Pittosporum viridiflorum Sims (Pittosporaceae): a review on a useful medicinal plant native to South Africa and tropical Africa

B. Madikizela, L.J. McGaw*

Phytomedicine Programme, Department of Paraclinical Sciences, University of Pretoria, Private Bag X04, Onderstepoort 0110, Pretoria, South Africa

*Corresponding author. Tel: +27 (012) 529 8351; fax: +27 (012) 529 8304
E-Mail: lyndy.mcgaw@up.ac.za

Abstract

Ethnopharmacological relevance: *Pittosporum viridiflorum* Sims, a Pittosporaceae species, is used extensively in African traditional medicine (ATM) by various tribes. This review is an appraisal of the information concerning the description, distribution, conservation status, traditional uses, phytochemistry, pharmacology and toxicology of this species with the aim of reconciling it with its traditional use.

Materials and methods: A wide-ranging literature search was conducted using database platforms such as Scopus, Google Scholar, Web of Science, ScienceDirect, PubMed and books including local reports and thesis submissions.

Results: Ten categories to which *P. viridiflorum* finds use in traditional medicine (TM) were found, and they include well-being, wounds, treatment of veterinary ailments, gastrointestinal and sexually transmitted diseases, kidney, circulatory and inflammatory disorders, as well as diseases such as cancer, tuberculosis, and malaria. Pharmacological tests conducted include those investigating antimicrobial, antidiarrhoeal, antimalarial, anticancer, anti-inflammatory, antioxidant and acaricidal properties. Promising activity was shown in a number of assays. Toxicological effects have also been reported from this species. However, it is recommended to conduct a detailed toxicological study, including genotoxicity, as this has not yet been evaluated. Compound(s) with antimalarial, anticancer and acaricidal properties have been isolated from *P. viridiflorum.*

Conclusions: The collective pharmacological and phytochemical properties of *P. viridiflorum* gives credence to the use of this plant species against various diseases in ATM, thus steering significant interest towards *in vivo* studies and clinical trials.

Keywords: *Pittosporum viridiflorum*, Pittosporaceae, anticancer, acaricidal, antimalarial, triterpenoids, glycosides.

List of abbreviations: ATCC = American Type Culture Collection; ATM = African traditional medicine; COX = cyclooxygenase; DCM = dichloromethane; EtOH= ethanol; GC = gas chromatography; GC/MS = gas chromatography-mass spectrometry; IC_{50} = 50% inhibitory concentration; LC_{50} = 50% lethal concentration; LOX = lipoxygenase; MeOH = methanol; MIC = minimum inhibitory concentration; MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; TM = traditional medicine; TCM = traditional Chinese medicine

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1. Introduction

The Pittosporaceae is a small family of flowering plants containing nine genera, with approximately 200-240 species largely restricted to Australia and limited to the paleotropics (Chandler et al., 2007; Sadashiva et al., 2013; El Dib et al., 2015). Seven of the Pittosporaceae genera are endemic to Australia, Indonesia and the Philippines (Manase et al., 2013). The origin of the Pittosporaceae is uncertain, with some researchers suggesting an Australian origin after its separation from Gondwana followed by dispersal, whereas others suggest vicariant dispersal from an east Gondwana origin (Chandler et al., 2007). The family has a wide range of habitats, from areas of great aridity, such as the Australian Nullarbor Plain, to wet montane areas of Queensland, New Caledonia and Hawaiian Islands, as well as alpines in New Zealand (Gemmill et al., 2002). From an economic perspective, *Hymenosporum* and *Pittosporum* are the two most commonly known Pittosporaceae genera, and they are used as cultivated ornamentals (El Dib et al., 2015). Australia is represented as the centre of prominence for *Hymenosporum*, however the genus also has a representation in New Guinea (Chandler et al., 2007). In addition to the Australian distribution of *Pittosporum*, the genus may be found in Africa, Asia, and the Hawaiian Islands (Chandler et al., 2007). It contains approximately 140-200 species comprising trees or shrubs that grow 2 to 30 m in height, and occasionally epiphytes (El Dib et al., 2015). The genus is Gondwanan, and its current distribution is in tropical and subtropical regions in Africa, Asia, New Zealand, Pacific Islands and Australia (Manase et al., 2013). *Pittosporum* is reported to be the only genus of the family present in Malagasy flora (Friis, 1987). The species of this genus are commonly known as “cheesewood” (El Dib et al., 2015). In Africa and Arabia *Pittosporum* is reported to be divided into 24 species (Friis, 1987).

The species from the *Pittosporum* genus are used widely as ornamentals in European gardens, New Zealand and Japan, and as a source of wood for charcoal, engraving and firewood, while some of them have medicinal purposes (Sadashiva et al., 2013). In terms of

the medicinal uses of *Pittosporum*, different parts of the species find use in traditional medicine (TM) as anti-inflammatory, antispasmodic, antimalarial and antimicrobial preparations, as narcotics, for chronic bronchitis, leprous affections, rheumatic and dropsical swellings, bruises, sciatica, chest infection, as well as certain skin diseases (Sadashiva et al., 2013). The seeds of some *Pittosporum* species are used in traditional Chinese medicine (TCM) as sedatives and cough-relieving remedies. Other species are famous in China for their analgesic and antidotal properties (Zhao et al., 2012). In India, the leaves, bark, roots and flowers are used as anti-inflammatory and antiseptic medications, in rheumatoid disorders, and as chewing sticks for oral care (Maya et al., 2014). The leaf, seed, fruit pulp and wood of Australian *Pittosporum* is used for bruises, muscle ache, sprains, cramps, coughs, colds, eczema, and pruritus or to induce lactation (Vesoul and Cock, 2011). With increasing interest in drug discovery from medicinal plants, several species of *Pittosporum* have been studied for pharmacological and phytochemical properties. Pharmacological studies revealed that crude extracts and compounds from this genus possess a wide spectrum of biological activities, such as antimicrobial, antidiarrhoeal, antimalarial and anti-inflammatory to mention a few. Among the compounds isolated from *Pittosporum*, essential oils, neolignan glycosides, and saponins have been considered as the main constituents (Sadashiva et al., 2013; Manase et al., 2013). Neolignan glycosides play an important role in resistance against opportunistic pathogens in plants and have pharmacological activities in mammalian cells (Gohari et al., 2011).

Pittosporum viridiflorum Sims, native to South Africa and further north into tropical Africa (van Wyk et al., 2009), is used widely in African traditional medicine (ATM) for the treatment of various diseases and ailments such as fever, malaria, and coughs to mention a few (Smith, 1895; Phillips, 1917; Watt and Breyer-Brandwijk, 1962; Starr et al., 2003). Pharmacological studies on different parts of *P. viridiflorum* have been conducted, and they include antimicrobial, antidiarrhoeal, antimalarial, antioxidant, anti-inflammatory, anticancer, acaricidal, and toxicity, with some providing credence for the use of this plant in ATM. Phytochemical research on *P. viridiflorum*, determining which secondary metabolites are present, quantifying and isolating them has been done. The present review summarizes pharmacological and phytochemical studies done previously on different parts of *P. viridiflorum*, with the aim of reconciling the traditional aspects of its usage with more recent discoveries in the pharmacology of the Pittosporaceae.

2. Description, distribution and conservation status of *P. viridiflorum*

The origin of the name *Pittosporum* is from two Greek words, "pitta" meaning "pitch" and "sporum" meaning "seed" (referring to the sticky seeds); and *viridiflorum* meaning "with green flowers" (www.plantzafrica.com). According to Smith (1966), the common Afrikaans name "kasuur" is a contraction of kaasuur, candle hour, which refers to the time the flowers exude their fragrance. *P. viridiflorum* has several synonyms (www.theplantlist.org, Klopper et al., 2006), including *P. abyssinicum* Delile, *P. abyssinicum* subsp. *cardiocarpum* (Cufod.) Cufod, *P. abyssinicum* subsp. *engleri* (Cufod.) Cufod, *P.* a*byssinicum* subsp. *fulvo-tomentosum* (Engl.) Cufod, *P. abyssinicum* subsp. *gilletii* Cufod P. *abyssinicum* subsp. *lanatum* (Hutch. & E.A. Bruce) Cufod., *P. abyssinicum* var. *angolense* Oliv., *P. cardiocarpum* Cufod., *P. engleri* Leonard ex Cufod, *P. lanatum* Flutch. & E.A. Bruce, Cufod., *P. lanatum* var. *engleri* (Cufod.), *P. cacondense* Exell & Mendonga, *P. viridiflorum feddeanum* (Pax) & Cuf., *P. mildbraedii* Engi., *P. rhodesicum* Cufod., *P. ripicolum* J. Leonard, P. *sinense* Desf., *P. ustulatum* Cufod., *P. ripicolum* subsp. *katangens*e J. Leonard, *P. viridiflorum* subsp. *afrorientale* (Cufod.) Cufod., *P. viridiflorum* subsp. *angolense* (Oliv.) Cufod., *P. viridiflorum* var. *malosanum* (Baker) Cufod., *P. viridiflorum* var. *afrorientale* Cufod., *P. antunesii* Engl., *P. commutatum* Putt., *P. dalzielii* Hutch., *P. feddeanum* Pax, *P. kapiriense* Cufod., *P. kruegeri* Engl., *P. lynesii* Cufod., *P. malosanum* Baker, *P. mannii* Hook.f., *P. mannii* subsp. *ripicola* (J.Léonard) Cufod., *P. quartinianum* Cufod., *P. rhodesicum* Cufod. *P. ripicola* J.Léonard, *P. ripicolum* subsp. *katangense* Leonard, *P. sinense* Desf., *P. spathicalyx* De Wild., *P. viridiflorum* var. *angolense* Cufod., *P. viridiflorum* var. *commutatum* (Putt.) Moeser ex Engler, *P. viridiflorum* subsp. *dalzielii* (Hutch.) Cufod., *P. viridiflorum* subsp. *feddeanum* (Pax) Cufod., *P. viridiflorum* var. *kruegeri* Engl., *P. viridiflorum* subsp. *malosanum* Cufod., *P. viridiflorum* subsp. *quartinianum* Cufod., *P. viridiflorum* subsp. *viridiflorum*, *P. viridiflorum* var. *viridiflorum* L., and *P. vosseleri* Engl. The species grows in a wide altitude range from deciduous forest to woodland, scrub, riverine fringe thicket and evergreen forest to rocky outcrops (Palgrave, 2002). *P. viridiflorum* is listed in South Africa as a protected plant species according to the National Forest Act of 1998, however, it is widely distributed in other African countries growing in mountains and littoral rainforest. In South Africa, *P. viridiflorum* may not be cut, disturbed, damaged (bark harvesting), destroyed or removed, collected, sold, purchased, and donated by any individual unless under a licence granted by the minister (DAAF, 2015).

The distribution of *P. viridiflorum* is in the eastern and western parts of South Africa up into tropical Africa and beyond to Arabia and India. In Africa, this species is found in Angola, Cameroon, Ethiopia, Somalia, Kenya, and South Africa where it is famous for its therapeutic potential (Watt and Breyer-Brandwijk, 1962; Hutchings et al., 1996; Amusan et al., 2002; Palgrave, 2002; Muthaura et al., 2007; Bekalo et al., 2009; Momeni et al., 2010; Van Wyk et al., 2011; Otang et al., 2014; Teka et al., 2015; Wandji et al., 2016). The plant has many common names where it is found, and is known as liankhuere in Angola, munati in Kenya, hunbosho or anbilbaye (Amharaic) in Ethiopia, alhowarradasbe in Saudi Arabia, bourangal (Bororo) in Cameroon, and as kasuur (Afrikaans) or kgalagangwo (Sotho) or umkhwenkwe (Xhosa) or umvusamvu (Zulu) in South Africa (Van Vuuren et al., 2013; Teka et al., 2015). This tree species is grown in various regions of the world as an ornamental plant with its fragrant flowers. It is cultivated in Europe, and St. Helena where it was introduced by the Dutch, in warm regions of the United States, and in Hawaii. *P. viridiflorum* was first collected from cultivated material on the Island, where it is now targeted for control as a potential threat (Starr et al., 2003).

P. viridiflorum is an attractive evergreen tree with varying sizes and shapes (Palgrave, 2002). It has a dense roundish to upright crown, often grows to about 10 m in height, and reaches 20-30 m in northern KwaZulu-Natal forests. The bark is greyish or brownish, smells sweet and tastes bitter due to liquorice (Palgrave, 2002; Van Wyk et al., 2011). Leaves of *P. viridiflorum* are variable in size, shiny dark green above and pale below producing a resinous smell when bruised (Venter and Venter, 2009). Its flowering season is from November to December, producing creamy yellow flowers with a strong scent making them irresistible to insects and insectivorous birds (Palgrave, 2002; Venter and Venter, 2009). Fruiting occurs from December to April, and the fruits are small with dark brown capsules (Figure 1) that release numerous bright red seeds when split, covered by a sticky slow drying resin (Venter and Venter, 2009; Van Wyk et al., 2011). The wood of *P. viridiflorum* is soft and pale with little difference between sapwood and heartwood (Venter and Venter, 2009).

Figure 1: *Pittosporum viridiflorum* (Photograph credit: Hyde, M.A., Wursten, B.T., Ballings, P., Coates Palgrave, M., 2017. Flora of Zimbabwe: http://www.zimbabweflora.co.zw).

3. Materials and methods

Database platforms were used to find information on the traditional use, pharmacology and phytochemistry of *P. viridiflorum*, including Scopus, Google Scholar, Web of Science, ScienceDirect, PubMed, PubChem and books including local reports and thesis submissions. All the synonyms of this plant, their traditional uses, pharmacological and phytochemistry studies were searched. The key words used included *P. viridiflorum* and all synonyms as mentioned above, medicinal uses, ethnobotanical studies, biological activities, pharmacology, secondary metabolites, phytochemistry, safety, toxicology and related words. The information was compiled and arranged as detailed in the various sections of this paper.

4. Results of literature search and discussion

4.1. Traditional uses

According to our knowledge, although *P. viridiflorum* is distributed in other continents beside Africa, there have been generally no reports of the medicinal use of this plant in the countries where it is found apart from those in Africa. However, in Portugal, although there is to date no report of the distribution of *P. viridiflorum*, the crushed whole plant is applied as a

poultice to repair stained or torn muscles, tendons and ligaments (Otang et al., 2014). *P. viridiflorum* has many uses in ATM, and is recorded in literature for curing various diseases and ailments. The bitter taste and sweet smell that is due to liquorice is reputed to align with medicinal properties (Palgrave, 2002). In this study ten categories were identified under which *P. viridiflorum* is used in traditional medicine (TM) (Table 1). Broadly, *P. viridiflorum* finds use in TM for well-being, gastrointestinal and respiratory tract infections, cancer, malaria, sexually transmitted diseases, veterinary ailments, circulatory ailments, kidney complaints, wounds and inflammation.

While the bark of *P. viridiflorum* is the most commonly utilised plant part in TM, leaves and roots are also utilised, although to a lesser extent. According to our findings, *P. viridiflorum* finds most traditional use in South Africa.

Table 1: Ethnobotanical usage of *P. viridiflorum* in African traditional medicine

Category of use	Traditional use description	References
Cancer	Root or bark decoctions or infusions are administered for several weeks in Eastern Cape, South Africa to cure cancer. Root powder administered orally for cancer.	Koduru et al., 2007
Circulatory	Stem bark and leaves are used in South Africa for high blood pressure. Leaves are used to treat tachycardia.	Muthaura et al., 2007; Wandji et al., 2016
Gastrointestinal	Leaves are used in Ethiopia as one of the main ingredients in some of the prescriptions for food poisoning, gastritis and indigestion. Stem bark against constipation. In South Africa leaves are used for gastric ulcers and intestinal disorders, whereas bark infusion mixed with *Kedrostis nana* var. *zeyheri* A. Meeuse is used for stomach cramps, and roasted bark or decoction for dysentery. Root and its bark decoctions are used as emetics and enemas for stomach problems. Roasted bark is used for dysentery. Smelling fresh crushed inner bark for ascariasis in Angola. In Cameroon, the dried bark is prescribed to children against stomach worm diseases.	Watt-Breyer, 1962; Hutchings et al., 1996; Muthaura et al., 2007; Bekalo et al., 2009; Momeni et al., 2010; Teka et al., 2015
Inflammation	Stem maceration is used for back and chest pain (infusion with *Brocchinia micrantha* (Baker) Mez and honey in Cameroon). Stem bark is used in Ethiopia for abdominal pains. Bark maceration or infusion for washing painful eyes in Angola. Powdery bark administered directly on the tooth daily for toothache in Swaziland. Leaves used in South Africa for pain and root infusions for body pain.	Mavi et al., 1985; Amusan et al., 2002; Muthaura et al., 2007; Focho et al., 2009; Van Wyk et al., 2009
Kidney complaints	Bark or leaves find use in Eastern Cape, South Africa for Kidney complaints.	Johnson, 1983
Malaria	In Kenya, stem bark decoction in goat bone soup is used as an effective antimalarial cure. The stem bark decoction is also used for malaria associated fever. In Cameroon, dried bark is used against malaria and as an antidote for insect bites.	Palgrave, 2002; Muthaura et al., 2007; Momeni et al., 2015, Wandji et al., 2016
Wounds	Root decoction is used as a wash for cut wounds in Eastern Cape Province of South Africa.	Van Wyk et al., 2009

Respiratory	In Ethiopia stem bark is used against slight cough, and leaves for pneumonia and tuberculosis (bark infusion taken orally in South Africa). Root or bark infusion for respiratory tract associated fever in Angola and chest complaints. Root infusion for chest complaints and cryptococcal meningitis. Bark infusion mixed with *Kedrostis nana* var. *zeyheri* A. Meeuse for influenza in South Africa.	Muthaura et al., 2007; Bekalo et al., 2009; Van Wyk et al., 2011;Teka et al., 2015
Sexually transmitted disease	The leaves or stem bark infusion or decoction are used in South Africa for syphilis and for treating opportunist fungal infections in Human Immuno-Deficiency Virus (HIV) positive people.	Muthaura et al., 2007; Otang et al., 2012; Wandji et al., 2016
Veterinary uses	In Ethiopia, powdered roots are used to increase lactation in cows, camels, goats and sheep. Bark decoction used in South Africa for gall and redwater sickness in cattle. Stem bark, root, and leaf infusions are used against livestock ticks in Kenya by direct application on the animal body surface.	Hutchings et al., 1996; Palgrave, 2002; Giday and Ameni, 2003; Van Wyk et al., 2011
Well-being	Stem bark is used for sexual failure and to increase appetite (in Ethiopia). Bark for preventing abortion in young women. Infusion of *P. viridiflorum*, *Brocchinia micrantha* (Baker) Mez and honey for amenorrhea in Cameroon. Stem bark decoction in goat bone soup used as a medicine for strength. Root infusions for dizziness. Root decoction is added to beer by Xhosa people in South Africa as an aphrodisiac. Chewing stick for oral hygiene in Ethiopia.	Mavi et al., 1985; Muthaura et al., 2007; Van Wyk et al., 2009; Otang et al., 2012; Bhat et al., 2014; Wandji et al., 2016

4.2. Phytochemistry and Pharmacological activity

The use of *P. viridiflorum* in ATM for treatment of various diseases and ailments has led to a series of investigations on its phytochemistry and pharmacological properties. Previous studies done on different parts of *P. viridiflorum* imply that this plant is phytochemically diverse and possesses a wide range of biological activities. The following section describes in greater detail the phytochemistry and pharmacological investigations conducted on *P. viridiflorum* (and synonyms) in different regions of the world.

4.2.1. Phytochemistry

In order to understand the pharmacological properties of a plant species, a detailed and extensive phytochemical investigation is required. Several species of *Pittosporum* have been studied for their secondary metabolites, and the genus is known as a good source of essential oils, and neolignan glycosides. The genins of these compounds were reported to be often substituted by sugars (a-L-arabinose and b-D-glucose) and specific groups like angeloyl, tigloyl, senecioyl or 2-methylbutenoyl groups. Triterpene saponins (senaciapittosides A and B from *P. senacia* Putt), pittoviridoside, sesquiterpene glycosides (undulatumosides A and B from *P. undulatum* Vent.), iridoid glycosides (6α-hydroxygeniposide), carotenoids (tobiraxanthins from *P. tobira* (Thunb.) W.T.Aiton) are some of the compounds isolated from *Pittosporum*.

In a study by Ramanandraibe et al. (2000) the essential oils of the leaves and fruit of *P. viridiflorum* var. *viridiflorum* synonym of *P. viridiflorum* collected in Madagascar were analysed, and compounds from the leaf (35 in total) and fruit oils (51 in total) were identified using GC and GC/MS techniques. The major constituents of sesquiterpene-rich leaf oil were reported to be α-cadinol (18.3%), and δ-cadinene (10.6%), whereas sabinene (13.2%), decanal (10.3%), and β-elemene (9.5%) were the main components of fruit oil containing aliphatic, mono and sesquiterpenoid compounds (Ramanandraibe et al., 2000). The presence of aliphatic hydrocarbons, alcohols, acids and aldehydes is a characteristic feature of oils from the Pittosporaceae.

A quantitative analysis of the total condensed tannin, free gallic acid, gallotannin, iridoid, phenolic and flavonoid contents of fresh and stored (for 12 years) *P. viridiflorum* (leaves and twigs) collected in KwaZulu-Natal, South Africa was determined by Amoo et al. (2012). No condensed tannins were detected in both fresh and stored plant material (leaves and twigs),

free gallic acid was present only in stored plant material, and there was no significant difference in gallotannins of both stored and fresh plant material (Amoo et al., 2012). The total iridoid, phenolic, and flavonoid contents of fresh leaves and twigs was reported to be significantly higher than that of the stored plant material extract (Amoo et al., 2012). Seasonal variations are known to have an effect on availability of plant secondary metabolites, thus there are several assumptions regarding the time and season for the collection of several plant parts, however, in the study by Amoo et al. (2012), the plant material used was collected in the same season, which eliminated the problem of seasonal variation. The absence of free gallic acid in fresh leaves and twigs, while present in stored ones could be due to compound reactivity that may lead to conversion or decomposition during storage, and this is problematic as it misleads quantitative data (Cronin et al., 1995). Higher total iridoid, phenolic, as well as flavonoid contents of fresh leaves and twigs compared to stored material could be attributed to the fact that some compounds are easily oxidised during the drying process (Cronin et al., 1995). There was a report of no correlation between the total phenolic and flavonoid content in the 70% acetone extract of *P. viridiflorum* leaves collected in Gauteng Province, South Africa (Adebayo et al., 2015).

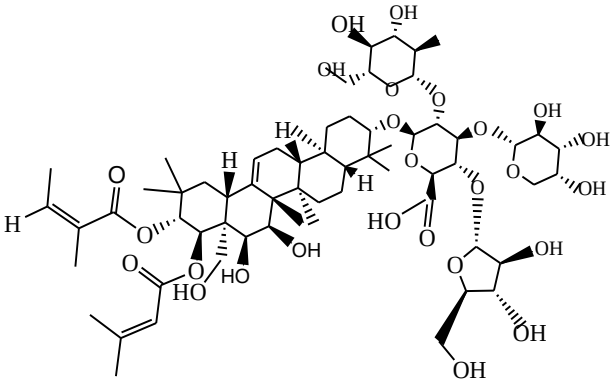
TLC analysis of the leaf methanol (MeOH) and water extracts of *P. viridiflorum* from Gauteng Province, South Africa was reported to show the presence of triterpene saponins (Adebayo et al., 2015) and this is in agreement with the results of Seo et al. (2002) from *P. viridiflorum* collected in the Antsiranana Province, Madagascar. The high performance liquid chromatography coupled with mass spectrometry (LCMS) analysis of both the aqueous and MeOH leaf extract of *P. viridiflorum* from western Cameroon (synonym *P. mannii*) showed the presence of pittoviridoside **1** and 1-O-rhamnopyranosyl-23-acetoxyimberic acid 29-methyl ester **8**, with the aqueous extract having a higher concentration of the compounds in a study by Wandj et al. (2016).

Presence of flavonoids, terpenoids, tannins, and phenolic compounds as well as absence of alkaloids, steroids and sterols was observed in the ethyl acetate fraction of *P. viridiflorum* (synonym *P. mannii*) stem bark from western Cameroon (Momeni et al., 2010). In another phytochemical study on *P. viridiflorum* leaf EtOH extract collected in Kenya, there was a report of the presence of saponins, flavonoids, phenols, triterpenes and phytosterols and absence of glycosides, carbohydrates, tannins, amino acids and alkaloids (Swamy et al., 2015). A phytochemical analysis of the stem bark of *P. viridiflorum* collected from Kerala state, India indicated saponins, flavonoids, phenols, carbohydrates, glycosides, amino acids, fixed oils, and fats (Sadashiva et al., 2013). However, Otang et al. (2012) found saponins, and

alkaloids, but little flavonoid or proanthocyanides in a quantitative phytochemical analysis of *P. viridiflorum* bark extract collected in Eastern Cape Province of South Africa. Momeni et al. (2010) noted a difference in the compound contents of *P. viridiflorum* collected in Cameroon compared to the same species collected in Madagascar, Kenya and Mali. Possibly geographical, seasonal or chemotype variations play a role in these discrepancies. The presence of saponins, flavonoids phenols and phytosterols shows the potential of plants to produce antioxidant, anticancer, anti-inflammatory, and antimicrobial activity. Saponins are known for their use in stopping bleeding, and in treating wounds by helping red blood cells to precipitate and coagulate. Flavonoids have been reported to have antibacterial activity against several bacterial strains, whereas phytosterols have antimicrobial activity towards *Staphylococcus aureus* and *Bacillus subtilis* (Swamy et al., 2015).

Chemical studies of *P. viridiflorum* have led to the isolation of several compounds, and these are illustrated in Figure 2. *Pittosporum* species are known to possess saponins. Triterpenoid saponins have been reported from the leaves of *P. phillyraeoides* DC. A new triterpenoid saponin, pittoviridoside, identified through spectral and GC analysis as 3-O-[β-D-glucopyranosyl (1→2)]-[α-arabinopyabosyl (1→3), [α-arabinofuranosyl (1→4)-β-D-glucopyranosyl-21-angeloyl-22-senecioylolean-12-en-3β, 15α, 16α, 21β, 22α, 28-hexol **1** was isolated from the leaf MeOH extract collected in Antsiranana, Madagascar (Seo et al., 2002). Surprisingly, the leaves of *P. viridiflorum* collected from Cameroon were reported to have pittoviridoside and 1-O-rhamnopyranosyl-23-acetoxyimberic acid 29-methyl ester, while only the former was isolated from the same species collected in Madagascar. Triterpenoid saponins were reported to have been isolated from *P. verticillatum* Bojer root bark extract (Manase et al., 2013). Interestingly, 21-O-β,β-dimethylacryloyl-22-O-angeloyl-R1-barrigenol although reported for the first time from *P. verticillatum*, the structural analog of this compound was characterised previously by Seo et al. (2002) in *P. viridiflorum* as 21-O-angeloyl-22-O-β,β-dimethylacryloyl-R1-barrigenol (Manase et al., 2013). Another new sesquiterpene glycoside and its two derivatives from the acetone extract of the fresh leaves of synonym *P. viridiflorum viridiflorum* collected in Antananarivo, Madagascar were isolated and identified as 4-*O*-β-D-glucopyranosylgermacra-1(10), 5-diene **2** and derivatives; 6'-acetyl-4-*O*-β-D-glucopyranosylgermacra-1(10), 5-diene **3** as well as 2'-*O*-acetyl-4-*O*-β-D-glucopyranosylgermacra-1(10), 5-diene **4** (Ramanandraibe et al., 2001). Three pentacyclic triterpenoids; oleanic acid **5**, 3, 22, 28-trihydroxyolean-12-ene 2 **6** and β-amyrin acetate **7** were isolated from the leaf MeOH extract *P. viridiflorum* collected from Kakamega rain

forest, Kenya (Nyabayo et al., 2015), again these compounds on the same plant extract prepared from species collected in Cameroon were not detected in a study by Wandj et al. (2016). Oleanic acid occurs widely in many plants as a free acid or aglycone for several plants (Wu et al., 2016). β-Amyrin acetate was isolated from *Alstonia boonei* De Wild stem bark (Okoye et al., 2014). A pentacyclic triterterpenoiol estersaponin identified as 1-0-{alpha-L-rhamnopyranosyl}-23-acetoxyimberbic acid 29-methyl ester **8** was isolated from the MeOH extract of the stem bark of *P. mannii* (synonym) collected in north west Cameroon (Nyongbela et al., 2013). This pentacyclic triterterpenoiol estersaponin is a derivative of imberbic acid, isolated previously from *Combretum sundaicum* Miq and *Lantana camara* L. leaves and flowers (Nyongbela et al., 2013). Pentacyclic triterpene saponins are the dominant secondary metabolites in several *Pittosporum* species (Bäcker et al., 2014).



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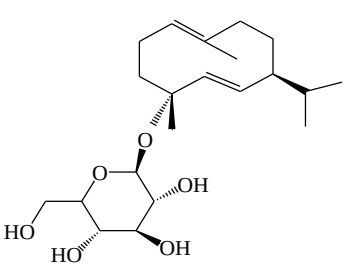

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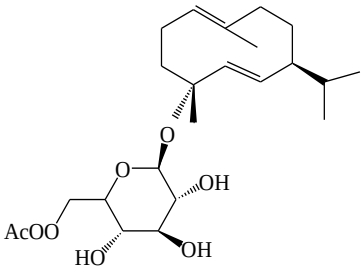

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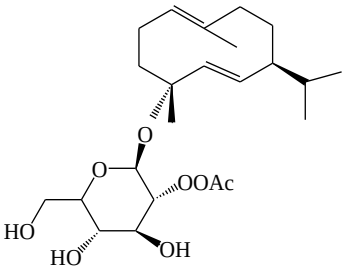

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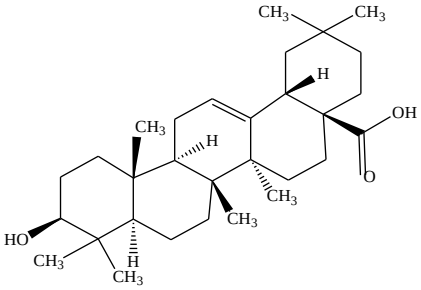

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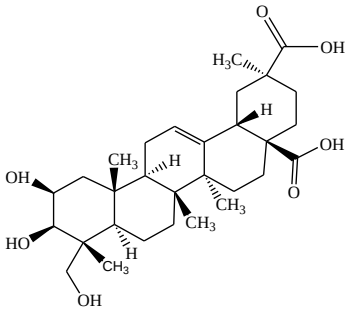

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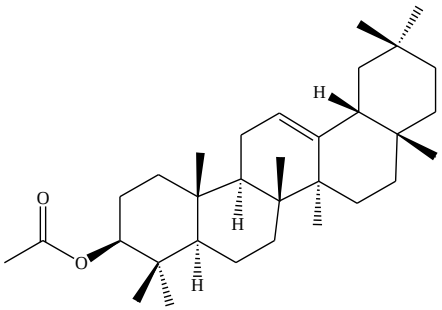

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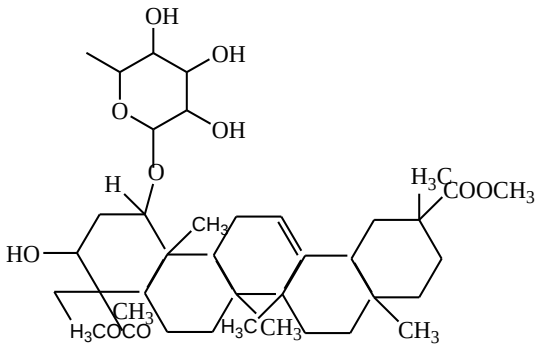

8

Figure 2: Compounds from *Pittosporum viridiflorum*: Pittoviridoside **1**, 4-*O*-D-glucopyranosylgermacra-1(10), 5-diene **2**, 6'-acetyl-4-*O*-β-D-glucopyranosylgermacra-1(10), 5-diene **3**, 2'-*O*-acetyl-4-*O*-β-D-glucopyranosylgermacra-1(10), 5-diene **4**, oleanic acid **5**, 3, 22, 28-trihydroxyolean-12-ene 2 **6**, β-amyrin acetate **7**, 1-0-{alpha-L-rhamnopyranosyl}-23-acetoxyimberbic acid 29-methyl ester **8**

4.2.2. Pharmacology
4.2.2.1. Antimicrobial

Given that *P. viridiflorum* is used in ATM to treat wounds and infectious diseases, several studies directed towards discovering the effect of *P. viridiflorum* on various bacterial and fungal pathogens have been conducted (Table 2). The antimicrobial methods previously used to determine the potential of different extracts from this plant include disc diffusion and microdilution. The 70% ethanol leaf extract of *P. viridiflorum* collected in Kenya was reported to have antibacterial activity against *Proteus vulgaris*, *Escherichia coli*, *Bacillus cereus*, and *Enterobacter aerogenes* with zones of inhibition greater than 8 mm; however it was inactive against *Salmonella typhimurium* (Swamy et al., 2014). This slightly validated the use of this plant against opportunistic bacterial infections. Furthermore, the MeOH leaf extract of *P. viridiflorum* collected from Gauteng, South Africa showed antimicrobial potential when tested against two rheumatoid triggers, *Proteus mirabilis* and *Proteus vulgaris*, with MIC values of 767 and 497 µg/ml respectively (Cock and van Vuuren, 2014). This gives credence to the traditional use of the leaves of this plant in South Africa against urinary tract and wound infections (Johnson, 1983; Van Wyk et al., 2009). Another study by Cock and van Vuuren (2015) aimed at evaluating the antimicrobial potential of the leaf MeOH and water extract against microbes associated with food spoilage and poisoning; the MeOH extract showed zones of inhibition against *Proteus mirabilis* and *Proteus vulgaris* with diameters of 8 and 6.7 mm respectively. However the leaf water extracts were inactive

against *Alcaligenes faecalis, Aeromonas hydrophila, Citrobacter freundii, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Pseudomonas fluorescens, Salmonella typhimurium, Serratia marcescens, Shigella sonnei, Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Aspergillus niger, Candida albicans* and *Rhizopus stolonifer* as they did not show zones of inhibition against these strains (Cock and van Vuuren, 2015). This might mean that pittoviridoside and 1-0-{alpha-L-rhamnopyranosyl}-23-acetoxyimberbic acid 29-methyl ester that were detected by Wandj et al. (2016) in the leaf water extract of this plant collected in Cameroon have no antimicrobial activity or that the extraction method used did not extract the compounds or that the compounds are not effective against the selected microorganisms. On the other hand, the assay (disc diffusion) chosen to test the antibacterial effect of the leaf water extract from this plant could have been ineffective as it depends on several factors that include diffusion rate of the plant extract, type of agar used and bacterial inoculum concentration tested (Hombach et al., 2013). Acetone extracts prepared from leaf material of *P. viridiflorum* collected in Mpumalanga, South Africa were reported to have low antibacterial activity against *Escherichia coli, Enterococcus faecalis, Pseudomonas aeruginosa* and *Staphylococcus aureus,* however the MIC values were not specified (Kaikabo, 2009). Good antibacterial inhibitory potential was reported from both the leaf and fruit essential oils of synonym *P. viridiflorum* Culofondis var. *viridiflorum* collected in Madagascar against almost all bacterial strains tested (*Streptococcus faecalis, Staphylococcus epidermis*, *Staphylococcus aureus*, and *Escherichia coli*) with zones of inhibition ranging from 11.0-18.3 mm, with the exception of *Pseudomonas aeruginosa*, as the fruit oil (7.8 mm) was not as active against this bacterial species (Ramanandraibe et al., 2000). One of the main constituents of *P. viridiflorum* fruit oil, α-cadinol isolated from *Diospyros discolor* Willd demonstrated antimicrobial properties against *Escherichia coli, Staphylococcus aureus* and *Staphylococcus epidermidis* (Su et al., 2015). Antifungal properties were reported for α-cadinol towards plant pathogenic fungi such as *Rhizoctonia solani, Fusarium oxysporum, Fusarium solani, Colletotrichum gloeosporiodes, Papulospora funerea* and *Ganoderma australe* with MIC values ranging from 11.7-51.9 µg/ml (Chang et al., 2008). The fruit oil sabinene isolated from *Oenanthe crocata* L. was reported to have antifungal activity towards 10 fungal strains with MIC values ranging between 0.16-0.64 µg/ml (Valente et al., 2013). Arunkumar et al. (2014) reported the antimicrobial activity of sabinene towards *Salmonella typhimurium, Aspergillus fumigatus, Escherichia coli, Candida albicans*, and *Trychophyton rubrum* with MIC values ranging from 3-25 µg/ml. The best antibacterial results were observed with sabinene towards *Salmonella*

typhimurium at 3 µg/ml. In an antifungal study by Otang (2013) with *P. viridiflorum* collected in the Eastern Cape, South Africa using both microdilution and disc diffusion methods against 10 fungal strains associated with HIV/AIDs, the lowest MIC was observed with the hexane extract against *Aspergillus fumigatus* (0.02 mg/ml). This to some extent verifies the ethnopharmacological use of this plant for treating opportunist fungal infections in HIV positive people in the Eastern Cape, South Africa.

Table 2: Reported antimicrobial results of extracts from different parts of *P. viridiflorum*

Extract	Method	Results, strains and MIC or zone of inhibition values	Place of plant collection	Reference
Leaf oil	Disk diffusion	*S. faecalis* = 13.6 mm, *S. epidermidis* = 13.3 mm; *S. aureus* = 18.3 mm; *P. aeruginosa* = 11.0 mm, *E. coli* = 13.0 mm	Madagascar	Ramanandraibe et al., 2000
Fruit oil	Disk diffusion	*S. faecalis* = 12.6 mm; *S. epidermidis* = 11.6 mm, *S. aureus* = 17.6 mm, *E. coli* = 13.0 mm *P. aeruginosa* = 7.8 mm	Madagascar	Ramanandraibe et al., 2000
Hexane bark	Microdilution	*B. subtilis* = 1.56 mg/ml, *S. aureus* = 3.13 mg/ml	South Africa	McGaw et al., 2000
EtOH bark	Microdilution	*S. aureus* = 1.56 mg/ml	South Africa	McGaw et al., 2000
DCM:MeOH stem	Microdilution	*B. cereus* = 4 mg/ml, *S. mutans* = 16 mg/ml, *S. aureus* = 6 mg/ml, *E. faecalis* = 1 mg/ml *L. acidophilus* = 8 mg/ml, *E. coli* = 4 mg/ml, *K. pneumoniae* = 8 mg/ml, *C. albicans* = 3 mg/ml, *C. neoformans* = 2 mg/ml	Ethiopia	van Vuuren and Viljoen, 2006
Water stem	Microdilution	*E. faecalis* = 8 mg/ml, *E. coli* = 4 mg/ml, *C. albicans* = 4 mg/ml, *C. neoformans* = 1 mg/ml		van Vuuren and Viljoen, 2006
DCM:MeOH leaf	Microdilution	Methicilin-resistant *S. aureus* = 8 mg/ml, gentamycin-resistant *S. aureus* = 8 mg/ml, *S. aureus* = 4 mg/ml, *S. epidermidis* = 8 mg/ml, *P. aeruginosa* = 8 mg/ml, *C. albicans* = 2 mg/ml, *B. agri* = 2 mg/ml, *P. acnes* 8 = mg/ml, *T. mentagrophytes* = 0.5 mg/ml, *M. canis* = 1mg/ml	South Africa	Mabona et al., 2013
Water leaf	Microdilution	Methicilin-resistant *S. aureus* = 8 mg/ml, gentamycin-resistant *S. aureus* = 4 mg/ml, *S. aureus* = 4 mg/ml, *S. epidermidis* = 4 mg/ml, *P. aeruginosa* >16 mg/ml, *C. albicans* = 2 mg/ml; *B. agri* = 8 mg/ml, *P. acnes* = 0.25 mg/ml, *T. mentagrophytes* = 1 mg/ml, *M.*	South Africa	Mabona et al., 2013

		canis = 1 mg/ml		
EtOH bark	Microdilution	*P. aeruginosa* = 100 µg/ml, *S. aureus* = 500 µg/ml, *P. mirabilis* = 200 µg/ml, *K. pneumonia* = 250 µg/ml, *E. coli* = 500 µg/ml	India	Sadashiva et al., 2013
MeOH leaf and twig (stored)	Microdilution	*S. aureus* = 2.08 mg/ml; *P. aeroginosa* = 6.25 mg/ml. *C. albicans* =0.78 mg/ml	South Africa	Amoo et al. 2013
MeOH leaf and twig fresh)	Microdilution	*S. aureus* = 1.56, *P. aeroginosa* = 6.25 mg/ml. *C. albicans* = 0.91 mg/ml	South Africa	Amoo et al. 2013
MeOH leaf	Microdilution	*P. mirabilis* = 767 µg/ml, *P. vulgaris* = 497 µg/ml	South Africa	Cock and van Vuuren, 2014
MeOH leaf	Disk diffusion	*P. mirabilis* = 8 mm, *P. vulgaris* =6.7 mm. [*A. faecalis*, *A. hydrophila*, *C. freundii*, *E. coli*, *K. pneumonia*, *P. aeruginosa*, *P. fluroscens*, *S. typhimurium*, *S. marcescens*, *S. sonnei*, *B. cereus*, *B. subtilis*, *S. aureus*, *S. epidermidis*, *A. niger*, *C. albicans*, *R. stolonifer*] = 0 mm	South Africa	Cock and van Vuuren, 2015
Water leaf	Disc diffusion	[*A. faecalis*, *A. hydrophila*, *C. freundii*, *E. coli*, *K. pneumonia*, *P. aeruginosa*, *P. fluroscens*, *P. mirabilis*, *P. vulgaris*, *S. typhimurium*, *S. marcescens*, *S. sonnei*, *B. cereus*, *B. subtilis*, *S. aureus*, *S. epidermidis*, *A. niger*, *C. albicans*, *R. stolonifer*] = 0 mm	South Africa	Cock and van Vuuren, 2015
EtOH leaf	Microdilution	[*E. faecalis*, *S. aureus*, *E. coli*, *P. aeruginosa*, *C. albicans*] > 512µg/ml	Ethiopia	Teka et al., 2015
Acetone Leaf	Microdilution	*B. anthracis* = 0.04 mg/ml	South Africa	Elisha et al., 2016

EtOH = ethanol, MeOH = methanol, DCM = dichloromethane, MIC= minimum inhibitory concentration, *S. faecalis = Streptococcus faecalis*, *S. epidermidis = Staphylococcus epidermidis*, *S. aureus = Staphylococcus aureus*, *P. aeruginosa = Pseudomonas aeruginosa*, *E. coli = Escherichia coli*, *B. subtilis = Bacillus subtilis*, *B. cereus = Bacillus cereus*, S. *mutans = Streptococcus mutans*; *E. faecalis = Enterococcus faecalis*, *L. acidophilus = Lactobacillus acidophilus*, *K. pneumoniae = Klebsiella pneumoniae*, *C. albicans = Candida albicans*, *C. neoformans = Cryptococcus neoformans*, methicilin-resistant *S. aureus* = methicilin-resistant *Staphylococcus aureus*, gentamycin-resistant *S. aureus* = gentamycin-resistant *Staphylococcus aureus*, *B. agri = Brevibacillus agri*, *P. acnes = Propionibacterium acnes*, *T. mentagrophytes = Trichophyton mentagrophytes*, *M. canis = Microsporum canis*, *P. mirabilis = Proteus mirabilis*, *P. vulgaris = Proteus vulgaris*, *A. faecalis = Alcaligenes faecalis*, *A. hydrophila = Aeromonas hydrophila*, *C. freundii = Citrobacter freundii*, *P. freundii =*; *S. typhimurium = Salmonella typhimurium*, *S. marcescens = Serratia marcescens*, *S. sonnei = Shigella sonnei*, *A. niger = Aspergillus niger*, *R. stolonifer = Rhizopus stolonifer*, *B. anthracis = Bacillus anthracis*, *P. fluorescens= Pseudomonas fluorescens*

The ethanol stem bark extract collected in India showed good antimicrobial potential against *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*

and *Klebsiella pneumoniae* with MIC values ranging from 100-500 µg/ml (Sadashiva et al., 2013); these findings could be attributed to the pentacyclic triterterpenoiol estersaponin present in this plant part. This needs to be explored further as other compounds responsible for this activity could be found in the EtOH stem bark extract. A plant extract showing MIC value of 1 mg/ml or less is regarded potent and worthy of being evaluated further for compounds which may serve as substitutes for known antibiotics which are not as effective as they used to be due to emergence of drug resistant strains. Furthermore, ethanol and hexane bark extracts of *P. viridiflorum* from KwaZulu-Natal, South Africa demonstrated some degree of antibacterial activity against *Bacillus subtilis* in a microdilution assay with MIC values of 1.56 and 3.13 mg/ml respectively; the ethanol extract also mildly inhibited *S. aureus* with MIC = 1.56 mg/ml (McGaw et al., 2000). The extracts appeared to be more active in the disc diffusion assay (McGaw et al., 2000). Higher inhibition of *Bacillus subtilis* and *Staphylococcus aureus* by the ethanol and hexane bark extracts in the disc diffusion assay when compared to the microdilution assay could be a result of different concentrations tested; 100 mg/ml in the disc diffusion (with one mg applied to each filter paper disc) and an initial concentration of 12.5 mg/ml in the microdilution assay.

A microdilution method was used to determine the antimicrobial potential of water and dichloromethane (DCM):MeOH (1:1) leaf extract collected in Gauteng, South Africa against 10 common skin pathogens. Both extracts showed MIC values ranging from 0.25 to greater than 16 mg/ml, and the lowest MIC value was observed against *Propionibacterium acnes* (water) and *Trichophyton mentagrophytes* (DCM:MeOH) with MIC = 0.25 and 0.5 mg/ml respectively (Mabona et al., 2013). The ethanol leaf extract was investigated for antimicrobial activity against one fungal and four bacterial strains from the American Type Culture Collection (ATCC). The leaf ethanol extract collected in south central Ethiopia was inactive against *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans* with MIC values >512 µg/ml (which was the highest concentration tested) (Teka et al. 2015). The DCM:MeOH (1:1) and water stem extracts of *P. viridiflorum* collected in Ethiopia were inactive against six bacterial and two fungal pathogens with MIC values ranging between 1 and 16 mg/ml (van Vuuren and Viljoen, 2006). When new and old leaves as well as twigs (50% MeOH extracts) of *P. viridiflorum* collected in KwaZulu-Natal, South Africa were tested for their antimicrobial potential, they did not show noteworthy inhibition against *Staphylococcus aureus* and *Pseudomonas aeruginosa* with MIC values ranging from 1.56 to >6.25 mg/ml, however both extracts were reported to have noteworthy antifungal activity against *Candida albicans* with MIC values of

0.78 and 0.91 mg/ml (Amoo et al., 2013). Leaf acetone extracts collected in Gauteng, South Africa showed good antibacterial activity against *Bacillus anthracis* Sterne strain with an MIC value of 0.04 mg/ml (Elisha et al., 2016). The ethnopharmacological use of *P. viridiflorum* against gastrointestinal and respiratory tract infections is ascribed to its antimicrobial activity towards both Gram-positive and Gram-negative bacterial strains associated with such ailments, however *in vivo* studies are needed to confirm this assertion. Although *P. viridiflorum* is known in TM for treating tuberculosis, there are no pharmacological studies that have been done to verify this property. Additionally, roots of *P. viridiflorum* are used as a wash for wounds, but there are no known reports of the antimicrobial evaluation of this plant part, and this area needs to be explored. There is a need for isolation of antimicrobial compounds from *P. viridiflorum* leaf and stem bark extracts as they have shown potential against various bacterial and fungal strains.

4.2.2.2. Antidiarrhoeal

P. viridiflorum finds use in the management of many gastrointestinal disorders in ATM, including diarrhoea. Research was done by Joseph et al. (2015) with the aim of evaluating *in vitro* the effects of the stem bark aqueous (hot water) extract of *P. viridiflorum* (collected in the western region of Cameroon, under the synonym *P. mannii*) at different concentrations (5-80 and 10-80µg/ml) on spontaneous contractile activity and contractions of isolated rat duodenum induced by carbachol, histamine, and potassium chloride in an organ bath assay. The aqueous stem bark extract significantly decreased the tonus and amplitude of spontaneous contractions of rat duodenum at 10-80 µg/ml concentration range, nonetheless at a higher concentration of 80 µg/ml, the same extract elicited a transient relaxation that was followed by a slight tonus increase, while the amplitude remained lower. This might be a result of different compounds from this extract showing different activities, with one acting as a relaxant, while the other acts as a contractor. Therefore, the aqueous extract should be administered cautiously as higher concentrations might worsen diarrhoeal conditions. The presence of atropine and promethazine at 0.713 and 0.5 µg/ml did not significantly affect the relaxant effect of the aqueous stem bark extract (Joseph et al., 2015). Carbachol and histamine-induced rat duodenum contractions were slightly but significantly inhibited by the aqueous extract in a concentration related manner of 20, 40 and 80 µg/ml, and the same extract (10-80 µg/ml) significantly induced a concentration dependent relaxation on potassium chloride-induced contraction of rat duodenum. This might suggest that the

mechanism of action of the aqueous extract of this plant is not specifically antihistaminic or anticholinergic and that it might relax the smooth muscles through inhibition of the influx of Ca^{2+}. To sum it all up, the aqueous extract of *P. viridiflorum* possesses antispasmodic and spasmodic effects at lower concentration, thus giving credence to its traditional use against diarrhoea and other gastrointestinal ailments. However, more studies *in vivo* on the mechanism of action, to determine whether the effect of the aqueous extract is a result of a histaminic or muscarinic receptor blockage, are required. Furthermore, compounds with antidiarrhoeal activity from this species should be isolated.

4.2.2.3. Antimalarial

Malaria is a major parasitic disease worldwide, specifically in Africa, and is responsible for approximately 2 to 3 million deaths yearly, killing mostly pregnant women and children less than 5 years old (Ramalhete et al., 2008). The common causative agent of human malaria, *Plasmodium falciparum,* has become highly resistant to known antimalarial drugs such as antifolates and chloroquine. Thus, antimalarial drugs with new modes of action are required urgently. Although artemisinin, a compound isolated from *Artemisia annua* L. has found wide application in treating malaria, studies at the Thai-Cambodian border have detected its reduced efficacy, thus raising concerns on development of resistance (Cui and Su, 2009). Worldwide, plants are widely used for traditional treatment of malaria, however, there is little scientific data to validate their traditionally claimed efficacies. In Africa, malaria is one of the top diseases that *P. viridiflorum* finds use in treating, and several studies have been conducted to determine the antiplasmodial properties of this plant in this region (Table 3). In a study in Kenya, leaf extracts of *P. viridiflorum* collected in the Eastern province were evaluated for *in vitro* antiplasmodial and *in vivo* antimalarial potential using *Plasmodium falciparum* D6 and W2, as well as mice infected with *Plasmodium berghei*. In that study, the antiplasmodial activity against *Plasmodium falciparum* D6 and W2 was considered high when the IC_{50} value was less than 5 µg/ml, moderate between 5-20 µg/ml, weak between 20-100 µg/ml, and inactive if more than 100 µg/ml. The MeOH leaf extract showed moderate antiplasmodial potential against *Plasmodium falciparum* D6 and W2 with IC_{50} values of 18.9 and 17.69 µg/ml, whereas the water extract demonstrated weak activity against D6 (IC_{50}=27.61 µg/ml) and was inactive against W2 (IC_{50}=224.27 µg/ml) (Muthaura et al., 2007). For *in vivo* antimalarial results, the leaf MeOH and water extracts showed 54.77% and 89.76% chemosuppression with 15 and 14 days of survival respectively (Muthaura et al.,

2007). In another antimalarial study of the MeOH leaves and root extracts of *P. lanatum* (synonym of *P. viridiflorum* collected in Nairobi, Kenya) against chloroquinoline resistant (V1/S) and sensitive (M24 and K39) *P. falciparum* strains, both extracts showed weak antimalarial activity with IC_{50} values ranging from 24.2-41.5 µg/ml (Wanyoike et al., 2004). *P. viridiflorum* stem bark water extract collected in Kenya showed weak antimalarial activity towards *P. falciparum* M24 (80 µg/ml) and K67 (30 µg/ml) (Gakunju et al., 1995). In South Africa, both whole plant and leaf/flower water extracts were inactive against *Plasmodium falciparum* D10 as their IC_{50} values were greater than 100 µg/ml (Clarkson et al., 2004). This is surprising as water is the most commonly used solvent for extraction in TM. The leaf/flower DCM, DCM: MeOH and MeOH extracts of *P. viridiflorum* showed weak antiplasmodial potential with IC_{50} values of 28, 47 and 70.5 µg/ml respectively. The whole plant MeOH extract demonstrated weak antiplasmodial activity with an IC_{50} value of 27.7 µg/ml (Clarkson et al., 2004). However, the DCM whole plant extract of *P. viridiflorum* was highly active against *Plasmodium falciparum* D10 with an IC_{50} value of 3 µg/ml, whereas the DCM: MeOH (1:1) showed moderate activity with an IC_{50} value of 10 µg/ml (Clarkson et al., 2004). *P. viridiflorum* collected in South Africa is one of 381 plants that were evaluated for antimalarial potential via insecticidal activity in research by Maharaj et al. (2011), which was done by exposing the *Anopheles arabiensis* mosquito on tiles with extract for 1 hour. *P. viridiflorum* root DCM extract was reported as causing the highest mortality (57%) of *Anopheles arabiensis* and was not significantly active compared to deltamethrin (the positive control); however, it was recommended for further antimalarial work against other causative agents of malaria. The ethyl acetate aerial part of *P. tobira* (a plant used in Mozambique against malaria) showed antimalarial activity towards *P. falciparum* 3D7 with IC_{50} value of 4.8 µg/ml (Ramalhete et al., 2008). This supports the interest in the *Pittosporum* genus as a source of antimalarial drugs. A compound, 1-0-{alpha-L-rhamnopyranosyl}-23-acetoxyimberbic acid 29-methyl ester **8** from the MeOH stem bark, demonstrated pronounced activity against both *Plasmodium falciparum* and *Leishmania donovani* parasites with IC_{50} values of 1.02 and 1.8 µg/ml respectively (Nyongbela et al., 2013), and was slightly less active than the positive controls artemisinin (0.80 µg/ml against *Plasmodium falciparum*) and miltefosine (0.16 µg/ml against *Leishmania donovani*). However, the activity of the compound supported the traditional use of this plant against malaria in ATM, and further studies on *in vivo* antimalarial activity and mechanism(s) of action of this compound are required. Antimalarial compounds from the whole plant DCM extract of *P. viridiflorum* should be isolated, and evaluated further for their potential in malaria therapy.

Table 3: Reported antimalarial results of extracts from different parts of *P. viridiflorum*

Extract	Strain tested and IC_{50} value	Place of plant collection	Reference
Water leaf/flower	*P. falciparum* D10 > 100µg/ml	South Africa	Clarkson et al., 2004
Water bark	*P. falciparum* K67 = 30 µg/ml; *P. falciparum* M24 = 80 µg/ml	Kenya	Gukunju, 1995
DCM leaf/flower	*P. falciparum* D10 = 28 µg/ml	South Africa	Clarkson et al., 2004
DCM:MeOH leaf/flower	*P. falciparum* D10 = 47 µg/ml	South Africa	Clarkson et al., 2004
MeOH leaf/flower	*P. falciparum* D10 = 70.5 µg/ml	South Africa	Clarkson et al., 2004
MeOH whole plant	*P. falciparum* D10 = 27.7 µg/ml	South Africa	Clarkson et al., 2004
DCM whole plant	*P. falciparum* D10 = 3 µg/ml	South Africa	Clarkson et al., 2004
DCM:MeOH whole plant	*P. falciparum* D10 = 10 µg/ml	South Africa	Clarkson et al., 2004
DCM root	*A. arabiensis* = 57% mortality	South Africa	Clarkson et al., 2004
MeOH leaf	*P. falciparum* D6 = 18.9 µg/ml, *P. falciparum* W2 = 17.69 µg/ml, *P. berghei* = 54.77%	Kenya	Muthaura et al., 2007
Water leaf	*P. falciparum* D6 = 27.61 µg/ml, *P. falciparum* W2 = 224.27 µg/ml, *P. berghei* =89.76%	Kenya	Muthaura et al., 2007
DCM bark	*P. falciparum* (K1) > 5.00 µg/ml, *L. donovani* = 21.70 µg/ml	Cameroon	Nyongbela et al., 2013
MeOH bark	*P. falciparum* (K1) = 4.30µg/ml, *L. donovani* = 8.60 µg/ml	Cameroon	Nyongbela et al., 2013
1-0-{alpha-L-rhamnopyranosyl}-23-acetoxyimberbic acid 29-methyl ester **8**	*P. falciparum* (K1) = 1.02 µg/ml, *L. donovani*= 1.8 µg/ml	Cameroon	Nyongbela et al., 2013

DCM = dichloromethane, MeOH = methanol, *A. arabiensis = Anopheles arabiensis*, *P. falciparum = Plasmodium falciparum*, *P. berghei = Plasmodium berghei*, *L. donovani = Leishmania donovani*, IC_{50} =50% inhibitory concentration

4.2.2.4. Acaricidal

Ticks are the vectors of several diseases afflicting livestock, as well as humans, and *Rhipicephalus appendiculatus* is among the main vectors of *Theileria parva*, a causative agent of East Coast Fever. Resistance of acari to acaricides, the existence of food residues, and environmental pollution has forced research to focus more on other sources such as medicinal plants to find a solution for the problems at hand. Plants are used to cure disease carried by ticks, and *P. viridiflorum* is among the plants that are mentioned occasionally for such purposes in ATM. Three pentacyclic triterpenoids (**5**, **6** and **7**) isolated from Kenyan *P. viridiflorum* were tested for *in vitro* larvicidal activity against *Rhipicephalus appendiculatus*. The compounds demonstrated a dose-dependent effect with compound **6** showing the best

effect followed by compound **7**, and lastly compound **5** had the least pronounced effect on the larvae, with this observation being made after 3 days of exposure (Nyabayo et al., 2015). Although the results supported the use of *P. viridiflorum* extract as an alternative acaricide, the larvicidal effects of the compounds (**5**, **6** and **7**) were lower than that of the commercial acaricide amitraz. A bioguided isolation technique was not followed when isolating acaricidal compounds from this plant, and this might mean that there could be other constituents with better activity. *P. tobira* is the only other *Pittosporum* species that has been evaluated for acaricidal activity, against a spider mite that affects vegetables and fruits. The leaf extract of this plant showed activity with an LC_{50} = 150 µg/ml towards *Trialeurodes urtiacae* (Moussa et al., 2010). Several other plants have been tested in Africa for acaricidal activity against *Rhipicephalus appendiculatus*. The fruits of *Solanum dasyphyllum* Schumach. & Thonn. (50 mg/ml) and the roots of *Neorautanenia mitis* (A.Rich.) Verdc (0.5 mg/l) showed 100% inhibition of the egg laying process, thus controlling tick production (Van Puyvelde et al., 1985). Roots and stem bark of *P.viridiflorum* find traditional use against ticks in Kenya, but these plant parts have not yet been evaluated for their anti-tick efficacy, therefore it would be ideal to test them.

4.2.2.5. Anticancer

Cancer is the second leading cause of death worldwide, responsible for about 8.2 million deaths and 14 million new cases in 2012 (Solowey et al., 2014). It is of great concern that in 2012, it was estimated that over the next two decades, the number of new cancer cases will rise by 12 million (Solowey et al., 2014). The currently available treatments for cancer include monoclonal antibodies, radiation, and surgical therapies, as well as chemotherapy, and immunotherapy. Due to high cancer-associated mortality rates, and side effects of chemotherapy and radiation, many patients seek alternative treatment. Plants have been used against cancer for a long time, and are believed to cure this disease without causing toxicity, as inaccurate as this view may be in reality. According to Kaur et al. (2011), more than 60% of cancer patients were reported to use herbs and vitamins in a survey conducted at Anderson Cancer Center, United States. Several anticancer agents are either directly or indirectly derived from natural sources, including plants. Examples of plant-derived anticancer drugs in clinical use include elliptinium, etoposide, homoharringtonine, paclitaxel, docetaxel, podophyllotoxin, teniposide, vinblastine and vincristine (Cragg and Newman, 2005). Despite the discovery of the currently available anticancer drugs, cancer still remains an aggressive

Table 4: Reported anticancer results of extracts from different parts of *P. viridiflorum*

Extract	Cell line	IC_{50} values	Place of collection	Reference
MeOH bark	Human ovarian cancer A2780	Not specified	Madagascar	Seo et al., 2002
80% MeOH bark	HL-60 leukaemia	5.15 µg	Austria	Poschner, 2013
Pittoviridoside	Human ovarian cancer A2780	10.1 µg/ml	Madagascar	Seo et al., 2002

MeOH = methanol, IC_{50} = 50% inhibitory concentration

human killer worldwide, promoting the search for new cancer therapies from plants due to a pressing need to develop effective therapy against this disease. Since *P. viridiflorum* is used in ATM against cancer, the leaf MeOH extract was tested for its potential as part of a continuing search for anticancer plants in Madagascar (Table 4). The extract was reported to have weak cytotoxicity towards the A2780 human ovarian cancer cell line; however IC_{50} values were not specified (Seo et al., 2002). A bioguided technique was conducted using the A2780 human ovarian cancer cell line and yeast assays in the hope of isolating anticancer compounds. The 70% and 80% aqueous MeOH extracts were reported to have bioactivity against 1138, 1140, 1353 and Sc7 yeast strains, and purified to give pittoviridoside. Pittoviridoside **1** from the leaves of *P. viridiflorum* had anticancer activity against the A2780 human ovarian cancer cell line with an IC_{50} value of 10.1 µg/ml (Seo et al., 2002). However, in that study, the positive controls used were not specified, thus preventing comparison of activity of pittoviridoside **1** (the isolated compound) with the anticancer drugs tested, to assess its effectiveness. Purified stem bark extract of synonym *P. mannii* showed anticancer effects with an IC_{50} value of 5.15 µg/ml against HL-60 leukaemia cells. However, the purified extract demonstrated massive necrosis in the treated HL-60 cells, and that was reputed to pittoviridoside **1** or another highly toxic unknown compound (Poschner, 2013). An essential oil found in the leaves of *P. viridiflorum*, β-elemene, is known to play an important role in enhancing effect of several anticancer drugs and in reducing the side effects of cancer treatment, and this could explain the continued therapeutic use of this plant in ATM (Chen et al., 2012). Decanal, a fruit oil constituent of *P. viridiflorum* showed anticancer activity towards Hela cells with an IC_{50} value of 4.57µg/ml (Lui et al., 2012), therefore studies aimed at evaluating the potential of this compound towards other cancer cells lines and its mechanism of action is required. A synthetic oleanic acid **5** derivative induced apoptosis and autophagy in MC7-breast cancer cells with an IC_{50} value of 24.9 µM (Wu et al., 2016). The root is the part reported to be used traditionally against cancer in South Africa, therefore evaluation of this plant part for anticancer properties might prove worthwhile.

4.2.2.6. Anti-inflammatory

Inflammation is an important biological process of the body that maintains homeostasis, thus fighting pathogens and repairing damaged tissues (Deepa and Renuka, 2014; Fürst and Zündorf, 2014). The same inflammatory process is also involved in the onset and maintenance of several disorders, in which case it is referred to as chronic inflammation (Fürst and Zündorf, 2014). Inflammation can result either from direct or indirect activation of nociceptive neurons by inflammatory mediators (Fürst and Zündorf, 2014). The most common class of medications against inflammation and related disorders is non-steroidal anti-inflammatory drugs (NSAIDs) used worldwide, with an estimated usage of more than 30 million per day (Shaik et al., 2015). However, besides the excellent anti-inflammatory potential of the NSAIDs, the severe side effects associated with them such as gastrointestinal ulceration, perforation, obstruction, and bleeding have limited their therapeutic usage.

Table 5 : Reported anti-inflammatory results of extracts from different parts of *P. viridiflorum*

Extract	Enzyme	IC_{50}/ % inhibition	Place of collection	Reference
Acetone leaf	15-LOX	30 µg/ml	South Africa	Adebayo et al., 2015
50% MeOH leaf and twig stored	COX-1	32.5%	South Africa	Amoo et al., 2012
50% MeOH leaf and twig stored	COX-2	0%	South Africa	Amoo et al., 2012
50% MeOH leaf and twig fresh	COX-1	22.5%	South Africa	Amoo et al., 2012
50% MeOH leaf and twig fresh	COX-2	32.0%	South Africa	Amoo et al., 2012

MeOH = methanol, 15-LOX =, COX-1=, COX-2=, IC_{50}/ % = 50% inhibitory concentration/ percentage

The majority of the African population relies on plants for alleviating inflammation and its associated disorders, and *P. viridiflorum* finds use in managing these conditions as well. Reports of the anti-inflammatory activity of *P. viridiflorum* found in literature are recorded in Table 5. Cyclooxygenase (COX) and lipoxygenase (LOX) enzymes were used to verify the use of *P. viridiflorum* collected in KwaZulu-Natal as an anti-inflammatory plant in Africa by Amoo et al. (2013) and Adebayo et al. (2015) respectively. Anti-inflammatory activity of the leaf acetone extract of *P. viridiflorum* collected in Gauteng against 15-LOX showed potential with an IC_{50} value of 30 µg/ml but the activity was not as good as that of the positive control, quercetin, which had an IC_{50} value of 8.75 µg/ml (Adebayo et al., 2015). Leaves and twigs (50% MeOH) extracts demonstrated low anti-inflammatory activity when tested against

COX-1 and 2 enzymes with percentage inhibition ranging between 0-35.5% (Amoo et al., 2013).

In an *in vivo* study conducted in Cameroon on the antinociceptive effects of leaves aqueous and MeOH extracts of *P. viridiflorum* (synonym *P. mannii*) in mice, both extracts suppressed non-inflammatory and inflammatory phases, suggesting that they may interfere with neurogenic and inflammatory mediators in both phases (Wandji et al., 2016). Furthermore, in an analgesic study, pretreatment of mice with naloxone, atropine, and yohimbine significantly inhibited the antinociceptive effect of the aqueous extract, however, potentiating the activity of the leaf MeOH extract (Wandji et al., 2016). The inflammatory symptoms on mice induced by acetic acid, formalin, capsaicin, glutamate and hot plate were significantly reduced by oral administration of leaf aqueous and MeOH extracts (Wandji et al., 2016). Although roots and stem bark of *P. viridiflor*um are reported to be used against pain, there are no reports of the evaluation of their anti-inflammatory activity. Reports of the isolation of anti-inflammatory compounds from a *Pittosporum* species have been made (Mendes et al., 2013). Previously isolated compounds from the fruit of *P. undulatum* identified as 4-guaien-11-ol, 5-guaien-11-ol, and undulatumoside were reported to have anti-inflammatory activity towards a murine monocytic macrophage cell line that was greater than that of indomethacin (Mendes et al., 2013). No anti-inflammatory compounds have been isolated from *P. viridiflorum* and it appears that no attempts have been made to isolate them. However, oleanic acid **5** and β-amyrin acetate **7,** compounds found in the leaves of *P. viridiflorum*, have been reported to have anti-inflammatory potential (Pollier and Goosens, 2012), and the activity of this plant could be due to these compounds and/or other unknown compounds. Additionally, oleanic acid **5** may be useful in modulating an immune response, however further studies to confirm the immunomodulatory potential of this compound are required. Sabinene, found in the fruit oil of this species, demonstrated anti-inflammatory activity towards tumor necrosis factor, interleukin 1 (1L)-1β, and IL-6 (Valente et al., 2013).

4.2.2.7. Antioxidant

Oxidative stress has been identified as the main cause of life-threatening disease development and progression (Kasote et al., 2015). The presence of antioxidants at lower concentrations than the substrate prevents or significantly delays oxidation of oxidizable substrates (Gupta and Sharma, 2006). Plants have been a source of antioxidants for a long

Table 6: Reported antioxidant results of extracts from different parts of *P. viridiflorum*

Extract	Assay	antioxidant IC_{50}/ % values in	Place of plant collection	Reference
EtoAc bark	DPPH	177.74 µg/ml	Cameroon	Momeni et al.,2010
EtoAc bark	$(ABTS)^{+}$	331.48 µg/ml	Cameroon	Momeni et al.,2010
Acetone bark	DPPH	0.22 µg/ml	South Africa	Otang et al., 2012
Acetone bark	NO	0.16 µg/ml	South Africa	Otang et al., 2012
Acetone bark	(H_2O_2)	0.13 µg/ml	South Africa	Otang et al., 2012
50% MeOH leaf and twig stored	DPPH	17.9 µg/ml	South Africa	Amoo et al., 2012
50% MeOH leaf and twig fresh	DPPH	17.5 µg/ml	South Africa	Amoo et al., 2012
50% MeOH leaf and twig stored	β-carotene	62.9%	South Africa	Amoo et al., 2012
50% MeOH leaf and twig fresh	β-carotene	39.1%	South Africa	Amoo et al., 2012

EtoAc = ethyl acetate, MeOH = methanol, IC_{50} = 50% inhibitory concentration; DPPH = 1,1-diphenyl-2-picryl-hydrazyl, (ABTS) = 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid, NO = nitric oxide, (H_2O_2) = hydrogen peroxide

time, and it is believed that almost all plants of medicinal importance have excellent antioxidant potential (Kasote et al., 2015). At present, there are about 19 *in vitro* and 10 *in vivo* techniques commonly used for determining antioxidant potential of plants (Kasote et al., 2015). It is reported that in the majority of *in vitro* assays, plants and compounds demonstrate antioxidant potential, and this is credited to their innate ability to synthesise non-enzymatic antioxidants (ascorbic acid and glutathione) and secondary metabolites (phenolic compounds) (Kasote et al., 2015). However, only a few plant species and compounds tested *in vitro* have been confirmed *in vivo* for their antioxidant potential. The bark of *P. viridiflorum* has been tested in different antioxidant assays (Table 6). The ethyl acetate extract of the stem bark (synonym *P. mannii* from Cameroon) exhibited strong free radical scavenging activities with IC_{50} value of 177.74 µg/ml in the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay using Trolox as positive control (Momeni et al., 2010). The IC_{50} value of *P. viridiflorum* stem bark in the 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) $(ABTS)^{+}$ test using Trolox as a standard was 331.48 µg/ml, strongly scavenging $ABTS^{+}$ in a dose dependent manner (Momeni et al., 2010). The bark acetone extract of *P. viridiflorum* (collected in Eastern Cape, South Africa) showed antioxidant potential in the DPPH, nitric oxide (NO), and hydrogen peroxide (H_2O_2) radical scavenging assays, giving credence to the use of this plant in the management of opportunistic fungal infections (OFIs) in human immunodeficiency virus (HIV) positive people in South Africa (Otang et al, 2012). Amoo et al. (2012) reported that the antioxidant potential of stored (for 12 years) and fresh twig or leaf material was

insignificantly different in the DPPH assay = 93.6 and 93.2%, respectively, however, it was significantly different against β-carotene = 62.9 and 39.1% respectively. Flavonoids and phenolics have been detected from *P. viridiflorum* collected in different parts of Africa, and these secondary metabolites are known to possess antioxidant potential (Stanković, 2011). Their mechanisms of action are through scavenging and chelating process. Therefore, the antioxidant potential of this plant may be attributed to phenolics and flavonoids present in different plant parts. Although the bark and leaves of *P. viridiflorum* collected in different regions in Africa have demonstrated antioxidant activity, there are no known reports of the compounds from this plant with such potential, therefore such studies are still required.

Table 7: Reported toxicity results of extracts from different parts of *P. viridiflorum*

Extract	Test organism	IC_{50}/LD_{50}/ % mortality values	Assay	Place of collection	Reference
Acetone bark	*A. salina*	>1 mg/ml	MTT	South Africa	Otang et al. 2013
Acetone leaf	Chang liver cells	246.95 µg/ml	MTT	South Africa	Otang et al. 2014
Hexane leaf	Chang liver cells	225.50 µg/ml	MTT	South Africa	Otang et al. 2014
Acetone leaf	Monkey kidney vero cells	54.6 µg/ml	MTT	South Africa	Elisha et al., 2016
Water leaf	*A. salina*	2.44 mg/ml	Brine shrimp	Cameroon	Wandji et al., 2016
MeOH leaf	*A. salina*	0.70 mg/ml	Brine shrimp	Cameroon	Wandji et al., 2016
DCM bark	Human bladder carcinoma cells	16.9 µg/ml	Fluorimetric	Cameroon	Nyongbela et al., 2013
MeOH bark	Human bladder carcinoma cells	>90.0 µg/ml	Fluorimetric	Cameroon	Nyongbela et al., 2013
Compound **8**	Human bladder carcinoma cells	15.9 µg/ml	Fluorimetric	Cameroon	Nyongbela et al., 2013
MeOH leaf	*A. franciscana*	0% mortality	Nauplii lethality	South Africa	Cock and Van Vuuren, 2015
Water leaf	*A. franciscana*	0% mortality	Nauplii lethality	South Africa	Cock and Van Vuuren, 2015
MeOH leaf	E6 Vero cells	18.08 µg/ml	MTT	Kenya	Muthaura et al., 2007
Water leaf	E6 Vero cells	69.21 µg/ml	MTT	Kenya	Muthaura et al., 2007
Water leaf	Swiss female mice	1000 mg /kg	Acute toxicity	Kenya	Muthaura et al., 2007
Water leaf	Swiss female mice	5000 mg/kg	Acute toxicity	Kenya	Muthaura et al., 2007
MeOH leaf	Swiss female mice	100 mg/kg	Acute toxicity	Kenya	Muthaura et al., 2007

MeOH = methanol, DCM = dichloromethane, compound 8 = 1-0-{alpha-L-rhamnopyranosyl}-23-acetoxyimberbic acid 29-methyl ester, IC_{50} = 50% inhibitory concentration, LD_{50} = the amount of a toxic agent that is sufficient to kill 50% of a population of animals, MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

4.2.2.8. Toxicity

Despite the long-term use of plants in TM therapy, there are still many concerns associated with their safety for human use, particularly in the long term. Medicinal plant toxicity research has demonstrated that a significant number of plants that were claimed to be safe have toxic effects. *P. viridiflorum* is widely used in ATM therapy, therefore it would be of value to determine the toxicological effects of the use of this plant. *In vitro* and *in vivo* toxicity studies have been conducted by different researchers on *P. viridiflorum* (Table 7).

According to the United States plant screening program, a crude extract or compound is generally considered to have *in vitro* cytotoxicity if its IC_{50} value is less or equal to 20 µg/ml for the former and 4 µg/ml for the latter (Graidist et al., 2015). The bark acetone extract collected in Eastern Cape, South Africa was non-toxic in a brine shrimp (*Artemia salina*) assay with LC_{50} values greater than 1 mg/ml, and was recommended for further exploration (Otang et al., 2013). The hexane and acetone leaf extracts demonstrated relatively weak toxicity against Chang liver cells, thus implying that they have a relatively low toxicity risk with IC_{50} values of 246.95 (acetone) and 225.50 (hexane) µg/ml compared to griseofulvin (positive control) = 9.02 µg/ml (Otang et al., 2014). Again, the leaf acetone extract collected in Gauteng South Africa was slightly toxic against the monkey kidney Vero cell line with an IC_{50} value of 54.6 µg/ml, however, the toxicity was lower than that of doxorubicin (positive control) = 8.3 µg/ml (Elisha et al., 2016). The inconsistencies of the toxicity of *P. viridiflorum* could be a result of the plant collected in different geographical areas reflecting enormous chemical variation across its range. Therefore, to conduct further research on this species, geographical variation should be considered, otherwise irrelevant species are more likely to be explored. This could also be a result of sensitivity to different cell lines (Chang and Vero) used as cytotoxicity test cells in these studies. Both aqueous and MeOH leaf extracts (in Cameroon) were reported to be non-toxic to *Artemia salina* larvae with IC_{50} values of 2.44 and 0.70 mg/ml respectively (Wandji et al., 2016). Cytotoxicity testing of the DCM and MeOH bark extracts as well as 1-0-{alpha-L-rhamnopyranosyl}-23-acetoxyimberbic acid 29-methyl ester **8** against human bladder carcinoma cells revealed slight toxicity of the compound (IC_{50} = 15 µg/ml) (Nyongbela et al., 2013). Cytotoxicity in *in vitro* studies does not mean toxicity *in vivo*. Therefore, *in vivo* cytotoxity evaluation of this compound is required. Toxicity was evaluated against *Artemia franciscana* as a test organism, and the leaf MeOH and water extracts from South Africa demonstrated zero mortality after 24 and 48 h of exposure (Cock and van Vuuren, 2015). However, the same

extracts (leaf water and MeOH from a Kenyan *P. viridiflorum*) were cytotoxic against Vero E6 cells with IC_{50} values of 69.21 and 18.08 µg/ml respectively (Muthaura et al., 2007). This further confirms the point above that *in vitro* toxicity does not necessarily equate to *in vivo* toxicity. Comparing *P. viridiflorum* to other *Pittosporum* species, *P. ochrosiaefolium* Bojer was reported to be cytotoxic towards brine shrimp larvae with an IC_{50} value of 17.8 µg/ml (Wanyoike et al., 2004). This suggests that *P. viridiflorum* may have cytotoxic compounds, which need to be explored.

In an *in vivo* acute toxicity study, the leaf water extract of *P. viridiflorum* demonstrated toxicity in mice with an LD_{50} of 1 000 mg/kg body weight, but the same extract was non-toxic when administered orally in a single dose level up to 5 000 mg/kg (Muthaura et al., 2007). In the same study, mice treated with *P. viridiflorum* MeOH leaf extract at 100 mg/kg were reported to have died within 24 h, however lower doses of 5 mg/kg were less toxic (Muthaura et al., 2007). Therefore, the water extract was less toxic than the MeOH extract. The cytotoxicity of *P. viridiflorum* has been attributed to pittoviridoside **1**. However, toxicity studies of the stem bark and root extracts as well as compounds of *P. viridiflorum* are required, including cytotoxicity and genotoxicity to evaluate if pittoviridoside **1** or other compounds are responsible for its side effects.

5. Conclusions

P. viridiflorum is an important representative of the Pittosporaceae family used widely in ATM for treatment of various ailments and diseases. The use of this species in ATM is very broad, ranging from well-being to treatment of wounds, kidney complaints, circulatory and inflammatory ailments, as well as diseases such as cancer, tuberculosis and malaria. There is a large body of work documenting the antimicrobial activity of *P. viridiflorum* against microorganisms associated with human infections. The leaf and fruit oils have shown potent antimicrobial activity against pathogens associated with gastrointestinal diseases (*Escherichia coli* and *Staphylococcus aureus*), urinary tract (*Streptococcus faecalis* and *Escherichia coli coli*) and respiratory tract infections (*Staphylococcus aureus*). The organic leaf and aqueous extracts from this plant have demonstrated antimicrobial activity against skin disease, urinary tract and wound infections as well as vulvovaginal candidiasis associated microorganisms which include *Trichophyton mentagrophytes*, *Candida albicans*, *Propionibacterium acnes*, *Proteus mirabilis* and *Proteus vulgaris*. The organic bark extract from this plant has shown antibacterial activity against urinary tract and pneumonia associated strains such as

Pseudomonas aeroginosa and *Klebsiella pneumoniae*. However, according to available literature, there are no reports yet of antimicrobial compounds isolated from *P. viridiflorum*.

Antimalarial activity of *P. viridiflorum* against various strains of *Anopheles arabiensis* and *Plasmodium falciparum* has been reported. An antimalarial compound has been isolated from the MeOH stem bark, nonetheless, further antimalarial research against other strains that cause malaria is required using the DCM whole extract of this plant as this has shown promising antimalarial activity against a *Plasmodium* species.

Organic extracts from leaf and bark of *P. viridiflorum* have shown antioxidant activity in the DPPH, $(ABTS)^{+}$, (H_2O_2), NO and β-carotene assays, however, there is still a need to isolate and identify compounds from this species with such activity. *P. viridiflorum* is reported for use against ethnoveterinary infections, syphilis, tuberculosis, and ascariasis, however, scientific reports from the literature to validate such claims are still pending. *P viridiflorum* needs to be screened for anthelmintic and mycobacterial activity. There is also a need to explore anti-inflammatory activity of the bark, root and stem of this plant as these plant parts have not yet been tested for their potential, but are used for treating various inflammatory ailments in ATM.

Eight compounds have been isolated from *P. viridiflorum*, with one antimalarial (1-0-{alpha-L-rhamnopyranosyl}-23-acetoxyimberbic acid 29-methyl ester **8**), one anticancer (pittoviridoside **1**), and three acaricidal pentacyclic triterpenoids (**5**, **6**, **7**), thus giving credence to the use of this plant in ATM against malaria, cancer and acari. Although *P. viridiflorum* extracts have demonstrated antimicrobial, anti-inflammatory, antidiarrhoeal, and antioxidant effects, no compounds demonstrating these properties have yet been isolated from this plant, and therefore this is an area that should be explored further.

The crude leaf MeOH extract showed high *in vitro* cytotoxicity towards Vero cells and *in vivo* toxicity at 100 mg/kg, killing test organisms 2 hr after dosing, so compounds responsible for toxicity from this plant species need to be identified. More efforts are required to determine the toxicological effects of using *P. viridiflorum,* for example mutagenicity testing in the Ames and comet assays. These will go a long way towards maximising the potential and realising the potential dangers of *P. viridiflorum* as an important ATM plant of the Pittosporaceae. Various extracts from different parts of *P. viridiflorum* revealed potent antimicrobial, anticancer, antimalarial, antioxidant, anti-inflammatory, and antidiarrhoeal properties thus highlighting the species as an important medicinal plant used in ATM. Discrepancies have been noted in the presence of phytochemicals from *P. viridiflorum*

collected in different geographical regions, therefore, further studies on this plant should be conducted using material from the same region where it was initially collected.

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Author contributions

BM (blnglmadikizela@gmail.com) conceptualised the study, conducted the literature survey and wrote the paper. LJM (lyndy.mcgaw@up.ac.za) supervised the work, and revised and submitted the manuscript.

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