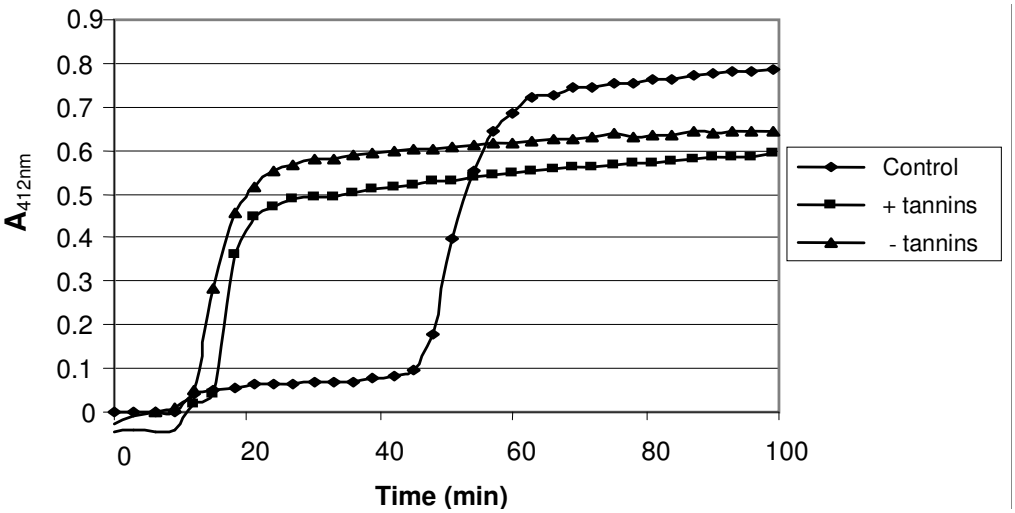

Figure 2. The effect of an aqueous *L. leonurus* root extract (7.5 mg/ml) on $CaCl_2$-induced clot formation. The inclusion of the aqueous extract to the assay is shown to decrease the time required for the formation of a fibrin clot and also decreases fibrin formation, compared to the control. After tannin removal, the effects are still visible, but to a lesser degree

augustata (Gibbs et al., 1983) and *T. lactifolia* L. (Ishida et al., 1988), which were found to display both properties.

No extracts were found to inhibit the $CaCl_2$-induced clotting time. Instead, it was found that a number of plant extracts decreased the clotting time. Furthermore, these extracts decreased fibrin formation. The action of these extracts may therefore be directed at fibrin formation. Since less fibrin is formed, clotting is completed in a shorter time frame. Although the aim was to identify extracts which would increase the clotting time, these extracts would be advantageous in that less dense clots would form. This property could also be beneficial in the treatment of cancer, since fibrin clot formation plays a vital role in masking tumours and promoting their attachment to the vascular endothelium, thereby aiding its progression (Gale and Gordon, 2001). The extracts having an effect in the presence of tannins included: the methanol extracts of *L. leonurus* (leaves and roots) and *T. capensis* (leaves and rhizome), and the aqueous extracts of *A. oppositifolia* (stems and roots), *L. leonurus* (leaves, stems and roots) and *T. capensis* (leaves and rhizome). After tannin removal, the aqueous *L. leonurus* root extract was the only sample to have an effect on the $CaCl_2$-induced clotting time assay (Figure 2). The *L. leonurus* extract (both before and after tannin removal) is shown to decrease the time required for the completion of clot formation, measured by the time taken from the beginning of the exponential increase in absorbance to the plateau phase. Fibrin formation, shown by the maximum absorbance obtained, is also shown to be decreased by

Table 2. IC_{50} values obtained for aqueous and organic root extracts of tannin-free *L. leonurus* (n=3).

Cell line tested	IC_{50} value (µg/ml)	
	Aqueous extract	Organic extract
HT-29	> 500	327.459 ± 5.871
A-549	> 500	269.023 ± 9.684
K562	> 500	145.901 ± 8.282
HL-60	> 500	62.474 ± 3.310
MCF-7	> 500	262.093 ± 8.156

the extract. Furthermore, the removal of tannins from the aqueous *L. leonurus* extract is shown to increase the clotting time and fibrin formation compared to the tannin-containing extract. An amount of 8.88 mg/ml extract was required (as compared to 5.91 mg/ml initially) to reduce the clotting time by 50% and decrease the fibrin formed by 19.06%. Tannins, therefore, had a small effect on the properties of the aqueous *L. leonurus* extract, but accounted for the effects of the remainder of the above-mentioned extracts.

Between 1940 and 2002, 40% of all anticancer drugs entering the market were natural products or derivatives thereof, with a further 8% consisting of natural product analogues. Since there exists a strong association between cancer and a state of hypercoagulation (Rickles and Falanga, 2001), the anticancer activity for extracts exhibiting anticoagulant activity was determined. As seen from Table 1, the tannin-free aqueous extract from *L. leonurus* root was the only extract that affected $CaCl_2$-induced clotting time. This extract was therefore tested for potential anticancer activity. The aqueous extract did not show any cytotoxicity against the cell lines tested (Table 2), with IC_{50} values in excess of 500 µg/ml. In contrast, the tannin-free organic extract was active against all cell lines tested, with IC_{50} values ranging from 62.474 ± 3.310 µg/ml to 327.459 ± 5.871 µg/ml. The most cytotoxic effect was noted against the HL-60 cell line, followed by the K562 cells, and the lowest cytotoxic effect was observed against the HT-29 cell line. The cytotoxic activity was thus attributable to a constituent within the extract other than tannins, although no significant effect on the $CaCl_2$-induced clotting time was noted for this extract.

The major chemical constituents of the extracts of *L. leonurus* include the diterpenoid labdane lactones, premarrubiin and marrubiin. Marrubiin is thought to be present in the extracts as an "artifact" of extraction, and is often present as the dominant constituent. Also present are tannins, quinines, saponins, alkaloids and triterpene steroids (Laonigro et al., 1979).

The cytotoxic effect of marrubiin on cell viability was thus determined. Due to the limited amount of standard available, marrubiin was tested at a single concentration of 50µg/ml. At this concentration, marrubiin only decreased cell viability by ± 10 to 25%, showing its weak

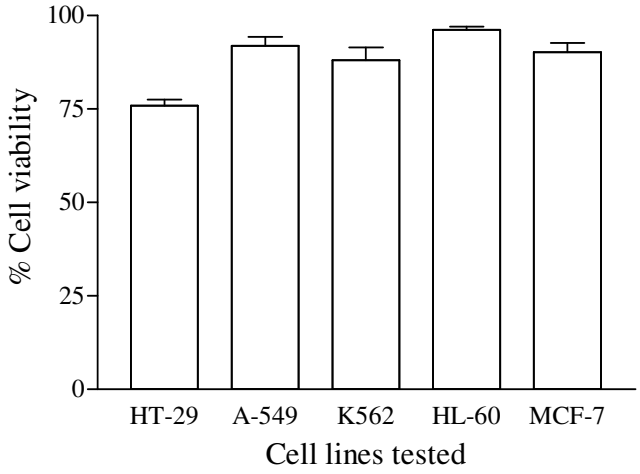

Figure 3.Cell viability of 5 cell lines after a 24 h exposure to 50 µg/ml marrubiin (n=3).

cytotoxic potential (Figure 3). The most cytotoxic effect was elicited against the HT-29 cells, with decreased cytotoxicity against the other cell lines tested. It is thus suggested that the cytotoxic effects of the organic extract are as a result of chemical constituents present other than marrubiin.

The anticancer activity of aqueous extracts of dried *Leonotis heterophyllus* Sweet was tested against seven cell lines (Chinwala et al., 2003). IC_{50} values obtained ranged from 8.0-40.0 mg/ml extract after a 48 h incubation period, which is significantly higher than the concentrations that were used in our study.

DISCUSSION

Initial screening of the selected plants indicated that many of these had antithrombotic and anticoagulant activity. However, the observed properties of the majority of these extracts were found to be attributable to tannins. After tannin removal, only a few aqueous extracts retained their activity. The *S. frutescens* leaf extract displayed antithrombotic activity, while leaf extracts of *G. superba* and *Z. aethiopica* retained their anticoagulant activity directed against thrombin-induced clotting after tannin removal. Only the *L. leonurus* root extract was found to decrease the $CaCl_2$- induced clotting time. Although tannins enhanced this activity, the anticoagulant property was retained after its removal.

Due to the anticoagulant effect of *L. leonurus*, its cytotoxicity against numerous cell lines was investigated. Contrary to the findings of the antithrombotic and anti-coagulant studies, it was found that the organic rather than the aqueous extracts displayed cytotoxic activity. The greatest cytotoxic effect was directed against HL-60 cells, an acute promyelocytic leukaemia cell line.

Although the identification of an extract having both antithrombotic/anticoagulant activity as well as a cytotoxic

effect would have been ideal, none of the plant extracts displayed both properties. However, plant extracts having definite antithrombotic/anticoagulant or cytotoxic activity not attributable to tannins or phenolic compounds were identified. Further testing will be required to identify the active components in the extracts.

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