Review article

A review on traditionally used South African medicinal plants, their secondary metabolites and their potential development into anticancer agents

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Abstract

Ethnopharmacological relevance

Approximately 70% of anticancer drugs were developed or derived from natural products or plants. Southern Africa boasts an enormous floral diversity with approximately 22,755 plant species with an estimated 3000 used as traditional medicines. In South Africa more than 27 million individuals rely on traditional medicine for healthcare. The use of South African plants for the treatment of cancer is poorly documented, however there is potential to develop anticancer agents from these plants. Limited ethnobotanical studies report the use of plants for cancer treatment in traditional medicine. Plants growing in tropical or subtropical regions, such as in South Africa, produce important secondary metabolites as a protective mechanism, which could be used to target various factors that play a key role in carcinogenesis.

Aims

The aim was to collate information from primary ethnobotanical studies on South African plants traditionally used for the treatment of cancer. Evaluation of literature focused on traditionally used plants that have been tested for their *in vitro* activity against cancer cells. Secondary metabolites, previously identified within these plant species, were also included for discussion

regarding their activity against cancer. The toxicity was evaluated to ascertain the therapeutic potential in further studies. Additionally, the aim was to highlight where a lack of reports were found regarding plant species with potential activity and to substantiate the need for further testing.

Materials and methods

A review of ethnobotanical surveys conducted in South Africa for plants used in the treatment of cancer was performed. Databases such as Science Direct, PubMed and Google Scholar, university repositories of master's dissertations and PhD theses, patents and books were used. Plant species showing significant to moderate activity were discussed regarding their toxicity. Compounds identified within these species were discussed for their activity against cancer cells and toxicity. Traditionally used plants which have not been scientifically validated for their activity against cancer were excluded.

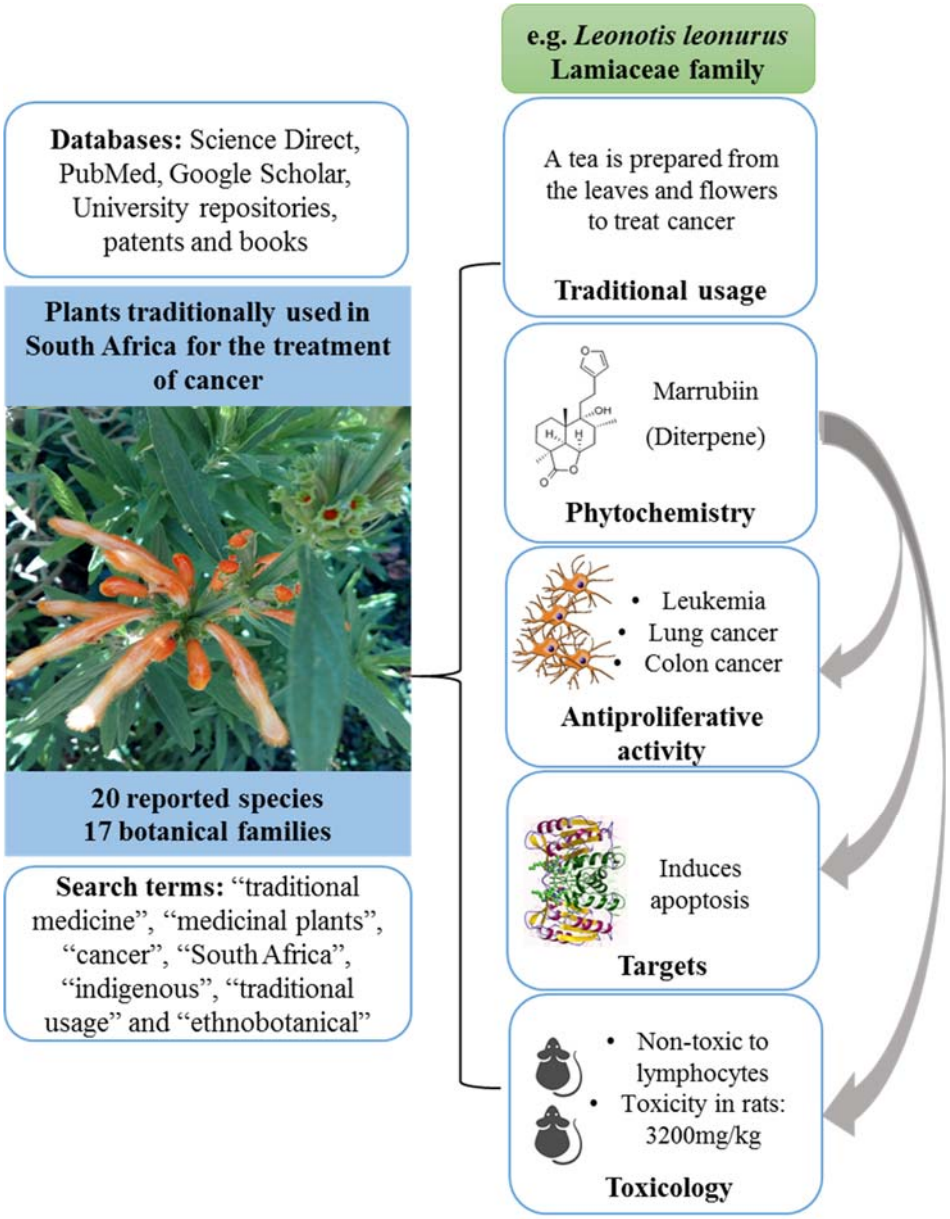
Results

Twenty plants were documented in ethnobotanical surveys as cancer treatments. Numerous scientific reports on the potential *in vitro* activity against cancer of these plants and the identification of secondary metabolites were found. Many of the secondary metabolites have not been tested for their activity against cancer cells or mode of action and should be considered for future studies. Lead candidates, such as the sutherlandiosides, sutherlandins, hypoxoside and pittoviridoside, were identified and should be further assessed. Toxicity studies should be included when testing plant extracts and/or secondary metabolites for their potential against cancer cells to give an indication of whether further analysis should be conducted.

Conclusion

There is a need to document plants used traditionally in South Africa for the treatment of cancer and to assess their safety and efficacy. Traditionally used plants have shown promising activity highlighting the importance of ethnobotanical studies and traditional knowledge. There are many opportunities to further assess these plants and secondary metabolites for their activity against cancer and their toxic effects. Pharmacokinetic studies are also not well documented within these plant extracts and should be included in studies when a lead candidate is identified.

Graphical abstract



Keywords: South Africa, Medicinal plants, Traditional usage, Natural products, Cancer

Abbreviations: American Type Culture Collection (ATCC), Cancer Association of South Africa (CANSA), European Collection of Authenticated Cell Cultures (ECACC), Fifty percent growth inhibition (GI_{50}), Fifty percent inhibitory concentration (IC_{50}), Fifty percent lethal concentration, (LC_{50}), Fifty percent lethal dose (LD_{50}), Nitric oxide (NO), Reactive oxygen species (ROS), Total growth inhibition (TGI), World Health Organization (WHO)

1. Introduction

Cancer remains one of the most devastating diseases, ranking second in the world as the leading cause of death, with approximately 8.8 million deaths reported in 2015. The World Health Organization (WHO) reported that nearly 70% of cancer deaths occur in low-and middle-income countries (WHO, 2018). During 2015, 17.5 million new cases were reported globally, which are predicted to increase to 23.6 million cases by 2030 (Global Burden of Disease and Cancer Collaboration, 2017; National Cancer Institute, 2018). According to the Cancer Association of South Africa (CANSA), approximately 115,000 South Africans are diagnosed with cancer each year. The most predominant cancer types in men include prostate, colorectal and lung cancer, whereas in female it is breast and cervical cancer (CANSA, 2019). In South Africa, numerous individuals make use of traditional medicine, particularly the use of plants, as their key source for medicine and healthcare (Mander, 1998). South Africa has an abundant amount of plant species, which have not yet been explored for their potential against cancer. Natural products obtained from plants, marine organisms and microorganisms, account for more than 60% of the currently used anticancer agents, of which approximately 25% have been sourced from plants and another 25% have been derived from plants (Juárez, 2014; Newman et al., 2002).

It is reported that of the 250,000 higher plant species in the world, only 5-15% have been examined for their bioactive compounds (Juárez, 2014). This indicates that there is a great opportunity for South African plants to be explored for their bioactive compounds and the potential activity of these compounds and medicinal plants against cancer.

The aim of this study was to identify plants traditionally used in South Africa for the treatment of cancer and to determine, through literature searches, whether these plants have been scientifically evaluated for their activity against cancer and whether promising secondary metabolites have been identified within these plants. Additionally, the objective was to highlight specific plant species and/ or secondary metabolites which showed activity against cancer in order to emphasize the need for their further evaluation.

2. Methodology

The literature search was conducted during the years 2017-2019, using various scientific databases such as Science Direct, PubMed and Google Scholar. The articles used for this review were not limited based on the year in which they were published. Scientifically published literature such as journals, patents, theses, books and book chapters were reviewed, which ranged from the year 1944-2019. Keywords that were included in the search engines were "traditional medicine", "medicinal plants", "cancer", "South Africa", "indigenous", "traditional usage" and "ethnobotanical". A total of over 400 scientific publications were reviewed to provide information regarding the traditional usage of medicinal plants and their potential into anticancer drug discovery. Furthermore, ethnobotanical surveys, which sourced first-hand information from community members, traditional healers, patients, herbalists and the elderly regarding plants traditionally used for the treatment of cancer in a specific area, were used in the literature search. Previously published reviews on the toxicity, *in vitro* cytotoxicity/ antiproliferative and *in vivo* anticancer potential of secondary metabolites were included, however these were only referenced and were not included in the discussion. Plants indigenous to South Africa were selected for this study, however literature searches on the potential activity against cancer cells and secondary metabolite isolation were not limited to plant material collected in South Africa and included studies conducted globally. Plants traditionally used in South Africa for the treatment of cancer, as summarized in Table 1, were further analysed only if significant to moderate *in vitro* activity against cancer cell lines was reported. Therefore, plants which did not show significant *in vitro* activity against cancer cells or plants which have not been reported for their activity were excluded from further discussion. In Table 1, the currently accepted species name is given, followed by the previous name or synonym as well as the vernacular names. All plant species names were validated using http://mpns.kew.org/mpns-portal and www.theplantlist.org.

3. Traditional usage of plants in South Africa for the treatment of cancer

The use of medicinal plants in South Africa as traditional remedies for cancer is not well documented. A limited amount of ethnobotanical studies have been conducted in South Africa regarding cancer treatments. Ethnobotanical studies have been performed in KwaZulu-Natal, the Eastern Cape and the Western Cape Provinces where interviews, questionnaires and general

conversations were held with traditional healers, community members, the elderly, patients and herbalists to gather information on plants used for cancer in these areas (Coopoosamy and Naidoo, 2012; Koduru et al., 2007a; Thring and Weitz, 2006). There are a few other reports by Pooley (1992); Semenya et al. (2013); Watt and Breyer-Brandwijk (1962) which documented the use of some medicinal plants for the treatment of cancer. Semenya et al. (2013), conducted an ethnobotanical study in the Limpopo Province, more specifically the Capricorn, Sekhukhune and Waterberg districts, for the use of medicinal plants for reproductive ailments, in which breast cancer was classified as a reproductive ailment.

A study conducted in Atteridgeville, Pretoria, aimed to provide an understanding of how traditional healers diagnosed and treated cancer. In most cases the diagnosis of cancer was related to numerous symptoms such as sores, growths and lumps which were often resistant to treatment, unusual discharge from the vagina which includes bleeding and infection, certain respiratory problems, unexpected weight loss and a drop in energy levels, dysuria in men and bloody diarrhea. In some cases, the traditional healers needed the help of a medical practitioner to diagnose cancer. Furthermore, it was noted that the traditional healers were aware of different cancer types and that it could be found on any part of the body. Additionally, it was noted that the type of treatment depended on the location of the cancer. For instance, cancer relating to sores would be treated using an ointment, whereas internal cancers were treated in a variety of different ways including drawing out lumps using a dressing prepared from leaves, washing the affected area using hot medicine, a blood cleanser using a mixture of herbs, inhalation of the medicine in various forms such as steam or in its dried form as well as the administration of a type of douche or enema. Cancer types which were known of but not limited to include cervical, colon, bladder, breast, lung and oesophageal cancer (Steyn and Muller, 2000). South African plants traditionally used for the treatment of cancer by various communities are described in Table 1. These have been reviewed from ethnobotanical surveys which conducted primary studies, through direct communication, to report medicinal plants traditionally used for the treatment of cancer in these areas.

Table 1

South African plants used for the treatment of cancer in traditional medicinal practices

Plant family	Species	Vernacular names	Traditional usage
Agapanthaceae	*Agapanthus africanus* (L.) Hoffmanns	English (agapanthus, African Lily, blue lily), Afrikaans (haakleli), Sotho (leta-la-phofu), Xhosa (isilakati), Zulu (icakathi, mathunga)	An aqueous infusion of the roots, which have been dried in the sun, is taken orally for cancer until the patient is cured (Koduru et al., 2007a).
Alliaceae	*Tulbaghia violacea* Harv.	English (wild garlic), Afrikaans (wildeknoflok, wildeknoffel), Xhosa (utswelane), Zulu (icinsini, isihaqa)	A decoction from the fresh bulbs is orally administered for several weeks to treat cancer (Koduru et al., 2007a).
Apocynaceae	*Raphionacme hirsuta* (E.Mey.) R.A. Dyer	English (false gentian, khadi-root), Afrikaans (khadiwortel, khadi), Manyika (nyakurumba), Southern Sotho (kxerenthsane), Xhosa (intsema), Zulu (umathangane, umathanjana)	Watt and Breyer-Brandwijk, 1962 reported that the Southern Sotho use the plant to treat internal tumours.
Combretaceae	*Combretum caffrum* (Eckl. & Zeyh.) Kuntze	English (Cape bushwillow), Rufiji (msugasugu), Swahili (mganda simba, mnyonyore)	The root bark is used for the treatment of cancer (Pettit et al., 1982).
Cornaceae	*Curtisia dentata* (Burm.f.) C.A. Sm.	English (lance wood, Cape assegai wood), Afrikaans (asgaai hout), Ndau (muchekamani), Nguni (umquixina), Northern Sotho (modula-tshwene), Shona (mubotjo, muchekamani, mupunguti), Swati (iliNcayi, isiNwati, umPoyi), Xhosa (omhlebe, umhlebe, uSirayi, umGzina), Zulu (umLahleni,	An aqueous decoction prepared from the crushed roots and bark are used for cancer. The decoction is taken orally each day until the symptoms are relieved (Koduru et al., 2007a).

		uMagunda, uMaginda, umBese, umPhephelelangeni), Venda (musagwe, mufhefhera)	
Euphorbiaceae	*Euphorbia ingens* E. Mey. ex Boiss	English (candelabra tree, candelabra euphorbia), Afrikaans (naboom), Karanga (mukonde), Manyika (mukonde), Ndebele (ikonze, uMhlohlo, uMhlonhlo), Nguni (uMhlondlo, uMhlontlo, uMthi-wamawele), Northern Sotho (mohlohlokgomo, mokgoto), Sepedi (mohlohlokgomo); Shangana (ishupa, mkonde), Shona (ikonze, mkonde, mukonde, mukondwe), Swati (unHlonhlo), Tswana (nkondze, nkonde), Zezuru (mukonde), Zulu (uMhlondlo, uMpapa, nkondze)	The latex is topically applied for external cancers until the cancer is healed (Koduru et al., 2007a). Watt and Breyer-Brandwijk, 1962 reported that the Sotho use the plant to treat cancer. The Bapedi ethnic group use freshly squeezed juice from the stem and apply it topically after bathing for the treatment of breast cancer (Semenya et al., 2013).
Fabaceae	*Lassertia frutescens* (L.) Goldblatt & J.C. Manning, formerly known as *Sutherlandia frutescens* (L.) R. Br	English (cancer bush), Afrikaans (kankerbossie, eendjies, gansbossie), Xhosa & Zulu (umnwele)	Decoctions prepared from the stems, leaves, flowers and seeds are administered orally to treat internal cancers. For external cancers, decoctions prepared from the different plant parts are applied topically (Koduru et al., 2007a). Watt and Breyer-Brandwijk, 1962 also reported that the plant is used for internal cancers. A tea prepared in 1L of water using fresh or dried leaves and stems, is taken each morning and evening (Thring and Weitz, 2006).

			The common name is derived from its use for the prevention and treatment of cancer (van Wyk and Wink, 2007).
Gunneraceae	*Gunnera perpensa* L.	English (river pumpkin), Afrikaans (wilde ramenas, rivierpampoen), Sotho (qobo), Swati (uqonho), Venda (rambola-vhadzimu, shambola-vhadzimu), Xhosa (iphuzi-lomlambo, ighobo), Zulu (uGobho, uGobhe)	An aqueous infusion or decoction prepared from the rhizomes is taken orally for a duration of three to four weeks to treat cancer (Koduru et al., 2007a).
Hyacinthaceae	*Eucomis autumnalis* (Mill.) Chitt formerly known as *Eucomis undulata* Ait.	English (pineapple flower, pineapple lily), Afrikaans (wildepynappel), Xhosa (ubuhlungu becanti, isithithibala, esimathunzi), Zulu (uMathunga, uKhokho, umakhandakantsele)	An aqueous or milk decoction prepared from the bulbs is taken orally for several weeks to treat cancer (Koduru et al., 2007a).
	Merwilla plumbea (Lindl.) Speta, formerly known as *Scilla natalensis* Planch.	English (wild squill), Afrikaans (blouslangkop), Sotho (kxerere, matunga), Zulu (inguduza)	The Southern Sotho inject a decoction as an enema for internal tumours (Watt and Breyer-Brandwijk, 1962). A decoction prepared from the bulbs is taken orally to treat cancer (Koduru et al., 2007a).
Hypoxidaceae	*Hypoxis argentea* Harv. ex Baker	English (small silver star-flower), Southern Sotho (leihlo-khomo le leholo, leihlo-la-kxomo-le-leholo, letsikitlane), Xhosa (ixalanxa), Zulu (inongwe)	An aqueous decoction of the crushed corms is taken orally for the treatment of cancer (Koduru et al., 2007a).

	Hypoxis colchicifolia Baker, formerly known as *Hypoxis latifolia* Hook.	English (broad-leaves hypoxis), Zulu (iLabatheka)	An aqueous decoction of the crushed corms is orally administered to treat cancer (Koduru et al., 2007a).
	Hypoxis hemerocallidea Fisch., C.A. Mey. &Avé-Lall.	English (star flower, yellow star), Afrikaans (sterblom, geelsterretjie, gifbol), Southern Sotho (moli kharatsa, lotsane), Tswana (tshuka), Xhosa (inongwe, ilabatheka, ixhalanxa, ikhubalo lezithunzela), Zulu (inkomfe, ilabatheka, inkomfe enkulu)	A decoction prepared from the crushed corms is taken orally to treat cancer (Koduru et al., 2007a). The corms and the plant of *Hypoxis* is used for cancer (Coopoosamy and Naidoo, 2012; Pooley, 1992).
Lamiaceae	*Leonotis leonurus* (L.) R. Br.	English (red dagga, wild dagga), Afrikaans (klipdagga, wildedagga, duiwelstabak), Lunyaneka (omupaya), Shona (ibetshule-badala, ilihambambeba, umhlahlampetu), Sotho (lebake, levake), Xhosa (umfincafincane), Zulu (imunyane, munyamunyane, twala-inoyani, umunyane)	A tea prepared from a handful of leaves and flowers is taken (25mL) each morning and night for the treatment of cancer (Thring and Weitz, 2006).
Pittosporaceae	*Pittosporum viridiflorum* Sims	English (cheesewood, white Cape beech), Afrikaans (kasuur, stinkbas, bosboekenhout, witboekenhout zeepbas), Kinga (nyamkowa), Manyika (mukwenkwe), Xhosa (umkwenkwe, umkhwenkwe), Zulu (umfasamvu, umkhwenkwe, umVusamvu)	Aqueous decoctions and infusions are prepared from the crushed roots and bark and administered orally for several weeks to treat cancer. Additionally the ground dried roots and bark can be taken orally with water (Koduru et al., 2007a).

Pteridaceae	*Cheilanthes contracta* (Kunze) Mett. ex Kuhn, formerly known as *Cheilanthes hirta* Sw.	English (parsley fern); Sotho (lehorometso, mahwane, mma-mawaneng); Zulu (inkomakoma)	In Natal the rhizomes are used as an ingredient for a cancer cure (Watt and Breyer-Brandwijk, 1962).
Ranunculaceae	*Knowltonia capensis* (L.) Huth	English (blistering leaves), Afrikaans (brandblare, kaatjie-drieblaar)	A decoction prepared from the crushed corms is taken orally to treat cancer (Koduru et al., 2007a).
Rutaceae	*Agathosma betulina* (P.J. Bergius) Pillans, formerly known as *Barosma betulilna* (P.J. Bergius) Bartl. & H.L. Wendl.	English (buchu, boegoe, mountain buchu, round-leaf buchu, rounds, short buchu), Afrikaans (boegoe)	The plant is used for the prevention of cancer (Thring and Weitz, 2006).
Solanaceae	*Solanum aculeastrum* Dunal	English (poison apple, bitter apple, Devil's apple, goat apple), Afrikaans (gifappel, bitterappel, bokappel), Manyika (dungwiza, mutura), Shona (dungwiza, mutura), Tswana (thola), Venda (murulwa), Xhosa (umthuma, itunga), Zezuru (dungwiza), Zulu (umthuma, mtuma)	In the Eastern Cape decoctions prepared from the fruits are administered orally, once a day, after filtration until the cancer is treated (Koduru et al., 2007a)
Ulmaceae	*Celtis africana* Burm.f.	English (white stinkwood), Afrikaans (witstinkhout), Lovedu (khirale, modutu), Shona (kumtuna, muguru, murima, musedaderere), Sotho (modutu, mohata-	An aqueous or milk infusion, prepared from the sun-dried roots, is taken orally for the treatment of cancer (Koduru et al., 2007a).

	khomo, molutu, motibadefate), Southern Tswana (modutu), Tlhokwa (mogotiri), Tswana (modutu), Venda (mpopano), Xhosa (umVumvu), Zulu (uSinga Iwesalukazi)

Considering the plants discussed in Table 1, 20 different plant species, from approximately three different provinces in South Africa have been documented for their use as cancer treatments. The plant species belonged to 17 different families, where the families with the largest number of plants species were from the Hypoxidaceae (3 plant species) and Hyacinthaceae (2 plant species) family. It should also be taken into account that due to the nature of traditional medicine, often symptoms of a specific disease are treated and the disease itself is not specified. For example, the treatment of cervical cancer could be related to genital sores, whereas skin cancer could be associated with skin wounds and lumps. Therefore, traditional medicine used for the treatment of cancer could potentially be linked to these types of symptoms and are not necessarily specified for cancer treatment. However, in this review, only plant species which were specified for their use against cancer were included.

Each of the traditional treatments for cancer listed in Table 1, apart from the study conducted by Semenya et al. (2013), do not specify the type of cancer that is treated. In some instances, the only mention is that the cancer is either internal or external. Although Semenya et al. (2013), was able to report that the treatment was for breast cancer, there was no indication of how the diagnosis was made. In contrast to South Africa, a wide range of medicinal plants have been used in North Africa for the treatment of cancer, furthermore the types of cancer for which the plant is used have been reported, as discussed in a review by Alves-Silva et al. (2017). In a study conducted by Nkosi and Sibiya (2018), it was found that some traditional healers in KwaZulu-Natal referred their patients to hospitals to first diagnose or confirm the type of cancer before initiating treatment. It is unclear whether the traditional healers are uncertain regarding the type of cancer that is being treated or whether it is protected indigenous knowledge and therefore is not stated. However, it should be prioritised that traditional and Western health care practitioners collaborate with regards to the treatment and diagnosis of a patients.

4. Activity of traditionally used medicinal plants against cancer cells

Traditional medicine has been described as “too valuable to be ignored in the research and development of modern drugs” (Yuan et al., 2016). Numerous drugs have been developed from plants or derived from plants for their therapeutic activity against cancer. Using an ethnomedicinal approach to select plants for testing in a biological model is a widely used selection method. Not only does selecting plants based on ethnomedicine or traditional

knowledge allow for a greater possibility of finding plants with *in vitro* and *in vivo* activity for the potential discovery of new drugs but it further increases the protection and preservation of traditional knowledge (Schwikkard and Mulholland, 2014).

There have been numerous studies on the use of South African plants and their potential cytotoxic/ antiproliferative activity against cancer cells. This is most often achieved by determining the viability of a cell population after the cells have been treated with the test substance. In numerous studies this is referred to as a cytotoxicity assay, which is defined as the ability of a test substance to interfere with the attachment of cells to a surface, effect the cell morphology, alter the rate of cell growth, cause cell death or the disintegration of cells using an *in vitro* model (Horváth, 1980). In many published articles the term "cytotoxicity" is interchanged with "antiproliferative" activity of a test substance, however there is a distinction between the two.

The US National Cancer Institute described the difference using two parameters; the fifty percent growth inhibition (GI_{50}) (antiproliferative) and the fifty percent lethal concentration (LC_{50}) (cytotoxic). The GI_{50} describes the ability of a substance to inhibit 50% growth of a cell population, and is defined by the following equation: $100 \times \frac{T-T0}{C-T0} = 50$. The LC_{50} however described the potential of a substance to cause 50% cell death and is defined by the following equation: $100 \times \frac{T-T0}{T0} = -50$, where T is the optical density of the cells after exposure to the test sunstance for a certain time ("X"); T0 is the optical density at time 0h, and C is the optical density of untreated cells after the same time period ("X") (NIH, 2015). Both these terms describe *in vitro* tests to determine the antiproliferative or cytotoxic effect of a substance.

According to the US National Cancer Institute Guidelines an extract is considered to have noteworthy *in vitro* activity against cancer cells if a fifty percent inhibitory concentration (IC_{50})/ GI_{50} value < 20 µg/mL is obtained after 48-72 h incubation with the extract (Boik, 2001). However, as stated by Kuete and Efferth (2015), there is a need to define thresholds for plant extracts and secondary metabolites which are tested against cancerous and non-tumorigenic cell lines in order to define them as significantly, moderately, low or non-toxic. Due to the lack of references defining these thresholds, Kuete and Efferth (2015) proposed thresholds, based on the

US National Cancer Institute threshold for a noteworthy plant extract. The following has been proposed:

Extracts are significantly (IC_{50} < 20 µg/mL), moderately (20 µg/mL < IC_{50} < 50 µg/mL), low (50 µg/mL< IC_{50} < 200 µg/mL) or non-toxic (IC_{50} > 200 µg/mL) when tested on cancerous cell lines, whereas extracts tested on non-tumorigenic cells are defined as significantly (IC_{50} < 100 µg/mL), moderately (100 µg/mL < IC_{50} < 300 µg/mL), low (300 µg/mL < IC_{50} < 1000 µg/mL) or non-toxic (IC_{50} > 1000 µg/mL). Where secondary metabolites are tested against cancerous cell lines, these are defined as significantly (IC_{50} < 4 µg/mL (or 10µM)), moderately (4 µg/mL (or 10µM) < IC_{50} < 20 µg/ml (or 50 µM)), low (20 µg/mL (or 50 µM) < IC_{50} < 100 µg/mL (or 250 µM)) or non-toxic (IC_{50} > 100 µg/mL (or 250 µM)), whereas on non-tumorigenic cell lines, secondary metabolites are defined as significantly (IC_{50} < 40 µg/mL (or 100µM)), moderately (40 µg/mL (or 100µM) < IC_{50} < 120 µg/mL (or 300 µM)), low (120 µg/mL (or 300 µM) < IC_{50} < 400 µg/mL (or 300 µM)) or non-toxic (IC_{50} > 400 µg/mL (or 1000 µM)) (Kuete and Efferth, 2015).

Another important aspect to take into account, is how selective a specific test substance is towards a cancerous cell line. This can be measured by calculating the selectivity index (SI) which compares the growth inhibitory effect of the test substance on cancerous as well as non-tumorigenic cell lines. The SI value is calculated by: the IC_{50} value of the test substance on non-tumorigenic cells/ the IC_{50} value of the test substance on cancerous cells, where a higher value (more than 1), indicates that a test substance is more targeted towards the cancerous cells (Badisa et al., 2010).

Cancer has six major hallmarks; evading apoptosis, insensitivity to antigrowth signals, self-sufficiency in growth signals, sustained angiogenesis, limitless replicative potential and tissue invasion and metastasis (Hanahan and Weinberg, 2000). The potential activity of a test substance against cancer, such as a plant extract or secondary metabolite, is not only associated with the cytotoxic or antiproliferative effect but is also related to the ability to inhibit mechanism concerning the above mentioned hallmarks. Some of these are described by Boik (2001) and Ediriweera et al (2018).

Many plants traditionally used in South Africa for the treatment of cancer, as mentioned in Table 1, have been tested for their possible activity against various cancer cell lines and have shown

noteworthy activity (Table 2). This highlights the potential of ethnobotanical selection and the importance of traditional knowledge in South Africa. In this review, plants which showed significant (IC_{50} <20 µg/mL) to moderate (20 µg/mL < IC_{50} < 50 µg/mL) activity were selected for further discussion (Kuete and Efferth, 2015). The plants tested by Charlson (1980) were also included as this study was performed using a rodent model (Table 2). Plants which did not show significant activity such as *Euphorbia ingens*, *Gunnera perpensa*, *Hypoxis hemerocallidea* and *Knowltonia capensis* as well as plants with no reported activity such as *Agapnthus africanus*, *Agathosma betulina*, *Combretum caffrum, Curtisia dentata*, *Hypoxis argentea* and *Merwilla plumbea* were excluded from further discussion. It is interesting to note that although there have been numerous compounds isolated from *C. caffrum*, which have shown significant activity against cancer cells, no reports on the activity of the crude extract were found. Possibly the most well-known compounds that have been found present within *C. caffrum* include the combretastatins (Pettit et al., 1987a; 1987b, 1995). Acacetin, which has also been isolated from *C. caffrum*, has shown promising activity (Pettit et al., 1987a). Other noteworthy compounds isolated from a 1:1 methylene chloride: methanol stem-wood extract include; 6,7-dihydroxy-2,3,4-trimethoxy-9,10-dihydrophenanthrene, 7-hydroxy-2.3,4,6-tetramethoxyphenanthrene, 2,7-dihydroxy-3,4,6-trimethox-9,10 dihydrophenanthrene and 7-hydroxy-2.3,4,6-tetramethox-9,10-dihydrophenanthrene, which have all shown significant activity against P-388 cells (Pettit et al., 1988).

Table 2

Traditionally used plants with activity against cancer cell lines

Plant family	Species	Extraction solvent	Plant part	Activity against cancer cell lines
Alliaceae	*Tulbaghia violacea* Harv.	Methanol	Bulbs	IC_{50}: 25 µg/mL on esophageal squamous carcinoma (SNO) cells; decrease in mitochondrial depolarization and p53 expression (Moonsamy, 2013).
Apocynaceae	*Raphionacme hirsuta* (E.Mey.) R.A. Dyer	50% Aqueous ethanol	Bulbs	Active against a rodent leukemia (P-388) cancer model (Charlson, 1980).
Fabaceae	*Lassertia frutescens* (L.) R. Br	Big Tree Health™ (butanol, chloroform, ethyl acetate methanol and water)	Capsules containing *L. frutescens* extract	Inhibition of 41.3-90.7% at 10 µL/mL against human prostate cancer (PC-3; LNCaP) cells using butanol, chloroform, ethyl acetate and methanol extracts (Chen, 2007).
Hyacinthaceae	*Eucomis autumnalis* (Mill.) Chitt formerly known as *Eucomis undulata* Ait.	Methanol	Roots	IC_{50}: 7.8 µg/mL against human hepatoma (Huh-7) cells; ethyl acetate and n-butanol partitions showed IC_{50} values of 28.5 and 39.3 µg/mL respectively against Huh-7 cells (Bisi-Johnson et al., 2011).
Hypoxidaceae	*Hypoxis colchicifolia* Bak	Methanol	Roots	IC_{50}: 24.4 µg/mL against Huh-7 cells (Bisi-Johnson et al., 2011).

Lamiaceae	*Leonotis leonurus* (L.) R. Br.	Methanol	Aerial	IC_{50}: 2.57 ± 0.37 and 8.6 ± 0.45 µg/mL against sensitive human leukemia cells (CCRF-CEM) and multidrug-resistant human leukemia cells (CEM/ADR5000) respectively (Saeed et al., 2016).
Pittosporaceae	*Pittosporum viridiflorum* Sims	Dichloromethane (DCM)	Bark	IC_{50}: 16.9 µg/mL against human colon cancer (HT-29) cells (Nyongbela et al., 2013).
Pteridaceae	*Cheilanthes contracta* (Kunze) Matt. Ex Kuhn	50% aqueous ethanol extract	Fresh rhizomes	Active against murine metastatic sarcoma (sarcoma 180) cells and inactive against murine lymphoid leukemia (L-1210) and murine lung carcinoma (Lewis) cells (Charlson, 1980).
Solanaceae	*Solanum aculeastrum* Dunal	Methanolic extract	Ripened fruits	IC_{50}: 32.88, 24.40, 47.11, 7.04, 27.94, 24.00, 12.97 and 20.11 µg/mL against human ovarian cancer (A2780), human colorectal carcinoma (CaCo-2), human prostate cancer (DU-145), human hepatoma (HepG2), human breast cancer (MCF-7, MDA-MB-231), human neuroblastoma (SH-SY5Y) and human breast cancer (SK-Br-3) cells respectively (Burger et al., 2018). IC_{50}: 17.1, 17.8 and 41.9 µg/mL against human cervical carcinoma

				(HeLa), MCF-7 and HT-29 cells (Koduru et al., 2006a). IC_{50}: 11.28 to 21.80 µg/mL against HepG2 cells using different viability assays (Cordier and Steenkamp, 2015).
		Aqueous	Fruits	IC_{50}: 28.4, 27.9 and 48.5 µg/mL against HeLa, MCF-7 and HT-29 cells respectively (Koduru et al., 2006a).
		Ethanol	Fruits	IC_{50}: 1.36 µg/mL against CCRF-CEM cells (Omosa et al., 2016).
		Hot aqueous	Fruits	IC_{50}: 22.02 to 63.4 µg/mL against HepG2 cells using different viability assays (Cordier and Steenkamp, 2015).
Ulmaceae	*Celtis africana* Burm.f.	80% ethanol (ethyl acetate partition)	Roots and bark	IC_{50}: 8.3 µg/mL against mouse lymphoma (L5178Y) cells. Novel isolated glucosphingolipid; IC_{50}: 7.8 µg/mL against L5178Y cells (Perveen et al., 2015)

An important aspect to take into account when conducting assays using plant extracts is to correctly authenticate the collected plant material and to deposit a voucher specimen in a recognized herbarium. Often species can be misidentified due to related species having several morphological similarities, such as the leaves, stems, seeds and fruits (Kiran et al., 2010). Additionally, when considering cell-based assays, it is important to choose the appropriate cell line for the researcher's application. These cell lines should be sourced from accredited companies such as the European Collection of Authenticates Cell Cultures (ECACC) or the American Type Culture Collection (ATCC) in order to ensure that you are working with authenticated, validated, characterized and mycoplasma-free cell lines. This is important so that misidentified or contaminated cell lines are not used for a specific study, which can invalidate results (Bácskay et al. 2017). Cell-based assays (including cell viability assays) should incorporate the correct controls including the following; an untreated cell control (no test substance added), a vehicle (solvent/medium used to dissolve the test substance) and a positive control (such as berberine, podophylotoxin, taxol, cisplatin etc.), in order to effectively compare known or expected observations with new test substances, ensuring that one can obtain reproducible results in each experiment. Additionally, appropriate technical and biological replicates should be included. Dose-responses are an essential manner in which to assess the efficacy of a test substance. This is done by testing at a range of appropriate concentrations (preferably 6-8) from which an IC_{50} value can be calculated. Reports which merely include a percentage cell viability tested at a single concentration should therefore be avoided as this cannot accurately determine the effect of a test substance on a cell population.

5. *In vitro* and *in vivo* toxicity

Toxicity testing is a crucial part in the discovery of new drugs in order to determine the safety of a substance. It can be performed *in vitro* using cells lines or through *in vivo* studies using animal models. As discussed in section 4, *in vitro* toxicity can be examined using non-tumorigenic cell lines where threshold, as described by Kuete and Efferth, (2015) can be used to determine whether a test substance can be classified as toxic or non-toxic. Toxicity testing in animal models requires ethics clearance in order to insure the most humane treatment of animals (El-Aal, 2014; Fenwick et al., 2009; Russell and Burch, 1959). *In vivo* toxicity of a test substance is generally

done using various testing procedures which are time and dose dependent. The 50% lethal dose (LD_{50}) is used to determine the degree of toxicity of a test substance, where an LD_{50} <1 mg/kg is extremely toxic, LD_{50} of 1-50 mg/kg is highly toxic, LD_{50} of 50-500 mg/kg is moderately toxic, LD_{50} of 500-5000 mg/kg is slightly toxic, LD_{50} of 5000-15000 mg/kg is practically non-toxic and an LD_{50} > 15,000 mg/kg is relatively harmless (Teke and Kuete, 2014). Furthermore, behavioural, biochemical, hematological, immunological and histological changes are all crucial elements in determining whether a substance has a toxic effect (Parasuraman, 2011). The mutagenic potential of a test substance should also be taken into consideration when determine the safety of a substance, which is the potential of a substance to cause gene mutations (deletions, insertions or point mutations) (Eastmond et al., 2009).

Plants which showed significant to moderate activity against cancer cells, as discussed in Table 2, were further analysed to determine whether *in vitro* or *in vivo* toxicity studies were conducted (Table 3). No information on the degree of toxicity of *Raphionacme hirsuta* was found, however it is reported to be poisonous even though the tubers and roots are used as a source of yeast to brew beer (Ngalo and Notten, 2018) and the bulbs are used to treat sexually transmitted diseases in the Limpopo Province (Mongalo and Makhafola, 2018). Additionally, no reports on the toxicity of *C. contracta* were found, however it has been reported that *Cheilanthes* species showed toxicity in goats, cattle, sheep, horses and pigs, and that the consumption thereof resulted in chronic anemia and mortality (Simmonds et al., 2000). *In vitro* toxicity of the plant extracts was described as significantly, moderately, low or non-toxic against non-tumorigenic cells as proposed by Kuete and Efferth (2015). Furthermore, the mutagenic or antimutagenic potential of the plants was included if reports were found.

Table 3

In vitro and *in vivo* toxicity of plants traditionally used in South Africa against cancer

Plant family	Species	*In vitro* toxicity	*In vivo* toxicity
Alliaceae	*Tulbaghia violacea* Harv.	Methanolic bulb, leaves and stalk extracts; moderate toxicity on human T lymphocytes (Jurkat) (IC_{50}: 216 to 256 µg/mL); induced DNA damage and apoptosis through reactive oxygen species (ROS) production as well as caspase 9 and caspase 3/7 activation (Mackenzie, 2012). Aqueous leaves and bulbs extracts; non-toxic to human fibroblasts (KMST7) (IC_{50} > 3mg/mL) (Saibu et al., 2015). Dichloromethane and 90% methanolic extracts were not mutagenic (Elgorashi et al., 2003).	Methanolic rhizome extract; no acute or sub-acute toxicity in Wistar rats (LD_{50} > 5000 mg/kg). No physiological or behavioral changes and liver/ kidney damage (Saibu et al., 2015). The green parts of the plant have been used as a condiment on meat and as an alternative to spinach (Watt and Breyer-Brandwijk, 1962).
Fabaceae	*Lassertia frutescens* (L.) R. Br	Aqueous extract inhibited epithelial mammary gland (MCF-12A) cell (54% viability at 10 mg/mL) and induced apoptosis, however this concentration is normally considered non-toxic (Stander et al., 2009). At 50 µg/mL, MCF-12A cells showed 100% cell growth (Steenkamp and Gouws, 2006). Aqueous leaf extract; non-toxic to human monocytes (THP-1) cells at 0.1-1.6 mg/mL (De Caires, 2011).	Aqueous and 80% ethanolic extracts induced bleeding, pericardial cyst formation, excess fluid and yolk sac oedema at varying concentration. No adverse effects were at 5 µg/mL (Chen et al., 2018). Ethanolic and aqueous aerial extracts; LD_{50}: 40.54 and 297.57 µg/mL on zebrafish (Zonyane et al., 2019).

		An aqueous leaf extract induced apoptosis in Chinese hamster ovarian (CHO) cells (at 3.5 mg/mL), however this is considered a non-toxic concentration (Chinkwo, 2005). Methanolic and an ethyl acetate extract; antimutagenic (Ntuli et al., 2018). Phyto-Nova Sutherlandia SU1^{TM} Immune Booster tablets extracted with 30% ethanol; non-toxic (IC_{50} 7.5 mg/mL) on human peripheral blood mononuclear cells (PBMCs) (Korb et al., 2010).	Consumption of 800 mg/day of *S. frutescens* leaf powder capsules did not cause adverse effects in human volunteers (Johnson et al., 2007). A dose of 2.5-5g of dry plant material per day of *S. frutescens* in the form of an infusion or decoction is considered a traditional dose (Van Wyk and Albrecht, 2008).
Hyacinthaceae	*Eucomis autumnalis* (Mill.) Chitt formerly known as *Eucomis undulata* Ait.	No *in vitro* toxicity reports were found.	Single dose (1-2kg) of bulbs, killed sheep on the day of administration (Van der Walt, 1944). Toxic when injected intravenously causing a weakened heartbeat, slow respiration and mortality (Watt and Breyer-Brandwijk, 1962). Ingestion of a decoction prepared from the bulbs, led a patient to suffer from stomachaches, diarrhea and oliguria, which disappeared after 10-12 days (Watt and Breyer-Brandwijk, 1962).

Hypoxidaceae	*Hypoxis colchicifolia* Bak	Dichloromethane bulb extract; non-mutagenic (Elgorashi et al., 2003). Methanolic corm extract; non-toxic on mouse myoblast muscle (C1C12) cells at 12.5-50 µg/mL (Cumbe, 2015).	No reports on *in vivo* toxicity were found.
Lamiaceae	*Leonotis leonurus* (L.) R. Br.	Leaf extracts; non-toxic on isolated human lymphocytes (Dlamini, 2015).	Aqueous shoot extracts; mortality in rats at 3200 mg/kg (acute toxicity) and changes in red blood cells at 1600 mg/kg (sub-acute toxicity) and 200 mg/kg (chronic toxicity) (Maphosa et al., 2008). Chloroform and 70% methanolic aerial extracts; non-toxic in rats (LD_{50} >5000 mg/100g) (acute toxicity). Both the extracts showed hepatoprotective effects in rats treated with paracetamol (El-Ansari et al., 2009). An aqueous leaf extract effected the blood system, kidney and liver functioning in rats at 500 mg/kg (Oyedemi et al., 2010).
Pittosporaceae	*Pittosporum viridiflorum* Sims	Acetone and hexane leaf extracts; moderate toxicity on human liver (Chang) cells (IC_{50} >200 µg/mL) (Otang et al., 2014).	Acetone and hexane bark extracts; low toxicity on brine shrimps (LD_{50} > 1mg/mL) (Otang et al., 2013).

		Acetone leaf extract; significant toxicity (IC_{50}: 57.4 µg/mL) on African Green monkey kidney (Vero) cells (Elisha et al., 2016). Methanol and aqueous leaf extracts; significant toxicity (IC_{50}: 18.08 and 69.21 µg/mL) on E6 Vero cells (Muthaura et al., 2007). Aqueous and acetone leaf extracts; moderate toxicity (IC_{50}: 129 and 165 µg/mL) on Madin Darby Canine Kidney (MDCK) cells, Ethanol and methanol extracts; significant toxicity (IC_{50}: 7-77 µg/mL) on MDCK cells (Mehrbod et al., 2018).	Aqueous leaf extract; LD_{50}:1000 mg/kg on Swiss female mice, at 100mg/kg a methanol leaf extract killed Swiss mice within 24h (Muthaura et al., 2007).
Solanaceae	*Solanum aculeastrum* Dunal	Methanolic leaf and fruit extracts; toxic on Vero cells (IC_{50}: 11.11 and 8.06 mg/mL). Aqueous leaf and fruit extracts; low toxicity (IC_{50}: 13.89 and 10.42 mg/mL) (Laban et al., 2015).	Fresh, dried and boiled berry extracts, administered to Wistar rats, caused a decrease in heart, liver and spleen weight and therefore could affect organ functioning (Aboyade et al., 2009).
Ulmaceae	*Celtis africana* Burm.f.	Dichloromethane and 90% methanolic root extracts were not mutagenic (Elgorashi et al., 2003). Ethyl acetate bark extract; low toxicity on human lung fibroblast (MRC-5) cells (17% inhibition at 10 µg/mL (Lacroix et al., 2011). Methanolic fruit extract; significant toxicity against mouse embryo fibroblast (3T3-L1) cells, mouse myoblasts (C2C12)	No reports of the *in vivo* toxicity were found.

cells, human endothelial (EA.hy.926) cells and human lymphoblasts (SC-1) cells (IC_{50}: 19.55, 91.18, 2.79 and 23.07 µg/mL) (Burger et al., 2018).

6. Phytochemistry

Numerous compounds have been isolated from plants in order to ascertain whether these have potential activity against cancer. Many of these compounds have shown preliminary *in vitro* activity against cancer as seen by their cytotoxic or antiproliferative potential and/ or by their effect on mechanism related to cancer cell proliferation, growth and metastasis. In some cases *in vivo* studies using tumor induced animal models or xenograft models have also been reported.

Newly identified drugs from plants are reported each year, however many of these have not been tested for their potential against cancer. A common challenge when working with secondary metabolites from plants is obtaining low yields. Furthermore, characterization and structure elucidation of the isolated secondary metabolites is required, often leaving insufficient amounts for further testing. This requires that larger quantities of plant material be collected and used for isolation, which is not always possible, due to low availability, conservation/ ecological related reasons and/ or seasonal variation and other similar related matters (Atanasov et al., 2015).

Even though these challenges remain, there have been numerous compounds successfully isolated from plants which have entered into clinical trials and have been approved for use against cancer. There however, still remains a need for the discovery of new drugs which can effectively inhibit mechanisms related to an increased growth, proliferation and metastasis of cancer, in order to prolong and improve quality of life.

In this review compounds with noteworthy activity, isolated from plant species discussed in Table 2, have been discussed. Furthermore, the toxicity of these compounds has been included (Table 4). Chemical structures of noteworthy compounds can be seen in Figure 1. No compounds were found to be reported from *Cheilanthes contracta* or *Raphionacme hirsute*, therefore these were not included in Table 4.

The activity of sutherlandin A and B to significantly inhibit the reactive oxygen species in LPS/IFNγ induced mouse macrophages correlated well with its chemopreventive effect, where these compounds are able to inhibit a process related to an increase in potential carcinogenesis. This was also seen for leoleorin A and B, which were able to inhibit nitric oxide (NO) production in microglial cells (Table 4).

Table 4

Noteworthy secondary metabolites isolated from plants traditionally used in South Africa against cancer and their toxicity

Plant family	Species	Compound/ IUPAC name (Type of compound)	Compounds with activity against cancer	Toxicity
Fabaceae	*Lassertia frutescens* (L.) R. Br	**Sutherlandioside B**/ (3α,7β,9β,24S)-3,7,24-Trihydroxy-1-oxo-9,19-cyclolanostan-25-yl β-D-glucopyranoside **(1)** **Sutherlandioside D**/(7β,9β,24S)-7,24-Dihydroxy-1-oxo-9,19-cyclolanost-2-en-25-yl β-D-glucopyranoside **(2)** (Cycloartance glycosides (triterpenoid))	**Sutherlandioside B and D** were isolated from a methanol leaves and stems extract of *L. frutescens* in an effort to identify and quantify biomarkers (Avula et al., 2010). Sutherlandioside D and B inhibited Gli-reporter activity in Shh Light II (JHU-68) cells (89% and 22% respectively) (Lin et al., 2016).	No reports were found
		Sutherlandin A/ quercetin 3-O-β-D-xylopyranosyl(1 → 2)-[6-O-(3-hydroxy-3-methylglutaroyl)]-β-D-glucopyranoside **(3)** **Sutherlandin B**/ quercetin 3-O-β-D-apiofuranosyl(1 → 2)-[6-O-(3-hydroxy-3-methylglutaroyl)]-β-D-glucopyranoside **(4)**	**Sutherlandin A and B** were first isolated from a methanolic leaf extract by Fu et al. (2009). Both inhibited reactive oxygen species in LPS/IFNγ induced mouse macrophages (RAW 264.7) (Lei et al., 2015).	No reports were found

		(Flavonol glycosides)		
Hypoxidaceae	*Hypoxis colchicifolia* Bak	**Hypoxoside**/ 4-{(1E)-5-[4-(β-L-Glucopyranosyloxy)-3-hydroxyphenyl]-1-penten-4-yn-1-yl}-2-hydroxyphenyl β-D-glucopyranoside **(5)** (Phenolic glycoside)	A dichloromethane bulb extract prepared from *H. colchicifolia*, yielded **hypoxoside** (first report from this species) (Bassey et al., 2014). IC_{50}: 26.46, 82.80 and 45.62 µg/mL against HeLa, HT-29 and MCF-7 cells; increased caspase3/7 in MCF-7 and HT-29 cells (Boukes, 2010). Patients with adenocarcinoma (9 patients), large-cell carcinoma (9 patients) and squamous cell carcinoma (9 patients); treated with hypoxoside, resulted in 19 patients surviving (avg. of 4 months; primary tumors and metastasis progressed); 5 survived (>1 year) and 1 survived (5 years; absent of cancer) (Smit et al., 1995).	IC_{50} = 19.31 µg/mL against PBMC's (Boukes, 2010). Patients taking 200mg capsules of extract (containing 50-55% hypoxoside), showed no toxic effects (Smit et al., 1995).
Lamiaceae	*Leonotis leonurus* (L.) R. Br.	**Leoleorin A**/ (8S,9R,10S)-15,16-epoxy-9α-hydroxylabda-5,13(16),14-trien-7-one **(6)** **Leoleorin B**/ 15,16-epoxylabda-5,8,13(16),14-tetraen-7-one **(7)** (Labdane diterpenoids)	**Leoleorin A and B** were isolated from the acetone leaf extract of *L. leonurus* and have been identified in *Lagopsis supina* (Li et al., 2014; Wu et al., 2013).	No reports were found

			Leoleorin A and B inhibited LPS induced NO production in microglial cells (BC-2) (IC_{50}: 14.9 and 34.0 µM) (Li et al., 2014).	
		Marrubiin/(2aS,5aS,6R,7R,8aR,8bR)-6-[2-(3-Furyl)ethyl]-6-hydroxy-2a,5a,7-trimethyldecahydro-2H-naphtho[1,8-bc]furan-2-one **(8)** (Diterpene)	**Marrubiin** was isolated for the first time from *L. leonurus* in a study by Rivett (1964). Inhibited human lung adenocarcinoma (A549), leukemia (HL-60 and K562), HT-29 and MCF-7 cells (10-15% at 50 µg/mL) (Kee et al., 2008)	Intraperitoneal injection and oral administration; LD_{50}: 115 and 370 mg/mL in rats (Krejčí and Zadina, 1959).
Pittosporaceae	*Pittosporum viridiflorum* Sims	**Pittoviridoside**/ (3β,15α,16α,21β,22α)-15,16,28-Trihydroxy-21-{[(2Z)-2-methyl-2-butenoyl]oxy}-22-[(3-methyl-2-butenoyl)oxy]olean-12-en-3-yl α-L-arabinofuranosyl-(1->4)-[α-D-arabinopyranosyl-(1 ->3)]-[β-D-glucopyranosyl-(1->2)]-β-D-glucopyranosiduronic acid **(9)** (Triterpenoid saponin)	**Pittoviridoside** was first isolated from a methanolic extract through bioassay guided fractionation (Seo et al., 2002). IC_{50}: 10.1 µg/mL against A2780 cells (Seo et al., 2002).	No reports were found

Solanaceae	*Solanum aculeastrum* Dunal	**Solamargine**/ (3β,22α,25R)-Spirosol-5-en-3-yl 6-deoxy-α-L-mannopyranosyl-(1->2)-[6-deoxy-α-L-mannopyranosyl-(1->4)]-β-D-glucopyranoside **(10)** (Steroidal glycoalkaloid)	**Solamargine** was isolated through bioassay guided fractionation from a methanolic fruit extract (Burger et al., 2018). Moderate activity; IC_{50}: 15.62 ± 1.45 and 18.59 ± 1.13μM against SH-SY5Y and SK-Br-3 cells (Burger et al., 2018).	IC_{50} = 20.25 and 8.30 μM against C2C12 and EA.hy.926 cells (Burger et al., 2018).
		Tomatidine/ (3β,5α,25S)-Spirosolan-3-ol **(11)** (Steroidal glycoalkaloid)	IC_{50} > 50 μM against A549 cells; inhibited cell invasion (48.7% at 40 μM); reduced MMP-2/-9 expression; inhibited ERK & Akt signaling pathways and NF-κβ activity (Yan et al., 2013). Toxic on human breast cancer cells (BT474) at 10 and 20 μM (Zhang et al., 2009).	No reports were found
Ulmaceae	*Celtis africana* Burm.f.	**Orientin**/ (1S)-1,5-Anhydro-1-[2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4H-chromen-8-yl]-D-glucitol **(12)** (Flavone)	**Orientin** was first isolated from *Celtis africana* by Perveen et al. (2011). Reduced levels of tumor markers CEA and CA19-9 in rats with colorectal cancer (Thangaraj and Vaiyapuri, 2017). Downregulated proliferative antigens (PCNA and Ki67), decreased expression of p65 Nf-κβ, TNF-α and IL-6.	Neuroprotective effects on neuroblastoma (SH-SY5Y) cells treated with 150 μM hydrogen peroxide; decreased

	Inhibition of iNOS and COX-2 expression (Thangaraj and Vaiyapuri, 2017). Inhibited esophageal cancer (EC-109) cells (at 5-8 μM); upregulated p53 expression, downregulated bcl-2 expression and induced apoptosis (An et al., 2015).	apoptosis; inactivated caspase 3/7 and caspase-9 (Law et al., 2014).

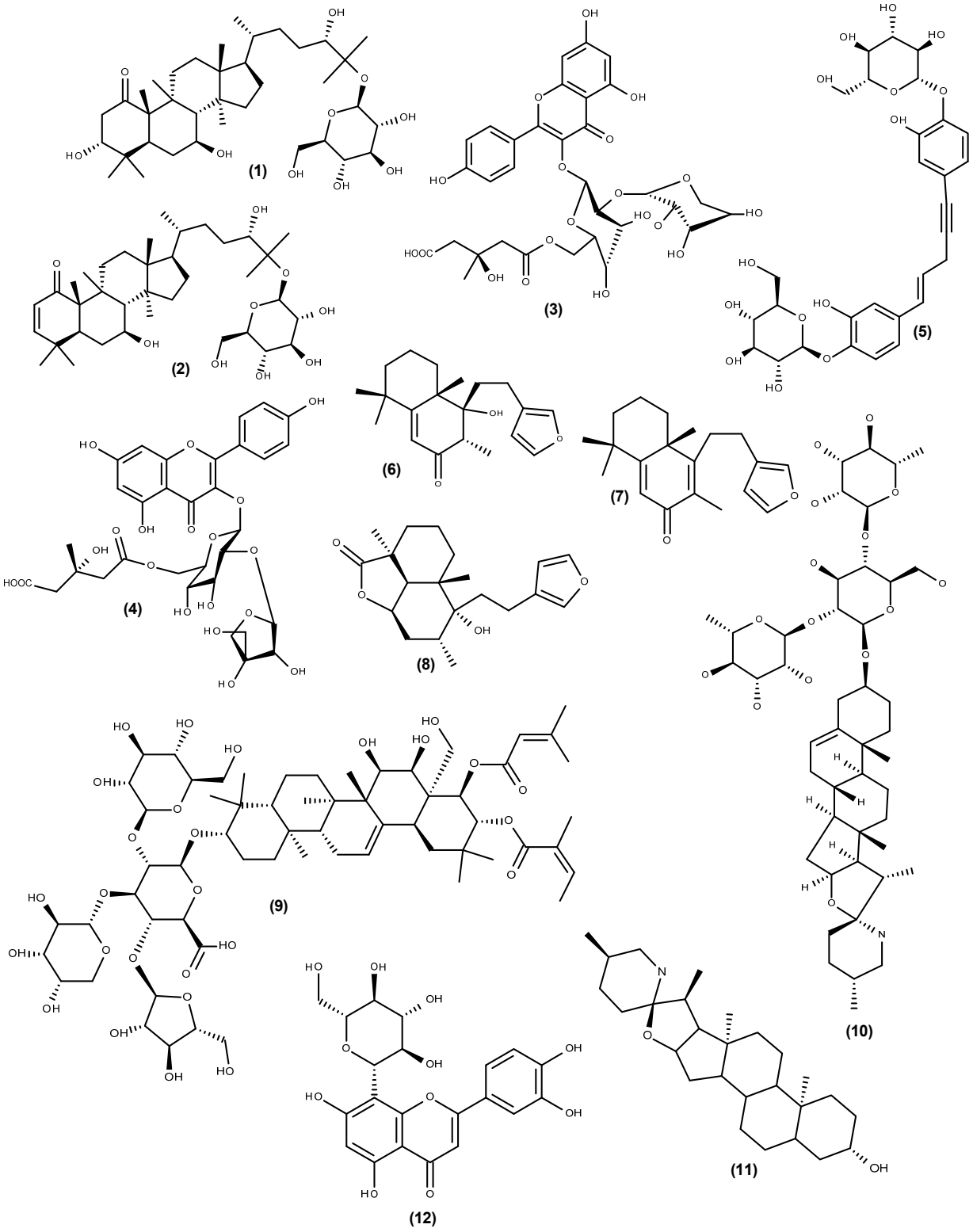


Fig. 1. Chemical structures of secondary metabolites isolated from species discussed in Table 4 which have shown *in vitro* and/ or *in vivo* activity against cancer

7. Discussion

In 2012, Lancet predicted an increase of 78% in cancer cases in South Africa by the year 2030 (Health 24, 2012). By 2014, which is the last report given by the National Cancer Registry, the cancer cases reached 115,000 per year, which is an increase from the approximate 74,500 reported in 2012 (CANSA, 2019). Even though the number of cancer cases is increasing dramatically in South Africa, and considering that approximately 27 million individuals rely on traditional medicine as their primary source of health care (Mander, 1998), only three ethnobotanical studies in South Africa have been reported which focus specifically on plants traditionally used for the treatment of cancer (Coopoosamy and Naidoo, 2012; Koduru et al., 2007a; Thring and Weitz, 2006). Furthermore, only 20 South African plants were found from primary studies to be reported for their traditional use to treat cancer. However, as stated by Cock et al. (2019), traditional medicine is often focused on using plants for the treatment of symptoms which could be related to a specific disease, and not necessarily targeting the disease itself. One report states that due to cancer having a complex set of signs and symptoms, reports on plants for the treatment of cancer are rare (Mongelli et al., 2000). Furthermore, it has been recommended that when selecting plants for the treatment of cancer or screening for activity against cancer cells, usages on symptoms and diseases related to cancer, such as, infections, inflammatory and skin disorders, immunological disorders as well as parasitic and viral diseases, should be taken into account (Steenkamp and Gouws, 2006).

Preparation of plants in traditional medicine are generally done in the form of decoctions, infusions, macerations and tinctures prepared mainly from water and in some cases from alcohol (Benzie and Wachtel-Galor, 2011). Fresh plant material is also used in the form of sap/ juice or a dressing prepared from the plant for direct application. In Table 1, traditional preparations for the treatment of cancer are mainly done in the form of decoctions and where specified water was used. However, in Table 2, plants which were evaluated scientifically for their activity against cancer and which showed activity, were mainly prepared using organic solvents such as methanol, ethanol and in some studies non/medium-polar dichloromethane extracts or extracts prepared with a mixture of water and ethanol were reported. The type of extraction solvents used can either lead to the extraction of polar compounds, non-polar compounds or a range of both and can therefore have an effect on the biological activity of a plant extract. Additionally,

Mander (1998), reported that the major plant parts used in traditional medicine included the roots (32.4%), bark (30.9%), bulbs (14.7%), and the least used was the whole plant (13.2%) as well as the leaves and stems (13.2%). This was also found to be true for the activity of the plants tested in Table 2, where the bulbs, roots, bark and fruits were mostly found to exhibit potential against cancer cells, however apart from the fruits, these are not sustainable parts to harvest as this could lead to the destruction of the entire plant. Therefore, focus has shifted to testing leaves of plants for their biological activity. In cases where significant biological activity is noted within a specific plant part which is not sustainable, such as the bark, roots or bulbs, cultivation of this plant should be considered. An example of this is the well-known anticancer compound, Taxol. Taxol was first isolated from the bark of the Pacific yew, a natural source which is being depleted. The synthesis of taxol has been a great challenge throughout the years with the aim to develop a synthetic version of taxol which is more sustainable, has a higher solubility when administered, a lower toxicity with a higher efficacy. A semisynthetic type of taxol has been developed from the needles and twigs of the *Taxus baccata*, which is a more sustainable source (Azu and Esses, 1997).

In this review it was noted that *R. hirsuta* and *C. contracta* were active when tested on *in vivo* tumor models performed in rats (Table 2) (Charlson, 1980). However, when a second extract was prepared from the same plants but collected at a different time period, the extracts were inactive against the same tumor models. This could potentially be due to chemical variations within the extracts based on the season in which these were harvested. There are no additional reports which confirm the pharmacological effect reported by Charlson (1980), furthermore no reports of secondary metabolites identified within these plants were found. Due to the lack of research conducted on these two plants, further biological testing should be considered, however it should be taken into account that both plants have been reported to have poisonous or toxic effects, however this has not yet been fully explored (Table 3).

A methanolic bulb extract prepared using *T. violacea,* showed moderate activity against esophageal squamous cancer (Table 2) and moderate toxicity was noted on T-lymphocytes, whereas a methanolic rhizome extract showed no toxicity in rats (Table 3). Numerous compounds have been isolated from *T. violacea* (Burton and Kayen, 1992; Krstin et al., 2018; Moodley et al., 2015; Pino et al., 2008), however many of these have not been tested for their

activity against cancer cells and therefore should be considered for further screening. A noteworthy compound which has been reported is methyl-α-D-glucopyranoside, which was able to induce apoptosis in MCF-7 and HeLa cells through DNA fragmentation and activation of caspase-3 (Lyantagaye, 2013), however this activity has not been further validated.

Several studies have been conducted on the potential activity of *L. futescens*, published as *S. frutescens*, against cancer cells, however many of them have shown that a high concentration is needed to induce a cytotoxic effect or to induce apoptosis in the cancer cells lines (Chinkwo, 2005; Stander et al, 2009; Steenkamp and Gouws, 2006). Significant inhibition however was noted when prostate cancer cells were treated with leaf extracts prepared using various solvents (Table 2). Furthermore, extracts prepared from the leaves have shown low toxicity on various non-tumorigenic cell lines, additionally the leaf powder showed no adverse effects in human trials (Table 3). Compounds isolated from *L. frutescens*, which have shown noteworthy activity against cancer include; sutherlandioside B and D as well as sutherlandin A and B (Table 4). These were first isolated and characterized in a study by Fu et al (2008) from a methanolic leaf extract. No toxicity studies on non-tumorigenic cell lines or in *in vivo* models have been reported for these compounds. Similar compounds have also been isolated, namely sutherlandioside A and C as well as suterlandin C and D, however, these have not been tested for their potential activity (Avula et al., 2010; Fu et al., 2008). As stated by Zonyane et al (2019), the biological activity of sutherlandins and sutherlandiosides has not yet been fully explored and more research is needed to determine their potential activity against cancer cell lines and to elucidate the mechanism of action.

A methanolic root extract prepared from *E. autumnalis* showed significant activity against human hepatoma cells (Table 2), however high toxicity of this plant was noted in animals (Table 3). In previous studies by Sidwell et al (1971) and Sidwell & Tamm (1970), numerous flavonoid compounds were isolated from *E. autumnalis*, of which only the homoisoflavones, autumnalin and 3,9-dihydro-autumnalin were tested for their activity against human gastric cancer (AGS) cells. These showed low to moderate activity with IC_{50} values of 74.6 and 30.5 µM for autumnalin and 3,9-dihydro-autumnalin respectively. Additionally, both these compounds showed moderate toxicity against normal human fibroblasts, both with IC_{50} values of 100 µM (Ebrahimi et al., 2015). Due to the highly toxic effects seen in animals when the plant was

ingested, further studies should be done on the isolation of compounds which could potentially show less toxicity in non-tumorigenic models while having significant activity against cancer cells.

Various *Hypoxis* species have been traditionally used for the treatment of cancer (Table 1), however a methanolic root extract prepared from *H. colchicifolia* was the only extract in this study which showed moderate toxicity on human hepatoma cells (Table 2). No toxicity studies on non-tumorigenic cell lines were conducted using the roots, however the bulbs/ corms showed no toxicity. Hypoxoside, isolated from the bulbs of *H. colchicifolia*, showed significant to moderate toxicity on various cancer cell lines, however significant toxicity was also noted on PBMCs (Table 4). On the contrary when hypoxoside was used in human clinical trials, no adverse side effects occurred (Table 4). Both *H. colchicifolia* and hypoxoside should be further tested in order to validate the activity against cancer cells and their toxicity, however these seem to be good candidates for further investigation. Numerous other compounds have been identified in *H. colchicifolia*; such as amabiloside, which did not show activity against various types of cancer cell lines (Likhitwitayawui et al., 1993). Dehydroxyhypoxoside A and B as well as bis-dehydroxyhypoxoside have additionally been isolated from the methanolic corm extract (Cumbe, 2015), however no reports on the activity against cancer cells were found and therefore should investigated.

Leonotis leonurus has been tested for several biological activities and has been tested on various different types of cancers, however only a methanolic extract prepared from the aerial parts showed significant activity against leukemia (Table 2). Various leaf extracts were non-toxic on lymphocytes, whereas *in vivo* studies showed that changes in the liver functioning and red blood cells occurred (Table 3). Numerous secondary metabolites have been isolated from various parts of the plant, including volatile compounds, which have been identified using GC-MS (Agnihotri et al., 2009; El-Ansari et al., 2009; He et al., 2012; McKenzie et al., 2006; Narukawa et al., 2015; Oyedeji et al., 2005; Pedro et al., 1991; Rivett, 1964; Wu et al., 2013). Vitexin, apigenin-7-O-β-glucoside (apigetrin), luteolin-7-O-β-glucoside, apigenin, luteolin, leoleorin A and B as well as marrubiin are noteworthy compounds isolated from *L. leonurus*. Several studies and reviews have documented the significant effects of these compounds against various types of cancer cell

lines (Ali et al., 2017; Aslam et al., 2015; He et al., 2016; Kahraman et al., 2012; Lin et al., 2008; Pan et al., 2003; Seelinger et al., 2008; Tuorkey, 2016; Yan et al., 2017).

An interesting compound that was isolated from a 70% ethanolic extract prepared from the flowering tops is geniposidic acid (Agnihotri et al., 2009). In a study by Lee (2018), geniposidic acid, at a concentration of 20 μM was found to have a protective effect on human keratinocytes (HaCat cells) by reducing apoptosis caused by UVB damage. It was furthermore able to suppress the arrest of the cell cycle in the G1 phase and the cleavage of caspase-3 caused by UVB, thereby inhibiting apoptosis in the HaCat cells.

Significant activity was observed against colon cancer cells when treated with a dichloromethane extract prepared from the bark of *P. viridifolrum* (Table 2). Toxicity studies revealed that extracts, prepared using different solvents, showed variable toxicity against non-cancerous cell lines, ranging from significantly to moderately toxic (Table 3). However, these were all prepared from the leaves. A hexane extract prepared from the bark however showed low toxicity on brine shrimps (Table 3). While the toxicity of the dichloromethane bark extract has not been reported, the hexane bark extract could give an indication of potential toxicity of the dichloromethane bark extract. A few compounds have been isolated from *P. viridiflorum* (Seo et al., 2002; Nyabayo et al., 2016; Ramanandraibe et al., 2001), however only beta-amyrin, which showed low toxicity against various cancer cell lines (Ding et al., 2009; Hata et al., 2002; Maiyo et al., 2016) and pittoviridoside (Seo et al., 2002), have been tested for their activity against cancer cells (Table 3). Pittoviridoside, isolated from a methanolic extract, showed moderate activity against ovarian cancer cells (Table 3) and should be considered for further analysis and toxicity studies.

Various fruit extracts prepared from *S. aculeastrum* have shown activity against different types of cancer cell lines (Table 2), however toxicity has also been observed on non-cancerous cell lines and *in vivo* studies revealed that the fruit caused changes in heart, liver and spleen weights when administered to rats (Table 3). This could be due to the steroidal alkaloids found within this species, such as solamargine, solasonine, tomatidine and solasodine (Burger et al., 2018; Koduru et al., 2007b; Wanyonyi et al., 2002; Wanyonyi et al., 2003). Other compounds isolated include lupeol and various volatile constituents (Kama-Kama et al., 2017; Koduru et al., 2006b; Wanyonyi et al., 2002; Wanyonyi et al., 2003). Noteworthy activity of solamargine was observed, however toxicity was also reported in non-tumorigenic cells (Table 4). Several reviews

on the activity of solamargine are available (Al Sinani and Eltayeb, 2017; Cham, 2017; Chauhan, 2018; Kalalinia and Karimi-Sani, 2017; Yuan et al., 2017). At moderately toxic concentrations, tomatidine was able to inhibit the invasive potential of lung cancer cells, however no reports on toxicity were found (Table 4). Extensive reviews including the *in vitro* activity, drug formulations and clinical trial outcomes are available for solasonine (Al Sinani and Eltayeb, 2017; Cham, 2017; Jiang et al., 2016).

An extract prepared from the roots and bark of *C. africana* showed significant activity in mouse lymphoma cells (Table 2), furthermore, no toxicity was observed when the bark was tested *in vitro* and the roots were not mutagenic, however the fruits showed high toxicity. No *in vivo* toxicity trials were reported to validate the *in vitro* toxicity (Table 3). In a study by Al-Taweel et al (2012), a number of compounds were isolated for the first time from *C. africana* of which oleanolic acid was one of the most noteworthy (Al-Taweel et al., 2012). Extensive reviews on the *in vitro* and *in vivo* potential of oleanolic acid have been published (Masullo et al., 2017; Patlolla and Rao, 2012; Zhong et al., 2017; Žiberna et al., 2017). Perveen et al (2011), furthermore, isolated two new C-glycosylflavonoids (celtiside A and B) as well as known compounds, vitexin, orientin, isoswertiajaponin, isoswetisin and 2-O-rhamnosyl vitexin for the first time from *C. africana*. Significant potential of orientin was observed both *in vitro* and *in vivo* (Table 4). Perveen et al (2015), additionally isolated a promising glucosphingolipid which showed moderate inhibition of mouse lymphoma cells with an IC_{50} value of 7.8 µg/mL, however the structure of this compound has not yet been published.

This literature review has surveyed 20 indigenous South African plant species used as traditional medicines for the treatment of cancer. Upon conducting the literature search, it was evident that only a few studies, relating to traditional cancer treatments in South Africa, have been conducted. Moreover, it was noted that there were no ethnobotanical surveys conducted in highly populated provinces such as Gauteng, Mpumalanga and Limpopo, where traditional medicine is common practice. This suggests that there is a large gap which needs to be filled in order to understand which plant species play an important role in traditional medicine for the treatment of cancer. Although the ethnobotanical reports documented specific plant parts and the preparation method used in the treatment, there was no mention regarding the type of cancer which was treated. As previously discussed, traditional treatments are often used for a specific symptom of a

disease and not necessarily to treat the disease itself, which could contribute towards the knowledge gap of traditional cancer treatments. Additionally, not all traditional healers are adequately trained in diagnosing the type of cancer which being treated, which adds to the uncertainty of cancer-type specific remedies. This highlights the need for traditional healers and Western health care practitioners to work together in order to adequately treat patients which rely primarily on traditional healthcare. The lack of reports which specify the type of cancer being treated could also be due to the protection of indigenous knowledge and therefore, is not always revealed.

Several plant species which are traditionally used for treating cancer have not been scientifically evaluated for their activity against cancer cells. These species include: *Agapanthus africanus*, *Agathosma betulina*, *Combretum caffrum, Curtisia dentata*, *Hypoxis argentea* and *Merwilla plumbea*. Additionally there is clear gap in research where extracts with significant antiproliferative activity have not been further investigated for their mechanism of action. In Table 1, the only extract to be further evaluated was *Tulbaghia violacea*, which decreased mitochondrial depolarization and p53 expression in oral squamous carcinoma cells. This emphasizes the need for further evaluation which could potentially identify novel modes of action. Furthermore, results were often reported as displaying significant activity against cancer cells, however this was not always the case, therefore it is important to note the concentrations at which these extracts were tested. In some studies extracts tested at concentrations >1000µg/mL were often described as showing significant activity, however these high concentrations are not considered noteworthy. This could be attributed to the lack of consensus on defined thresholds which classify extracts or compounds as showing significant, moderate or non-toxic effects against cancerous/ non-cancerous cell lines. It was further noted that the use of the terms cytotoxic and antiproliferative activity are often used interchangeably, however the cytotoxic activity relates to the ability to cause cell death whereas the antiproliferative activity inhibits the growth/ proliferation of cells which after a prolonged period can lead to cell death.

Most of the evaluated species, apart from *Celtis africana, Cheilanthes contracta, Eucomis autumnalis, Hypoxis colchicifolia* and *Raphionacme hirsute*, have been evaluated to some extent for their toxic potential, however the toxicity was not necessarily reported on the same extract (plant parts and extraction solvent) which showed significant biological activity against cancer

cells, which highlights the need to test active extracts for toxicity in order to obtain a better understanding regarding their overall therapeutic potential and selectivity towards cancerous cells.

Although there have been numerous compounds identified from these plant species, many of these were not identified through bio-assay guided fractionation correlating their biological activity against cancer cells but were identified through investigation of other biological activities (not antiproliferative/ cytotoxicity) or studies focused on phytochemical investigation or profiling of extracts by identifying biomarkers. These compounds were then identified in additional studies to have significant activity against cancer cells. This emphasizes a major concern in which plants with significant biological activity are not further evaluated for their mechanism of action and identification of bioactive compounds responsible for the activity, resulting in a large knowledge gap with regards to these plant species. The compounds identified within the discussed plant species, which showed significant activity against cancer cells, have not been fully evaluated for their toxicity.

Lastly when considering the methodology used to identify biologically active plant extracts or compounds the following was noted: the described methodology for determining cell viability often no mention of the appropriate controls, such an untreated cells, vehicle/ solvent controls, and also failed to provide the number of replicates performed within an experiment. Each assay should ideally be performed with a minimum of three independent repeats, and should include each test/ concentration/ control in triplicate within an experiment in order to verify the reliability and the statistical significance of the obtained results. In addition, the correct calculation and interpretation of results using statistical programs is a crucial aspect. Lastly, the correct authentication of plant material and cell lines used within a study is of high importance in order to obtain reproducible and valid results.

8. Conclusion

South Africa has a great biodiversity and an abundance of plant species of which only a few have been documented for their use in traditional medicine for the treatment of cancer. Even though there are only a few ethnobotanical reports, most of these plants have shown *in vitro* activity against cancer cells and some have shown antitumor effects *in vivo*. Numerous secondary

metabolites have been identified within some of these plants, with a few showing promising activity.

There is a lack of reports of some of the plant extracts for activity against cancer cells, such as *R. hirsuta* and *C. contracta*, which should be further explored. Secondary metabolites such as the sutherlandiosides and sutherlandins (from *L. frutescens*), hypoxoside (from *H. colchicifolia*) and pittoviridoside (from *P. viridiflorum*) should be considered as good candidates for further biological testing and mechanistic studies relating to cancer. Compounds identified in *T. violacea* should be evaluated for potential activity against various types of cancer cell lines, whereas additional compound isolation should be considered for *E. autumnalis*. Due to the lack of reports on *C. africana*, additional studies should be considered.

This review highlights the importance of selecting plants, for screening against cancer cell lines, based on traditional knowledge and the scientific validation of traditionally used anticancer herbal remedies in the potential discovery of new anticancer agents. Additionally, this review emphasizes the importance of further research of many of the South African plants for activity against cancer cells and the role that these plants can have in the possible further development of anticancer drugs.

List of compounds: sutherlandioside B and D, sutherlandin A and B, hypoxoside, leoleroin A and B, marrubiin, pittoviridoside, solamargine, tomatidine, orientin.

List of cell lines: African Green monkey kidney cells (Vero, Vero E6), hamster ovarian cells (CHO), human breast cancer (BT474, MCF-7, MDA-MB-231, Sk-Br-3), human cervical cancer (HeLa, KB (contaminant of HeLa cells)), human colon cancer (CaCo2, HCT-116DR, HCT-8, HCT-15, HT-29), human endothelial cells (EA.hy.826), human epithelial mammary gland cells (MCF-12A), human esophageal cancer (EC-109, SNO), human fibroblasts (KMST7), human gastric epithelial cells (HE-17), human hepatoma (HepG2, Huh-7), human leukemia (BC, CCRF-CEM (sensitive), CEM/ADR 5000 (multidrug-resistant), SC-1 (lymphoblasts)), human liver cells (Chang), HL-60, K562), human lung cancer (A549, Lewis, NCI-H187), human lung fibroblasts (MRC-5, TIG-1), human melanoma (SK-Mel-2, UACC-62), human monocytes (THP-1), human neuroblastoma (SH-SY5Y), human ovarian cancer (A2780, SK-OV-3), human prostate cancer (DU-145, LNCAP (androgen-sensitive), PC-3), human renal carcinoma (TK-10), human T-lymphocytes (Jurkat), human umbilical vein endothelial cells (HUVEC), Madin Darby canine

kidney cells (MDCK), microglial cells (BV-2), murine embryo fibroblasts (3T3-4), murine leukemia (P-388), murine lymphoid leukemia (L5178Y, L-1210), murine macrophages (Raw 264.7), murine melanoma (B16), murine metastatic sarcoma (Sarcoma 18), murine myoblasts (C1C12, C2C12), peripheral blood mononuclear cells (PBMCs), Shh Light II cells (JHU-68).

Author contribution

D. Twilley and S. Rademan designed the study and co-wrote the manuscript; N. Lall reviewed and edited the final draft. All authors have read and approved the final draft.

Declarations of interest

The authors declare that there are no conflicts of interest

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