***In vitro* cytotoxicity, antioxidant and anti-inflammatory activities of *Pittosporum viridiflorum* Sims and *Hypoxis colchicifolia* Baker used traditionally against cancer in Eastern Cape, South Africa**

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Highlights

• *Pittosporum viridiflorum* leaf acetone extracts were cytotoxic against four cancer cells.
• *Pittosporum viridiflorum* leaf water extract had anticancer potential on breast cancer cells.
• Excellent 15-LOX inhibition shown by leaf acetone extract of *P. viridiflorum*
• *Hypoxis colchicifolia* had weak anticancer, anti-inflammatory and antioxidant potential.
• *Hypoxis colchicifolia* and *P. viridiflorum* were not toxic to Vero and bovine dermis cells.

Abstract

Cancer is one of the leading causes of death both in developed and developing countries and was responsible for the loss of 9.6 million lives in 2018 alone. Development of drug resistant strains of cancer has hampered cancer therapy. Worldwide, plants have been reported to play a major role in the traditional treatment of cancer. Irinotecan, paclitaxel, and vincristine are some of the examples of plant derived compounds that are used as anticancer agents. This study evaluated the cytotoxicity, anti-inflammatory, and antioxidant activities of *Pittosporum viridiflorum* Sims and *Hypoxis colchicifolia* Baker, two plants used in South African traditional medicine for treating cancer. Acetone, ethanol, and water (hot and cold) extracts were screened against four human cancer lines (breast MCF-7, colorectal Caco-2, lung A549 and cervical

Hela) for anticancer potential. Two normal cells; monkey kidney Vero and bovine dermis were used to test for cytotoxicity, and *Salmonella typhimurium* TA98 and TA100 were used to determine genotoxicity of the samples. The acetone extract of *P. viridiflorum* had significant cytotoxicity activity towards the cancer cells (MCF7, Caco-2, A549 and Hela) with LC_{50} values ranging from 3.16-26.87 µg/ml. The same extract had noteworthy anti-inflammatory activity against 15-lipoxygenase with IC_{50} value=5.01 µg/ml and was not toxic against Vero and bovine dermis cells nor was it genotoxic. This warrants further extensive study to determine the mechanism of action of anticancer and anti-inflammatory activities as well as to identify specific compounds responsible for the observed activities.

Keywords: Anticancer; anti-inflammatory; antioxidant; cytotoxicity; medicinal plants; MTT assay

1. Introduction

Cancer is a group of diseases involving uncontrolled growth of abnormal cells that have the potential to invade and spread to other body parts (Dantu et al., 2012; Dai et al., 2016). Imbalances in the body result in growth of these abnormal cells, and cancer may be treated by correcting the imbalance. Internal factors (such as inherited mutations, hormones, immune conditions, and mutations) and external factors (which include tobacco, infectious organisms, chemicals, and radiation) may act together or sequentially to initiate or promote cancer development (Chandra and Nagani, 2013; Khazir et al., 2014). Worldwide, there are over 100 different cancers affecting humans with each classified by the cell type that is initially affected (Shaikh et al., 2014). Breast, prostrate, lung, colorectal, cervix and stomach cancers are reported as the most common cancers worldwide, with colorectal cancer noted to have the

highest incidence and mortality rates (Jacob-Herrera et al., 2016). In South Africa, the rates of cancer are increasing with breast cancer reported as the second leading form of cancer among South African women, and the most common type in women worldwide (Madhuri and Pandey, 2009). Cancer is one of the most aggressive human killers in the world, reported to have killed 9.6 million people in 2018 alone (WHO, 2018). It is estimated that by 2020 new cancer cases will reach 15 million, and 70% will be in developing countries (Kuete et al., 2016).

Many efforts have been made for cancer treatment, and they include immunomodulation, surgical removal of tumors, radiation and chemotherapy (Tagne et al., 2014). However, the currently used standard cancer treatment methods have not successfully fulfilled their expectations, despite their cost, as they have side effects, thus reducing the quality of life dramatically (Solowey et al., 2014). Although breast cancer can be treated using herceptin (a biological drug), this drug is very expensive, and its activity is limited to certain types of tumors. Additionally, cancer cells have acquired drug resistance not only to a single but to several drugs, and this is one of the major problems in the management of this disease (Saeed et al., 2016). This phenomenon is defined as cross-resistance of cancer cells to the cytostatic and cytotoxic activity of several and diverse anticancer drugs in terms of structure or functionality and molecular target (Saeed et al., 2016). This has created a demand for development of effective, and yet affordable anticancer drugs.

Lipoxygenases (LOXs) are inflammatory enzymes associated with numerous human cancer types such as prostate, lung, breast, and colon (Sacan and Turhan, 2014). Therefore, finding new LOX inhibitors for anticancer therapy is very important. Furthermore, it is increasingly recognised that oxidative stress that occurs because of an imbalance between the formation and neutralization of pro-oxidants causes many of today's diseases including cancer (Shaik et al., 2014; Karker et al., 2016). Free radicals seek stability by pairing with biological macromolecules (DNA, proteins, and lipids) in healthy human cells causing lipid peroxidation,

DNA and protein damage, thus initiating oxidative stress (Karker et al., 2016). These changes contribute to cancer and inflammatory diseases. Oxidation and free radicals might be imperative in carcinogenesis at multiple tumor sites as all cells are exposed to oxidative stress (Shaik et al., 2014).

Research reports worldwide indicate that cancer patients resort to medicinal plants as a simultaneous resource or an alternative to find cure, and more than 3 000 species with anticancer properties are acknowledged (Jacob-Herrera et al., 2016). The US National Cancer Institute recognized the potential of natural products as sources of anticancer drugs in the 1950s and has since contributed to novel naturally occurring anticancer drug discovery (Fouche et al., 2008). Vincristine, irinotecan, etoposide and paclitaxel serve as some of the remarkable examples of pharmaceuticals from natural products, providing justification for the search of new molecules in nature (Kuete et al., 2011). Although the natural product approach to discovery of anticancer agents has been successful worldwide, in South Africa there are still only few reports on medicinal plants used in cancer therapy irrespective of the fact that some plants from this region are known to exhibit cytotoxicity towards cancer cells (Fouche et al., 2008).

Secondary metabolites from plants with antioxidant activity assist in alleviating oxidative stress by preventing free radical generation or by scavenging free radicals. Therefore, drug candidates with antioxidant and anti-inflammatory activities are favoured anticancer agents.

This study aimed to determine the cytotoxicity towards cancer cells, antioxidant, and anti-inflammatory activities of extracts of two plants used in the traditional treatment of cancer in South Africa. *Pittosporum viridiflorum* Sims (Pittosporaceae) and *Hypoxis colchicifolia* Baker (Hypoxidaceae) are amongst the plants documented in Eastern Cape, South Africa in traditional cancer therapy (Koduru et al., 2007). *H. colchicifolia* is one of the most sought after *Hypoxis* species in African traditional medicine for preparation of herbal remedies, teas, and tinctures

(Mills et al., 2005). The application of *Hypoxis* in traditional anticancer therapy is one of the most prominent uses of the plant species. The corm decoction is administered orally for treatment of cancer by the Xhosa people (Koduru et al., 2007). Infusions and decoctions of corms are used as convalescent and strengthening tonics in children and adults for tuberculosis, prostatic hypertrophy, and testicular tumours (Mukuka, 2010). The "rootstocks" are used by the Zulu people to treat urinary tract infections, heart weaknesses, nervous disorders and internal tumors (Mills et al., 2005; Mukuka, 2010). *P. viridiflorum* finds use in traditional medicine for the treatment of cardiovascular diseases, cancer, chest pains and abdominal pains (Madikizela and McGaw, 2017). These two plants were selected for this study based on their traditional use in cancer therapy, and the assays conducted were chosen against the background that several studies have revealed that many antioxidant compounds have anti-inflammatory and anticancer activities (Koduru et al., 2007, Sekerler eta al., 2018). Additionally, plant derived extracts or compounds with anti-inflammatory and antioxidant activities to support their anticancer efficacy have enhanced potential for development as effective treatments or adjuncts to cancer treatment (Shaik et al., 2014).

2. Materials and methods

2.1. Plant material collection and extraction

H. colchicifolia (corms) and *P. viridiflorum* (leaves and bark) were collected in Pietermaritzburg and Pretoria, South Africa, respectively. Voucher specimens were prepared and deposited at the H.G.W.J. Schweickerdt Herbarium (PRU) at the University of Pretoria for identification. The voucher specimen number for *P. viridiflorum* is PRU 120025, and for *H. colchicifolia* is PRU 124355. The collected plant material (corms and bark first cut into small pieces) was air-dried at room temperature (25 ºC), and ground into powder. Acetone, 70% ethanol, and water (cold and hot) were used for extract preparation. Powdered plant material

(2 g) was extracted with 40 ml of the solvent by sonicating for 1 h. The hot water extract was prepared by extracting 2 g of dried material in 30 ml of distilled water and boiling for 5 min, cooled for 1 h at room temperature, and then filtered. The extracts were then filtered through Whatman no.1 filter paper, dried and kept at 8ºC until use.

2.2. The cytotoxicity assay

Cytotoxicity of the extracts was determined using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-2H-tetrazolium bromide (MTT) colorimetric assay according to the method of Mosmann (1983). Four human cancer cell lines were used; epitheloid cervix (HeLa), epithelial colorectal (Caco-2), breast (MCF-7), and alveolar basal epithelial (A549) adenocarcinoma. Two normal cells; Vero African monkey kidney and bovine dermis (BD) were used in this study. The human cancer cells were grown in Dulbecco's Modified Eagle's (DMEM, HyClone, Thermo Scientific, Aalst, Belgium) supplemented with 0.4 mM L-glutamine, sodium pyruvate and 10% foetal bovine serum (FBS, HyClone, Thermo Scientific) for a week. Minimal Essential Medium (MEM) supplemented with 0.4 mM L-glutamine, sodium pyruvate and 5% FBS was used to grow Vero and BD cells. Briefly, 100 µl of the cells were inoculated at plating densities based on growth characteristics of each cell in 96 well microtitre plates and incubated at 37 ºC in a 5% CO_2 incubator for 24 h. Following 24 h incubation of cells, 100 µl of extract were added and the plates were incubated for 48 h at 37 ºC in a 5% CO_2 incubator. The microtitre plates were removed from the incubator and washed with 200 µl of phosphate buffered saline (PBS) after the removal of the supernatant, which was then replaced by addition of 200 µl of the medium (DMEM for cancer cells and MEM for normal cells) and 30 µl of 5 mg/ml MTT. The microtitre plates were incubated for 4 h at 37ºC. After incubation, the supernatant was removed and 50 µl of dimethylsulfoxide (DMSO) were added to each well, and a microplate reader was used to measure the amount of MTT reduction at a wavelength of 570 nm and

reference wavelength of 630 nm. The experiment was repeated 3 times. To calculate percentage viability the following formula was used:

$$\text{Percentage cell viability} = \left\{\frac{\text{Mean absorbance of sample}}{\text{Mean absorbance of control}}\right\} x100$$

The LC_{50} values were calculated from the graph plotted as inhibition percentage against the concentration.

2.3. The 15-lipoxygenase inhibitory assay

The LOX inhibitory assay measures the conversion of linoleic acid to linoleic hydroperoxide, in the presence of the LOX enzyme. The linoleic hydroperoxide is responsible for oxidation of Fe^{2+} in the FOX reagent to Fe^{3+} ions, which interact with acidified xylenol orange to give a colored Fe^{3+} -dye complex that absorbs a wavelength of 560 nm. An extract that contains inhibitors of LOX will reduce Fe^{3+}-dye formation complexes. The 15-lipoxygenase (15-LOX) inhibitory assay was done in a 96 well microtitre plate according to Pinto et al. (2007) with slight modifications. The 15-LOX enzyme (40 µl) was incubated with 20 µl extracts (1 mg/ml) at room temperature for 5 min. Then 40 µl linoleic acid (final concentration, 140 mM) in borate buffer (50 mM, pH 7.4) was added to all wells except the blanks (background). The mixture was incubated at 25ºC in the dark for 20 min. FOX reagent (40 µl) consisting of sulphuric acid (30 mM), xylenol orange (100 mM), iron (II) sulfate (100 mM) in methanol/water (9:1) was added to all the wells to stop the reaction. For the negative control, only 15-LOX solution and buffer were pipetted into the wells, and quercetin (1 mg/ml) was used as a positive control. After 30 min of incubation, linoleic acid (40 µl) was added to the blanks, and the complex Fe^{3+}/xylenol orange formation was measured using a microplate

reader at 560 nm. The lipoxygenase inhibitory activity was determined by calculating the percentage of inhibition of hydroperoxide production using the following formula:

% inhibition = [(absorbance of control - absorbance of blank) - (absorbance of sample - absorbance of blank)/ (absorbance of control - absorbance of blank)] × 100.

2.4. The 2,2-azino-bis (3-ethylben- zothiazoline-6-sulfonic acid) diammonium salt (ABTS) antioxidant assay

The quantitative 2,2-azino-bis (3-ethylben- zothiazoline-6-sulfonic acid) diammonium salt (ABTS) assay measures the relative ability of the tested sample to scavenge the ABTS generated in aqueous phase, as compared to the water soluble vitamin E (trolox), and this is measured at a wavelength of 734 nm (Shalaby and Shanab, 2013). ABTS radical scavenging capacity of the extracts was determined according to Re et al. (1999) in a 96 well microtitre plate. Briefly, methanol (40 µl) was added to all the wells, followed by 40 µl of extract (10 mg/ml) in the first two wells which was then serially diluted down the wells. ABTS (160 µl) was added in all the wells and the mixture was incubated at room temperature in the dark for 30 min. Methanol was used as a negative control, and extracts without ABTS as blanks, whereas trolox and ascorbic acid (1 mg/ml) were used as positive controls. The absorbance was measured at 734 nm using a microplate reader after 30 min of incubation in the dark. The experiment was repeated three times, and the percentage of ABTS inhibition was calculated using the following formula:

$$(\%)\text{inhibition} = \left\{\frac{(Absorbance\ of\ control - Absorbance\ of\ blank) - (Absorbance\ of sample - Absorbance\ of\ blank)}{(Absorbance\ of\ control - Absorbance\ of\ blank)}\right\} X100$$

The results were expressed as IC_{50}, which is the concentration of the test samples that resulted in 50 % radical scavenging activity plotted on a graph.

2.5. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant scavenging assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay involves the reduction of the stable purple DPPH radical to yellow DPPH-H with an absorption wavelength of 517 nm by the evaluated plant extract or compound. DPPH radical scavenging activity of the extracts was determined according to Brand-Williams et al. (1995) in a 96 well microtitre plate. Briefly, 40 µl of methanol was added to all the wells, and the extract (10 mg/ml) was added in the first four wells and then serially diluted down the wells. One hundred and sixty microlitres of DPPH at a concentration of 25 µg/ml in methanol was added in the first two columns, and 160 µl of methanol was added in columns 3 and 4 as blanks. Ascorbic acid and trolox (1 mg/ml) were used as positive controls, whereas methanol was used as a negative control. After 30 min of incubation in the dark at room temperature, the absorbance was measured at 517 nm using a microplate reader. The experiment was repeated three times. The results were calculated as the percentage reduction of the initial DPPH absorption in relation to the control group. The following formula was used to determine the concentration of extract that reduced DPPH colour by 50 % (IC_{50}):

Scavenging capacity (%) = 100 - [(absorbance of the sample - absorbance of the sample blank) × 100/ (absorbance of control) - (absorbance of control blank)].

2.6. Genotoxicity assay

Genotoxicity of extracts was determined using the *Salmonella* microsome assay, following the procedure of Maron and Ames (1983) modified by Mortelmans and Zeiger (2000). Two S*almonella typhimurium* strains (TA100 and TA98) were used as test organisms, and the assay was done without metabolic activation. Three concentrations of extracts (0.5, 0.05 and 0.005 mg/ml) prepared in 10% DMSO were tested. Water, and 10% DMSO were used as negative controls, whereas 4-nitroquinoline-1-oxide (4-NQO) at a concentration of 2 µg/ml was used as

a positive control. All samples were tested in triplicate. The results were expressed as the mean (±standard error) number of the revertant colonies per plate.

3. Results and discussion

3.1. Cytotoxicity of the extracts

The results for cytotoxicity activity of the tested crude extracts against four human cancer cell lines and two normal cells are summarized in Table 1 as LC_{50} values in µg/ml. The LC_{50} values of the extracts tested ranged from 3.16 to 503.00 µg/ml against cancer cells. The parameter of the National Cancer Institute (NCI) to consider a plant crude extract as toxic when the LC_{50} value is ≤ 30 µg/ml after 72 h treatment was followed (Steenkamp and Gouws, 2006; Saeed et al., 2016). Four extracts showed interesting anticancer activity with LC_{50} values below 30 µg/ml, indicating cytotoxicity towards at least one cancer cell line. *P. viridiflorum* leaf acetone extracts exhibited noteworthy anticancer activity against all cancer cells tested, with LC_{50} values ranging from 3.16 -26.87 µg/ml. Sesquiterpene glycosides, with their 6'-O-acetyl and 2'-O-acetyl derivatives from the acetone leaf extract could be responsible for the anticancer activity observed in the present study (Ramanandraibe et al., 2001). An essential oil component, β-elemene reported from the leaves of *P. viridiflorum,* is known for reducing side effects of anticancer drugs and in enhancing their therapeutic potential (Chen et al., 2012). The ethanol extract of both the leaves and bark of *P. viridiflorum* showed interesting anticancer activity against breast, cervix and colorectal cancer cells with LC_{50} values ranging from 13.28 to 23.37 µg/ml. The pentacyclic acids previously isolated from the methanol leaf extract of this plant could be responsible for the observed anticancer activity of the ethanol leaf extract (Nyabayo et al., 2015). The methanol bark extract of *P. viridiflorum* was cytotoxic towards HL-60 leukaemia cells (LC_{50}=5.15 µg/ml) in a study by Poschner et al. (2003). Seo et al. (2002) reported that the leaf methanol extract showed weak cytotoxicity towards A2780 human

ovarian cancer cells in their study, but isolated a compound (pittoviridoside) with anticancer potential (LC_{50}= 10.1 µg/ml). The cold water extracts of *P. viridiflorum* (leaves) showed interesting cytotoxic activity towards MCF-7 cells with an LC_{50} value=10.15 µg/ml, which provides some support for the traditional use of this plant in anticancer therapy. The bioactivity of a plant is not often determined by a single compound, but could be a result of a combination of compounds interacting together giving it therapeutic potential, hence the noteworthy anticancer activity observed in this study on the extracts.

Although the most prominent use of *Hypoxis* plant species is traditional anticancer therapy, for *H. colchicifolia*, all the extracts showed weak anticancer potential with LC_{50} values ranging from 50.00 to 251.95 µg/ml. The water extract of the same plant was reported to have shown no toxicity towards human liver carcinoma cells (HepG2) (Verschaeve et al., 2013). According to literature, several compounds have been isolated from *H. colchicifolia* and they include phenolic glycosides (3-hydroxy-4-*O*-β-D-glucopyranosyl benzaldehyde and 1,5-bis(3,4-dihydroxyphenyl)1-2-dihydroxy-4-pentyne-2-p-coumoroyl-β-gluco-pyranoside), pentenyne-bis-glucosidophenol, hypoxoside, rooperol, and a mixture of dehydroxyhypoxide A and B (Cumbe, 2015, Patel, 2016). Antitumor activity has been reported on pentenyne-bis-glucosidophenol (Verschaeve et al., 2013). Hypoxoside was reported to have cured the lung cancer of a patient in phase 1 clinical trials and no toxic effects were observed clinically or biochemically (Smit et al., 1995). However, according to Verschaeve et al. (2013), hypoxoside has no anticancer activity and it is converted to rooperol (which is cytotoxic) upon hydrolysis, hence the anticancer activity. Further compounds with anticancer activity from *H. colchicifolia* need to be isolated, therefore a bioguided isolation technique could produce better results. It is possible that metabolism of compounds in the plant material needs to happen *in vivo* before an anticancer effect is noted.

Concerning the medical impact of these cancers, the anticancer activity of *P. viridiflorum* could be considered as very important. As highlighted in our previous study, none of the extracts tested for both *H. colchicifolia* and *P. viridiflorum* showed toxicity towards normal cells (Vero and bovine dermis). In the present study, the water extracts of *P. viridiflorum* bark and leaves were not cytotoxic towards these same normal cells. Additionally, weak cytotoxicity towards Chang liver cells was reported in study by *P. viridiflorum* leaves acetone (IC_{50}=246.95 µg/ml) and hexane (IC_{50}=225.50 µg/ml) extracts, therefore posing less toxicity risk (Otang et al., 2014).

3.2. Antioxidant activities of the extracts

Several methods are used to determine the antioxidant activities of natural products with varying results. DPPH and ABTS are the two most commonly used free radicals to assess *in vitro* the antioxidant activity of plant extracts (Shalaby and Shanab, 2013). The radical scavenging potential of twelve extracts from two plants is illustrated in Table 2. Antioxidant activity of an extract or compound is inversely proportional to its IC_{50} value, meaning the lower the IC_{50} value the stronger the antioxidant activity. The extracts demonstrated varying degrees of antioxidant activity in the assays with IC_{50} values ranging from 2.42-249.18 µg/ml. *P. viridiflorum* bark acetone extract had the best antioxidant activity in both the DPPH (IC_{50}=2.42 µg/ml) and ABTS **(**4.31 µg/ml) assays and it was more than that of trolox. In a study done by Otang et al. (2012) determining the antioxidant potential of *P. viridiflorum* using different assays (DPPH, nitric oxide scavenging, reducing power, and hydrogen peroxide), strong antioxidant activity of the bark acetone extract was reported with IC_{50} values ranging from 0.13-0.26 µg/ml, but less than that of the positive control (vitamin C). Strong antioxidant activity of the bark ethyl acetate extract of *P. manni* (synonym of *P. viridiflorum*) with IC_{50} values at least 3 times more than trolox in DPPH (IC_{50}=177.49 µg/ml) and ABTS (IC_{50}=331.48

μg/ml) assays was reported in a study by Momeni et al. (2010). In this study, the bark extracts of *P. viridiflorum* showed much greater antioxidant activity than the leaf extracts in both DPPH and ABTS assays. Phenolic compounds are known for their strong antioxidant activity, however the antioxidant effects differ among the phenols. The strongest antioxidant activity for *H. colchicifolia* was observed for the hot water extract with IC_{50} value=12.18 μg/ml in the ABTS assay. All the extracts of *H. colchicifolia* showed weak DPPH scavenging activity with IC_{50} values ranging from 19.758-249 μg/ml. However, it was interesting to note that the water extracts of *H. colchicifolia* displayed more scavenging activity than both the acetone and ethanol extracts.

3.3. 15-LOX inhibitory activity

The results of 15-LOX inhibitory effects of twelve extracts are depicted in Table 2. A lower IC_{50} value is associated with higher 15-LOX inhibitory activity. The IC_{50} values of 15-LOX inhibition by the extracts ranged from 5.01-99.86 μg/ml. The leaf acetone extract of *P. viridiflorum* showed the best activity against the 15-LOX enzyme with IC_{50} = 5.01 μg/ml which was notably lower than that of the positive control quercetin (13.44 μg/ml). The extracts of *H. colchicifolia* did not show noteworthy anti-inflammatory activity as their IC_{50} values were more than that of quercetin. Surprisingly the hot water extract of this plant showed better activity than acetone and 70% ethanol extracts. Our study is in accordance with a previous study by Aremu et al. (2010) where *H. colchicifolia* ethanol bulb extract did not show anti-inflammatory activity against cyclooxygenase 1 and 2 (COX 1 and 2) although different parameters were used to check the activity. However, in the same study, petroleum ether, dichloromethane, and water extracts of *H. colchicifolia* were reported to have anti-inflammatory activity against COX-1 and COX-2 enzymes. Therefore, *H. colchicifoli*a bulb should be explored further for the presence of anti-inflammatory compounds.

3.4. Genotoxicity results

Although *H. colchicifolia* and *P. viridiflorum* are used for therapeutic purposes in traditional medicine, that does not mean that they are safe, as they may be hazardous in the long term. Research has shown that plants are potentially toxic in both *in vitro* and *in vivo* studies. Therefore, the genotoxicity effect of extracts was tested in this study in the Ames test without metabolic activation (detects direct mutagen detection). The results are presented in Table 3 as the number of revertant colonies per plate. The gene safety of a test sample is primarily confirmed through the bacterial-based Ames test. *S. typhimurium* TA98 and TA100 were selected for this study because of their sensitivity and ability to detect a large proportion of known mutagens (Verschaeve and Van Staden, 2008). For an extract to be considered genotoxic, it must show a dose-dependant increase in the number of revertant colonies, or the revertants must be equal to or more than twice the negative control (Maron and Ames, 1983). Previously, we determined genotoxicity of *H. colchicifolia* (bulb acetone, ethanol, water, and hot water) and *P. viridiflorum* (bark acetone, ethanol and water, and leaf acetone and ethanol) extracts (Madikizela and McGaw et al., 2018). When analysing our previous results and the current findings, almost all the extracts showed no genotoxicity against *S. typhimurium* strains tested as they did not show a dose-dependent increase in the number of revertant colonies, nor were colonies equal to or more than twice those of the negative control. However, the bark (acetone and hot water) and leaf (70% ethanol and hot water) extracts of *P. viridiflorum*, and bulb (70% ethanol and hot water) extracts of *H. colchicifolia* showed a dose-dependent increase in the number of revertant colonies when tested against *S. typhimurium* TA100 indicating that they could be genotoxic. The water extract of *H. colchicifolia* was negative in micronucleus and comet genotoxicity assays (Verschaeve et al., 2013). The extract of *H. colchicifolia* (dichloromethane) showed no genotoxicity against both *S. typhimurium* TA98 and TA100 in a previous study by Elgorashi et al. (2003). In the comet and micronucleus assay the

dichloromethane and methanol extracts were reported to have no genotoxicity (Taylor et al., 2003).

P. viridiflorum (leaf water extract) exhibited genotoxicity as the number of revertants were more than twice the negative control at a concentration of 5 mg/ml against *S. typhimurium* TA100. For more in depth genotoxicity studies, subsequent tests should be conducted such as the micronucleus assay, comet assay, and with other strains (*S. typhimurium* TA102, TA104, TA1535, TA1537) including testing for genotoxicity with metabolic activation of the test substances.

4. Conclusion

P. viridiflorum leaf acetone extract displayed interesting cytotoxicity activity towards all cancer cells tested in the study, and anti-inflammatory potential towards 15-LOX enzyme, which warrants further studies on the plant. Anticancer and antioxidant activities exhibited by the leaf ethanol extracts of *P. viridiflorum* were interesting and provides some credence for the traditional use of this plant in cancer treatment. Thus, this provides a strong motivation to determine if the plant could be a source of compounds active against MCF-7/Caco-2/HeLa cells. Both *H. colchicifolia* and *P. viridiflorum,* plant species with importance in traditional anticancer therapy, can be considered as a source for isolation, identification and development of anticancer, anti-inflammatory and antioxidant agents. It would be extremely useful to identify compounds with multiple useful biological activities. Further research should be aimed at isolating and identifying specific bioactive molecules responsible for the observed anticancer, anti-inflammatory and antioxidant activities and the precise mechanisms of action.

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Author contributions

BM conceptualized the study, did the experimental work, and drafted the manuscript. LM supervised the work, provided facilities and finances, and edited and submitted the manuscript.

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Table 1: *In vitro* cytotoxicity results against four cancerous and two normal cell lines. Some of the cytotoxicity results (those highlighted in bold) towards normal cells are published in Madikizela and McGaw (2018).

Plants species	Extract	Cell lines tested					
		A549	**Caco-2**	**HeLa**	**MCF-7**	**Vero**	**Bovine dermis**
		LC_{50} in µg/ml					
H. colchicifolia (bulb)	Acetone	60.02±0.10	70.23±1.25	61.83±0.02	63.09±0.18	**690.00±1.02**	**280.00±0.02**
	Ethanol	50.00±0.29	123.53±0.23	96.86±1.88	121.45±1.01	**890.00±0.45**	**140.00±0.04**
	Cold water	205.04±1.06	219.16±0.92	315.29±0.01	225.32±1.43	**2480.00±0.02**	**190.00±1.04**
	Hot water	240.00±1.76	145.20±1.34	251.95±0.30	210.21±0.04	**390.00±0.44**	**1360.00±0.07**
P. viridiflorum (bark)	Acetone	34.60±0.01	33.87±1.01	40.85±0.34	48.61±1.16	**470.00±0.87**	**1590.00±0.50**
	Ethanol	64.00±0.27	16.65±0.23	13.28±0.22	23.37±1.18	**910.00±1.28**	**370.00±0.67**
	Cold water	330.00±2.00	387.98±0.67	468.03±0.63	503.27±0.02	**1444.00±1.80**	**1410.00±0.08**
	Hot water	203.92±1.18	369.60±1.26	316.34±1.21	474.78±1.00	268.96±1.26	1000.00±0.67
P. viridiflorum (leaves)	Acetone	3.16±1.02	26.87±0.01	3.57±0.03	25.47±0.02	**964.84±0.82**	**40.00±1.22**
	Ethanol	60.05±1.97	21.39±1.27	21.14±1.29	15.93±1.06	**946.39±1.37**	**80.00±0.37**
	Cold water	77.41±0.08	80.07±1.03	50.79/66.37	10.20±0.73	934.70±1.33	49.21±0.10
	Hot water	69.00±1.02	66.49±0.20	57.03±1.46	70.46±1.27	977.04±1.94	94.94±1.08
Doxorubicin	-	0.98±	3.20±		2.07±	0.007±0.50	0.23±0.07

Table 2: Antioxidant activity in the DPPH and ABTS assays, and anti-inflammatory activity of crude extracts against 15-LOX enzyme represented as IC_{50} values

Plant species	Extracts	ABTS	DPPH	LOX inhibition
		IC_{50} values in µg/ml		
H. colchicifolia (bulb)	Acetone	217.02±0.07	218.10±1.65	99.86±1.21
	Ethanol	153.04±0.01	249.18±0.11	72.93±0.01
	Water	18.94±0.00	19.75±1.92	76.54±0.24
	Hot water	12.18±0.01	22.30±2.11	37.16±0.02
P. viridiflorum (leaves)	Acetone	71.18±0.06	76.20±0.09	5.01±0.01
	Ethanol	31.27±0.00	38.91±0.15	26.01±0.09
	Water	28.35±0.02	44.50±0.11	65.16±0.04
	Hot water	28.35±0.03	44.55±0.01	44.20±0.00
P. viridiflorum (bark)	Acetone	4.31±0.27	2.42±0.00	28.56±0.52
	Ethanol	9.16±0.01	5.17±0.01	29.05±0.07
	Water	8.24±0.03	16.07±0.01	13.78±1.74
	Hot water	7.34±0.00	16.07±0.02	20.59±0.67
Ascorbic acid		3.24±0.60	4.04±0.57	-
Trolox		8.03±0.02	7.13±0.18	-
Quercetin		-	-	13.44±0.01

DPPH=2,2-diphenyl-1-picrylhydrazyl, ABTS=2,2-azino-bis (3-ethylben- zothiazoline-6-sulfonic acid) diammonium salt, LOX=15-Lipooxygenase, -=not tested

Table 3: Genotoxicity against *Salmonella typhimurium* strains

Plant species	Extract	*Salmonella typhimurium* strains					
		TA 98			TA 100		
		5 mg/ml	0.5 mg/ml	0.05 mg/ml	5 mg/ml	0.5 mg/ml	0.05 mg/ml
P. viridiflorum	Bark hot water	35.00±1.00	18.67±0.67	21.00±0.58	373.33±1.33	270.33±1.78	376.33±1.83
P. viridiflorum	Leaf water	23.67±0.88	29.33±0.88	28.67±0.67	443.33±1.02	376.00±0.00	328.67±1.33
P. viridiflorum	Leaf hot water	29.67±0.67	29.00±0.00	24.67±0.33	360.67±0.33	373.67±1.19	318.33±0.33
4NQO	235.00±0.58				889.67±0.33		
10% DMSO	25.00±0.57				219.33±1.45		
Water	30.67±1.33				220.67±1.20		

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