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Toxicity and phytochemical properties of southern African medicinal plants used traditionally to treat pain and inflammatory ailments

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A B S T R A C T

The harmful side effects associated with conventional anti-inflammatory pharmaceuticals has focussed attention on medicinal plants as alternative therapeutics. However, the use of medicinal plants is predominantly based on ethnobotanical knowledge and often lacks toxicity data and safety validation. A comprehensive review of the toxicity and phytochemical properties of southern African medicinal plants used for inflammation and pain-related ailments was undertaken. Various ethnobotanical books and search engines including Science direct, Sci-Finder, Google Scholar and Scopus were used to determine which medicinal plants have been screened for toxicity. A total of 117 medicinal plants have previously been evaluated by toxicity screening. The predominant toxicity test used was the MTT reduction assay (109 species), followed by the brine shrimp lethality assay (57 species), and the XTT assay (24 species). The Ames test, which was used to screen 20 species, was the most frequently used assay to determine mutagenic properties of the plant extracts. The top five most commonly screened cell lines were Vero monkey kidney cells (46 plant extracts), RAW 264.7 macrophages (23 extracts), human breast cancer cells (MCF-7, 12) (21 extracts), mouse fibroblast (3T3) (18 extracts) and Graham cells (HEK-293) (17 extracts). Over 80% of the tested medicinal plant extracts were found to be non-toxic. Although many species have been evaluated for their toxicity properties, plant extracts are often tested using one or two assays and also lack diversity in terms of choice of cell lines used for screening. Phytochemical studies remain scarce and substantial further work is required.

1. Introduction

Inflammation and pain related disorders are a global concern that affect the well-being of many individuals. The frequent use of medications, high health care expenses, increased unemployment rates and disability are among the major challenges facing patients with inflammatory pain disorders (Turk, 2002; Breivik et al., 2006; Gaskin and Richard, 2012; Nahin, 2015). It was estimated in 2012 that over 100 million adults in the United States were affected by chronic pain (Nahin, 2015). The value of lost work due to pain was estimated to range from $299 to $335 billion annually during that period (Gaskin and Richard, 2012). Furthermore, in the United States, the overall economic cost of chronic pain ranged from $560 to $635 billion in the year 2010 (Gaskin and Richard, 2012). In southern Africa, limb pain and headaches are the most prevalent form of chronic pain (Tsang et al., 2008; Kamerman et al., 2020). People suffering from chronic pain are also more prone to mental disorders. Indeed, a study conducted in fifteen European countries showed that 21% of participants suffering from chronic pain were also diagnosed with depression (Breivik et al., 2006). Of the 4839 participants in a European study who suffered from chronic pain, 59% had experienced chronic pain for two to fifteen years (Breivik et al., 2006). Pain detection is challenging since the instruments that are used to assess pain intensity are limited to determine pain that is experienced within the last week (Breivik et al., 2008). In addition, pain sensitisation varies greatly across individuals, making pain quantification complex.

Non-steroidal anti-inflammatory drugs (NSAID) are the most widely used medications for pain and inflammatory related disorders (Pahwa et al., 2019). However, the severity of side effects results in an estimated 107,000 patients hospitalised annually for NSAID-related gastrointestinal complications, and at least 16,500 NSAID-related deaths each year among rheumatoid arthritis patients alone (Singh, 1998; Conaghan, 2012). These harmful side effects have attracted considerable research interest globally into the potential of natural products. Studies on toxicity and safety validation are imperative as the use of medicinal plants in both developed and developing

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countries continues to rise (Makunga et al., 2008; Lazarou and Heinrich, 2019). In the United States, about 44% of the total self-care costs for complementary and alternative medicine (CAM) ($14.8 billion) was used for purchasing non-vitamin, non-mineral and natural products in 2007 (Barnes et al., 2009). Furthermore, the U.S. population spent about $33.9 billion on CAM products and practitioners, compared to $11.9 billion on healthcare practitioners (Barnes et al., 2009). The prevalence of use of CAMs in the USA increased from 32% to 64% of the adult population between 1997 and 2002, and by a further 29% in 2007 (Su and Li, 2011). The use of CAMs to treat back and joint pain also increased substantially between 2002 and 2007 in the US (Barnes et al., 2009). This interest of herbal remedies in developed countries, particularly in Australia (Zhang et al., 2008; Holst et al., 2011), America (Agra et al., 2007; Barnes et al., 2009), and several European countries (Lazarou and Heinrich, 2019) is perceived to be useful for maintaining a healthy lifestyle, rather than only as a medication.

Various scientific studies worldwide have explored the ethnopharmacological validation of herbal remedies. The development of therapeutic agents from medicinal plants has led to the discovery of many effective and widely used analgesic and anti-inflammatory drugs. These include the universal anti-inflammatory drug, acetylsalicyclic acid, and a glycoside of methyl salicylate (spiraein), which have been used traditionally since 400 BC for treating pain and fever (Rao and Knaus, 2008; Van Wyk and Wink, 2017). The alkaloid, quinine, was isolated in the 17th century from the bark of various South American species of the genus *Cinchona*, including *Cinchona officinalis* L. (Misra et al., 2008). In addition to its potent antimalarial properties, chloroquine has also been shown to regulate inflammatory autoimmune responses (Park et al., 2019) and is effective as an antitumour agent (Goel and Gerriets, 2019). Another alkaloid (morphine) that is isolated from *Papaver somniferum* L. is widely used as a potent analgesic agent (Brook et al., 2017). More commonly it is converted to the less addictive codeine and is widely used to treat pain (Van Wyk and Wink, 2017). Although multiple studies have investigated the anti-inflammatory and analgesic activity of crude extracts and isolated compounds (De Sousa, 2011), major challenges exist in validating their safety and effectiveness in human trials (Fürst and Zündorf, 2014). While the rate of medicinal plant use is increasing worldwide, the production of alternative therapeutics by pharmaceutical companies remains steady (Fabricant and Farnsworth, 2001; Yuan et al., 2016). During 1991, in the United States, it was reported that for every 10,000 bioactive compounds isolated, only 10 reach human trials and only one compound on average is approved for commercialisation by the US Food and Drug Administration (Fabricant and Farnsworth, 2001). The lack of pharmacodynamics and pharmacokinetics studies of traditional medicinal plants is the dominant factor contributing to drug trial failures (Fasinu et al., 2012; Heinrich et al., 2020).

The limited volume of ethnopharmacological data and scientific validation of medicinal plants is particularly apparent for African plants. It was only after 1995 that a rapid increase of scientific investigations of southern African medicinal plants was observed (Van Wyk, 2008). Indeed, 96% of the total publications between 2001-2017 in the South African Journal of Botany were based on medicinal plant research (Viljoen et al., 2019). However, only 9% of these experiments were conducted *in vivo* (Viljoen et al., 2019). The sub-Saharan region has approximately 25% of the world's higher plants (Van Wyk, 2008). Yet, African traditional medicine is the most poorly documented of the known traditional healing systems (Van Wyk and Wink, 2017), despite being the oldest (Cragg and Newman, 2013).

While the long-term use of traditional medicinal plants has been accompanied with the assumption of safety and limited side effects (Lazarou and Heinrich, 2019), scientific findings have identified multiple toxins in medicinal plant preparations (Van Wyk and Wink, 2015). Medicinal plant poisoning may also be influenced by environmental factors, with heavy metal contamination being the most prevalent in western countries (Ernst, 2002). In South Africa, a 5-year forensic study of traditional medicinal plant poisoning detected 4% of cases resulting from heavy metal contamination (Stewart et al., 1999). A substance is considered toxic when it interferes with the normal metabolic functioning of the cell and/or creates substantial unwanted side effects (Wink and Van Wyk, 2008; McGaw et al., 2014). Commonly reported classes of toxic secondary metabolites in plants may include phorbol esters, cyanogenic glycosides, lectins, and pyrrolizidine alkaloids (Wink and Van Wyk, 2008; Ndhlala et al., 2013; Kristanc and Kreft, 2016). However, medicinal plants are used worldwide as antimicrobial, anti-cancer, anti-inflammatory, analgesic and cardiovascular agents, despite often containing poisonous substances that are classified as extremely or highly hazardous (Wink and Van Wyk, 2008; Kristanc and Kreft, 2016).

Toxicity studies of southern African medicinal plants against normal cell lines are limited (Adamu et al., 2012; Hurinanthan, 2013; Naidoo et al., 2013; Adebayo et al., 2015; Mzindle, 2017; Mongalo et al., 2018; Ghuman et al., 2019). The most recent review on toxicity of African medicinal plants against normal cells identified about 400 species (McGaw et al., 2014) of 5400 recorded African medicinal plants which have been screened against cell lines (Neuwinger, 2000). Most of these plant species have been subjected to one or two biological assays and often are screened against only a few different cell lines (McGaw et al., 2014). Mutagenic and carcinogenic properties of African medicinal plants have also received relatively little attention (Elgorashi et al., 2001; Reid et al., 2006; Mulaudzi et al., 2013; Kuete, 2014). An inventory of poisonous plants of the world by Wink and Van Wyk, (2008) documented few (about 1%) poisonous chemical constituents from a pool of about 150 000 phytochemicals. To date, relatively little information is available on the toxic properties of medicinal plants in both developed and developing countries (Kuete, 2014; Van Wyk and Wink, 2015; Kristanc and Kreft, 2016). More work is required to identify the toxic properties of medicinal plants and to evaluate their safety indices.

Medicinal plants are widely used for the treatment of pain and inflammatory disorders, with approximately 80% of the global population estimated to use plants for this purpose (Uritu et al., 2018). A recent review by Khumalo et al. (2021) identified 555 medicinal plant species that are used specifically to treat pain and inflammation in southern Africa. Indeed, ten of top fifteen commercially significant medicinal plant in the southern African region are used to treat pain and inflammatory disorders (Van Wyk, 2008). These plants include *Agathosma betulina* (P.J. Bergius) Pillans, *Aloe ferox* Mill., *Artemisia afra* Jacq. ex Willd., *Harpagophytum procumbens* (Burch.) DC ex Meisn., *Hypoxis hemerocallidea* Fisch., C.A. Mey. & Avé-Lall., *Lippia javanica* (Burm.f.) Spreng, *Siphonochilus aethiopicus* (Schweif.) B.L. Burt, *Sutherlandia frutescens* (L.) R. Br., *Warburgia salutaris* (G. Bertol.) Chiov. and *Xysmalobium undulatum* (L.) W.T. Aiton (Van Wyk, 2008; Street and Prinsloo, 2013). Although these species are commercially important, most have only been subjected to basic inflammatory and toxicity screening assays. Indeed, *Harpagophytum procumbens* and *Aloe ferox* are potent anti-inflammatory agents that are commercialised in European countries, North America, Australia and Africa (Stewart and Cole, 2005; Makunga et al., 2008; Van Wyk, 2011; Chen et al., 2012; Street and Prinsloo, 2013; Mncwangi et al., 2012). However, relatively little *in vivo* toxicity data is available on these species. A recent publication on the *in vivo* genotoxic effects of *Aloe ferox* further highlighted the lack of such studies on this species (Galli et al., 2021).

The bark and leaves of *Warburgia salutaris* are widely used medicinally for various disorders, including inflammation, febrile complaints, rheumatism, sexually transmitted infections, malaria, toothache and backache, as well as for respiratory, gastrointestinal and dermatological disorders (Pujol, 1990; Mabogo, 1990; Hutchings et al., 1996; Grace et al., 2003; Van Wyk and Gericke, 2018). The biological and chemical properties of this plant have also been relatively well studied (Mashimbye et al., 1999; Rabe and Van Staden, 2000; Madikane et al., 2007; Mabona et al., 2013; Akhalwaya et al., 2018; Khumalo et al., 2019), although *in vivo* acute toxicity studies are not available. *Lippia javanica* is also widely used to treat multiple ailments (Hutchings et al., 1996; Van Wyk and Gericke, 2018). However, acute toxicity studies have revealed potential neurotoxic effects of *L. javanica* extracts (Lukwa et al, 1994; Madzimure et al., 2011). With many southern African inhabitants continuing to use medicinal plants on a regular basis, toxicity investigation of these herbal remedies is imperative.

It is estimated that there are over 28 million consumers of medicinal plant products in South Africa (Mander et al., 2007). Commercialised plant products are harvested and sold directly from the wild, often with no processing or toxicity evaluations (Grace et al., 2003; Khumalo, 2018). This review summarises the known toxicity effects of southern African medicinal plants that are used traditionally to treat pain and inflammation. The medicinal plants examined in this review were identified in a recent study, which identified 555 plant species used traditionally to treat pain and inflammation in seven countries of the southern African region (Khumalo et al., (2022). That study reported that only approximately 200 of those species had previously been screened for anti-inflammatory and analgesic properties, and highlighted the lack of toxicity data accompanying the biological activity of plant extracts.

2. Materials and methods

The aim of this study was to document the toxicity and phytochemical properties of southern African medicinal plants used traditionally to treat inflammation and pain-related ailments. Various ethnobotanical books, thesis publications and search engines (including Google-Scholar, PubMed, Scopus and Science-Direct electronic databases) were used to identify original scientific research papers. The following terms were used as filters and were searched both alone and in combinations: “Southern Africa”, “pain”, “inflammation” “anti-inflammatory”, “brine shrimp”, “cytotoxity”, and “plant poisons”.. The initial aim was to record cytotoxic properties of plant species applied therapeutically for pain and inflammatory disorders in southern Africa. Most of these species are native southern African plants. A further literature review was undertaken on the recorded plant species to identify those species reported for phytochemical properties.

2.1. Eligibility criteria

Peer reviewed journal articles and other literature were searched using specific key words, based on their relevance to the study. Abstract reading was used to determine the relevance of the study. The cited publications included in this study were all full text manuscripts and were thoroughly read to ensure that eligibility criteria were met.

2.1.1. Inclusion criteria

The following inclusion criteria for eligibility of the study were considered:

- Publications written in English and prior to August 2021 were used in this review.
- This review study is non-biased and without any taxonomic preference.
- In terms of ethnobotanical literature surveys, to be included in this review the plant species must be recorded to be used specifically for pain and inflammatory disorders, instead of just being recorded to treat individual non-specific symptoms.
- In terms of biological activity, only plant species that are used traditionally for pain and inflammation were included, rather than randomly selected plants or taxonomically related species.
- In terms of phytochemical properties, only studies reporting individual compounds were included, rather than those screened to identify the presence of classes of secondary metabolites.

2.1.2. Exclusion criteria

The following criteria were used to exclude some studies:

- Where name changes and families of plant species were encountered, particularly in older publications, the websites The Plant List (http://www.theplantlist.org/) and South African National Biodiversity Institute (SANBI) (http://www.sanbi.org) were used to confirm the correct or current nomenclature of the species. A final check was made using POWO (2022), (https://powo.science.kew.org/)
- Plant species that are used to treat symptoms of inflammation such as fever, colds and coughs as well as allergies, without specifically stating that they are used to treat inflammation, were excluded from this study.
- Introduced and exotic species were excluded, unless they were extensively used as part of southern African traditional medicine.

2.2. Data collection

A thorough literature search of publications on the southern African medicinal plants used therapeutically for pain and inflammation was undertaken and included in this study. Also, *in vivo* and *in vitro* biological screening of southern African medicinal plants specifically to treat pain and inflammatory disorders were included, regardless of the study origin.

The following data was collected:

- Plant name and families for each species recorded in the individual publications.
- Vernacular names of different ethnic groups were collected from individual publications and from the SANBI red list website.
- The plant part used, method of preparation and mode of administration were recorded (where available).

Microsoft Excel was used for statistical data analysis.

3. Results

A literature search identified 117 medicinal plant species (Table 1) with reported anti-inflammatory activity that have previously been screened for toxicity. The list includes both native southern African plant species and naturalised exotics. Fabaceae is the most represented family (18 species), followed by Euphorbiaceae and Asteraceae with five species each. Eighty-seven percent of the plant extracts screened were non-toxic when tested against normal cell lines. Thirteen percent of the extracts exhibited cytotoxic properties, of which 8% were toxic in cancer cells and 5% in normal cell lines. The Vero monkey cell line was found to be the most susceptible in multiple studies (Yelani et al., 2010; McGaw et al., 2014; Elisha et al., 2016; Yaouba et al., 2018). The highest toxicity against Vero cells (LC_{50} of 2.38 μg/mL) was noted from the acetone extract of *Maesa lanceolata* Forssk., (Elisha et al., 2016), closely followed by the roots of *Lannea schweinfurthii* Engl., with LC_{50} of 7.36 μg/mL (Yaouba et al., 2018). Quinones isolated from the methanol extracts of *M. lanceolata* also displayed potent toxicity against human cervix carcinoma cancer cells, with IC_{50} values between 5 nM and 12.5 μM (Seumo et al.,

Table 1
Cytotoxicity of southern African medicinal plants traditionally used to treat pain and inflammation.

Species	Plant part and extract	Toxicity assay used	Toxicity properties	References
Abrus precatorius L. subsp. *africanus* Verdc. Fabaceae	Ethanol extract of the leaves and stems	Ames test (*Salmonella typhimurium* TA98 strain)	Non-genotoxic with 22.6% (leaf) and 20.33% (stem) at 500 μg/mL	Madikizela et al. (2014)
	Ethanol extract of aerial parts	XTT toxicity assay	LC_{50} of 118.5 μg/mL against Vero cells	Tshikalange et al. (2016)
	Isolated compounds from methanol root extract	MTT toxicity assay	Toxic against cancer cell lines with LC_{50} values between 1 and 33 μM for human squamous cell carcinoma (CAL-27), human colorectal adenocarcinoma (Caco-2), and human lung cell carcinoma (NCI–H460)	Okoro et al. (2021)
Acokanthera oppositifolia (Lam.) Codd Apocynaceae	50% methanol extract of the roots	Ames assay (*Salmonella typhimurium* strain TA1535)	Mutagenic effect without metabolic activation at 5000 μg/mL	Kuete (2014)
	Organic extracts of the leaves	MTT toxicity assay	Cell line: RAW 264.7 macrophages; LC_{50} of 32.5 μg/mL	Adebayo et al. (2019)
Adansonia digitata L. Malvaceae	Organic and aqueous extracts of the leaves	Ames assay (*Salmonella typhimurium* strains TA98 and TA100)	Non-mutagenic at 500 μg/mL (with and without S9 metabolic activation)	Mulaudzi et al. (2013)
	The methanol leaf extract	MTT toxicity assay	Cell line: RAW 264.7 macrophages; LC_{50} of >100 μg/mL	Ayele et al. (2013); McGaw et al. (2014)
	The methanol leaf extract	MTT toxicity assay	LC_{50} of 70. 0 mg/mL on Vero monkey kidney cells	Lall and Kishore (2014)
Agathosma betulina (P.J. Bergius) Pillans Rutaceae	Methanol: dichloromethane (1:1) extract of the leaves	MTT cell viability assay	Cell line: Graham cells; LC_{50} of 100. 0 mg/mL	Lall and Kishore (2014)
	Methanol and aqueous extract of the leaves	Brine shrimp lethality assay	Non-toxic with percentage mortality of <10% after 48h exposure (concentration of 2 mg/mL)	Cock and Van Vuuren (2015)
Albizia adianthifolia (Schumach.) W. Wight Fabaceae	Aqueous extract of the bark	MTT cell viability assay	Cell line: Graham cells, human embryonic kidney cells (HEK-293); Non-toxic (110% at 100 μg/mL)	Naidoo et al. (2013)
Aloe arborescens Mill. Asphodelaceae	The ethanol leaf extract	MTT toxicity assay	Cell line: Melanocyte cells; non-toxic up to 25 mg/mL	Lall and Kishore (2014)
	1:1 Methanol and water extract of the leaves	MTT toxicity assay	Cell line: Vero cells; 80% viability at 100 μg/mL	Ghuman et al. (2019)
	Water and acid soluble polysaccharide fractions	Sulforhodamine-B cytotoxicity assay	Cytotoxic against HepG2 human liver cancer cells, with LC_{50} of 26.1 μg/mL (water soluble) and 21.4 μg/mL (acid soluble)	Nazeam et al. (2017)
Aloe ferox Mill. Asphodelaceae	Aqueous extract of the roots and leaves	Brine shrimp lethality assay	LD_{50} of 900 μg/mL	Abosede et al. (2015)
	Leaf gel and whole leaf extract	MTT toxicity assay	>80 % viability at 400 μg/mL in normal human keratinocyte cells	Fox et al. (2017)
	1:1 Methanol and water extract of the leaves	MTT toxicity assay	Cell line: Vero cells; >80% viability at 100 μg/mL	Ghuman et al. (2019)
Amaranthus hybridus L. subsp. *cruentus* (L.) Thell Amaranthaceae	Whole plant extract	Brine shrimp lethality assay	LC_{50} of 116.1 μg/mL	McGaw et al. (2014)
	Methanol and aqueous extract of the leaves	Brine shrimp lethality assay	Non-toxic with percentage mortality of <50% after 48h exposure (concentration of 2 mg/mL)	Cock and Van Vuuren (2015)
	Methanol and aqueous extract of the leaves	MTT toxicity assay	Cell line: Mouse fibroblast (3T3 NIH); cell viability of >50% at 1000 μg/mL	Mzindle (2017)
Antidesma venosum E. Mey. ex Tul. Phyllanthaceae	Aqueous extract of the roots	Acute systematic toxicity test	LC_{50} of 25.4 μg/mL against HeLa cells and 44.00 μg/mL in MCF-12A cells	Steenkamp et al. (2009)
	Dichloromethane extract of the leaves and twigs	Micronucleus and Comet tests (human white blood cells)	Positive results in micronucleus test, twigs tested positive in both tests without S9	Kuete (2014)
Artemisia afra Jacq. ex Willd. Asteraceae	Ethanol and aqueous extract	MTT toxicity assay	LC_{50} values of 18.2 against U937 and 31.8 μg/mL in HeLa cancer cells. LC_{50} of >250 μg/mL from aqueous extract in both cell line	Spies et al. (2013)
	Ethanol extract of the plant	XTT cell proliferation kit	Cell line: fibroblasts; 20% viability at 100 μg/mL	More et al. (2012)
	Chloroform fraction of the leaves	LDH leakage assay	Toxic with LC_{50} of <10 μg/mL HepG2 cells	Moyo et al. (2019)
	Aqueous and ethanol extracts of the leaves	MTT toxicity assay		Mzindle (2017)

(*continued*)

Table 1 (*Continued*)

Species	Plant part and extract	Toxicity assay used	Toxicity properties	References
Asystasia gangetica (L.) T. Anderson Acanthaceae			Cell line: Mouse fibroblast; cell viability of >50% at 1000 μg/mL	
Bidens pilosa L. Asteraceae	Aqueous extract of the leaves	MTT cell viability assay	Cell line: Graham cells, HEK-293; non-toxic (112% at 100 μg/mL)	Naidoo et al. (2013)
	Aqueous extract of the leaves	XTT cell viability assay	Non-toxic against MT-4 cell line; LC_{50} of 100 μg/mL	Hurinanthan (2013)
	Aqueous and ethanol extract of the leaves	MTT cell viability assay	Cell line: Mouse fibroblast; non-toxic with cell viability of >100% at 1000 μg/mL	Mzindle (2017)
Bolusanthus speciosus (Bolus) Harms Fabaceae	Acetone leaf extract	MTT toxicity assay	Non-toxic against Vero cells with LC_{50} of 52.8 μg/mL	Elisha et al. (2016)
Bridelia micrantha (Hochst.) Baill. Phyllanthaceae	Methanol extract of the leaves	Brine shrimp lethality assay	LC_{50} of >90.00 mg/mL	Ajaiyeoba et al (2006)
	Aqueous extract of the bark	Acute systematic toxicity test	Toxic in HeLa cells with LC_{50} of 8.9 μg/mL and 24.2 μg/mL in MCF-12A cells	Steenkamp et al. (2009)
Bulbophyllum scaberulum (Rolfe) Bolus Orchidaceae	Ethanol root extract	Ames test (*Salmonella typhimurium* TA98 strain)	Mutagenic effect without metabolic activation at 50 μg/mL	Chinsamy et al. (2014)
Burkea africana Hook. Fabaceae	Aqueous and methanol extract of the bark	Neutral red uptake assay	LC_{50} of 42.5 μg/mL in C2C12 myoblasts and 84.5 μg/mL in 3T3-L1 pre adipocytes. Non-toxic in normal human dermal fibroblasts and U937 human pro-monocytic cell line with LC_{50} of >100 μg/mL	Cordier et al. (2013)
	Acetone leaf extract	MTT cell viability assay	Cell line: RAW 264.7 macrophages; 53.7% cell viability at 50 μg/mL	Dzoyem and Eloff (2015)
	Ethanol extract of the bark	Cytopathic effect inhibition assay	Non-toxic in Madin Darby canine kidney cells	Mair et al. (2018)
Calpurnia aurea (Aiton) Benth. subsp. *aurea* Fabaceae	Acetone leaf extract	MTT toxicity assay	Toxic against Vero cells with LC_{50} of 13.6 μg/mL	Elisha et al. (2016)
Carpobrotus dimidiatus (Haw.) L. Bolus Aizoaceae	Methanol leaf extract	XTT cell viability assay	Non-toxic against MT-4 cell line; LC_{50} of 113 μg/mL	Hurinanthan (2013)
	Aqueous and methanol extracts of the leaves	MTT cell toxicity assay	Cell line: Mouse fibroblast; >50% viability at 1000 μg/mL	Mzindle (2017)
	50% methanol extract of the leaves	MTT toxicity assay	Non-toxic in RAW 264.7 and Vero cells with LC_{50} of >1000 μg/mL; LC_{50} of 826.1 μg/mL in HepG2 liver cell line	Mulaudzi et al. (2019)
Carpobrotus edulis (L.) L.Bolus subsp. *edulis* Aizoaceae	Aqueous and methanol extracts of the fruit	Brine shrimp lethality assay	Non-toxic with percentage mortality of <20% after 48h (concentration of 2 mg/mL)	Cock and Van Vuuren (2015)
	1:1 Methanol and dichloromethane leaf extract	Brine shrimp lethality assay	Percentage mortality of 48% after 48h (concentration of 1 mg/mL)	Akhalwaya et al. (2018)
	Aqueous leaf extract	MTT toxicity assay	Cell line: RAW 264.7 macrophages; LC_{50} of > 300.0 μg/mL. Vero kidney cells; LC_{50} of 309.2 μg/mL and LC_{50} of 297.5 μg/mL in HepG2 liver cell line	Mulaudzi et al. (2019)
	Acetone extract of the leaves	MTT cell viability assay	Cell line: RAW 264.7 macrophages; 92% cell viability at 100 μg/mL	Ondua et al. (2019)
Centella asiatica (L.) Urb. Apiaceae	Dichloromethane extract of the whole plant	Alamar Blue fluorescent cytotoxicity assay	Cell line; Rat skeletal myoblast L6; LC_{50} of 82.6 μg/mL	Irungu et al. (2007); McGaw et al. (2014)
	Acetone extract of the leaves Aqueous leaf extract	MTT cell viability assay	Cell line: MCF-7; LC_{50} of 53.6 μg/ml; 81.8 in Caco-2 and 46.4 μg/ml in A549 cell lines LC_{50} of > 100 μg/mL in MCF-7, Caco-2, and A549 cells	Soyingbe et al. (2018)
Chenopodium album L. Amaranthaceae	Aqueous and methanol extracts of aerial parts	XTT cell viability assay	Non-toxic against MT-4 cell line; LC_{50} of >100 μg/mL	Hurinanthan (2013)
Clausena anisata (Willd.) Hook.f. ex Benth. Rutaceae	The ethanol leaf extract	Acute toxicity assay	LD_{50} of 393.7 mg/kg in albino rats	Lall and Kishore (2014)
	Acetone extract of the leaves	MTT toxicity assay	Cell line: Vero cells; LD_{50} of 53.0 μg/mL	Adamu et al. (2012); McGaw et al. (2014)
	Acetone extract of the leaves	MTT toxicity assay	LC_{50} of 23.0 μg/mL in Vero cell line	Adebayo et al., (2015)
	1:1 Methanol and dichloromethane extract of leaves and stems	Brine shrimp lethality assay	Stems; LC_{50} of 120 μg/mL at 48h Leaves; LC_{50} of 130 μg/mL at 48h	Akhalwaya et al. (2018)
Clematis brachiata Thunb. Ranunculaceae	Aqueous extract of the leaves	MTT cell viability assay	Cell line: HEK-293; non-toxic (100% at 100 μg/mL)	Naidoo et al. (2013)

(*continued*)

Table 1 (*Continued*)

Species	Plant part and extract	Toxicity assay used	Toxicity properties	References
	Ethanol leaf extract	XTT toxicity assay	Non-toxic in human melanoma (A375), epidermoid carcinoma (A431), HeLa and HEK-293 cell lines with LC_{50} of >200 μg/mL	Twilley et al. (2017)
	1:1 Methanol and dichloromethane extract of the leaves and stems	Brine shrimp lethality assay	Non-toxic with percentage mortality of 31.6% at 48h (leaves) and 46.1% at 48h (stem)	Akhalwaya et al. (2018)
Cleome monophylla L. Cleomaceae	Aqueous root extract	XTT cell viability assay	Non-toxic against MT-4 cell line; LC_{50} of 109 μg/mL	Hurinanthan (2013)
	Aqueous and methanol extracts of the leaves	MTT toxicity assay	Cell line: Mouse fibroblast; non-toxic with cell viability of >100% at 50 μg/mL	Mzindle (2017)
Combretum zeyheri Sond. Combretaceae	Methanol extract of the roots	Brine shrimp lethality assay	LC_{50} of >100 μg/mL	Lima et al. (2012)
	Acetone leaf extract	MTT toxicity assay	Cell line: RAW 264.7 macrophages; 55.9% cell viability at 50 μg/mL	Dzoyem and Eloff (2015)
**Conyza canadensis* (L.) Cronquist Asteraceae	Organic extracts of aerial parts	MTT toxicity assay	Cell line: MRC-5; LC_{50} of 250.00 μg/mL	McGaw et al. (2014)
	Organic extracts of the leaves	MTT toxicity assay	Cell line: RAW 264.7; LC_{50} of 17.6 μg/mL	Adebayo et al. (2019)
Cotyledon orbiculata L. Crassulaceae	1:1 Methanol and dichloromethane extract of the leaves	Brine shrimp lethality assay	Non-toxic with percentage mortality of 35.2% at 48h	Akhalwaya et al. (2018)
	Acetone extract of the leaves	MTT cell viability assay	Cell line: RAW 264.7 macrophages; 78.3% cell viability at 50 μg/mL	Ondua et al. (2019)
Cremaspora triflora (Thonn.) K. Schum. subsp. *triflora* Rubiaceae	Acetone leaf extract	MTT toxicity assay	Non-toxic against Vero cells with LC_{50} of 57.4 μg/mL	Elisha et al. (2016)
Crinum moorei Hook. f. Amaryllidaceae	Methanol extract of the plant	MTT toxicity assay	LC_{50} of 90.5 μg/mL in U251; 82.3 μg/mL in U87 glioblastoma cells; and LC_{50} of >100 μg/mL SH-SY5Y neuroblastoma cells	Omoruyi et al. (2021)
Croton gratissimus Burch. Euphorbiaceae	1:1 Methanol and dichloromethane extract of stem and leaves	Brine shrimp lethality assay	Non-toxic with percentage mortality of 42.7% at 48h (stem). LC_{50} of 103 μg/mL at 48h (leaves)	Akhalwaya et al. (2018)
	Acetone extract of the leaves	MTT toxicity assay	Cancer cell lines: LC_{50} of 97.4 μg/mL in A549, LC_{50} of 74.0 μg/mL against Caco-2, LC_{50} of 78.2 μg/mL in HeLa and LC_{50} of 83.7 μg/mL in MCF-7 cells. Non-toxic against Vero cells with LC_{50} of 462.8 μg/mL	Njoya et al. (2018)
Croton sylvaticus Hochst. Euphorbiaceae	Ethanol extract of the leaves Isolated compounds from the leaves	Brine shrimp lethality assay	Toxic, with LC_{50} of 29.7 μg/mL, LC_{50} of 0.074 μg/mL (julocrotine), LC_{50} of 308 μg/mL (lupeol), LC_{50} of 312 μg/mL (penduliflaworosin)	Kapingu et al. (2005)
	Dichloromethane and methanol extract of aerial parts	Micronucleus test (human lymphocyte cultures)	Extracts induced micronuclei without exogenous metabolic activation S9 (concentration not reported)	Kuete (2014)
	Acetone extract of the leaves	MTT toxicity assay	Cancer cell lines: LC_{50} of 32.7 μg/mL in A549, LC_{50} of 150.6 μg/mL in Caco-2, LC_{50} of 169.0 μg/mL in HeLa, and LC_{50} of 13.1 μg/mL in MCF-7 cells. Extract was toxic in Vero cells with LC_{50} of 27.9 μg/mL	Njoya et al. (2018)
Cryptocarya latifolia Sond. Lauraceae	Dichloromethane extract of the bark	Brine shrimp lethality assay	Non-toxic with percentage mortality of 30.88% at 48h (concentration of 2 mg/mL)	Khumalo et al. (2021)
Datura stramonium L. Solanaceae	Methanol and chloroform extracts of the leaves	Brine shrimp lethality assay	Extracts showed cytotoxicity with LC_{50} of 21.6 μg/mL	Khalighi et al. (2012)
	Leaves	brine shrimp lethality assay	LC_{50} of 39 μg/mL	McGaw et al. (2014)
	Dichloromethane and methanol extracts of the seeds and leaves	Ames test (*Salmonella typhimurium* strain)	Mutagenic effect by increasing the number of His1 revertants with Umu-C test in the absence of S9	Kuete (2014)
Dichrostachys cinerea (L.) Wight & Arn. Fabaceae	Methanol extract of the leaves	XTT cell viability assay	Non-toxic against MT-4 cell line; LC_{50} of 106 μg/mL	Hurinanthan (2013)
	Methanol extract of the leaves	MTT cell viability assay	Non-toxic in mouse fibroblast cells with cell viability of 205% at 1000 μg/mL	Mzindle (2017)
		Brine shrimp lethality assay		Akhalwaya et al. (2018)

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Table 1 (*Continued*)

Species	Plant part and extract	Toxicity assay used	Toxicity properties	References
	1:1 Methanol and dichloromethane Extract of stems		Non-toxic with percentage mortality of 15% at 48h (concentration of 1 mg/mL)	
Diospyros lycioides Desf. Ebenaceae	Acetone extract of the leaves	MTT cell viability assay	Cell line: MCF-7 cells, percentage mortality of >70% at 50 μg/mL after 48h	Pilane et al. (2015)
Diospyros mespiliformis Hochst. ex A. DC. Ebenaceae	Ethanol extract of the roots and leaves	XTT toxicity assay	LC_{50} of 89.5 μg/mL against Vero cells	Tshikalange et al. (2016)
Dombeya rotundifolia (Hochst.) Planch. Malvaceae	Dichloromethane extract of the bark	Brine shrimp lethality assay	Non-toxic with percentage mortality of 7.84% (24h) and 7.8 % (48h) (concentration of 2 mg/mL)	Khumalo et al. (2021)
Ehretia rigida (Thunb.) Druce Boraginaceae	Acetone leaf extract	MTT cell viability assay	Cell line: RAW 264.7 macrophages; 34.2% cell viability at 50 μg/mL	Dzoyem and Eloff (2015)
Ekebergia capensis Sparrm. Meliaceae	Aqueous extracts of the leaves	XTT cell viability assay	Non-toxic against MT-4 cell line; LC_{50} of 100 μg/mL	Hurinanthan (2013)
	Organic extracts of the leaves	MTT toxicity assay	Toxic in Vero cells; LC_{50} of 13-27 μg/mL	McGaw et al. (2014)
	Aqueous and methanol extracts of leaves	MTT toxicity assay	Non-toxic in mouse fibroblast cells with cell viability of >150% at 1000 μg/mL	Mzindle (2017)
	Aqueous extract of the bark	Ames test(*Salmonella typhimurium* strain TA98)	Weakly increases the number of His1 revertant colonies with and without S91 activation	Kuete (2014)
	Methanol extract of the bark	Brine shrimp lethality assay	Non-toxic with percentage mortality of 19.2% at 48h (concentration of 2 mg/mL)	Khumalo et al. (2021)
Elaeodendron croceum (Thunb.) DC. Celastraceae	Acetone extract of the leaves 20-hydroxy-20-epi-tingenone and tingenone	XTT toxicity assay XTT toxicity assay	Non-toxic in MCF-7; LC_{50} of 126.4 μg/mL; toxic in Vero cells with LC_{50} of 56.1 μg/mL Compounds were cytotoxic in HeLa, SNO and MCF-7 cancer cells with LC_{50} between 2.4 and 0.4 μM	Yelani et al. 2010
	Acetone extract of the leaves	MTT toxicity assay	Toxic against Vero cells with LC_{50} of 5.2 μg/mL	Elisha et al. (2016)
	Aqueous solution of silver nanoparticles with the aqueous barks and leaves extracts	MTT toxicity assay	LC_{50} of 18.3 μg/mL in MDA-MB-231 and LC_{50} of 8.3 μg/mL against MCF-7 cells (Leaves) LC_{50} of 31.0 μg/mL in MDA-MB-231 and LC_{50} of 16.5 μg/mL in MCF-7 cells (Bark)	Odeyemi et al. (2020)
Elephantorrhiza elephantina (Burch.) Skeels Fabaceae	Aqueous root extract	Acute toxicity assay	Non-toxic in rats up to 1600 mg/kg body weight	Lall and Kishore (2014)
	Aqueous extract of the bulb	MTT toxicity assay	Non-toxic in RAW 264.7 macrophages and Vero cells with >100% cell viability at 50 μg/mL; and >80% at 125 μg/mL in U937 cells	Sigidi et al. (2017)
Emex australis Steinh. Polygonaceae	Aqueous extracts of the leaves	XTT toxicity assay	Non-toxic against MT-4 cell line; LC_{50} of 91 μg/mL	Hurinanthan (2013)
Englerophytum magalismontanum (Sond.) T.D.Penn. Sapotaceae	Acetone leaf extract	MTT cell viability assay	Cell line: RAW 264.7 macrophages; non-toxic with 98.2% cell viability at 50 μg/mL	Dzoyem and Eloff (2015)
	1:1 Methanol and dichloromethane extract of stems	Brine shrimp lethality assay	Percentage mortality of 42.6% at 48h (concentration of 1 mg/mL)	Akhalwaya et al. (2018)
Erythrina caffra Thunb. Fabaceae	Aqueous extracts of the seeds	Brine shrimp lethality assay	Non-toxic with LC_{50} value of 630.0 μg/mL	Lewis (1995)
	Methanol extract of the bark	Micronucleus test (human lymphocyte cultures)	Induced micronuclei without exogenous metabolic activation S9 (concentration not reported)	Kuete (2014)
	Isolated compounds caffraisoflavan I, caffraisoflavan II, erystagallin A, erythrabyssin I, erythrabyssin II, abyssinone II, abyssinone IV and abyssinone V	Cell line toxicity assay	Five compounds exhibited cytotoxic effects at 6.2 – 12.5 μg/mL in HEK-293 cells and MES-SA, a human uterine sarcoma epithelial cell line	El-Masry et al. (2014)
Erythrina lysistemon Hutch. Fabaceae	Acetone extract of the stem bark	Cell line toxicity test	Cell line: Monkey kidney cells; LC_{50} of 69.0 μg/mL	McGaw et al. (2014)
	1:1 Methanol and dichloromethane extract of stems	Brine shrimp lethality assay	Non-toxic with percentage mortality of 16.3% at 48h (concentration of 1 mg/mL)	Akhalwaya et al. (2018)

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Table 1 (*Continued*)

Species	Plant part and extract	Toxicity assay used	Toxicity properties	References
	Dichloromethane and methanol extracts of bark	Brine shrimp lethality assay	Non-toxic with percentage mortality of 37.0% at 48h (MeOH) and 16.6% at 48h (DCM extract)	Khumalo (2018)
	Seven isoflavone derivatives isolated from the bark	Brine shrimp lethality assay	All compounds were non-toxic with percentage mortality between 14% and 23.5% at 48h	Sadrgove et al. (2020)
Erythrophleum lasianthum Corbishley Fabaceae	Aqueous extract of the leaves	Brine shrimp lethality assay	Non-toxic with LC_{50} of 2000 μg/mL at 24h	Lewis (1995)
	Ethanol leaf extract	XTT toxicity assay	Non-toxic against HeLa and HEK-293 cell lines with LC_{50} of >200 μg/mL. Toxic against A375 (LC_{50} of 58.6 μg/mL)	Twilley et al. (2017)
	Leaf extract	MTT toxicity and RTCA assays	Extracts were non-toxic in mononuclear leukocytes, U937 macrophage, Vero kidney and liver cell lines	Maroyi (2019)
Euclea undulata Thunb. Ebenaceae	Acetone leaf extract	MTT cell viability assay	Cell line: RAW 264.7 macrophages; non-toxic with 84.9% cell viability at 50 μg/mL	Dzoyem and Eloff (2015)
Eucomis autumnalis (Mill.) Chitt Hyacinthaceae	Methanol extract of the bulbs	MTT toxicity assay	Non-toxic in HEK293 cells with LC_{50} of 6011 μg/mL	Ayeni et al. (2019)
	1:1 Methanol and water extract of the leaves and bulbs	MTT toxicity assay	Cell line: Vero cells; >50% viability at 100 μg/mL	Ghuman et al. (2019)
Eulophia petersii (Rchb.f.) Rchb.f. Orchidaceae	Petroleum ether extract of the bulb	Ames test (*Salmonella typhimurium* TA98 strain)	Mutagenic potential without metabolic activation at 5000 μg/mL.	Chinsamy et al. (2014)
Ficus elastica Roxb. ex Hornem. Moraceae	Acetone extract of the leaves	MTT toxicity assay	Cell line: RAW 264.7 macrophages; 86.6% cell viability at 50 μg/mL	Ondua et al. (2019)
Ficus sur Forssk. (= *Ficus capensis* Thunb.) Moraceae	Methanol extract of the fruit	MTT toxicity assay	Cell line: RAW 264.7 macrophages; LC_{50} of >100 μg/mL	Ayele et al. (2013)
	Aqueous extract of the leaves	XTT cell viability assay	Non-toxic against MT-4 cell line; LC_{50} of 71 μg/mL	Hurinanthan (2013)
	Aqueous and methanol extracts of the leaves	MTT toxicity assay	Non-toxic in mouse fibroblast cells with cell viability of >150% at 1000 μg/mL	Mzindle (2017)
	Ethanol extract of the bark and roots	The Ames test (*Salmonella typhimurium* strain TA98)	Non-genotoxic with 28% (bark) and 22.6% (roots) at 500 μg/mL	Madikizela et al. (2014)
Gomphocarpus fruticosus (L.) Aiton f. subsp. *fruticosus* Apocynaceae	Leaf ethanol extract	XTT toxicity assay	Non-toxic in A375, A431 and HEK-293 cancer cell lines with LC_{50} of >200 μg/mL. Toxic in HeLa cells with LC_{50} of 51 μg/mL	Twilley et al. (2017)
Gunnera perpensa L. Gunneraceae	Aqueous and methanol extracts of the leaves	XTT toxicity assay	Non-toxic against MT-4 cell line; LC_{50} of >100 μg/mL	Hurinanthan (2013)
	The methanol extract of rhizome	Brine shrimp lethality test	LC_{50} of 137.6 mg/mL	Lall and Kishore (2014)
	Aqueous and methanol extract of the leaves	MTT toxicity assay	Non-toxic in mouse fibroblast cell line with cell viability of >100% at 1000 μg/mL	Mzindle (2017)
Gymnosporia senegalensis (Lam.) Loes. Celastraceae	Unidentified fractions from the dichloromethane extract of leaves	WST-8 cell proliferation assay	Cell line: RAW264.7 macrophages; Percentage inhibition of >80% at 250 μg/mL	Makgatho et al. (2018)
	Methanol extract of the leaves	MTT cell proliferation assay	Isolated compounds, (2S)-1-O-(4′Z,7′Z,10′Z -octadecatrienoyl) glycerol, quercetin 3-O-α-L-arabinofuranoside, β-sitosterol glucoside and β-sitosterol displayed cell viability between 50-70% at 50μM in colon cancer (DLD1), MCF-7, and gastric cancer (MKN45) cells	Tatsimo et al. (2019)
Harpagophytum procumbens (Burch.) DC. ex Meisn. Pedaliaceae	Ethanol extract of the roots	Tetracolour one assay	Cell line: RAW 267.4 macrophages; cell viability of 96.9% at 200 μg/mL	Inaba et al. (2010)
	The ethanol root extract	Acute toxicity assay	LD_{50} of 13.5 g/kg body weight	Lall and Kishore (2014)
	Aqueous extract of the roots	MTT toxicity assay	Non-toxic against mouse myoblast C2C12 cells with >100% cell viability at 500 μg/mL, and <100% at 100μg/mL in human colorectal adenocarcinoma HCT116 cell lines	Locatelli et al. (2017)
		Brine shrimp lethality assay	LC_{50} of >20 mg/mL	Recinella et al. (2020)

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Table 1 (*Continued*)

Species	Plant part and extract	Toxicity assay used	Toxicity properties	References
	Aqueous extract of the whole plant			
Harpephyllum caffrum Bernh. Anacardiaceae	The leaf ethanol extract	Acute toxicity assay	LD_{50} of up to 10 g/kg b. wt. in male albino rats	Shabana et al. (2011)
	Water-methanol (1:3) leaf extract	MTT toxicity assay	Cell line: human keratinocyte cells; LC_{50} of 50 mg/mL	Lall and Kishore (2014)
	1:1 Methanol and dichloromethane leaf extract	MTT toxicity assay	LC_{50} of 190 μg/mL in *Bovine dermis*; LC_{50} of 290 μg/mL in Vero cell line	Mongalo et al. (2018)
	Methanol extract of the bark	Brine shrimp lethality assay	Non-toxic with percentage mortality of 13.6% at 48h (concentration of 2 mg/mL)	Khumalo (2018)
Helichrysum nudifolium (L.) Less. var. *pilosellum* (L.f.) Beentje Asteraceae	Methanol and dichloromethane extract of the leaves	The Ames assay (*Salmonella typhimurium* strains TA98 and TA 100)	Non-mutagenic (with and without S9 metabolic activation)	Reid et al. (2006)
	1:1 Chloroform and methanol extract of aerial parts	Sulforhodamine B assay	Cell viability of 73% in Graham cells; 83% in SF-268 glioblastoma cells and 35% in MCF-7 at 100 μg/mL	Lourens et al. (2011)
Heteromorpha arborescens (Spreng.) Cham. & Schltdl. Apiaceae	Acetone extract of the leaves	MTT toxicity assay	Vero monkey kidney cells; LC_{50} of 43 μg/mL	Adamu et al. (2012)
	Acetone leaf extract	MTT toxicity assay	Non-toxic against Vero cells with LC_{50} of 81 μg/mL	Elisha et al. (2016)
	Dichloromethane and methanol extracts of the bark, leaves and twigs	Micronucleus test Comet test (human lymphocyte cultures)	The DCM extract of twigs and bark, methanol leaf extract induced micronuclei without S9. Mutagenic effect of DCM leaf extract in comet test without S9	Kuete (2014)
Hypericum roeperianum G.W. Schimp. ex A. Rich. Hypericaceae	Acetone leaf extract	MTT toxicity assay	Cell line: Vero cells with LC_{50} of 66.2 μg/mL	Elisha et al. (2016)
	Methanol bark extract and isolated compounds	Resazurin reduction assay	LC50 values ranged from 11.43 μg/mL in leukaemia cancer cell line and 26.7 μg/mL against HCT116 cells. Compounds displayed cytotoxicity against all nine tested cancer cell lines	Guefack et al. (2020)
Hypoxis hemerocallidea Fisch., C. A. Mey. & Avé-Lall. Hypoxidaceae	Methanol and dichloromethane extracts of bulbs and leaves	The Ames assay (*Salmonella typhimurium* strains TA98 and TA 100)	Non-mutagenic (with and without S9 metabolic activation)	Reid et al. (2006)
	Aqueous extract of the corm	MTT cell viability assay	Non-toxic in Graham cells, 104% cell growth at 100 μg/mL	Naidoo et al. (2013)
	Organic extracts of the bulbs	MTT toxicity assay	LC_{50} of 210.9 and 95.5μg/mL for PE and MeOH extracts respectively	Mwinga et al. (2019)
	Methanol extract of bark	Micronucleus test (human lymphocyte cultures)	Induced micronuclei without S9 (concentration not reported)	Kuete (2014)
Jatropha curcas L. Euphorbiaceae	Methanol extract of the leaves	Alamar blue assay	Cell-line: Rat skeletal myoblast cells; non-toxic with LC_{50} of >450.0 μg/mL	McGaw et al. (2014)
	Acetone leaf extract	MTT cell viability assay	Cell line: RAW 264.7 macrophages; 71.4% cell viability at 50 μg/mL	Dzoyem and Eloff (2015)
Kigelia africana (Lam.) Benth. Bignoniaceae	Ethanol extracts of fruits, seeds, and roots	Brine shrimp lethality assay	Fruits and seeds; LC_{50} of 124.0 μg/mL Root LC_{50} of 593 μg/mL	Gadir (2012)
	Aqueous extract of the leaves	MTT cell viability assay	Potentially toxic in Graham cells with 78% cell growth at 100 μg/mL	Naidoo et al. (2013)
	Ethyl acetate extract of the bark	Alamar blue assay	Cell line: LLC/MK2 monkey kidney cells; LC_{50} of 125 μg/mL	McGaw et al. (2014)
	Methanol leaf extract	Acute toxicity test	Safe up to 3000 mg/kg dosage in swiss albino mice	Lall and Kishore (2014)
	Aqueous and methanol extract of the leaves	Brine shrimp lethality assay	Non-toxic with percentage mortality of <50% after 48h exposure (concentration of 2 mg/mL)	Cock and Van Vuuren (2015)
Lannea schweinfurthii Engl. Anacardiaceae	Methanol extract of the roots	Tryphan blue exclusion assay Acute toxicity assay	Cell line: Vero E6; LC_{50} of 225.2 μg/mL LD_{50} of >5000 mg/kg body weight in mice	Gathirwa et al. (2008)
	Ethanol root extract	MTT and neutral red uptake assays	Non-toxic in both assays with LC_{50} of >100 μg/mL against SH-SY5Y cells	Adewusia et al. (2013)

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Table 1 (*Continued*)

Species	Plant part and extract	Toxicity assay used	Toxicity properties	References
	Root extract 3-((E)-nonadec-16′-enyl) phenol	MTT cytotoxicity assay	Toxic against Vero cells (LC_{50} =7.3 μg/mL). Toxic against Vero cells LC_{50} = 16.1 μg/mL	Yaouba et al. (2018)
Leonotis leonurus (L.) R.Br. Lamiaceae	70% methanol and chloroform extract of aerial parts	Acute toxicity test	Non-toxic; LD_{50} value >5000 mg/100 g body weight	El-Ansari et al. (2009)
	Aqueous leaf extract	XTT cell viability assay	Non-toxic against MT-4 cell line; LC_{50} of 101 μg/mL	Hurinanthan (2013)
	Aqueous and methanol extract of the leaves	MTT cell viability assay	Non-toxic in mouse fibroblast, cell viability of >150% at 1000 μg/mL	Mzindle (2017)
Leucaena leucocephala (Lam) DeWit Fabaceae	Acetone leaf extract	MTT cell viability assay	Cell line: RAW 264.7 macrophages; 76.2% cell viability at 50 μg/mL	Dzoyem and Eloff (2015)
Lippia javanica (Burm.f.) Spreng Verbenaceae	Methanol extract of the roots	Brine shrimp lethality assay	LC_{50} of 1138.00 μg/mL	McGaw et al. (2014)
	Methanol extract of the leaves	Brine shrimp lethality assay	LC_{50} of 2237 μg/mL (concentration of 2 mg/mL)	Cock and Van Vuuren (2015)
	Acetone leaf extract	MTT cell viability assay	Cell line: RAW 264.7 macrophages; 56.2% cell viability at 50 μg/mL	Dzoyem and Eloff (2015)
Maesa lanceolata Forssk. Maesaceae	Acetone extract of the leaves	MTT toxicity assay	Non-toxic in Vero monkey kidney cells; LD_{50} of 104 μg/mL	Adamu et al. (2012)
	Acetone leaf extract	MTT toxicity assay	Toxic against Vero cells with LC_{50} of 2.3 μg/mL	Elisha et al. (2016)
Melianthus comosus Vahl Melianthaceae	Acetone extract of the leaves	Brine shrimp lethality assay	Non-toxic against *Artemia salina* (concentration not specified)	McGaw et al. (2005)
	Ethanol extract of the leaves	XTT toxicity assay	Cell line: Vero cells; LC_{50} of 51.4 μg/mL	Heyman et al. (2009)
	Six isolated compounds from the leaves	Resazurin reduction assay	All compounds were toxic in cancer cells. Compound 1 was the most potent, with LC_{50} values of 0.07 μM in CCRF-CEM, 0.06 μM in CEM/ADR5000 and 0.36 μM against MCF-7	Bedane, et al. (2020)
Merwilla plumbea (Lindl.) Speta [=*Scilla natalensis* Planch.] Asparagaceae	Dichloromethane and methanol extracts of the bark	Micronucleus test	Induced micronuclei without S9 (human lymphocyte cultures) (concentration not reported)	Kuete (2014)
Morus mesozygia Stapf ex A. Chev. Moraceae	Acetone leaf extract	MTT cell viability assay	Cell line: RAW 264.7 macrophages; non-toxic with 61% cell viability at 50 μg/mL	Dzoyem and Eloff (2015)
	Acetone leaf extract	MTT toxicity assay	Cell line: Vero cells; LC_{50} of 40.7 μg/mL	Elisha et al. (2016)
Ocotea bullata (Burch.) E. Mey. in Drége Lauraceae	Methanol extract of the bark	Comet assay (human lymphocyte cultures)	DNA damage without exogenous metabolic activation S9 (concentration not reported)	Kuete (2014)
	Aqueous extract of the bark	MTT cell toxicity assay	Non-toxic to Vero cells with an LC_{50} of 380 μg/mL	Ogundajo et al. (2018)
Peltophorum africanum Sond. Fabaceae	Organic and aqueous extract of the bark	The Ames assay (*Salmonella typhimurium* strains TA98 and TA100)	Non-mutagenic at 500 μg/mL (with and without S9 metabolic activation)	Mulaudzi et al. (2013)
	Aqueous extract of the roots	MTT cell viability assay	Cell line: Graham cells, HEK-293; non-toxic (98% cell growth at 100 μg/mL)	Naidoo et al. (2013)
	Acetone extract of the leaves	MTT cell viability assay	Cell line: Vero Monkey kidney cell; LC_{50} of 103 μg/mL	Adebayo et al. (2015)
	Ethanol extract of the roots	XTT toxicity assay	Non-toxic with LC_{50} of 133 μg/mL against Vero cells	Tshikalange et al. (2016)
Pentanisia prunelloides (Klotzsch ex Eckl. & Zeyh.) Walp. Rubiaceae	The ethanol leaf extract	MTT toxicity assay	Non-toxic on monkey kidney cells with LC_{50} up to 31 mg/mL	Lall and Kishore (2014)
	Ethanol extract of the leaves and roots	The Ames test (*Salmonella typhimurium* strain (TA98)	Non-genotoxic with 22% (leaf) and 27.6% (root) at 500 μg/mL	Madikizela et al. (2014)
	Aqueous extracts of the leaves and rhizomes	Acute toxicity test	Non-toxic, LD_{50} of ≥5000 mg/kg orally	Miya et al. (2016)
Physalis viscosa L. Solanaceae	Methanol extracts of the leaves	XTT cell viability assay	Toxic against MT-4 cells, with a LC_{50} of 34 μg/mL	Hurinanthan (2013)
	Aqueous and methanol extract of the leaves	MTT cell viability assay	Non-toxic in mouse fibroblasts, cell viability of >100% at 1000 μg/mL	Mzindle (2017)
Pittosporum viridiflorum Sims Pittosporaceae	Methanol and aqueous extract of the leaves	Brine shrimp lethality assay	Non-toxic with percentage mortality of <20% after 48h exposure (concentration of 2 mg/mL)	Cock and Van Vuuren (2015)
	Dichloromethane extract of the bark	Brine shrimp lethality assay		Khumalo et al. (2021)

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Table 1 (*Continued*)

Species	Plant part and extract	Toxicity assay used	Toxicity properties	References
			Percentage mortality of 17.9% at 48h (concentration of 2 mg/mL)	
	Acetone leaf extract	MTT toxicity assay	Non-toxic against Vero cells with LC_{50} of 54.6 μg/mL	Elisha et al. (2016)
Plantago lanceolata L. Plantaginaceae	Organic extract of aerial parts	Sulforhodamine B (SRB) assay	LC_{50} values of 172.3 μg/mL in HeLa; 142.8 μg/mL in MCF-7; 405.5 μg/mL in HT-29 and 551.7 μg/mL against MRC-5 cell lines	Beara et al. (2012)
	Organic extract of the leaves	MTT toxicity assay	Cell line: RAW 264.7; LC_{50} of 44.1 μg/mL	Adebayo et al. (2018)
Plumbago auriculata Lam. Plumbaginaceae	Ethanol root extract	MTT toxicity assay	Cell line: HGE-17; non-toxic, LC_{50} of 278.5 μg/mL	Paul et al. (2013)
	Dichloromethane and methanol extracts of the twigs, fruits and leaves	Micronucleus and Comet assays (human lymphocyte cultures)	Organ- and solvent-dependant DNA damage without S9 (concentration not reported)	Kuete (2014)
Portulaca oleracea L. Portulacaceae	Aqueous leaf extract	XTT cell viability assay	Non-toxic against MT-4 cell line; LC_{50} of >100 μg/mL	Hurinanthan (2013)
	Aqueous and methanol leaf extracts	MTT toxicity assay	Non-toxic in mouse fibroblast, cell viability of >100% at 1000 μg/mL	Mzindle (2017)
Prunus africana (Hook.f.) Kalkman Rosaceae	Aqueous stembark extract	MTT toxicity assay	Cell line: Vero E6 LC_{50} of 104.1 μg/mL	McGaw et al. (2014)
	Dichloromethane extract of the twigs and methanol extract of the leaves	Micronucleus and Comet tests (human lymphocyte cultures)	The twigs exhibited DNA damage without exogenous metabolic activation (S9). The leaves showed mutagenic effect (concentration not reported)	Kuete (2014)
	Dichloromethane extract of the bark	Brine shrimp lethality assay	Percentage mortality of 32% at 48h (concentration of 2 mg/mL)	Khumalo (2018)
Pseudodictamnus africanus (L.) Salmaki & Siadati [=*Ballota africana* (L.) Benth.] Lamiaceae	Aqueous and methanol extracts of the leaves	Brine shrimp lethality assay	Non-toxic with percentage mortality of <20% after 48h exposure (concentration of 2 mg/mL)	Cock and Van Vuuren (2015)
Ptaeroxylon obliquum (Thunb.) Radlk. Rutaceae	Methanol and aqueous extract of the leaves	Brine shrimp lethality assay	Percentage mortality of <10% after 48h exposure (concentration of 2 mg/mL)	Cock and Van Vuuren (2015)
Pterocarpus angolensis DC. Fabaceae	Organic and aqueous extracts of the bark and leaves	The Ames assay (*Salmonella typhimurium* strain TA98 and TA100)	Non-mutagenic at 500 μg/mL (with and without S9 metabolic activation)	Mulaudzi et al. (2013)
	Bark and leaves	Brine shrimp lethality assay	LC_{50} of 1400-1500 μg/mL (bark) LC_{50} of 3600 -3800 μg/mL (leaf)	McGaw et al. (2014)
Ricinus communis L. Euphorbiaceae	Stem and leaf extracts	Brine shrimp lethality assay	LC_{50} of 1400-1500 μg/mL	McGaw et al. (2014)
	Aqueous and organic extracts of the leaves	MTT toxicity assay	LC_{50} of 120 μg/mL in *Bovine dermis*; LC_{50} of 110 μg/mL against Vero cell line	Mongalo et al. (2018)
	Dichloromethane and methanol extracts of the roots	Micronucleus assay (human lymphocyte cultures)	Toxic effect of DCM extract and DNA damage of methanol extract without exogenous metabolic activation S9 (concentration not reported)	Kuete (2014)
Rotheca myricoides (Hochst.) Steane & Mabb. [=*Clerodendrum myricoides* (Hochst.) R. Br. ex Vatke] Lamiaceae	Methanol and dichloromethane extract of the roots	Ames assay (*Salmonella typhimurium* strains TA98 and TA 100)	Non-mutagenic effect (with and without S9 metabolic activation)	Reid et al. (2006)
	Dichloromethane extract of the rootbark	Alamar Blue fluorescent cytotoxicity assay	Cell line: Rat skeletal myoblast L6; LC50 of >90.0 μg/mL	Irungu et al. (2007); McGaw et al. (2014)
	Methanol extract of the rootbark	Acute toxicity assay	Safe up to 2000 mg/kg dosage in mice	Welu et al. (2020)
Senecio serratuloides DC. Asteraceae	Aqueous extract of the leaves	MTT cell viability assay	Cell line: Graham cells, HEK-293. Non-toxic (112% cell growth at 100 μg/mL)	Naidoo et al. (2013)
	Dichloromethane extract of the leaves	Micronucleus test (human lymphocyte cultures)	Toxic effect without exogenous metabolic activation S9 (concentration not reported)	Kuete (2014)
Senna occidentalis (L.) Link Fabaceae	Aqueous leaf extract	XTT toxicity assay	Non-toxic against MT-4 cell line; LC_{50} of >100 μg/mL	Hurinanthan (2013)
	Aqueous and methanol extract of the leaves	MTT cell toxicity assay	Cell line: Mouse fibroblast; Non-toxic, cell viability of >150% at 1000 μg/mL	Mzindle (2017)
	Ethanol root extract	XTT toxicity assay		Tshikalange et al. (2016)

(*continued*)

Table 1 (*Continued*)

Species	Plant part and extract	Toxicity assay used	Toxicity properties	References
			LC_{50} of 70.8 μg/mL against Vero cells	
Senna petersiana (Bolle) Lock Fabaceae	Organic extracts of the seeds	Cell line toxicity assay	Cell line: Monkey kidney cells; LC_{50} of 24 μg/mL	McGaw et al. (2014)
Sclerocarya birrea (A. Rich.) Hochst. Anacardiaceae	Methanol extract of the bark	Tryphan blue exclusion assay Acute toxicity assay	Cell line: Vero E6; LC_{50} of 225.2 μg/mL. LD_{50} of >5000 mg/kg body weight in mice	Gathirwa et al. (2008)
	Aqueous extract of the bark	MTT cell viability assay	Cell line: Graham cells, (102% cell growth at 100 μg/mL)	Naidoo et al. (2013)
	Methanol and aqueous extracts of the leaves	Brine shrimp lethality assay	Non-toxic with percentage mortality of <10% after 48h exposure (concentration of 2 mg/mL)	Cock and Van Vuuren (2015)
Searsia chirindensis (Baker f.) Moffett Anacardiaceae	Methanol and dichloromethane extract of the leaves	The Ames assay (*Salmonella typhimurium* strains TA98 and TA 100)	Non-mutagenic effect (with and without S9 metabolic activation)	Reid et al. (2006)
Solanum nigrum L. Solanaceae	Seven isolated compounds	MTT toxicity assay	Degalactotigonin was cytotoxic in all cancer cell lines (HepG2, NCI-H460, MCF-7, SF-268) with LC_{50} values of 0.2–4.4 μM.	Zhou et al. (2006)
	Methanol extract of the leaves	MTT toxicity assay	Cell line: RAW 264.7 macrophages; LC_{50} of >100 μg/mL	Ayele et al. (2013); McGaw et al. (2014)
Solanum panduriforme E. Mey. Solanaceae	1:1 Methanol and dichloromethane extract of the leaves	MTT toxicity assay	LC_{50} of 180 μg/mL in *Bovine dermis*; LC_{50} of 120 μg/mL in Vero cell line	Mongalo et al. (2018)
Spirostachys africana Sond. Euphorbiaceae	Dichloromethane and methanol extracts of leaves, bark and twigs	Micronucleus and comet tests (human lymphocyte cells)	Induced micronuclei without addition of a metabolizing enzyme solution (S9 mix) at 500 and 100 mg/mL Extract showed mutagenic effect without S9	Kuete (2014)
	Organic extracts of leaves and stems	Brine shrimp lethality assay	LC_{50} of 385 μg/mL at 48h (leaf) LC_{50} of 277 μg/mL at 48h (stem)	Akhalwaya et al. (2018)
Sutherlandia frutescens (L.) R. Br. Fabaceae	Methanol and dichloromethane extracts of the leaves	The Ames assay (*Salmonella typhimurium* strains TA98 and TA100)	Mutagenic effect with metabolic activation	Reid et al. (2006)
Syzygium cordatum Hochst. ex Krauss Myrtaceae	Aqueous and methanol extract of the bark	Neutral red uptake assay	LC_{50} of >100 μg/mL in human dermal fibroblasts and U937 human pro-monocytic cell line. LC_{50} of 95.6 μg/mL in C2C12 myoblasts, and 74.6 μg/mL in 3T3-L1	Cordier et al. (2013)
	Aqueous extract of the bark	MTT cell viability assay	Non-toxic in Graham cells, HEK-293 with 104% cell growth at 100 μg/mL	Naidoo et al. (2013)
	1:1 Methanol and Dichloromethane bark extract	MTT toxicity assay	Cell line: Human kidney epithelial cells; LC_{50} of 26.8 μg/mL	McGaw et al. (2014)
	Methanol and aqueous extracts of the bark	Brine shrimp lethality assay	Non-toxic with percentage mortality of <20% after 48h exposure (concentration of 2 mg/mL)	Cock and Van Vuuren (2015)
	Methanol bark extract	Brine shrimp lethality assay	Non-toxic with percentage mortality of 20% at 48h (concentration of 2 mg/mL)	Khumalo (2018)
Tecomaria capensis (Thunb.) Spach Bignoniaceae	1:1 Methanol and dichloromethane extract of the stems	Brine shrimp lethality assay	Non-toxic with percentage mortality of 44.1% at 48h (concentration of 1 mg/mL)	Akhalwaya et al. (2018)
Terminalia sericea Burch. ex DC. Combretaceae	Ethanol root extract	MTT and neutral red uptake assays	Non-toxic in both assays with LC_{50} of >95 μg/mL in SH-SY5Y cells	Adewusia et al. (2013)
	Methanol and aqueous extract of the leaves	Brine shrimp lethality assay	Non-toxic with percentage mortality of <10% after 48h exposure (concentration of 2 mg/mL)	Cock and Van Vuuren (2015)
Terminalia prunioides M.A. Lawson Combretaceae	Methanol and aqueous extracts of the leaves	Brine shrimp lethality assay	Non-toxic with percentage mortality of <10% after 48h exposure (concentration of 2 mg/mL)	Cock and Van Vuuren (2015)
Tetradenia riparia (Hochst.) Codd Laminaceae	Methanol extract of the leaves	XTT cell viability assay	Non-toxic against MT-4 cells; LC_{50} of >100 μg/mL	Hurinanthan (2013)
	Methanol extract of the fruits	Comet test (human lymphocyte cultures)	DNA damage without S9 (concentration not reported)	Kuete (2014)

(*continued*)

Table 1 (*Continued*)

Species	Plant part and extract	Toxicity assay used	Toxicity properties	References
	Aqueous and methanol extracts of the leaves	MTT cell toxicity assay	Cell line: Mouse fibroblast; Non-toxic, cell viability of >150% at 1000 μg/mL	Mzindle (2017)
	Organic extract of the leaves	Brine shrimp lethality assay	LC_{50} of 229 μg/mL at 48h	Akhalwaya et al. (2018)
	1:1 Methanol and water extract of the stem	MTT cell toxicity assay	Cell line: Vero cells; >50% viability at 100 μg/mL	Ghuman et al. (2019)
Trichilia dregeana Sond Meliaceae	Aqueous extract of the leaves	MTT cell viability assay	Non-toxic in Graham cells; (100% cell growth at 100 μg/mL)	Naidoo et al. (2013)
	Methanol and aqueous extracts of the leaves	Brine shrimp lethality assay	Non-toxic with percentage mortality of <10% after 48h exposure (concentration of 2 mg/mL)	Cock and Van Vuuren (2015)
Trichilia emetica Vahl Meliaceae	Dichloromethane extract of the bark	Monkey kidney cell toxicity test	Cell line: Monkey kidney cells; LC_{50} of 50 μg/mL	Prozesky et al. (2001)
	Aqueous root extract	MTT reduction assay	Non-toxic, LC_{50} = 41000mg/mL	Lall and Kishore (2014)
	Methanol and water extracts of the leaves	MTT toxicity assay	Cell lines: J774, WI38; non-toxic with LC_{50} of >100 μg/mL	McGaw et al. (2014)
	Dichloromethane and methanol extracts of the bark and leaves	Micronucleus and comet assay	Appearance of micronuclei in bark. DNA damage of leaf methanol extract in Comet test without S9 (human lymphocyte cultures)	Kuete (2014)
Tulbaghia violacea Harv. Amaryllidaceae	Methanol and dichloromethane extracts of the bulbs and leaves	The Ames assay (*Salmonella typhimurium* strains TA98 and TA100)	Non-mutagenic (with and without S9)	Reid et al. (2006)
	Methanol extract of the bark and leaves	Micronucleus assay	Induced micronuclei without S9 (human lymphocyte cultures) (concentration not reported)	Kuete (2014)
	Aqueous and methanol extracts of the leaves and roots	Brine shrimp lethality assay	LC_{50} of 718 μg/mL (leaf) and 716 μg/mL (roots) at 48h	Cock and Van Vuuren (2015)
	Aqueous and methanol extracts of the leaves	MTT cell toxicity assay	Cell line: Mouse fibroblast; Non-toxic with cell viability of >100% at 1000 μg/mL	Mzindle (2017)
Turraea floribunda Hochst. Meliaceae	Dichloromethane and methanol extracts of the bark and leaves	Comet and micronucleus assays (human lymphocyte cultures)	Mutagenic effect in comet test without exogenous metabolic activation (S9). Appearance of micronuclei without S9 (concentration not reported)	Kuete (2014)
Typha capensis (Rohrb.) N.E.Br. Typhaceae	Acetone extract of the leaves	MTT toxicity assay	Cell line: RAW 264.7 macrophages; cell viability of 100% at 12.5 μg/mL	Ondua et al. (2019)
Vachellia nilotica (DC.) Kyal. & Boatwr. subsp. *kraussiana* (Benth.) Kyal. & Boatwr. Fabaceae	Ethanol extract of the bark and fruits	MTT toxicity assay	LC_{50} of 65.2 μg/mL in 3T3 cell line	McGaw et al. (2014)
Vachellia sieberiana (DC.) Kyal. & Boatwr. var. *woodii* (Burtt Davy) Kyal. & Boatwr. Fabaceae	Methanol and dichloromethane extract of the bark	The Ames assay (*Salmonella typhimurium* strains TA98 and TA100)	Extracts tested negative for mutagenic effect	Elgorashi et al. (2001)
Volkameria glabra (E.Mey.) Mabb. & Y.W.Yuan [=*Clerodendrum glabrum* E.Mey.] Lamiaceae	Isolated compounds from bark; β-amyrin palmitate, glutinol, lupeol-3-palmitate, lupeol and stigmasterol	MTT toxicity assay	All the isolated compounds were non-toxic in MCF-7 hormone receptor positive breast cancer cell line with LC_{50} of >500 μM	Teclegeorgish et al. (2020)
Warburgia salutaris (G.Bertol.) Chiov. Canellaceae	Dichloromethane extract of the bark	Brine shrimp lethality assay	Non-toxic with percentage mortality of 22.3% at 48h (concentration of 2 mg/mL)	Khumalo (2018)
	Organic extract of the bark	Brine shrimp lethality assay	LC_{50} of 164 μg/mL at 48h	Akhalwaya et al. (2018)
	Aqueous extract of the leaves	MTT toxicity assay	Non-toxic with LC_{50} of >100 μg/mL in MCF-7, Caco-2, and A549 cell lines; LC_{50} of 77.5 μg/mL in HeLa cells	Soyingbe et al. (2018)
	1:1 Methanol and dichloromethane extract	MTT toxicity assay	LC_{50} of 680 μg/mL in *Bovine dermis*; toxic in Vero cell line with LC_{50} of 10 μg/mL	Mongalo et al. (2018)
	Iso-mukaadial acetate isolated from the bark	Crystal violet assay	Cell line: L6 rat myoblast, 98.4% viability at 3 μg/mL	Msomi et al., (2019)
Xysmalobium undulatum (L.) W.T. Aiton Apocynaceae	Methanol and dichloromethane extracts of the leaves	The Ames assay (*Salmonella typhimurium* strains TA98 and TA100)	Non-mutagenic effect (with and without S9 metabolic activation)	Reid et al. (2006)
	Aqueous extract of the plant	*In vitro* 3D spheroid model Acute toxicity test	Moderate hepatoxicity against HepG2 cell line after 21 days Moderate toxicity with at	Calitz et al. (2019)

(*continued*)

Table 1 (*Continued*)

Species	Plant part and extract	Toxicity assay used	Toxicity properties	References
			250 mg/kg dosage in Sprague Dawley rats	
Zantedeschia aethiopica (L.) Spreng. Araceae	1:1 Methanol and water extract of the stems	MTT toxicity assay	Cell line: Vero cells; >60% viability at 100 μg/mL	Ghuman et al. (2019)
Zanthoxylum capense (Thunb.) Harv. Rutaceae	Acetone extract of the leaves	MTT toxicity assay	LD_{50} of 8.0 μg/mL in Vero cell line	Adamu et al. (2012)
	Dichloromethane extract of the roots	MTT toxicity assay	Cell Line: Human acute monocytic leukemia and THP-1; LC_{50} of 45.70 μg/mL	McGaw et al. (2014)
	Acetone extract of the leaves	MTT toxicity assay	Cell line: Vero monkey kidney cells; non-toxic with LC_{50} > 1000 μg/mL	Adebayo et al. (2015)
	1:1 Methanol and dichloromethane extract of the leaves and stems	Brine shrimp lethality assay	Percentage mortality of 46.3% at 48 h (leaves) and 9.1% at 48h (stems) (concentration of 1 mg/mL)	Akhalwaya et al. (2018)
Ziziphus mucronata Willd. Rhamnaceae	Ethanol extract of the roots	MTT and neutral red uptake assays	Non-toxic in both assays with LC_{50} of >100 μg/mL against SH-SY5Y cells	Adewusia et al. (2013)
	1:1 Methanol and dichloromethane extract of the leaves	MTT toxicity assay	LC_{50} of 100 μg/mL in *Bovine dermis*; LC_{50} of 220 μg/mL in Vero cells	Mongalo et al. (2018)
	Dichloromethane and methanol leaf extracts	Micronucleus and Comet tests (human lymphocyte cultures) Ames test	Appearance of micronuclei in both assays without exogenous metabolic activation (S9). DNA damage from DCM extract. Mutagenic effect with metabolic activation from the methanol extract (concentration not reported)	Kuete (2014)
	Leaf extracts	Brine shrimp lethality assay	LC_{50} of 900 μg/mL	McGaw et al. (2014)
Ziziphus rivularis Codd Rhamnaceae	Acetone leaf extract	MTT cell viability assay	Cell line: RAW 264.7 macrophages; non-toxic with 71.6% cell viability at 50 μg/mL	Dzoyem and Eloff (2015)

* Naturalised exotic species

2022). In the brine shrimp lethality assay, 95% of species were reported to be non-toxic. To evaluate the mutagenic and carcinogenic properties of medicinal plant extracts, the Ames, Comet, and micronuclei assays were used (Elgorashi et al., 2001; Reid et al., 2006; Mulaudzi et al., 2013; Kuete, 2014). About 40% of the tested plant extracts exhibited mutagenic effects in a dose dependent manner. *In vivo* acute toxicity assays still remain relatively unexplored, with less than 10% of medicinal plants screened in these assays. Furthermore, very few chronic toxicity studies of plant extracts were reported.

Although a substantial number of species have been subjected to toxicity studies, most of these have only been evaluated using one or two biological assays. Therefore, it is often not possible to develop comprehensive conclusions on their overall cytotoxity properties. Medicinal plants that were screened using multiple assays include *Clausena anisata* (Willd.) Hook.f. ex Benth., *Rotheca myricoides* (Hochst.) Steane & Mabb. [=*Clerodendrum myricoides* (Hochst.) R.Br. ex Vatke], *Ekebergia capensis* Sparrm., *Gunnera perpensa* L., *Harpagophytum procumbens, Hypoxis hemerocallidea, Kigelia africana* (Lam.) Benth., *Melianthus comosus* Vahl, *Peltophorum africanum* Sond., *Sclerocarya birrea* (A. Rich.) Hochst., *Syzygium cordatum* Hochst. ex Krauss, *Tetradenia riparia* (Hochst.) Codd, *Trichilia emetica* Vahl, *Tulbaghia violacea* Harv., and *Ziziphus mucronata* Willd. In some cases, several authors have screened similar plants against the same type of cell line. While their findings are generally comparable and generally reveal their lack of toxicity, these results may not be inclusive of the overall toxicity of the plants. Screening against several different cell lines is required. Indeed, only a few species (*Carpobrotus edulis, S. cordatum, Z. mucronata,* and *Erythrophleum lasianthum*) were screened against multiple cell lines. These medicinal plants are widely available and affordable at informal (muthi) markets in South Africa, which serve over 27 million consumers annually, including those from the neighbouring countries Zimbabwe, Mozambique, and Swaziland (Mander et al., 2007; Williams and Whiting, 2016; Khumalo, 2018). Safety validation studies of important southern African plants are not well documented (McGaw et al., 2014; Kuete, 2014). The bark of *Erythrophleum lasianthum* is used as a snuff to relieve headaches (Pujol, 1990; Hutchings et al., 1996; Grace et al., 2003; Khumalo, 2018; Mhlongo and Van Wyk, 2019), and has been harvested for muthi market trade from as early as 1946 [Gerstner (1946), as cited by Williams et al. (2013)]. Although traders and consumers are aware of the toxic properties of the bark of this species, it is remains actively traded at the Johannesburg muthi markets (Khumalo, 2018). The active toxic principles include two diterpenoid alkaloids: cassaine and erythrophleine (Grace et al., 2003; Van Wyk et al., 2009; Ndhlala et al., 2013; Maroyi, 2019). Erythrophleine is a potent analgesic and vasoconstrictor and is believed to contribute to the therapeutic effects of this plant (Van Wyk et al., 2009). However, studies reporting *in vivo* acute toxicity of this species were not found in the literature.

In addition to the analgesic and anti-inflammatory properties of medicinal plants recorded in this review, various other traditional uses and biological activities have also been reported. Species such as *Peltophorum africanum, Kigelia africana, Sclerocarya birrea and Hypoxis hemerocallidea* are sold and widely used to treat sexually transmitted infections (Pujol, 1990, Hutchings et al., 1996, Von Koenen, 2001; Grace et al., 2003; Van Wyk et al., 2009; Magwede et al., 2018). Basic *in vivo* studies have reported noteworthy anti-HIV activities for these plant species (Prinsloo et al., 2018). However, *in vivo* toxicity studies of these species remain underexplored. The pharmacological properties of *Hypoxis hemerocallidea* have been well documented. The anticancer, anti-HIV and anti-inflammatory properties of this plant are associated with the compound rooperol, which has also been investigated in clinical trials (Van Wyk et al., 2009). Toxicity and safety validation studies are of particular concern as many southern African inhabitants prefer to consult traditional healers and use herbal remedies rather than using allopathic medications (Tshikalange et al., 2005; Mander et al., 2007; Khumalo, 2018).

For several medicinal plant extracts, the anti-inflammatory activity may have been influenced by the toxic properties of the plants. These include *Abrus precatorius* L. subsp. *africanus, Acokanthera oppositifolia, Crinum moorei, Elaeodendron croceum, Erythrophleum lasianthum, Ricinus communis* and *Senecio serratuloides*. Plant extracts from these species exhibit noteworthy inhibitory activity against the inflammatory cyclooxygenases (COX) and lipoxygenases (LOX) enzymes, despite displaying substantial toxicity (Khumalo et al., 2021). In addition, *Bulbophyllum scaberulum* (Rolfe) Bolus var. *scaberulum, Combretum kraussii* Hochst., *Synadenium cupulare* (Boiss.) L.C. Wheeler and *Vachellia sieberiana* (DC.) Kyal. & Boatwr. var. *woodii* (Burtt Davy) Kyal. & Boatwr., (Jäger et al., 1996; Eldeen et al., 2005; Chinsamy et al., 2014; Khumalo et al., 2021) exhibit potent anti-inflammatory activity by modulating COX enzyme activity. However, there is a lack of toxicity data for these species in the literature and their safety of should be examined as a priority.

Fig. 1 shows the southern African medicinal plant parts used to treat pain and inflammatory disorders that have been most frequently evaluated for toxicity. The leaves were the most frequently tested plant parts (58%), followed by bark (26%) and roots (10%). The fruits, seeds, and bulbs were the least screened plant parts, accounting for only 2% of the total evaluations each.

Leaves of southern African medicinal plants were most frequently screened for anti-inflammatory activity (Elgorashi and McGaw, 2019). The leaf material is sustainable for conservation purposes and may therefore be prefered to large scale production. However, several studies have screened different plant parts than those used traditionally for toxicity, which does not allow the therapies to be validated for toxicity and pharmacological potential. Correct plant identification and testing of the plant part used medicinally is crucial for scientific validation studies (Heinrich et al., 2020). Different plant parts may contain different phytochemicals (Evert, 2006; Van Wyk and Wink, 2015), and therefore testing the part used therapeutically is imperative. For example, the poisonous bark of *Erythrophleum lasianthum* is widely used traditionally as an analgesic. However, various authors have screened the leaves, which were reported to be non-toxic (Lewis, 1995; Twilley et al., 2017; Maroyi, 2019). The bark of *Zanthoxylum capense* is popularly used traditionally to treat inflammatory pain (Pujol, 1990, Hutchings et al., 1996, Van Wyk et al., 2009; Mhlongo and Van Wyk, 2019; Khumalo et al., 2021). Additionally, multiple authors have screened the leaves and roots of this species toxicity (Adamu et al., 2012; McGaw et al., 2014; Adebayo et al., 2015; Akhalwaya et al., 2018).

Fig. 2 shows a comparison of the solvent extracts of southern African medicinal plants with anti-inflammatory properties that are commonly evaluated for toxicity. Methanol and water are the most frequently used solvents for extraction. Surprisingly, ethanol was among the least tested solvent extracts. These findings contrast with the frequently used solvents for evaluating the anti-inflammatory and analgesic properties, which reported ethanol as the preferred solvent, with 59% of medicinal plants screened, followed by acetone (38%) and methanol (34%) (Khumalo et al., 2021). Notably, the toxicity activity evaluations were not performed in the same study as anti-inflammatory activity, making the calculation of the safety/therapeutic indices of these medicinal plants unachievable.

Aqueous extracts mimic the traditional method of medicine preparation as many medicinal plants that are used to treat pain and inflammation are traditionally prepared as decoctions or infusions (Mabona and Van Vuuren, 2013; Maroyi, 2011, Bruschi et al., 2011; Magwede et al., 2018; Cock and Van Vuuren, 2020). Decoctions and infusions are often taken orally to treat internal inflammatory ailments, or applied externally as a rub, press or a wash to relieve body pains and swellings (Khumalo et al., 2021). The potential of methanol as a suitable solvent is based on its ability to extract a wide diversity of both polar and non-polar components from plant material (Evert, 2006). Very little information is available on the toxicity properties of essential oils from medicinal plants. Toxicity of plant species may also be influenced by individual compounds within the crude extracts. Interestingly, less than 5% of the toxicity studies evaluated the toxicity of individual bioactive compounds (Table 1) and evaluation of the toxicity of the individual compounds is required. Furthermore, in cases where individual compounds were tested, most of the studies screened against cancer cell lines rather than normal cells (Zhou et al., 2006; Gamal-Eldeen et al., 2007; Yelani et al., 2010; Xuan and Khanh, 2016; Teclegeorgish et al., 2020).

Fig. 3 summarises the most commonly used toxicological screening assays for evaluating southern African medicinal plants used to treat pain and inflammatory disorders. The findings from this review show that a total of 117 medicinal plant species have previously been evaluated by toxicity screening in at least one assay system.

The most frequently used toxicity assay was the MTT (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay with 109 species tested, followed by the brine shrimp lethality (57 species), and the XTT assays (sodium 3ʹ-[1- (phenylaminocarbonyl)- 3,4-tetrazolium]-bis (4-methoxy6-nitro) benzene sulfonic acid hydrate)

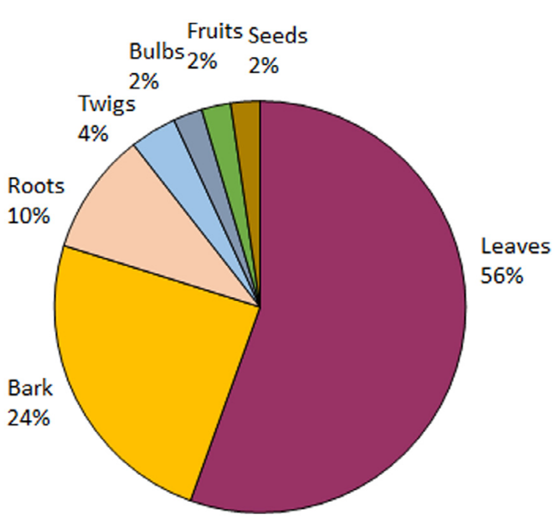

Fig. 1. Percentages of plant parts tested for cytotoxicity properties.

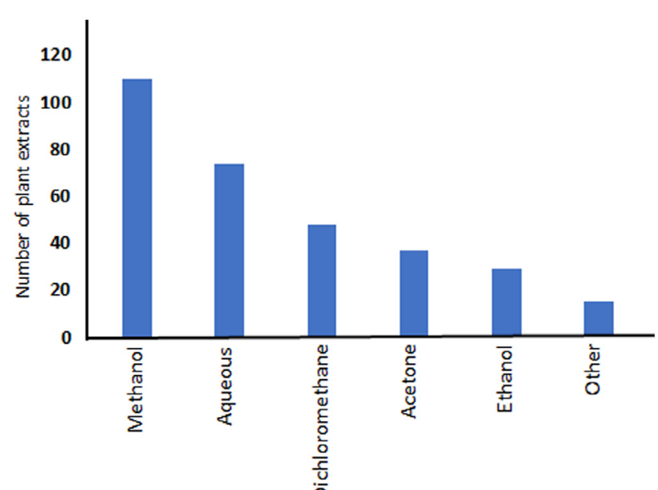

Fig. 2. The most frequently used plant extracts for cytotoxicity screening assays.

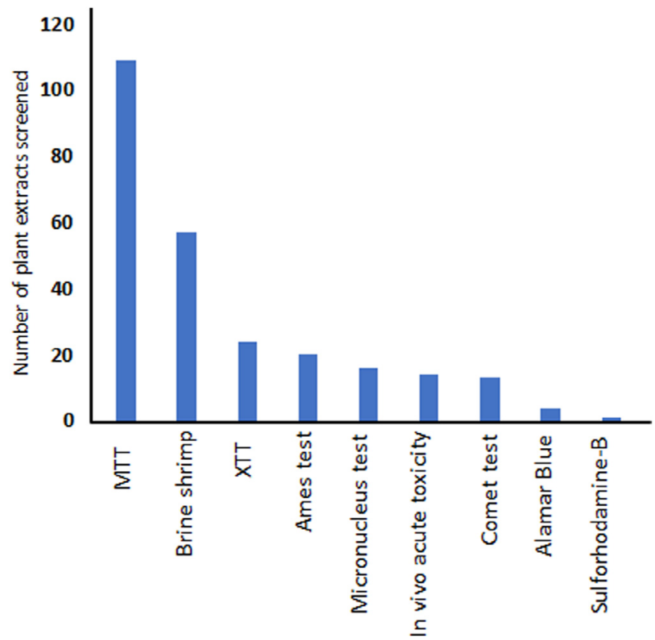

Fig. 3. The most frequently used biological assays for cytotoxicity studies.

(24 species). The Ames test, which was used to screen 20 species, was most frequently used assay to determine mutagenic properties of the plant extracts. Interestingly, none of the plant species identified in this review were screened for toxicity properties using the MTS assay 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium). Whilst this assay is similar to the MTT assay and both detect the production of reducing equivalents in actively respiring cells, the MTS assay has an advantage as the formazan products that are released by viable cells are directly soluble in cell culture medium, thereby providing consistent results (Riss et al., 2016). Thus, it is surprising that we were unable to find any previous toxicity studies that used that method.

The top 15 cell lines used for evaluating toxicity properties of southern African medicinal plants traditionally used to treat pain and inflammation are shown in Fig. 4. *Abrus precatorius* L., *Croton gratissimus* Burch., *Croton sylvaticus* Hochst., *Elaeodendron croceum* (Thunb.) DC., and *Plantago lanceolata* L. were most frequently screened for toxicity against cancer cell lines (such as Caco-2, HT-29, MCF-7-12, and HeLa), rather than normal cells (Beara et al., 2012; Yelani et al., 2010; Njoya et al., 2018; Okoro et al., 2021). The top five most screened cell lines were Vero monkey kidney cells (46 plant extracts), RAW 264.7 macrophages (23 extracts), human breast cancer cells (MCF-7, 12) (21 extracts), mouse fibroblast (3T3) (18 extracts) and Graham cells with 17 extracts.

The southern African medicinal plants that have been explored for their chemical properties include *Aloe ferox, Bolusanthus speciosus* (Bolus) Harms, *Agathosma betulina, Burkea africana* Hook., *Croton sylvaticus, Erythrina caffra* Thunb., *Harpagophytum procumbens, Hypericum roeperianum* G.W. Schimp. ex A. Rich., *Jatropha curcas* L., *Kigelia africana, Leucaena leucocephala* (Lam) DeWit, *Lippia javanica, Peltophorum africanum, Merwilla plumbea* (Lindl.) Speta (=*Scilla natalensis* Planch.), *Solanum nigrum* L., *Terminalia sericea* Burch. ex DC., *Turraea floribunda* Hochst., and *Warburgia salutaris*.

4. Discussion

The interest in alternative therapeutics from herbal remedies in developed countries has increased in recent years (Kristanc and Kreft, 2016; Lazarou and Heinrich, 2019). Although conventional pharmaceuticals undergo rigorous toxicity trials prior to their use (McGaw et al., 2014; Kristanc and Kreft, 2016), reports of their toxic side effects have also increased drastically over the years. Approximately 230,000 cases of allopathic medication-related hospitalisations were recorded annually in Australia (Roughead et al., 2013). The most common cases of medication-related hospitalisations include the use of antirheumatics, corticosteroids and antihypertensives. The reported toxicities and harmful side effects from medication intake include gastrointestinal ulcers and bleeding, osteoporosis and cardiovascular disorders (Trelle et al., 2011; Roughead et al., 2013). Moreover, the frequent use of NSAID's was reported to be among the most common causes of drug-induced liver injury (Leise et al., 2014).

In developed countries, the adverse effects (hospitalisations, admission to intensive care unit and death) associated with medical errors remain a public concern (Garrouste-Orgeas et al., 2012). An estimated 850,000 cases of medical errors associated with conventional pharmaceuticals were recorded per year in the United Kingdom, whilst similar errors have caused over 98,000 deaths annually in the United States (Ash et al., 2004). Accidental poisoning by

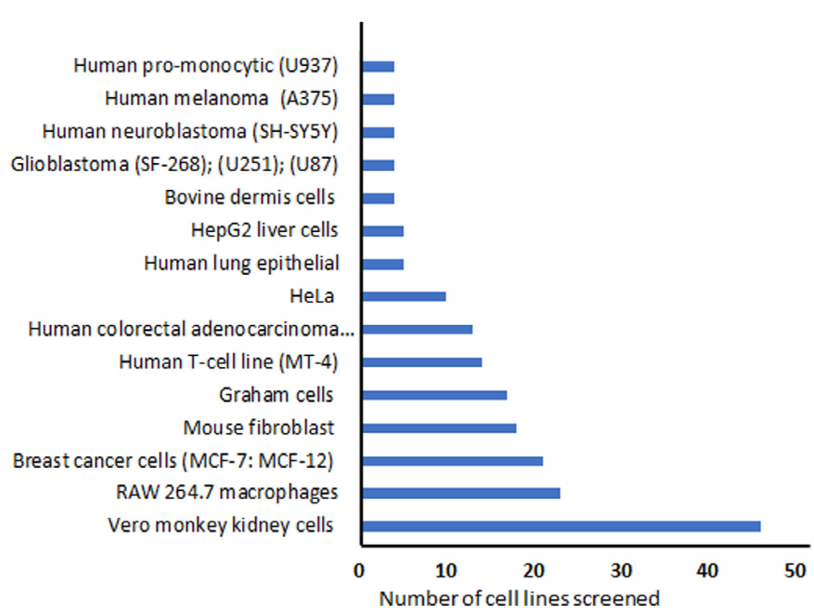

Fig. 4. The most represented cell lines for cytotoxicity screening of southern African plant extracts.

medicinal plants is most frequently associated with incorrect species identification or use of the incorrect plant part, incorrect dosage, as well as the method and the mode of administration (Ndhlala et al., 2013). This type of poisoning is relatively common in the southern African region and is largely connected with the informal trade of medicinal plants and consultations with traditional healers. This contrasts with the traditional Chinese healing system, which has long been practiced as a professional medical sector, prescribing herbal remedies for over 5,000 years (Yuan et al., 2016). Poisoning due to incorrect plant use is a common phenomenon and is mostly attributed to the incorrect identification, sharing of a vernacular name between unrelated species, and/or having one species with several names. This continues to be a major challenge facing ethnobotanical surveys by various authors (Pujol, 1990; Hutchings et al., 1996; Grace et al., 2003; De Wet and Ngubane, 2014; Khumalo, 2018; Mhlongo and Van Wyk, 2019). These inconsistencies pose a serious threat to consumers. For example, popularly sold medicinal barks of the same genus often have only one trade/vernacular name. These include two *Erythrina* species that are known as *umnsinsi*, three *Cryptocarya* species (*umkhondweni*), and two *Croton* species are referred to as *umahlabekufeni* (Grace et al., 2003; Khumalo et al., 2019). Aerial parts of *C. sylvaticus* and the bark extract of *Erythrina caffra* have shown mutagenic properties in micronucleus tests (Kuete, 2014). Furthermore, isolated compounds from the bark *of E. caffra* exhibit substantial cytotoxic effects (LC_{50} of 6.25 – 12.50 μg/mL) in HEK-293 cells and MES-SA human uterine sarcoma epithelial cell lines (El-Masry et al., 2014). In addition, acetone extracts of the leaves from *C. sylvaticus* display toxicity in normal and cancer cell lines (Njoya et al., 2018). These findings demonstrate that there may be very different toxicity profiles between species of the same genus with a shared trade name. The most common side effects associated with medicinal plant poisoning include neurotoxicity, gastrointestinal tract disturbances, kidney, and liver failure, as well as skin irritation and swelling associated with topical application (Watt and Breyer-Brandwijk, 1962; Wink and Van Wyk, 2008; Ndhlala et al., 2013).

Some plant toxins are associated with life-threatening conditions in humans, affecting critical organs and key functions such as the central nervous system. The prolonged use of herbal remedies by local people through self-medication or advice from traditional healers may be more associated with maintaining and/or enhancing a healthy lifestyle rather than treating a specific ailment (Whiting et al., 2002; Khumalo, 2018). Prolonged consumption of a herbal remedy plays a critical role in chronic toxicity, which may become untraceable over time (Fasinu et al., 2012; Ndhlala et al., 2013). Cases of patients that developed chronic hepatotoxicity due to the prolonged consumption of herbal preparations have been reported in developed countries such as America and Australia. Indeed, patients in Australia have have presented with jaundice and liver abnormalities following prolonged medicinal plant use (Whiting et al., 2002). In the United States, a study was conducted during 2001-2002 in Oregon Health and Science University, Portland, and the Portland Veteran Affairs Medical Centre to compare the negative impact of herbal supplement intake with cases of fulminant hepatic failure (Estes et al., 2003). Their results showed no significant differences in deaths and survival rates between the two groups studied (herbal supplement use and no history of herbal supplement use). Furthermore, in 50% cases of hepatic toxicity cases observed in patients with no history of herbal supplement use, acetaminophen consumption was the leading cause (Estes et al., 2003). Fatal and non-fatal chronic and acute liver poisoning (hepatic sinusoidal obstruction syndrome, also known as veno-occlusive disease) caused by pyrrolizidine alkaloids from *Senecio* species are regularly reported (e.g., Van Schalkwyk et al., 2021). Our review highlights the need to not only test the toxicity of herbal monotherapies, but to also evaluate their toxicities in combination with other therapies.

Different cell lines exhibit organ-specific toxicity (McGaw et al., 2014; Gadaga and Tagwireyi, 2014). Most of the toxicity activities of plant extracts recorded in this review were tested in only one type of assay, predominantly the MTT assay. Although, some species were screened against several different cell lines, those results are often not comparable. While *in vitro* lethality studies on human cell lines were found to correlate with those observed in rodents (McGaw et al., 2014), cell line models still have multiple limitations. Cell line models are limited to the determination of lethality, without considering other potential side effects. Furthermore, while many southern African medicinal plants that are used to treat pain and inflammation have been subjected to acute toxicity screening, very few studies have evaluated chronic toxicity. This is important because inflammatory disorders are often chronic and frequent consumption of medication over a long period of time is anticipated. Chronic toxicity studies of medicinal plants are imperative in order to assess the cumulative toxicity of extracts, behavioural changes, and chronic side effects (Gadaga and Tagwireyi, 2014).

In the southern African region, a four-year survey (1981-1985) conducted at the Ga-Rankuwa hospital in Johannesburg into acute poisoning cases reported that of the 1306 patients admitted, traditional herbal remedies were the second leading cause, with 204 cases, after accidental ingestion of paraffin (kerosine), with 760 cases (Joubert, 1990). However, traditional medicinal plants were the leading cause of mortality, with 31 deaths reported (51.7%), followed by paraffin with 26.7% of the overall 60 deaths recorded (Joubert, 1990). Another study carried out between 1990-1995 at the same hospital, revealed similar trends (Scott, 2003). Of the 4000 patients admitted, paraffin was also the leading cause of poisoning in that study (60% cases), while medicinal plants were responsible for only 7.8% cases. However, the highest incidents of death (57.5% cases) were from the consumption of traditional remedies (Scott, 2003). Similar results were also observed in a 10-year (1980-1989) study conducted in Zimbabwe's six main city hospitals, which evaluated poisoning in 2873 children up to 15 years of age (Scott, 2003). The toxic medicinal plant species associated with the highest mortality rates in those studies were three exotic species: *Datura stramonium* L., *Jatropha curcas* L., and *Ricinus communis* L.; as well as the non-endemic species *Callilepis laureola* DC., *Artemisia afra* Jacq. ex Willd. var. *afra* and *Mentha longifolia* (L.) L., (Scott, 2003). While the consumers of these herbal remedies are generally aware of the toxic properties of the plants, these species are still widely used and traded at the informal muthi markets of southern Africa, without adequate regulation and control (Fanell et al., 2004; Ndhlala et al., 2013).

Jatropha curcas L. is a medicinal herb from tropical America that has been introduced to Asian and African countries (Rakshit et al., 2008; Van Wyk and Gericke, 2018). The symptoms associated with the consumption of this plant include diarrhoea, abdominal pains and vomiting (Ndhlala et al., 2013). Diterpene alkaloids isolated from the plant are responsible for many of the cytotoxic properties of this species. While several studies have investigated the toxic effects of *Jatropha curcas,* they screened against cancer cell lines (Oskoueian et al., 2011). Three new diterpenoids, identified as *epi*-jatrophol, jatrophaldehyde and *epi*-jatrophaldehyde isolated from the rootbark exhibit antitumor properties (Zhang et al., 2012). Twelve rhamnofolane diterpenoids including jatrophalactone, jatrophalone and jatrophadiketone, as well as curcusones A–D were identified as the major constituents of the roots and these compounds exhibited potent inhibitory effects against various cancer cell lines, with LC_{50} values ranging from 1.36 to 4.70 μM (Liu et al., 2012, 2013). In addition to medicinal benefits, diterpene alkaloids can cause gastrointestinal irritation, severe cardiotoxicity and arrhythmia, heart seizure and death (Van Wyk et al., 2009; Ye et al., 2021). Phorbol esters also contribute to the cytotoxic properties of this plant *in vivo* (Rakshit et al., 2008; Li et al., 2010). No significant abnormal changes were evident in the organs at the lowest dose (21.26 mg/kg body mass). However, visible lesions were found in lungs and kidneys, with diffused haemorrhages in the lung, and glomerular sclerosis and atrophy in the kidney at higher doses. Additionally, multiple abruptions of cardiac muscle fibres and anachromasis of cortical neurons were noted at the highest dose of 36 mg/kg body mass (Li et al., 2010). Very few studies have investigated the cytotoxic properties of this plant extract in normal cell lines. Substantially more studies are required as this plant is still widely consumed as a medicinal herb to treat stomach ailments in southern Africa (Van Wyk and Gericke, 2018).

The leaves of *Artemisia afra* are applied externally to treat inflammation and backache; placed on a tooth cavity to relieve pain; steam from the leaves is inhaled to treat headaches, and unspecified parts of this plant are used to relieve earache (Khumalo et al., 2021). Biological activities reported for the plant include antiviral, antimalarial, cardiovascular, and pulmonary effects (Van Wyk et al., 2009; Mjiqiza et al., 2013; Moyo et al., 2019). This plant is widely used and popularly sold at the South African informal markets. Although it has been listed among the top fifteen species of significant commercial interest in southern Africa (Van Wyk, 2008; Street and Prinsloo, 2013), *Artemisia afra* extracts have cytotoxic, narcotic and sedative effects (Van Wyk et al., 2009). The reported toxic and hallucinogenic effects are associated with the compound thujone (Van Wyk et al., 2009). Symptoms observed from the consumption of the leaves include vomiting, dizziness, cramps and seizures (Ndhlala et al., 2013). The toxicity and phytochemical properties of this species have been extensively explored. The aqueous extract of *A. afra* extracts are non-toxic in mice when administered acutely, and showed low chronic toxicity properties (Mukinda et al., 2007). However, in high doses, the extract may have a hepatoprotective effect (Mukinda et al., 2007). In addition, a study by Eshetu et al. (2016) that evaluated toxic effects of aqueous leaf extracts of *A. afra* on the liver and kidney in Wistar rats, also found that acute and chronic oral administration was not toxic to the kidneys of mice. However, liver sections of male and female rats showed minor periportal mononuclear leukocytic infiltration (Eshetu et al., 2016). Further toxicity studies focusing on traditional medicinal plants are a priority.

5. Conclusions

Findings from this review showed that 117 medicinal plants that are traditionally used to treat pain and inflammation have previously been evaluated for toxicity. Over 80% of the tested plants were non-cytotoxic towards the cell lines they were tested against. However, a substantial number of medicinal plants screened for mutagenic properties exhibited mutagenic effects. In cases where the species have been investigated for their toxicity properties, many of these are *in vitro* studies and were often conducted using only one or two biological screening assays. Additionally, there is a limited number of studies that have evaluated toxicity towards normal cell lines, with Vero cells being most frequently screened, followed by macrophages and breast cancer cells. Toxicity of plant extracts can be influenced by the type of cell line screened. Thus further studies against different cell lines are required for all plant species to better evaluate the toxicity and safety of these plant species. *In vivo* studies account for less than 10% of the overall toxicity studies. In cases in which *in vivo* toxicity evaluations have been conducted, the liver and kidney are generally the focus of the study. Different classes of secondary metabolites of plant extracts affect different organs, including the central nervous system, cardiovascular system, the liver and other essential organs. None of the species in this review were screened against those organs for safety validation of the plant extracts and individual compounds. Additionally, where plants have been tested for toxicity, the evaluations are often not undertaken in parallel with anti-inflammatory assays and it is therefore not possible to determine a therapeiutic index. Future studies should evaluate the toxicity of plant extracts in combination with their bioactivity evaluations, and the therapeutic index should be reported. Earlier studies investigating the phytochemical properties of the plants focused on the isolation and characterisation of individual compounds, without evaluating their associated biological activities. Thus, multidisciplinary studies involving the ethnobotany, biological and toxicity screening, as well as phytochemical investigation of herbal remedies are crucial for safety and efficacy validation.

Declaration of Competing Interest

All authors declare no conflicts of interest.

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