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THE ANTI-PROLIFERATIVE AND ANTIOXIDANT ACTIVITY OF FOUR INDIGENOUS SOUTH AFRICAN PLANTS.

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Abstract

Background: Cancer is a major cause of death worldwide. Limitations of current cancer therapies necessitate the search for new anticancer drugs. Plants represent an immeasurable source of bioactive compounds for drug discovery. The objective of this study was to assess the anti-proliferative and antioxidant potential of four indigenous South African plants commonly used in traditional medicine.
Materials and Methods: The anti-proliferative activity of the plant extracts were assessed by the 2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2*H*-Tetrazolium-5-Carboxanilide (XTT) assay on A431; HaCat; HeLa; MCF-7 and UCT-Mel 1 cells and sulforhodamine-B (SRB) assay on HCT-116 and HCT-15 cell lines. Antioxidant activity was determined using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH), nitric oxide (NO) and superoxide scavenging assays.
Results: Three of the plant extracts (*Combretum molle*fruit, *Euclea crispa* subsp. *crispa* leaves and stems and *Sideroxylon inerme* leaves and stems showed anti-proliferative activity on the A431 cells with IC_{50}values ranging between 26.9 - 46.7 µg/ml. The *Euclea crispa* subsp. *crispa* extract also showed anti-proliferative activity on the MCF-7 cell line (45.7 µg/ml). All of the plant extracts (*Combretum molle* leaves and fruit, *Euclea crispa* subsp. *crispa* leaves and stems, *Sideroxylon inerme* leaves and stems and *Terminalia prunioides* leaves and stems) showed DPPH scavenging activity with IC_{50} values ranging from 1.8 µg/ml to 11.5 µg/ml.
Conclusion: These results indicate that the active extracts of *Combretum molle*, *Euclea crispa* subsp. *Crispa* and *Sideroxylon inerme* warrant further investigation to determine the mechanism of anti-proliferative activity against cancerous cells. These plant extracts also show potential for further evaluation in the prevention and treatment of cancer.

Key words: South African plants, Traditional medicine, Anti-proliferative activity, Antioxidant activity.

Abbreviations: ATCC: American Type Culture Collection; **CANSA**: Cancer Association of South Africa; **DMEM**: Dulbecco's Modified Eagles Medium; **DMSO**: Dimethyl sulfoxide; **DNA**: Deoxyribonucleic acid; **DPPH**: 2, 2-diphenyl-1-picrylhydrazyl; **EMEM**: Eagle's Minimum Essential Medium; **FBS**: Fetal Bovine Serum; **IC_{50}**: Fifty percent inhibitory concentration; **MD**: Maryland; **NaOH**: Sodium hydroxide; **NBT**: Nitrotetrazolium Blue chloride; **NCCS**: National Centre for Cell Science; **NCI**: National Cancer Institute; **NO**: Nitric oxide; **ROS**: Reactive Oxygen Species; **RSA**: Republic of South Africa; **SD**: Standard deviation; **SRB**: sulforhodamine-B; **USA**: United States of America; **WHO**: World Health Organization; **XTT**: 2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5-Carboxanilide.

Introduction

Cancer is one of the major causes of death due to non-communicable diseases world- wide.
In 2012 alone, cancer was identified as the causal agent of more than 8.2 million deaths. In addition, the incidence and mortality rates have shown an increasing trend in Africa, Asia and Central and South America. According to the World

Health Organization report, seventy percent of cancer deaths have occurred in these countries (WHO, 2014). In South Africa, more than 100,000 cases are reported each year. The six most prevalent cancers found among South African men are prostate cancer, lung cancer, colorectal cancer, esophageal cancer, Kaposi sarcoma and cancers of which the site of origin within the body is not known. The six most prevalent cancers found among South African women are breast cancer, cervical cancer, colorectal cancer, Kaposi sarcoma, melanoma and cancers of which the site of origin within the body is not known (CANSA, 2013).

Conventional cancer treatment involves various aspects to either treat the disease itself or the symptoms of the disease or both. In general, surgical removal of cancerous tissue, radiotherapy and chemotherapy are employed for the treatment of cancers. Limitations of current therapies including adverse side effects and lowered efficacy due to drug resistance, which warrants the search for new drugs (NCI cancer treatment research, 2017).

Many anticancer agents in use today, originate from natural resources such as animals, microorganisms and plants (Nobili et al., 2009). Herbal medicine forms a big part of many traditional medicine systems. The knowledge of these traditional medicine systems have provided key information for the discovery of anticancer agents from plants. Traditional medicine further forms a very important source of affordable and readily accessible health care system for most people in developing countries (Falkenberg et al., 2002).

Antioxidants are compounds that scavenge reactive oxygen species (ROS). Since ROS can cause DNA damage, which can lead to cancer development; antioxidants are believed to possess chemopreventive abilities. Well known plant derived antioxidants include ‘quercetin’; ‘resveratrol’; ‘curcumin’ and ‘catechins’. Many epidemiology studies have shown that cancer incidence is low in countries with high levels of antioxidant rich plant consumption and as such employing antioxidants as a nutritional supplement might aid in cancer prevention (Borek, 1997; NCI, 2014).

The plants (*Combretum molle* R. Br. Ex G. Don, *Euclea crispa* subsp. *Crispa* (Thunb.) Gürke, *Sideroxylon inerme* L. and *Terminalia prunioides* M.A. Lawson) selected for this study have been used traditionally as anticancer agents and the available data ascribed anticancer properties to the compounds isolated from these plants. Table 1 gives the traditional usage; compounds isolated and reported anticancer activity of the plants.

Materials and methods

Materials

The A431, HeLa, and MCF-7 cell lines were obtained from American Type Tissue Collection (ATCC), MD, USA, whereas the HCT-116 and HCT-15 cell lines were obtained from NCCS Pune, India. Prof Davids, University of Cape Town, Cape Town, RSA, kindly donated the UCT-Mel 1 and HaCat cell lines. Foetal bovine serum (FBS) and antibiotics were purchased from Separations (Pty) Ltd. (Randburg, Johannesburg, RSA). The XTT cell proliferation kit II, sulforhodamine-B (SRB), DPPH, ascorbic acid, Griess reagent, sodium nitroprusside, Nitrotetrazolium Blue chloride (NBT), sodium hydroxide (NaOH), quercetin and all other materials were of analytical grade and were acquired from Sigma-Aldrich (Missouri, USA).

Plant collection and identification

All plant materials were collected during 2013 in Nelspruit, Mpumalanga. The plants were identified by the HGJW Schweickerdt Herbarium at the University of Pretoria and given herbarium specimen (PRU) numbers. *Combretum molle* R. Br. Ex G. Don leaves and fruit (PRU 120569), *Euclea crispa*subsp. *Crispa* (Thunb.) Gürke leaves, stems (PRU 120536), *Sideroxylon inerme* L. leaves, stems (PRU 120537), and *Terminalia prunioides* M.A. Lawson leaves and stems (PRU 120508).

Extraction of plants

The dried aerial parts of the plant were mechanically ground to a fine powder. The powdered plant material of each plant was extracted with ethanol for 48h and thereafter for another 24h using fresh solvent. A Buchner funnel was used to filter the solutes and which was subsequently evaporated by a vacuum rotary evaporator. The percentage yield of the plant extracts were calculated for the formula:

$$\% \text{ Yield} = \frac{\text{Extract weight (g)}}{\text{Powdered weight (g)}} \times 100$$

Cell culturing

The human epidermoid carcinoma (A431), metastatic melanoma (UCT-Mel 1), colorectal carcinoma (HCT-116 and HCT-15) and keratinocytes (HaCat) cell lines were maintained in culture flasks containing Dulbecco’s Modified Eagles Medium (DMEM). The human breast adenocarcinoma (MCF-7), and cervix adenocarcinoma (HeLa) cell lines were maintained in Eagle’s Minimum Essential Medium (EMEM). The complete media for all the cell lines were comprised of

the respective media supplemented with 10 % FBS and 1 % antibiotics. The antibiotic mixture consisted of 100 U/ml penicillin, 100 µg/ml streptomycin, and 250 µg/L fungizone. All of the cell lines were grown at standard growth conditions (37°C in a humidified incubator set at 5% CO_2) and sub-cultured when they had reached 100 % confluence.

Cell proliferation assay

XTT cell proliferation kit II

The anti-proliferative activity of the samples was measured by the XTT method using the Cell Proliferation Kit II (Sigma-Aldrich, Missouri, USA). The assay was performed according to the method by Zheng et al., 2001.1×10^4 cells were seeded (in 100 µl) in a 96-well microtiter plate and incubated for 24h at standard growth conditions to allow for cell attachment. The plant extracts were assessed at concentrations ranging from 3.1µg/ml – 400 µg/ml. The vehicle control wells were exposed to 2% DMSO. Actinomycin D (concentrations ranging between 3.91×10^{-4} µg/ml - 0.05 µg/ml) was used as positive control. Plant extracts in medium without cells were used as blank colour controls. The microtiter plates were incubated for 72h. Subsequently, 50 µl of XTT reagent (0.3 mg/ml) was added and the plate was further incubated for another 2 h. A multi-well plate reader (BIO-TEK Power-Wave XS) was used to measure the absorbance of the colour complex at 490 nm with a reference wavelength set at 690 nm.

Sulforhodamine-B assay (SRB) assay

The anti-cancer activity was measured according to the method by Madhunapantula et al., 2008. In brief,100 µl of HCT-116 and HCT-15 cells were plated in a 96-well plate at a density of 0.5×10^4 cells/ml. After 48h incubation at standard growth conditions, the cells were exposed to the extracts at concentrations ranging between 0-200 µg/mL for 72h.

The SRB assay was performed as specified by Skehan et al., 1990 to determine the cell viability. Experimentally, cells were fixed in 1/4th volume of cold 50% (w/v) TCA at 4˚C for 1 h. Thereafter, the media was decanted and the wells washed with water (200 µl × 4 times) to remove any remaining TCA and serum proteins. The plates were dried and then incubated with 100 µl 0.4 % SRB for 30 min to stain the cellular proteins. Quick washing with 1 % acetic acid (200 µl× 4 times) removed any unbound SRB while, the bound SRB was solubilized in 10.0mM Tris base solution (100 µl/well). A multimode plate reader was used to measure the absorbance at a wavelength of 490 nm.

Antioxidant assays

DPPH

The DPPH scavenging activity of the extracts were measured following the method by Du Toit et al., 2001. The plant extracts and positive control, vitamin C, were evaluated at concentrations ranging from 0.78 µg/ml to 100 µg/ml. DPPH ethanolic solution (0.04 M) was added to each sample well, whereas distilled water was added to the negative color control wells. The plates were incubated at room temperature in the dark for 30 minutes. Following the incubation period, the absorbance was measured using a BIO-TEK Power-Wave XS multiplate reader at a wavelength of 515 nm.

Nitric oxide

The nitric oxide scavenging potential of the samples was determined by utilizing sodium nitroprusside as a nitric oxide generator and Greiss reagent as the detector. The method by Mayuret al., 2010 was followed to determine the scavenging activity of the samples. The samples and Vitamin C, positive control, were evaluated at concentrations ranging from 15.6 µg/ml to 2000 µg/ml. Sodium nitroprusside solution (0.01 M) was added to each well and incubated for 90 min, in light at room temperature. Subsequently, Griess reagent solution (1:1) was added to each well. For the negative color controls, distilled water was added instead of Griess reagent. The absorbance values of the samples were read using a multi-well plate reader (BIO-TEK Power-Wave XS) set at a wavelength of 546 nm.

Superoxide

The method by Hyland et al., 1983 was used to determine the superoxide scavenging activity of the extracts, which, involves the use of alkaline DMSO to generate superoxide anions. In short, 100 µl of alkaline DMSO (5 mM NaOH) was added to all the wells of a 96-well microtiter plate. Serial dilutions were made to final concentrations which ranged from 3.90625 µg/ml – 500 µg/ml. Next, 10 µl of NBT (10 mg NBT, 10 ml DMSO) was added to all the sample wells, while10 µl of DMSO was added to the color control wells. The absorbance of the plates were read using a multi-well plate reader (BIO-TEK Power-Wave XS) set at a wavelength of 560 nm.

Table 1: Traditional usage, the compounds isolated and biological activity tested of the plants selected for the study.

Plant	Plant part	Traditional usage	Anticancer activity reported	Compounds isolated	Reference
Combretum molle R. Br. Ex G. Don	Fruit	Aid in childbirth.	-	-	Watt & Breyer-Brandwijk, 1962
	Leaves	Wound dressing; antidiarrheal; anthelminthic; dropsy; chest complaints; and as an aid in childbirth.	-	-	Drummond & Coates-Palgrave, 1973; Haerdi, 1964; Kokwaro, 1976; Kerharo, 1974
	Stem bark	Angina and stomach problems.	-	-	Kerharo, 1974; Watt & Breyer-Brandwijk, 1962
	Roots	Wound dressing; hookworm; snakebites; leprosy; general body swellings; fever; stomach pains; constipation; sterility and abortion.	-	-	Drummond & Coates-Palgrave, 1973; Watt & Breyer-Brandwijk, 1962; Kokwaro, 1976; Chhabra et al., 1989
	Stem	-	Cytotoxicity	-	Fyhrquist et al. 2006
	Leaves	-	Cytotoxicity	-	Fyhrquist et al. 2006
	Leaves	-	Anti-inflammatory	-	McGaw et al., 2001
	-	-	-	Combretene A; Combretene B	Bahar et al. 2004
	-	-	-	β-D-glucopyranosyl 2α,3β,6β-trihydroxy-23-galloylolean-12-en-28-oate; Combregenin; Arjungenin; Arjunglucoside I; Combreglucoside	Kemvoufo et al. 2008
	-	-	-	Sericoside; Arjunglucoside II; Punicalin	Asres et al. 2001
	-	-	-	Mollic acid; Mollic acid 3ß-O-glucoside; Mollic acid 3ß-O-arabinoside; Mollic acid 3ß-O-Xyloside	Pegel & Rogers, 1985
	-	-	-	2,6-dihydroxy-2,3,6-	Kovács et al. 2008

				trimethoxyphenanthrene; 3,6-dihydroxy-2,4,7-trimethoxyphenanthrene; 2,6-dihydroxy-4,7-trimethoxy-9,10-dihydrophenanthrene; 6,7-dihydroxy-2,3,4-trimethoxy-9,10-dihydrophenanthrene	
	-	-	-	3,4-dihydroxy-4,5-dimethoxybibenzyl	Letcher et al. 1972
Euclea crispa subsp. *crispa* (Thunb.) Gürke	Roots	Coughs	-	-	Maroyi, 2013
	Unspecified parts	Melanoma skin cancer	-	-	Gramham et al. 2000
	Leaves	-	-	Hyperoside; quercitrin; epicatechin; (+)- catechins; gallocatechin	Pretorius et al. 2003
	Root bark	-	-	Lupeol; botulin; oleanolic acid	Sibanda et al. 1992
Sideroxylon inerme L.	Bark	Skin hyperpigmentation; gall sickness in stock and red water in cattle	Antioxidant activity; cytotoxicity	Epigallocatechin gallate; procyanidin B1.	Momtaza et al. 2008;
	Stem bark	Emetic	-	-	Chhabra et al. 1993
	Roots	Conjunctivitis; hernia; coughs; and paralysis	-	-	Chhabra et al. 1993
	Bark	Tonics to calves and goats	-	Cinnamic acid, kaempferol and leucanthocyanidins	Hutchings et al., 1996
Terminalia prunioides M.A. Lawson	Unspecified parts	Fungal infections	-	-	Fyhrquist, 2007
		Skin diseases	-	-	Neuwinger, 1996

Statistical analysis

A minimum of three experimental repeats were performed and each experiment was performed in triplicate to calculate the fifty percent inhibitory concentrations (IC_{50}) of the samples. One-way Anova was used to evaluate the significant difference between the plant extracts and the positive controls for the cell proliferation and antioxidant assays. The IC_{50} values and one-way Anova analysis (Turkey method) were done by using GraphPad prism 4 software.

Results and Discussion

Plant extraction yield

The ground plant material was extracted with ethanol and the percentage yield for each sample was calculated. The plant material weight and yield percentage results are given in table 2. The leaves extract from *C. molle* had the highest percentage yield (41%), whereas the *C. molle* fruit extract had the lowest percentage yield (6.5%). This finding could indicate high variability in the chemical composition of the different plant parts of the *C. molle* tree. *E. crispa* subsp. *crispa* had the second highest percentage yield (20%) followed by *S. inerme* (18%) and *T. prunioides* (14%). It would have been expected that the plant with the highest powdered material weight, *E. crispa* subsp. *crispa*, would also yield the highest percentage yield. Although ethanol is considered a more polar solvent, it does have the ability to extract non-polar compounds to a certain extent. Some of the plants might very well contain more non-polar compounds than polar compounds, which could denote why the weight of the powdered material is not directly proportional to the percentage yield among different plant species.

Table 2: Weights and percentage yield results for the plant extracts.

Plant	Powdered weight (g)	Extract weight (g)	% Yield
Combretum molle R. Br. Ex G. Don (leaves)	22.4	9.2	41
Combretum molle R. Br. Ex G. Don (fruits)	25.6	1.7	6.5
Euclea crispa subsp. *crispa* (Thunb.) Gürke (leaves and stems)	43.3	8.6	20
Sideroxylon inerme L. (leaves and stems)	13.3	2.4	18
Terminalia prunioides M.A. Lawson (leaves and stems)	22.1	3.2	14

Anti-proliferative activity

The anti-proliferative activity of the plant extracts was evaluated against the A431, HCT-116. HCT-15; HeLa; MCF-7 and UCT-Mel 1 cancerous cell lines. In addition, the anti-proliferative activity of these samples was evaluated against a normal phenotype cell line, the HaCat cell line. The XTT colorimetric assay was used to evaluate the anti-proliferative activity of the plant extracts against a range of cell lines. Mitochondrial dehydrogenase, an enzyme present in viable cells, reduces the yellow coloured water soluble form of XTT to an orange coloured insoluble formazan product (ATCC XTT Cell proliferation assay kit instruction manual, 2011). The results are given in table 3 as IC_{50} values, which denotes the concentration at which fifty percent of the cell proliferation and growth of the cells inhibited. Overall, the plant extracts showed the highest anti-proliferative activity on the A431 human epidermoid carcinoma cell line, with the *C. molle* fruit extract having activity with a low IC_{50} value of 23.2 µg/ml. The *C. molle* fruit extract has also shown to have noteworthy inhibitory effects on the growth and proliferation of some of the other cancerous cell lines including the HeLa, MCF-7 and UCT-Mel 1 cell lines with IC_{50} values found to be ranging from 48.7 to 51.3 µg/ml. Although these results show the *in vitro* potential, of the *C. molle* fruit extract to inhibit the growth and proliferation of cancerous cells, the low IC_{50} value of 45.9 µg/ml obtained for the normal HaCat cell line indicates that the extract might be more toxic towards the normal cells than those cancerous cells.

The leaf and fruit extracts of *C. molle* showed growth inhibitory effects on the HCT- 15 human Duke's type C (lymph node metastasis) (Frederiksen et al., 2003), colorectal adenocarcinoma at low IC_{50} values of 14.9 µg/ml and 24.2 µg/ml, respectively. This finding is curious when considering that no activity was found for the leaf and fruit extracts of *C. molle* on the HCT-116 which is also a human colorectal carcinoma cell line (Duke's type D- liver metastasis) (Ahmed et al., 2013). A study by Ahmed et al., 2013 showed that there are some gene mutation variant differences among the HCT-15 and HCT-116 cell lines. The HCT-15 cell line has gene mutation variants E545K and D549N for the PI3KCA gene, whereas the HCT-116 cell line showed a H4107R gene mutation variant. The HCT-15 cell line also had a S241F gene mutation variant for the TP53 gene while the HCT-116 cell line had no mutation variants for

this gene, displaying the wild type variant. The findings of the study showed that there are differences between cancerous cell lines even though the cell lines originate from the same type of tissue and disease. Therefore, it might be a possibility to find different activities of a particular sample on different cell lines originating from the same type of tissue and disease.

Table 3: Effect of the extracts on the cell proliferation of various cell lines after 72h treatment.

Treatment	IC_{50} (µg/ml) ± SD						
	A431	HaCat	HCT-15	HCT-116	HeLa	MCF-7	UCT-Mel 1
Combretum molle R. Br. Ex G. Don Fruit extract	23.2 ± 0.8***	45.9 ± 7.0***	24.2 ± 0.028***	>200	48.7 ± 8.0***	50.4 ± 0.6***	51.3 ± 0.1***
Combretum molle R. Br. Ex G. Don Leaf extract	68.6 ± 4.0***	104.3 ± 0.3***	14.9 ± 0.054***	>200	>400	71.8 ± 1.0***	112.2 ± 0.8***
Euclea crispa subsp. *crispa* (Thunb.) Gürke	41.8 ± 0.4***	167.2 ± 4.0***	125.0 ± 0.034***	148.5 ± 8.9***	100.3 ± 6.0***	45.7 ± 7.0***	70.9 ± 3.0***
Sideroxylon inerme L.	46.7 ± 2.0	119.2 ± 0.8	N/D	137.2 ± 3.0***	>400	93.1 ± 6.0***	90.1 ± 3.0***
Terminalia prunioides M.A. Lawson	158.6 ± 0.05***	>400	N/D	>200	>400	140.6 ± 7.0***	140.4 ± 8.0***
Actinomycin D[a]	0.28 ± 0.018	0.6 ± 1.8 × 10^2	-	-	2.2 × 10^3 ± 5.0	1.7 × 10^3 ± 5.0	2.7 × 10^2 ± 4 × 10^4
Oxaliplatin[b]			41 ± 4.2	15.40 ± 5.2			

**P-value < 0.01; ***P-value < 0.001
A431: Human epidermoid carcinoma cell line
HaCat: Human keratinocyte cell line
HCT-116: Human colorectal carcinoma cell line
HeLa: Human cervical adenocarcinoma cell line
MCF-7: Human breast adenocarcinoma cell line
UCT-Mel 1: Human pigmented malignant melanoma cell line
IC_{50}: Fifty percent inhibitory concentration
SD: Standard deviation
N/D: Not determined
[a]: Positive control for the A431; HaCat; HeLa; MCF-7 and UCT-Mel 1 cell lines
[b]: Positive control for the HCT-15 and HCT-116 cell line.

The *E. crispa* extract and the *S. inerme* extract showed promising activity on the A431 cellline with IC_{50} values of 41.8 µg/ml and 46.7 µg/ml, respectively. The *E. crispa* extract also showed anti-proliferative activity on the MCF-7 breast adenocarcinoma cell line with a low IC_{50} value of 45.7µg/ml. Given that the IC_{50} value found for the *E. crispa* extract on the HaCat cell line is high (167.2 µg/ml), it therefore indicates a much better safety margin than the *C. molle* fruit extract. As with *the E. crispa* extract, the *S. inerme* extract was found to have a relatively good safety margin due to its high IC_{50} value of 119.2 µg/ml found against the HaCat cell line, when considering its activity on the A431 cell line. The *C. molle* leaf extract high to low anti-proliferative activity on the HCT-15, A431, MCF-7, HaCat and UCT-Mel 1 cell lines, while no activity was found against the HCT-116 or HeLa cell lines, up to the highest concentrations evaluated. A study by Fyhrquist et al., 2006 showed that a methanol leaf extract of *C. molle* had growth inhibitory activity on the T24 bladder cancer cell line with an IC_{50} value of 27.7 µg/ml. The research by Fyhrquist et al., 2006 also indicated that a methanol root extract of *C. molle* had similar activity to the methanolic leaf extract of *C. molle* on the T24 bladder cancer cell line, while only moderate growth inhibitory activity was found for both of the extracts against the HeLa and MCF-7 cell lines.

Antioxidant activity

The antioxidant potential of the extracts was determined by evaluating their capacity to scavenge the DPPH free radical, the nitric oxide reactive nitrogen species and the superoxide reactive oxygen species. The IC_{50} values are shown in table 4 and the dose-response curves of the extracts for the DPPH and nitric oxide antioxidant assays are given in figure 1 and 2, respectively. All the plant extracts tested in this study showed scavenging activity for the DPPH radical at low concentrations and scavenging activity of the nitric oxide reactive nitrogen species and the superoxide reactive oxygen species at high concentrations. Since these three molecules are different from one another, it was expected that each plant

extract would react differently to each radical. The *Terminalia prunioides* (1.8 µg/ml)and *Combretum molle* (1.9 µg/ml)leaf extracts have shown exceptional DPPH radical scavenging activity as compared with the positive control, vitamin C (1.9 µg/ml). A study by Masoko & Elof (2007) have shown that some of the extracts of *Combretum molle* and *Terminalia prunioides* do have DPPH scavenging activity. This study employed the use of a qualitative DPPH assay in which the leaf acetone extract showed strong DPPH scavenging activity where as the methanol extract of *Terminalia prunioides* and the acetone and methanol leaf extracts of *Combretum molle* has moderate DPPH scavenging activity. In the case of both plants, the hexane and dichloromethane leaf extracts showed no DPPH scavenging activity. The present study also showed that the ethanolic fruit extract of *Combretum molle* possess DPPH scavenging activity at a low IC_{50} value of 5.1 µg/ml.

Table 4: The antioxidant activity of plant extracts for the DPPH, nitric oxide and superoxide scavenging assays.

Sample	IC_{50} (µg/ml) ± SD		
	DPPH	Nitric oxide	Superoxide
Combretum molle R. Br. Ex G. Don Fruit extract	5.1±0.05***	180.3±1.2***	166.7±1.5***
Combretum molle R. Br. Ex G. Don Leaf extract	1.9±0.006	77.46±0.3***	124.4±3.9***
Euclea crispa subsp. *crispa* (Thunb.) Gürke	2.5±0.02***	99.92±0.9***	164.6±13.2***
Sideroxylon inerme L.	11.5±0.04***	131.5±0.4***	115.6±15.6***
Terminalia prunioides M.A. Lawson	1.8±0.007**	86.13±0.2***	135.9±10.5***
Vitamin C[a] Quercetin[b]	1.9±0.005	62.74±0.9	17.35±2.8

**P-value < 0.01; ***P-value < 0.001
DPPH: 2,2-diphenyl-1-picrylhydrazyl
IC_{50}: Fifty percent inhibitory concentration
SD: Standard deviation
[a]: Positive control for the DPPH and Nitric oxide scavenging assays
[b]: Positive control for the Superoxide scavenging assay

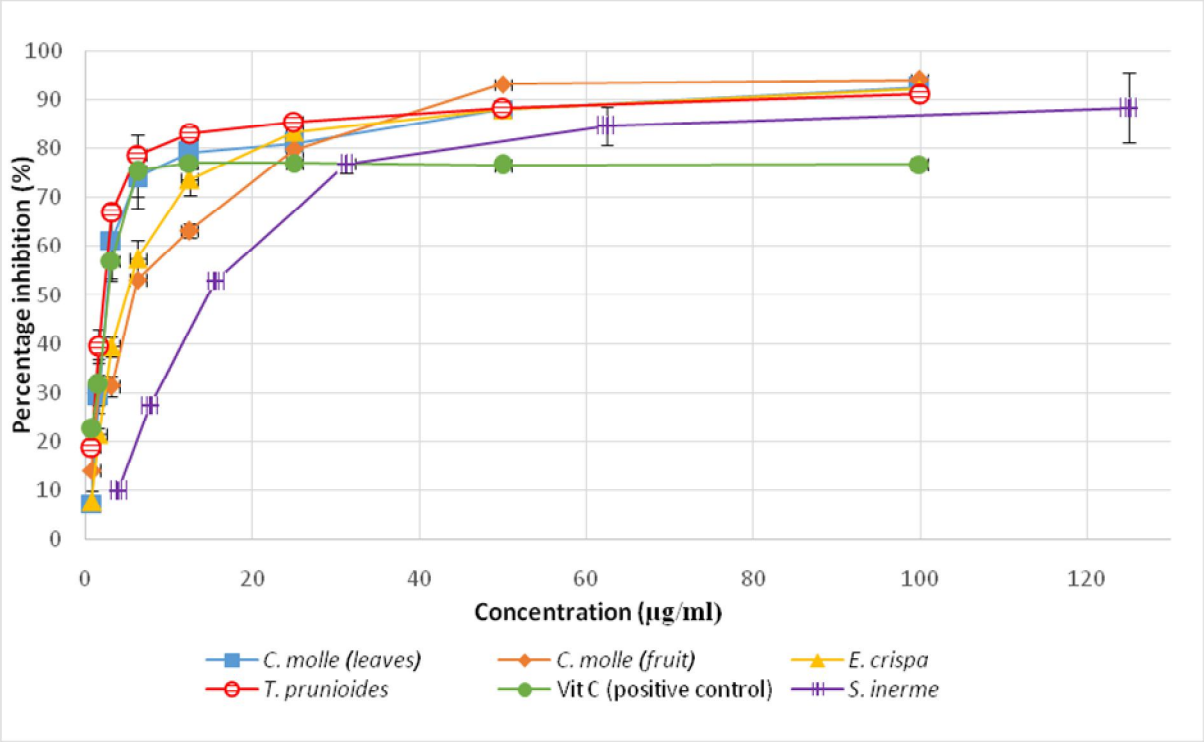

Figure 1: The dose-response curves of the inhibition of DPPH free radicals by the ethanolic plant extracts, *C. molle* (leaves); *C. molle* (fruit); *E. crispa*; *S. inerme*; and *T. prunioides*, and the positive control, Vitamin C.

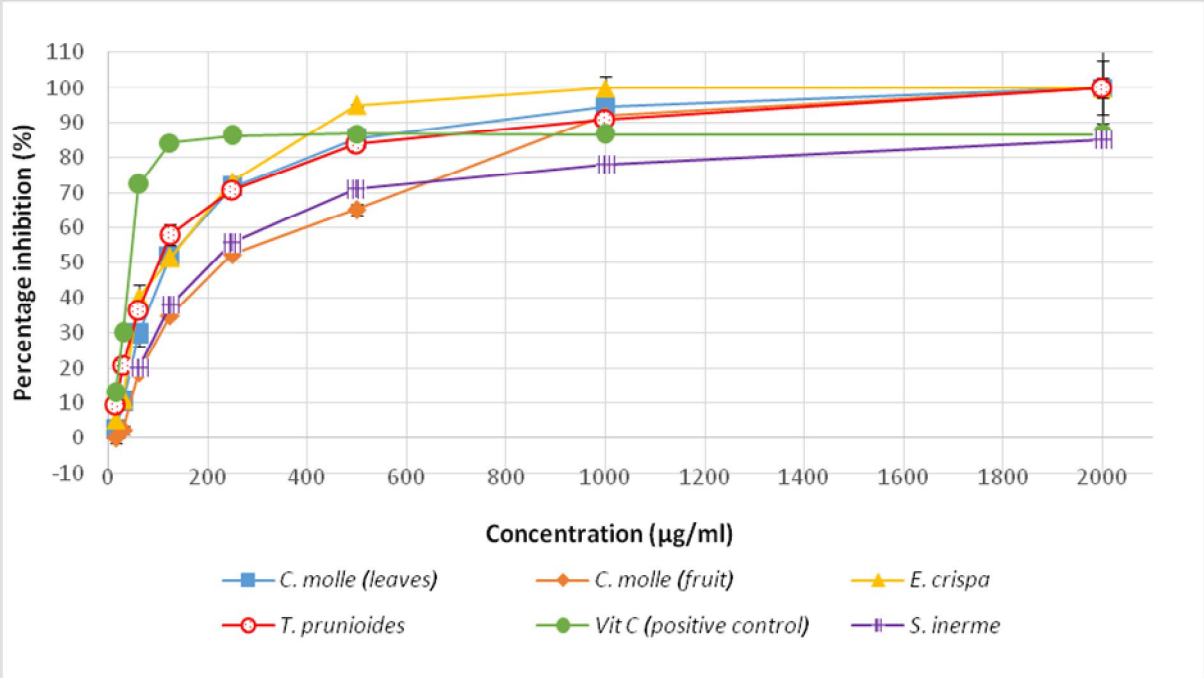

Figure 2: The dose-response curves of the inhibition of Nitric oxide reactive nitric species by the ethanolic plant extracts, *C. molle* (leaves); *C. molle* (fruit); *E. crispa*; *S. inerme*; and *T. prunioides*, and the positive control, Vitamin C.

The *Euclea crispa* extract also showed DPPH scavenging activity at an IC_{50} value of 2.5 µg/ml. Although this result compares well to a study by Shahid (2012) in which the IC_{50} values for DPPH scavenging activity from various extracts of *Euclea crispa* were shown to range from 0.84 µg/ml to 4.7 µg/ml, another study by Shahid (2012) indicated an IC_{50} value of 134.46 µg/ml for a hexane extract of *Euclea crispa*. The *Sideroxylon inerme* extract showed the activity at the highest concentration among the plant evaluated with anIC_{50}value of 11.5 µg/ml, though a study conducted by Momtaz et al., 2008 obtained an IC_{50} value of 1.54 µg/ml for the methanolic bark extract of *Sideroxylon inerme*. Differences in results obtained between research studies could be attributed to the use of different plant parts and solvents. Although the extracts did not show scavenging activity in the nitric oxide and superoxide assays at low concentrations, the extracts did indicate to have scavenging potentials comparable to that of Vitamin C for the DPPH free radical. This activity found in this study suggests that further research on the antioxidant activity of these extracts should be conducted to provide a basis for their possible use as chemopreventive agents.

Conclusion

This study was conducted to evaluate the *in vitro* anti-cancer and chemopreventive potential of four indigenous South African plants commonly used in traditional medicine. The results indicated that three of the plant extracts, *Combretum molle* fruit extract; *Euclea crispa* subsp. *crispa* and *Sideroxylon inerme*, had anti-proliferative activity on the A431 cell line at low concentrations. The leaf and fruit extracts of *Combretum molle* were observed to have potent growth inhibitory activity on the HCT-15 cell line. The *Euclea crispa* subsp. *crispa* extract showed anti-proliferative activity on the MCF-7 cell line at a low concentration. All the extracts showed antioxidant potential by scavenging of the DPPH free radical. As such, this study provides the initial evidence of the potential of these extracts as anti-proliferative agents of cancerous cells and their possible chemopreventive activity via their antioxidant properties.

Conflict of interest: Authors declare that this research presents no conflict of interests.

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