

# **INFLUENCE OF ENVIRONMENTAL PARAMETERS ON EFFICACY OF HERBAL MEDICINES**

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## Declaration

The research work that generated data used in this PhD thesis was carried out in the Vegetable and Ornamental Plant Institute of the Agricultural Research Council (ARC), Experimental Farm and laboratory (Phytomedicine Programme at Onderstepoort) of the University of Pretoria, between 2003 and 2010, under the supervision of Professor Kobus N. Eloff.

I declare that all the work outlined in the thesis submitted to the University of Pretoria for the degree of Doctor of Philosophy is a result of my own effort, and that the work of others cited in this thesis is duly acknowledged, and this work has not in any form been previously submitted by me for a degree or any other qualification at this or other academic institutions.



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PhD CANDIDATE

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*“...but with God all things are possible”*. Matthew 19:26.

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## Abstract

It is evident that herbal medicines continue to be the mainstay of healthcare systems and source of livelihoods of many local communities in South Africa and other developing countries. As a result, there is an overwhelming dependence on medicinal products harvested from natural populations. This dependence has led to local extinction of some important medicinal plants that include *Warburgia salutaris* and *Cassine transvaalensis* in South Africa. Cultivation has great potential to relieve the pressure on natural populations. However, some traditional practitioners and scientists believe that cultivation may weaken medicinal properties and that increased secondary metabolites may form only under stress conditions, respectively. This is certainly true in some cases especially where infections with pathogens, browsing by herbivores or competition takes place in nature. It is however not clear how true this is with environmental stresses. The overall aim of this study was to evaluate to what degree different environmental conditions influenced antimicrobial and antioxidant activities of plants cultivated outside their natural environment.

In order to address the aim of the study, exploratory and in-depth studies were undertaken. The exploratory study comprised long-lived *Combretum collinum* Fresen. (Combretaceae), *Terminalia sericea* Burch. ex DC. (Combretaceae) and *Sclerocarya birrea* (A. Rich.) Hochst. (Anacardiaceae). Short-lived herbaceous *Tulbaghia violacea* Harv. (Alliaceae) and *Hypoxis hemerocallidea* Fish., C.A.Mey. & Avé-Lall. (Hypoxidaceae), were included as part of the exploratory study. The in depth studies were further undertaken, also with short-lived herbaceous *Leonotis dysophylla* Benth. (Lamiaceae), *Bulbine frutescens* (L.) Willd. (Asphodelaceae) and *T. violacea*. Acetone leaf extracts of all plants were studied for antimicrobial activity against bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Enterococcus faecalis*) and fungi (*Candida albicans*, *Cryptococcus neoformans* and *Aspergillus fumigatus*). Extracts were also studied for antioxidant activity against Trolox and L-ascorbic acid standard oxidants using 2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-2-picryl-hydrazyl (DPPH) free radicals, respectively.

The exploratory study tested the effect of different rates of annual rainfall ( $\geq 870$  mm/year, 651 mm/year and 484 mm/year) on the antibacterial activity of *C. collinum*, *T. sericea* and *S. birrea* growing in nature.

The minimum inhibitory concentration (MIC) of acetone extracts of air-dried leaves was determined by using microplate serial dilution technique. Thin layer chromatography (TLC) and bioautography determined chemical constituents and antibacterial activity of extracts, respectively. The majority of extracts had low MIC values, which indicated good antibacterial activity against test bacteria (MIC of 240 µg/ml - 60 µg/ml). Leaf extracts of *C. collinum* and *S. birrea* against *S. aureus* (range of 390 – 100 µg/ml), *E. coli* (310 -70 µg/ml) and *P. aeruginosa* (520 - 70 µg/ml) had antibacterial activity increased significantly with low rate of annual rainfall. However, extracts of *T. sericea* against *P. aeruginosa* (240 - 100 µg/ml) and *E. faecalis* (150 - 820 µg/ml) had antibacterial activity significantly increased and decreased, respectively. Extracts of *C. collinum* and *S. birrea* against *E. faecalis* as well as *T. sericea* against *S. aureus* and *E. coli* did not show any clear correlation between activity and different rates of annual rainfall. Inconsistent results suggest that other factors in nature such as genetic variability, age difference, pathogens, herbivores or allelopathy (competition) might have influenced the antibacterial activity of extracts. The results indicate that the antimicrobial activity of plants growing in nature may be highly variable.

In order to eliminate possible effect of those factors common in nature, another exploratory study was undertaken using clone *T. violacea* and *H. hemerocallidea* of similar age (Chapter 3). Plants were grown under controlled conditions that included irrigation with 1000 ml of distilled water in intervals of 3, 14 and 21 days outside natural environment. Dry mass of all plants was reduced significantly ( $P \leq 0.05$ ) with watering interval of 21 days, which indicated the effect of water stress. Air-dried leaves of all plants were finely ground and extracted with acetone. Extracts had good antibacterial activity as attested by low MIC values ( $< 1$  mg/ml) across watering intervals. Differences in the antibacterial activity of the extracts against test bacterial between water treatments were not statistically significant ( $P \leq 0.05$ ). Furthermore, there was no clear correlation between the activity of extracts and water treatments in terms of the MIC and total activity values or chemical constituents. The results in general suggest that cultivation under optimal watering intervals may not necessarily weaken the biological activity of extracts.

To complement the above findings, in depth studies were also undertaken with clone *L. dysophylla*, *T. violaceae* and *B. frutescens* of similar age growing under controlled conditions outside natural environment. The studies determined the influence of a wide range of water (50 ml – 500 ml) and temperature (15°C and 30°C) treatments on antibacterial, antifungal and antioxidant of extracts. With

the exception of a crassulacean acid metabolism (CAM) plant, *B. frutescens*, transpiration, dry mass and leaf areas of the other two plants were reduced significantly ( $P \leq 0.05$ ) under high temperature of 30°C and lowest water supply of 50 ml. Acetone leaf extracts had some biological activity. Differences in the majority of antibacterial and antifungal activities of extracts between water and temperature treatments were not statistically significant. With the exception of the influence of temperature, the majority of the antioxidant activity of extracts was almost similar between water treatments. However, the significant reduction of the antioxidant activity of all extracts under high temperature of 30°C was indicative of great sensitivity to high temperatures.

The overall findings suggest that the biological activity of plants is more likely to vary widely in nature than under controlled conditions outside the natural environment. This is an indication that natural environment cannot always guarantee high and stable biological activity. As a result, beliefs by some traditional practitioners and scientists that cultivation weakens medicinal properties and good secondary metabolites form only under stress, respectively, cannot be widely substantiated. Therefore, the study encourages cultivation of medicinal plants. It has potential to optimise yield of biomass production, and ensure uniform and quality biological activity as well as reduce misidentification.

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## List of Acronyms

ABTS:	2,2-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)
AIDS:	Acquired immunodeficiency syndrome
ANOVA:	Analysis of variance
AOXA:	Antioxidant activity
ARC:	Agricultural Research Council
BEA:	Benzene:ethyl acetate:ammonia (non-polar mobile system)
CAM:	Crassulacean Acid Metabolism
CEF:	Chloroform/ethyl acetate/formic acid (intermediate mobile system)
COHRED:	Council on Health Research for Development
DPPH:	2,2, diphenyl-2-picryl-hydrazyl
EMW:	Ethyl acetate:methanol:water (polar mobile system)
FAO:	Food and Agriculture Organization
GTZ:	Gesellschaft für Technische Zusammenarbeit (German Company for International Cooperation)
HIV:	Human immunodeficiency virus
IC <sub>50</sub> :	Inhibitory concentration (concentration of the sample leading to 50% reduction of the initial DPPH concentration)
INT:	Iodonitrotetrazolium

MIC:	Minimum Inhibitory Concentration
NEPAD:	New Partnership for Africa's Development
R <sub>f</sub> :	Retardation factor
ROS:	Reactive oxygen species
SADC:	Southern African Developing Countries
SD:	Standard deviation
SE:	Standard error
TA:	Total activity
TEAC:	Trolox equivalent antioxidant capacity
TLC:	Thin Layer Chromatography
TMPSSP:	eThekweni Medicinal Plant Sector Support Programme
TRAFFIC:	Trade Records Analysis of Flora and Fauna in Commerce
UV:	Ultraviolet
VOPI:	Vegetable and Ornamental Plant Institute of the ARC
WHO:	World Health Organization