

DECLARATION

I, the undersigned, declare that this thesis submitted to the University of Fort Hare for the degree of Doctor of Philosophy in Microbiology in the Faculty of Science and Agriculture, School of Science, and the work contained herein is my original work with exemption to the citations and that this work has not been submitted at any other university in partial or entirety for the award of any degree.

Name: _____

Signature: _____

Date: _____

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DEDICATIONS

**This thesis is dedicated to my soul mate and wife (Adeola); my beloved daughter (Precious)
and my wonderful son (Bethel), to God be the glory.**

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GENERAL ABSTRACT

General Abstract

Helichrysum longifolium and *H. pedunculatum* belong to the Astereaceae family and are used extensively in folkloric medicine in South Africa to manage stress-related ailments and as dressings for wounds normally encountered in circumcision rites, bruises, cuts and sores. The *in vitro* antibacterial time-kill studies, the synergistic potentials, the phytochemical screenings and antioxidant potentials as well as the isolation of the bioactive compounds from the extracts of these two plants were carried out in this study.

The *in vitro* antibacterial activities and time kill regimes of crude extracts of *H. pedunculatum* was assessed. The extracts was active against both Gram positive and Gram negative bacteria tested at a concentration of 10 mg/ml. Minimum Inhibitory Concentration (MIC) values for all the susceptible bacteria ranged between 0.1 – 35 mg/ml. The average log reduction in viable cell count in time kill assay ranged between 0.17 Log₁₀ to 6.37 Log₁₀ cfu/ml after 6 h of interaction, and between 0.14 Log₁₀ and 6.99 Log₁₀ cfu/ml after 12 h interaction in 1 × MIC and 2 × MIC of the extract. The effect of the aqueous extract was only bacteriostatic on both reference and environmental strains and the clinical isolates were outrightly resistant to aqueous extract. This is worrisome and this could be one reason why, there is an incidence of high death rate resulting from circumcision wounds infection even after treating such wounds with *H. pedunculatum* leaf.

In vitro antibacterial time kill studies of extracts of *H. longifolium* was assessed. All test bacteria were susceptible to the methanol extract, while none was susceptible to the aqueous extract. Two of the test bacteria were susceptible to the ethyl acetate extract, while ten and seven were susceptible to the acetone and chloroform extracts respectively at the test concentration of 5 mg/ml. The minimum inhibitory concentrations (MICs) ranged between 0.1 and 5.0 mg/ml, while minimum bactericidal concentrations (MBCs) ranged between 1.0 and >5 mg/ml for all the

extracts. Average log reductions in viable cell counts for all the extracts ranged between 0.1 Log₁₀ and 7.5 Log₁₀ cfu/ml after 12 h interaction at 1 × MIC and 2 × MIC. Most of the extracts were rapidly bactericidal at 2 × MIC achieving a complete elimination of most of the test organisms within 12 h exposure time.

The effect of combinations of the crude extracts of *H. pedunculatum* leaves and eight antibiotics was investigated by means of checkerboard and time-kill methods. In the checkerboard method, synergies of between 45.83-56.81% were observed and this is independent of Gram reaction, with combinations in the aqueous extract yielding largely antagonistic interactions (18.75%). The time kill assay also detected synergy that is independent of Gram reaction with a $\geq 3\text{Log}_{10}$ potentiation of the bactericidal activity of the test antibiotics. We conclude that the crude leaf extracts of *H. pedunculatum* could be potential source of broad spectrum antibiotics resistance modulating compounds.

The interactions between crude extracts of *H. longifolium* in combination with six first-line antibiotics using both the time-kill and the checkerboard methods were carried out. The time-kill method revealed the highest bactericidal activity exemplified by a 6.7 Log₁₀ reduction in cell density against *Salmonella* sp. when the extract and Penicillin G are combined at $\frac{1}{2} \times \text{MIC}$. Synergistic response constituted about 65%, while indifference and antagonism constituted about 28.33% and 6.67% in the time kill assay, respectively. The checkerboard method also revealed that the extracts improved bactericidal effects of the antibiotics. About 61.67% of all the interactions were synergistic, while indifference interactions constituted about 26.67% and antagonistic interactions was observed in approximately 11.66%.

The *in vitro* antioxidant property and phytochemical constituents of the aqueous crude leaf extracts of *H. longifolium* and *H. pedunculatum* was investigated. The scavenging activity on superoxide anions, DPPH, H₂O₂, NO and ABTS; and the reducing power were determined, as well as the flavonoid, proanthocyanidin and phenolic contents of the extracts. The extracts exhibited scavenging activity in all radicals tested due to the presence of relatively high total phenol and flavonoids contents in the extracts. Our findings suggest that *H. longifolium* and *H. pedunculatum* are endowed with antioxidant phytochemicals and could serve as a base for future drugs.

Bioactivity-guided fractionation of the leaves of *H. longifolium* and *H. pedunculatum* yielded two known compounds. From the n-hexane fraction of *H. longifolium* a compound was isolated (Stigmasterol) and from the ethyl acetate fraction of *H. pedunculatum* another compound (β -sitosterol) was isolated. The compounds were isolated and identified using various techniques. The antimicrobial, anti-inflammatory, antioxidant, analgesic and anti-pyretic activities of these compounds have been reported in literatures.

In general, the experiments and tests conducted in this study appear to have justified the folkloric medicinal uses of *H. longifolium* and *H. pedunculatum* for the treatment of stress related ailments and wound infections and make a substantial contribution to the knowledge base of the use of herbal medicine for the treatment of the microbial infections.