Identification of Commonly Used Traditional Medicines by Planar Chromatography for Quality Control Purposes

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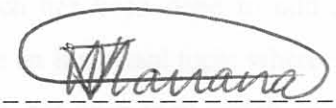
Declaration

I declare that this dissertation is my own unaided work conducted under the supervision of Prof. J. N. Eloff. It is submitted to the Department of Pharmacology, Faculty of Health Sciences, University of Pretoria, Pretoria, for the Degree of Magister Scientiae. It has not been submitted before for any degree or examination in any other University.



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Abstract

South Africa contains more than 9% of the world higher plant species diversity, of which many are used to treat human and livestock health problems. Unfortunately, the motivation to put medicinal plants on the market is frequently not to provide an essential service but to make money. In some overseas countries falsification of selling plant materials adulterated with cheaper products by dishonest traders is a problem. In South Africa a number of patients have become ill or died after using wrong plant or dose. It is frequently difficult or impossible to identify a plant from the root, bulb or bark form sold in most markets. Thus far, not much has been done to address this problem. Quality control of medicinal plants is therefore an important topic wherever traditional or herbal medicines are used. Planar chromatography has been used widely to verify the identity of phytomedicines used in the western herbal industry. Thin layer chromatography (TLC) atlases are available for many herbal medicines. This study proposes to use TLC to verify the identity of plant species of the bark and bulb material sold in Pretoria informal market.

After interviewing herbal traders and healers, plants were selected on the basis of availability, cost, toxicity and accessibility. Reference plant materials based on the traditional name provided were obtained from the Pretoria National Botanical Garden and Agricultural Research Council. Powdered material of the market and reference species were extracted with three solvents (ethanol, acetone and hexane) and final crude extracts separated in three TLC systems (polar, intermediate and non-polar systems) and the compound composition was detected using three spray reagents. As an indication of biological activity, the antibacterial activity of the selected materials was determined using four bacteria by bioautography and minimum inhibitory concentration methods.

In general, the plant materials sold by different traders in Pretoria had a similar chemical profile to the reference samples, although there were variations in the chemical profile of the same species from different areas. Although TLC technique is useful in the identification of traditional plants, it is not able to differentiate closely related plant species due to the similarities in chemical compositions and slight variation. Environmental factors did not have a major impact on the on the chemical composition of *Artemisia afra*. In conclusion, identification of traditional medicines by planar chromatography is possible, although it may be complicated by chemical variation and geographic differences. Planar chromatography can also be used to determine the magnitude of adulteration in markets that sell African traditional medicines and to determine the identity of illegally collected over-

exploited plant medicines. It therefore appears that plants sold in the Pretoria Traditional medicine market are correctly identified.

Samevatting

Suid-Afrika beskik oor meer as 9% van die wêreld se hoërplant spesies. Baie van hierdie plante word gebruik vir behandeling van mens- en diersiektes. Dikwels is die doel van verskaffers van medisinale plante eerder om geld te maak en nie om 'n essensiële diens te lewer nie. Omdat dit prakties onmoontlik is om gemaalde plantprodukte te identifiseer, het dit dikwels in die buiteland gebeur dat die etiket van westerse kruiemedisyne nie ooreenstem met die plantmateriaal in die houer nie. In Suid-Afrika is pasiente al vergiftig omdat die verkeerde plant of dosis gebruik is. Dit is dikwels onmoontlik om 'n plant van die wortel, bol of bas wat verkoop word, te identifiseer. Min is tot dusver gedoen om die omvang van die probleem aan te spreek. Gehaltebeheer van tradisionele medisinale plante is gevolglik 'n belangrik onderwerp. Dunlaag chromatografie [DLC] is al in die buiteland gebruik vir die identifisering van westerse kruiemedisyne en DLC atlasse is beskikbaar. In hierdie studie is beplan om DLC te gebruik om te bepaal tot watter mate die plante wat in Pretoria verkoop word, korrek geïdentifiseer is.

Na onderhoude met verkopers en gebruikers van tradisionele plantmedisyne, is plante uitgesoek op basis van beskikbaarheid, koste, toksisiteit en gemak om te versamel. Verwysings plantmateriaal is vanaf die Pretoria Nasionale Botaniese Tuin en Landbou-navorsingsraad verkry. Gemaalde materiaal is ge-ekstraheer met drie ekstraheermiddels met verskillende polariteite en geskei deur silika gel dunlaagchromatografie. As maatstaf van biologiese aktiwiteit is antibakteriese aktiwiteit bepaal deur minimum inhiberende konsentrasie te bepaal met vier bakterieë. Deur bio-outografie is die verskeidenheid en eienskappe van die inhiberende verbindings ook bepaal.

Oor die algemeen het die chemiese profiel en antibakteriese aktiwiteit van die materiaal wat verkoop is, ooreengestem met die verwysingsmateriaal. Hoewel DLC bruikbaar is in die identifikasie van verskillende plante werk dit nie goed vir na-verwante plante nie. Omgewings faktore het nie 'n baie groot invloed gehad op die chemiese samestelling van *Artemisia afra* nie. DLC was ook bruikbaar om plante wat bedreig is en onwettig versamel is te identiseer vir moontlike vervolging van oortreders. Dit blyk dus dat plante wat op die Pretoria tradisionele medisyne mark verkoop word, korrek geïdentifiseer is.

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Table of Contents

List of figures

List of tables

Abbreviations

µl:	Micro liter
A:	Agricultural Research Council sample
AA:	Acetone-acetone extract
ACBB:	*Acacia caffra* Pretoria National Botanical Garden sample
Ace:	Acetone
ACMB:	*Acacia caffra* market sample
AE:	ARC ethanol extract
AH:	ARC hexane extract
ArAg:	*Artemisia afra* private garden sample
ArAml:	*Artemisia afra* maize land sample
ArARC:	*Artemisia afra* Agricultural Research Council sample
ArAveld:	*Artemisia afra* veldt sample
ArBB:	*Artemisia afra* Pretoria National Botanical Garden sample
ArBG:	*Artemisia afra* Pretoria National Botanical Garden sample
ARC:	Agricultural Research Council
ArDg:	*Artemisia afra* market sample
B:	Pretoria National Botanical Garden sample
BA:	PNBG acetone extract
BAC:	PNBG *Acacia caffra*
BAK:	PNBG *Acacia karoo*
BAM:	PNBG *Acacia Montana*
BE:	PNBG ethanol extract
BEA:	Benzene, ethanol and ammonium in ratio of 9:1:0.1 respectively
BH:	PNBG hexane extract
BHAb:	*Boophane haemanthoides* Agricultural Research Council sample
BHMb:	*Boophane haemanthoides* market sample
BPA:	PNBG *Peltophorum africanum*
CEF:	Chloroform, ethyl acetate and formic acid in ratio of 5:4:1 respectively
CMM:	Raw Chinese Medicinal Material
COX-1:	Cyclo-oxygenase-1
CPM:	Chinese Proprietary Medicine
CSBB:	*Croton sylvaticus* Pretoria National Botanical Garden sample

CSMB:	*Croton sylvaticus* market sample
CTM:	Chinese Traditional Medicine
D:	Fertilized soil sample
E. coli:	*Escherichia coli.*
EA:	Ethanol-acetone extract
EE:	Ethanol-ethanol extract
EMW:	Ethyl acetate, methanol and water in ratio of 10:1.35:1 respectively
Entero:	*Enterococcus faecalis*
EtOH:	Ethanol
GC:	Gas chromatography
H:	Shade sample
HA:	Hexane-acetone extract
Hex/Hax:	Hexane
INT:	ρ-iodonitrotetralium violet
J:	Pyrethrum-treated sample
L:	Home garden sample
LC:	Liquid chromatography
M:	Maize land sample
MA:	Market acetone extracts
MAC:	Market *Acacia caffra*
ME:	Market ethanol extract
mg/ml:	Milligram per milliliter
MH:	Market hexane extract
MIC:	Minimum inhibitory concentration
MPA:	Market *Peltophorum africanum*
N:	Seed source sample
NP/PEG:	1% Diphenylboric acid –2 amino ethyl ester in methanol/ 5% Polyethylene glycol
PABB:	*Peltophorum africanum* Pretoria National Botanical Garden sample
PAMB:	*Peltophorum africanum* market sample
PNBG:	Pretoria National Botanical Garden
Psuedo:	*Psuedonomas aeruginosa*

RA:	Reference acetone extract
RE:	Reference ethanol extract
R_f value:	A ratio of the distance from the origin to the center of the separated zone divided by the distance from the origin to the solvent front.
RH:	Reference hexane extract
RP:	Reverse phase
rpm:	Revolutions per minute
S:	SeSotho
SATM:	South African Traditional Medicine
Staph:	*Staphylococcus aureus*
T:	Private garden sample
TLC:	Thin layer chromatography
UV:	Ultraviolet
V:	Veldt sample
WSBE:	*Warburgia salutaris* Pretoria National Botanical Garden sample
WSLE:	*Warburgia salutaris* Pretoria National Botanical Garden leaf sample
WSMB:	*Warburgia salutaris* market sample
Z:	IsiZulu

Conference presentations

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2. J Manana and J. N. Eloff [Paper] January 2001. The use of planar chromatography to identify commonly used herbal medicines in the Pretoria area. Conference South African Association of Botanists. Rand Afrikaans University, Johannesburg, South Africa.
3. J. N. Eloff and J. Manana [Poster] April 2002 Analysis of plant metabolomic of traditional medicines. First international Plant Metabolomics Congress, Wageningen, Netherlands.
4. J. Manana and J. N. Eloff [Poster] July 2002. The use of planar chromatography to evaluate traditional medicines in the Pretoria area. Indigenous Plant Use Form, Pine Lodge, George, South Africa.
5. J. N. Eloff and J. Manana [Poster] January 2003. The use of planar chromatography to identify commonly used herbal medicines in the Pretoria area. South African Association of Botanists and The international society of Ethnopharmacology conference. Pretoria University, Pretoria, South Africa