Traditional usage and biological activity of *Plectranthus madagascariensis* and its varieties: a review

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Abstract

Ethnopharmacological relevance: *Plectranthus madagascariensis* (Pers.) Benth. is an indigenous aromatic South African plant species that are traditionally used to treat various dermatological and respiratory ailments.

Aim of the study: Three varieties of *P. madagascariensis* exist in South Africa, namely, *Plectranthus aliciae* (Codd) van Jaarsv. & T.J. Edwards, *Plectranthus ramosior* (Benth.) Van Jaarsv. and *Plectranthus madagascariensis* (Pers.) Benth var. *madagascariensis*. This article summarizes the documented ethnobotanical uses and research which has been conducted to date on the chemical constituents and biological effects of *P. madagascariensis* and its varieties. This review aimed to investigate and highlight the lack scientific reports of the potential activity of these varieties based on their traditional usage and to emphasise the need for further investigation of the benefits of *P. madagascariensis* and its varieties.

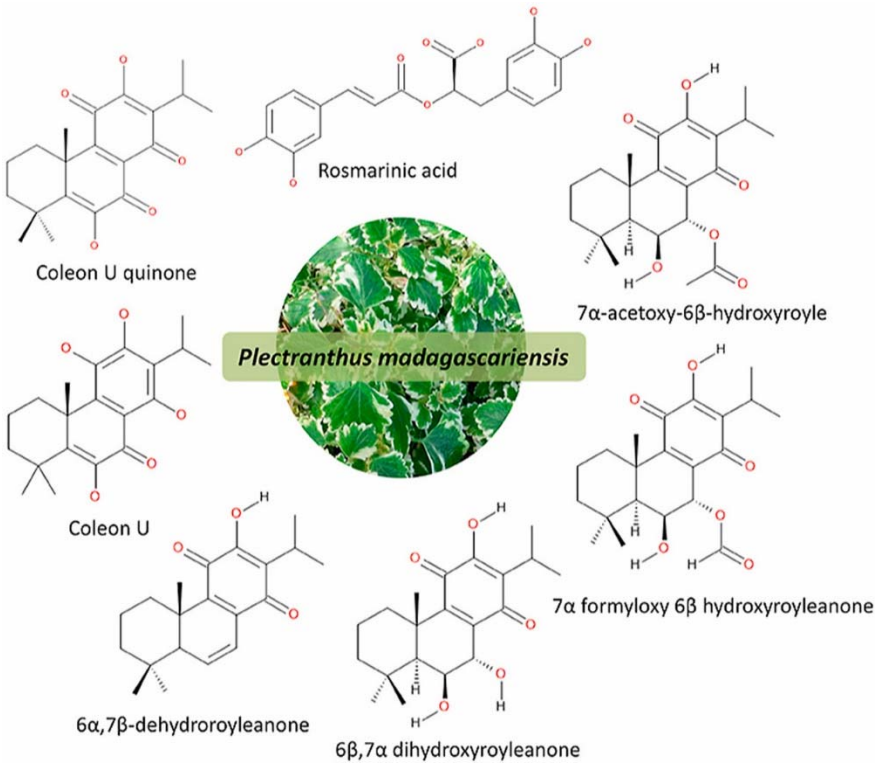
Materials and Methods: Extensive database retrieval using platforms not limited to but including Google Scholar, ScienceDirect and PubMed, was performed by using keywords such as "*Plectranthus madagascariensis*" "*Plectranthus madagascariensis* var. aliciae", "*Plectranthus* aliciae", "*Plectranthus ramosior*", "*Plectranthus madagascariensis* var. ramosior" and "*Plectranthus hirtus"* In addition, relevant books and digital documentation were consulted to collect all available scientific literature to provide a comprehensive review.

Results: Several studies have reported on the traditional usage of *P. madagascariensis* for the treatment of diseases related to the respiratory system such as coughs, colds and asthma and dermatological disorders related to wounds and inflammation. No records were found on the traditional usage of *P. madagascariensis* varieties to treat other maladies, however, *P. ramosior* has been reported to be used as a toxin for fishing. In literature, seven major phytochemical compounds have been identified in *P. madagascariensis*. Its extract and essential oil have been reported to have polyphenols, abietane diterpenes and abietane diterpenes with a quinone moiety as constituents. *Plectranthus madagascariensis* and its major phytochemicals have been reported to target various biological targets. The report on the antibacterial activity of *P. madagascariensis* against tuberculosis and wound infections has been consistent and correlates with its documented traditional usage of the plant. Literature has been found on the antibacterial activity of *P. aliciae* targeting bacteria associated with wound infections and lung cancer cells. No further literature on the biological activity of the other *P. madagascariensis* varieties has been found. Other noteworthy

biological activities reported in the literature of *P. madagascariensis* and identified phytochemicals include their activities against Alzheimer's disease and cancer, especially against breast cancer and this has not been linked to the traditional usage of the plant.

Conclusion: *Plectranthus madagascariensis* and its compounds have been proven to be effective in treating a range of maladies. Based on the extensive literature on this plant, it can be concluded that numerous *in vitro* pharmacological activities of *P. madagascariensis* have been reported. However, there is a lack of information available for this species with regards to its *in vivo* data including both pre-clinical and clinical studies. Since the extract of *P. madagascariensis* and its isolated compounds have displayed noteworthy anticancer potential, we recommend further investigation of pharmacokinetic studies to be included in future research.

Graphical abstract



Keywords*: Plectranthus madagascariensis*, *Plectranthus madagascariensis* var. aliciae, *Plectranthus aliciae*, *Plectranthus madagascariensis* var. madagascariensis, *Plectranthus hirtus*, Lamiaceae, Ethnobotanical use, Phytochemicals, Biological activity

1. Introduction

Plectranthus madagascariensis (Pers.) Benth. is a semi-succulent, aromatic, ground cover plant belonging to the Lamiaceae family. It grows up to one meter high and is widely distributed in KwaZulu-Natal and the Eastern Cape where it grows in dry rocky outcrops, forest margins and shaded subtropical thickets. The oppositely arranged oval leaves are distinctively variegated with white margins that are toothed at the edges (three to seven teeth). The leaves are slightly hairy above and below the blade that is between 35-40 mm long. The inflorescence is a 125 mm erect raceme with four to six flowers at each node. Between February and November small white, mauve or purple tubular flowers between seven and 18 mm long emerge. The small seeds are 1 mm in diameter. The genus largely relies on pollination by various fly species such the *Stenobasipteron* spp and bee species such as *Pachymelus limbatus* and *Amegilla caelestina*. Studies have confirmed that butterflies rarely pollinate *P. madagascariensis* and that the species is not dependant on this relationship (Harrower, 2014; Latti, 2019; Potgieter et al., 1999; Random Harvest, 2020; Van Jaarsveld and Edwards, 1997).

It is difficult to distinguish between *Plectranthus* species and its closely related species due to similarities in morphological features that can be identified between the various species. This has resulted in taxonomic difficulties related to the naming of various species belonging to the *Plectranthus* genera. In South Africa, *Plectranthus madagascariensis* (Pers.) Benth var. *madagascariensis*, syn. *Plectranthus hirtus* Benth. is classified as an indigenous South African plant and a variety of *P. madagascariensis* (Pers.) Benth from which *P. madagascariensis* 'Lynne' is a well-known cultivar. The endemic *Plectranthus aliciae* (Codd) van Jaarsv. & T.J. Edwards (syn. *Plectranthus madagascariensis* var. *aliciae* Codd) and *Plectranthus ramosior* (Benth.) Van Jaarsv., (syn. *Plectranthus madagascariensis* var. *ramosior* Benth its synonym *Plectranthus hadiensis* (Forssk.) Schweinf. ex Sprenger and *Plectranthus madagascariensis* (Pers.) Benth.) are considered varieties of *P. madagascariensis*. Although substantial literature on *Plectranthus madagascariensis* is available, articles documenting the differences and similarities amongst the three varieties of *P. madagascariensis* are in limited supply. Morphological similarities between these plants could potentially contribute to the misidentification of the plant varieties, resulting in literature incorrectly referencing *P. madagascariensis* (Codd, 1975; Foden and Potter, 2005a, 2005b; Lukhoba et al., 2006; Matlamela and Kamundi, 2006; The Plant List, 2012).

This study summarizes and reviews the documented ethnobotanical uses and research that had been conducted to date on the chemical constituents and biological effects of *P. madagascariensis* and its varieties.

1.1.Traditional medicinal uses

Rice et al. (2011) reported that *Plectranthus* species from southern Africa is traditionally used to treat 10 out of 13 disease hallmarks as reported by Cook (1995). *Plectranthus* species are traditionally used to treat diseases associated with the respiratory system, the central nervous system, the skin, the digestive system, the liver and is also used in the treatment of infections, pain, inflammation, fever and cancer (Matias et al., 2019b). Lukhoba et al. (2006) reported the traditional and ethnobotanical use of 62 *Plectranthus* species in Africa, America, Asia, Australasia and the pacific. The top three traditional uses of *Plectranthus* species was for digestive, respiratory and skin diseases. Other reported traditional uses of *Plectranthus* species include pain, inflammation, infections and fevers, muscle contractions, neurological disorders and blood circulation. *Plectranthus* species are divided into two clades namely Clade 1 (formerly related to the *Coleus* genus) that are known for their medicinal potential and Clade 2 (*Plectranthus* Clade). Three hundred *Plectranthus* species are found in Africa of which 50 can be found in southern Africa and Madagascar, 25% of which are from Clade 1. Twelve *Plectranthus* species are traditionally used in southern Africa. However, synonyms are highly prevalent in the genus making it extremely difficult to gather and combine information on the ethnobotanical use of the genus (Lukhoba et al., 2006; RiceRice et al., 2011). It is, therefore, possible that certain traditional knowledge has been lost or that information was incorrectly reported for a specific *Plectranthus* species. The possibility should not be excluded that reported traditional uses of *P. madagascariensis* are the only traditional usage of the species and the traditional usage of the synonymous species and their varieties should also be taken into consideration.

Traditionally *P. madagascariensis* is used by the Zulu and Xhosa communities to treat respiratory and dermatological disorders. For respiratory problems such as coughs, colds and asthma, the whole plant including the roots are used and administered either as an enema or a decoction (plant material is boiled with water) and an infusion (boiling water is added to the plant material) that can be administered orally. Cutaneous wounds and scabies are treated with crushed leaves applied directly to the affected area (Hutchings et al., 1996; Pereira et al., 2015; Rabe and van Staden,

1998; Rice et al., 2011). Mahomoodally and Priyamka (2014) reported the traditional usage of *P. madagascariensis* as a popular folk medicine in Mauritius. The juice extract from the leaves are warmed with honey and lemon juice and administered orally twice a day to treat cough, flu, bronchitis and asthma. It is believed that the Egyptians made a perfume called Kyphi that is translated to "welcome to the gods" that contained *P. madagascariensis* and was used to induce hypnotic states (González-Minero and Bravo-Díaz, 2018). *Plectranthus hadiensis*, a synonym of *P. ramosior* is used as a poison for fishing (Rice et al., 2011).

Taking the top three traditional uses of *Plectranthus* species into consideration namely digestive, respiratory and skin disorders only two of these uses have been reported for *P. madagascariensis*. Although not reported as a traditional usage, further investigations of *P. madagascariensis* and its varieties on digestive disorders are recommended. Furthermore, *P. ramosior* previously classified as *P. madagascariensis* var. ramosior and a synonym for *P. hadiensis* could potentially be traditionally used as a poison, therefore, toxicity studies are extremely important when conducting research on *P. madagascariensis* and its varieties.

2. Methodology

Extensive database retrieval for ethnobotanical uses, biological activities reported for *P. madagascariensis*, its varieties and secondary metabolites using platforms not limited to but including Google Scholar, ScienceDirect and PubMed up to June 2020 was performed. In addition, several PhD and MSc dissertations were also reviewed. Many books were consulted on the traditional usage of *Plectranthus* species and *P. madagascariensis* in South Africa. The keywords used in the search engines regarding plants were "*Plectranthus madagascariensis*" "*Plectranthus madagascariensis* var. aliciae", "*Plectranthus* aliciae", "*Plectranthus ramosior*", "*Plectranthus madagascariensis* var. ramosior", "*Plectranthus hirtus"* and "*Coleus madagascariensis*". In addition, a separate search was performed on the secondary metabolites identified in *P. madagascariensis* using the keywords "*Plectranthus* compounds", "*Plectranthus madagascariensis* compounds", "rosmarinic acid", "coleon U", "coleon U quinone", "6β,7α-dihydroxyroyleanone", "7α-formyloxy-6β-hydroxyroyleanone" and "7α-acetoxy-6β hydroxyroyleanone". No criteria were excluded from the search engines.

The plant names were confirmed using the South African National Biodiversity Institute's Red List of South African Plants (http://redlist.sanbi.org/), PlantZAfrica (http://pza.sanbi.org/), The Plant List (www.theplantlist.org) and Kew science (https://mpns.science.kew.org/mpns-portal/).

3. Phytochemistry

Phytochemical studies on some *Plectranthus* species have confirmed high concentrations of di- and tri-terpenes in these plant species. Reports have indicated that *Plectranthus* species commonly contain abietane diterpenes of the coleon and royleanone type as well abietanoid quinone methides with significant cytotoxic and antiproliferative activity (Matias et al., 2019a, 2019b; Pereira et al., 2015).

Five major components have been isolated from the methanolic extract of *P. madagascariensis*, namely the polyphenol, rosmarinic acid (A) and the abietane diterpenes coleon U (B), 6β,7α-dihydroxyroyleanone (C), 7α-formyloxy-6β-hydroxyroyleanone (D) and 7α-acetoxy-6β hydroxyroyleanone (E) (Figure 1). The extract of *P. madagascariensis* also contained coleon U quinone (F) as the main component in addition to the above-mentioned compounds. It is believed that coleon U quinone is the oxidised form of coleon U (Figure 1) (Garcia et al., 2019b; Kubínová et al., 2014; Matias et al., 2019a, 2019b).

The Lamiaceae family consists of various genera, such as *Mentha* (mint), *Ocimum* (basil) and *Salvia* (sage) which are known for their aromatic characteristics. The essential oil of *P. madagascariensis* is rich in 6,7-dehydroroyleanone (DHR) (Figure 1, G), an abietane diterpene with a quinone moiety (Pereira et al., 2015; Sitarek et al., 2020). Garcia et al. (2018) was successful in isolating and identifying DHR from *P. madagascariensis*. The leaves were subjected to hydrodistillation using a Clevenger apparatus and high-pressure extraction. The highest yield was confirmed for hydrodistillation with a yield of 18.55±2.00% (% DHR weight/hydrodistillate extracts weight). DHR was isolated from the essential oil through flash chromatography with a yield of 20.4% (w/w). Nuclear magnetic resonance (NMR) was carried out and the NMR spectrum was compared with literature to structurally identify DHR.

A total oil yield of 0.1% v/w was isolated from the leaves of *P. madagascariensis* through hydrodistillation using a Clevenger apparatus (Ascensao et al., 1998). Gas chromatography-mass

spectrometry (GC-MS) analysis done by Ascensao et al. (1998) determined that 86.6% of the essential oil from the leaves are composed of DHR.

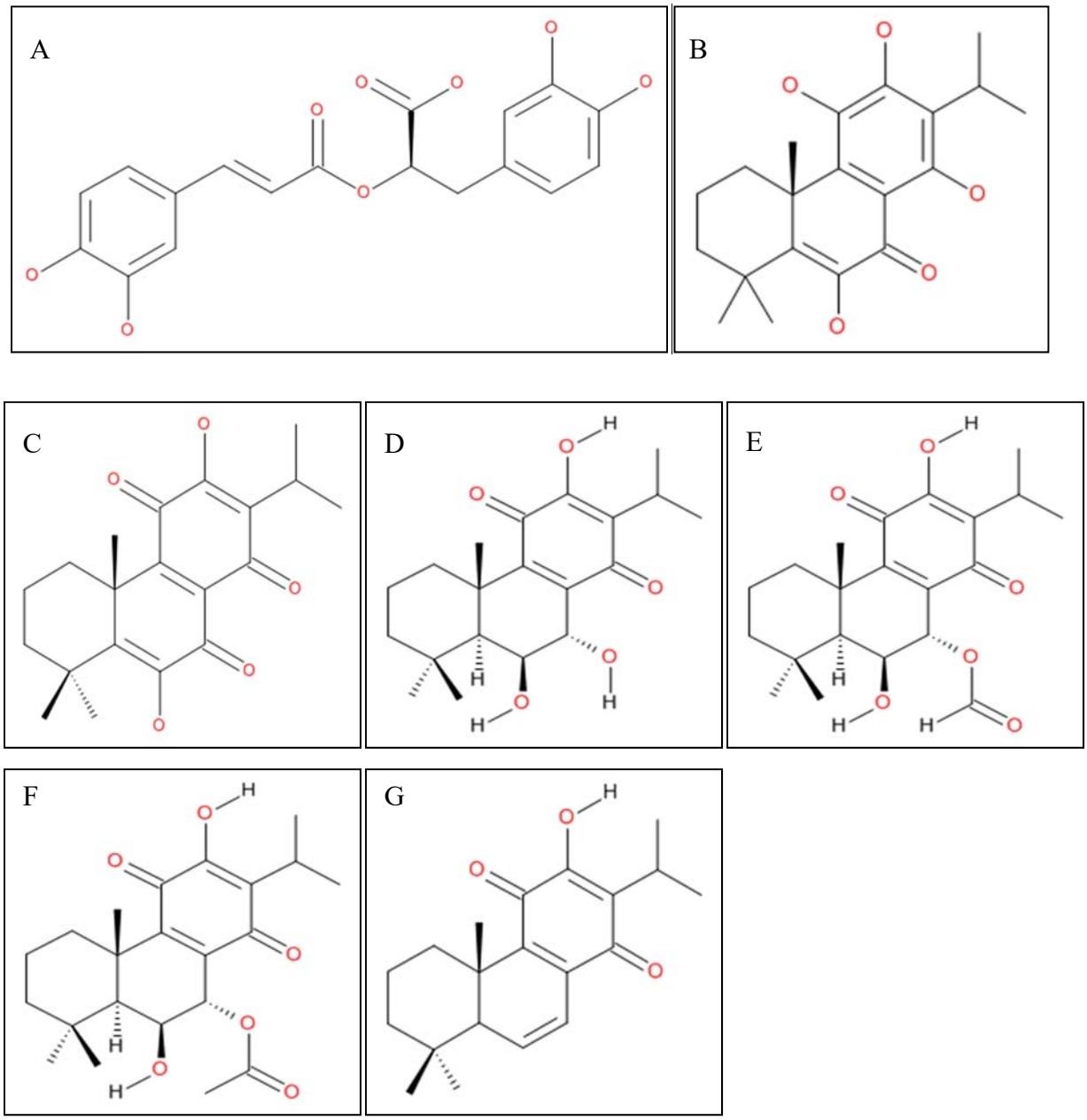


Figure 1: Chemical structures of compounds isolated from *Plectranthus madagascariensis*. (A) Rosmarinic acid, (B) Coleon U, (C) 6β,7α-dihydroxyroyleanone, (D) 7α-formyloxy-6β-hydroxyroyleanone, (E) 7α-acetoxy-6β-hydroxyroyleanone, (F) Coleon U quinone, (G) 6,7-dehydroroyleanone

4. Biological activity of the extracts and secondary metabolites

Plants from the Lamiaceae family are well known for their antibacterial activity and are a promising source for new antibacterial treatments. Several species from the *Plectranthus* genus

have been confirmed to treat several diseases that are related to their use in traditional medicine. The biological activity of *P. madagascariensis* and its varieties are documented in the following section.

4.1 Antimicrobial and antimycobacterial activity

Several *Plectranthus* species including *P. madagascariensis* are used in traditional medicine to treat various respiratory diseases such as asthma, bronchitis and coughs. The *Plectranthus* species; *P. barbatus* and *P. bojeri* have demonstrated to be effective for treating pneumonia. Part of these effects could be due to the antibacterial activity of the Lamiaceae family and *Plectranthus* genus. *Plectranthus amboinicus* has previously been confirmed to have antibacterial activity against *Mycobacterium tuberculosis*, a bacteria associated with tuberculosis. (Lukhoba et al., 2006; Nguta et al., 2016; Rijo et al., 2010).

Pereira et al. (2015) and Matias et al. (2019a) investigated the antimicrobial activity of *P. madagascariensis* extract using various methods. The acetone extract was found to be significantly active against *Bacillus subtilis,* methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus epidermidis* associated with skin infections, wounds and *Mycobacterium smegmatis*, a model bacterium for tuberculosis activity. Minimum inhibitory concentration (MIC) values of *P. madagascariensis* against *B. subtilis, M. smegmatis, S. aureus* and *S. epidermidis* was confirmed to be 3.91µg/mL, 31.25 µg/mL, 3.91 µg/mL and 7.81 µg/mL respectively. The positive controls vancomycin, amphotericin B and norfloxacin were included in the study, however, the MIC values were not provided in this article.

In a similar study, the acetone extracts of *P. madagascariensis* 'Lynne' and *P. aliciae* were tested to determine their MIC and minimum bactericidal concentration (MBC). The activity was determined against two Gram-positive bacteria, *Enterococcus faecalis* (ATCC 29212) and *S. aureus* (ATCC 25923). *Plectranthus madagascariensis* 'Lynne' displayed MIC values of 15.6 µg/mL and 125 µg/mL against *E. faecalis* and *S. aureus* respectively. On the other hand, *P. aliciae* had the same MIC value against *E. faecalis* but was slightly more active against *S. aureus* with a MIC of 62.5 µg/mL. Both extracts had a minimum bactericidal concentration (MBC) of 125 µg/mL against *S. aureus* and *P. aliciae* against *E. faecalis*. No MBC was observed for *P. madagascariensis* 'Lynne' against *E. faecalis* at the highest concentration tested (125 µg/mL)

(Garcia et al., 2019a). An aqueous extract of *P. madagascariensis* prepared using microwave extraction was confirmed to have an MIC value of 40 µg/mL against *S. epidermidis* (Rijo et al., 2014b).

Furthermore, the acetone extract of *P. madagascariensis* was confirmed to inhibit the Gram-negative bacteria, *Pseudomonas syringae* and *Klebsiella pneumoniae* at MIC values of 40 µg/mL and 3.91 µg/mL respectively. The acetone extract did not inhibit the yeasts *Candida albicans* and *Saccharomyces cerevisiae* (Matias et al., 2019a; Wellsow et al., 2006).

Kubínová et al. (2014) identified four compounds present in an extract of *P. madagascariensis* and investigated the antibacterial activity of these compounds. The four compounds identified were coleon U quinone, 7α-acetoxy-6β-hydroxyroyleanone, 6β,7α-dihydroxyroyleanone and rosmarinic acid. No antibacterial activity was found for rosmarinic acid against the Gram-positive bacteria *E. faecalis* (ATCC 29212) and *S. aureus* (ATCC 29213) and the Gram-negative bacteria *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). Noteworthy antibacterial activity was reported for coleon U quinone against *S. aureus* and *E. faecalis* with a MIC of 0.5 µg/mL and 8 µg/mL respectively. Coleon U has shown antibacterial activity against *B. subtilis* (IMI347329) and *P. syringae* (IMI347448) with MIC values of 3.13 µg/mL and 6.25 µg/mL respectively. The oxidised form of coleon U, coleon U quinone, inhibited the growth of *B. subtilis* at an MIC value of 25 µg/mL and *P. syringae* at an MIC value of 3.12 µg/mL (Wellsow et al., 2006). Antimicrobial reports on coleon U isolated from *Plectranthus grandidentatus* displayed MIC values of 0.98 µg/mL and 31.25 µg/mL against MRSA and vancomycin-resistant *E. faecalis* respectively (González, 2015).

Kubínová et al. (2014) reported moderate antibacterial activity for 6β,7α dihydroxyroyleanone and 7α-acetoxy-6β-hydroxyroyleanone against *S. aureus* and *E. faecalis* with MIC values ranging between 16 µg/mL and 32 µg/mL. No antibacterial activity was found against the Gram-negative bacteria, *E. coli* and *P. aeruginosa*, for any of the compounds.

The antibacterial activity of compound; 7α-acetoxy-6β-hydroxyroyleanone was investigated against methicillin-sensitive *S. aureus* ATCC 25924, ATCC 43866 and ATCC 700699 (MSSA) and MRSA strains CIP 106760 and FFHB 29593. MIC values between 15.63 µg/mL and 31.25 µg/mL were observed against MSSA and 7.81 µg/mL against MRSA strains. Antibacterial

activity against vancomycin-sensitive *E. faecalis* FFHB 427483, *E. casseliflavus* ATCC 49996, *E. faecium* FFHB 435628 and low-level vancomycin-resistant *E. faecalis* ATCC 51299 (VRE) at MIC values between 7.81 µg/mL and 15.63 µg/mL was observed (Rijo et al., 2014a).

The compounds 6β,7α-dihydroxyroyleanone, 7α-acetoxy-6β-hydroxyroyleanone and 6,7-dehydroroyleanone were investigated for their anti-mycobacterial activity against two mycobacterial strains namely the *M. tuberculosis* strain H37Rv (ATCC 27294) and the MDR clinical isolate, strain 02TBDM039EP097. Furthermore, the cytotoxicity of the compounds on African green monkey kidney epithelial cells (Vero) and mouse embryonic fibroblast cells (3T3) was evaluated. Compound 7α-acetoxy-6β-hydroxyroyleanone exhibited noteworthy anti-mycobacterial activity against both bacterial strains with a MIC value of 25 µg/mL against H37Rv and 3.12 µg/mL against MDR. The compound 7α-acetoxy-6β-hydroxyroyleanone was found to be more active against the MDR-*M. tuberculosis* strain than the anti-tuberculosis drugs rifampicin and isoniazid with MIC values of 16 mg/ml and 4 mg/ml respectively. No anti-mycobacterial activity was observed for 6β,7α-dihydroxyroyleanone and 6,7-dehydroroyleanone against H37Rv at the highest concentration tested of 25 µg/mL. However, 6β,7α-dihydroxyroyleanone inhibited MDR at a MIC of 12.5 µg/mL and 6,7-dehydroroyleanone at a MIC below 12.5 µg/mL. The study hypothesised that the noteworthy anti-mycobacterial activity against the MDR-*M. tuberculosis strain* was due to the 7α-AcO group at the B ring of the abovementioned compounds (Rijo et al., 2010).

The cytotoxicity of 7α-acetoxy-6β-hydroxyroyleanone was confirmed against 3T3 and Vero cell lines. At a 50% growth inhibition concentration (GI_{50}) of 12.96 µg/mL and 12.80 µg/mL, 7α-acetoxy-6β-hydroxyroyleanone inhibited the growth of 3T3 and Vero cell lines respectively, revealing significant cytotoxicity. However, the compound was confirmed to be selective towards the MDR strain versus both cells lines, with selectivity indexes of 3.2 against 3T3 and 6.62 against Vero cell lines (Rijo et al., 2010). The selectivity index is a ratio calculation to determine if a test substance is more targeted towards one biological target in comparison to another. A selectivity index is calculated by dividing the IC_{50} of one target with another. A selectivity index greater than one is an indication that the sample is more targeted towards the denominator biological target (Peña-Morán et al., 2016). Therefore, although 7α-acetoxy-6β-hydroxyroyleanone was

significantly cytotoxic on the tested cell lines the compound is more targeted towards inhibiting the MDR strain.

The essential oil of *P. madagascariensis* was tested for its growth inhibitory potential against several Gram-positive and Gram-negative bacteria that included *Micrococcus* species, *B. subtilis*, *S. aureus* and *Yersinia enterocolitica*. The essential oil was confirmed to be most active towards the Gram-positive bacteria *S. aureus* and the *Micrococcus* sp. with inhibition zones of 7.5 mm and 12.5 mm respectively when 10 µL of the essential oil was tested using the agar-diffusion assay (Ascensao et al., 1998).

In a study on DHR isolated from the essential oil of *Tetradenia riparia*, DHR was confirmed to inhibit *M. tuberculosis* H37R together with antibiotic-resistant and susceptible isolates at an MIC value of 31.2 µg/mL. Cytotoxicity studies on murine macrophages confirmed that DHR was selective towards targeting the bacteria with a selectivity index of 7.9. The authors noted that DHR could be a potential anti-tuberculosis drug candidate (Baldin et al., 2018).

Lipid-based drug delivery systems such as phytosomes are attractive nanocarrier systems to enhance the delivery and availability of low-soluble drugs to the target site. These systems encapsulate both hydrophilic (molecules that dissolve in gastrointestinal fluids and blood) and lipophilic (molecules that cross biological membranes) molecules and prevent premature degradation in the body (Danaei et al., 2018; Matias, 2016). In a study by Matias (2016), phytosomes (PS) and chitosan-coated phytosomes (ChiPS) were prepared from the acetone extract of *P. madagascariensis*. Chitosan is a polysaccharide with proven bacteriostatic activity. Particles with a diameter of 191.3±75.3 nm and 1082±363 nm and polydispersity index (PDI) of 0.243±0.18 and 0.22±0.10 for PS and ChiPS were formed (Matias et al., 2015). The PDI is an indication of the size distribution of particles in a given sample. For pharmaceutical applications, a PDI of 0.3 and less is desired (Danaei et al., 2018). The particle size for PS fell within the ideal range for transdermal drug delivery systems. Matias (2016) set out to determine the *in vitro* antibacterial activity and cytotoxicity of PS and ChiPS and *in vivo* irritancy potential of ChiPS. At a concentration of 1 mg/mL, ChiPS displayed a zone of inhibition of 17 mm against *S. epidermidis* (ATCC 12228) that was comparable to the inhibition of the pure compound 6β, 7α-dihydroxyroyleanone, *P. madagascariensis* extract and the positive control vancomycin with

inhibition diameters of 19 mm, 21 mm and 22 mm respectively. The chitosan control (not loaded with *P. madagascariensis* extract) displayed an inhibition zone of 8 mm. This indicates that the antibacterial activity of chitosan-coated phytosomes is increased with the addition of *P. madagascariensis* to concentrations comparable to the extract and positive control. The antibacterial activity of PS and ChiPS was tested against *S. aureus* (ATCC 25923), MRSA (CIP 106760) and *S. epidermidis* (ATCC 12228) using the broth microdilution assay against the extract and positive control vancomycin.The phytosomes (PS) displayed no increase in antibacterial activity compared to the extract. The chitosan coated phytosomes (ChiPS) displayed a fourfold increase in antibacterial activity against *S. aureus* (ATCC 25923). However, no control for chitosan phytosomes (not loaded with the extract) was included in this study. Therefore, it is unclear if the antibacterial activity observed was because of the synergistic activity of chitosan and *P. madagascariensis* as seen in the zone inhibition study or if the activity was due to the known antibacterial activity of chitosan.

4.2 Anti-cancer activity

Several *Plectranthus* species have demonstrated noteworthy anti-cancer and antitumor activity. *Plectranthus amboinicus, P. hadiensis* and *P. barbatus* are known to have cytotoxic effects on cancer cells. The *Plectranthus madagascariensis* acetone extract was investigated for its cytotoxic effect against several cancer cell lines. At a concentration of 15 µg/mL, the acetone extract of *P. madagascariensis* inhibited 20.13% of breast cancer cell growth (MDA-MB-231). The IC_{50} of the acetone extract was determined to be 64.52 µg/mL and is considered to have low cytotoxic activity (Matias et al., 2019a).

The cytotoxic activity of the acetone extracts of *P. madagascariensis* 'Lynne' and *P. aliciae* against three types of cancer including human colon (HCT116), non-small cell lung carcinoma (NCI-H460) and breast adenocarcinoma (MCF-7) was determined. *Plectranthus madagascariensis* 'Lynne' displayed GI_{50} values of 3.47±0.15 µg/mL and 5.39±0.48 µg/mL against MCF-7 and NCI-H460 cell lines respectively and a GI_{50} value between 5 µg/mL and 10 µg/mL against HCT116. *Plectranthus aliciae* was active towards HCT116, MCF-7 and NCI-H460 between 15 µg/mL and 20 µg/mL (Garcia et al., 2019a). The acetone extracts of *P.*

madagascariensis and *P. aliciae* are considered to have significant cytotoxic activity against HCT116, NCI-H460 and MCF-7 cell lines.

A study by Brito et al. (2018) on the cytotoxic activity of *P. madagascariensis* revealed that the aqueous extract inhibited cell growth at IC_{50} values of 1.7±0.3 mg/mL against MCF-7 cells and 1.5±0.2 mg/mL against human liver cancer cells (HepG2) cells and is considered to be non-cytotoxic.

Anti-cancer studies done by Matias et al. (2019b) determined that the two abietane diterpenes 6β,7α-dihydroxyroyleanone and 7α-acetoxy-6β-hydroxyroyleanone were effective and selective towards the lung cancer cell line NCI-H460 with cytostatic activity at 25.0±2.0 μM and 3.1±0.4 μM respectively. Matias et al. (2019b) hypothesized that the presence of lipophilic substituents at positions 6 and 7 of abietane diterpenes are required for cytotoxic activity.

The anticancer activity of 6β,7α-dihydroxyroyleanone, 7α-formyloxy-6β-hydroxyroyleanone, 7α-acetoxy-6β-hydroxyroyleanone and coleon U isolated from *P. madagascariensis* against breast cancer cell lines (MDA-MB-231, MCF-7) and a colon cancer cell line (HCT116) was determined. Although none of the compounds affected the growth of MDA-MB-231, 6β,7α-dihydroxyroyleanone, 7α-formyloxy-6β-hydroxyroyleanone, 7α-acetoxy-6β-hydroxyroyleanone and coleon U inhibited MCF-7 cells at a GI_{50} of 26.0±0.6 μM, 7.9±0.8 μM, 6.4±0.4 μM and 5.5±0.8 μM respectively. The positive control doxorubicin inhibited MCF-7 growth at a GI_{50} value of 0.16±0.0018 μM. The compound 7α-formyloxy-6β-hydroxyroyleanone inhibited the growth of the colon cancer cell line HCT116 at a GI_{50} of 7.9±1.2 μM compared to the positive control doxorubicin with a GI_{50} of 0.125±0.0013 μM (Matias et al., 2019b)

Anti-cancer studies by Sitarek et al. (2020) on human primary H7PX glioma cells found that 7α-acetoxy-6β-hydroxyroyleanone inhibited cell growth at an IC_{50} below 25 μg/mL. At the highest concentration of 100 μg/mL, 6β,7α-dihydroxyroyleanone inhibited 20% of H7PX glioma cell growth.

The compound 6,7-dehydroroyleanone isolated from the essential oil of *P. madagascariensis* was found to inhibit cell growth of human cervix carcinoma cell line (KB-3-1) at an IC_{50} value of 30

μM compared to the positive controls cryptophycin-52 with an IC_{50} value of 1.3×10^{-5} μM and griseofulvin with an IC_{50} value of 19 μM (Abdissa et al., 2017; Garcia et al., 2019b).

Rosmarinic acid was confirmed to inhibit the growth of MCF-7 and HepG2 (human liver cancer cell line) cells at IC_{50} values of 0.5±0.1 mg/mL and 3.4±0.9 mg/mL respectively (Brito et al., 2018).

The compound DHR induced apoptosis in primary H7PX glioma cells. Apoptosis is a form of cell death in which cells undergo morphological changes, resulting in cell death. Current cancer therapies such as chemo-and-radiotherapy induce apoptosis in cancer cells. In addition, DHR was found to induce G2/M cell cycle arrest and increase H2A.X phosphorylation (Sitarek et al., 2020).

Anti-cancer studies conducted by Garcia et al. (2018) revealed the cytotoxic activity of DHR against human lymphoid leukaemia cells (MOLT-3) and the human tumour cell line HL-60. The IC_{50} value obtained for DHR cytotoxicity against MOLT-3 cells were 5.4±0.3 μM and 4.46 μM against HL-60 cells as determined by Kusumoto et al. (2014). These results were compared to the cytotoxicity of a standard therapeutic drug, etoposide, with an IC_{50} value of 0.3±0.1 μM and 0.4±0.1 μM on MOLT-3 and HL-60 respectively. Moderate activity was observed against non-small-cell lung carcinoma cells A549, NCI-H460 and NCI-H460/R and with IC_{50} values of 30 μM, 14 μM and 11 μM respectively. In addition, DHR was tested for its cytotoxicity against human embryonal bronchial epithelial cells MRC-5 with an IC_{50} value of 24 μM, indicating that DHR is slightly more selective towards killing cancer cells without inducing toxic effects on normal cells. Furthermore, Garcia et al. (2018) set out to discover the mechanism of action by which DHR inhibit cell proliferation. They determined that DHR does not target microtubules, but was able to evade P-glycoprotein mediated mechanisms of resistance at 5 μM compared to Dex-VER tested at 5 μM, a known P-glycoprotein inhibitor in NCI-H460/R cells. These results support the data reported by Sitarek et al. (2020) which confirmed the ability of DHR to activate caspases-3 and -9, triggering apoptosis and the release of pro-apoptotic factors involved in activating the apoptotic pathway.

Due to their chemical composition, DHR and 7α-acetoxy-6β-hydroxyroyleanone (Figure 1) have been identified as attractive lead compounds for derivatization (Isca et al., 2020). Derivatives of these compounds were investigated for enhanced cytotoxic activity and targeting the P-glycoprotein using molecular docking. P-glycoproteins is involved in the transfer of drugs out of

the cell, enabling cancer cells to resist lethal doses of cytotoxic drugs resulting in multidrug-resistant cancer. The molecular docking study on the interaction between the above-mentioned compound derivatives and P-glycoprotein revealed that the presence of aromatic moieties increased the binding affinities of the compounds to the protein. The molecular docking studies confirmed that one benzoyloxy moiety is needed at position C-6 and chemical moieties smaller than unsubstituted benzoyl rings at position C-12 to effectively bind to the M-site of the protein pump (Isca et al., 2020).

The use of gold for the synthesis of nanostructured materials is increasing. This is due to the relatively low toxicity to biological systems, optical properties, large service area-to-volume ratio and conformational flexibility of gold, making it ideal for nanoparticle formation (De Freitas et al., 2018).

Garcia et al. (2018) prepared hybrid nanoparticles to improve the cytotoxic activity and targeted delivery of DHR. Nanoparticles were prepared from a 20 μM acetone solution of DHR. In a 1:1 (v/v) ratio, the acetonic DHR solution was mixed with hybrid polymeric-gold nanoparticle solution and was stirred at 800 rpm for 24 h in the dark. The nanoparticles were characterized by dynamic light scattering (DLS), measuring the PDI and diameter of the nanoparticles. A PDI value of 0.1 and a particle diameter of 281.1 nm was detected, indicating narrow size distribution (PDI) ideal for pharmaceutical applications (Li and Mattei, 2019). On the other hand, the DHR-conjugated hybrid nanoparticles were characterized through atomic force microscopy (AFM) and were confirmed to be small spherical particles with a size of 19.05±1.77 nm. HPLC-DAD determined a 98.57±0.23% conjugation efficiency of the DHR nanosystem.

The anti-cancer potential of DHR hybrid nanoparticles was assessed on human non-small cell lung carcinoma (NCI-H460) and multidrug-resistant lung cancer cells (NCI-H460/R). Hybrid nanoparticles without DHR displayed no inhibition of the cells at the highest concentration tested of 50 μg/mL. However, DHR nanoparticles significantly increased the activity of DHR in both cells lines. DHR nanoparticles displayed an eightfold decrease in the IC_{50} against NCI-H460 from 4.10±0.61 μg/mL to 0.53±0.06 μg/mL and a fivefold decrease in the IC_{50} against and NCI-H460/R from 3.18±0.32 μg/mL to 0.65±0.18 μg/mL compared to DHR alone (Garcia et al., 2018).

Although anti-cancer activity was observed for the DHR nanoparticles, more research is required to optimize the size of the DHR nanoparticle to be effectively transported to the lungs.

4.3 Antioxidant activity

During inflammation, macrophages release pro-inflammatory factors such as free radicals and nitric oxide that can contribute to cellular damage of the tissue. Although controversy exists on testing plant extracts for their free-radical-scavenging activity, it is still a widely used practice. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay is extensively used as a chemical assay to determine the potential free radical scavenging ability of a plant extract. However, it is suggested that the DPPH assay is supported by other antioxidant assays such as the ABTS (Ascorbic acid, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt), Nitric oxide (NO) and FRAP (ferric-reducing antioxidant power) free-radical-scavenging assays (Lalhminghlui and Jagetia, 2018).

The antioxidant activity of *P. madagascariensis* acetone extract was screened to determine the free-radical-scavenging ability of the extract using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Matias et al. (2019a) concluded that a methanolic extract of *P. madagascariensis* obtained through ultrasound-assisted extraction and maceration at a tested concentration of 100 µg/mL scavenged 89.0% and 64.8% of the DPPH free radicals respectively. The antioxidant activity was due to high concentrations of polyphenols in the extract as determined by High-Performance Liquid Chromatography with a Diode Array Detector (HPLC-DAD). The acetone extract, on the other hand, scavenged less than 50% of the free radicals at 100 µg/mL. In a similar study, the methanol extract of *P. madagascariensis* scavenged 48.4±1.23% of the DPPH free radicals at a concentration of 100 µg/mL (Andrade, 2016). Studies performed by Rijo et al. (2014b) and Brito et al. (2018) on the aqueous extract of *P. madagascariensis* produced similar DPPH free-radical scavenging activities with IC_{50} values of 41.66 µg/mL and 45.4±2.2 µg/mL respectively. The antioxidant activity could be due to rosmarinic acid that is a major compound in the species that scavenged DPPH free-radicals at an IC_{50} value of 2.8±0.1 µg/mL, as reported in an earlier study by Brito et al. (2018).

4.4 Anti-diabetic activity

Several enzymes play a significant role in the onset of diabetes mellitus. These enzymes include α-glucosidase, aldose reductase and phosphoenolpyruvate carboxykinase (PEPCK). The intestinal enzyme, α-glucosidase is a carbohydrate-hydrolase responsible for the release of α-glucose from large carbohydrates. Inhibition of α-glucosidase causes a decrease in α-linkage cleavage at the anomeric centre in a sugar molecule resulting in a reduction of glucose absorbance. Furthermore, α-glucosidase is associated with the onset of several diseases such as diabetes, cancer and viral infections. Studies have confirmed that the enzyme aldose reductase is linked to several diabetic complications. The enzyme affects the patient's sight by damaging the eye tissue and affecting the peripheral nervous system and organs such as the kidneys. Aldose reductase catalyses the reduction of glucose and aldehydes to sorbitol and is a key enzyme in the polyol pathway which contributes to various complications in diabetic patients. Phosphoenolpyruvate carboxykinase (PEPCK) is a key enzyme in the gluconeogenesis metabolic pathway responsible for the sustained release of glucose during fasting. In patients with diabetes, PEPCK is overproduced resulting in the overexpression of the gluconeogenesis pathway in the liver and kidneys, resulting in hyperglycaemia. Glucose transporter type 4 (GLUT-4) is a glucose transporter protein and is regulated by insulin levels. In addition, insulin resistance has been linked to impaired GLUT-4 translocation in patients suffering from diabetes (Gómez-Valadés et al., 2006; Ha et al., 2012; Kubínová et al., 2014; Simmons, 2017).

Rosmarinic acid isolated from a *P. madagascariensis* methanolic extract was confirmed to inhibit α-glucosidase at an IC_{50} value of 33.0±4.6 μM. This was comparable to quercetin previously confirmed to have an IC_{50} value of 26.7 μM (Ha et al., 2012; Kubínová et al., 2014). A study by Ha et al. (2012) reported the inhibitory activity of rosmarinic acid against aldose reductase with an IC_{50} value of 11.2 μM. *In vivo* studies conducted by Runtuwene et al. (2016) studied the effect of rosmarinic acid on insulin levels and the balance between insulin and glucose levels in the blood of induced type-1 and type-2 diabetic rats. Runtuwene et al. (2016) confirmed rosmarinic acid was effective in reducing the expression of the PEPCK enzyme and increasing GLUT-4 protein expression, therefore reducing hyperglycemia and insulin sensitivity at a dose of 200 mg/kg. However, Runtuwene et al. (2016) suggested further *in vivo* studies in humans since the dosage of

rosmarinic acid could differ for humans. This indicates the potential of rosmarinic acid as a potential treatment for diabetes mellitus and various allergy treatments.

4.5 Alzheimer's Disease

Alzheimer's disease (AD) is defined by Hase et al. (2018) as a "neurodegenerative disease-causing cognitive dysfunction such as memory impairment and disorientation". Various targets present themselves as potential treatments for AD. Acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) are enzymes found in healthy brains. AChE and BuChE are responsible for controlling acetylcholine (ACh) levels in the brain. However, patients suffering from AD have demonstrated an increase in BuChE levels in their brain. Patients affected by AD experience altered levels of AChE and low levels of ACh. Therefore, AChE inhibitors have been proven to improve ACh levels and improve cholinergic neuron function that is damaged during AD progression. Furthermore, the accumulation of amyloid-β (Aβ) protein in the brain has been linked to the formation of neuritic plaques that contribute to AD. The most abundant Aβ peptides are Aβ 1–40 and Aβ1–42. Therefore, Aβ-peptides, AChE and BuChE have become attractive therapeutic strategies for AD (Dos Santos et al., 2018; Hase et al., 2018; Kubínová et al., 2014).

An aqueous extract from the aerial parts of *P. madagascariensis* 'Lynne' inhibited AChE at an IC_{50} value of 440±80 μg/mL and alcohol dehydrogenase (ADH) at an IC_{50} value of 67±23 μg/mL. The inhibitory activity was said to be due to the presence of 83 μg/mg of rosmarinic acid in the extract. Rosmarinic acid inhibited AChE and ADH at IC_{50} values of 100±30 μg/mL and 19±4 μg/mL respectively (Brito et al., 2018).

Kubínová et al. (2014), reported 6β,7α dihydroxyroyleanone, 7α-acetoxy-6β-hydroxyroyleanone and coleon U quinone to have IC_{50} values of 287.3±9.9 μM, 256.4±5.9 μM and 290.7±3.5 μM respectively against BuChE. This was comparable to the IC_{50} value of 168.0±4.8 μM of the positive control, galantamine. The observed activity was hypothesized to be due to the presence of ester bonds in the compounds.

In vitro and *in vivo* studies by Hase et al. (2018) found that while the permeability of rosmarinic acid into the brain is low, the compound was able to reduce Aβ aggregation, by increasing monoamine secretion in 5-month-old female Tg2576 mice at 0.5% after 11 days of daily feeding. This study supported the *in vitro* results obtained by Ono et al. (2004) that confirmed the ability

of rosmarinic acid to inhibit the accumulation of Aβ-peptides, the formation of Aβ-fibrils and destabilization of preformed Aβ-fibrils.

4.6 Anti-tyrosinase, anti-collagenase and anti-elastase activity

Tyrosinase is an enzyme that plays a key role in the production of melanin in the skin. When overproduced, tyrosinase causes an overproduction of melanin, resulting in hyperpigmentation of the skin. A methanolic extract prepared from the fresh leaves of *P. madagascariensis* displayed an IC_{50} value of 23.99 µg/mL against the tyrosinase enzyme. The positive control, kojic acid, inhibited the tyrosinase enzyme at an IC_{50} value of 3.607 µg/mL. The researchers classified *P. madagascariensis* methanolic extract, as a noteworthy tyrosinase inhibitor (Etsassala, 2016).

Rosmarinic acid inhibited 47.87±1.41% of the tyrosinase enzyme at a concentration of 50 µg/mL compared to the positive control, kojic acid, with an IC_{50} value of 5.71 µg/mL. The compounds 7α-acetoxy-6β-hydroxyroyleanone and 6β,7α-dihydroxyroyleanone inhibited 46.62±4.74% and 14.73±2.02% of the tyrosinase enzyme at 50 µg/mL, respectively. Anti-tyrosinase studies confirmed DHR inhibited 75.72±3.61% of the enzyme at 50 µg/mL (Andrade, 2016). Rosmarinic acid and 7α-acetoxy-6β-hydroxyroyleanone are considered moderate tyrosinase inhibitors.

Elastase and collagenase are the two main enzymes responsible for breaking down the two major components of connective tissue in the skin, elastin and collagen. The breakdown of elastin and collagen results in loss of skin elasticity resulting in sagging skin and the formation of wrinkles. The percentage elastase inhibitory activity of 7α-acetoxy-6β-hydroxyroyleanone and 6β,7α-dihydroxyroyleanone was 29.26±2.75% and 39.16±5.18% respectively for the two compounds when tested at 100 µg/mL. The positive control ursolic acid inhibited 69.85±3.65% of the elastase enzyme at 100 µg/mL. At a concentration of 100 µg/mL rosmarinic acid exhibited 44.78±4.53% inhibition of collagenase. The positive control epigallocatechin gallate inhibited 93.09 ± 5.27% of the enzyme activity at 100 µg/mL. At 100 µg/mL, the compounds 7α-acetoxy-6β-hydroxyroyleanone and 6β,7α-dihydroxyroyleanone inhibited 33.45±3.25% and 24.04±3.02% of the collagenase enzyme respectively while DHR inhibited 38.33±4.40% and 60.63±9.68% of the elastase and collagenase enzymes respectively (Andrade, 2016).

4.7 Targeting pain and inflammation

Plectranthus madagascariensis is used traditionally as an application for wounds and scabies, which are often painful and inflamed lesions. Inflammation is characterised by, swelling (edema), pain, redness, heat and tissue loss (Chen et al., 2018; Lukhoba et al., 2006). Several *Plectranthus* species including *P. amboinicus*, *P. barbatus* and *P. hadiensis* have been confirmed to target inflammation, suggesting that *P. madagascariensis* and its phytochemical constituents may have potential anti-inflammatory activity.

Several researchers have investigated the anti-inflammatory potential of rosmarinic acid using animal models. Lucarini et al. (2013) investigated the potential of rosmarinic acid isolated from *Rosmarinus officinalis* L. (Lamiaceae) targeting factors associated with inflammation on male Swiss albino mice. At an oral dosage of 40 mg/kg, rosmarinic acid reduced paw edema by 61% after three hours compared to the positive control, indomethacin which inhibited 53.83% of edema at 10 mg/kg. Rosmarinic acid was able to reduce 38.3% of formalin-induced pain in mice after 15-30 minutes at a dosage of 20 mg/kg compared to morphine that inhibited 55.8% of formalin-induced pain at a dosage of 4 mg/kg.

4.8 Antifeedant activity

Wellsow et al. (2006) determined the antifeedant activity of several *Plectranthus* species against *Spodoptera littoralis*, known by its three common names, the African cotton leafworm, the Mediterranean brocade and lastly the Egyptian cotton leafworm. *Plectranthus madagascariensis* acetone extract and isolated compound coleon U was determined to be phagostimulants. However, the oxidised form of coleon U, coleon U quinone, had significant antifeedant activity at 100 ppm with a 50% feeding index (FI_{50}) value of 91 ppm.

5. Toxicity

Before a substance is applied or administered *in vivo* for human safety and efficacy studies, it is important to determine the relative toxicity and safety of the test substance. Toxicity studies are performed in two ways, the first uses non-cancerous cell lines *in vitro* and the second uses animal models such as mice, rats, brine shrimp or zebrafish (Arome and Chinedu, 2013; Twilley et al., 2020).

The toxicity of *P. madagascariensis* 'Lynne' and *P. aliciae* acetone extracts were determined against *Artemia salina* 'brine shrimp' due to the ease and inexpensive nature of the assay. Toxic effects at 50% lethal concentration (LC_{50}) were observed at 91.7 µg/mL and 53.48 µg/mL for *P. madagascariensis* 'Lynne' and *P. aliciae* respectively. At 100 ppm, *P. madagascariensis* 'Lynne' displayed a mortality rate of 9.67±0.93% and *P. aliciae* a mortality rate of 16.71±1.01% (Garcia et al., 2019a).

Since *P. madagascariensis* and *P. aliciae* acetone extracts displayed LC_{50} values between 91.7 µg/mL and 53.48 µg/mL respectively this indicated an LD_{50} of more than 2500 mg/kg in mice. Therefore, although the samples were considered toxic with the brine shrimp toxicity assay, moderate toxicity was observed when tested in mice.

Rijo et al. (2014b) confirmed that an aqueous extract of *P. madagascariensis* had low toxicity towards the human keratinocyte (HaCaT) cell line at the highest concentration of 500 µg/mL after 24 hours. No toxicity data was found for *P. ramosior* or *P. madagascariensis* var. *madagascariensis*. Similar toxicity data was observed for the acetone extracts of *P. madagascariensis* and *P. aliciae* on brine shrimp. This could be an indication that the toxicity between the varieties is not significantly different. Toxicity studies on an aqueous extract *Plectranthus amboinicus* (Lour) Spreng at the highest tested concentration of 10 000 mg/kg revealed no acute toxicity in mice. Subacute toxicity studies revealed an increase in kidney function parameters which are related to the dosage. While the aqueous extract of *P. madagascariensis* appears safe to use, based on the IC_{50} data, caution should be taken at higher concentrations, particularly when ingested (Asiimwe et al., 2014).

Cytotoxicity studies on HaCaT cells compared the extract of *P. madagascariensis* and ChiPS to determine changes in the toxicity of the extract. It was confirmed that the cytotoxicity of *P. madagascariensis* was lowered when combined with chitosan in comparison with the extract alone with IC_{50} values of 85.87 µg/mL and 56.77 µg/mL respectively (Matias, 2016).

In vivo acute and sub-chronic irritation studies were conducted on male hairless Sho® SCID mice to determine the irritancy potential of *P. madagascariensis* extract and ChiPS compared to a known skin irritant, Sodium Laureth Sulfate (SLS). Negligible irritation at 5% in carboxymethyl

cellulose hydrophilic gel was observed for the extract and ChiPS compared to 5% SLS that caused mild irritation (Matias, 2016).

6. Discussion

Researchers regularly select plants based on their traditional usage to test the extracts and their secondary metabolites against the claimed disease targets or novel targets. Traditional knowledge directs researchers to find plant extracts and compounds with positive biological activity. This highlights the importance of preserving ethnomedicinal knowledge (Patwardhan, 2005; Twilley et al., 2020).

Three varieties of *P. madagascariensis* are indigenous to South Africa, namely, *P. aliciae*, *P. ramosior* and *P. madagascariensis* var. *madagascariensis*. Lukhoba et al. (2006) reported the extensive use of synonyms in *Plectranthus* species that is evident from the species discussed in this review. The prevalence of synonyms of plant species can result in traditional knowledge being incorrectly documented. When investigating the top three traditional uses of *Plectranthus* species namely respiratory, skin and digestive disorders and comparing it to the traditional uses of *P. madagascariensis* only two of these disorders are documented. Although studies were conducted on the skin and respiratory disorders, no study investigated the use of *P. madagascariensis*, its varieties or isolated compounds on disorders of the digestive system. Since species in the genus is traditionally used to treat digestive disorders, the possibility exists that *P. madagascariensis* could have been used to treat digestive disorders. When investigating the traditional usage of a species either to determine the biological activity of the species for an ethnobotanical evaluation, it is important to take into consideration the traditional uses of the synonymous plants and the varieties of these species. This is also where voucher specimen numbers and the inclusion of these voucher numbers are of utmost importance in scientific articles.

Due to the morphological similarities between the studied *Plectranthus* species, the possibility exists that *P. madagascariensis* and its varieties have not only been misidentified by researchers but also traditional health practitioners making use of these species. Therefore, *P. madagascariensis* and its varieties could potentially have different traditional uses than those that are currently documented. Various articles have discussed the difficulty of identifying *Plectranthus* species to the untrained eye due to morphological similarities and the lack of criteria

to differentiate the species from one another. Evidence of misidentification could potentially be found in an article by Matias et al. (2019b) referencing an image of *P. madagascariensis* which lacks the characteristic variegated white leaf margins. The reviewed articles seldom reported the herbarium voucher specimen numbers. This could be problematic since vouchers serve as crucial supporting material for publications to ensure the reproducibility of the data. Vouchers are important for authenticating the taxonomy of a species, identifying geographical locations of the species and for ecological, environmental and genetic studies (Culley, 2013). For a genus such as *Plectranthus* where confusion between the species can easily occur due to morphological similarities, voucher specimens are crucial to ensure that the correct species is identified. Due to the lack of voucher specimens of *P. madagascariensis* and its varieties, it should be taken into consideration the misidentification of the species that could result in variable data between studies.

No reports of *P. madagascariensis* considered the synonyms and varieties of the species when conducting investigations of biological activities. Biological and phytochemical studies mostly focused only on *P. madagascariensis* and one study on *P. aliciae*. No study was found on *P. ramosior* or *P. madagascariensis* var. *madagascariensis* that could potentially give information on the similarities and differences in the biological activity of these varieties. Although *P. madagascariensis* is not considered toxic, *P. hadiensis*, a synonym of *P. ramosior* is used as a poison that could potentially be related to the toxicity observed in mice studies (Lukhoba et al., 2006).

Toxicity studies were only reported for the acetone extracts of *P. madagascariensis* and *P. aliciae* and the aqueous extract of *P. madagascariensis*. The acetone extracts of *P. madagascariensis* and *P. aliciae* were confirmed as being potentially toxic when ingested. However, the aqueous extract of *P. madagascariensis* was confirmed to have low toxicity towards HaCaT cells. No toxicity studies were reported on the compounds isolated from *P. madagascariensis* or the essential oil of the plant using HaCaT cells or animal models. Toxicity studies on plant extracts and secondary metabolites are useful to determine if further analysis of the samples should be conducted. Twilley et al. (2020) suggested that when the safety of a plant extract or secondary metabolite is being investigated the mutagenicity of the extract or compound should be determined. Mutagenicity refers to the potential of a test substance to cause permanent changes in genetic material and DNA damage in cells (Mortelmans and Zeiger, 2000). No study investigated the mutagenic potential of

P. madagascariensis, its varieties or the identified secondary metabolites that could provide useful information on the safety of the samples.

Cytotoxicity and toxicity studies were mostly performed on *P. madagascariensis* and *P. aliciae* acetone extracts. No cytotoxicity studies were reported for *P. ramosior* or *P. madagascariensis* var. *madagascariensis*. *Plectranthus madagascariensis* and *P. aliciae* were found to have similar significant cytotoxic activity against human breast (MCF-7) and human colon (HCT116) adenocarcinoma cells. Interestingly, compounds isolated from *P. madagascariensis* 6β,7α-dihydroxyroyleanone, 7α-formyloxy-6β-hydroxyroyleanone, 7α-acetoxy-6β-hydroxyroyleanone, rosmarinic acid and coleon U had moderate to significant cytotoxic activity against MCF-7, HCT116, human cervix carcinoma cell line (KB-3-1) and the human liver cancer cells (HepG2). Although these compounds have not yet been identified in *P. aliciae*, based on the similar activities between the extracts of these two species, the compounds of *P. madagascariensis*, could be present in *P. aliciae* and amongst the other varieties too. However, this is merely an assumption and the isolation and identification of compounds from the different varieties remains elusive. The secondary metabolites DHR, 6β,7α-dihydroxyroyleanone and 7α-acetoxy-6β-hydroxyroyleanone isolated from *P. madagascariensis* have been confirmed to be selective towards NCI-H460 lung carcinoma cells with moderate and significant cytotoxic effects respectively. DHR isolated from the essential oil of *P. madagascariensis* was confirmed to target multiple lung cancer cell lines including resistant NCI-H460 lung carcinoma cells. These compounds could be the reason for the significant cytotoxic activity of *P. madagascariensis* and *P. aliciae* against NCI-H460 lung carcinoma cells. None of the studies investigated the selectivity of the extracts or compounds for cancerous cell lines. Current cancer treatments not only target cancerous cells but also non-cancerous cells. Calculating the selectivity index could indicate if a sample is selective towards cancerous cells rather than non-cancerous cells (Peña-Morán et al., 2016).

Traditionally *P. madagascariensis* is used to treat respiratory ailments such as coughs, bronchitis and asthma (Huthings et al., 1996; Pereira et al., 2015; Rabe and van Staden, 1998; Rice et al., 2011). However, it is not clear whether these symptoms were confused with that of lung cancer and tuberculosis. No reports indicate the traditional usage of *P. madagascariensis* or the varieties thereof for lung cancer or tuberculosis. From these studies, it is clear that *P. madagascariensis* and its varieties could potentially be used for the treatment of lung cancer and tuberculosis or could

have traditionally been used to treat the symptoms of these diseases without the knowledge of the traditional users. However, no biological studies were found to investigate the plant extracts or compounds for asthma, or the bacteria involved in the onset of bronchitis such as *Bordetella pertussis, Chlamydia pneumonia*, *Haemophilus influenza, Moraxella catarrhalis, Mycoplasma pneumonia* and *Streptococcus pneumonia* (Sethi, 2020; Worrall, 2008). Furthermore, an aqueous root extract of *P. madagascariensis* and an extract prepared for the whole plant have been documented to be traditionally used for respiratory disorders. From the manuscripts reviewed herein, the aerial parts of the plants were used to conduct these studies and mostly extracted with acetone, ethanol or methanol. Several studies have confirmed that different plant parts have significant differences in biological activity. This is largely due to the fact that different secondary metabolites are present either exclusively in a certain plant part or are found in various concentrations in different plant parts. When traditional knowledge is adapted to confirm the activity *in vitro* or *in vivo* in biological studies it is important to include the ethnobotanical method in the studies to confirm and document the results. Several articles reviewed did not consider the traditional usage of *P. madagascariensis*. Therefore the traditional usage of *P. madagascariensis* for respiratory diseases and the way it is traditionally administered has not yet been fully explored, leaving a gap between traditional knowledge and scientific validation that needs to be filled.

The use of nanosystems for the treatment of lung cancer and other respiratory diseases should also not be forgotten. DHR nanoparticles were able to significantly decrease the IC_{50} value against NCI-H460 lung carcinoma cells (Garcia et al., 2018). However, no other studies have investigated the potential of the *P. madagascariensis* extract, and compound, 6β,7α-dihydroxyroyleanone and 7α-acetoxy-6β-hydroxyroyleanone loaded nanoparticles for the treatment of lung cancer or other respiratory ailments.

The antibacterial activity of the Lamiaceae family can be attributed to phenolic secondary metabolites present in the extract and the essential oil of the plant. Antibiotic resistance has become a concern and the World Health Organisation has classified resistance as a global threat to human health. Antibiotic resistance occurs when bacteria become resistant to antibiotics resulting in longer treatment times and higher medical costs as a result of misuse of antibiotics (WHO, 2020). Antibiotic resistance is found in respiratory maladies such as tuberculosis and wound infections such as prosthetic implant-associated infections. As mentioned, *P. madagascariensis* is

traditionally used to treat respiratory ailments such as coughs. However, the cause of the symptom has not been reported in the literature and could be due to bacterial lung infections caused by *Haemophilus* species, *M. tuberculosis*, *K. pneumoniae, Streptococcus pneumoniae* and *S. aureus* (Speert, 2006). *Mycobacterium tuberculosis* (MDR), resistant *K. pneumoniae,* and methicillin-resistant *S. aureus* (MRSA) strains are becoming more frequent and harder to treat with current antibiotics. The acetone extract of *P. madagascariensis* was found to be active against both Gram-positive and Gram-negative bacteria associated with respiratory diseases such as *M. smegmatis*, a model bacterium for *M. tuberculosis* research, MRSA and *K. pneumoniae*. In a similar study, the acetone extract of *P. aliciae* was confirmed to inhibit *S. aureus* growth at a lower concentration than the acetone extract *P. madagascariensis*. Although these species are morphologically similar, differences in antibacterial activity was observed. This could be due to a difference in the concentration of secondary metabolites or the type of compounds present in the two species. No reports on the antibacterial activity of *P. ramosior* or *P. madagascariensis* var. *madagascariensis* were found and the anti-mycobacterial activity of *P. aliciae* is yet to be investigated. Traditionally *P. madagascariensis* plant material is prepared either as a decoction or an infusion when treating respiratory diseases. However, none of the studies investigated an aqueous extract for the inhibition of bacteria associated with lung infections or cytotoxicity against lung cancer cells. An aqueous extract of *P. madagascariensis* was confirmed to significantly inhibit *S. epidermis* that is associated with wound infections. Due to the significant antibacterial activity of the aqueous extract further studies of the extract on bacteria associated with respiratory infections could be beneficial. The noteworthy anti-mycobacterial activity of *P. madagascariensis* could be attributed to the abietane diterpenes 6β,7α-dihydroxyroyleanone, 7α-acetoxy-6β-hydroxyroyleanone and 6,7-dehydroroyleanone present in the plant. Diterpenoids are known to have antimicrobial activity that is evident from the noteworthy anti-mycobacterial activity of the extracts against MDR *M. tuberculosis* due to the 7α-AcO group at the B ring.

Traditionally the crushed leaves of *P. madagascariensis* are used to treat wounds and scabies. Several factors are associated with wound healing as described by Robson (1997) as a series of event that includes “coagulation, inflammation, matrix synthesis and deposition, angiogenesis, fibroplasia, epithelialization, contraction, and remodelling”. However, when there is an abnormality in the wound healing process, bacteria can cause infections, which are more difficult to treat especially when the bacteria are resistant to conventional treatment strategies. Bacteria that

are associated with bacterial wound infections and nosocomial wound infections are normally *Staphylococcal* species such as *S. epidermidis* and *S. aureus*, and others such as *P. aeruginosa, E. faecalis* and *E. coli* (Parr et al., 1999). From the studies investigated only the acetone extracts of *P. madagascariensis* and *P. aliciae* were investigated for their antibacterial activity against some of the bacteria associated with wound infections. Interestingly, only *P. aliciae* was bactericidal against *E. faecalis* and slightly more active against *S. aureus* than *P. madagascariensis*. However, none of the studies investigated the antibacterial activity of *P. madagascariensis* and *P. aliciae* on *E. coli* and *P. aeruginosa* and the extracts of *P. ramosior* or *P. madagascariensis* var. *madagascariensis* on any of the bacteria associated with wounds. Furthermore, only the aqueous extract of *P. madagascariensis* was tested for its antibacterial activity against *S. epidermidis*. More studies can be done on the antibacterial activity of these *Plectranthus* species on bacteria associated with wound infections. The essential oil of *P. madagascariensis* was confirmed to target only the Gram-positive bacterium *S. aureus* using the agar disc-diffusion assay. Several factors such as the bacterial species and type of antibiotic determine the sensitivity of the agar disc-diffusion assay. Studies have confirmed that although the agar disc-diffusion assay is consistent to the broth microdilution assay between 71% and 90% of the time, it cannot be used as a sole method to determine the antibacterial activity of a test substance (Dickert et al., 1981). Limitations of the agar-diffusion method include the inability to distinguish between the bactericidal and bacteriostatic activity of the test substance. Furthermore, although it is a fast and inexpensive method it is difficult to quantify the amount of the test substance that diffused into the agar medium and the MIC cannot be calculated (Balouiri et al., 2016). The biological variations that the microdilution assay takes into consideration should also be considered. An established microdilution method is available to determine the MIC value of essential oils as described by Kamatou et al. (2006) that should be considered when testing the antibacterial activity of essential oils. DHR, isolated from the essential oil of *P. madagascariensis* has been confirmed to have moderate antibacterial activity against *M. tuberculosis*, however, no tests were done on other Gram-positive or Gram-negative bacteria providing new opportunities for further research of this compound. Coleon U quinone isolated from *P. madagascariensis* was confirmed to inhibit *S. aureus* and *E. faecalis* that are associated with wound infections at noteworthy concentrations. Furthermore, coleon U and 7α-acetoxy-6β-hydroxyroyleanone displayed moderate to noteworthy antibacterial activity against resistant *S. aureus* and *E. faecalis*. Although the compounds

6β,7α-dihydroxyroyleanone, 7α-acetoxy-6β-hydroxyroyleanone displayed moderate antibacterial activity against Gram-positive bacteria, none of the compounds isolated from *P. madagascariensis* inhibited the Gram-negative bacteria *E. coli* and *P. aeruginosa*. Matias (2016) set out to develop phytosomes that are loaded with acetonic *P. madagascariensis* extract and chitosan-coated phytosomes containing the acetonic *P. madagascariensis* extract. Agar disc-diffusion studies confirmed that the antibacterial activity of *P. madagascariensis* acetone extract was increased when the phytosomes was coated with chitosan compared to a chitosan-coated phytosomes without the extract. The antibacterial activity of the phytosomes was tested using the microdilution assay and was confirmed to have increased antibacterial activity and reduced toxicity and irritancy potential, however, the chitosan-coated phytosomes control was omitted from the study. When considering the methodologies used to identify the biological activities of the plant extract several articles failed to mention the activity of the positive and negative controls. This is vital information that should be included in biological assays and the reporting of such assays. The relevant controls and their results should be mentioned to ensure that the experiment can be repeated and controls are available to which the success of current and future assays can be measured (Choudhary, 2017).

Although some efforts have been made to develop nanoparticle delivery systems for *P. madagascariensis*, these studies are not complete with crucial information such as the activity of the controls which were missing. Furthermore, no delivery systems have been considered for the other *Plectranthus* species discussed in this review article. Rosmarinic acid is a major compound present in the Lamiaceae family and *Plectranthus* species including *P. madagascariensis*. Rosmarinic acid displayed no antibacterial activity on Gram-positive or Gram-negative bacteria. However, this secondary metabolite has been confirmed to target pain and reduce inflammation in *in vivo* mice studies that were comparable to that of the positive control, indomethacin. Taking all the information regarding wound infections and the traditional usage of *P. madagascariensis* for the treatment of wounds into consideration, *P. madagascariensis, P. aliciae* and their isolated compounds have noteworthy antibacterial activity especially against Gram-positive bacteria that is associated with wound infections. Studies on the anti-inflammatory activity of the extracts, the potential of the extracts targeting pain, matrix synthesis and tissue remodelling is absent from the literature that can tie the traditional usage of the plant with its biological activity together. Therefore, future studies on the extracts, their compounds, and the delivery systems of these actives for wound remodelling are recommended. Furthermore, several articles have published on

the anti-inflammatory potential of rosmarinic acid using animal models. The use of animal models is increasingly becoming more difficult due to ethical processes. Several *in vitro* anti-inflammatory models are available to determine the anti-inflammatory activity of reviewed *Plectranthus* species and their secondary metabolites. Flow cytometry is a rapid and useful tool to determine the anti-inflammatory potential of several inflammatory markers simultaneously and should be considered when investigating the anti-inflammatory activity of *P. madagascariensis*, its varieties and secondary metabolites.

The release of free radicals has been associated with inflammation that can contribute to tissue damage; an increased inflammatory response can contribute to a range of diseases. Although the antioxidant activity of a natural product cannot be studied alone to confirm its activity, it can give some information on the free radical scavenging potential of a natural compound. A research article by Amorati and Valgimigli (2015) set out to discuss the advantages and limitations of current antioxidant assays. The DPPH antioxidant assay is the most utilised antioxidant method due to the inexpensive nature and ease of the assay. The endpoint of the assay is normally measured after 30 minutes as with the manuscripts reviewed. Amorati and Valgimigli (2015) acknowledged the potential of the assay, however, pointed out that there are flaws in the assay. Amorati and Valgimigli (2015) proposed a kinetic reading approach since the reactivity rate of natural products is different from one another. Through using the kinetic reading approach, the data will reflect the true rate constants of the actives instead of a single-point reading where this is not possible. Furthermore, solvents such as ethanol and methanol (polar protic solvents) are usually used to perform DPPH antioxidant assays. Studies have confirmed phenolic compounds react faster with DPPH when the compounds were dissolved in polar protic solvents resulting in a skewed data of the actual antioxidant capacity. Although noteworthy antioxidant activity was observed for *P. madagascariensis* and isolated compound, rosmarinic acid, it is not clear if the data presented is a true reflection of the free radical scavenging potential of the extract and compound and could further be explored using more sensitive and reliable antioxidant assays. *In vitro* cell-based antioxidant assays are more sensitive and comprehensive since compounds not necessarily involved in free radical scavenging but compounds that are involved in the antioxidant pathway and protecting cells against oxidative damage can be identified (Da Silva et al., 2016).

It was interesting to note that *P. madagascariensis* and its isolated compounds were found to be active against several enzymes involved in maladies such as diabetes, skin-hyperpigmentation and skin ageing not related to the reported traditional usage of the plant. Traditionally an aqueous extract from the aerial parts of several *Plectranthus* species is used to treat neurological disorders such as epilepsy, meningitis, convulsions, mental retardation and depression (Lukhoba et al., 2006). The extract and secondary metabolites of *P. madagascariensis* has shown to target the enzymes related to the onset of Alzheimer's disease. Although the traditional usage of *Plectranthus* species for Alzheimer's disease has not been documented for *P. madagascariensis* or its varieties it does show potential to treat Alzheimer's disease and potentially other unexplored neurological disorders. The compounds 7α-acetoxy-6β-hydroxyroyleanone and 6β,7α-dihydroxyroyleanone showed moderate anti-elastase and collagenase activity associated with ageing. *Plectranthus madagascariensis* extracts displayed noteworthy tyrosinase, alcohol dehydrogenase and acetylcholinesterase activity. The compounds isolated from *P. madagascariensis* were confirmed to have noteworthy PEPCK, α-glucosidase, butyrylcholinesterase and acetylcholinesterase activity. However, no study focused on the enzyme inhibitory activity of *P. aliciae*, *P. ramosior* or *P. madagascariensis* var. *madagascariensis* and their secondary metabolites. These species have potential to target a range of enzymes related to cancer, asthma, diabetes and Alzheimer's diseases that are related to the traditional usage of the plant in some cases but have not fully been explored. Furthermore, the research articles reviewed did not explore the type of enzyme inhibition in any of the studies to confirm competitive, non-competitive or allosteric competitive inhibition of the extracts or secondary metabolites against the enzymes investigated. This is something that can further be explored to provide vital information when developing therapeutic agents for these maladies.

Lastly, the reviewed studies that isolated compounds from *P. madagascariensis* only focused on isolating, identifying and determining the biological activity of seven secondary metabolites (Figure 1). *Plectranthus* species are known to contain a wide range of compounds that have demonstrated noteworthy medical and economic potential. Although, some researchers have set out to investigate the phytochemicals of *Plectranthus* species this field is still largely unexplored (Abdel-Mogib et al., 2002). No studies were found on the isolation and identification of compounds from the variety species. Studies on the phytochemical profiles and biological activities have revealed variety species either contain different secondary metabolites or the same

compounds but in varying concentrations (Wang et al., 2017). This difference in phytochemical compounds between varieties could have different biological activities and should further be explored. Several isolation methods and equipment are available for rapid identification of compounds as discussed in an article by Altemimi et al. (2017). HPLC coupled with a mass spectrophotometer provides a rapid and accurate method for the identification of compounds when pure compounds are unavailable and could be considered for the identification of more compounds in *P. madagascariensis* and its varieties.

7. Conclusion

Plectranthus species are known for their medicinal potential in treating various maladies. *Plectranthus madagascariensis* and its varieties have been used as a medicinal plant in South Africa for centuries, all sharing similar common names. However, research on these varieties indigenous and endemic to South Africa remains largely unexplored. Due to the morphological similarities between *P. madagascariensis* and its varieties, the possibility exists that researchers unknowingly test variety species rather than *P. madagascariensis.* Lukhoba et al. (2006) has also mentioned the difficulty in discriminating between morphological similar *Plectranthus* species and could contribute to discrepancies in data. Currently, there is a need for information on the morphological differences between these species to clearly distinguish between *P. madagascariensis* and its varieties. *Plectranthus madagascariensis* has a long history for being traditionally used in wound healing which is supported by the antibacterial and anti-inflammatory potential of the plant extract and secondary metabolites. Rosmarinic acid is a major compound of *P. madagascariensis*. The anti-inflammatory and antioxidant activity of rosmarinic acid could be linked to the traditional usage of *P. madagascariensis* for wound care. The ability of rosmarinic acid to reduce swelling and relieve pain could explain the traditional usage of *P. madagascariensis* in the treatment of skin ailments. Furthermore, an aqueous crude extract of *P. madagascariensis* displayed antibacterial activity towards bacteria associated with wounds and antioxidant activity that is in accordance with the traditional preparation and use of the plant. However, further mechanistic studies on wound healing and inflammatory studies remain elusive. In addition, no research on skin-related diseases such as acne vulgaris, scabies and eczema were observed. With the anti-inflammatory, antibacterial and wound healing activity of *P. madagascariensis* confirmed, other skin related diseases could be a potential target for *P. madagascariensis*. Regarding the

traditional usage of *P. madagascariensis* treating respiratory ailments, compounds isolated from the extract has proven cytotoxicity towards lung cancer cells and target *M. tuberculosis*, a bacteria that is the cause of tuberculosis and is under the top ten causes of deaths worldwide according to the World Health Organization (2018). However, no research focused on the use of *P. madagascariensis* or its isolated compounds for the treatments of asthma or other respiratory-related diseases such as bronchitis that could be a potential target for further research. In addition to rosmarinic acid, six major compounds namely coleon U, 6β,7α dihydroxyroyleanone, 7α-formyloxy-6β-hydroxyroyleanone, 7α-acetoxy-6β-hydroxyroyleanone, coleon U quinone, and 6,7-dehydroroyleanone (isolated from the essential oil) have been proven *in vitro* to have noteworthy antibacterial, anti-cancer, anti-diabetic potential and targeting Alzheimer's disease. These isolated compounds of *P. madagascariensis* have proven to have the potential to be developed into therapeutics targeting these life-threatening diseases. However, no *in vivo* human studies are available for these disease targets, opening a door for further human studies. Several researchers have set out to incorporate *P. madagascariensis* extracts and compounds in nano-delivery-systems, however, the biological activity of these systems is largely unanswered. These delivery systems could contribute to the development the pharmaceutical industry and improve public health, but more research in this area is required. The phytochemical and biological data reviewed suggest the potential use of *P. madagascariensis* and its varieties in the cosmeceutical and pharmaceutical fields. Lastly, toxicity and mutagenic studies are lacking for the species and their secondary metabolites to determine the safety of the samples.

This review examines the potential use of *P. madagascariensis* and its varieties that are indigenous to South Africa based on its traditional usage and scientific validation studies to target various diseases. Additionally, this review highlights research opportunities for these species to ultimately develop safe and useful products with medicinal and economic potential.

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9. References

Abdel-Mogib, M., Albar, H., Batterjee, S., 2002. Chemistry of the genus *Plectranthus*. Molecules 7, 271–301. doi:10.3390/70200271

Abdissa, N., Frese, M., Sewald, N., 2017. Antimicrobial Abietane-Type Diterpenoids from *Plectranthus punctatus*. Molecules 22, 1919. doi:10.3390/molecules22111919

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D.G., Lightfoot, D.A., 2017. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. Plants 6. doi:10.3390/plants6040042

Amorati, R., Valgimigli, L., 2015. Advantages and limitations of common testing methods for antioxidants. Free Radic. Res. 49, 633–649. doi:10.3109/10715762.2014.996146

Andrade, J.E. da C.M., 2016. Unravelling new ethnopharmacological roles of *Plectranthus* species: biological activity screening. University of Lusófona, Lisboa.

Arome, D., Chinedu, E., 2013. The importance of toxicity testing. J. Pharm. Biosci. 4, 146–148.

Ascensao, L., Figueiredo, A.C., Barroso, J.G., Pedro, L.G., Schripsema, J., Deans, S.G., Scheffer, J.J.C., 1998. *Plectranthus madagascariensis*: Morphology of the glandular trichomes, essential oil composition, and its biological activity. Int. J. Plant Sci. 159, 31–38. doi:10.1086/297518

Asiimwe, S., Borg-Karlsson, A.-K., Azeem, M., Maud Mugisha, K., Namutebi, A., James Gakunga, N., 2014. Chemical composition and toxicological evaluation of the aqueous leaf extracts of *Plectranthus amboinicus* Lour. Spreng. Int. J. Pharm. Sci. Invent. ISSN 3, 19–27.

Baldin, V.P., Scodro, R.B. de L., Lopes-Ortiz, M.A., de Almeida, A.L., Gazim, Z.C., Ferarrese, L., Faiões, V. dos S., Torres-Santos, E.C., Pires, C.T.A., Caleffi-Ferracioli, K.R., Siqueira, V.L.D., Cortez, D.A.G., Cardoso, R.F., 2018. Anti-Mycobacterium tuberculosis activity of essential oil and 6,7-dehydroroyleanone isolated from leaves of *Tetradenia riparia* (Hochst.) Codd (Lamiaceae). Phytomedicine 47, 34–39. doi:10.1016/j.phymed.2018.04.043

Balouiri, M., Sadiki, M., Ibnsouda, S.K., 2016. Methods for *in vitro* evaluating antimicrobial activity: A review. J. Pharm. Anal. doi:10.1016/j.jpha.2015.11.005

Brito, E., Gomes, E., Falé, P.L., Borges, C., Pacheco, R., Teixeira, V., Machuqueiro, M., Ascensão, L., Serralheiro, M.L.M., 2018. Bioactivities of decoctions from *Plectranthus* species related to their traditional use on the treatment of digestive problems and alcohol intoxication. J. Ethnopharmacol. 220, 147–154. doi:10.1016/j.jep.2018.04.006

Centers for Disease Control and Prevention, 2019. Tuberculosis (TB) Disease: Symptoms and Risk Factors | Features | CDC [WWW Document]. URL https://www.cdc.gov/features/tbsymptoms/index.html (accessed 5.4.20).

Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., Li, Y., Wang, X., Zhao, L., 2018. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget. doi:10.18632/oncotarget.23208

Choudhary, A., 2017. Importance of Negative and Positive Controls in Microbial Analysis [WWW Document]. Pharm. Guidel. URL https://www.pharmaguideline.com/2017/05/importance-of-negative-and-positive-controls.html#gsc.tab=0 (accessed 8.10.20).

Codd, L.E., 1975. *Plectranthus* (Labiatae) and allied genera in Southern Africa. Bothalia 11, 371–442. doi:10.4102/abc.v11i4.1482

Cook, F.E.M., 1995. Economic botany data collection standard., Economic botany data collection standard. Royal Botanic Gardens (Kew).

Culley, T.M., 2013. Why Vouchers Matter in Botanical Research. Appl. Plant Sci. 1, 1300076. doi:10.3732/apps.1300076

Da Silva, L.C.N., Filho, C.M.B., De Paula, R.A., E Silva, C.S., De Souza, L.I.O., Da Silva, M.V., dos Santos Correia, M.T., De Figueiredo, R.C.B.Q., 2016. *In vitro* cell-based assays for evaluation of antioxidant potential of plant-derived products. Free Radic. Res. 50, 801–812. doi:10.1080/10715762.2016.1193668

Danaei, M., Dehghankhold, M., Ataei, S., Hasanzadeh Davarani, F., Javanmard, R., Dokhani, A., Khorasani, S., Mozafari, M., 2018. Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. Pharmaceutics 10, 57. doi:10.3390/pharmaceutics10020057

De Freitas, L.F., Varca, G.H.C., Batista, J.G.D.S., Lugão, A.B., 2018. An overview of the synthesis of gold nanoparticles using radiation technologies. Nanomaterials 8, 939. doi:10.3390/nano8110939

Dickert, H., Machka, K., Braveny, I., 1981. The uses and limitations of disc diffusion in the antibiotic sensitivity testing of bacteria. Infection 9, 18–24. doi:10.1007/BF01640803

Dos Santos, T.C., Gomes, T.M., Pinto, B.A.S., Camara, A.L., De Andrade Paes, A.M., 2018. Naturally occurring acetylcholinesterase inhibitors and their potential use for Alzheimer's disease therapy. Front. Pharmacol. doi:10.3389/fphar.2018.01192

Etsassala, N.G.E.R., 2016. Detection of selective tyrosinase inhibitors from some South African plant extracts of Lamiaceae family. University of the Western Cape, Cape Town.

Foden, W., Potter, L., 2005a. *Plectranthus madagascariensis* (Pers.) Benth. var. madagascariensis [WWW Document]. URL http://redlist.sanbi.org/species.php?species=1671-49 (accessed 3.29.20).

Foden, W., Potter, L., 2005b. *Plectranthus ramosior* (Benth.) Van Jaarsv. [WWW Document]. URL http://redlist.sanbi.org/species.php?species=1671-158 (accessed 3.29.20).

Garcia, C., Ntungwe, E., Rebelo, A., Bessa, C., Stankovic, T., Dinic, J., Díaz-Lanza, A., Reis, C.P., Roberto, A., Pereira, P., Cebola, M.J., Saraiva, L., Pesic, M., Duarte, N., Rijo, P., 2019a. Parvifloron D from *Plectranthus strigosus*: Cytotoxicity screening of *Plectranthus* spp. extracts. Biomolecules 9, 616. doi:10.3390/biom9100616

Garcia, C., Silva, C.O., Monteiro, C.M., Nicolai, M., Viana, A., Andrade, J.M., Barasoain, I., Stankovic, T., Quintana, J., Hernández, I., González, I., Estévez, F., Díaz-Lanza, A.M., Reis, C.P., Afonso, C.A.M., Pesic, M., Rijo, P., 2018. Anticancer properties of the abietane diterpene 6, 7-dehydroroyleanone obtained by optimized extraction. Future Med. Chem. 10,

1177–1189. doi:10.4155/fmc-2017-0239

Garcia, C., Teodósio, C., Oliveira, C., Oliveira, C., Díaz-Lanza, A., Reis, C., Duarte, N., Rijo, P., 2019b. Naturally occurring *Plectranthus*-derived diterpenes with antitumoral activities. Curr. Pharm. Des. 24, 4207–4236. doi:10.2174/1381612825666190115144241

Gómez-Valadés, A.G., Vidal-Alabró, A., Molas, M., Boada, J., Bermúdez, J., Bartrons, R., Perales, J.C., 2006. Overcoming diabetes-induced hyperglycemia through inhibition of hepatic phosphoenolpyruvate carboxykinase (GTP) with RNAi. Mol. Ther. 13, 401–410. doi:10.1016/j.ymthe.2005.08.026

González-Minero, F., Bravo-Díaz, L., 2018. The use of plants in skin-care products, cosmetics and fragrances: Past and Present. Cosmetics 5, 50. doi:10.3390/cosmetics5030050

González, M.A., 2015. Aromatic abietane diterpenoids: Their biological activity and synthesis. Nat. Prod. Rep. 32, 684–704. doi:10.1039/c4np00110a

Ha, T.J., Lee, J.H., Lee, M.H., Lee, B.W., Kwon, H.S., Park, C.H., Shim, K.B., Kim, H.T., Baek, I.Y., Jang, D.S., 2012. Isolation and identification of phenolic compounds from the seeds of *Perilla frutescens* (L.) and their inhibitory activities against α-glucosidase and aldose reductase. Food Chem. 135, 1397–1403. doi:10.1016/j.foodchem.2012.05.104

Harrower, A., 2014. P*lectranthus madagascariensis* [WWW Document]. PlantZAfrica. URL http://pza.sanbi.org/plectranthus-madagascariensis (accessed 3.29.20).

Hase, T., shishido, S., Yamamoto, S., Yamashita, R., Nukima, H., taira, S., toyoda, T., Abe, K., Hamaguchi, T., Ono, K., Noguchi-shinohara, M., Yamada, M., Kobayashi, S., 2018. Rosmarinic acid suppresses Alzheimer's disease development by reducing amyloid β aggregation by increasing monoamine secretion. Sci. Rep. 9, 1–13. doi:10.1038/s41598-019-45168-1

Hutchings, A., Scott, A.H., University of Zululand., National Botanical Institute (South Africa), 1996. Zulu medicinal plants: an inventory. University of Natal Press.

Isca, V.M.S., Ferreira, R.J., Garcia, C., Monteiro, C.M., Dinic, J., Holmstedt, S., André, V.,

Pesic, M., dos Santos, D.J.V.A., Candeias, N.R., Afonso, C.A.M., Rijo, P., 2020. Molecular docking studies of royleanone diterpenoids from *Plectranthus* spp. as P-glycoprotein inhibitors . ACS Med. Chem. Lett. doi:10.1021/acsmedchemlett.9b00642

Kamatou, G.P.P., Van Zyl, R.L., Van Vuuren, S.F., Viljoen, A.M., Figueiredo, A.C., Barroso, J.G., Pedro, L.G., Tilney, P.M., 2006. Chemical composition, leaf trichome types and biological activities of the essential oils of four related *Salvia* species indigenous to southern Africa. J. Essent. Oil Res. 18, 72–79. doi:10.1080/10412905.2006.12067125

Kubínová, R., Pořízková, R., Navrátilová, A., Farsa, O., Hanáková, Z., Bačinská, A., Čížek, A., Valentová, M., 2014. Antimicrobial and enzyme inhibitory activities of the constituents of *Plectranthus madagascariensis* (Pers.) Benth. J. Enzyme Inhib. Med. Chem. 29, 749–752. doi:10.3109/14756366.2013.848204

Kusumoto, N., Aburai, N., Ashitani, T., Takahashi, K., Kimura, K., 2014. Pharmacological prospects of oxygenated abietane-type diterpenoids from taxodium distichum cones. Adv. Biol. Chem. 4, 109–115. doi:10.4236/abc.2014.42015

Lalhminghlui, K., Jagetia, G.C., 2018. Evaluation of the free-radical scavenging and antioxidant activities of Chilauni, Schima wallichii Korth *in vitro*. Futur. Sci. OA 4, 272. doi:10.4155/fsoa-2017-0086

Latti, I., 2019. Photos of South African Plants - Category: *Plectranthus* - Image: *Plectranthus madagascariensis* [WWW Document]. Oper. Wildflower. URL https://www.operationwildflower.org.za/index.php/albums/genera-m-z/plectranthus/plectranthus-madagascariensis-1050 (accessed 3.29.20).

Li, T., Mattei, A., 2019. Pharmaceutical crystals: science and engineering - Tonglei Li, Alessandra Mattei - Google Books, First edition. ed. Wiley & Sons Inc.

Lucarini, R., Bernardes, W.A., Ferreira, D.S., Tozatti, M.G., Furtado, R., Bastos, J.K., Pauletti, P.M., Januário, A.H., Andrade Silva, M.L., Cunha, W.R., 2013. *In vivo* analgesic and anti-inflammatory activities of *Rosmarinus officinalis* aqueous extracts, rosmarinic acid and its acetyl ester derivative. Pharm. Biol. 51, 1087–1090. doi:10.3109/13880209.2013.776613

Lukhoba, C.W., Simmonds, M.S.J., Paton, A.J., 2006. *Plectranthus*: A review of ethnobotanical uses. J. Ethnopharmacol. doi:10.1016/j.jep.2005.09.011

Mahomoodally, M.F., Priyamka, D., 2014. A quantitative ethnopharmacological documentation of natural pharmacological agents used by pediatric patients in Mauritius. Biomed Res. Int. doi:10.1155/2014/136757

Matias, D., 2016. Isolation, modeling and phytosome forms of antimicrobial and antiproliferative compounds from *Plectranthus* spp. | Request PDF. University of Alcalá, Alcalá de Henares.

Matias, D., Nicolai, M., Fernandes, A.S., Saraiva, N., Almeida, J., Saraiva, L., Faustino, C., Díaz-Lanza, A.M., Reis, C.P., Rijo, P., 2019a. Comparison study of different extracts of *Plectranthus madagascariensis*, *P. neochilus* and the rare *P. porcatus* (Lamiaceae): chemical characterization, antioxidant, antimicrobial and cytotoxic activities. Biomolecules 9, 179. doi:10.3390/biom9050179

Matias, D., Nicolai, M., Saraiva, L., Pinheiro, R., Faustino, C., Diaz Lanza, A., Pinto Reis, C., Stankovic, T., Dinic, J., Pesic, M., Rijo, P., 2019b. Cytotoxic activity of royleanone diterpenes from *Plectranthus madagascariensis* Benth. ACS Omega 4, 8094–8103. doi:10.1021/acsomega.9b00512

Matias, D., Roque, L., Simões, M. de F., Diaz-Lanza, A., Rijo, P., P. Reis, C., 2015. *Plectranthus madagascariensis* phytosomes: formulation optimization. J. Biomed. Biopharm. Res. 12, 223–231. doi:10.19277/bbr.12.2.119

Matlamela, P.F., Kamundi, D.., 2006. *Plectranthus aliciae* (Codd) Van Jaarsv. & T.J. Edwards. [WWW Document]. URL http://redlist.sanbi.org/species.php?species=1671-120 (accessed 3.27.20).

Mortelmans, K., Zeiger, E., 2000. The Ames *Salmonella*/microsome mutagenicity assay. Mutat. Res. - Fundam. Mol. Mech. Mutagen. 455, 29–60. doi:10.1016/S0027-5107(00)00064-6

Nguta, J.M., Appiah-Opong, R., Nyarko, A.K., Yeboah-Manu, D., Addo, P.G.A., Otchere, I., Kissi-Twum, A., 2016. Antimycobacterial and cytotoxic activity of selected medicinal plant extracts. J. Ethnopharmacol. 182, 10–15. doi:10.1016/j.jep.2016.02.010

Ono, K., Hasegawa, K., Naiki, H., Yamada, M., 2004. Curcumin sas potent anti-amyloidogenic effects for Alzheimer's β-Amyloid fibrils *in vitro*. J. Neurosci. Res. 75, 742–750. doi:10.1002/jnr.20025

Parr, A.M., Zoutman, D.E., Davidson, J.S.D., 1999. Antimicrobial activity of lidocaine against bacteria associated with nosocomial wound infection. Ann. Plast. Surg. 43, 239–245. doi:10.1097/00000637-199909000-00003

Patwardhan, B., 2005. Ethnopharmacology and drug discovery. J. Ethnopharmacol. 100, 50–52. doi:10.1016/j.jep.2005.06.006

Peña-Morán, O.A., Villarreal, M.L., Álvarez-Berber, L., Meneses-Acosta, A., Rodríguez-López, V., 2016. Cytotoxicity, post-treatment recovery, and selectivity analysis of naturally occurring podophyllotoxins from *Bursera fagaroides* var. fagaroides on breast cancer cell lines. Molecules 21. doi:10.3390/molecules21081013

Pereira, M., Matias, D., Pereira, F., Reis, C.P., Simões, M.F., Rijo, P., 2015. Antimicrobial screening of *Plectranthus madagascariensis* and *P. neochilus* extract. Biomed. Biopharm. Res. 12, 127–138.

Potgieter, C.J., Edwards, T.J., Miller, R.M., Van Staden, J., 1999. Pollination of seven *Plectranthus* spp. (Lamiaceae) in southern Natal, South Africa. Plant Syst. Evol. 218, 99–112. doi:10.1007/BF01087038

Rabe, T., van Staden, J., 1998. Screening of *Plectranthus* species for antibacterial activity. South African J. Bot. 64, 62–65.

Random Harvest, 2020. *Plectranthus madagascariensis* aliciae [WWW Document]. URL https://www.randomharvest.co.za/South-African-Indigenous-Plants/Show-Plant/PlantId/314?Plant=Plectranthus madagascariensis aliciae (accessed 3.29.20).

Rice, L.J., Brits, G.J., Potgieter, C.J., Van Staden, J., 2011. *Plectranthus*: A plant for the future? South African J. Bot. 77, 947–959. doi:10.1016/j.sajb.2011.07.001

Rijo, P., Duarte, A., Francisco, A.P., Semedo-Lemsaddek, T., Simões, M.F., 2014a. *In vitro*

antimicrobial activity of royleanone derivatives against Gram-positive bacterial pathogens. Phyther. Res. 28, 76–81. doi:10.1002/ptr.4961

Rijo, P., Matias, D., Fernandes, A.S., Simões, M.F., Nicolai, M., Reis, C.P., 2014b. Antimicrobial plant extracts encapsulated into polymeric beads for potential application on the skin. Polymers (Basel). 6, 479–490. doi:10.3390/polym6020479

Rijo, P., Simões, M.F., Francisco, A.P., Rojas, R., Gilman, R.H., Vaisberg, A.J., Rodríguez, B., Moiteiro, C., 2010. Antimycobacterial metabolites from *Plectranthus*: royleanone derivatives against *Mycobacterium tuberculosis* strains. Chem. Biodivers. 7, 922–932. doi:10.1002/cbdv.200900099

Robson, M.C., 1997. Wound infection: A failure of wound healing caused by an imbalance of bacteria. Surg. Clin. North Am. 77, 637–650. doi:10.1016/S0039-6109(05)70572-7

Runtuwene, J., Cheng, K.-C., Amitani 1 Akinori Morinaga, M., Takimoto, Y., Harold Ralph Kairupan, B., Inui, A., 2016. Rosmarinic acid ameliorates hyperglycemia and insulin sensitivity in diabetic rats, potentially by modulating the expression of PEPCK and GLUT4. Drug Des. Devel. Ther. 10–2193. doi:10.2147/DDDT.S108539

Sethi, S., 2020. Acute Bronchitis - Lung and Airway Disorders - MSD Manual Consumer Version [WWW Document]. URL https://www.msdmanuals.com/home/lung-and-airway-disorders/acute-bronchitis/acute-bronchitis (accessed 10.12.20).

Simmons, R.A., 2017. Cell glucose transport and glucose handling during fetal and neonatal development, in: Polin, R.A., Abman, S.H., Rowitch, D.H., Benitz, W.E., Fox, W.W. (Eds.), Fetal and Neonatal Physiology. Elsevier, p. 428–435.e3. doi:10.1016/b978-0-323-35214-7.00043-3

Sitarek, P., Toma, M., Ntungwe, E., Kowalczyk, T., Skała, E., Wieczfinska, J., Śliwiński, T., Rijo, P., 2020. Insight the biological activities of selected abietane diterpenes isolated from *Plectranthus* spp. Biomolecules 10. doi:10.3390/biom10020194

Speert, D.P., 2006. Bacterial infections of the lung in normal and immunodeficient patients. Novartis Found. Symp. 279, 51.

The Plant List, 2012. *Plectranthus madagascariensis* var. ramosior Benth. [WWW Document]. URL http://www.theplantlist.org/tpl1.1/record/kew-158265 (accessed 7.3.20).

Twilley, D., Rademan, S., Lall, N., 2020. A review on traditionally used South African medicinal plants, their secondary metabolites and their potential development into anticancer agents. J. Ethnopharmacol. doi:10.1016/j.jep.2020.113101

Van Jaarsveld, E.J., Edwards, T.J., 1997. Notes on *Plectranthus* (Lamiaceae) from southern Africa. Bothalia 27, 1–6. doi:10.4102/abc.v27i1.647

Wang, H., Guo, X., Hu, X., Li, T., Fu, X., Liu, R.H., 2017. Comparison of phytochemical profiles, antioxidant and cellular antioxidant activities of different varieties of blueberry (*Vaccinium* spp.). Food Chem. 217, 773–781. doi:10.1016/j.foodchem.2016.09.002

Wellsow, J., Grayer, R.J., Veitch, N.C., Kokubun, T., Lelli, R., Kite, G.C., Simmonds, M.S.J., 2006. Insect-antifeedant and antibacterial activity of diterpenoids from species of *Plectranthus*. Phytochemistry 67, 1818–1825. doi:10.1016/j.phytochem.2006.02.018

WHO, 2020. Antibiotic resistance [WWW Document]. URL https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance (accessed 8.1.20).

World Health Organization, 2018. The top 10 causes of death [WWW Document]. URL https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death (accessed 4.21.20).

Worrall, G., 2008. Acute bronchitis. Can. Fam. Physician 54, 238–239.