Declaration

I hereby declare that the MSc thesis *VARIATION IN CHEMICAL COMPOSITION OF HARPAGOPHYTUM SPECIES AS A FUNCTION OF AGE AND LOCALITY* that I submit to the University of the Free State is my own independent work and has never been previously submitted for a qualification at/in another University or faculty.

I cede my copyright in the thesis to the University of the Free State.

MAFEREKA FRANCIS TYSON MOSOABISANE

DATE: 14 December 2009

VARIATION IN CHEMICAL COMPOSITION OF *HARPAGOPHYTUM* SPECIES AS FUNCTION OF AGE AND LOCALITY

Thesis submitted in fulfilment of the requirements for the degree

Master of Science

in the

Department of Chemistry
Faculty of Agricultural and Natural Science

at the
University of the Free State
Bloemfontein

by

MAFEREKA FRANCIS TYSON MOSOABISANE

Supervisor: Prof. J. H. van der Westhuizen
Co-supervisors: Dr. G. Kemp and Dr. P. C. Zietsman

December 2009

ACKNOWLEDGEMENTS

I wish to express my special thanks to those who have been part of this long journey. Most of them left lasting impression and deserve more than my gratitude.

Firstly, I thank God for grace, strength and blessings to finish this study.

To my supervisor Prof. J.H. van der Westhuizen for his guidance, assistance, perseverance and invaluable advice to become an innovative researcher.

Co-supervisor Dr. G. Kemp for the guidance, assistant and the analytical skills I learned from him.

To Dr. P. C. Zietsman for all the field work we have done together.

I thank North West Department of Agric Conservation and Environment and all the harvesters living in Cassel, Ganyesa and Moswana who sacrificed their time to help us in digging of the plants. My tour to Kalahari Desert has been wonderful through their assistance.

To Prof. H. F. Steyn from the Statistical Consultation Service of the North West University at Potchefstroom, your help with the statistical analysis is much appreciated.

For the financial support I got from RTFP, University of the Free State (Cluster) and Mimosa Central Co-operative Ltd.

The staff and co-students who made the laboratory environment better in the Chemistry Department. I really thank everyone who made the difference and gave constant encouragement to finish this study.

A special thanks to my parents ‘Mabatho and Motlelentoa for their continued love, support and interest in my studies. My family members who have always been strive for my success.

M. F. T. Mosoabisane

Table of contents

9. Appendix A: Raw data of the analysed samples by HPLC

10. Appendix B: Additional statistical analysis for chapter 4

11. Appendix C: Additional statistical analysis for chapter 5

12. Appendix D: Additional statistical analysis for chapter 6

13. Appendix E: Additional statistical analysis for chapter 7

List of abbreviations

Ach	Acetylcholine
ANOVA	Univariate analysis of variance
APCI	Atomic Pressure Chemical Ionization
$BaCl_2$	Barium Chloride
BCL	Bicuculline
C-18	Carbon 18
CAP	Caprivi
CASS	Cassel
CHO	Carbohydrate
CO_2	Carbon dioxide
COX-2	Cyclooxygenase-2
DAD	Diode Array Detector
DC	Devil's Claw
df	Degrees of freedom
DIC	Diclofenac
ESI	Electron Spray Ionization
F	F Test in statistic
FERRO	Ferrolands
GABAergic	Gamma-Amino butyric Acid
GAN	Ganyesa
GPS	Global positioning System
HepG2	Hepatocellular Carcinoma
HPLC	High Performance Liquid Chromatography
HPTLC	High Performance Thin Layer Chromatography
IC_{50}	Inhibitory Concentration of 50%
iNOS	Inducible Nitric Oxide Synthase
IL-6	Interleukin-6 inhibitor
IL-1β	Interleukin 1β
K1	Chloroquine-resistant
LC	Liquid Chromatography
LPS	Lipopolysaccharide

LT	Cysteinyl-Leukotriene
MAKGA	Makgabeng
MANOVA	Multivariate analysis of variance
MEOH	Methanol
Min	Minutes
M-NR	Molopo Nature Reserve
MPS	Multidimensional Pain Scale
MOS	Moswana
MS	Mass Spectrometer
Ms	Mean square in statistics
NF-κB	Nuclear Factor Kappa Beta
NIR-FT	Near Infrared Fourier Transformer
NIRS	Near Infrared Spectroscopy
NMR	Nuclear Magnetic Resonance
NSAID	Non-Steroidal Anti-inflammatory Drugs
NWDACE	North West Department of Agric Conservation and Environment
P	Probability
PCT	Picrotoxin
PGE_2	Prostaglandin E_2
PTFE	Precise Time and Frequency Equipment
PTZ	Pentylenetetrazole
% RSD	Percentage Relative Standard Deviation
SHDC	Sustainable Harvested Devil’s Claw
SPE	Solid Phase Extraction
SPRING	Springbokfontein
SS	Sum of squares
Terra F	Terra Firma
TNFα	Tumor Necrosis Factor-Alpha
TPA	12-O-Tetradecanoylphorbol-B-acetate
TX	Thromboxane
UV	Ultra Violet
VAS	Visual Analogue Scale
WOMAC	Western Ontario and McMaster Osteoarthritis index
Zim	Zimbabwe

List of tables

List of figures

Summary (English)

Dried tubers, growing on secondary roots of *Harpagophytum procumbens* subsp. *procumbens* are widely used as an analgesic and anti-inflammatory medicine against arthritis and other chronic pain conditions. Tubers are harvested in large quantities from South Africa, Botswana and Namibia. The dried material is exported to European countries. This industry makes a big contribution to poverty alleviation in rural areas. The large quantities exported (about 600 tons per annum), has raised concerns about the sustainability of wild harvesting. Apart from threatening the plant with extinction, overexploitation of the resource will also threaten the economic survival of rural communities.

This thesis aims to study the variation in chemical composition between samples from different regions, species, harvesting regimes and age of tubers. Only tubers from secondary roots are removed and a large percentage of harvested plants survive. This thesis is part of a bigger study of which the aim is to determine the optimum wild harvesting regime resulting in the best yield of the active ingredient in a sustainable harvesting industry.

Plant material (tubers) was collected from December 2007 to February 2008 in the North West Province, Limpopo Province, Namibia, Caprivi in Zimbabwe. In the North West Province, plant materials were collected in 6 different areas and/or farms: Cassel, Ganyesa, Moswana, Molopo Nature Reserve, Terra firma and Lafras. Samples from Cassel represents tubers from the same plants with different inter harvest periods (one to five years).

The freshly collected tubers were sliced, sun dried and analysed with HPLC-UV for six different analytes (harpagide, harpagoside, 8-*p*-coumaroylharpagide, verbascoside, isoverbascoside and 6-acetylacteoside). These analytes can be divided into two structurally related groups.

An analytical method was developed to quantify the six analytes routinely. The method is based on water and methanol as eluent and a reverse phase analytical column. A stepwise isocratic procedure (3% MeOH for 1 min, 50% MeOH for 20 min, column cleanup with 95% MeOH for 5 min and regeneration with 3% MeOH for 5 min) was found to be the best for our

purposes. The method was validated for specificity, accuracy, precision, robustness and linearity. Internal standards were used for calibration.

The data from more than one thousand analyzed samples were statistically processed using StatSoft, inc. (2008), STATISTICA, version 8.0. to answer the following questions:

1. Are there meaningful differences in chemical composition between populations from the five different inter harvest periods (1 to 5 years) at Cassel in the North West Province (Are there meaningful differences in chemical composition of tubers that are one, two, three, four or five years old?).
2. Are there meaningful differences in chemical composition between the six populations from the North West Province (Cassel, Ganayesa, Moswana, Terra Firma, Lafras and Molopo Nature Reserve)?
3 Are there meaningful differences in the chemical composition of populations from North West Province and Namibia?
4. Are there meaningful differences in the chemical composition of populations from North West Province/ Namibia (*H. procumbens* subsp. *procumbens*, from the Northern Province (*H. zeyheri* subsp. *zeyheri*) and from Zimbabwe (Victoria Falls) (*H. zeyheri* subsp. *sublobatum*)?

Factor analysis of the six variables (harpagide, harpagoside, 8-*p*-coumaroylharpagide, verbascoside, isoverbascoside and 6-acetylacteoside) yielded two, three or four factors depending on the level of degree of variance that we required. Multivariate and univariate analysis (MANOVA and ANOVA) of normal and transformed data indicated highly significant differences. Levine’s test was used to test homogeneity of variances.

In correlation analysis all six variables were used because the factors did match clusters based on the chemical structure of the six variables. In all four experiments significant variations were observed and described.

Cluster analysis, using scatter plots of three factors (factor 1: verbascoside, isoverbascoside and 6-acetylacteoside, factor 2: harpagide and factor 3: 8-*p*-coumaroylharpagide) identified three distinct populations. These three populations are from three different geographical regions and correspond with the corresponding taxonomic classification of the different populations. (*H. zeyheri* subsp. *zeyheri* from the Limpopo Province, *H. zeyheri* subsp.

sublobatum from Zimbabwe (Victoria Falls) and Captivi and *H. procumbens* subsp. *procumbens* from the North West province and Namibia.

Summary (Afrikaans)

Gedroogte sekondêre wortels van *Harpagophytum procumbens* subsp. *procumbens* (Burch.) de Candolle ex Meissner word algemeen vir medisinale gebruik geneem vir pynverligtende en anti-inflamatoriese werking teen artitis en ander chroniese pyn toestande. Die plant groei wild in veral Suid Afrika, Botswana en Namibïe waar die oes en verkope hiervan 'n groot bydrae maak tot armoede verligting in arm afgeleë gebiede. Na raming word tot 600 ton gedroogte wortels per jaar na Europa uitgevoer en ontstaan die vrese dat hierdie wild groeiende plant se volhoubare ontginning bedreig word. Nie alleen kan oorontginning die plant uitwis nie, maar dit kan die ekonomiese oorlewing van arm gemeenskappe bedreig wat hierop berus as bron van inkomste.

Die doel van hierdie projek was om die chemiese samestelling te ontleed van a) wortels uit verskillende streke, b) wortels van verskillende *Harpagophytum* spesies, c) die gereeldheid van oesting en d) plant ouderdom. Hierdie tesis vorm deel van 'n groter projek om die oes kondisies te bepaal wat sal lei tot die produksie van wortels met verhoogde vlakke van aktiewe komponente met medisinale waarde, met 'n oog op volhoubare verbouing van die plant.

Plant materiaal is tussen Desember 2007 en Februarie 2008 versamel in die Noord Wes Provinsie, Limpopo Provinsie, Namibia, Caprivi en Zimbabwe. Die plante is oopgegrawe en slegs die sywortels is versigtig geoes om nie die voortbestaan van die plant te benadeel nie. Die vesamelde wortels is verwerk en met HPLC geanaliseer en die vlakke van ses verskillende analiete (harpagied, harpagosied, 8-*p*-rumaroiehylharpagied, verbaskosied, isoverbaskosied en 6-asetielakteosied) is gekwantifiseer.

Meer as 'n duisend datapunte is statisties ontleed om te bepaal of daar betekenisvolle verskille is in die chemiese samestelling van sywortels is wat:

van een tot vyf jaar oud is vanaf dieselfde lokaliteit?
vanuit ses verskillends populasies in die Noord Wes Provinsie?
vaniut die Noord Wes Provinsie en Namibia?

vanuit die Noord Wes Provinsie/Namibie, die Limpopo Provinsie en Zimbabwe?

Die rekenaar program, *Statistica*, is gebruik vir die analisering van die data en het ingesluit die faktor analise van die ses veranderlikes (die ses geanaliseerde analiete) wat twee, drie en vier faktore onderskeidelik opgelewer het. Multivariate en univariate analises van ongetransformeerde en getransformeerde data het hoogs betekenisvolle verskille uitgewys, terwyl korrelasie analises betekenisvolle variasies uitgewys het. Kluster analises het drie diskrete populasies uitgewys wat korreleer met die diskrete geografiese areas waar materiaal versamel is asook die verskillende spesies wat in hierdie areas aangetref word.

1. Introduction

Dried tubers, growing on secondary roots of *Harpagophytum procumbens* subsp. *procumbens* are widely used as an analgesic and anti-inflammatory medicine against arthritis and other chronic pain conditions. Tubers are harvested in large quantities from South Africa, Botswana and Namibia. The dried material is exported to European countries. This industry makes a big contribution to poverty alleviation in rural areas. The large quantities exported (about 600 tons per annum), have raised concerns about the sustainability of wild harvesting. Apart from threatening the plant with extinction, overexploitation of the resource will also threaten the economic survival of rural communities. *H. zeyheri* (Decne.) subsp. *sublobatum* is sometimes regarded as an alternative to *H. procumbens,* despite the fact that some populations contain less harpagoside.

This thesis aims to study the variation in chemical composition between samples from different regions, species, harvesting regimes and age of tubers. Only tubers from secondary roots are removed and a large percentage of harvested plants survive. This thesis is part of a bigger study of which the aim is to determine the optimum wild harvesting regime resulting in the best yield of the active ingredient in a sustainable harvesting industry.

This study aims primarily to determine the chemical variation within local populations and between different populations as part of a bigger study to determine the chemical variation over the entire population range. The second aim is to differentiate between *Harpagophytum* species by analyzing the chemical composition statistically. The results are important to maximise sustainable production of the active ingredient from wild populations and to ensure a long term continued harvesting.

2. Literature review

2.1 Introduction

Dried secondary roots of *Harpagophytum procumbens* subsp. *procumbens* (Burch.) de Candolle ex Meissner (Ihlenfeldt and H. Hartman[1]) are widely used to treat arthritis and as a tonic by indigenous people in the countries around the Kalahari region in Southern Africa. Wild plants are harvested and exported in large quantities to European countries. This has raised concerns in the past about the sustainability of wild harvesting of *H. procumbens* which is part of natural resource management in the African continent[2].

The putative analgesic and anti-inflammatory properties are usually ascribed to iridoid glucosides (mainly harpagoside with smaller quantities of harpagide)[3]. Commercial products contain between 0.5 and 3% harpagoside. The extracts are often more potent than pure iridoids indicates that other compounds may play a role[4].

2.2 Ecology and biology

Harpagophytum de Candolle ex Meissene (Pedaliaceae) is commonly known as grapple, wood spider, kamaku, kanako, sengaparile or Devil’s claw. The vernacular name of the plant is derived from the appearance of the fruit (figure **2.1a**) which has numerous long arms with hooked thorns[5]. This is a weedy, prostrate perennial plant with opposite or sub opposite leaves and creeping stems spreading from large tuberous rootstock (figure **2.1b**)[5]. The plant is a geophyte. It grows in summer and dies back during winter. There are two species of the plant namely *H. procumbens* and *H. zeyheri*. *H. procumbens* has two subspecies: *H. procumbens* subsp. *procumbens* and *H. procumbens* subsp. *transvaalense*[1]. *H. zeyheri* has three subspecies: *H. zeyheri* subsp. *schijffii*[1], *H. zeyheri* subsp. *sublobatum* (Engler)[1] and *H. zeyheri* subsp. *zeyheri* Decaisne.

The tubular flowers of both species are similar in appearance and range in colour from pale yellow (almost white) to violet or dark violet[5,6] (figure **2.2**). *Harpagophytum* species bear flowers and leaves during summer[7]. In *H. procumbens*, the arms of the fruit are longer than its width and, in *H. zeyheri*, the arms are mostly shorter than the width of the fruit or sometimes as long[8]. It is believed that the fruit is dispersed by animals[9].

H. procumbens plants sprout shortly after the first summer rains, (usually from October) and flower from November to April. Fruits are borne from December until the end of April when the plants start to die back as reported by Zietsman and Pelser[10].

H. procumbens is cross-pollinated by carpenter bees. Numerous other insects such as beetles and weevils have been observed entering the tubular flowers but are regarded as nectar and pollen robbers as they are too small to act as pollinators to pollinate the flowers[10].

Both species have black oblong seeds[5]. Seed germination is unpredictable and slow, requiring optimal conditions in terms of the soil and moisture. The seeds are well adapted for drought conditions. About twenty-five percent of seeds released make contact with the soil[11]. In addition, the seeds have a slow respiration rate, allowing them to survive under the dry conditions of the Kalahari for 20 years. In contrast, a high germination rate of 54% was achieved after seeds from Glen Agricultural College (Bloemfontein) were germinated at a constant temperature of 25°C[12]. The seeds were also found not to be light sensitive[12].

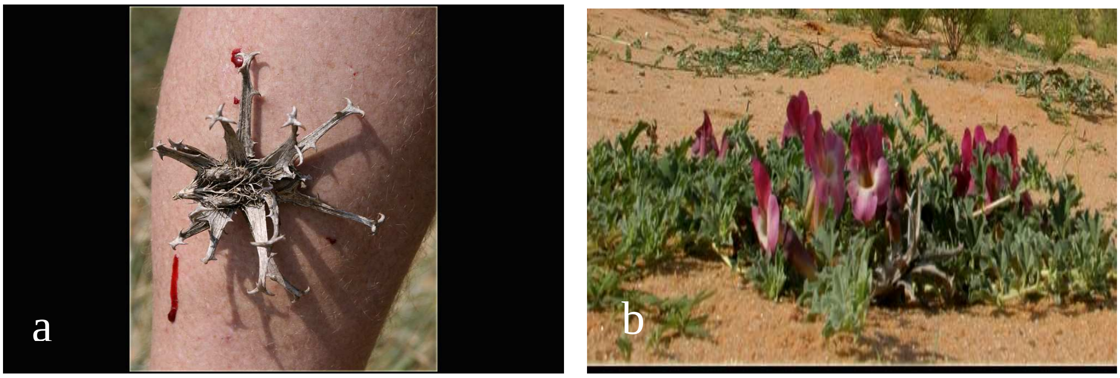


Figure **2.1:** **a** Shows the fruit and **b** shows the *H. procumbens* on top of the soil. (Photos by Dr. Zietsman)

Figure **2.2:** *H. procumbens* leaves and flowers. (Photos by Dr. Zietsman)

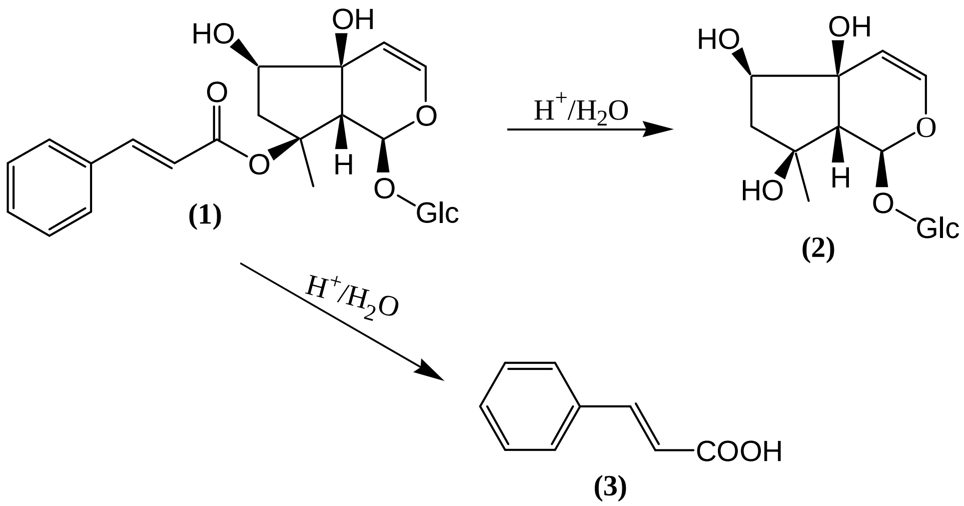
Secondary roots branch from tap root (called the "mother tuber" by harvesters). Tubers are formed in the secondary roots and quite often they develop into a chain of tubers[6]. Secondary roots store 46% stachyose which helps the plant to survive during droughts[7].

The tap roots can grow as deep as 90 cm with secondary tubers as long as 25cm and up to 6cm thick[7]. Hachfeld and Schippmann[12] reported that *H. procumbens* usually grow in open and overgrazed areas with low annual rain fall and deep red sandy soil as found in the Kalahari savanna. They also claimed that *Harpagophytum* species are often found in areas where the grass cover is below 25% and the herb cover below 20%. In favourable soil and a suitable habitat the plant distribution is very patchy, making it difficult to estimate the density of *H. procumbens*[11].

2.3 Chemical composition of *H. procumbens* extracts.

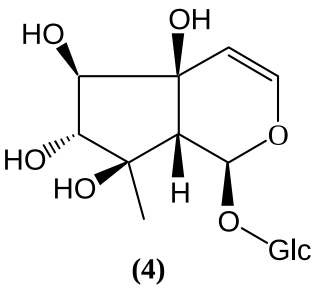
According to the available literature, 26 compounds have so far been isolated from *H. procumbens*. Most of these compounds are iridoid glycosides (1). An iridoid is a monoterpene composed of a cyclopentane ring that is fused to a six-membered oxygen enol ether (2). The iridoids in *H. procumbens* are enol ethers and their glycosides and/or cinnamic acid esters.

Tunmann and Lux[13] first isolated harpagoside and harpagide with methanol from the dried roots of *H. procumbens* in 1962. No structure was given. Lichti and von Wartburg[14] assigned the structure to harpagoside **(1)**. Acid hydrolysis of harpagoside yielded harpagide **(2)** and cinnamic acid **(3)** (Scheme 1).

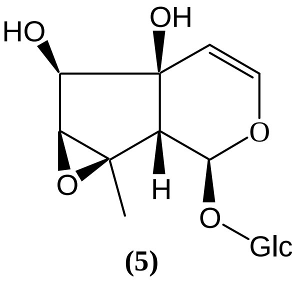


Scheme 1

Tunmann and Hammer[15] proposed the structure **(4)** for procumbide in 1968 which had been isolated by Tunmann and Stierstorfer[16] in 1964. It was only in 1979[17] that the structure was confirmed by nuclear magnetic resonance (NMR) to be structure **(5)**.

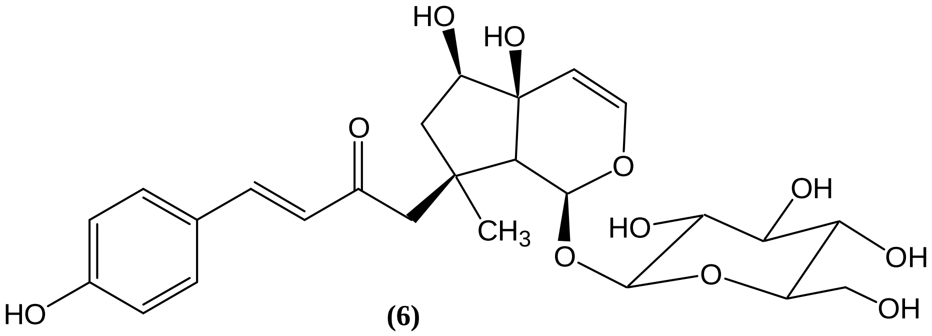


(4)

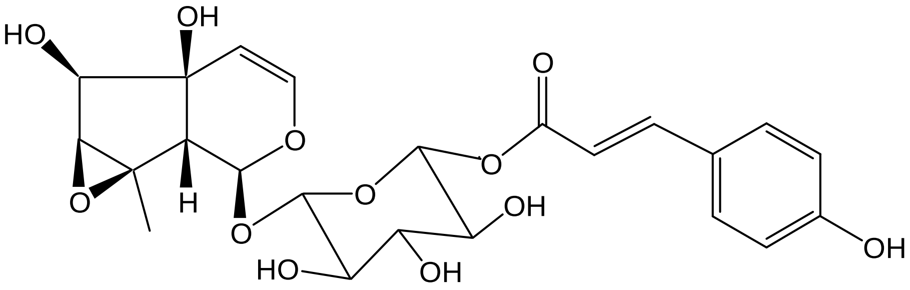


(5)

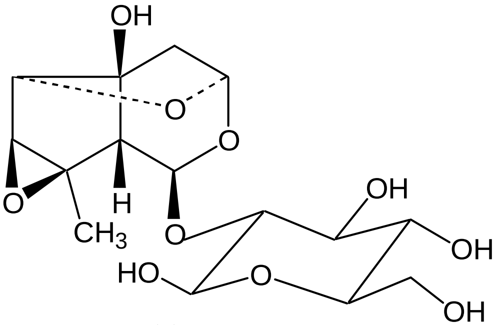
From a methanolic extract, Kikuchi and co-workers[18] obtained 8-p-coumaroylharpagide **(6)**, 6'-O-(p-coumaroyl)-procumbide **(7)** and a novel procumboside **(8)** from *H. procumbens*, as well as the known iridoid glycoside, harpagide **(2)**, harpagoside **(1)** and procumbide **(5)**.



(6)

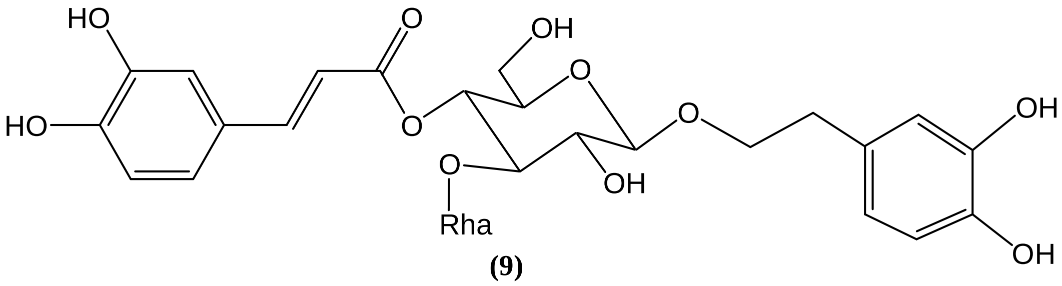


(7)

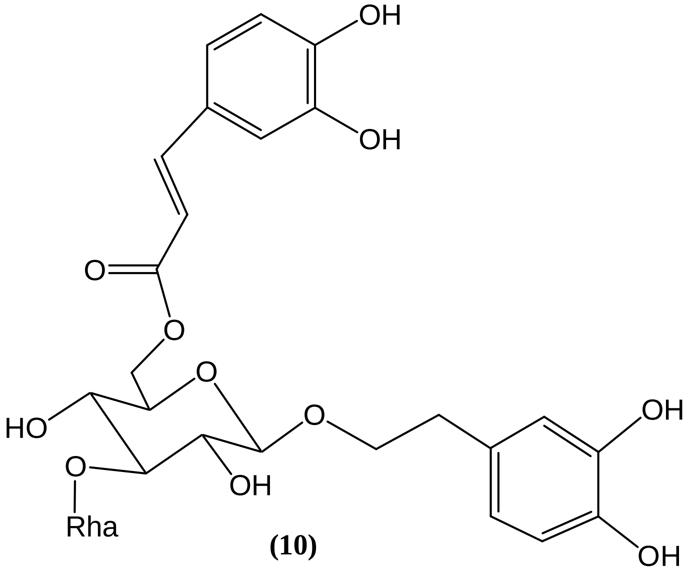


(8)

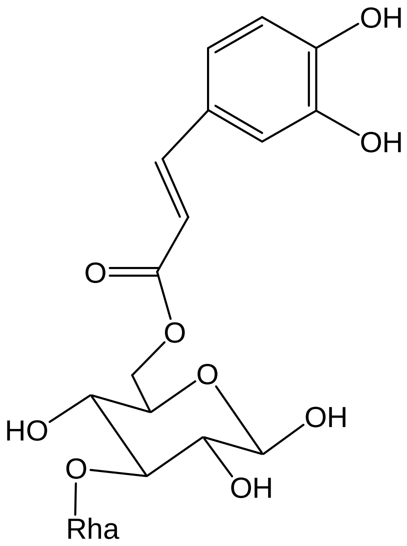
Ferreira and co-workers[19] isolated acteoside **(9)** (also known as verbascoside) and isoacteoside **(10)** (also known as isoverbascoside) from an acetone extract of dried secondary roots tubers. They also isolated a novel bioside **(11)** of which the structure was confirmed through synthesis. They also synthesized the two compounds **(9)** and **(10)** from bioside **(11)**. The biosides are esters of cinnamic acid and a sugar, mostly glucose.



(9)

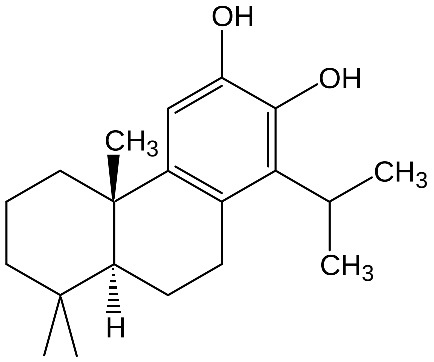


(10)

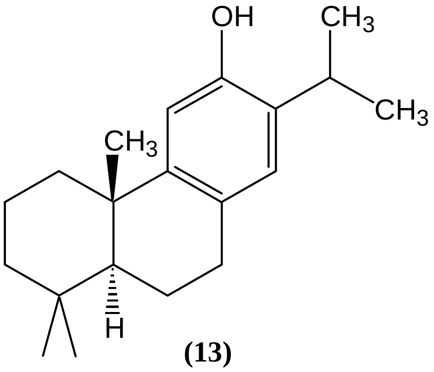


(11)

From a bioassay-guided fractionation of the petroleum ether tuber extract of *H. procumbens* two diterpenes, (+)-8,11,13-totaratriene-12,13-diol **(12)** and (+)-8,11,13-abietatrien-12-ol or ferruginol **(13)** were collected by Clarkson and co-workers [20].

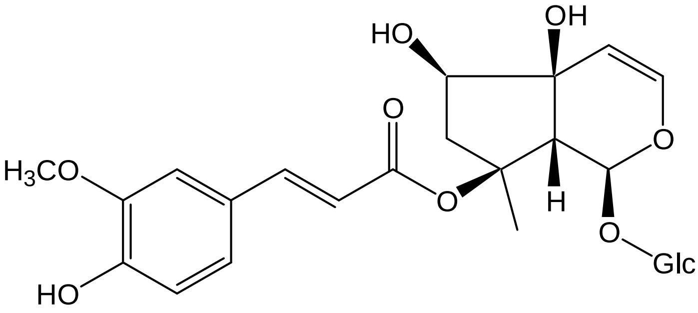


(12)

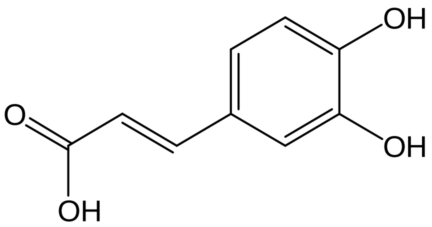


(13)

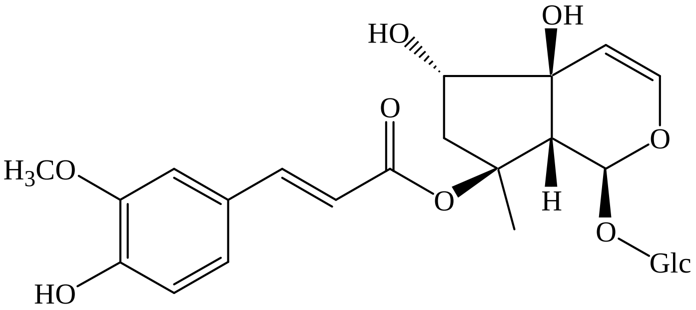
Boje and co-workers[21] isolated 10 compounds from a *H. procumbens* water extract: harpagoside **(1)**, 8-*p*-coumaroylmyoporoside **(5)**, acteoside **(9)**, isoacteoside **(10)**, cinnamic acid **(3)**, 8-feruloylharpagide **(14)**, caffeic acid **(15)**, 8-cinnamoylmyoporoside **(16)**, pagoside **(17)**, and 6'-O-acetylacteoside **(18)**. The last five compounds were purified from *H. procumbens* for the first time, while the latter three were new natural products. They found the same compounds, with the exception of 6'-O-acetylacteoside **(18),** in *H. zeyheri*.



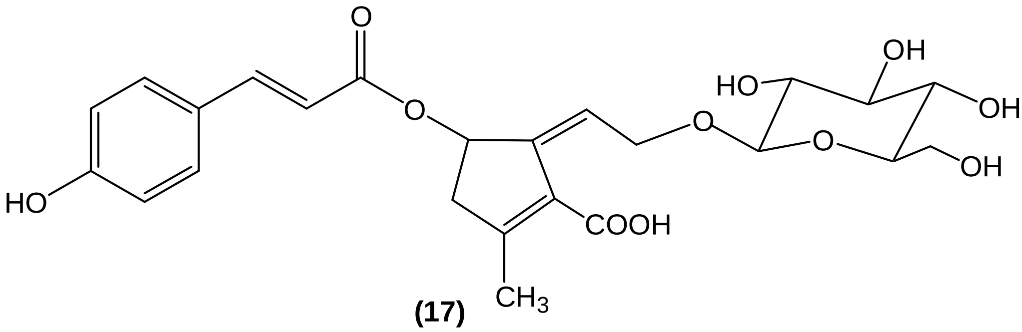
(14)



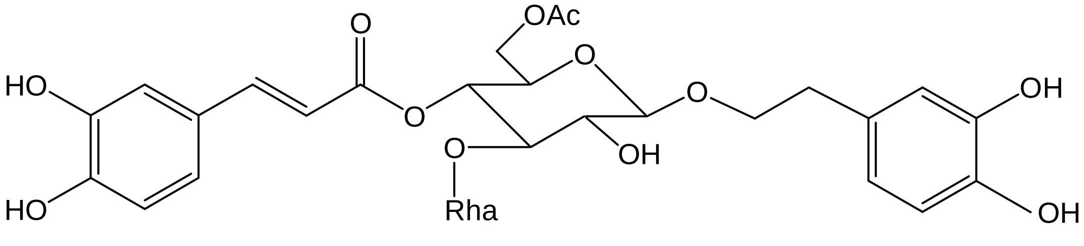
(15)



(16)

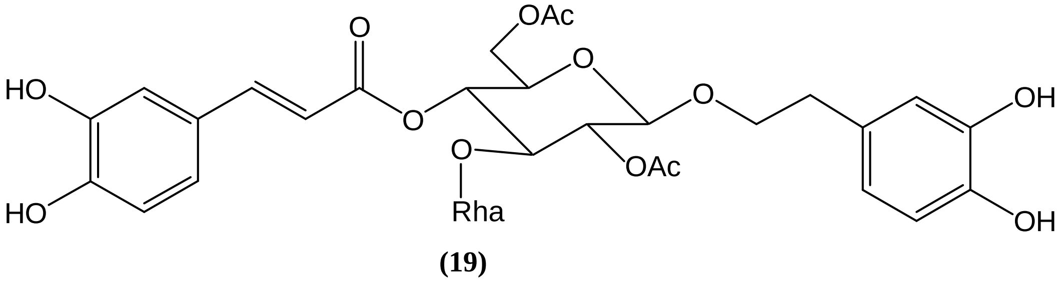


(17)



(18)

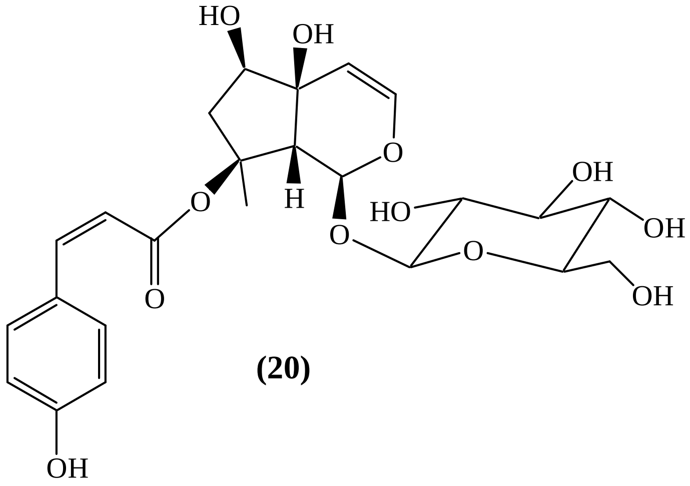
Munkombwe[22] obtained two acetyl phenolic glycosides, 6'-O-acetylacteoside **(18)** and 2,6'-diacetylacteoside **(19)** from an acetonitrile/water extract of a commercially available *H. procumbens* extract.



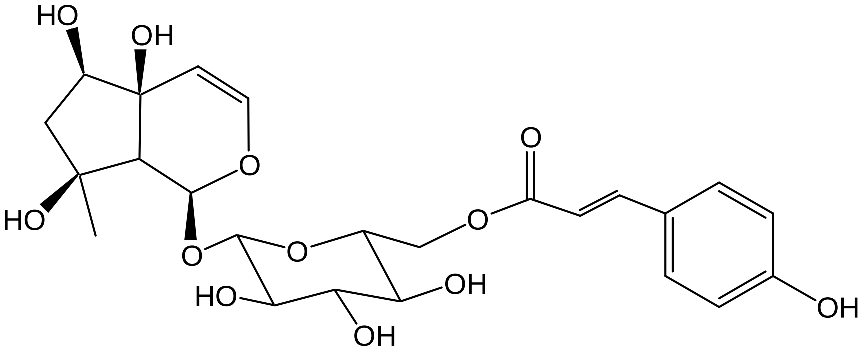
(19)

Seger and co-workers[23] identified the new natural product 8-Z-(*p*-coumaroyl)harpagide **(20)**, the known (E) isomer **(6)** and two new constituents, 6'-(Z)-(*p*-coumaroyl)harpagide

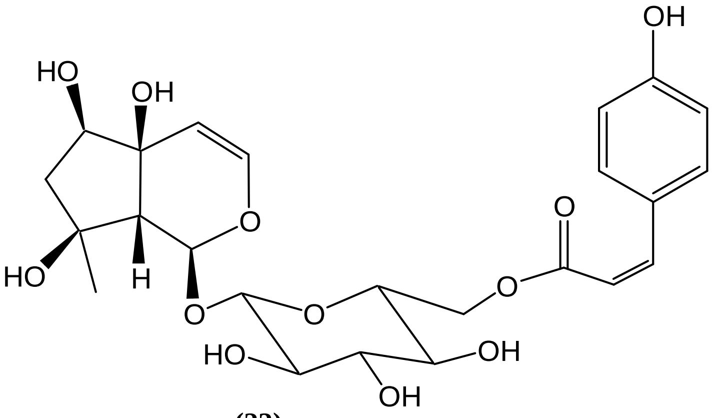
(21) and 6'-(E)-(*p*-coumaroyl)harpagide **(22)** from *H. procumbens* using liquid chromatography coupled to diode array detector mass spectrometer and solid phase extraction and NMR (LC-DAD-MS/SPE-NMR). The co-existence of (Z) and (E) cinnamoyl derivatives of iridoids was attributed to the light-induced isomerization processes of *p*-methoxycinnamoyl derivatives[24].



(20)

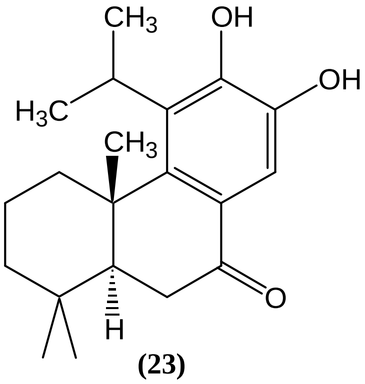


(21)

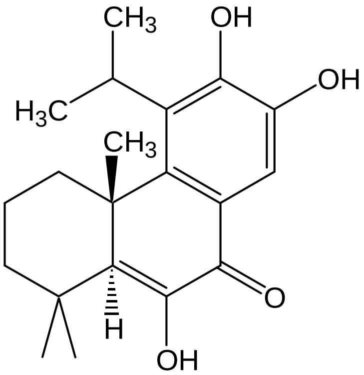


(22)

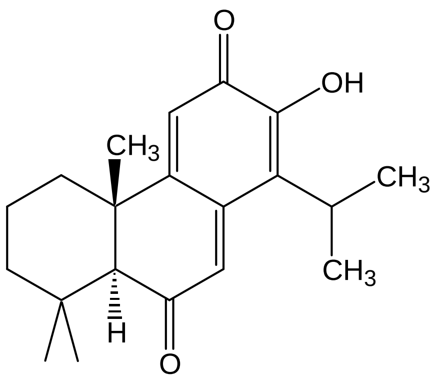
Clarkson and co-workers[25] identified 15 compounds in ethanol and petroleum ether extracts of *H. procumbens*. Four of the 15 compounds were isolated for the first time, 12,13-dihydroxychina-8,11,13-trien-7-one **(23)**, 6,12,13-trihydroxychina-5,8,11,13-tetraen-7-one **(24)**, 13-hydroxytotara-7,9,13-trien-6,12-dione / maytenoquine **(25)** and a Diels-Alder dimer **(26)**.



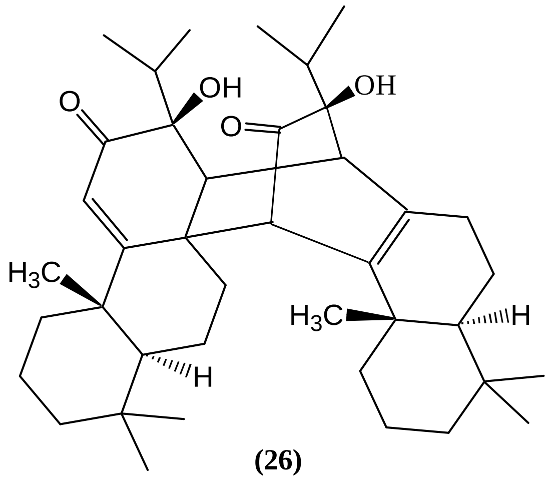
(23)



(24)



(25)



(26)

2.4 Commercialisation

The potential therapeutic value of *H. procumbens* was obtained from the Khoisan people by G. H. Mehnert, a German farmer living in Namibia in the 1920s, who sent samples to his homeland for chemical analysis[26]. The commercial harvest of this medicinal plant started only in the 1960s[8] when the Namibian company, Harpago Proprietor Limited, started to export dried tubers of *H. procumbens* to the German Company called Erwin Hagen Naturheilmittel in 1962. Export figures are only available from 1973[10].

The chain of supply begins with the harvesters, the exact number of whom is unknown[8]. Most of the harvesting is conducted by people living in rural areas, with between 10 000 and 15 000 harvesters relying solely on the sales of the dried tubers as a source of income[27].

Export of *H. procumbens* from Africa to Europe is significant and constantly increasing from the three main supplying countries, South Africa, Botswana and Namibia, with the latter having been the major exporter since 1973[9].

A study conducted in 2006 by Strobach and Cole[2] on the export data of these 3 countries showed that the majority of dried *H. procumbens* were exported to Germany and France. Germany ranks third among countries that buy this medicinal plant with sales of approximately €30M. Table **2.1** shows data that were collected from 1973 to 2004 on *H. procumbens* exported from South Africa, Botswana and Namibia.

Each exporting country regulates trade in of *Harpagophytum* with its own set of laws for the harvesters, suppliers (middlemen) and the pharmaceutical companies. In Namibia, the *Harpagophytum* species are protected under the Nature Conservation Ordinance[28] of 1975, and so permits are required to harvest, collect and transport them. In South Africa[10], permits are required on the provincial level to harvest and export *H. procumbens*. In Botswana, protection is offered under the Agricultural Resources Conservation Act of 1977 which requires trading and export permits to control pressure on natural populations of *H. procumbens*[10].

There is an increase in demand for *H. procumbens* due to an increase in the number of people suffering from different forms of arthritis and other locomotive disorders. This demand has brought greater opportunities for those who are involved in harvesting and trade but it has also vastly increased pressures on this natural resource[10]. In 2003 there were 57 *H. procumbens*-based drugs produced by 46 different companies[29]. It has been estimated that 650 tonnes of dried tubers are needed from 8-11 million plants[30].

Although no records are kept, *H. zeyheri* is sometimes also harvested and added to dried *H. procumbens* roots for trade[8]. Because buyers do not buy *H. zeyheri* roots knowingly or willingly, it is difficult to assess the impact of this on the market. It is generally agreed that acceptance of *H. zeyheri* would be beneficial for harvesters because then they could harvest any *Harpagophytum* species for sale.

Table **2.1:** Data of all the exports from 1973 to 2004

Total exports of *H. procumbens* (in kg) from Namibia, Botswana and South Africa (data from Nott[29], Steward and Cole 2005[6]). nd = no data available

Year	Namibia	Botswana	South Africa	Total Exports
1973	28,161	nd	nd	nd
1974	nd	nd	nd	nd
1975	180,000	nd	nd	180,000
1976	180,000	nd	nd	180,000
1977	190,000	nd	nd	190,000
1978	nd	nd	nd	nd
1979	nd	nd	nd	nd
1980	nd	nd	nd	nd
1981	84,350	nd	nd	84,350
1982	133,619	nd	nd	133,619
1983	124,291	nd	nd	124,291
1984	107,800	nd	nd	107,800
1985	183,370	nd	nd	183,370
1986	91,078	nd	nd	91,078
1987	nd	nd	nd	nd
1988	nd	nd	nd	nd
1989	nd	nd	nd	nd
1990	nd	nd	nd	nd
1991	20,000	nd	nd	nd
1992	95,000	10,719	nd	105,719
1993	70,000	3,278	nd	73,278
1994	160,000	24,437	nd	184,437
1995	284,409	45,633	nd	330,042
1996	313,652	nd	nd	313,652

1997	251,091	5,493	nd	256,584
1998	613,336	501	nd	613,837
1999	604,335	2,050	6,936	613,321
2000	379,740	nd	341	380,081
2001	726,333	33,506	31,112	790,951
2002	1,018,616	29,608	20,619	1068,843
2003	457,485	3,084	4,500	465,069
2004	283,142	42,025	nd	325,167
Total (kg)	4,932,139	200,334	63,508	5,195,981

2.5 Pharmacology

The pharmacology of *H. procumbens* goes back to 1958 when Zorn[32] found anti-arthritic and antiphlogistic activity after oral administration of *H. procumbens* aqueous secondary tuber extract in animal models. Schruffer[33] evaluated 50 human patients after an aqueous *H. procumbens* extract treatment and concluded that 1230 mg of the extract was equally or more effective than 1230 mg of phenylbutazone, a drug used for arthritis. In a placebo-controlled study by Guyader[34], an extract showed a significant decrease in pain after three weeks among 50 arthritic patients tested. A significant decrease in the severity of pain and an increase in spinal and cofexomoral mobility were some of the results recorded by Lecomte[35] in a double-blind trial with 89 patients suffering from articular pain.

On the other hand, Grahame and Robinson[36] recorded negative results when testing *H. procumbens* extracts for use in carrageenin foot swelling and adjuvant-induced arthritis. Twelve patients suffering from seronegative rheumatic arthritis, seropositive arthritis and psoriatic arthropathy were given *H. procumbens* tablets containing 410 mg of an aqueous extract and evaluated over a six week period. Only 4 patients showed an improvement in the parameters that were measured (pain: on a 0-3 scale; early morning stiffness: on a 1-3 scale; grip strength; blood count etc).

The efficacy of pain relief from dried *H. procumbens* root extract was assessed by Pigent and Lecompte[37] on a 30 day controlled study in 100 patients suffering from different rheumatic conditions. A daily dose of 2460 mg dry extract was administered and the results were compared to those of a placebo[38]. After 30 days the patients taking the *H. procumbens* extract showed an improvement in diarrhoea and gastritis compared to the patients on the placebo.

Fiebich and co-workers[39] showed that inflammatory diseases such as rheumatoid arthritis could be prevented by a 60% ethanolic extract of *H. procumbens*. LPS-induced TNFα synthesis was inhibited. LPS-induced cytokines IL-6 and IL-1β and the prostanoid PGE_2 in human monocytes were also inhibited with a *Harpagophytum*-containing drug

(SteiHap 69) at concentrations 100 μg/mL in excess. They also found that pure harpagoside and harpagide failed to inhibit LPS-induced TNFα-release.

One hundred and twenty-two patients suffering from osteoarthritis of the knee and hip were randomly treated with Harpadol® (a herbal medicine containing 435 mg of cryoground powdered *H. procumbens*) and with diacerhein (a non-steroidal anti-inflammatory drug used for arthritis) in a double-blind clinical study by Chantre and co-workers[40]. On completion of the study, patients using Harpadol® showed fewer adverse effects with similar efficacy to diacerhein and both drugs sharing a progressive and significant reduction in the Lequesne functional index.

Frerick and co-workers[41] conducted a clinical trial on 46 patients suffering from osteoarthritis of the hip. The patients were given 2 tablets each of either 60% ethanolic dry *H. procumbens* extract, ibuprofen or placebo tablets. Out of 71% of *H. procumbens* patients that completed the treatment, 20% (which was considered a clinically relevant response rate) of patients showed improvement in the severity of pain, and 52% of the patients taking *H. procumbens* were able to go through the study without using rescue therapy in comparison to 36% in the placebo group.

The Western Ontario and McMaster Osteoarthritis index (WOMAC) and 10 cm Visual Analogue Scale (VAS) pain scale were used in an uncontrolled multicentre drug surveillance study by Wegener and Lupke[42] to assess the efficacy of *H. procumbens* on 75 patients suffering from osteoarthritis of hip or knee for approximately 12 weeks. After assessment, data from the WOMAC index showed a significant improvement: 23.8% for the pain subscale, 22.2% for the stiffness subscale and 23.1% for the physical function subscale. The VAS pain score was decreased significantly as follows: 22.6% for worst pain, 25.2% average pain, 25.8% for the actual pain and 24.5% for the total pain score.

A total of 130 patients suffering from non-radicular back pain were treated with *H. procumbens* extract (LI 174) over a period of 8 weeks in a clinical study by Luandahn and Walper[43]. The clinical effectiveness and tolerance of the *H. procumbens* extract (LI

174) was judged according to the Multidimensional Pain Scale (MPS), Arhus back pain index and two parameters (finger-floor distance and Schober's sign) evaluating the mobility of the lumbar spine. Significant improvement of pain symptoms and mobility of the patient's spine during treatment was observed with no serious side effects. It was concluded that the extract is an excellent alternative for the treatment of chronic back pain.

The aqueous extract of *H. procumbens* was used by Mahomed and Ojewole[44] to determine its analgesic effect in mice, as well as its anti-inflammatory and anti-diabetic effects in rats. Diclofenac (DIC, 100 mg/kg) and Chlorpropamide were used as reference agents for comparison. *H. procumbens* achieved analgesia against induced nociceptive pain stimuli in mice, dose-related reductions in induced inflammation of the rat hind paw oedema and achieved significant reductions of glucose concentration in blood of the fasted normal and fasted diabetic rats. The aqueous extract of two species, *H. procumbens* and *H. zeyheri*, show dose-dependent inhibition of carrageenan-induced oedema in rats' paws and their analgesic and inflammatory properties were the same[45].

Anti-inflammatory and analgesic effects of an aqueous extract of *H. procumbens* in mice and rats were evaluated by Lanhers and co-workers[46]. The crude *H. procumbens* extract in the carrageenan-induced oedema test showed positive anti-inflammatory results, while purified harpagoside did not confirm the anti-inflammatory activity as it exerted no protective effects on carrageenan-induced oedema when tested alone. They also tested the crude extract after treating it with 0.1 N hydrochloric acid similar to the physio-chemical conditions found in the stomach. The acid treatment destroyed the anti-inflammatory and analgesic effects of *H. procumbens* and harpagoside used at 400 mg/kg.

In support of Lanhers' work, Soulimani and co-workers[47] demonstrated that an aqueous extract of *H. procumbens* undergoes a low pH induced transition in the stomach and a loss of activity while intraduodenal administration of the same extract helped in reducing the carrageen-induced transition by 65%.

Tests done on normotensive rats by Circosta and co-workers[4] with a methanolic extract of *H. procumbens* showed a decrease in the heart rate, a dose-dependent reduction of the arterial blood pressure, and protection against arrhythmias induced by aconitine. They also tested purified harpagoside and compared it to the crude extract activity on the above medical condition. It was found that the purified harpagoside had less of an effect than crude *H. procumbens* extract. This introduced possibilities in the search for other constituents that may act in synergy.

The inhibitory activity of aqueous extracts of *H. procumbens* and *H. zeyheri* as well as ten compounds isolated from *H. procumbens* were tested on human neutrophile elastase by Boje and co-workers[21]. Inhibition, with a weak dose-dependency, was observed at very low IC_{50} values. An IC_{50} of 542 µg/mL was found for the aqueous extract of *H. procumbens* and 1012µg/mL for *H. zeyheri*. 6-*o*-acetylateoside inhibited the enzyme with an IC_{50} of 47 µg/mL, isoacteoside with 179 µg/mL, 8-*p*-coumaroylharpagide with 179 µg/mL, pagoside with 154 µg/mL and caffeic acid as (a reference compound), with an IC_{50} of 86 µg/mL. The values for acteoside, harpagide and cinnamic acid were higher than 300 µg/mL.

Whitehouse and co-workers[48] screened for efficacy with standard preclinical screening methods. They found that, at higher than recommended doses for human, *H. procumbens* extract was ineffective in reducing oedema of the rats' foot that had been induced by mycobacterium butyric or λ-carrageenan. The extract did not act as an in-vitro inhibitor of prostaglandin synthetase. They suggest that there was no evidence for anti-inflammatory activity in the treatment of arthritic disease with *H. procumbens* when compared to the antiarthritic and anti-inflammatory analgesic drugs type. Moussard and co-workers[49] also claimed no evidence of anti-inflammatory efficacy. Therefore, they concluded that *H. procumbens* lacks biochemical effects on arachidonic acid metabolism similar to NSAID-like effect on whole blood eicosanoid production in humans.

The anti-inflammatory and analgesic properties of *H. procumbens* may be related to its anti-oxidant activity that helps in deactivation of oxidative free radicals. Bhattacharya investigated the anti-oxidant activity of an ethanolic *H. procumbens* extract and deprenyl (a standard anti-oxidant) in terms of their effects on catalase, superoxide dismutase, glutathione peroxidase and lipid peroxidase activities in the frontal cortex and striatum in the brain of a rat. *H. procumbens* showed a dose-related increase in catalase, superoxide dismutase, and glutathione peroxidase activities in both brain areas after 7 days treatment. They concluded that *H. procumbens* exerts significant anti-oxidant activity. This may explain why it cures rheumatoid arthritis, an oxidative free-radical induced disease[50].

The antiplasmodial activity of *H. procumbens* petroleum ether root extract was also investigated against a chloroquine-resistant (K1) and sensitive (D10) strain of plasmodium falciparum. Low cytotoxicity in two mammalian cell lines (CHO and HepG2)[20] was observed. Two diterpenes compounds isolated from this extract, (+)-8,11,13-totaratriene-12,13-diol (12) and (+)-8,11,13-abietatrien-12-ol or ferruginol (13), were reported to display a significant in vitro antiplasmodial activity against K1 and D10.

Mahomed and Ojewole[51] studied the anticonvulsant activity of *H. procumbens* secondary root aqueous extract against pentylenetetrazole (PTZ), picrotoxin (PCT) and bicuculline (BCL) induced seizures in mice. Two anticonvulsant drugs, phenobarbitone and diazepam, were used as reference standards for comparison. They found that *H. procumbens* has anticonvulsant activity via enhancing GABAergic neurotransmission in the brain. The average convulsion duration was reduced. The plant was found to depress the central nervous system. They concluded that these plants show pharmaceutical properties which lead to folkloric and ethno-medical uses of the extract in the treatment, management and/or control of childhood convulsions and epilepsy.

Tippler and co-workers[52] have done in vitro studies to demonstrate the anti-inflammatory efficacy of *H. procumbens* by preincubation of an extract in human whole blood anticoagulated with heparin before addition of the ionophore A23187. Cysteinyl-leukotriene (LT) and thromboxane (TX) B2 release into plasma was inhibited by an extract containing 7.3% harpagoside at IC_{50} of 9.2 μM and 55.3 μM, respectively. However, there was no inhibition when the whole blood was preincubated with percollisolated neutrophils in buffer. They concluded that there was a biotransformation of *H. procumbens* extracts and harpagoside in plasma before inhibition of eicosanoid biosynthesis. Tests were done also by Loew and co-workers[53] on Cysteinyl-LT and TXB_2 with different fractions of *H. procumbens* along with human male studies for pharmacokinetic studies. Their observations strongly relate the harpagoside content to the inhibition of leukotriene biosynthesis. They suggested that an extract might be separated into fractions that have different pharmacological effects.

Inducible nitric oxide synthase (iNOS) and Cyclooxygenase-2 (COX-2) are enzymes that mediate inflammatory processes and have been associated with pathogenesis. The methanol extract of *H. procumbens* was reported to inhibit 12-O-tetradecanoylphorbol-13-acetate (TPA) induced COX-2 expression in human breast epithelia cells[54]. An alcohol-water extract of *H. procumbens*[55] suppressed nitrite formation by 80% which inhibited iNOS expression and nuclear NF-κB translocation (a transcription factor responsible for regulating COX-2 expression). The water extract of *H. procumbens* was also shown to suppress COX-2 and iNOS pathways by inhibiting lipopolysaccharide (LPS) in L929 Cells[56].

Transcription factors NF-κB and AP-1 (independently or combined) regulate COX-2 expression in a mouse skin. In a study by Kundu and co-workers[58] the inhibitory effects of *H. procumbens* was tested on NF-κB activation which regulates upstream kinase ERK and on TPA-induced activation of AP-1. It was found that the *H. procumbens* methanolic extract failed to inhibit TPA-stimulated DNA binding of NF-κb but managed to diminish TPA-induced activation of AP-1 responsible for COX-2 expression in human mammary epithelial cells.

In vitro studies of the crude methanolic extract of *H. procumbens*' secondary roots and its active components, harpagide and harpagoside on smooth muscle by Occhiuto et al[58] demonstrated complex interaction between the active compounds in the drug and the mechanism that regulate the calcium in the cells. Harpagoside reduced the response to the agonists that act on smooth muscle (Ach and $BaCl_2$) and cause arthritis. At lower doses, harpagide acted as sensibilizer of a cholinergic response and at higher doses antagonized the response of cholinergic receptors. The harpagide two-phase effect in terms of dose dependence explains why lower doses of crude extract exert stronger action against Ach response than the higher doses.

Abdelouahab and Heard[59] studied the expression of epidermal COX-2 from freshly excised porcine skin. They compared an ethanol extract of *H. procumbens* and the four major individual components, harpagoside, harpagide, 8-coumaroylharpagide and verbascoside with an ibuprofen standard. They also studied a combination of harpagide, 8-coumaroylharpagide and verbascoside in the absence and presence of harpagoside. They found that harpagoside is the key anti-inflammatory constituent of *H. procumbens*. Pure 8-coumaroylharpagide is one of the most effective constituents of tubers as it leads to the lowest level of COX-2 production. Verbascoside plays an adjunct role. Harpagide is a pro-inflammatory that promotes COX-2 expression (two fold increase in COX-2 expression) and could thus result in an increased inflammatory response. They concluded that the overall activity of *H. procumbens* extract depends on the precise proportions of the compound present in the extract. According to them, this may explain the variable therapeutic responses that are often observed. The pharmacopeia standardization of an *H. procumbens* extract, based upon harpagoside only is an inadequate yardstick for the potential effect of the extract on inflammation.

Grant and co-workers[60] wrote a review article to cover all the clinical trials that were done up to year 2006 about biological and potential therapeutic actions of *Harpagophytum procumbens*.

2.6 Chemical analysis and quality control

Increased demand for *H. procumbens* extract has led to shortages of raw material from traditional collection areas. This has stimulated the expansion of collection in new areas and adulteration with *H. zeyheri*. Reliable analytical methods are required not only to compare the chemical composition of *H. procumbens* from different regions (which may be more than a thousand kilometers apart) but also to detect adulteration with *H. zeyheri*.

Baghdikian and co-workers[45], using C-18 reverse phase chromatography with a water/methanol gradient, found that harpagoside occurs in both *H. zeyheri* (mean content of 1.09%) and *H. procumbens* (mean content of 1.85%). *H. zeyheri* contains about 0.75% 8-*p*-coumaroylharpagide and *H. procumbens* about 0.05%. They concluded that the harpagoside/8-*p*-coumaroylharpagide ratio is a reliable indicator to distinguish between *H. procumbens* (ratio of between 20 and 38) and *H. zeyheri* (ratio of between 1 and 2).

Eich and co-workers[61] using a Supelcosil LC-18 reverse phase column and two gradient systems, consisting of methanol/water or acetonitrile/water, found results similar to those of Baghdikian and co-workers[45]. They collected plants from a larger area and found harpagoside/8-*p*-coumaroylharpagide ratios of between 17 and 47 for *H. procumbens*. They concluded that the sum of harpagoside and 8-*p*-coumaroylharpagide constitutes more than 99% of the iridoids in the samples. The percentage of 8-*p*-coumaroylharpagide of total iridoid content instead of the harpagoside/8-*p*-coumaroylharpagide could be a more reliable indicator to distinguish between the two species. *H. zeyheri* has between 30.9 and 61.4% 8-*p*-coumaroylharpagide and *H. procumbens* has between 2.0 and 5.7%. They suggested a limiting value of 8% 8-*p*-coumaroylharpagide in commercial preparations.

Guillerault[62] and co-workers also developed an HPLC method to determine harpagide, 8-*p*-coumaroylharpagide and harpagoside in *H. procumbens* drugs and commercial extracts. They used a linear gradient system of methanol and water as the mobile phase on a reserve phase C18 column for the total runtime of 50 min.

Schmidt[63] developed a fast HPLC method based on a monolithic reverse phase column to determine harpagoside and 8-*p*-coumaroylharpagide concentrations in *H. procumbens* extracts. The run time was reduced from 30 min to 5 min.

Günther and Schmidt[64] developed a high performance thin layer chromatographic (HPTLC) method to analyse carbon dioxide (CO_2) extracts of the secondary roots of *H. procumbens*. They found a close correlation with HPLC methods. They extracted 30% more harpagoside but found that the CO_2 extract was less pure, containing more lipophilic materials. This is explained by the less polar nature of liquid CO_2 compared with methanol. They found HPTLC to provide similar results compared to an HPLC method with the exception that it is less time-consuming in respect to sample pre-treatment with less consumption of the solvent.

NIR-FT-(nuclear infrared-Fourier transformer) Raman spectroscopy was used as a rapid and reliable analytical tool for the identification and quantification of harpagoside in ethanolic *H. procumbens* extracts and pharmaceutical products derived from it by Baranska and co-workers[65]. The strongest band at 1634 cm^{-1} was used to identify harpagoside in the extracts of *H. procumbens*. They also identify harpagoside in situ in slices of freeze-dried *H. procumbens* roots and concluded that a significantly higher harpagoside concentration occurs in the outer part of the root.

The European pharmacopoeia[66] prescribes the identification, testing and assay for an extract of *H. procumbens*. It requires not less than 1.2% harpagoside with reference to the dried drug. Its identification involves examination of the dried powder under a microscope and silica gel TLC of the methanolic extract. It should contain no starch (no blue colour with an iodine solution) and should contain less than 12% moisture. The extract is quantified by reverse-phase chromatography, using an octadecylsilyl silica gel (5 μm) column, a mobile phase consisting of equal volumes of water and methanol, a flow rate of 1.5 mL/min (100 mm by 4 mm column), methyl cinnamate as internal standard and UV detection at 278 nm.

Joubert and co-workers[67] investigated the effect of drying conditions on harpagoside levels and the use of near infra-red spectroscopy for rapid quantification of iridoids. They concluded that tunnel drying was comparable to freeze drying in maintaining harpagoside levels and recommended it for commercial purposes. Sun-dried plant material led to a significantly lower harpagoside level. This was attributed to the slower removal of water compared to freeze drying, combined with temperatures that allow enzymatic activity and enzymatic degradation of harpagoside.

2.7 Harvesting and cultivation

Many harvesters and their families living in the rural areas of Namibia, South Africa and Botswana rely on the collection of *H. procumbens* as a source of income to support themselves[68]. The increase in demand for *H. procumbens* automatically increases pressures on the resources[8]. Therefore, harvesting sustainably is crucial because it can avoid over-exploitation which could lead to complete eradication of the plant[2].

H. procumbens is considered to be in higher demand by international companies than on local markets and concerns were raised for over-exploitation of this important medicinal plant which rural people use for their health care and income[2].

Traditional sustainable harvesting of *H. procumbens* has been practiced by the ethnic groups like the San people[10], who dug around the plant and cut off the secondary roots while leaving the taproot to develop new tubers. In some areas where traditional knowledge is lacking, the whole plant is removed instead of only cutting off the roots with tubers. It was concluded that a plant requires at least 3 to 4 years before new tubers are developed[10].

Low prices that are paid to harvesters are a major drawback to sustainable harvesting, as harvesters must harvest as many plants as possible in one location for a decent return of their labour[7]. Poor prices discourage conservation strategies by harvesters[68].

Several projects have been conducted in the past on the control of trade and sustainable harvesting to ensure a sustainable benefit from *H. procumbens*. Concerns were raised at the CITES eleventh Conference of parties (Cop 11)[2] held in Gigiri (Kenya) in April 2000. Germany proposed that the species be listed on Appendix II but there was a lack of scientific data regarding the population and ecology of the plant as well as on the impact of harvesting. More research would be required before decisions could be taken to list the plant on Appendix II[2].

A project called Sustainable Harvested Devil's Claw (SHDC) was launched in Namibia. Despite its limited scope, it demonstrated that sharing benefits contributes towards good resource conservation[8]. The project demonstrates benefits by ensuring fair prices, creating options, making information available and providing general support to harvesters who take the responsibility for the resource management. It has also shown the importance of legalizing the traditional knowledge and extending the message of the best practices around the whole Kalahari region.

Strocbach and Cole[2] combined traditional knowledge and scientific research to investigate the influence of the fluctuating rainfall on the *H. procumbens,* to find a simple and reliable method to establish an annual harvesting quota in the areas with a potential harvesting and finally to make recommendations for more effective management of the resources. They also suggested that *H. procumbens* needs 3 to 4 years to develop new tubers to compensate for the fluctuating rainfall and disturbances such as the competition for moisture from dense herbs or the encroachment of shrubs.

Kumba and co-workers[69] recommended that the *H. procumbens* plant be replanted into their natural habitat for better sustainable harvesting. In this conservation strategy only tubers from secondary roots are dug out during harvesting. Accidentally uprooted taproots should be replanted in situ and seeds that are found in the surrounding area should be covered with soil. This ensures that plants regenerate again or are replaced by newly germinated seedlings after harvesting. The holes that are created should also be covered with soil.

In South Africa sustainable harvesting is done with the help of the North West Department of Agricultural Conservation and Environment (NWDACE)[70] that initiated a *H. procumbens* Harvesting Project that aims to train harvesters in sustainable harvesting, monitor plant populations to avoid over-exploitation and facilitate sales to buyers. There have been unsustainable practices in areas where the NWDACE is not doing its duty or areas not included in the project[70]. Despite this, the NWDACE plays an important role in ensuring sustainable harvesting in the North West Province of South Africa.

Lombard[71] reported that despite the extreme poverty of the people harvesting these plants and the difficult labour involved with the poor infrastructure that prevails in the areas, communities are able and willing to maintain their resources.

Cultivation would be an alternative to wild harvesting of the naturally growing *H. procumbens* plants and has been considered in the past to compensate for species that have already been over-exploited due to the poor harvesting practices[69].

A successful cultivation project was established by Levieille and co-workers[72] who produced plantlets from nodal cuttings which were transferred into sterile vermiculite with nutrient solution without a carbon source. After being exposed to a reduced humidity and micro propagated into soil, these plants grew into mature fertile plants bearing flowers, fruits and tuberised secondary roots, similar to the tubers of wild plants. However, the plants cultivated by nodal cuttings cannot be harvested more than once, as they do not produce primary roots.

Cultivation has been achieved by a rain-feed system[73] in an agricultural suitable environment. Schneider and co-workers[74] used different propagation method of seeds, nodal cuttings, transplanting primary tuber from locations where they were unwanted by farmers and in vitro cultivation processes. Transplanting taproots produced fast-growing plants with good stability against unexpected changes in the weather whereas seed propagation had low rates of natural germination and lower survival rate of seedlings even though the method was not very expensive and sustainable. The nodal cutting method of propagation was found to be successful in the propagation of single plants but required expensive irrigation, making it uneconomical. Propagation through the *in vitro* method produced an unlimited number of new plants but the method was very expensive[74].

Kumba and co-workers[69] reported on cultivation processes that were undertaken by the University of Namibia, the Ministry of Agriculture, Water and Rural Development and local farmers at Okakarara district in Namibia and an experimental station. In the project, both tap root and tubers were harvested in the wild and replanted separately. New shoots were regenerated and subsequently developed into new plants at all planting locations where replanting was done. Since all the participants in the project took good care of their plants, a sense of ownership developed. Cultivation of *H. procumbens* was accepted by communal farmers in Okakarara district and could be a short-term option in the cultivation of wild collections.

Unfortunately cultivation by means of cuttings needs large quantities of water for irrigation, as confirmed by von Willert and Sanders[75]. They established experiments in the cultivation of *H. procumbens* without the use of irrigation or artificial fertilizers. In the experiment they found that cultivated plants sprouted earlier in the season, grew faster and produced ten times more tubers than wild plants, even though the harpagoside content was the same in both cultivated and wild plants.

The commercial cultivation of *H. procumbens* has generated a lot of debate about its negative or positive effects on harvesters that rely on wild harvesting and small-scale rural farmers[76]. Large supplies of cultivated plant material could have a negative impact on people relying on the sales of the wild plants because they could suffer serious financial problems. In contrast, the cultivation methods that are being developed are possibly suitable for areas with a more favourable climate, less labour and inadequate infrastructure. However, cultivation efforts could also have positive effects if harvesters have the opportunity to increase the resource base, thereby ensuring a sustainable crop.

More importantly, cultivation efforts could provide an opportunity to restore areas that were unsustainably harvested[76].

2.8 References

1. Ihlenfelt, H. D.; Hartman, H. Die Gattung *Harpagophytum* (Burch.) DC. Ex Meissner (monographie der Afrikanischen Pedaliaceae). Mitteilungen Staatsinstitut fuer Allgemeine Botanik (Hamburg). **1970**, 13, 15-16.
2. Strohbach, M.; Cole, D. Population dynamics and sustainable harvesting of the medicinal plant *Harpagophytum procumbens* in Namibia. Federal Agency for Nature conservation, **2006**, 203, 1-59.
3. Eichler, O.; Koch, C. Antiphylogistic, analgetic and spasmolytic effects of harpagoside glycoside from *Harpagophytum procumbens* roots. *Arzneim.-Forsc*. **1970**, 20, 107-109.
4. Circosta, C.; Occhiuto, F.; Ragusa, S.; Trovato, A.; Tumina, G.; Briguglio, F.; De Pasquale, A. A drug used in traditional medicine: *Harpagophytum procumbens* DC. II. Cardiovascular activity. *J. Ethnopharm.* **1984**, 11, 259-274.
5. Van Wyk, B. E; Oudsthoorn, B; Gericke, N. Medicinal Plants of South Africa, Briza Publications, Pretoria. **1997**, 144.
6. Watt, J. M.; Breyer-Brandwijk, M. G. Medicinal and Poisons Plants of Southern and Eastern Africa, 2nd Edition, E&S Livingstone Ltd, London. **1962**, 830-831.
7. Schneider, E. Sustainable use in semi-wild populations of *Harpagophytum procumbens* in Namibia. *Med. Plant Conser.* **1997**, 4, 7-9.
8. Ernst, W. H. O.; Tietema, T.; Veenedaal, E. M.; Masene, R. Dormancy, germination and seedling growth of two Kalaharian perennials of the genus *Harpagophytum* (Pedaliaceae). *J. Tropical Ecol.* **1988**, 4, 185-198.
9. Stewart, K. M.; Cole, D. The commercial harvest of devil's claw (*Harpagophytum* spp.) in Southern Africa: The devil's in the details. *J. Ethnopharm.* **2005**, 100, 225-236.
10. Zietsman, P. C.; Pelser, A. Sustainable harvesting of Devil's claw (*Harpagophytum procumbens*): A community based conversation approach. *Nat. Sci.* **2004**, 20, 1-18.

11. Hachfeld, F.; Schippmann, U. *Proceed. D.C. Conf.* **2002**, 157-163.
12. Hacfeld, B.; Schippmann, U. *Med. Plant Conser.* **2001**, 6, 4-9.
13. Tunmann, P.; Lux, R. Zur Kenntnis der inhaltsstoffe aus der wurzel von *Harpagophytum Procumbens* DC. *Dtsch. Apoth. Ztg.* **1962**, 102, 1274 – 1275.
14. Lichti, H.; Von Wartburg, A. Zur Kontitution von harpagoside (1). *Tetrahedron lett.* **1964**, 15, 835-843.
15. Tunmann, P.; Hammer, H. E. Inhaltsstoffe aus der wurzel von *Harpagophytum Procumbens* D.C. *Justus Liebigs Ann.* **1968**, 712, 138-145.
16. Tunmann, P.; Stierstorfer, N. Zur kenntnis der inhaltsstoffe aus der wurzel von *Harpagophytum procumbens. Tetrahedron lett.* **1964**, 25, 1697-1699.
17. Bendall, M. R.; Ford, C. W.; Thomas, D. M. The structure of procumbide. *Aust. J. Chem.* **1979**, 32, 2085-2091.
18. Kikuchi, T.; Matsuda, A.; Kubo, Y.; Namba, T. New iridoid glycosides from *Harpagophytum procumbens* D.C. *Chem. Pharm. Bull.* **1983**, 31, 2296-2301.
19. Burger, J. F. W.; Brandt, E. V.; Ferreira, D. Iridoid and phenolic glycosides from *Harpagophytum procumbens*. *Phytochem.* **1987**, 26, 1453-1457.
20. Clarkson, C.; Campbell, W. E.; Smith, P. J. In vitro antiplasmodial activity of abietane and totarane diterpenes isolated from *Harpagophytum procumbens* (Devil's claw). *Planta Med.* **2003**, 69, 720-724.
21. Boje, K.; Lechtenberg, M.; Nahrstedt, A. New and known iridoid- and phenylethanoid glycosides from *Harpagophytum procumbens* and their in vitro inhibition of human leukocyte elastase. *Planta Med*. **2003**, 69, 820-825.
22. Munkombwe, N. M. Acetylated phenolic glycosides from *Harpagophytum procumbens*. *Phytochem.* **2003**, 62, 1231-1234.
23. Seger, C.; Godejohann, M.; Tseng, L.; Spraul, M.; Girtler, A.; Sturm, S.; Stuppner, H. LC-DAD-MS/SPE-NMR Hyphenation. A Tool for the Analysis of Pharmaceutically Used Plant Extracts: Identification of Isobaric Iridoid Glycoside Regioisomers from *Harpagophytum procumbens*. *Anal. Chem.* **2005**, 77, 878-885.
24. Cogne, A. L.; Queiroz, E. F.; Wolfender, J. L.; Marston, A.; Mavi, S.; Hostettmann, K. On-line identification of unstable catalpol derivatives from *Jamesbrittenia fodina* by LC-MS and LC-NMR. *Phytochem. Anal*. **2003**, 14, 67-73

25. Clarkson, C.; Staerk, D.; Hansen, H. S.; Smith, J. P.; Jaroszewski, J. W. Identification of major and minor constituents of *Harpagophytum procumbens* (Devil's claw) using HPLC-SPE-NMR and HPLC-ESIMS/APCIMS. *J. Nat. Prod.* **2006**, 69, 1280-1288.
26. Wegener, T. Devil's claw: from African traditional remedy to modern analgesic and anti-inflammatory. *Herbal Gram.*, **2000**, 50, 47-54.
27. Cole, D.; Lombard, C. The sustainable harvested Devil's claw Project in Namibia. Centre for research information and action in Africa. Windhoek, Namibia, **2000**.
28. Marshall, N. T. Searching for a cure. Conservation of medicinal wildlife resources in East and Southern Africa. Traffic international. **1998**
29. Kathe, W.; Barsch, F.; Honnef, S. Trade in Devil's claw (*Harpagophytum* spp.) in Germany-status, trends and certification. Prepared for the Food and Agriculture Organisation of the United Nations, Non-Wood Forest Products Programme. **2003**.
30. Von Willert, D. J.; Sanders, J.; In: Breckle, S. W.; Schweizer, B.; Frangmeier, A. Devil's claw: Conservation through Cultivation. Results of Wordwide Ecological studies. Proceedings of the Second Symposium of the A.S.W Schimper Foundation. **2004**, 27-44.
31. Nott, K. A survey of the harvesting and export of *Harpagophytum procumbens* and *H. zeyheri* in SWA/Namibia. Etosha Ecological institute, Okaukuejo, **1986**.
32. Zorn, B. Uber die antiarthritische wirkung der *Harpagophytum* wurtzel: vorlaufige mittleilung. *Z Rheumatol*, **1958**, 17, 134-138.
33. Schrüffer, H. Salus Teufelskralle-Tabletten. Ein fortshritt in der nichtsteroidalen antirheumatischen Therapie. *Die Medizinische Publikation*, **1980**, 1, 1-8.
34. Guyder, M. Les plantes antirhumatismales. Etude historique et pharmacologique, et etude Clinique du nebulisat d' *Harpagophytum procumbens* DC chez 50 patients arthrosique suivis en service hospitalier [Dissertation]. Universite Pierre et Marie Curie, **1984**.
35. Lecomte, A.; Costa, J. P. *Harpagophytum* dans l'arthrose: Etude en double insu contre placebo. *Le Magazine*, **1992**, 15, 27-30.
36. Grahame, R.; Robinson, B. V. Devil's claw (*Harpagophytum procumbens*): pharmacological and clinical studies. *Ann. Rheum. Dis*. **1981**, 40, 632.

37. Pigent, M.; Lecompte, A. Etude des effects de l'*harpagophytum* en rhumatologie degenerative. 37 *Le Magazine*, **1990**, 10, 1-10.
38. Ribbat, J. M.; Schakau, D. Behandluing chronisch aktivieter Schmerzen am Bewegungsapparat. *Natur. Med*, **2001**, 16, 23-30.
39. Fiebich, B. L.; Heinrich, M.; Hiller, K. O.; Kammerer, N. Inhibition of TNF-synthesis in LPS-stimulated primary human monocytes by *Harpagophytum* extract SteiHap 69. *Phytomed.* **2001**, 8, 28-30.
40. Chantre, P.; Cappelaere, A.; Leblan, D.; Guedon, D.; Vandermander, J.; Fournie, B. Efficacy and tolerance of *Harpagophytum procumbens* versus diacerhein in treatment of osteoarthritis. *Phytomed.* **2000**, 7, 177-183.
41. Frerick, H.; Biller, A.; Schmidt, U. Stufenschema bei coxarthrose. *Der Kassenart*, **2001**, 5(34), 41
42. Wegener, T.; Lupke, N. P. Treatment of patients with arthrosis of hip or knee with an aqueous extract of Devil's claw (*Harpagophytum procumbens* DC.). *Phytother. Res.* **2003**, 17(10), 1165-1172.
43. Laudahn, D.; Walper, A. Efficacy and tolerance of *Harpagophytum* extract LI 174 in patients with chronic non-dicular back pain. *Phytother. Res.* **2001**, 15, 621-624.
44. Mahomed, I. M.; Ojewole, J. A. O. Analgesic, anti-inflammatory and anti-diabetic properties of *Harpagophytum procumbens* DC (Pedaliaceae) secondary root aqueous extract. *Phytother.* **2004**, 18, 982-989.
45. Baghdikian, B.; Lanhers, M. C.; Fleurentin, J.; Ollivier, E.; Maillard, C.; Balansard, G. Mortier, F. An analytical study, anti-inflammatory and analgesic effects of *Harpagophytum procumbens* and *Harpagophytum zeyheri*. *Planta Med.* **1997**, 58, 171-176.
46. Lanhers, M. C.; Fleurentin, J.; Mortier, F.; Annie, V.; Younos, C. Anti-inflammatory and analgesic effects of an aqueous extract of *Harpagophytum procumbens*. *Planta Med.* **1992**, 58, 117-123.
47. Soulimani, R.; Younos, C.; Mortier, F.; Derrieu, C. The role of stomach digestion on the pharmacological activity plant extracts, using an example extracts of *Harpagophytum procumbens*. *Can. J. Physiol. Pharmacol.* **1994**, 1532-1536.

48. Whitehouse, L. W.; Znamirowska, M.; Paul, C. J. Devil's claw (*Harpagophytum procumbens*): no evidence for anti-inflammatory activity in the treatment of arthritic disease. *Can. Med. Assoc. J.* **1983**, 129, 249-251.
49. Moussard, C.; Alber, D.; Toubin, M. M.; Thevenon, N.; Henry, J. C. A drug in traditional medicine, *Harpagophytum procumbens*: No evidence for NSAID-like effect on whole blood eicosanoid production in human. *Prostag. Leuk. Essen. Fat. Acids,* **1992**, 46, 283-286.
50. Bhattacharya, A.; Bhattacharya, S. K. Anti-oxidant activity of *Harpagophyum procumbens* (Devil's claw). *Brit. J. Phytother.* **1998**, 5, 68-74.
51. Mahomed, I. M.; Ojewole, J. A. O. Anticonvulsant activity of *Harpagophytum procumbens* DC [Pedaliaceae] secondary root aqueous extract in mice. *Brain Res. Bull.* **2006**, 69, 57-62.
52. Tippler, B.; Syrovets, T.; Planza, N.; Loew, D.; Simmet, T. *Harpagophytum procumbens* DC used in traditional medicine inhibits eicosanoid biosynthesis in human whole blood. *Int. J. Tiss. Reac.* **1997**, 7, 101-102.
53. Loew, D.; Möllerfeld, J.; Schrödter, A.; Puttkammer, S.; Kaszkin, M. Investigations on the pharmacokinetic properties of *Harpagophytum* extracts and their effects on eicosanoid biosynthesis in vitro and ex vivo. *Clinic. Pharmacol. Ther.* **2001**, 69, 356-363.
54. Na, H.; Mossanda, K. S.; Lee, J.; Surh, Y. Inhibition of phorbol ester-induced COX-2 expression by some edible African plants. *Biofac.* **2004**, 21, 149-153.
55. Kaszkin, M.; Beck, K. F.; Koch, E.; Erdelmeier, C.; Kusch, S.; Pfeilschifter, J.; Loew, D. Downregulation of iNOS expression in rat mesangial cells by special extracts of *H. procumbens* derives from harpagoside (1)-dependent and independent effects. *Phytomed.* **2004**, 11, 585-595.
56. Jang, M.; Lim, S.; Han, S.; Park, H.; Shin, I.; Kim, J.; Kim, N.; Lee, J.; Kim, K.; Kim, C. *Harpagophytum procumbens* suppresses lipopolysaccharide-stimulated expressions of cyclooxygenase-2 and inducible nitric oxide synthase in fibroblast cell line L929. *J. Pharmacol. Sci.* **2003**, 93, 367-371.
57. Kundu, K. J.; Mossanda, S. K.; Na, H.; Surh, Y. Inhibitory effects of the extracts of Sutherlandia frutescens (L) R. Br. and *Harpagophytum procumbens* DC. on phorbol

ester-induced COX-2 expression in mouse skin: AP-1 and CREB as potential upstream targets. *Cancer Lett.* **2005**, 218, 21-31.

58. Occhiuto, F.; Circosta, C.; Ragusa, S.; Ficarra, P.; De Pasquale, R. C. A drug used in traditional medicine: *Harpagophytum procumbens* DC. IV. Effects on some isolated muscle preparations. *J. Ethnopharm.* **1985**, 13, 201-208.
59. Abdelouahab N.; Heard, C. Effect of the major glycosides of *Harpagophytum procumbens* (Devil's claw) on epidermal cyclooxygenase-2 (COX-2) in vitro. *J. Nat. Prod.* **2008**, 71, 746-749.
60. Grant, L.; McBean, D. E.; Warnock, A. M. A review of the biological and potential therapeutic actions of *Harpagophytum procumbens*. *Phytother. Res.* **2007**, 21, 199-209.
61. Eich, J.; Schmidt, M.; Betti, G. HPLC analysis of iridoid compounds of *Harpagophytum* taxa: Quality control pharmaceutical drug material. *Pharm. Pharmacol. Lett.* **1998**, 2, 75-78.
62. Guillerault, L.; Ollivier, E.; Elias, R.; Balansard, G. Determination of harpagide, 8-p-coumaroyl-harpagide, and harpagoside (1) by HPLC in *Harpagophytum procumbens* drugs and in a commercial extract. *J. Liq. Chrom.* **1994,** 17, 2951-2960.
63. Schmidt, A. H. Validation of a fast-HPLC method for the separation of iridoid glycosides to distinguish between the *Harpagophytum* species. *J. Liq. Chrom. Rel. Techn.* **2005**, 28, 2339-2347.
64. Günther, M.; Schmidt, P. C. Comparison between HPLC and HPTLC-densitometry for the determination of harpagoside (1) from *Harpagophytum procumbens* CO_2-extracts. *J. Pharmacol. Biome. Anal.* **2005**, 37, 817-821.
65. Baranska, M.; Schulz, H.; Siuda, R.; Strehle, M. A.; Rösch, P.; Popp, J.; Joubert, E.; Manley, M. Quality control of *Harpagophytum procumbens* and its related phytopharmaceutical products by means of NIR-FT-Raman spectroscopy. *Biopolym.* **2005**, 77, 1-8.
66. European pharmacopeia, 5th Edition, Appendix 3, *D.C. Monogr.* **2005**. 1401.
67. Joubert, E.; Manley, M; Gray, B. R.; Schulz, H. Rapid measurement and evaluation of the effect of drying conditions of harpagoside (1) content in *Harpagophytum procumbens* (Devil's claw) root. *J. Agri. Food Chem.* **2005**, 53, 3493-3502.

68. Wynberg, R. Achieving a fair and sustainable trade in devi's claw (*Harpagophytum* spp.). In: Sunderland, T.; Ndoye, O. (Eds.), Forest Products, Livelihoods and Conservation: Case studies of non- timber Forest Research, Bogor Barat, Indonesia, **2004**, 2, 53-72.
69. Kumba, F. F.; Kaurivi, J. Z. U.; Katjivena, H. A project of indigenous communities in Namibia to cultivate *Harpagophytum procumbens*. *Med. Plant Conser.* **2002**, 8, 24-27.
70. Raimondo, D.; Newton, D.; Fell, C.; Donaldson, J.; Dickson, B. Devil's Claw (*Harpagophytum* spp. In South Africa): Conservation and Livelihoods issues. *Traffic Bull.* **2005**, 20, 98-112.
71. Lombard, C. The sustainable harvested Devil's claw project in Namibia. *Med. Plant Conser.* **2000**, 6, 9-10.
72. Levieille, G.; Wilson, G.; Robin, J. R.; Cambornac, M. In-vitro micro-propagation of fertile plants of *Harpagophytum procumbens* and *H. zeyheri* (Devil's Claw). *Med. Plant Conser.* **2000**, 6, 10-11.
73. Olivier, G.; Sander, J.; Von Willert, D. Can crops be cultivated in the Kalahari without irrigation? A solution. Prepared for the Kalahari *Harpagophytum* Project, South Africa. **2000**.
74. Schneider, E.; Sanders, J.; Von Willert, D. Sustainable use by cultivation combined with controlled harvesting in semi-wild populations. Devil's claw (*H. procumbens)* from Southern Africa. Medicinal and Aromatic Plants, Springer, Netherlands, **2006**, 181-202.
75. Von Willert, D. J; Sanders, J. Devil's claw: conservation through cultivation. In: Breckle, S.W.; Schweizer, B.; Fangmeier, A. eds. Results of worldwide ecological studies: proceedings of the 2nd symposium of the A.F.W. Schimper-Foundation, 23-25 October. Hohenhem, Verlag Günter Heimbach, Stuttgart, German. **2002**, 27-44.
76. Cole. D. The impact of certification on the sustainable use of Devil's claw (*Harpagophytum procumbens*) in Namibia. Unpublished report prepared for the food and agricultural Organisation of the United Nations, Non-wood Forest Products Programme. **2003**.

3. Experimental methods

3.1 Collection of plant material

Plant material was collected from December 2007 to February 2008 in the North West Province, Limpopo Province, Namibia, the Caprivi Strip and around the Victoria Falls in Zimbabwe (figure **3.1** and **3.2**) as is described below.

Roots collected from the North West Province and Namibia were *H. procumbens* subsp. *procumbens*, plants from Limpopo Province were *H. zeyheri* subsp. *zeyheri* while plants from Zimbabwe and the Caprivi area were *H. zeyheri* subsp. *sublobatum* (according to taxonomy).

In the North West Province, roots were collected in 6 different areas; Cassel, Ganyesa, Moswana, Molopo Nature Reserve, Terra Firma and Lafras. Samples from Cassel represent roots with different ages from 5 different sub-locations, all with different inter-harvest periods. Inter-harvest periods of one, two, three, four and five years represent lateral roots that have been allowed to develop since harvesting one, two, three, four or five years ago and consequently represent lateral roots that are one, two, three, four or five years old respectively. Plant material collected at Ganyesa and Moswana were harvested at 3 sub-locations and samples from each of these populations were also of varying age.

Each collection at Cassel, Ganyesa and Moswana represents a population that is spread over an area of about one hectare. The different populations were between one and three kilometres apart. Ganyesa is about 100 km from Cassel and about 30 km from Moswana. The small population of 25 plants in the Molopo Nature Reserve represents a virgin population that has never been subjected to root harvesting before. The small population from Terra Firma (28 plants) come from a grazing area that was treated with herbicide to control bush encroachment about twenty years ago.

Roots from North West were dug out and the plants were marked for future reference using small metal rods. Tubers from each plant were bagged, labelled and GPS coordinates recorded for each location (figure **3.3** and **3.4**).

Roots collected from Namibia, Zimbabwe, the Caprivi Strip and the Limpopo Province were received as dried sliced samples.

The different collections can be summarised as follows:
Zimbabwe. *H. zeyheri* subsp. *sublobatum*. 12 different plants.
Caprivi Strip. *H. zeyheri* subsp. *sublobatum*. 6 different plants.
Namibia. *H. procumbens* subsp. *procumbens*. 5 different plants.
Limpopo Province:
Ferrolands. *H. zeyheri* subsp. *zeyheri*. 27 different plants.
Makgabeng. *H. zeyheri* subsp. *zeyheri*. 41 different plants.
Springbokfontein. *H. zeyheri* subsp. *zeyheri*. 25 different plants.
Northwest Province:
Molopo Nature Reserve. *H. procumbens* subsp. *procumbens*. 24 different plants.
Terra Firma. *H. procumbens* subsp. *procumbens*. 30 different plants.
Lafras. *H. procumbens* subsp. *procumbens*. 8 different plants.
Cassel. *H. procumbens* subsp. *procumbens*. Five different ages. 117 different plants.
Ganyesa. *H. procumbens* subsp. *procumbens*. Three different ages. 70 different plants.
Moswana. *H. procumbens* subsp. *procumbens*. Three different ages. 68 different plants.

Figure **3.1:** Areas in which tubers of secondary roots were collected in Southern Africa (shaded in green).

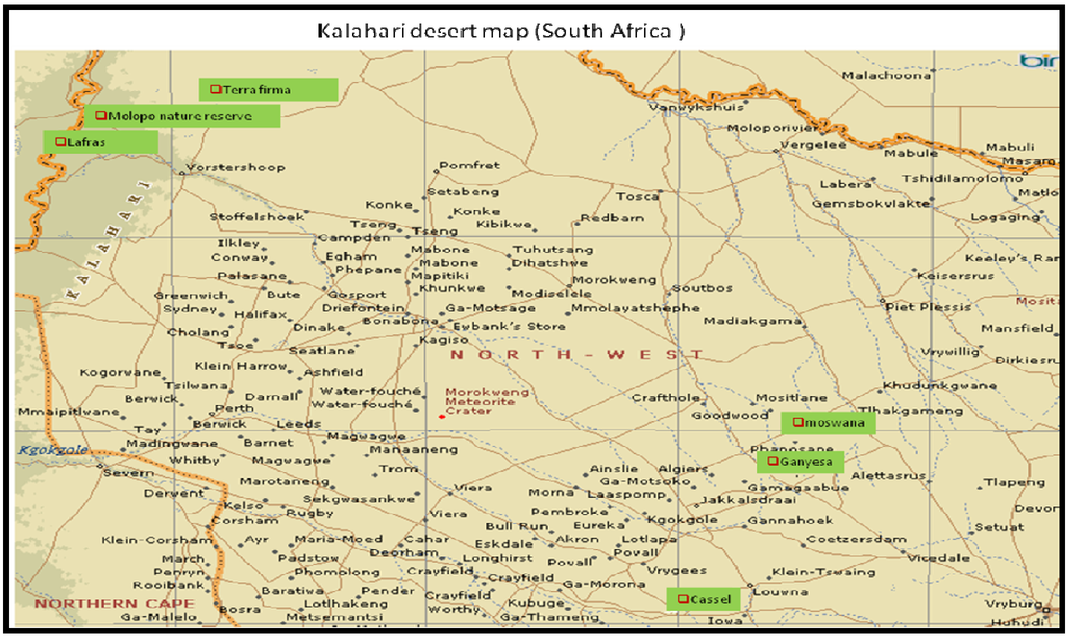


Figure **3.2:** Areas where roots were collected in the North West Province (shaded in green).

Figure **3.3:** The collection team from the North West Department of Agricultural Conservation and Environment.

Figure **3.4:** Conditions in the North West Province collection areas.

The secondary tubers were collected by either digging a hole around the plant leaving the tap root intact (figure **3.5a-d)**, or carefully removing the entire plant, harvesting the tubers followed by replanting the tap root (figure **3.6** and **3.7**).

The freshly collected roots were sliced, sun dried (figure **3.8**) and analysed with HPLC-UV for six different chemical entities (harpagide, harpagoside, 8-p-coumaroylharpaide, verbascoside, isoverbascoside and 6-acetylacteoside) according to the procedure described below.

3.2 Chemical analysis of samples

3.2.1 Method development and validation

a. Chemicals

Methanol, HPLC-gradient grade was obtained from Sigma-Aldrich (Munich, Germany). All reference standards were obtained from Phytolab (Hamburg, Germany).

b. HPLC Equipment

The analysis of extracts was performed using Agilent technologies 1200 series HPLC-system (Agilent, USA) with a solvent degasser, binary pump, auto sampler, and UV/Vis detector.

Figure **3.5:** Digging around the plant for secondary tubers only (**a-d**).

Figure **3.6:** The whole plant was removed and the primary root was replanted.

Figure **3.7:** The whole plant was removed.

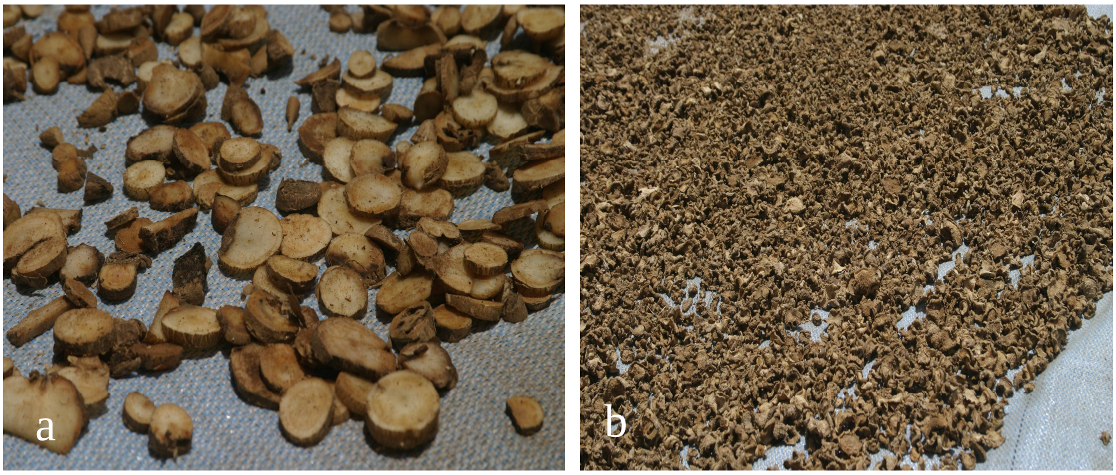


Figure **3.8:** **a** Sliced tubers. **b** Sun dried tubers.

c. Chromatographic conditions

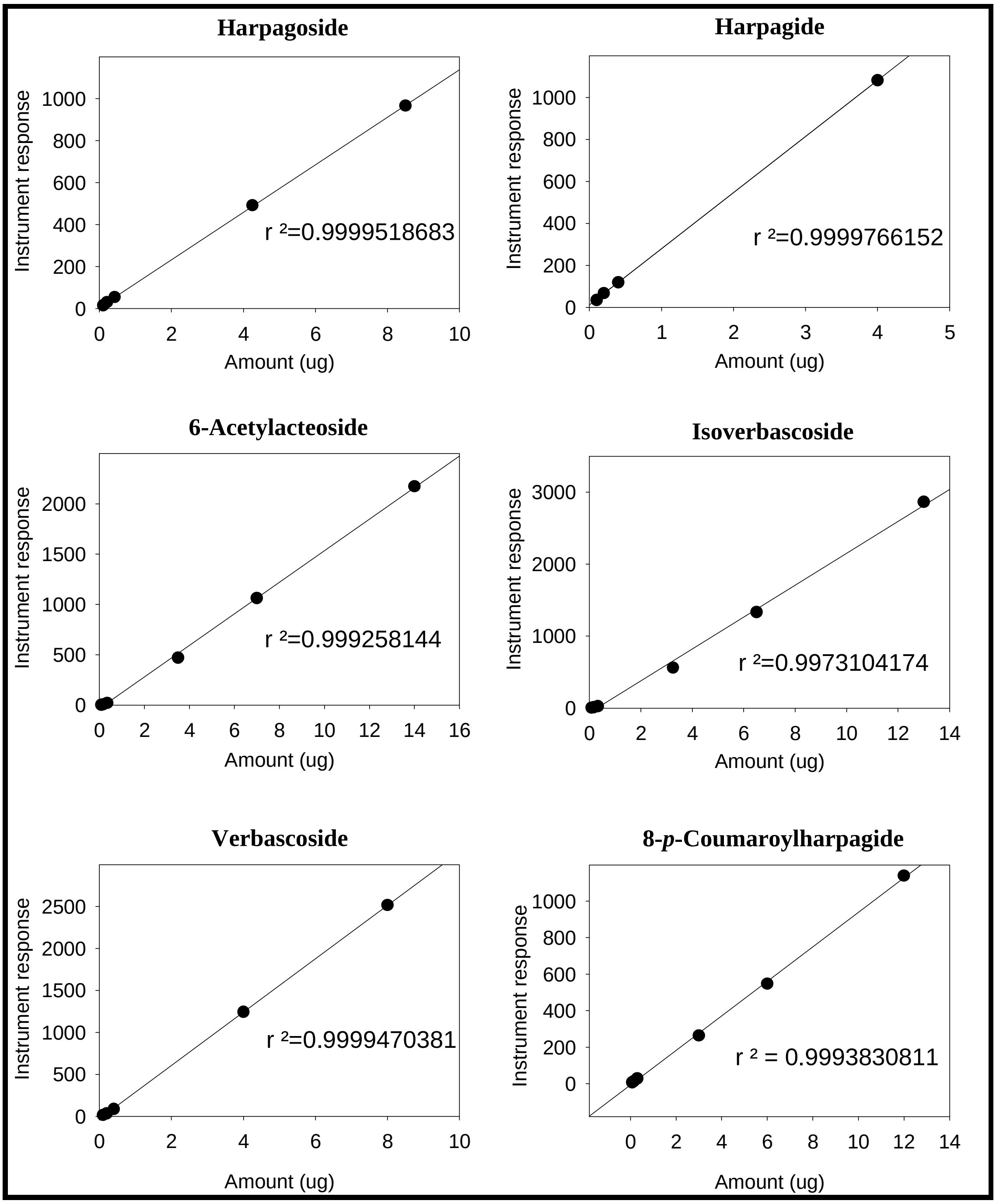
Analysis was performed at 30 ^{0}C on a Zorbax Eclipse XDB-C18 column (150 mm x 4.6 mm, 5 µm, Agilent). The separation was done at 1 mL/min with isocratic steps starting with 3% MeOH for 1 min, 50% MeOH for 20 min followed by column cleanup at 95% for MeOH 5 min and regeneration with 3% MeOH for 5 min. The UV/Vis detector was programmed to change the absorbance wavelength according to each eluting analyte that had been determined by the reference standards beforehand. The wavelength was programmed as follows: 205 nm at 3.6 minutes, 330 nm at 4.1 minutes, and 278 nm at 16 minutes. All analyses were performed in triplicate and the relative standard deviation of peak intensity was calculated.

d. Calibration curves

Stock solutions of each of the reference standards were prepared in MeOH and diluted to generate a 5 level calibration range (Table **3.1)**. Each standard solution was measured in triplicate (figure **3.9).**

e. Extraction procedure

Since the extraction method is very simple, only the incubation temperature and length of incubation was investigated on the extracted amounts. The extraction procedure for the optimization was repeated at 2 temperatures (30 ^{0}C and 60 ^{0}C) at different lengths of time, from 30 min up to 120 min (figure **3.10)**.



Harpagoside

Harpagide

6-Acetylacteoside

Isoverbascoside

Verbascoside

8-*p*-Coumaroylharpagide

Figure **3.9**: Calibration curves for all the reference standards that were used.

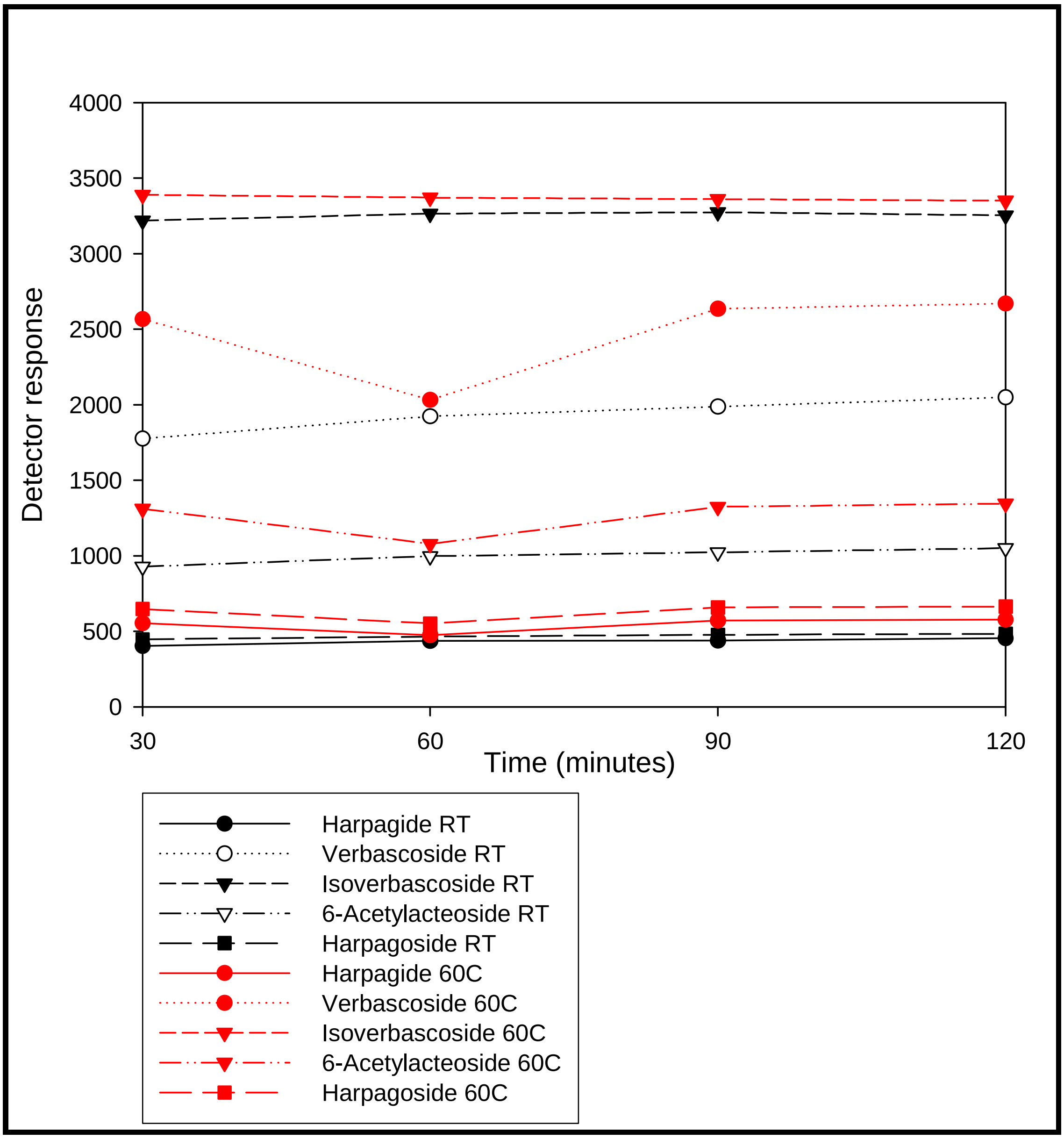


Figure **3.10:** The effect of the extraction temperature and the length of incubation on the amount extracted.

f. Sample preparation.

The final sample protocol was as follows. Crude sun-dried *H. procumbens* samples were ground to a fine powder in a coffee bean grinder. From this powder, 0.5 g was extracted with 20 mL methanol at 60 ^{0}C for 30 minutes. Extracts were made in triplicate from each sample (of plant material) and filtered through a 0.45 µm-PTFE membrane syringe cartridge (Gelman Sciences, Dreieich, Germany) into the vials and transferred to the HPLC auto sampler rack for analysis.

g. Moisture content

Since the amount of moisture present in the samples affects the concentration of each analyte, it was necessary to determine the moisture content. Moisture content was determined from three different samples of *H. procumbens*. Crude sun-dried samples were ground to a fine powder in a coffee grinder and dried under vacuum at 60 ^{0}C with a moisture absorber (Phosphorus pentoxide). The weight of each sample was recorded at various time intervals and the moisture content determined (figure **3.11)**. The average moisture content was 8.7% which is within the accepted limit of less than 12% as recommended by commercial companies.

h. Method validation

The validation of the method developed was done in accordance with the ICH-Guidelines Q2 (R1)[1]. The following characteristics were examined: specificity, linearity, precision, accuracy and robustness.

Specificity

The individual peaks from each of the reference standards were clearly separated from each other. Analyte identification in the plant material was performed by comparing the retention times with those of the reference standards (Figure **3.12**).

Table **3.1:** Calibration curves and concentration range used for the reference standards

Reference standard	**curve**	**Concentration range (ng/µL)**	**Regression value (R^2)**
Harpagide	y = 267.58x + 11.19	10 – 400	0.9999766
Verbascoside	y = 317.97x – 28.84	10 – 800	0.9999470
Isoverbascoside	y = 221.62x – 61.01	8 – 1300	0.9973104
6-Acetylacteoside	y = 156.67x – 32.15	8 – 1400	0.9992581
8-*p*-Coumaroylharpagide	y = 945.99x - 5.91	7 – 1200	0.9993831
Harpagoside	y = 113.21x + 5.83	10 – 850	0.9999519

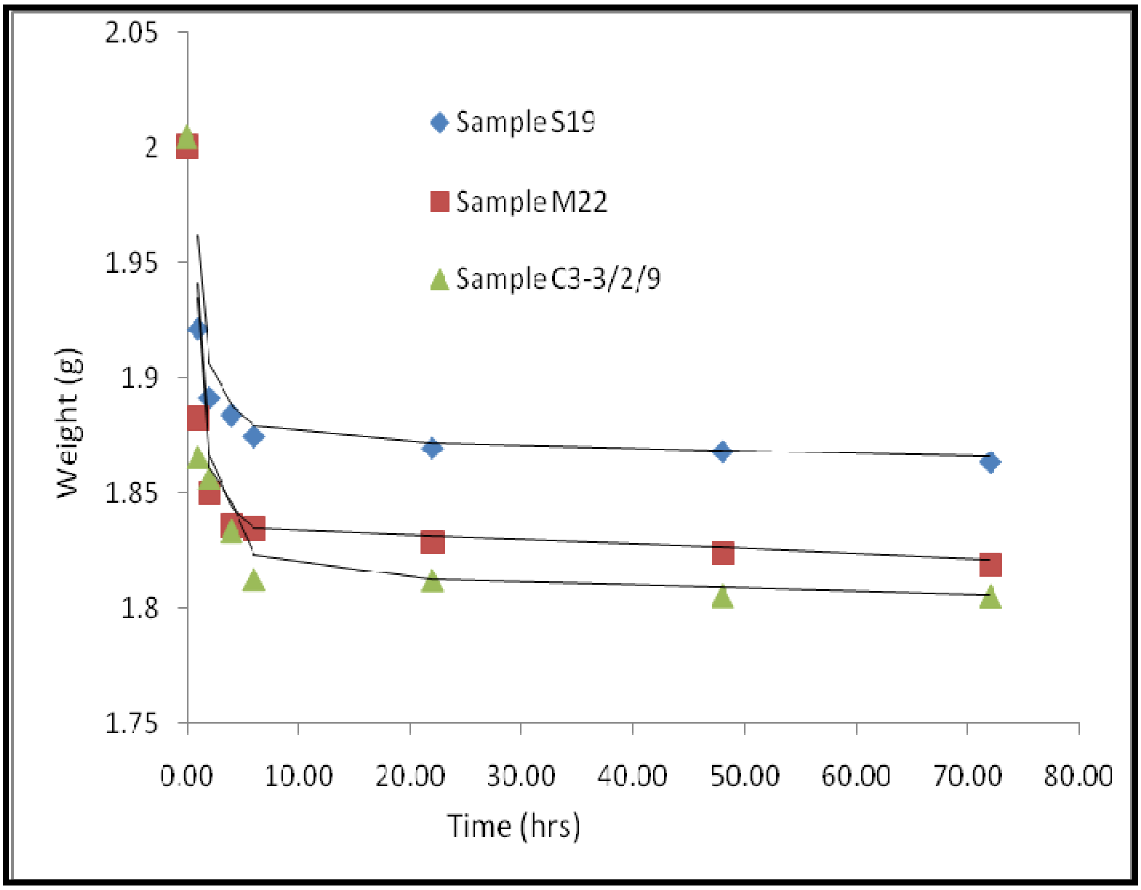


Figure **3.11:** Drying weight of each sample verses time.

Linearity

The linearity of each of the six analytes was measured over a concentration range > 40 times as listed in Table **3.1**. The regression equations (Table **3.1**) were determined by plotting the peak height (y) against amount (x) of each standard injected. The coefficient of correlation (r^2) with values exceeding 0.997 in all cases demonstrated the very good relationship between peak height and amount injected (Table **3.1**).

Precision

Precision was performed at two different levels – intra-day and inter-day repeatability. This was assessed in 5 replicates of the standard reference solutions and the percentage of relative standard deviation (%RSD) was calculated. The %RSD for the intra-day repeatability ranged from 0.1% to 3.0% depending on the analyte. The %RSD for inter-day repeatability over the 48 hours test period ranged between 1.0% and 3.5%. Retention time deviations were always less or equal to 0.2 minutes for all analytes.

Accuracy

The accuracy of the method was determined through recovery assays by spiking the blank matrix with a fixed amount of each of the 6 analytes. The recovery of the analytes varied with all being above 90%.

Robustness

To test the robustness of the method, different factors were evaluated during the study. These factors include: varying the incubation temperature during the extraction process; different operators doing the extraction; as well as operating the HPLC instrument and changing to a new column of the same specifications. Although no significant difference was observed when varying these factors, it was decided to follow the developed method carefully where possible.

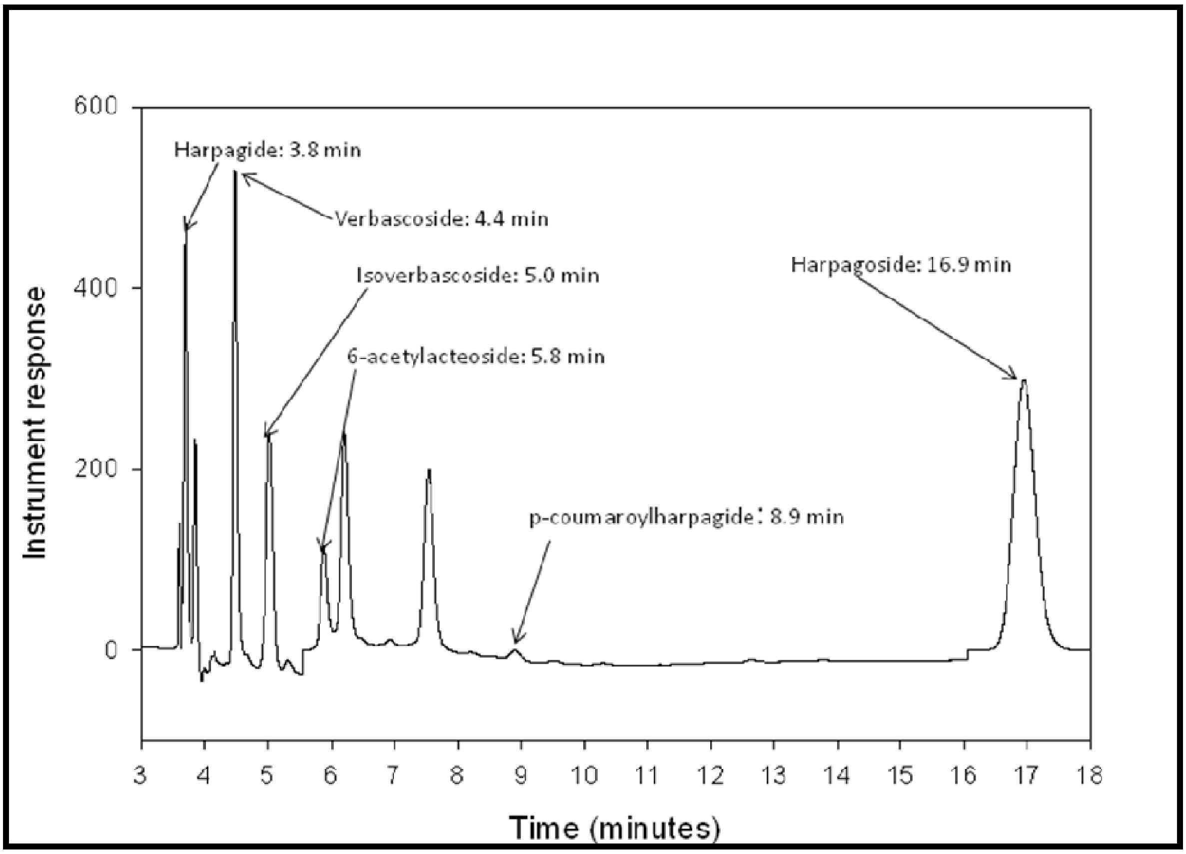


Figure **3.12:** Chromatogram of an actual methanolic extract. The retention time of each compound is indicated on the x-axis. Instrument response (peak height) relates to the concentration of each analyte.

3.2.2 Analysis of samples

The samples were analysed according to the method developed and described in section 3.2.1. Initially all samples were analyzed in triplicate. Due to time and other constraints and the close correlation between the triplicate values, some samples were analysed in duplicate. The results of all samples that were analysed by HPLC are given in Appendix **A**.

3.3 Data analysis

The raw data in Appendix **A** were analysed in collaboration with Prof. H. S. Steyn from the Statistical Consultation Service of the North West University at Potchefstroom campus using StatSoft, inc. (2008). STATISTICA (data analysis software system), version 8.0. Results are discussed in chapter 4, 5, 6 and 7. The interpretations and explanations were based on the multivariate statistical analysis theory that is found in the following text books: MULTIVARIATE STASTICAL ANALYSIS, a conceptual introduction by Kachigan[2] and MATHEMATICAL STATISTICS and DATA ANALYSIS by Rice[3].

3.4 References

1. ICH, Topic Q2 (R1) – Validation of analytical procedures: Text and Methodology; International conference on harmonization of technical requirements for registration of pharmaceuticals for human use, **2005**, 1-13.
2. Kachigan, S. K. Multivariate Statistical Analysis: A conceptual introduction, 2nd Edition, Radius Press, New York. **1991**, 117-269.
3. Rice, J. A. Mathematical Statistics and Data Analysis, 2nd Edition, Wadsworth Publishing Company, California. **1995**, 444-470.

4. Analysis of Cassel data (Five different inter harvest periods)

4.1 Purpose of this study

In this study we aim to answer the following questions using our analytical results (in Appendix **A**):

1. Are there meaningful differences in the chemical composition between populations from the five different inter harvest periods (1 to 5 years) at Cassel in the North West Province (Are there meaningful differences in composition of secondary roots (tubers) that are one, two, three, four or five years old?).
2. Are there meaningful differences in the chemical composition between the six populations from the North West Province (Cassel, Ganayesa, Moswana, Terra Firma, Lafras and Molopo Nature Reserve)?
3 Are there meaningful differences in the chemical composition of populations from North West Province and Namibia?
4. Are there meaningful differences in the chemical composition of populations from North West Province/ Namibia (*Harpagophytum procumbens* subsp. *procumbens*, from the Limpopo Province (*Harpagophytum zeyheri* subsp. *zeyheri*) and from Zimbabwe (*Harpagophytum zeyheri* subsp. *sublobatum*)?

In this chapter we analyse the data from the five different inter harvest periods from Cassel in North West Province. We can restate question 1 above as a hypothesis: “There are significant differences in the chemical composition between populations from Cassel representing five different inter harvest periods (1 to 5 years)”.

4.2 Statistical analysis

4.2.1 Factor analysis

Factor analysis with the principal component method (followed by a Varimax-rotation) identifies two factors. Factor 1 (eigenvalue 2.42) and factor 2 (eigenvalue 1.56) explain 40% and 26 % of the total variance (66% cumulatively). Factor 1 consists of verbascoside, isoverbascoside and acetylacteoside and factor 2 consists of coumaroylharpagide and harpagoside (Table **4.1**). Surprisingly harpagide is not included in factor 2 despite its close structural relationship with harpagoside and coumaroylharpagide. Intriguingly harpagide is also not biologically active whilst two components of factor 2 (coumaroylharpagide and harpagoside) are considered to be. Since the communality of harpagide (0.23) is relatively low (Table **4.2**), it seems not to be statistically part of the data structure.

Table **4.1:** Factor analysis on Cassel-data

Variable	Factor loadings (Varimax raw) (DKdata Averages. Sta) Extraction: Principal components (Marked loadings are >.700000) Include condition: locality = ‘CASS’	
	Factor 1	Factor 2
Harpagide	0.656965	-0.196558
Verbascoside	**0.739769**	0.131205
Isoverbascoside	**0.866900**	-0.019610
Acetylacteoside	**0.753272**	0.299476
Coumaroylharpagide	0.304869	**0.831455**
Harpagoside	-0.166781	**0.847292**
Expl. Var.	2.418557	1.555141
Prp. Totl	0.403093	0.259190

Table **4.2:** Communalities from Cassel data

Variable	Communalities (DKdata Averages. Sta) Extraction: Principal components Rotation: Unrotated. Include condition: locality = 'CASS'		
	From 1 Factor	From 2 Factors	Multiple R-Square
Harpagide	0.326667	0.470238	0.230755
Verbascoside	0.556494	0.564473	0.383903
Isoverbascoside	0.678695	0.751901	0.565358
Acetylacteoside	0.652512	0.657104	0.512100
Coumaroylharpagide	0.284336	0.784262	0.450678
Harpagoside	0.007485	0.745720	0.294175

4.2.2 Analysis of Variance

Due to the fact that factor analysis excludes harpagide from the structurally closely related 8-coumaroylharpagide and harpagoside factor, all six variables were used (chemical constituents) in correlation analysis.

A multivariate analysis of variance (MANOVA) (table **4.3**) on the 6 chemical constituents indicates highly significant differences between the different inter-harvesting periods (one to five years, indicated as 'occasions' (p = 0.00013 for the Wilks test and eta-square=0.14) that cannot be attributed to random differences.

Univariate analyses of variance (ANOVA) were performed on each of the chemical constituents and results are displayed in Table **4.4a** to **4.4f**.

Levene's test for homogeneity of variances in chemical composition as a function of inter harvest interval (Table **4.5**) for Cassel indicates that the homogeneity of variances exists for each of the compositions (p>0.05) which is the assumption made in the ANOVA.

Table **4.3:** MANOVA Multivariate Test for Cassel

	Multivariate test of Significance, Effect Sizes, and Powers (DKdata Averages. Sta) Sigma-restricted parameterization Effective hypothesis decomposition Include condition: locality = ‘CASS’								
Effect	Test	Value	F	Effect df	Error df	P	Partial eta-squared	Non-centrality	Observed power (alpha=0.05)
Intercept	Wilks	0.06491	256.9199	6	107.000	0.00000	0.935093	1541.520	1.000000
Occasion	Wilks	0.59200	2.5302	24	374.4884	0.000126	0.139527	60.724	0.999468

Table **4.4a:** ANOVA for harpagide Cassel data

Sources of variation	Degrees of freedom	Harpagide SS	Harpagide MS	Harpagide F	Harpagide P
Intercept	1	620.1031	620.1031	403.5417	0.000000
Occasion	11	11.1086	2.7772	1.8073	0.132372
Error	421	172.1050	1.5367		
Total	432	183.2136			

Table **4.4b:** ANOVA for verbascoside Cassel data

Sources of variation	Degrees of freedom	Verbascoside SS	Verbascoside MS	Verbascoside F	Verbascoside P
Intercept	1	711.6872	711.6872	289.1339	0.000000
Occasion	11	37.4367	9.3592	3.8023	0.006157
Error	421	275.6818	2.4614		
Total	432	313.1185			

Table **4.4c:** ANOVA for isoverbascoside Cassel data

Sources of variation	Degrees of freedom	Isoverbacoside SS	Isoverbacoside MS	Isoverbacoside F	Isoverbacoside P
Intercept	1	2638.937	2638.937	156.5499	0.000000
Occasion	11	13.214	3.304	0.1960	0.940035
Error	421	1887.967	16.857		
Total	432	1901.181			

Table **4.4d:** ANOVA for acetylacteoside Cassel data

Sources of variation	Degrees of freedom	Acetylacteoside SS	Acetylacteoside MS	Acetylacteoside F	Acetylacteoside P
Intercept	1	220.2142	220.2142	2487.018	0.000000
Occasion	11	16.7965	1.5270	17.245	0.000000
Error	421	421	0.0885		
Total	432	432			

Table **4.4e:** ANOVA for coumaroylharpagide Cassel data

Sources of variation	Degrees of freedom	Coumaroylharpagide SS	Coumaroylharpagide MS	Coumaroylharpagide F	Coumaroylharpagide P
Intercept	1	0.272249	0.272249	718.8376	0.000000
Occasion	11	0.011635	0.002909	7.6801	0.000017
Error	421	0.042418	0.000379		
Total	432	0.054053			

Table **4.4f:** ANOVA for harpagoside Cassel data

Sources of variation	Degrees of freedom	Harpagoside SS	Harpagoside MS	Harpagoside F	Harpagoside P
Intercept	1	7523.297	7523.297	720.4148	0.000000
Occasion	11	62.935	15.734	1.5066	0.205058
Error	421	1169.617	10.443		
Total	432	1232.552			

Table **4.5:** Levene's test for homogeneity of variances on Cassel data

	Levene's Test for Homogeneity of Variances (Dkdata Averages. Sta) Effect: Occasion Degrees of freedom for all F's: 4, 112 Include condition: locality= 'CASS'			
	MS Effect	**MS Error**	**F**	**P**
Harpagide	0.310768	0.67072	0.463336	0.762499
Verbascoside	1.567735	1.07227	1.462072	0.218518
Isoverbascoside	5.122012	10.0103	0.511673	0.727258
Acetylacteoside	0.614584	0.81962	0.749844	0.560143
Coumaroylharpagide	0.000150	0.00016	0.923484	0.453010
Harpagoside	1.971375	3.83553	0.513977	0.725580

4.2.3 Multiple comparisons

The p-values of multiple comparisons using the Tukey HSD method between the five inter harvest periods (year one to five) for the six variables (six chemical constituents) respectively are indicated in tables **4.6a** to **4.6f**.

Table **4.6a:** Multiple comparisons between inter harvest intervals for mean harpagide content

	Unequal N HSD; Variable Harpagide (DKdata Avareges.sta) Approximate Probabilities for Post Hoc Tests Error: Between MS = 1.5367, df = 112.00 Include condition: locality = 'CASS'					
Cell No.	occasion	{1} 2.3066	{2} 2.5824	{3} 2.6248	{4} 1.7747	{5} 2.2294
1	11		0.947230	0.913851	0.614168	0.999638
2	12	0.947230		0.999964	0.183842	0.869978
3	13	0.913851	0.999964		0.129799	0.803676
4	14	0.614168	0.183842	0.129799		0.709772
5	15	0.999638	0.869978	0.803676	0.709772	

Table **4.6b:** Multiple comparisons between inter harvest intervals for mean verbascoside content

Cell No.	Unequal N HSD; Variable Verbascoside (DKdata Avareges.sta) Approximate Probabilities for Post Hoc Tests Error: Between MS = 2.4614, df = 112.00 Include condition: locality = ‘CASS’					
	occasion	{1} 2.6199	{2} 2.2355	{3} 3.4895	{4} 1.9973	{5} 1.9969
1	11		0.926342	0.357162	0.681759	0.681305
2	12	0.926342		0.058777	0.985780	0.985702
3	13	0.357162	0.058777		0.011410	0.011384
4	14	0.681759	0.985780	0.011410		1.000000
5	15	0.681305	0.985702	0.011384	1.00000	

Table **4.6c:** Multiple comparisons between inter harvest intervals for mean isoverbascoside content.

Cell No.	Unequal N HSD; Variable Isoverbascoside (DKdata Avareges.sta) Approximate Probabilities for Post Hoc Tests Error: Between MS = 16.857, df = 112.00 Include condition: locality = ‘CASS’					
	occasion	{1} 4.6383	{2} 4.3632	{3} 5.3731	{4} 4.6674	{5} 4.7185
1	11		0.999518	0.975852	1.000000	0.999996
2	12	0.999518		0.919524	0.999163	0.998425
3	13	0.975852	0.919524		0.975577	0.981507
4	14	1.000000	0.999163	0.975577		0.999999
5	15	0.999996	0.998425	0.981507	0.999999	

Table **4.6d:** Multiple comparisons between inter harvest intervals for mean acetylacteoside content.

	Unequal N HSD; Variable Acetylacteoside (DKdata Avareges.sta) Approximate Probabilities for Post Hoc Tests Error: Between MS = 1.9485, df = 112.00 Include condition: locality = ‘CASS’					
Cell No.	occasion	{1} 2.1937	{2} 2.0258	{3} 3.2190	{4} 2.3484	{5} 2.4468
1	11		0.994639	0.113410	0.996129	0.974672
2	12	0.994639		0.035811	0.934894	0.844402
3	13	0.113410	0.035811		0.202509	0.314827
4	14	0.996129	0.934894	0.202509		0.999266
5	15	0.974672	0.844402	0.314827	0.999266	

Table **4.6e:** Multiple comparisons between inter harvest intervals for mean coumaroylharpagide content.

	Unequal N HSD; Variable Coumaroylharpagide (DKdata Avareges.sta) Approximate Probabilities for Post Hoc Tests Error: Between MS = 0.00038, df = 112.00 Include condition: locality = ‘CASS’					
Cell No.	occasion	{1} 0.04116	{2} 0.04792	{3} 0.06745	{4} 0.04329	{5} 0.04151
1	11		0.779299	0.000275	0.996325	0.999998
2	12	0.779299		0.008129	0.928507	0.797826
3	13	0.000275	0.008129		0.000458	0.000205
4	14	0.996325	0.928507	0.000458		0.997826
5	15	0.999998	0.797826	0.000205	0.997826	

Table **4.6f:** Multiple comparisons between inter harvest intervals for mean harpagoside content.

Cell No.	Unequal N HSD; Variable Harpagoside (DKdata Avareges.sta) Approximate Probabilities for Post Hoc Tests Error: Between MS = 10.443, df = 112.00 Include condition: locality = ‘CASS’					
	occasion	{1} 7.0904	{2} 7.5980	{3} 8.5849	{4} 7.7086	{5} 9.1363
1	11		0.985146	0.542955	0.969175	0.227564
2	12	0.985146		0.838358	0.934894	0.491587
3	13	0.542955	0.838358		0.880995	0.976221
4	14	0.969175	0.934894	0.880995		0.545143
5	15	0.227564	0.491587	0.976221	0.545143	

4.3 Average chemical composition

Table **4.7** gives the average harpagide, harpagoside, verbascoside, 6-acetylacteoside, isoverbascoside and coumaroylharpagide with standard deviation for each of the five inter harvest periods at Cassel.

Table **4.7:** Averages of chemical constituents in terms of amounts per sample (Cassel data)

Locality	Average concentration of each chemical compound present in the samples from different localities					
	Harpagide (μg/g)	Verbascoside (μg/g)	Isoverbascoside (μg/g)	Acetylacteoside (μg/g)	Coumaroylharpagide (μg/g)	Harpagoside (μg/g)
CASS 1	2.31	2.62	4.64	2.19	0.04	7.09
St. deviation	1.28	1.42	3.51	1.00	0.01	2.75
CASS 2	2.58	2.24	4.36	2.03	0.05	7.60
St. deviation	1.37	1.18	4.43	1.26	0.02	3.76
CASS 3	2.62	3.49	5.37	3.22	0.07	8.58
St. deviation	1.01	2.02	2.30	1.47	0.02	2.87
CASS 4	1.77	2.00	4.67	2.35	0.04	7.71
St. deviation	0.91	1.63	5.78	1.28	0.02	3.16
CASS 5	2.23	2.00	4.72	2.45	0.04	9.14
St. deviation	1.53	1.45	3.65	1.80	0.02	3.49

4.4 Conclusions (Cassel Data Analysis)

From tables **4.6a to 4.6f** we make the following conclusions (the red figures indicate significant variation):

1. Harpagide shows no significant variation from year to year (Table **4.6a**).

2. Verbascoside shows significant variation between year three, four and five (Table **4.6b**).

3. Isoverbascoside shows no significant changes from year to year (Table **4.6c**).

4. Acetylacteoside shows significant differences between year two and year three (Table **4.6d**).

5. Coumaroylharpagide shows significant variation from year to year and three year old plants seem to differ significantly from the rest (Table **4.6e**). This is the most variable component.

6. Harpagoside does not seem to be affected by age. This is an important observation as harpagoside is the biologically active ingredient which determines quality and commercial value (Table **4.6f**).

5. Analysis of North West data (Six different localities in the North West Province)

5.1 Purpose of the study in chapter 5

As mention in chapter 4 the purpose here is to answer the following question using our results in Appendix **A.**

Are there meaningful differences in chemical composition between the six populations from the North West Province (Cassel, Ganayesa, Moswana, Terra Firma, Lafras and Molopo Nature Reserve)?

In this chapter we analysed the data from the six different populations in the North West Province. The question above is restated as a hypothesis: “There are significant differences in the chemical composition between six different populations in the North West Province (Cassel, Ganayesa, Moswana, Terra Firma, Lafras and Molopo Nature Reserve)”. In contrast with chapter 4, where all the collections were done on the same area, the populations represented in this chapter are up to 100 km apart.

5.2 Statistical analysis

5.2.1 Factor analysis

Factor analysis with principal component method (followed by a Varimax-rotation) identifies two factors. Factor 1 (eigenvalue 2.38) 39.3% and factor 2 (eigenvalue 1.38) explains 62.7% of total variance (Table **5.1a**). Addition of a third factor (eigenvalues 0.923) explains 78% of the total variance (Table **5.1b**).

Table **5.1a:** Factor analysis on North West data (two factors)

Variable	Factor loadings (Varimax raw) (DKdata Averages. Sta) Extraction: Principal components (Marked loadings are >.700000)	
	Factor 1	Factor 2
Harpagide	0.344280	0.319214
Verbascoside	**0.805416**	-0.002848
Isoverbascoside	**0.872889**	0.004190
Acetylacteoside	**0.808781**	0.096819
Coumaroylharpagide	0.299102	**0.782464**
Harpagoside	-0.177723	**0.856904**
Expl. Var.	2.304333	1.457831
Prp. Totl	0.384055	0.242972

Table **5.1b:** Factor analysis on North West data (three factors)

Variable	Factor loadings (Varimax raw) (DKdata Averages. Sta) Extraction: Principal components (Marked loadings are >.700000)		
	Factor 1	Factor 2	Factor 3
Harpagide	0.093567	0.077671	**0.980564**
Verbascoside	**0.829762**	0.014975	0.022945
Isoverbascoside	**0.812722**	-0.058470	0.331476
Acetylacteoside	**0.844790**	0.125042	-0.002485
Coumaroylharpagide	0.310822	**0.786760**	0.119163
Harpagoside	-0.157455	**0.871933**	0.035683
Expl. Var.	2.192848	1.404569	1.087388
Prp. Totl	0.365475	0.234095	0.181231

Table **5.2:** Communalities on North West data

	Communalities (DKdata Averages. Sta) Extraction: Principal components Rotation: Unrotated.			
Variable	From factor 1	From factor 2	From factor 3	Multiple R-Square
Harpagide	0.176522	0.220426	0.976293	0.152990
Verbascoside	0.596172	0.648702	0.689255	0.395678
Isoverbascoside	0.703671	0.761952	0.773812	0.528730
Acetylacteoside	0.645412	0.663501	0.729312	0.455969
Coumaroylharpagide	0.257095	0.701712	0.729801	0.296648
Harpagoside	0.004954	0.765869	0.786332	0.218316

5.2.2 Analysis of Variance

Due to the fact that factor analysis excludes harpagide from the structurally closely related 8-coumaroylharpagide and harpagoside factor, and also because it appears significant in factor 3, all six variables were used (chemical constituents) in correlation analysis.

A multivariate analysis of variance (MANOVA) (table **5.3a**) and transformed data (table **5.3b**) on the 6 chemical constituents indicates highly significant differences between the different localities (Cassel, Ganyesa, Moswana, Molopo nature Reserve, Lafras and Terra firma, indicated as ‘locality’ ($p<0.001$) when comparing the 6 variables means multivariately. However, the homogeneity of variances doesn’t hold in 5 of 6 variables (table **5.4a**) and even on the transformed data homogeneity does not hold for 4 variables (table **5.4b**).

Table **5.3a:** The MANOVA: multivariate test on North West Data

	Multivariate test of Significance, Effect Sizes, and Powers (DKdata Averages. Sta) Sigma-restricted parameterization Effective hypothesis decomposition								
Effect	Test	Value	F	Effect df	Error df	P	Partial eta-squared	Non-centrality	Observed power (alpha=0.05)
Intercept	Wilks	0.149179	290.8708	6	306	0.00	0.850821	1745.225	1.000000
locality	Wilks	0.438249	9.3605	30	1226	0.00	0.186364	280.816	1.000000

Table **5.3b:** The MANOVA multivariate test on transformed North West data

	Multivariate test of Significance, Effect Sizes, and Powers (DKdata Averages. Sta) Sigma-restricted parameterization Effective hypothesis decomposition								
Effect	Test	Value	F	Effect df	Error df	P	Partial eta-squared	Non-centrality	Observed power (alpha=0.05)
Intercept	Wilks	0.009537	5296.313	6	306	0.00	0.990463	31777.88	1.000000
locality	Wilks	0.41153	10.156	30	1226	0.00	0.199048	304.68	1.000000

Table **5.4a:** The homogeneity of variances

	Levene's Test for Homogeneity of Variances (DKdata AVERAGES.sta) Effect: Locality Degrees of freedom for all F's: 5, 311			
	MS Effect	**MS Error**	**F**	**P**
Harpagide	1.90325	0.646085	2.94583	0.012937
Verbascoside	25.72929	2.091202	12.30359	0.000000
Isoverbascoside	1.36579	8.527444	0.16016	0.976801
Acetylacteoside	14.24225	1.677418	8.49058	0.000000
Coumaroylharpagide	0.00050	0.000150	3.31551	0.006222
Harpagoside	20.44854	5.936451	3.44457	0.004807

Table **5.4b:** The homogeneity of variances for transformed data

	Levene's Test for Homogeneity of Variances (DKdata AVERAGES.sta) Effect: Locality Degrees of freedom for all F's: 5, 311			
	MS Effect	**MS Error**	**F**	**P**
Harpagide	0.297995	0.116859	2.550042	0.027917
Verbascoside	0.683486	0.167705	4.075527	0.001344
Isoverbascoside	0.196171	0.143967	1.362610	0.238166
Acetylacteoside	0.408046	0.179493	2.273322	0.047236
Coumaroylharpagide	0.097124	0.041738	2.326995	0.042694
Harpagoside	0.357719	0.182847	1.956379	0.084907

5.2.3 Multiple comparisons

The p-values of multiple comparisons using the Tukey HSD method between each pair of localities Cassel, Ganyesa, Moswana, Molopo Nature Reserve, Lafras and Terra firma for the six variables (chemical constituents) respectively are indicated in tables **5.5a** to **5.5f**. Table **5.6** gives the inter-correlations on which the factor analysis was based.

Table **5.5a:** Multiple comparisons between localities in North West Province for mean harpagide content

	Unequal N HSD; variable harpagide (DKdata averages_trans1.sta) Approximate Probabilities for Post Hoc Tests Error: Between MS = 0.29202, df = 311.00 Include condition: locality = 'North West'						
Cell no.	Locality	{1} 0.74151	{2} 0.74362	{3} 0.58375	{4} 0.78534	{5} 0.99375	{6} 0.47847
1	CASS		1.000000	0.530239	0.999766	0.937919	0.411248
2	GAN	1.000000		0.515117	0.999816	0.940013	0.401864
3	MOS	0.530239	0.515117		0.789499	0.653008	0.974882
4	M-NR	0.999766	0.999816	0.789499		0.972339	0.361386
5	Lafras	0.937919	0.940013	0.653008	0.972339		0.397671
6	Terra Firma	0.411248	0.401864	0.974882	0.361386	0.397671	

(Cassel = CASS, Ganyesa = GAN, Moswana = MOS, Molopo Nature Reserve = M-NR)

Table **5.5b:** Multiple comparisons between localities in North West Province for mean verbascocide

	Unequal N HSD; variable Verbascoside (DKdata averages_trans1.sta) Approximate Probabilities for Post Hoc Tests Error: Between MS = 0.50141, df = 311.00 Include condition: locality = ‘North West’						
Cell no.	Locality	{1} 0.75838	{2} 0.90072	{3} 0.83828	{4} -0.1568	{5} 2.1615	{6} 1.0558
1	CASS		0.842254	0.986381	0.000126	0.001053	0.580639
2	GAN	0.842254		0.995651	0.000023	0.004986	0.958342
3	MOS	0.986381	0.995651		0.000035	0.002567	0.841947
4	M-NR	0.000126	0.000023	0.000035		0.000020	0.000020
5	Lafras	0.001053	0.004986	0.002567	0.000020		0.022113
6	Terra Firma	0.580639	0.958342	0.841947	0.000020	0.022113	

(Cassel = CASS, Ganyesa = GAN, Moswana = MOS, Molopo Nature Reserve = M-NR)

Table **5.5c:** Multiple comparisons between localities in North West Province for mean isoverbascoside content

	Unequal N HSD; variable Isoverbascoside (DKdata averages_trans1.sta) Approximate Probabilities for Post Hoc Tests Error: Between MS = 0.37832, df = 311.00 Include condition: locality = ‘North West’						
Cell no.	Locality	{1} 1.3162	{2} 1.5220	{3} 1.4985	{4} 1.0690	{5} 1.7257	{6} 1.4193
1	CASS		0.354002	0.513261	0.731762	0.767473	0.987193
2	GAN	0.354002		0.999924	0.109545	0.985961	0.987384
3	MOS	0.513261	0.999924		0.149653	0.977087	0.996241
4	M-NR	0.731762	0.109545	0.149653		0.268923	0.357982
5	Lafras	0.767473	0.985961	0.977087	0.268923		0.919260
6	Terra Firma	0.987193	0.987384	0.996241	0.357982	0.919260	

(Cassel = CASS, Ganyesa = GAN, Moswana = MOS, Molopo Nature Reserve = M-NR)

Table **5.5d:** Multiple comparisons between localities in North West Province for mean 6-acetylacteoside content

	Unequal N HSD; variable 6-Acetylacteoside (DKdata averages_trans1.sta) Approximate Probabilities for Post Hoc Tests Error: Between MS = 0.50517, df = 311.00 Include condition: locality = 'North West'						
Cell no.	Locality	{1} 0.80810	{2} 1.0393	{3} 1.4246	{4} 0.43226	{5} 1.2225	{6} 1.3578
1	CASS		0.386991	0.000026	0.445098	0.853077	0.032747
2	GAN	0.386991		0.019626	0.036509	0.995601	0.508197
3	MOS	0.000026	0.019626		0.000038	0.993030	0.999170
4	M-NR	0.445098	0.036509	0.000038		0.226761	0.000110
5	Lafras	0.853077	0.995601	0.993030	0.226761		0.998966
6	Terra Firma	0.032747	0.508197	0.999170	0.000110	0.998966	

(Cassel = CASS, Ganyesa = GAN, Moswana = MOS, Molopo Nature Reserve = M-NR)

Table **5.5e:** Multiple comparisons between localities in North West Province for mean coumarylharpagide content

	Unequal N HSD; variable Coumaroyharpagide (DKdata averages_trans1.sta) Approximate Probabilities for Post Hoc Tests Error: Between MS = 0.11227, df = 311.00 Include condition: locality = 'North West'						
Cell no.	Locality	{1} -2.859	{2} -2.910	{3} -2.872	{4} -2.677	{5} -2.346	{6} -2.910
1	CASS		0.947044	0.999910	0.411146	0. 026577	0.991694
2	GAN	0.947044		0.986737	0.152186	0.009880	1.000000
3	MOS	0.999910	0.986737		0.329222	0.020738	0.997992
4	M-NR	0.411146	0.152186	0.329222		0.356501	0.151408
5	Lafras	0. 026577	0.009880	0.020738	0.356501		0.009835
6	Terra Firma	0.991694	1.000000	0.997992	0.151408	0.009835	

(Cassel = CASS, Ganyesa = GAN, Moswana = MOS, Molopo Nature Reserve = M-NR)

Table **5.5f:** Multiple comparisons between localities in North West Province for mean harpagoside content

	Unequal N HSD; variable Harpagoside (DKdata averages_trans1.sta) Approximate Probabilities for Post Hoc Tests Error: Between MS = 0.11227, df = 311.00 Include condition: locality = ‘North West’						
Cell no.	Locality	{1} -2.859	{2} -2.910	{3} -2.872	{4} -2.677	{5} -2.346	{6} -2.910
1	CASS		0.000356	0.928699	0.969114	0.999932	0.000020
2	GAN	0.000356		0.017374	0.543208	0.832407	0.001418
3	MOS	0.928699	0.017374		0.999934	0.999997	0.000020
4	M-NR	0.969114	0.543208	0.999934		0.999896	0.000023
5	Lafras	0.999932	0.832407	0.999997	0.999896		0.016312
6	Terra Firma	0.000020	0.001418	0.000020	0.000023	0.016312	

(Cassel = CASS, Ganyesa = GAN, Moswana = MOS, Molopo Nature Reserve = M-NR)

Table **5.6:** Inter-correlations on which factor analyses were based

	Correlations (DKdata AVERAGES.sta) Casewise deletion of MD N=317					
locality	Harpagide	Verbascoside	Isoverbascoside	Acetylacteoside	Coumaroyl harpagide	Harpagoside
CASS	1.00	0.13	0.33	0.12	0.20	0.08
GAN	0.13	1.00	0.56	0.51	0.27	-0.10
MOS	0.33	0.56	1.00	0.62	0.19	-0.08
M-NR	0.12	0.51	0.62	1.00	0.28	0.01
Lafras	0.20	0.27	0.19	0.28	1.00	0.41
Terra Firma	0.08	-0.10	-0.08	0.01	0.41	1.00

(Cassel = CASS, Ganyesa = GAN, Moswana = MOS, Molopo Nature Reserve = M-NR)

5.3 Average chemical composition

Table **5.7** gives the average harpagide, harpagoside, verbascoside, 6-acetylacteoside, isoverascoside and coumaroylharpagide with standard deviation for each of the six locations in the North West Province.

Table **5.7:** Averages of chemical constituents from each area

Locality	Average concentration of each chemical compound present in the samples from different localities					
	Harpagide (µg/g)	Verbascoside (µg/g)	Isoverbascoside (µg/g)	Acetylacteoside (µg/g)	Coumaroylharpagide (µg/g)	Harpagoside (µg/g)
CASS	2.23	2.00	4.72	2.45	0.04	9.14
St. deviation	0.34	0.63	0.37	0.46	0.01	0.82
GAN	3.21	2.67	5.75	2.98	0.04	4.88
St. deviation	0.76	0.62	0.40	0.12	0.01	0.93
MOS	1.47	2.00	5.23	3.83	0.04	7.18
St. deviation	0.74	1.80	1.87	2.28	0.01	1.94
M-NR	2.45	0.97	3.92	1.80	0.06	7.09
St. deviation	1.38	0.48	2.91	1.19	0.02	1.96
Lafras	2.65	8.50	6.71	3.75	0.08	7.36
St. deviation	0.56	5.83	4.05	2.12	0.01	1.61
Terra Firma	1.67	2.90	4.92	3.88	0.04	3.46
St. deviation	0.49	1.01	2.62	1.96	0.01	1.47

(Cassel = CASS, Ganyesa = GAN, Moswana = MOS, Molopo Nature Reserve = M-NR)

5.4 Conclusions (North West Data Analysis)

From tables **5.5a** to **5.5f** (the red figures indicate significant variation) we make the following conclusions:

1. Harpagide shows no significant variation between localities (Table **5.5a**).

2. Verbascoside shows significant differences in means between Molopo Nature Reserve and all other localities and also between, Lafras and all other localities (table **5.5b**).

3. Isoverbascoside shows no significant differences between localities (Table **5.5c**).

4. Acetylacteoside shows significant differences in means between Ganyesa and both Molopo Nature Reserve and Moswana, also differences between Cassel and both Molopo Nature Reserve and Terra Firma, while both Moswana and Terra Firma also differ significantly from Molopo Nature Reserve (table **5.5d**). No clear pattern is evident.

5. Coumaroylharpagide shows significant variation between Lafras and all the localities in North West except Molopo Nature Reserve (Table **5.5e**). Lafras has a higher average concentration (7.4 μg/g) than all the North West localities, followed by Molopo Nature reserve (table **5.7**). Molopo Nature reserve is geographically very close to Lafras whilst the other localities are further geographically. It may be worthwhile to investigate this further.

6. Harpagoside shows significant variation between localities. Terra Firma stands out in being significantly different from all the other localities in North West Province (table **5.5f**). This can be discerned by inspecting the concentration mean of harpagoside in Terra Firma plant materials (which is the smallest) in table **5.7** that give the average composition of each of the six North West Province localities. There are also significant differences between both Moswana and Cassel with Ganyesa (table **5.5f**).

6. Analysis of North West and Namibia data

6.1 The aim of study in Chapter 6

In this study, we aim to answer the following question using our results in Appendix **A**.

Are there meaningful differences in the chemical composition of populations from North West Province and Namibia?

In this chapter, the data from the six different populations in the North West Province and Namibia were analysed and compared to each other. We restate the question above as a hypothesis: “There are significant differences in the chemical composition between six different populations in the North West Province (Cassel, Ganayesa, Moswana, Terra Firma, Lafras and Molopo Nature Reserve) and Namibia populations. In contrast with chapter 5, where the populations are about 100 km apart, this chapter analyses data from locations of two countries (South Africa and Namibia) that are about 1000 km apart.

6.2 Statistical analysis

6.2.1 Factor analysis

Factor analysis with principal component method (followed by a Varimax-rotation) identifies two factors. Factor 1 (eigenvalue 2.39) 39.9% and factor 2 (eigenvalue 1.41) explains 63.4% of total variance (Table **6.1a**). Addition of third factor (eigenvalue 0.87) explains 78% of the total variance (Table **6.1b**).

Table **6.1a:** Factor analysis on North West and Namibia data (two factors)

Variable	Factor loadings (Varimax raw) (DKdata Averages. Sta) Extraction: Principal components (Marked loadings are >.700000)	
	Factor 1	Factor 2
Harpagide	0.291277	0.553999
Verbascoside	0.805196	-0.070017
Isoverbascoside	0.866944	0.112497
Acetylacteoside	0.811032	0.131690
Coumaroylharpagide	0.250395	0.795099
Harpagoside	-0.210228	0.760739
Expl. Var.	2.249440	1.552721
Prp. Total	0.374907	0.258787

Table **6.1b:** Factor analysis on North West and Namibia data (three factors)

Variable	Factor loadings (Varimax raw) (DKdata Averages. Sta) Extraction: Principal components (Marked loadings are >.700000)		
	Factor 1	Factor 2	Factor 3
Harpagide	0.083856	0.060800	0.960214
Verbascoside	0.828322	-0.066559	-0.012525
Isoverbascoside	0.812506	-0.062248	0.328706
Acetylacteoside	0.852099	0.142818	0.032427
Coumaroylharpagide	0.249774	0.672930	0.427520
Harpagoside	-0.088131	0.909642	-0.028365
Expl. Var.	2.149543	1.312682	1.214845
Prp. Total	0.358257	0.218780	0.202474

Table **6.2:** Communalities on North West and Namibia data

Variable	Communalities (DKdata Averages. Sta) Extraction: Principal components Rotation: Unrotated. Exclude condition: locality = 'Zim' or locality = 'Limpopo Province'			
	From factor 1	From factor 2	From factor 3	Multiple R-Square
Harpagide	0.231502	0.391757	0.932740	0.22577
Verbascoside	0.514199	0.653243	0.690705	0.36082
Isoverbascoside	0.712306	0.764247	0.772089	0.53170
Acetylacteoside	0.639479	0.675114	0.747521	0.46101
Coumaroylharpagide	0.286935	0.694881	0.697996	0.29520
Harpagoside	0.009403	0.622919	0.836020	0.16870

6.2.2 Analysis of Variance

Factor analysis excludes harpagide from the structurally closely related 8-coumaroylharpagide and harpagoside in factor 1, and since it appears significant in factor 3 all six variables were used (chemical constituents) in correlation analysis.

A multivariate analysis of variance (MANOVA) (table **6.3a**) and transformed data (table **6.3b**) on the 6 chemical constituents indicates highly significant differences between the different localities (Cassel, Ganyesa, Moswana, Molopo nature Reserve, Lafras, Terra firma and Namibia, indicated as 'locality' ($p<0.001$ for the Wilks test) when comparing the 6 variable means multivariately. The homogeneity of variances does not hold for three of the six variables (table **6.4**)

Table **6.3a:** The MANOVA multivariate test on North West and Namibia Data

	Multivariate test of Significance, Effect Sizes, and Powers (DKdata Averages. Sta) Sigma-restricted parameterization Effective hypothesis decomposition								
Effect	Test	Value	F	Effect df	Error df	P	Partial eta-squared	Non-centrality	Observed power (alpha=0.05)
Intercept	Wilks	0.188293	222.7288	6	310.000	0.00	0.811707	1336.373	1.000000
locality	Wilks	0.347062	10.3254	36	1364.066	0.00	0.214149	371.716	1.000000

Table **6.3b:** The MANOVA Multivariate Test on transformed North West and Namibia data

	Multivariate test of Significance, Effect Sizes, and Powers (DKdata Averages. Sta) Sigma-restricted parameterization Effective hypothesis decomposition								
Effect	Test	Value	F	Effect df	Error df	P	Partial eta-squared	Non-centrality	Observed power (alpha=0.05)
Intercept	Wilks	0.075750	630.4002	6	310.000	0.00	0.924250	3782.401	1.000000
locality	Wilks	0.347538	10.3104	30	1364.066	0.00	0.213903	371.174	1.000000

Table **6.4:** The homogeneity of variances

	Levene's Test for Homogeneity of Variances (DKdata AVERAGES.sta) Effect: Locality Degrees of freedom for all F's: 6, 315 Excluded locality = ‘Zim’ or locality = ‘Limpopo Province’			
	MS Effect	**MS Error**	**F**	**P**
Harpagide	0.071879	0.032845	2.188413	0.043911
Verbascoside	0.143820	0.034594	4.157394	0.000489
Isoverbascoside	0.105763	0.075748	1.396238	0.215568
Acetylacteoside	0.157309	0.083414	1.885880	0.082766
Coumaroylharpagide	0.000668	0.000095	7.023944	0.000000
Harpagoside	0.338510	0.196173	1.725569	0.114461

Transformed univariate analyses of variance (ANOVA) done for each of the chemical variables and results are displayed in table **6.5a** to **6.5f**.

Table **6.5a:** ANOVA for harpagide

Sources of variation	Degrees of freedom	Harpagide SS	Harpagide MS	Harpagide F	Harpagide P
Intercept	1	103.0656	103.0656	1211.506	0.000000
Occasion	6	2.4297	0.4050	4.760	0.000116
Error	316	26.7978	0.0851		
Total	322	29.2275			

Table **6.5b:** ANOVA for verbascoside

Sources of variation	Degrees of freedom	Verbascoside SS	Verbascoside MS	Verbascoside F	Verbascoside P
Intercept	1	101.1620	101.1620	903.5872	0.000000
Occasion	6	9.2493	1.5416	13.7693	0.000000
Error	316	35.2661	0.1120		
Total	322	44.5155			

Table **6.5c:** ANOVA for isoverbascoside

Sources of variation	Degrees of freedom	Isoverbascoside SS	Isoverbascoside MS	Isoverbascoside F	Isoverbascoside P
Intercept	1	234.2417	234.2417	1147.912	0.000000
Occasion	6	4.0598	0.6766	3.316	0.003519
Error	316	64.2786	0.2041		
Total	322	68.3384			

Table **6.5d:** ANOVA for acetylacteoside

Sources of variation	Degrees of freedom	Acetylacteoside SS	Acetylacteoside MS	Acetylacteoside F	Acetylacteoside P
Intercept	1	156.5757	156.5757	650.2177	0.000000
Occasion	6	14.0557	2.3426	9.7283	0.000000
Error	316	75.8536	0.2408		
Total	322	89.9093			

Table **6.5e:** ANOVA for coumaroylharpagide

Sources of variation	Degrees of freedom	Coumaroylharpagide SS	Coumaroylharpagide MS	Coumaroylharpagide F	Coumaroylharpagide P
Intercept	1	0.192386	0.192386	747.5206	0.000000
Occasion	6	0.018132	0.003022	11.7418	0.000000
Error	316	0.081070	0.000257		
Total	322	0.099202			

Table **6.5f:** ANOVA for harpagoside

Sources of variation	Degrees of freedom	Harpagoside SS	Harpagoside MS	Harpagoside F	Harpagoside P
Intercept	1	810.5662	810.5662	1862.903	0.000000
Occasion	6	36.4286	6.0714	13.954	0.000000
Error	316	137.0594	0.4351		
Total	322	173.4880			

6.2.3 Multiple comparisons

The p-values of multiple comparisons using the Tukey HSD method between each pair of localities Cassel, Ganyesa, Moswana, Molopo Nature Reserve, Lafras, Terra Firma and Namibia for the six variables respectively are indicated in tables **6.6a** to **6.6f**. Homogeneous groups of localities from pair wise comparisons of the chemical constituents are shown in simplified form in tables **6.7a** to **6.7f**.

Table **6.6a:** Multiple comparisons between localities in North West Province and Namibia for mean harpagide content

Cell no.	Unequal N HSD; variable harpagide (DKdata averages_trans1.sta) Approximate Probabilities for Post Hoc Tests Error: Between MS = 0.29202, df = 311.00							
	Locality	{1} 0.89504	{2} 0. 89829	{3} 1.4016	{4} 0.81354	{5} 1.0355	{6} 1.03547	{7} 0.75220
1	CASS		1.000000	0.087214	0.663290	0.999955	0.961822	0.482450
2	GAN	1.000000		0.091308	0.619911	0.999981	0.965970	0.453680
3	Namibia	0.087214	0.091308		0.024212	0.121404	0.424480	0.007878
4	MOS	0.663290	0.619911	0.024212		0.872268	0.731803	0.983580
5	M-NR	0.999955	0.999981	0.121404	0.872268		0.985316	0.425177
6	Lafras	0.961822	0.965970	0.424480	0.731803	0.985316		0.45204
7	Terra Firma	0.482450	0.453680	0.007878	0.983580	0.425177	0.45204	

(Cassel = CASS, Ganyesa = GAN, Moswana = MOS, Molopo Nature Reserve = M-NR)

Table **6.6b:** Multiple comparisons between localities in North West Province and Namibia for mean verbascocide

Cell no.	Unequal N HSD; variable Verbascoside (DKdata averages_trans1.sta) Approximate Probabilities for Post Hoc Tests Error: Between MS = 0.50141, df = 311.00							
	Locality	{1} 0.92123	{2} 0.99630	{3} 0.64396	{4} 0.96134	{5} 0.49082	{6} 1.5690	{7} 1.0703
1	CASS		0.839298	0.847470	0.992692	0.000188	0.002086	0.598659
2	GAN	0.839298		0.639594	0.996554	0.000029	0.011070	0.978703
3	Namibia	0.847470	0.639594		0.745150	0.991196	0.000266	0.405137
4	MOS	0.992692	0.996554	0.745150		0.000046	0.005230	0.869504
5	M-NR	0.000188	0.000029	0.991196	0.000046		0.000026	0.000026
6	Lafras	0.002086	0.011070	0.000266	0.005230	0.000026		0.045509
7	Terra Firma	0.598659	0.978703	0.405137	0.869504	0.000026	0.045509	

(Cassel = CASS, Ganyesa = GAN, Moswana = MOS, Molopo Nature Reserve = M-NR)

Table **6.6c:** Multiple comparisons between localities in North West Province and Namibia for mean isoverbascoside content

Cell no.	Unequal N HSD; variable Isoverbascoside (DKdata averages_trans1.sta) Approximate Probabilities for Post Hoc Tests Error: Between MS = 0.37832, df = 311.00							
	Locality	{1} 1.3098	{2} 1.4664	{3} 1.7629	{4} 1.4477	{5} 1.1326	{6} 1.6156	{7} 1.3888
1	CASS		0.382031	0.691359	0.560953	0.823635	0.826113	0.993824
2	GAN	0.382031		0.945307	0.999984	0.138412	0.994628	0.994403
3	Namibia	0.691359	0.945307		0.927393	0.291885	0.998646	0.847824
4	MOS	0.560953	0.999984	0.927393		0.191272	0.98985	0.998792
5	M-NR	0.823635	0.138412	0.291885	0.191272		0.329843	0.437215
6	Lafras	0.826113	0.994628	0.998646	0.98985	0.329843		0.953254
	Terra Firma	0.993824	0.994403	0.847824	0.998792	0.437215	0.953254	

(Cassel = CASS, Ganyesa = GAN, Moswana = MOS, Molopo Nature Reserve = M-NR)

Table **6.6d:** Multiple comparisons between localities in North West Province and Namibia for mean 6-acetylacteoside content

Cell no.	Unequal N HSD; variable 6-acetylacteoside (DKdata averages_trans1.sta) Approximate Probabilities for Post Hoc Tests Error: Between MS = 0.37832, df = 311.00							
	Locality	{1} 0.97790	{2} 1.1392	{3} 1.3920	{4} 1.4085	{5} 0.72359	{6} 1.2749	{7} 1.3609
1	CASS		0.450820	0.836004	0.000031	0.551247	0.890376	0.040203
2	GAN	0.450820		0.983583	0.023238	0.052155	0.997986	0.582088
3	Namibia	0.836004	0.983583		1.000000	0.321249	0.999776	1.000000
4	MOS	0.000031	0.023238	1.000000		0.000051	0.998160	0.999781
5	M-NR	0.551247	0.052155	0.321249	0.000051		0.270350	0.000158
6	Lafras	0.890376	0.997986	0.999776	0.998160	0.270350		0.999854
	Terra Firma	0.040203	0.582088	1.000000	0.999781	0.000158	0.999854	

(Cassel = CASS, Ganyesa = GAN, Moswana = MOS, Molopo Nature Reserve = M-NR)

Table **6.6e:** Multiple comparisons between localities in North West Province and Namibia for mean coumaroylharpagide content

Unequal N HSD; variable coumarylharpagide (DKdata averages_trans1.sta)
Approximate Probabilities for Post Hoc Tests
Error: Between MS = 0.37832, df = 311.00

Cell no.	Locality	{1} 0.03105	{2} 0.02812	{3} 0.07694	{4} 0.03039	{5} 0.03951	{6} 0.05645	{7} 0.02767
1	CASS		0.933652	0.000144	0.999984	0.529638	0.025925	0.983267
2	GAN	0.933652		0.000054	0.982292	0.173959	0.007559	1.000000
3	Namibia	0.000144	0.000054		0.000112	0.004234	0.401985	0.000048
4	MOS	0.999984	0.982292	0.000112		0.434374	0.019924	0.994722
5	M-NR	0.529638	0.173959	0.004234	0.434374		0.345832	0.139072
6	Lafras	0.025925	0.007559	0.401985	0.019924	0.345832		0.006151
	Terra Firma	0.983267	1.000000	0.000048	0.994722	0.139072	0.006151	

(Cassel = CASS, Ganyesa = GAN, Moswana = MOS, Molopo Nature Reserve = M-NR)

Table **6.6f:** Multiple comparisons between localities in North West Province and Namibia for mean harpagoside content

Unequal N HSD; variable harpagoside (DKdata averages_trans1.sta)
Approximate Probabilities for Post Hoc Tests
Error: Between MS = 0.37832, df = 311.00

Cell no.	Locality	{1} 2.9444	{2} 2.4746	{3} 3.0976	{4} 2.8358	{5} 2.7927	{6} 2.8705	{7} 1.8167
1	CASS		0.000517	0.999808	0.962528	0.985372	0.999990	0.000026
2	GAN	0.000517		0.749137	0.023809	0.636154	0.894305	0.002160
3	Namibia	0.999808	0.749137		0.995949	0.990721	0.998165	0.034863
4	MOS	0.962528	0.023809	0.995949		0.999989	1.000000	0.000026
5	M-NR	0.985372	0.636154	0.990721	0.999989		0.999986	0.000031
6	Lafras	0.999990	0.894305	0.998165	1.000000	0.999986		0.023625
	Terra Firma	0.000026	0.002160	0.034863	0.000026	0.000031	0.023625	

(Cassel = CASS, Ganyesa = GAN, Moswana = MOS, Molopo Nature Reserve = M-NR)

Table **6.7a:** Homogeneous groups of localities from pair wise comparisons for harpagide

Cell No.	Unequal N HSD; variable harpagide (DKdata AVER_tot_tran.sta) Homogenous Groups, alpha = 0.05000 Error: Between MS = 0.08507, df = 315.00			
	Locality1	Harpagide Mean	1	2
7	Terra Firma	0.752199	****	
4	MOS	0.813540	****	
1	CASS	0.895037	****	****
2	GAN	0.898290	****	****
5	M-NR	0.919205	****	****
6	Lafras	1.035466	****	****
3	Namibia	1.401557		

(Cassel = CASS, Ganyesa = GAN, Moswana = MOS, Molopo Nature Reserve = M-NR)

Table **6.7b:** Homogeneous groups of localities from pair wise comparisons for verbascoside

Cell No.	Unequal N HSD; variable verbascoside (DKdata AVER_tot_tran.sta) Homogenous Groups, alpha = 0.05000 Error: Between MS = 0.11196, df = 315.00				
	Locality1	Verbascoside Mean	1	2	3
5	M-NR	0.490818		****	
3	Namibia	0.643962	****	****	
1	CASS	0.921231	****		
4	MOS	0.961343	****		
2	GAN	0.996302	****		
7	Terra Firma	1.070319	****		
6	Lafras	1.569015			****

(Cassel = CASS, Ganyesa = GAN, Moswana = MOS, Molopo Nature Reserve = M-NR)

Table **6.7c:** Homogeneous groups of localities from pair wise comparisons for isoverbascoside

Cell No.	Unequal N HSD; variable isoverbascoside (DKdata AVER_tot_tran.sta) Homogenous Groups, alpha = 0.05000 Error: Between MS = 0.20406, df = 315.00		
	Locality1	Isoverbascoside Mean	1
5	M-NR	1.132554	****
1	CASS	1.309757	****
7	Terra Firma	1.388790	****
4	MOS	1.447736	****
2	GAN	1.466405	****
6	Lafras	1.615578	****
3	Namibia	1.762905	****

(Cassel = CASS, Ganyesa = GAN, Moswana = MOS, Molopo Nature Reserve = M-NR)

Table **6.7d:** Homogeneous groups of localities from pair wise comparisons for 6-acetylateoside

Cell No.	Unequal N HSD; variable 6-acetylacteoside (DKdata AVER_tot_tran.sta) Homogenous Groups, alpha = 0.05000 Error: Between MS = 0.24080, df = 315.00				
	Locality1	6-Acetylacateoside Mean	1	2	3
5	M-NR	0.723587	****		
1	CASS	0.977901	****		
2	GAN	1.139168	****	****	
6	Lafras	1.274923	****	****	****
7	Terra Firma	1.360905		****	****
3	Namibia	1.391951	****	****	****
4	MOS	1.408514			****

(Cassel = CASS, Ganyesa = GAN, Moswana = MOS, Molopo Nature Reserve = M-NR)

Table **6.7e:** Homogeneous groups of localities from pair wise comparisons for coumaroylharpagide

Cell No.	Unequal N HSD; variable coumaroylharpagide (DKdata AVER_tot_tran.sta) Homogenous Groups, alpha = 0.05000 Error: Between MS = 0.00026, df = 315.00				
	Locality1	Coumaroylharpagide Mean	1	2	3
7	Terra Firma	0.027666	****		
2	GAN	0.028119	****		
4	MOS	0.030393	****		
1	CASS	0.031052	****		
5	M-NR	0.039513	****	****	
6	Lafras	0.056448		****	****
3	Namibia	0.076940			****

(Cassel = CASS, Ganyesa = GAN, Moswana = MOS, Molopo Nature Reserve = M-NR)

Table **6.7f:** Homogeneous groups of localities from pair wise comparisons for harpagoside

Cell No.	Unequal N HSD; variable harpagoside (DKdata AVER_tot_tran.sta) Homogenous Groups, alpha = 0.05000 Error: Between MS = 0.43511, df = 315.00				
	Locality1	Harpagoside Mean	1	2	3
7	Terra Firma	1.816675			****
2	GAN	2.474642		****	
5	M-NR	2.792663	****	****	
4	MOS	2.835838	****		
6	Lafras	2.870536	****	****	
1	CASS	2.944354	****		
3	Namibia	3.097551	****	****	

(Cassel = CASS, Ganyesa = GAN, Moswana = MOS, Molopo Nature Reserve = M-NR)

Table **6.8** gives the inter-correlations on which the factor analysis for all variables was based.

Table **6.8:** Inter-correlations between variables

	Correlations (DKdata AVERAGES.sta) Marked correlations are significant at $p < .05000$ N=322 (Casewise deletion of missing data)							
Variable	Means	Std.Dev.	Harpagide	Verbascoside	Isoverbascoside	Acetylacteoside	Coumaroylharpagide	Harpagoside
Harpagide	2.267062	1.314032	1.000000	0.100343	0.337246	0.141023	0.358300	0.096410
Verbascoside	2.724433	2.338129	0.100343	1.000000	0.550083	0.506727	0.171228	-0.103122
Isoverbascoside	5.184933	3.886790	0.337246	0.550083	1.000000	0.629557	0.301798	-0.063903
Acetylacteoside	3.160607	2.193718	0.141023	0.506727	0.629557	1.000000	0.279093	0.026345
Coumaroylharpagide	0.050063	0.029687	0.358300	0.171228	0.228504	0.279093	1.000000	0.365116
Harpagoside	7.013742	3.592230	0.096410	-0.103122	-0.063903	0.026345	0.365116	1.000000

6.3 Average chemical composition

Table **6.9** gives the average harpagide, harpagoside, verbascoside, 6-acetylacteoside, isoverascoside and coumaroylharpagide with standard deviation for each of the seven locations in the North West Province and Namibia.

Table **6.9:** Averages of chemical components from each area (North West and Namibia)

Locality	Average concentration of each chemical compound present in the samples from different localities					
	Harpagide (µg/g)	Verbascoside (µg/g)	Isoverbascoside (µg/g)	Acetylacteoside (µg/g)	Coumaroylharpagide (µg/g)	Harpagoside (µg/g)
CASS	2.23	2.00	4.72	2.45	0.04	9.14
St. deviation	0.34	0.63	0.37	0.46	0.01	0.82
GAN	3.21	2.67	4.75	2.98	0.04	4.88
St. deviation	0.76	0.62	0.40	0.12	0.01	0.93
Namibia	5.67	1.32	7.73	4.14	0.15	8.85
St. deviation	3.08	0.70	3.94	2.49	0.16	4.02
MOS	1.47	2.00	5.23	3.83	0.04	7.18
St. deviation	0.74	1.80	1.87	2.28	0.01	1.94
M-NR	2.45	0.97	3.92	1.80	0.06	7.09
St. deviation	1.38	0.48	2.91	1.19	0.02	1.96
Lafras	2.65	8.50	6.71	3.75	0.08	7.36
St. deviation	0.56	5.83	4.05	2.12	0.01	1.61
Terra Firma	1.67	2.90	4.92	3.88	0.04	3.46
St. deviation	0.49	1.01	2.62	1.96	0.01	1.47

(Cassel = CASS, Ganyesa = GAN, Moswana = MOS, Molopo Nature Reserve = M-NR)

6.4 Conclusions (North West and Namibia Data Analysis)

From tables **6.5a** to **6.5f** (the red figures indicate significant variation) we make the following conclusions:

1. As concluded in chapter 4, harpagide shows not significant variation between the six North West localities. The Namibia harpagide shows significant variation with only Moswana and Terra Firma from the North West populations (Table **6.5a**).

2. Verbascoside from Namibia population shows significant variation with Lafras population only. Lafras and Molopo Nature Reserve show a significant variation with all the other populations in North West as was described in Chapter 4 (table **6.5b**).

3. Isoverbascoside shows no significant differences between localities (Table **6.5c**).

4. Acetylacteoside shows no significant variation between Namibia and North West. This compound varies significantly between Moswana, Molopo Nature Reserve, Cassel, Ganyesa and Terra firma (table **6.5d**). No clear pattern is evident.

5. Coumaroylharpagide shows significant difference between Namibia and all the North West except Lafras (Table **6.5e**). Lafras coumaroylharpagide shows significant variation with all the North West localities except Molopo Nature Reserve. Terra Firma varies significantly with both Lafras and Namibia.

6. Terra Firma’s harpagoside stands out in being significantly different from all other localities in North West and Namibia (Table **6.5f**). This correlates with a significantly lower harpagoside concentration in the Terra Firma population (3.45 μg/g) as compared with the other populations (4.88 to 9.14). Harpagoside in plant material from Ganyesa shows significant variation with Moswana and Cassel.

7. Analysis of data from North West Province, Namibia, Caprivi, Zimbabwe and Limpopo Province (all data)

7.1 Purpose of the study in chapter 7

In this chapter we aim to answer the question below using our results shown in Appendix **A**.

Are there meaningful differences in the chemical composition of populations from North West Province/Namibia (*H. procumbens* subsp. *procumbens*), Limpopo Province (*H. zeyheri* subsp. *zeyheri*), Caprivi and Zimbabwe (Victoria Falls) (*H. zeyheri* subsp. *sublobatum*)?

In this chapter we analysed the data from North West Province/Namibia (*H. procumbens* subsp. *procumbens*, from the Limpopo Province (*H. zeyheri* subsp. *zeyheri*) and from Zimbabwe (Victoria Falls) (*H. zeyheri* subsp. *sublobatum*). We restate question above as a hypothesis: "There are significant differences in the chemical composition between collections from the North West Province, Namibia (*H. procumbens* subsp. *procumbens*), the Limpopo Province (*H. zeyheri* subsp. *zeyheri*), Caprivi and from Victoria Falls in Zimbabwe (*H. zeyheri* subsp. *sublobatum*)?

7.2 Statistical analysis

7.2.1 Factor analysis

Factor analysis with principal component method (followed by a Varimax-rotation) could identify two, three or four factors. Two factors could explain 59.8% (**7.1a**), three factors 74.5% (**7.1b**) and four factors of 88.4% (**7.1c**) of the total variance. In the four factor analysis the eigenvalues were as follows: Factor 1 (eigenvalue 2.38), factor 2 (eigenvalue 1.20), factor 3 (eigenvalue 0.89) and factor 4 (eigenvalue 0.83).

Table **7.1a:** Factor analysis on data from North West Province, Namibia, Caprivi, Zimbabwe and Limpopo Province (two factors)

Variable	Factor loadings (Varimax raw) (DKdata Averages. Sta) Extraction: Principal components (Marked loadings are >.700000)	
	Factor 1	Factor 2
Harpagide	-0.019667	0.787968
Verbascoside	0.818781	-0.118397
Isoverbascoside	0.866602	0.218889
Acetylacteoside	0.843721	-0.161451
Coumaroylharpagide	-0.004094	0.705442
Harpagoside	0.494849	0.022666
Expl. Var.	2.378547	1.207051
Prp. Totl	0.396424	0.201175

Table **7.1b:** Factor analysis on data from North West Province, Namibia, Caprivi, Zimbabwe and Limpopo Province (three factors)

Variable	Factor loadings (Varimax raw) (DKdata Averages. Sta) Extraction: Principal components (Marked loadings are >.700000)		
	Factor 1	Factor 2	Factor 3
Harpagide	0.035285	-0.919066	0.132530
Verbascoside	0.830478	0.065956	-0.091355
Isoverbascoside	0.873960	0.187279	0.132327
Acetylacteoside	0.848040	0.138687	-0.071526
Coumaroylharpagide	-0.080488	-0.198914	0.857711
Harpagoside	0.417541	0.381520	0.495417
Expl. Var.	2.354735	1.088465	1.029643
Prp. Totl	0.392456	0.181411	0.171607

Table **7.1c:** Factor analysis on data from North West Province, Namibia, Caprivi, Zimbabwe and Limpopo Province (four factors)

Variable	Factor Loadings (Varimax raw) (DKdata AVERAGES_tot.sta) Extraction: Principal component (Marked loadings are >.700000)			
	Factor 1	Factor 2	Factor 3	Factor 4
Harpagide	-0.015468	0.985763	0.070362	-0.02444
Verbascoside	0.867167	0.114803	-0.023036	0.026720
Isoverbascoside	0.838052	-0.232889	0.078580	0.231923
Acetylacteoside	0.841041	0.126119	-0.084832	0.165314
Coumaroylharpagide	-0.011937	-0.067768	0.996210	0.008121
Harpagoside	0.152953	0.024346	0.007936	0.983514
Expl.Var	2.185436	1.060238	1.011350	1.049795
Prp.Totl	0.364239	0.176706	0.168558	0.174966

Table **7.2:** Communalities on data from North West Province, Namibia, Caprivi, Zimbabwe and Limpopo Province (four factors)

Variable	Communalities (DKdata AVERAGES_tot.sta) Extraction: Principal components Rotation: Unrotated				
	From Factor 1	From Factor 2	From Factor 3	From factor 4	Multiple R-Square
Harpagide	0.002875	0.621280	0.863492	0.977517	0.133401
Verbascoside	0.677532	0.684420	0.702390	0.766402	0.463739
Isoverbascoside	0.733355	0.737932	0.743521	0.757781	0.499628
Acetylacteoside	0.722324	0.737932	0.743521	0.757781	0.499628
Coumaroylharpagide	0.001190	0.497664	0.781714	0.997235	0.040016
Harpagoside	0.243455	0.245389	0.565336	0.991350	0.119194

Tables **7.3a to 7.3f** give the homogenous pair wise comparisons in groups for each and every chemical variable.

Table **7.3a:** Homogeneous groups of localities from pair wise comparisons for harpagide

Cell No.	Unequal N HSD; variable Harpagide (DKdata AVER_tot_tran.sta) Homogenous Groups, alpha = 0.05000 Error: Between MS = 0.08855, df = 421.00						
	Locality1	Harpagide Mean	1	2	3	4	5
8	Terra Firma	0.765577	****				
5	MOS	0.831348	****				
1	CASS	0.915265	****	****			
2	GAN	0.918686	****	****			
6	M-NR	0.940990	****	****	****		
9	CAP	1.013362	****	****	****	****	
12	SPRING	1.031232	****	****	****	****	
7	Lafras	1.060366	****	****	****	****	
11	MAKGA	1.210025			****	****	
10	FERRO	1.301578				****	
4	Namibia	1.452387		****	****	****	****
3	Zim.	1.740287					****

(Cassel = CASS, Ganyesa = GAN, Moswana = MOS, Caprivi = CAP, Ferrolands = FERRO, Makgabeng = MAKHA, Springbokfontein = SPRING, Molopo Nature Reserve = M-NR)

Table **7.3b:** Homogeneous groups of localities from pair wise comparisons with verbascoside

Cell No.	Unequal N HSD; variable Verbascoside (DKdata AVER_tot_tran.sta) Homogenous Groups, alpha = 0.05000 Error: Between MS = 0.05768, df = 421.00						
	Locality1	Verbascoside Mean	1	2	3	4	5
10	FERRO	0.161616		****			
11	MAKGA	0.162149		****			
12	SPRING	0.333850		****	****		
6	M-NR	0.511170			****	****	
4	Namibia	0.636917	****	****	****	****	
9	CAP	0.691277	****		****	****	
3	Zim.	0.711611	****			****	
1	CASS	0.852825	****				
5	MOS	0.879648	****				
2	GAN	0.908601	****				
8	Terra Firma	0.971353	****				****
7	Lafras	1.320272					****

(Cassel = CASS, Ganyesa = GAN, Moswana = MOS, Caprivi = CAP, Ferrolands = FERRO, Makgabeng = MAKHA, Springbokfontein = SPRING, Molopo Nature Reserve = M-NR)

Table **7.3c:** Homogeneous groups of localities from pair wise comparisons with isoverbascoside

Cell No.	Unequal N HSD; variable Isoverbascoside (DKdata AVER_tot_tran.sta) Homogenous Groups, alpha = 0.05000 Error: Between MS = 0.09953, df = 421.00				
	Locality1	Isoverbascoside Mean	1	2	3
10	FERRO	0.235579			
11	MAKGA	0.322913			
12	SPRING	0.376214			
6	M-NR	1.110021	****		
1	CASS	1.246954	****		****
8	Terra Firma	1.309302	****		****
5	Lafras	1.353595	****	****	****
2	MOS	1.368678	****	****	
9	GAN	1.388725	****	****	
7	CAP	1.482503	****	****	
4	Namibia	1.595711	****	****	
3	Zim	1.755297		****	

(Cassel = CASS, Ganyesa = GAN, Moswana = MOS, Caprivi = CAP, Ferrolands = FERRO, Makgabeng = MAKHA, Springbokfontein = SPRING, Molopo Nature Reserve = M-NR)

Table **7.3d:** Homogeneous groups of localities from pair wise comparisons with acetylacteoside

Cell No.	Unequal N HSD; variable Acetylacteoside (DKdata AVER_tot_tran.sta) Homogenous Groups, alpha = 0.05000 Error: Between MS = 0.06571, df = 421.00							
	Locality1	Acetylacteoside Mean	1	2	3	4	5	6
10	FERRO	0.228108				****		
3	Zim	0.273506				****	****	
11	MAKGA	0.449018				****	****	
12	SPRING	0.599282					****	****
9	CAP	0.647715	****			****	****	****
6	M-NR	0.790135	****					****
1	CASS	0.936948	****	****				
2	GAN	1.025795	****	****	****			
7	Lafras	1.096706	****	****	****			
8	Terra Firma	1.148424		****	****			
4	Namibia	1.162477	****	****	****			
5	MOS	1.166595			****			

(Cassel = CASS, Ganyesa = GAN, Moswana = MOS, Caprivi = CAP, Ferrolands = FERRO, Makgabeng = MAKHA, Springbokfontein = SPRING, Molopo Nature Reserve = M-NR)

Table **7.3e:** Homogeneous groups of localities from pair wise comparisons with coumaroylharpagide

Cell No.		Unequal N HSD; variable Coumaroylharpagide (DKdata AVER_tot_tran.sta) Homogenous Groups, alpha = 0.05000 Error: Between MS = 0.00020, df = 421.00						
	Locality1	Coumaroyl harpagide Mean	1	2	3	4	5	6
8	Terra Firma	0.027666	****					
2	GAN	0.028119	****					
5	MOS	0.030393	****					
1	CASS	0.031052	****		****			
6	M-NR	0.039513	****	****	****			
11	MAKGA	0.043541		****				
12	SPRING	0.043601		****	****			
10	FERRO	0.044199		****		****		
7	Lafras	0.056448		****		****		
4	Namibia	0.076940						
3	Zim	0.151778					****	
9	CAP	0.196950						****

(Cassel = CASS, Ganyesa = GAN, Moswana = MOS, Caprivi = CAP, Ferrolands = FERRO, Makgabeng = MAKHA, Springbokfontein = SPRING, Molopo Nature Reserve = M-NR)

Table **7.3f:** Homogeneous groups of localities from pair wise comparisons with harpagoside

Cell No.	Unequal N HSD; variable Harpagoside (DKdata AVER_tot_tran.sta) Homogenous Groups, alpha = 0.05000 Error: Between MS = 0.31638, df = 421.00						
	Locality1	Harpagide Mean	1	2	3	4	5
11	MAKGA	0.005056				****	
12	SPRING	0.011833				****	
10	FERRO	0.107641				****	
8	Terra Firma	1.797713					****
9	CAP	1.928997	****	****			****
2	GAN	2.440752		****			
6	M-NR	2.751772	****	****			
5	MOS	2.792504	****		****		
7	Lafras	2.827956	****	****	****		
1	CASS	2.898584	****		****		
4	Namibia	3.047515	****	****	****		
3	Zim	3.542236			****		

(Cassel = CASS, Ganyesa = GAN, Moswana = MOS, Caprivi = CAP, Ferrolands = FERRO, Makgabeng = MAKHA, Springbokfontein = SPRING, Molopo Nature Reserve = M-NR)

7.2.2 Analysis of Variance

A multivariate analysis of variance (MANOVA) (table **7.4a**) on the 6 chemical compositions indicates highly significant differences between the different 12 localities (Cassel, Ganyesa, Moswana, Molopo nature Reserve, Lafras and Terra firma, Caprivi, Springbokfontein, Makgabeng, Ferrolands, Namibia and Zimbabwe indicated as ‘locality’ (p<0.001 for the Wilks test and eta-square=0.14) when using 6 variables to compare 12 localities. Table **7.4b** show the transformed data of MANOVA.

Table **7.4a:** MANOVA on 6 variables to compare l2 localities

	Multivariate Tests of Significance, Effect Sizes, and Powers (DKdata AVERAGES_tot.sta) Sigma-restricted parameterization Effective hypothesis decomposition								
Effect	Test	Value	F	Effect df	Error df	P	Partial eta-squared	Non-centrality	Observed power (alpha=0.05)
Intercept	Wilks	0.147763	399.8859	6	416.000	0.00	0.852237	2399.315	1.000000
locality	Wilks	0.019166	36.9866	66	2231.409	0.00	0.522440	2441.113	1.000000

Table **7.4b:** MANOVA on 6 transformed variables to compare l2 localities

	Multivariate Tests of Significance, Effect Sizes, and Powers (DKdata AVERAGES_tot.sta) Sigma-restricted parameterization Effective hypothesis decomposition								
Effect	Test	Value	F	Effect df	Error df	P	Partial eta-squared	Non-centrality	Observed power (alpha=0.05)
Intercept	Wilks	0.061243	1062.768	6	416.000	0.00	0.938757	6376.606	1.000000
locality	Wilks	0.006566	52.678	66	2231.409	0.00	0.609082	3476.716	1.000000

Levene’s test on homogeneity of variances does not hold for all the variables in this chapter (table **7.5**). Transformed univariate analyses of variance (ANOVA) were performed on each of the chemical variable and results are displayed in table **7.6a** to **7.6f**.

Table **7.5:** Levene’s test for homogeneity of variances

	Levene’s Test for Homogeneity of Variances (Dkdata Averages. Sta) Effect: Occasion Degrees of freedom for all F’s: 4, 112			
	MS Effect	**MS Error**	**F**	**P**
Harpagide	0.069950	0.034978	1.99983	0.027056
Verbascoside	0.136182	0.018549	7.34185	0.000000
Isoverbascoside	0.144037	0.036225	3.97618	0.000016
Acetylacteoside	0.147412	0.022685	6.49825	0.000000
Coumaroylharpagide	0.001192	0.000076	14.75043	0.000000
Harpagoside	1.459754	0.141050	10.34923	0.000000

Table **7.6a:** ANOVA for harpagide

Sources of variation	Degrees of freedom	Harpagide SS	Harpagide MS	Harpagide F	Harpagide P
Intercept	1	220.2142	220.2142	2487.018	0.000000
Occasion	11	16.7965	1.5270	17.245	0.000000
Error	421	421	0.0885		
Total	432	432			

Table **7.6b:** ANOVA for verbascoside

Sources of variation	Degrees of freedom	Verbascoside SS	Verbascoside MS	Verbascoside F	Verbascoside P
Intercept	1	84.00948	84.00948	1456.415	0.000000
Occasion	11	37.05320	3.36847	58.397	0.000000
Error	421	24.28428	0.05768		
Total	432	61.33748			

Table **7.6c:** ANOVA for isoverbascoside

Sources of variation	Degrees of freedom	Isoverbascoside SS	Isoverbascoside MS	Isoverbascoside F	Isoverbascoside P
Intercept	1	84.00948	84.00948	1456.415	0.000000
Occasion	11	37.05320	3.36847	58.397	0.000000
Error	421	24.28428	0.05768		
Total	432	61.33748			

Table **7.6d:** ANOVA for acetylacteoside

Sources of variation	Degrees of freedom	Acetylacteoside SS	Acetylacteoside MS	Acetylacteoside F	Acetylacteoside P
Intercept	1	114.9861	114.9861	1749.817	0.000000
Occasion	11	36.3086	3.3008	50.230	0.000000
Error	421	27.6653	0.0657		
Total	432	63.9739			

Table **7.6e:** ANOVA for coumaroylharpagide

Sources of variation	Degrees of freedom	Coumaroylharpagide SS	Coumaroylharpagide MS	Coumaroylharpagide F	Coumaroylharpagide P
Intercept	1	0.751882	0.751882	3685.629	0.000000
Occasion	11	0.338125	0.030739	150.677	0.000000
Error	421	0.085886	0.000204		
Total	432	0.424011			

Table **7.6f:** ANOVA for harpagoside

Sources of variation	Degrees of freedom	Harpagoside SS	Harpagoside MS	Harpagoside F	Harpagoside P
Intercept	1	0.751882	0.751882	3685.629	0.000000
Occasion	11	0.338125	0.030739	150.677	0.000000
Error	421	0.085886	0.000204		
Total	432	0.424011			

In table **7.7** the cumulative frequencies and percentages of all the plants analysed are given per locality.

Table **7.7:** Frequencies of plants per locality

Variable	Frequency table: Locality (DKdata AVERAGES_tot.sta)			
	Count	Cumulative Count	Percent	Cumulative Percent
CASS	117	117	27.02079	27.0208
GAN	70	187	16.16628	43.1871
MOS	68	255	14.70439	58.8915
Zimbabwe	12	267	2.77136	61.6628
Namibia	5	272	1.15473	62.8176
M-NR	24	296	5.54273	68.3603
Lafras	8	304	1.84758	70.2079
Terra Firma	30	334	6.92841	77.1363
CAP	6	340	1.38568	78.5219
FERRO	27	367	6.23557	84.7575
MAKGA	41	408	9.46882	94.2263
SPRING	25	433	4.77367	100.000
Missing	0	433	0.00000	100.000

(Cassel = CASS, Ganyesa = GAN, Moswana = MOS, Caprivi = CAP, Ferrolands = FERRO, Makgabeng = MAKHA, Springbokfontein = SPRING, Molopo Nature Reserve = M-NR)

7.2.3 Multiple comparisons

Table **7.8** gives the inter-correlations on which the factor analysis was based. The p-values of multiple comparisons using the Tukey HSD method between each pair of localities Cassel, Ganyesa, Moswana, Molopo Nature Reserve, Lafras, Terra Firma, Namibia, Zimbabwe, Caprivi and Limpopo Province for the six variables respectively are indicated in tables **7.9a** to **7.9f**.

Table **7.8:** Inter-correlations between variables

Variable	Correlations (DKdata AVERAGES.sta) Marked correlations are significant at $p < 0.05000$ N=334 (Casewise deletion of missing data)							
	Means	Std.Dev.	Harpagide	Verbascoside	Isoverbascoside	Acetylacteoside	Coumaroyl harpagide	Harpagoside
Harpagide	2.469043	1.692159	1.000000	0.014889	0.389391	-0.024522	0.674418	0.220090
Verbascoside	2.684376	2.310089	0.014889	1.000000	0.513220	0.508611	-0.006876	-0.117612
Isoverbascoside	5.352647	3.932376	0.389391	0.513220	0.513220	0.544014	0.301798	-0.002019
Acetylacteoside	3.065276	2.213982	-0.02452	0.508611	0.508611	1.000000	-0.072841	-0.027373
Coumaroylharpagide	0.061446	0.069037	0.674418	-0.006876	0.301798	-0.072841	1.000000	0.375632
Harpagoside	7.175405	3.668521	0.220090	-0.117612	-0.002019	-0.027373	0.375632	1.000000

Table **7.9a:** Multiple comparisons between localities from all data for mean harpagide content

Cell No.	Unequal N HSD; variable harpagide (DKdata AVER_tot_tran.sta) Approximate Probabilities for Post Hoc Tests Error: Between MS = 0.08855, df = 421.00												
	Locality	{1} 0.91526	{2} 0.91869	{3} 1.7403	{4} 1.4524	{5} 0.83135	{6} 0.94099	{7} 1.0604	{8} 0.76558	{9} 1.0134	{10} 1.3016	{11} 1.2100	{12} 1.0312
1	CASS		1.000000	0.000018	0.157765	0.892625	1.000000	0.998193	0.728517	0.999990	0.000129	0.000460	0.967847
2	GAN	1.000000		0.000018	0.164936	0.863455	1.000000	0.998546	0.698594	0.999993	0.000155	0.000580	0.974322
3	Zim	0.000018	0.000018		0.932613	0.000018	0.000018	0.000314	0.000018	0.001420	0.016001	0.000783	0.000018
4	Namibia	0.157765	0.164936	0.932613		0.045283	0.217444	0.635079	0.013966	0.452935	0.999715	0.980793	0.521771
5	MOS	0.892625	0.863455	0.000018	0.045283		0.982088	0.929764	0.999463	0.996213	0.000018	0.000018	0.423211
6	M-NR	1.000000	1.000000	0.000018	0.217444	0.982088		0.999711	0.664315	1.000000	0.001634	0.075042	0.996484
7	Lafras	0.998193	0.998546	0.000314	0.635079	0.929764	0.999711		0.706402	1.000000	0.901701	0.997610	1.000000
8	Terra F	0.728517	0.698594	0.000018	0.013966	0.999463	0.664315	0.706402		0.955174	0.000018	0.000018	0.069909
9	CAP	0.999990	0.999993	0.001420	0.452935	0.996213	1.000000	1.000000	0.955174		0.878697	0.992630	0.992630
10	FERRO	0.000129	0.000155	0.016001	0.999715	0.000018	0.001634	0.901701	0.000018	0.878697		0.993371	0.059265
11	MAKGA	0.000460	0.000580	0.000783	0.980793	0.000018	0.075042	0.997610	0.000018	0.992630	0.993371		0.605120
12	SPRING	0.967847	0.974322	0.000018	0.521771	0.423211	0.996484	1.000000	0.069909	0.992630	0.059265	0.605120	

(Cassel = CASS, Ganyesa = GAN, Moswana = MOS, Caprivi = CAP, Ferrolands = FERRO, Makgabeng = MAKHA, Springbokfontein = SPRING, Molopo Nature Reserve = M-NR)

Table **7.9b:** Multiple comparisons between localities from all data for mean verbascoside content

Cell No.	Unequal N HSD; variable verbascoside (DKdata AVER_tot_tran.sta) Approximate Probabilities for Post Hoc Tests Error: Between MS = 0.05768, df = 421.00												
	Locality	{1} 0.85283	{2} 0.90860	{3} 0.71161	{4} 0.63692	{5} 0.87965	{6} 0.51117	{7} 1.3203	{8} 0.97135	{9} 0.69128	{10} 0.16162	{11} 0.16215	{12} 0.33385
1	CASS		0.968528	0.955628	0.959626	0.999964	0.000067	0.005614	0.752408	0.991458	0.000018	0.000018	0.000018
2	GAN	0.968528		0.687386	0.824654	0.999921	0.000018	0.029984	0.997478	0.920881	0.000018	0.000018	0.000018
3	Zimbabwe	0.955628	0.687386		0.999998	0.862347	0.000041	0.000041	0.252304	1.000000	0.000019	0.000019	0.006551
4	Namibia	0.959626	0.824654	0.999998		0.910303	0.999610	0.000432	0.548317	1.000000	0.075667	0.076434	0.696944
5	MOS	0.999964	0.999921	0.862347	0.910303		0.000024	0.013001	0.946541	0.971074	0.000018	0.000018	0.000018
6	M-NR	0.000067	0.000018	0.000041	0.999610	0.000024		0.000018	0.000018	0.979466	0.000045	0.000046	0.304199
7	Lafras	0.005614	0.029984	0.000041	0.000432	0.013001	0.000018		0.138701	0.000365	0.000018	0.000018	0.000018
8	Terra Firma	0.752408	0.997478	0.252304	0.548317	0.946541	0.000018	0.138701		0.679920	0.000018	0.000018	0.000018
9	CAP	0.991458	0.920881	1.000000	1.000000	0.971074	0.979466	0.000365	0.679920		0.007432	0.007541	0.292314
10	FERRO	0.000018	0.000018	0.000019	0.075667	0.000018	0.000045	0.000018	0.000018	0.007432		1.000000	0.317605
11	MAKGA	0.000018	0.000018	0.000019	0.076434	0.000018	0.000046	0.000018	0.000018	0.007541	1.000000		0.322424
12	SPRING	0.000018	0.000018	0.006551	0.696944	0.000018	0.304199	0.000018	0.000018	0.292314	0.317605	0.322424	

(Cassel = CASS, Ganyesa = GAN, Moswana = MOS, Caprivi = CAP, Ferrolands = FERRO, Makgabeng = MAKHA, Springbokfontein = SPRING, Molopo Nature Reserve = M-NR)

Table **7.9c:** Multiple comparisons between localities from all data for mean isoverbascoside content

Cell No.	Unequal N HSD; variable isoverbascoside (DKdata AVER_tot_tran.sta) Approximate Probabilities for Post Hoc Tests Error: Between MS = 0.09953, df = 421.00												
	Locality	{1} 1.2470	{2} 1.3687	{3} 1.7553	{4} 1.5957	{5} 1.3536	{6} 1.3536	{7} 1.4825	{8} 1.3093	{9} 1.3887	{10} 0. 23558	{11} 0. 32291	{12} 0. 37621
1	CASS		0.489088	0.004539	0.845815	0.713409	0.940072	0.942834	0.999818	0.999785	0.000018	0.000018	0.000018
2	GAN	0.489088		0.107872	0.992997	1.000000	0.163255	0.999898	0.999887	1.000000	0.000018	0.000018	0.000018
3	Zimbabwe	0.004539	0.107872		0.999720	0.077924	0.000050	0.854940	0.026724	0.685022	0.000018	0.000018	0.000018
4	Namibia	0.845815	0.992997	0.999720		0.988055	0.382686	0.999991	0.956681	0.996855	0.000018	0.000018	0.000018
5	MOS	0.713409	1.000000	0.077924	0.988055		0.238956	0.999655	0.999994	1.000000	0.000018	0.000018	0.000018
6	M-NR	0.940072	0.163255	0.000050	0.382686	0.238956		0.432756	0.558327	0.932520	0.000018	0.000018	0.000018
7	Lafras	0.942834	0.999898	0.854940	0.999991	0.999655	0.432756		0.994834	0.999997	0.000018	0.000018	0.000018
8	Terra Firma	0.999818	0.999887	0.026724	0.956681	0.999994	0.558327	0.994834		0.999999	0.000018	0.000018	0.000018
9	CAP	0.999785	1.000000	0.685022	0.996855	1.000000	0.932520	0.999997	0.999999		0.000018	0.000018	0.000019
10	FERRO	0.000018	0.000018	0.000018	0.000018	0.000018	0.000018	0.000018	0.000018	0.000018		0.997361	0.917957
11	MAKGA	0.000018	0.000018	0.000018	0.000018	0.000018	0.000018	0.000018	0.000018	0.000018	0.997361		0.999985
12	SPRING	0.000018	0.000018	0.000018	0.000018	0.000018	0.000018	0.000018	0.000018	0.000019	0.917957	0.999985	

(Cassel = CASS, Ganyesa = GAN, Moswana = MOS, Caprivi = CAP, Ferrolands = FERRO, Makgabeng = MAKHA, Springbokfontein = SPRING, Molopo Nature Reserve = M-NR)

Table **7.9d:** Multiple comparisons between localities from all data for mean 6-acetylacteoside content

Cell No.	Unequal N HSD; variable 6-acetylacteoside (DKdata AVER_tot_tran.sta) Approximate Probabilities for Post Hoc Tests Error: Between MS = 0.06571, df = 421.00												
	Locality	{1} 0.93695	{2} 1.0258	{3} 0.27351	{4} 1.1625	{5} 1.1666	{6} 0.79013	{7} 1.0967	{8} 1.1484	{9} 0.64772	{10} 0.22811	{11} 0.44902	{12} 0.59928
1	CASS		0.658369	0.000018	0.965501	0.000028	1.000000	0.985165	0.062366	0.724560	0.000018	0.000018	0.000212
2	GAN	0.658369		0.000018	0.999536	0.060963	0.064350	0.999993	0.788410	0.305996	0.000018	0.000018	0.000018
3	Zimbabwe	0.000018	0.000018		0.000020	0.000018	0.000065	0.000018	0.000018	0.321914	0.999999	0.878929	0.079255
4	Namibia	0.965501	0.999536	0.000020		1.000000	0.478924	1.000000	0.956681	0.066185	0.000018	0.000018	0.025753
5	MOS	0.000028	0.060963	0.000018	1.000000		0.000039	0.999994	1.000000	0.023098	0.000018	0.000018	0.000018
6	M-NR	1.000000	0.064350	0.000065	0.478924	0.000039		0.411462	0.000095	0.998400	0.000018	0.000018	0.291499
7	Lafras	0.985165	0.999993	0.000018	1.000000	0.999994	0.411462		1.000000	0.098916	0.000018	0.000018	0.005876
8	Terra Firma	0.062366	0.788410	0.000018	0.956681	1.000000	0.000095	1.000000		0.034743	0.000018	0.000018	0.000018
9	CAP	0.724560	0.305996	0.321914	0.066185	0.023098	0.998400	0.098916	0.034743		0.165216	0.973537	1.000000
10	FERRO	0.000018	0.000018	0.999999	0.000018	0.000018	0.000018	0.000018	0.000018	0.165216		0.067901	0.000036
11	MAKGA	0.000018	0.000018	0.878929	0.000018	0.000018	0.000018	0.000018	0.000018	0.973537	0.067901		0.642685
12	SPRING	0.000212	0.000018	0.079255	0.025753	0.000018	0.291499	0.005876	0.000018	1.000000	0.000036	0.642685	

(Cassel = CASS, Ganyesa = GAN, Moswana = MOS, Caprivi = CAP, Ferrolands = FERRO, Makgabeng = MAKHA, Springbokfontein = SPRING, Molopo Nature Reserve = M-NR)

Table **7.9e:** Multiple comparisons between localities from all data for mean coumaroylharpagide content

Cell No.	Unequal N HSD; variable coumaroylharpagide (DKdata AVER_tot_tran.sta) Approximate Probabilities for Post Hoc Tests Error: Between MS = 0.00020, df = 421.00												
	Locality	{1} 0.03105	{2} 0.02812	{3} 0.15178	{4} 0.07694	{5} 0.03039	{6} 0.03951	{7} 0.05645	{8} 0.02767	{9} 0.19695	{10} 0.04420	{11} 0.04354	{12} 0.04360
1	CASS		0.000106	0.178476	1.000000	0.994764	0.999100	1.000000	0.000018	0.112607	0.000018	0.000018	0.000018
2	GAN	0.000106		0.000115	0.866143	0.014115	0.749794	0.968033	0.000593	0.918010	0.000018	0.000018	0.000018
3	Zimbabwe	0.178476	0.000115		0.965571	0.050471	0.028606	0.314943	0.000018	0.000058	0.000018	0.000018	0.000018
4	Namibia	1.000000	0.866143	0.965571		0.999904	0.999594	0.999979	0.022528	0.072432	0.000018	0.000018	0.000018
5	MOS	0.994764	0.014115	0.050471	0.999904		1.000000	1.000000	0.000018	0.247015	0.000018	0.000018	0.000018
6	M-NR	0.999100	0.749794	0.028606	0.999594	1.000000		1.000000	0.000018	0.318724	0.000018	0.000018	0.000018
7	Lafras	1.000000	0.968033	0.314943	0.999979	1.000000	1.000000		0.013286	0.193666	0.000018	0.000018	0.000018
8	Terra Firma	0.000018	0.000593	0.000018	0.022528	0.000018	0.000018	0.013286		1.000000	0.000018	0.000018	0.000018
9	CAP	0.999785	1.000000	0.685022	0.996855	1.000000	0.318724	0.193666	1.000000		0.000018	0.000018	0.000018
10	FERRO	0.000018	0.000018	0.000018	0.000018	0.000018	0.000018	0.000018	0.000018	0.000018		0.999951	0.999984
11	MAKGA	0.000018	0.000018	0.000018	0.000018	0.000018	0.000018	0.000018	0.000018	0.000018	1.000000		1.000000
12	SPRING	0.000018	0.000018	0.000018	0.000018	0.000018	0.000018	0.000018	0.000018	0.000019	0.317605	0.322424	

(Cassel = CASS, Ganyesa = GAN, Moswana = MOS, Caprivi = CAP, Ferrolands = FERRO, Makgabeng = MAKHA, Springbokfontein = SPRING, Molopo Nature Reserve = M-NR)

Table **7.9f:** Multiple comparisons between localities from all data for mean harpagoside content

Cell No.	Unequal N HSD; variable harpagoside (DKdata AVER_tot_tran.sta) Approximate Probabilities for Post Hoc Tests Error: Between MS = 0.31638, df = 421.00												
	Locality	{1} 2.8986	{2} 2.4408	{3} 3.5422	{4} 3.0475	{5} 2.7925	{6} 2.7518	{7} 2.8280	{8} 1.7977	{9} 1.9290	{10} 0.10764	{11} 0.00506	{12} 0.01183
1	CASS		0.489088	0.004539	0.845815	0.713409	0.940072	0.942834	0.999818	0.999785	0.000018	0.000018	0.000018
2	GAN	0.489088		0.107872	0.992997	1.000000	0.163255	0.999898	0.999887	1.000000	0.000018	0.000018	0.000018
3	Zimbabwe	0.004539	0.107872		0.999720	0.077924	0.000050	0.854940	0.026724	0.685022	0.000018	0.000018	0.000018
4	Namibia	0.845815	0.992997	0.999720		0.988055	0.382686	0.999991	0.956681	0.996855	0.000018	0.000018	0.000018
5	MOS	0.713409	1.000000	0.077924	0.988055		0.238956	0.999655	0.999994	1.000000	0.000018	0.000018	0.000018
6	M-NR	0.940072	0.163255	0.000050	0.382686	0.238956		0.432756	0.558327	0.932520	0.000018	0.000018	0.000018
7	Lafras	0.942834	0.999898	0.854940	0.999991	0.999655	0.432756		0.994834	0.999997	0.000018	0.000018	0.000018
8	Terra Firma	0.999818	0.999887	0.026724	0.956681	0.999994	0.558327	0.994834		0.999999	0.000018	0.000018	0.000018
9	CAP	0.999785	1.000000	0.685022	0.996855	1.000000	0.932520	0.999997	0.999999		0.000018	0.000018	0.000019
10	FERRO	0.000018	0.000018	0.000018	0.000018	0.000018	0.000018	0.000018	0.000018	0.000018		0.997361	0.917957
11	MAKGA	0.000018	0.000018	0.000018	0.000018	0.000018	0.000018	0.000018	0.000018	0.000018	0.997361		0.999985
12	SPRING	0.000018	0.000018	0.000018	0.000018	0.000018	0.000018	0.000018	0.000018	0.000019	0.917957	0.999985	

(Cassel = CASS, Ganyesa = GAN, Moswana = MOS, Caprivi = CAP, Ferrolands = FERRO, Makgabeng = MAKHA, Springbokfontein = SPRING, Molopo Nature Reserve = M-NR)

7.2.4 Cluster analysis

In figure **7.1** factor 1 and factor 2 by visual inspection clearly distinguish all the Zimbabwe/ Caprivi plants from the rest of the plants materials and the Limpopo (Ferrolands, Springbokfontein and Makgabeng) plant materials are also fairly well separated. In figure **7.2** (factor 1 and 3) all the Limpopo plant materials are distinguish from the rest of the plant materials. Lastly the cluster analysis using factor 3 and 2 distinguishes Zimbabwe / Caprivi plant materials from the rest (figure **7.3**).

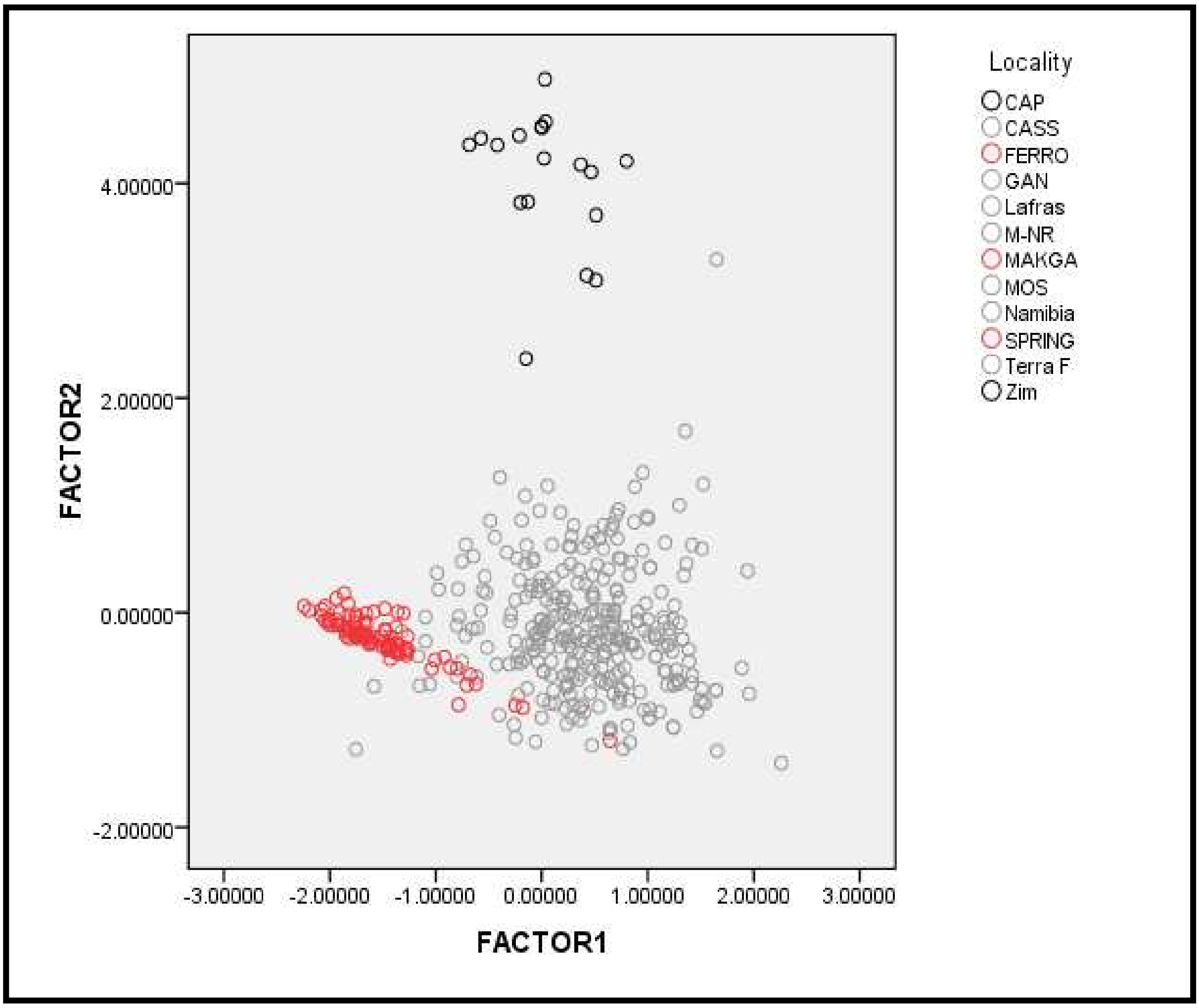


Figure **7.1**: Cluster analysis using factor 1 and 2.

.

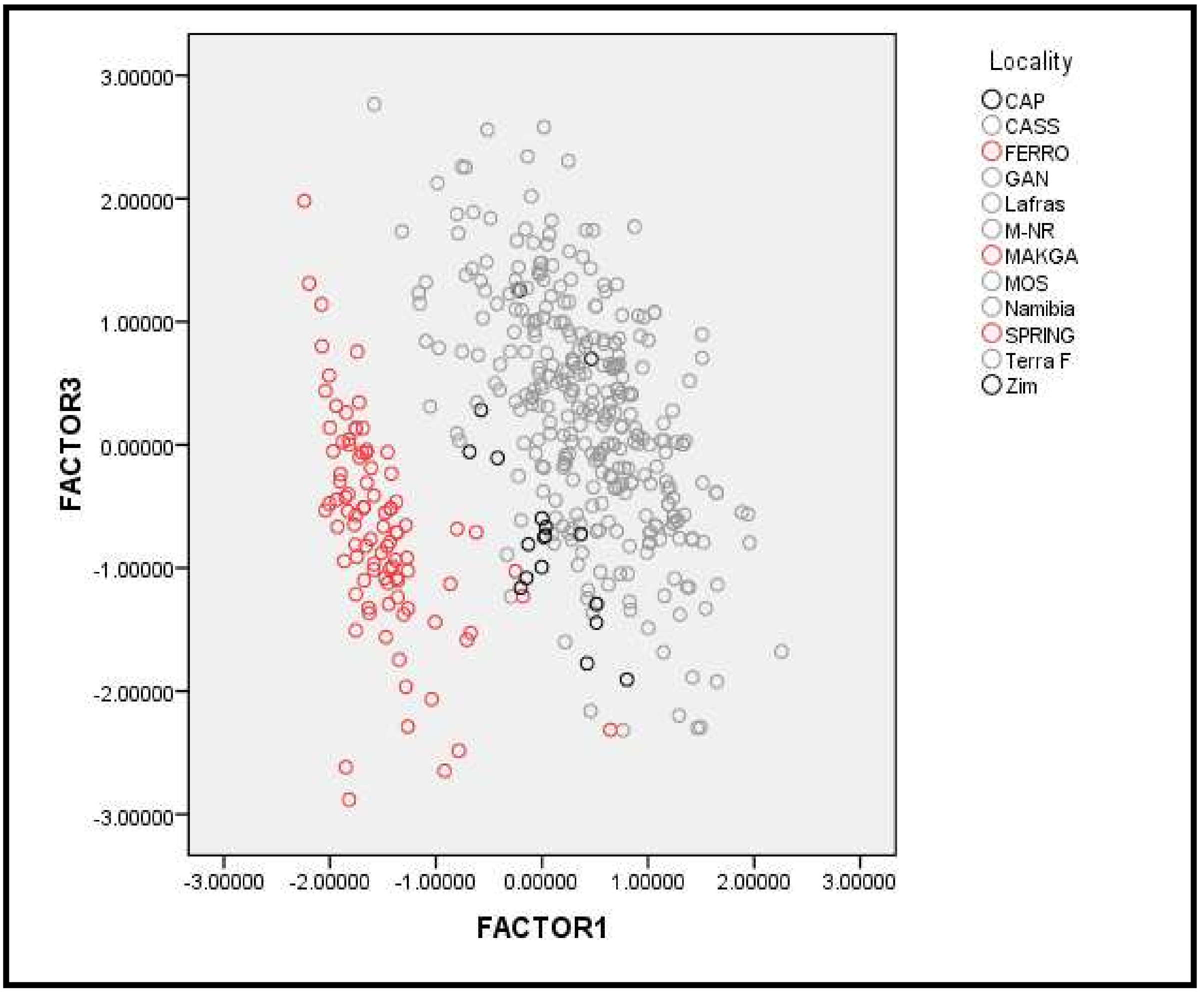


Figure **7.2**: Cluster analysis using factor 1 and 3.

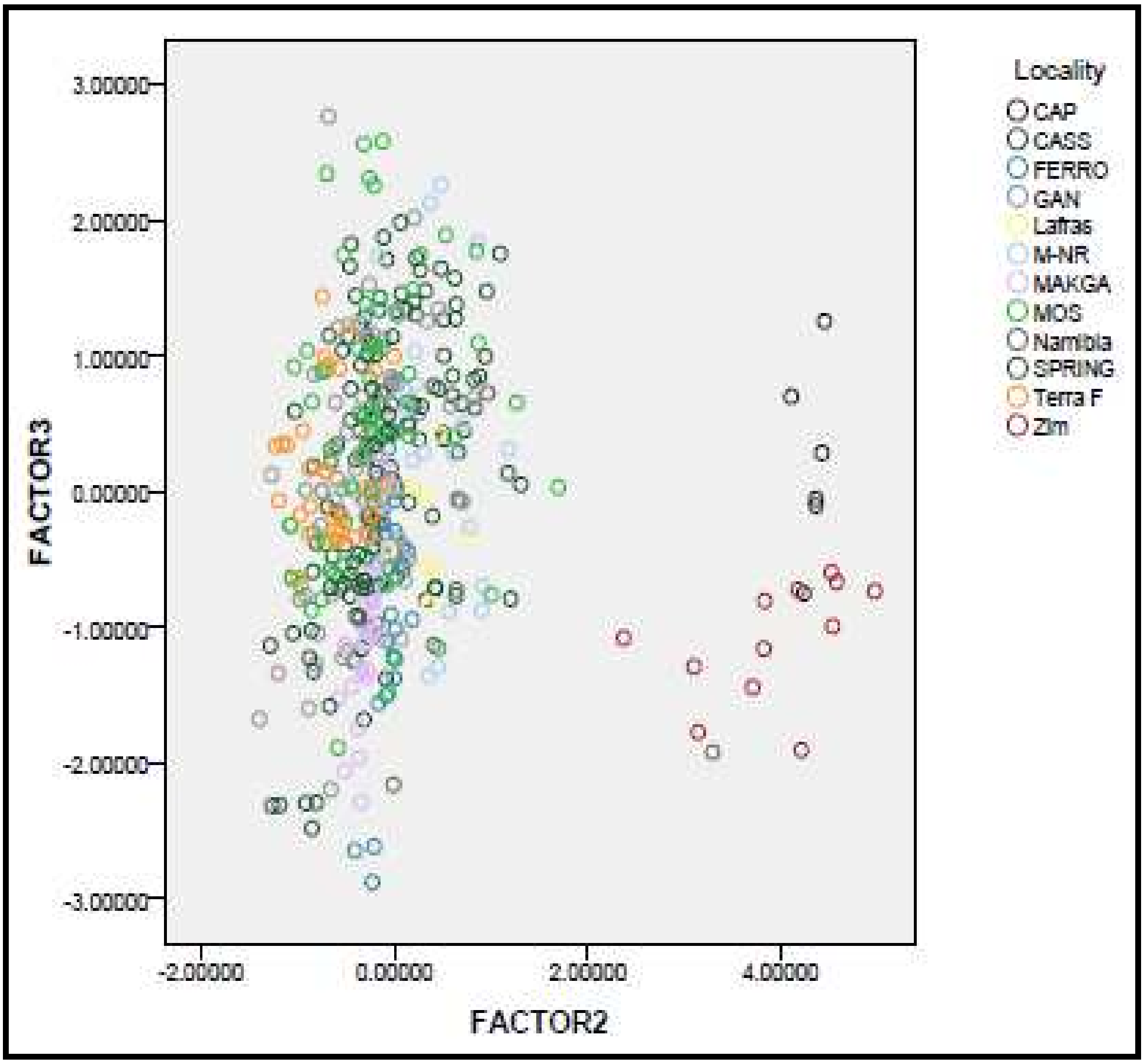


Figure **7.3**: Cluster analysis using factor 2 and 3.

7.3 Average chemical composition

Table **7.10** gives the averages of all six variables in terms of concentration amounts with standard deviation for each of the 12 locations of the analysed samples.

Table **7.10:** Averages of chemical components in terms of amounts per sample (all data)

Locality	Average concentration of each chemical compound present in the samples from different localities					
	Harpagide (µg/g)	Verbascoside (µg/g)	Isoverbascoside (µg/g)	Acetylacteoside (µg/g)	Coumaroylharpagide (µg/g)	Harpagoside (µg/g)
CASS	2.23	2.00	4.72	2.45	0.04	9.14
St. deviation	0.34	0.63	0.37	0.46	0.01	0.82
GAN	3.21	2.67	4.75	2.98	0.04	4.88
St. deviation	0.76	0.62	0.40	0.12	0.01	0.93
MOS	1.47	2.00	5.23	3.83	0.04	7.18
St. deviation	0.74	1.80	1.87	2.28	0.01	1.94
M-NR	2.45	0.97	3.92	1.80	0.06	7.09
St. deviation	1.38	0.48	2.91	1.19	0.02	1.96
Lafras	2.65	8.50	6.71	3.75	0.08	7.36
St. deviation	0.56	5.83	4.05	2.12	0.01	1.61
Terra Firma	1.67	2.90	4.92	3.88	0.04	3.46
St. deviation	0.49	1.01	2.62	1.96	0.01	1.47
Namibia	5.67	1.32	7.73	4.14	0.15	8.85
St. deviation	3.08	0.70	3.94	2.49	0.16	4.02
Zimbabwe	7.89	1.61	9.85	0.51	0.37	11.51
St. deviation	1.75	0.84	2.09	0.74	0.11	3.09
CAP	2.59	1.67	5.38	1.12	2.06	3.74
St. deviation	1.03	1.29	2.57	0.32	0.97	0.58
FERRO	4.48	0.29	0.59	0.27	0.06	0.08
St. deviation	3.05	0.16	0.30	0.11	0.00	0.11
MAKGA	3.55	0.28	0.72	0.66	0.06	0.00
St. deviation	1.42	0.13	0.37	0.31	0.00	0.00
SPRING	2.76	0.73	0.83	1.32	0.06	0.00
St. deviation	1.42	0.92	0.46	1.81	0.00	0.00

(Cassel = CASS, Ganyesa = GAN, Moswana = MOS, Caprivi = CAP, Ferrolands = FERRO, Makgabeng = MAKHA, Springbokfontein = SPRING)

7.4 Conclusions (all Data Analysis)

From these tables **7.9a** to **7.9f** (the red figures indicate significant variation) we make the following conclusions:

1. Harpagide from Zimbabwe shows a significant variation when compare to all other samples, except Namibia (Table **7.9a**). Two of the three samples from Limpopo Province (Ferrolands and Makgabeng) show a similar significant variation from all samples with the exception of Namibia, Lafras and Caprivi. The third sample from the Northern Province (SPRING), in contrast, only differs from the Zimbabwe sample (the sample that differs from all other samples).

2. Verbascoside from Zimbabwe differs significantly only from M-NR and Lafras. The three localities in the Limpopo province (SPRING, FERRO, MAKGA) differ significantly from most other localities except for Namibia (table **7.9b**) and for M-NR and CAP (the last two are not as significant as the rest). The significant differences between Lafras and M-NR and most other locations were discussed in chapter 3.

3. Isoverbascoside in the Limpopo Province sample shows significant differences from all other localities (table **7.9c**).

4. Acetylacteoside in the Limpopo Province sample shows significant differences from all other samples except for Zimbabwe and Caprivi (table **7.9d**). The same applies to Zimbabwe that differs from all other samples, except those from Caprivi and Limpopo province. Caprivi only differs from MOS and Terra Firma.

5. Coumaroylharpagide in the Limpopo Province samples shows significant differences from all other localities. The same applies to Terra Firma samples that differ from all other samples (table **7.9e**).

6. Harpagoside in the Limpopo Province samples shows significant differences from all other localities. Zimbabwe differs significant from CASS, M-NR and Terra-Firma. Caprivi significantly only differs from the Limpopo localities (table **7.9f**).

In conclusion, Limpopo differs significantly from all other samples in harpagide, verbascoside, isoverbascoside, 6-acetylacteoside, coumaroylharpagide and harpagoside. It differs in all the chemical components that we analysed. The only exception is harpagide in the SPRING sample that only differs significantly from the Zimbabwe sample.

Zimbabwe shows significant differences from all other populations only in terms of harpagide. It however differs from the Limpopo samples in all the chemical components that we analysed as already discussed above. It differs in terms of harpagoside from a few North West populations (Cassel, Molopo Nature Reserve and Terra Firma).

Caprivi does not seem to differ significantly from the other samples, except from the Limpopo samples as discussed above. Interestingly it differs significantly from Zimbabwe in harpagide content (but in none of the other chemical components).

Graphical representations of principal component factors neatly classify the different populations into three groups that correspond with their taxonomic classification.

1. Group 1 corresponds with *H. zeyheri* subsp. *sublobatum*. These are the populations from Caprivi and Zimbabwe. Plotting factor 1 (verbascoside, isoverbascoside and acetylacteoside) versus factor 2 (harpagide) (figure **7.1**) (black circles) and factor 1 versus factor 3 (coumaroylharpagide) (figure **7.3**) (black and red circles) clearly distinguishes the Caprivi and Zimbabwe populations from the rest. Factor 2 versus factor 3 (figure **7.2**) fails to detect these populations.

2. Group 2 corresponds with H. *zeyheri* subsp. *zeyheri* from the Limpopo populations. This cluster can be clearly seen in figure **7.2** (factor 1) (verbascoside, isoverbascoside and 6-acetylacteoside) versus factor 3 (coumaroylharpagide) (red circles). Factor 1 versus factor 2 (Figure **7.1**) shows reasonable separation (red circles). Some overlap between red circles and the rest is observed. Figure **7.3** (factor 2 versus factor 3) shows no clearly separated cluster for this group.

3. Group 3 corresponds to *H. procumbens* subsp. *procumbens* from Namibia and North West Province. This group is clearly distinguishable in figure **7.1** (factor 1 (verbascoside,

isoverbascoside and 6-acetylacteoside) versus factor 2 (harpagide)). In figure **7.2** it overlaps with the Zimbabwe/Caprivi cluster and in figure 3 it overlaps with the Limpopo cluster.

8. Conclusion and future work

Multivariate and univariate analysis (MANOVA and ANOVA) of the six variables (harpagide, harpagoside, coumaroylharpagide, verbascoside, isoverbascoside and acetylacteoside) of normal and transformed data indicated highly significant differences between populations and age groups. Factor analysis yielded two, three or four factors depending on the level of degree of variance that we indicated.

Correlation analysis used all six variables because the factors did not match factors based on the chemical structure of the six variables. Correlation analysis indicated the following:

1. There are meaningful differences in chemical composition of tubers that are one, two, three, four or five years old. Significantly, the active ingredient harpagoside does not seem to be affected by plant age.
2. There are meaningful differences in chemical composition between the six populations from the North West Province (Cassel, Ganayesa, Moswana, Terra Firma, Lafras and Molopo Nature Reserve).
3 There are meaningful differences in the chemical composition of populations from North West Province and Namibia.
4. There are meaningful differences in the chemical composition of populations from North West Province/ Namibia (*H. procumbens* subsp. *procumbens*, from the Northern Province (*H. zeyheri* subsp. *zeyheri*) and from Zimbabwe (Victoria Falls) (*H. zeyheri* subsp. *sublobatum*).

Cluster analysis, using scatter plots of three factors (factor 1: verbascoside, isoverbascoside and 6-acetylacteoside, factor 2: harpagide and factor 3: coumaroylharpagide) identified three distinct populations. These three populations are from three different geographical regions and correspond to the corresponding taxonomic classification of the different populations. (*H. zeyheri* subsp. *zeyheri* from the Limpopo Province, *H. zeyheri* subsp. *sublobatum* from Zimbabwe (Victoria Falls) and Caprivi and *H. procumbens* subsp. *procumbens* from the North West province and Namibia.

Future work will depend on the availability of plant material that we did not have access to. Samples from Botswana should be added to our analysis.

Before a more exact pattern of chemical characteristics can be described the subspecies not included in this study should be investigated. These are *H. procumbens* subsp. *transvaalense* and *H. zeyheri* subsp. *schijffii*. Both subspecies occur in Limpopo Province.

Appendix A: Raw data of the analysed samples by HPLC

Cassel 1 data

Plant name	Locality	Rep.	Harpagid(%)	Verbasc.(%)	Isoverbasc.(%)	6-Acetylac.(%)	Coumaroy.(%)	Harpagos(%)
C1-07-01	CASS1	1	0.401	0.58	0.505	0.401	0.006	1.028
C1-07-01	CASS1	2	0.376	0.566	0.501	0.396	0.006	1.000
C1-07-01	CASS1	3	0.377	0.578	0.513	0.405	0.006	1.005
C1-07-02	CASS1	1	0.493	0.624	0.531	0.299	0.01	1.294
C1-07-02	CASS1	2	0.462	0.585	0.488	0.285	0.01	1.239
C1-07-02	CASS1	3	0.483	0.603	0.5	0.29	0.01	1.271
C1-07-03	CASS1	1	0.272	0.096	0.338	0.22	0.013	1.536
C1-07-03	CASS1	2	0.264	0.099	0.355	0.218	0.012	1.428
C1-07-03	CASS1	3	0.278	0.1	0.357	0.221	0.012	1.446
C1-07-04	CASS1	1	0.733	0.204	1.129	0.58	0.009	1.486
C1-07-04	CASS1	2	0.771	0.21	1.19	0.603	0.009	1.544
C1-07-04	CASS1	3	0.751	0.207	1.176	0.592	0.009	1.482
C1-07-05	CASS1	1	0.252	0.375	1.729	0.593	0.007	1.977
C1-07-05	CASS1	2	0.491	0.386	1.775	0.651	0.007	1.886
C1-07-05	CASS1	3	0.504	0.386	1.827	0.654	0.007	1.854
C1-07-06	CASS1	1	0.806	1.027	2.96	1.025	0.007	1.216
C1-07-06	CASS1	2	0.797	0.995	2.873	1.016	0.007	1.208
C1-07-06	CASS1	3	0.789	1.017	2.807	1.013	0.007	1.249
C1-07-07	CASS1	1	0.306	0.341	0.356	0.202	0.01	2.124
C1-07-07	CASS1	2	0.312	0.338	0.35	0.2	0.011	2.202
C1-07-07	CASS1	3	0.303	0.333	0.354	0.2	0.01	2.137
C1-07-09	CASS1	1	0.289	0.262	0.434	0.326	0.006	1.572
C1-07-09	CASS1	2	0.292	0.272	0.431	0.317	0.006	1.486
C1-07-09	CASS1	3	0.302	0.287	0.435	0.318	0.006	1.501
C1-07-10	CASS1	1	0.809	0.671	2.552	0.682	0.008	1.779
C1-07-10	CASS1	2	0.897	0.751	2.897	0.753	0.008	1.739
C1-07-10	CASS1	3	0.909	0.772	2.996	0.761	0.008	1.781
C1-07-11	CASS1	1	0.211	0.595	0.668	0.203	0.007	1.597
C1-07-11	CASS1	2	0.218	0.613	0.662	0.202	0.008	1.647
C1-07-11	CASS1	3	0.217	0.631	0.685	0.203	0.008	1.613
C1-07-12	CASS1	1	0.256	0.206	0.625	0.379	0.015	2.288
C1-07-12	CASS1	2	0.26	0.205	0.632	0.377	0.016	2.285
C1-07-12	CASS1	3	0.253	0.203	0.627	0.374	0.015	2.248
C1-07-13	CASS1	1	0.344	0.54	0.876	0.49	0.008	1.872
C1-07-13	CASS1	2	0.343	0.531	0.854	0.482	0.008	1.888
C1-07-13	CASS1	3	0.342	0.538	0.859	0.483	0.008	1.881
C1-07-14	CASS1	1	0.67	0.492	1.041	0.254	0.008	0.665
C1-07-14	CASS1	2	0.656	0.487	1.046	0.259	0.007	0.641
C1-07-14	CASS1	3	0.635	0.482	0.985	0.247	0.007	0.62
C1-07-16	CASS1	1	0.371	1.109	0.94	0.456	0.009	1.731
C1-07-16	CASS1	2	0.369	1.086	0.916	0.456	0.009	1.746
C1-07-16	CASS1	3	0.374	1.095	0.918	0.46	0.009	1.729
C1-07-18	CASS1	1	0.423	0.53	0.507	0.261	0.003	1.02

C1-07-18	CASS1	2	0.41	0.516	0.476	0.254	0.003	1.019
C1-07-18	CASS1	3	0.421	0.524	0.502	0.263	0.003	1.041
C1-07-19	CASS1	1	0.199	0.336	0.421	0.465	0.009	1.362
C1-07-19	CASS1	2	0.202	0.341	0.419	0.479	0.009	1.417
C1-07-19	CASS1	3	0.205	0.346	0.425	0.474	0.009	1.39
C1-07-20	CASS1	1	1.157	1.104	0.975	0.42	0.006	0.201
C1-07-20	CASS1	2	1.193	1.128	0.983	0.417	0.006	0.192
C1-07-20	CASS1	3	1.257	1.12	1.107	0.478	0.007	0.239
C1-07-21	CASS1	1	0.265	0.197	0.525	0.313	0.006	1.271
C1-07-21	CASS1	2	0.263	0.196	0.523	0.309	0.006	1.249
C1-07-22	CASS1	1	0.342	0.74	0.649	0.665	0.013	1.956
C1-07-22	CASS1	2	0.344	0.739	0.647	0.664	0.013	1.995
C1-07-22	CASS1	3	0.347	0.746	0.655	0.677	0.013	2.009
C1-07-23	CASS1	1	0.626	0.34	0.871	0.332	0.007	0.724
C1-07-23	CASS1	2	0.651	0.35	0.902	0.34	0.007	0.72
C1-07-23	CASS1	3	0.677	0.379	0.902	0.349	0.007	0.817
C1-07-24	CASS1	1	0.386	0.512	0.842	0.446	0.008	1.965
C1-07-24	CASS1	2	0.415	0.554	0.881	0.466	0.009	2.058
C1-07-24	CASS1	3	0.423	0.564	0.912	0.481	0.009	2.101
C1-07-25	CASS1	1	0.237	0.533	0.621	0.52	0.005	0.543
C1-07-25	CASS1	2	0.254	0.57	0.642	0.53	0.005	0.574
C1-07-25	CASS1	3	0.246	0.543	0.619	0.518	0.005	0.554

Cassel 2 data

Plant name	Locality	Rep.	Harpagid(%)	Verbasc.(%)	Isoverbasc.(%)	6-Acetylac.(%)	Coumaroy.(%)	Harpagos(%)
C2-07-01	CASS2	1	0.505	0.508	0.957	0.207	0.008	0.938
C2-07-01	CASS2	2	0.516	0.517	0.966	0.212	0.008	0.934
C2-07-01	CASS2	3	0.508	0.509	0.948	0.211	0.008	0.958
C2-07-02	CASS2	1	0.554	0.497	1.167	0.649	0.021	2.846
C2-07-02	CASS2	2	0.549	0.496	1.151	0.612	0.021	2.77
C2-07-02	CASS2	3	0.551	0.5	1.156	0.617	0.021	2.815
C2-07-03	CASS2	1	0.462	0.334	0.551	0.549	0.007	0.95
C2-07-03	CASS2	2	0.469	0.328	0.55	0.544	0.006	0.96
C2-07-03	CASS2	3	0.47	0.328	0.562	0.558	0.007	0.979
C2-07-05	CASS2	1	0.558	0.626	0.336	0.277	0.006	0.761
C2-07-05	CASS2	2	0.548	0.615	0.329	0.277	0.006	0.742
C2-07-05	CASS2	3	0.554	0.635	0.332	0.276	0.006	0.734
C2-07-06	CASS2	1	0.366	0.754	1.343	0.124	0.008	1.584
C2-07-06	CASS2	2	0.365	0.761	1.332	0.121	0.008	1.549
C2-07-06	CASS2	3	0.371	0.757	1.331	0.123	0.008	1.588
C2-07-07	CASS2	1	0.377	0.289	0.595	0.589	0.012	2.474
C2-07-07	CASS2	2	0.373	0.279	0.585	0.576	0.012	2.433
C2-07-07	CASS2	3	0.377	0.291	0.601	0.591	0.012	2.451
C2-07-08	CASS2	1	0.431	0.853	0.726	0.378	0.01	0.894
C2-07-08	CASS2	2	0.424	0.814	0.692	0.368	0.01	0.902
C2-07-08	CASS2	3	0.448	0.889	0.768	0.395	0.01	0.908

C2-07-09	CASS2	1	0.478	1.145	0.882	0.567	0.012	1.768
C2-07-09	CASS2	2	0.455	1.086	0.848	0.543	0.011	1.709
C2-07-09	CASS2	3	0.48	1.141	0.867	0.559	0.011	1.771
C2-07-10	CASS2	1	0.215	0.167	0.214	0.101	0.005	0.516
C2-07-10	CASS2	2	0.232	0.175	0.229	0.102	0.005	0.528
C2-07-10	CASS2	3	0.221	0.173	0.227	0.101	0.002	0.522
C2-07-11	CASS2	1	0.263	0.136	0.308	0.164	0.008	1.422
C2-07-11	CASS2	2	0.188	0.134	0.306	0.164	0.008	1.455
C2-07-11	CASS2	3	0.191	0.137	0.311	0.166	0.008	1.435
C2-07-12	CASS2	1	0.46	0.352	0.469	0.277	0.008	1.77
C2-07-12	CASS2	2	0.427	0.337	0.467	0.267	0.007	1.692
C2-07-12	CASS2	3	0.426	0.335	0.456	0.266	0.007	1.695
C2-07-14	CASS2	1	0.479	0.346	0.364	0.265	0.011	1.664
C2-07-14	CASS2	2	0.463	0.338	0.364	0.264	0.011	1.624
C2-07-14	CASS2	3	0.466	0.344	0.373	0.27	0.011	1.667
C2-07-15	CASS2	1	1.086	0.389	2.701	0.854	0.011	1.103
C2-07-15	CASS2	2	1.073	0.391	2.674	0.86	0.011	1.109
C2-07-15	CASS2	3	1.079	0.391	2.724	0.859	0.011	1.092
C2-07-16	CASS2	1	0.634	0.317	0.515	0.367	0.01	2.081
C2-07-16	CASS2	2	0.623	0.307	0.501	0.359	0.01	2.077
C2-07-16	CASS2	3	0.64	0.327	0.514	0.367	0.01	2.058
C2-07-17	CASS2	1	0.265	0.223	0.328	0.244	0.004	1.263
C2-07-17	CASS2	2	0.27	0.226	0.341	0.252	0.004	1.284
C2-07-17	CASS2	3	0.27	0.225	0.334	0.249	0.004	1.27
C2-07-18	CASS2	1	0.452	0.572	0.7	0.221	0.014	1.952
C2-07-18	CASS2	2	0.447	0.587	0.711	0.221	0.014	1.946
C2-07-18	CASS2	3	0.457	0.587	0.725	0.228	0.014	1.949
C2-07-19	CASS2	1	0.44	0.513	0.567	0.372	0.007	0.732
C2-07-19	CASS2	2	0.45	0.521	0.582	0.374	0.008	0.737
C2-07-19	CASS2	3	0.471	0.548	0.596	0.386	0.008	0.759
C2-07-20	CASS2	1	0.39	0.25	0.488	0.246	0.011	1.811
C2-07-21	CASS2	1	0.4	0.343	0.496	0.277	0.008	1.079
C2-07-21	CASS2	2	0.4	0.337	0.56	0.297	0.008	1.039
C2-07-21	CASS2	3	0.399	0.346	0.572	0.308	0.008	1.021
C2-07-22	CASS2	1	0.45	0.497	0.652	0.364	0.011	1.143
C2-07-22	CASS2	2	0.445	0.491	0.661	0.355	0.011	1.128
C2-07-23	CASS2	1	0.867	0.224	0.443	0.299	0.008	3.593
C2-07-23	CASS2	2	0.898	0.22	0.445	0.3	0.008	3.703
C2-07-23	CASS2	3	0.896	0.23	0.458	0.307	0.008	3.707
C2-07-24	CASS2	1	1.407	0.6	4.165	0.884	0.007	1.023
C2-07-24	CASS2	2	1.439	0.61	4.219	0.894	0.007	1.008
C2-07-24	CASS2	3	1.416	0.604	4.16	0.89	0.007	1.018
C2-07-25	CASS2	1	0.374	0.358	1.035	1.061	0.017	1.835
C2-07-25	CASS2	2	0.382	0.36	1.047	1.064	0.017	1.913
C2-07-25	CASS2	3	0.366	0.34	0.999	1.011	0.017	1.797

Cassel 3 data

Plant name	Locality	Rep.	Harpagid(%)	Verbasc.(%)	Isoverbasc.(%)	6-Acetylac.(%)	Coumaroy.(%)	Harpagos(%)
C3-07-01	CASS3	1	0.081	0.691	0.713	0.606	0.009	1.148
C3-07-01	CASS3	2	0.506	0.71	0.715	0.645	0.01	1.078
C3-07-01	CASS3	3	0.492	0.687	0.706	0.659	0.009	1.058
C3-07-02	CASS3	1	0.924	0.319	0.828	0.464	0.009	0.963
C3-07-02	CASS3	2	0.931	0.321	0.841	0.474	0.009	0.963
C3-07-02	CASS3	3	0.96	0.326	0.871	0.48	0.009	0.987
C3-07-03	CASS3	1	0.782	1.102	1.269	0.382	0.013	1.928
C3-07-03	CASS3	2	0.801	1.134	1.335	0.395	0.014	2.014
C3-07-03	CASS3	3	0.774	1.121	1.326	0.391	0.014	1.947
C3-07-04	CASS3	1	0.783	1.134	1.341	0.395	0.014	1.943
C3-07-04	CASS3	2	0.785	1.133	1.336	0.394	0.014	1.972
C3-07-04	CASS3	3	0.768	1.098	1.285	0.381	0.013	1.919
C3-07-05	CASS3	1	0.615	1.36	0.822	0.657	0.015	1.383
C3-07-05	CASS3	2	0.615	1.327	0.807	0.668	0.015	1.382
C3-07-05	CASS3	3	0.614	1.311	0.8	0.663	0.015	1.379
C3-07-06	CASS3	1	0.343	0.355	0.651	0.378	0.012	1.342
C3-07-06	CASS3	2	0.334	0.329	0.607	0.36	0.011	1.256
C3-07-06	CASS3	3	0.342	0.344	0.63	0.368	0.012	1.298
C3-07-07	CASS3	1	0.432	0.802	0.775	0.339	0.017	1.466
C3-07-07	CASS3	2	0.426	0.783	0.755	0.331	0.017	1.435
C3-07-07	CASS3	3	0.405	0.749	0.716	0.318	0.016	1.369
C3-07-08	CASS3	1	0.414	0.618	0.631	0.826	0.017	2.081
C3-07-08	CASS3	2	0.405	0.594	0.618	0.81	0.017	2.07
C3-07-08	CASS3	3	0.414	0.605	0.628	0.829	0.017	2.081
C3-07-09	CASS3	1	0.901	1.003	1.945	0.817	0.014	1.174
C3-07-09	CASS3	2	0.883	0.983	1.894	0.839	0.014	1.153
C3-07-09	CASS3	3	0.862	0.958	1.835	0.813	0.014	1.113
C3-07-10	CASS3	1	0.306	0.369	0.995	0.661	0.015	2.045
C3-07-10	CASS3	2	0.311	0.381	0.934	0.652	0.015	1.957
C3-07-10	CASS3	3	0.323	0.413	0.996	0.679	0.016	2.125
C3-07-11	CASS3	1	0.643	1.855	1.496	1.069	0.024	1.389
C3-07-11	CASS3	2	0.623	1.792	1.451	1.031	0.023	1.333
C3-07-11	CASS3	3	0.612	1.778	1.437	1.026	0.023	1.312
C3-07-12	CASS3	1	0.509	0.733	0.929	0.787	0.011	1.587
C3-07-12	CASS3	2	0.535	0.78	1.024	0.863	0.011	1.671
C3-07-12	CASS3	3	0.508	0.76	0.958	0.81	0.011	1.595
C3-07-13	CASS3	1	0.452	0.601	2.309	1.245	0.013	1.077
C3-07-13	CASS3	2	0.44	0.611	2.303	1.245	0.013	1.057
C3-07-13	CASS3	3	0.433	0.602	2.283	1.245	0.013	1.048
C3-07-14	CASS3	1	0.427	0.398	0.689	0.279	0.009	1.829
C3-07-14	CASS3	2	0.423	0.399	0.662	0.262	0.009	1.882
C3-07-14	CASS3	3	0.442	0.424	0.699	0.273	0.009	1.963
C3-07-15	CASS3	1	0.734	1.281	0.557	0.604	0.006	0.945
C3-07-15	CASS3	2	0.727	1.315	0.552	0.6	0.006	0.931
C3-07-15	CASS3	3	0.725	1.271	0.554	0.592	0.006	0.92

C3-07-16	CASS3	1	0.467	0.892	1.139	0.724	0.012	0.813
C3-07-16	CASS3	2	0.455	0.869	1.133	0.708	0.011	0.81
C3-07-16	CASS3	3	0.467	0.891	1.102	0.703	0.011	0.816
C3-07-17	CASS3	1	0.396	0.204	0.754	0.305	0.012	1.58
C3-07-17	CASS3	2	0.393	0.202	0.769	0.316	0.012	1.495
C3-07-17	CASS3	3	0.4	0.196	0.739	0.312	0.012	1.536
C3-07-18	CASS3	1	0.293	0.848	1.262	0.753	0.02	2.127
C3-07-18	CASS3	2	0.291	0.892	1.328	0.787	0.02	2.117
C3-07-18	CASS3	3	0.287	0.868	1.285	0.767	0.02	2.101
C3-07-19	CASS3	1	0.405	0.594	1.585	1.055	0.011	1.997
C3-07-19	CASS3	2	0.415	0.6	1.599	1.05	0.011	2.033
C3-07-19	CASS3	3	0.41	0.596	1.583	1.039	0.011	2.019
C3-07-20	CASS3	1	0.65	0.546	1.55	1.28	0.012	1.621
C3-07-20	CASS3	2	0.628	0.54	1.516	1.253	0.012	1.592
C3-07-20	CASS3	3	0.637	0.545	1.553	1.29	0.012	1.651
C3-07-21	CASS3	1	0.281	0.225	0.348	0.245	0.007	2.238
C3-07-21	CASS3	2	0.298	0.243	0.365	0.256	0.007	2.276
C3-07-21	CASS3	3	0.302	0.239	0.364	0.257	0.007	2.37
C3-07-22	CASS3	1	0.361	0.56	0.883	0.562	0.018	2.037
C3-07-22	CASS3	2	0.374	0.577	0.926	0.588	0.019	2.15
C3-07-22	CASS3	3	0.368	0.57	0.912	0.576	0.018	2.051
C3-07-23	CASS3	1	0.65	0.459	1.187	0.665	0.021	3.632
C3-07-23	CASS3	2	0.639	0.448	1.161	0.65	0.021	3.531
C3-07-23	CASS3	3	0.634	0.446	1.149	0.667	0.02	3.526
C3-07-24	CASS3	1	0.718	0.447	1.02	0.407	0.01	1.605
C3-07-24	CASS3	2	0.76	0.471	1.077	0.425	0.01	1.664
C3-07-24	CASS3	3	0.731	0.443	1.028	0.434	0.01	1.572
C3-07-25	CASS3	1	0.33	0.275	1.302	0.656	0.017	2.17
C3-07-25	CASS3	2	0.33	0.274	1.294	0.652	0.017	2.184
C3-07-25	CASS3	3	0.331	0.278	1.326	0.666	0.017	2.184

Cassel 4 data

Plant name	Locality	Rep.	Harpagid(%)	Verbasc.(%)	Isoverbasc.(%)	6-Acetylac.(%)	Coumaroy.(%)	Harpagos(%)
C4-07-01	CASS4	1	0.342	1.388	2.231	1.166	0.017	2.124
C4-07-01	CASS4	2	0.356	1.379	2.303	1.195	0.017	2.187
C4-07-01	CASS4	3	0.355	1.351	2.253	1.179	0.017	2.199
C4-07-02	CASS4	1	0.277	0.61	0.692	1.848	0.025	2.424
C4-07-02	CASS4	2	0.289	0.645	0.713	1.888	0.025	2.443
C4-07-02	CASS4	3	0.301	0.635	0.713	1.931	0.025	2.465
C4-07-03	CASS4	1	1.078	0.584	2.626	1.274	0.013	1.957
C4-07-03	CASS4	2	1.089	0.585	2.622	1.253	0.013	1.884
C4-07-03	CASS4	3	1.1	0.586	2.662	1.272	0.014	1.95
C4-07-04	CASS4	1	0.669	0.445	1.037	1.177	0.016	2.485
C4-07-04	CASS4	2	0.73	0.465	1.041	1.205	0.017	2.743
C4-07-04	CASS4	3	0.696	0.418	0.945	1.101	0.016	2.627
C4-07-05	CASS4	1	1.384	0.327	1.104	0.575	0.013	1.633
C4-07-05	CASS4	2	1.387	0.317	1.165	0.58	0.013	1.653

C4-07-05	CASS4	3	1.41	0.324	1.206	0.591	0.013	1.678
C4-07-06	CASS4	1	0.384	0.562	0.697	0.659	0.008	3.283
C4-07-06	CASS4	2	0.385	0.552	0.686	0.648	0.008	3.269
C4-07-06	CASS4	3	0.395	0.565	0.7	0.669	0.009	3.329
C4-07-07	CASS4	1	0.784	0.86	0.857	0.493	0.012	2.404
C4-07-07	CASS4	2	0.78	0.852	0.847	0.47	0.012	2.425
C4-07-07	CASS4	3	0.785	0.846	0.853	0.492	0.013	2.404
C4-07-08	CASS4	1	0.397	0.568	1.19	0.472	0.009	2.35
C4-07-08	CASS4	2	0.404	0.576	1.175	0.476	0.009	2.394
C4-07-08	CASS4	3	0.412	0.57	1.186	0.473	0.009	2.379
C4-07-09	CASS4	1	0.416	0.691	1.01	0.629	0.017	4.274
C4-07-09	CASS4	2	0.444	0.716	1.035	0.642	0.018	4.538
C4-07-09	CASS4	3	0.446	0.749	1.095	0.678	0.018	4.45
C4-07-10	CASS4	1	0.771	0.76	1.13	0.584	0.029	5.708
C4-07-10	CASS4	2	0.748	0.742	1.106	0.576	0.029	5.539
C4-07-10	CASS4	3	0.746	0.74	1.103	0.573	0.029	5.49
C4-07-11	CASS4	1	1.144	0.534	1.971	0.536	0.008	1.328
C4-07-11	CASS4	2	1.232	0.555	2.125	0.55	0.008	1.351
C4-07-11	CASS4	3	1.216	0.558	2.103	0.621	0.008	1.381
C4-07-12	CASS4	1	0.735	0.738	1.654	1.604	0.018	4.922
C4-07-12	CASS4	2	0.749	0.729	1.63	1.573	0.018	4.972
C4-07-12	CASS4	3	0.74	0.739	1.603	1.551	0.018	4.959
C4-07-13	CASS4	1	0.522	0.241	0.928	0.355	0.012	2.852
C4-07-13	CASS4	2	0.534	0.264	0.964	0.369	0.012	2.945
C4-07-13	CASS4	3	0.535	0.255	0.974	0.368	0.012	2.9
C4-07-14	CASS4	1	1.036	0.521	2.228	1.469	0.017	2.19
C4-07-14	CASS4	2	1.029	0.509	2.326	1.516	0.018	2.187
C4-07-14	CASS4	3	0.988	0.552	2.229	1.43	0.017	2.085
C4-07-15	CASS4	1	0.844	1.068	0.889	0.656	0.014	2.539
C4-07-15	CASS4	2	0.832	1.072	0.864	0.632	0.014	2.501
C4-07-15	CASS4	3	0.888	1.054	0.953	0.691	0.015	2.6
C4-07-17	CASS4	1	0.47	0.997	1.501	1.756	0.024	4.935
C4-07-17	CASS4	2	0.457	1.029	1.49	1.721	0.023	4.792
C4-07-17	CASS4	3	0.473	1.057	1.505	1.747	0.024	4.857
C4-07-18	CASS4	1	0.391	0.836	1.312	0.847	0.015	2.406
C4-07-18	CASS4	2	0.424	0.886	1.377	0.882	0.015	2.497
C4-07-18	CASS4	3	0.405	0.83	1.294	0.844	0.015	2.442
C4-07-19	CASS4	1	0.453	0.257	1.233	0.78	0.032	4.981
C4-07-19	CASS4	2	0.455	0.261	1.239	0.782	0.032	5.021
C4-07-19	CASS4	3	0.458	0.254	1.246	0.787	0.032	5.005
C4-07-20	CASS4	1	1.301	2.696	11.838	1.893	0.008	1.385
C4-07-20	CASS4	2	1.275	2.7	11.814	1.897	0.008	1.385
C4-07-20	CASS4	3	1.305	2.704	11.824	1.884	0.008	1.396
C4-07-21	CASS4	1	1.603	2.772	4.908	1.469	0.049	4.666
C4-07-21	CASS4	2	1.599	2.711	4.914	1.46	0.049	4.668
C4-07-21	CASS4	3	1.551	2.767	4.928	1.447	0.049	4.568
C4-07-22	CASS4	1	0.415	0.57	1.193	0.479	0.014	4.557
C4-07-22	CASS4	2	0.483	0.567	1.167	0.483	0.021	4.505

C4-07-22	CASS4	3	0.49	0.575	1.194	0.481	0.021	4.589
C4-07-23	CASS4	1	0.494	0.356	1.126	1.123	0.021	3.042
C4-07-23	CASS4	2	0.41	0.358	1.136	1.119	0.014	3.034
C4-07-23	CASS4	3	0.415	0.347	1.122	1.132	0.014	3.075
C4-07-24	CASS4	1	0.812	0.419	0.558	0.35	0.012	2.94
C4-07-24	CASS4	2	0.812	0.394	0.556	0.347	0.012	2.935
C4-07-24	CASS4	3	0.775	0.409	0.543	0.34	0.012	2.829
C4-07-25	CASS4	1	0.335	0.314	0.643	0.296	0.011	2.298
C4-07-25	CASS4	2	0.356	0.331	0.67	0.302	0.011	2.384
C4-07-25	CASS4	3	0.355	0.325	0.665	0.296	0.011	2.418

Cassel 5 data

Plant name	Locality	Rep.	Harpagid(%)	Verbasc.(%)	Isoverbasc.(%)	6-Acetylac.(%)	Coumaroy.(%)	Harpagos(%)
C5-07-01	CASS5	1	0.526	0.273	0.522	0.334	0.005	1.179
C5-07-01	CASS5	2	0.579	0.27	0.533	0.337	0.005	1.155
C5-07-01	CASS5	3	0.574	0.258	0.534	0.339	0.005	1.175
C5-07-02	CASS5	1	0.307	0.215	0.415	0.333	0.005	1.661
C5-07-02	CASS5	2	0.335	0.295	0.46	0.369	0.005	1.819
C5-07-02	CASS5	3	0.318	0.253	0.441	0.352	0.005	1.718
C5-07-03	CASS5	1	0.564	1.07	1.528	0.503	0.015	2.675
C5-07-03	CASS5	2	0.571	1.049	1.501	0.495	0.014	2.635
C5-07-03	CASS5	3	0.578	1.096	1.561	0.503	0.015	2.741
C5-07-04	CASS5	1	0.405	0.295	0.258	0.142	0.005	0.714
C5-07-04	CASS5	2	0.41	0.301	0.264	0.144	0.005	0.722
C5-07-04	CASS5	3	0.407	0.299	0.262	0.144	0.005	0.723
C5-07-05	CASS5	1	1.281	0.573	2.849	1.358	0.009	0.787
C5-07-05	CASS5	2	1.302	0.575	2.841	1.362	0.009	0.804
C5-07-05	CASS5	3	1.322	0.58	2.899	1.387	0.009	0.797
C5-07-06	CASS5	1	0.35	0.241	0.494	0.31	0.005	2.318
C5-07-06	CASS5	2	0.338	0.228	0.466	0.291	0.005	2.264
C5-07-06	CASS5	3	0.342	0.233	0.473	0.295	0.005	2.272
C5-07-07	CASS5	1	0.383	0.723	1.153	0.395	0.014	2.33
C5-07-07	CASS5	2	0.374	0.696	1.089	0.385	0.014	2.275
C5-07-07	CASS5	3	0.377	0.721	1.133	0.382	0.014	2.308
C5-07-08	CASS5	1	0.43	0.364	0.419	0.282	0.006	1.723
C5-07-08	CASS5	2	0.423	0.335	0.429	0.291	0.006	1.706
C5-07-08	CASS5	3	0.322	0.346	0.403	0.273	0.006	1.702
C5-07-09	CASS5	1	1.113	0.274	0.435	0.496	0.008	2.19
C5-07-09	CASS5	2	1.104	0.263	0.421	0.493	0.008	2.274
C5-07-09	CASS5	3	1.077	0.265	0.424	0.463	0.008	2.245
C5-07-10	CASS5	1	0.285	0.19	0.614	0.396	0.003	1.756
C5-07-10	CASS5	2	0.287	0.203	0.649	0.411	0.003	1.733
C5-07-10	CASS5	3	0.296	0.196	0.621	0.398	0.003	1.804
C5-07-11	CASS5	1	0.287	0.218	0.635	0.234	0.011	2.045
C5-07-11	CASS5	2	0.286	0.222	0.642	0.233	0.011	2.044
C5-07-11	CASS5	3	0.302	0.197	0.631	0.271	0.012	2.07
C5-07-12	CASS5	1	0.341	0.266	0.432	0.252	0.005	3.533

C5-07-12	CASS5	2	0.333	0.253	0.416	0.239	0.005	3.578
C5-07-12	CASS5	3	0.322	0.253	0.419	0.243	0.005	3.495
C5-07-13	CASS5	1	0.309	0.167	0.313	0.167	0.005	0.689
C5-07-13	CASS5	2	0.326	0.165	0.332	0.176	0.006	0.698
C5-07-13	CASS5	3	0.308	0.166	0.313	0.167	0.005	0.671
C5-07-14	CASS5	1	0.327	0.37	0.622	0.65	0.01	1.934
C5-07-14	CASS5	2	0.326	0.37	0.627	0.611	0.01	1.938
C5-07-14	CASS5	3	0.319	0.364	0.614	0.599	0.009	1.893
C5-07-15	CASS5	1	0.297	1.368	2.208	0.896	0.007	1.899
C5-07-15	CASS5	2	0.292	1.308	2.153	0.866	0.007	1.795
C5-07-15	CASS5	3	0.296	1.357	2.228	0.905	0.007	1.844
C5-07-17	CASS5	1	1.16	0.278	0.466	0.191	0.008	2.609
C5-07-17	CASS5	2	1.21	0.285	0.483	0.194	0.008	2.757
C5-07-17	CASS5	3	1.179	0.282	0.47	0.192	0.01	2.706
C5-07-18	CASS5	1	-0.017	0.273	0.673	0.304	0.01	1.843
C5-07-18	CASS5	2	0.336	0.274	0.689	0.322	0.01	1.801
C5-07-18	CASS5	3	0.34	0.273	0.684	0.325	0.01	1.768
C5-07-19	CASS5	1	0.411	0.271	0.751	0.33	0.01	1.701
C5-07-19	CASS5	2	0.42	0.274	0.767	0.335	0.01	1.731
C5-07-19	CASS5	3	0.398	0.268	0.743	0.327	0.007	1.674
C5-07-20	CASS5	1	0.345	0.209	0.787	0.544	0.007	1.31
C5-07-20	CASS5	2	0.357	0.211	0.81	0.56	0.007	1.352
C5-07-20	CASS5	3	0.348	0.209	0.798	0.546	0.01	1.328
C5-07-21	CASS5	1	0.198	0.269	0.918	0.488	0.01	1.915
C5-07-21	CASS5	2	0.201	0.267	0.939	0.496	0.01	1.932
C5-07-21	CASS5	3	0.203	0.287	0.968	0.515	0.022	1.926
C5-07-22	CASS5	1	0.313	0.626	2.745	1.676	0.022	2.881
C5-07-22	CASS5	2	0.312	0.628	2.765	1.679	0.022	2.861
C5-07-22	CASS5	3	0.311	0.618	2.725	1.671	0.006	2.864
C5-07-23	CASS5	1	0.153	0.286	0.736	0.46	0.006	1.625
C5-07-23	CASS5	2	0.151	0.274	0.71	0.452	0.005	1.562
C5-07-23	CASS5	3	0.148	0.279	0.711	0.443	0.002	1.557
C5-07-24	CASS5	1	0.43	0.527	1.517	0.536	0.009	1.168
C5-07-24	CASS5	2	0.423	0.532	1.522	0.521	0.009	1.155
C5-07-24	CASS5	3	0.435	0.542	1.559	0.534	0.009	1.188
C5-07-25	CASS5	1	0.228	0.275	1.153	0.487	0.007	1.339
C5-07-25	CASS5	2	0.227	0.261	1.129	0.481	0.007	1.354
C5-07-25	CASS5	3	0.217	0.25	1.054	0.458	0.006	1.325

Ganyesa 1 data

Plant name	Locality	Rep.	Harpagid(%)	Verbasc.(%)	Isoverbasc.(%)	6-Acetylac.(%)	Coumaroy.(%)	Harpagos(%)
G1-07-01	GAN1	1	0.346	1.111	1.05	0.418	0.057	0.862
G1-07-01	GAN1	2	0.319	1.085	1.014	0.424	0.055	0.82
G1-07-01	GAN1	3	0.318	1.061	1.01	0.403	0.054	0.812
G1-07-02	GAN1	1	0.388	0.811	0.862	0.387	0.038	1.16
G1-07-02	GAN1	2	0.393	0.821	0.879	0.394	0.038	1.177
G1-07-02	GAN1	3	0.409	0.835	0.875	0.39	0.038	1.204

G1-07-03	GAN1	1	0.22	0.494	1.272	0.847	0.026	1.147
G1-07-03	GAN1	2	0.223	0.488	1.208	0.827	0.025	1.127
G1-07-03	GAN1	3	0.226	0.511	1.29	0.861	0.026	1.205
G1-07-04	GAN1	1	0.411	1.011	2.005	1.514	0.039	1.632
G1-07-04	GAN1	2	0.387	0.939	1.83	0.724	0.037	1.558
G1-07-04	GAN1	3	0.392	0.616	1.892	0.741	0.038	1.597
G1-07-05	GAN1	1	0.319	0.645	1.446	0.844	0.046	1.381
G1-07-05	GAN1	2	0.33	0.625	1.47	0.879	0.047	1.403
G1-07-05	GAN1	3	0.328	0.132	1.451	0.877	0.047	1.41
G1-07-06	GAN1	1	0.291	0.132	0.458	0.218	0.047	1.542
G1-07-06	GAN1	2	0.284	0.123	0.437	0.212	0.047	1.54
G1-07-06	GAN1	3	0.284	0.125	0.44	0.211	0.046	1.539
G1-07-07	GAN1	1	0.25	0.511	1.466	0.724	0.038	0.882
G1-07-07	GAN1	2	0.233	0.478	1.338	0.688	0.037	0.828
G1-07-07	GAN1	3	0.243	0.491	1.416	0.703	0.037	0.85
G1-07-08	GAN1	1	0.482	0.365	0.39	0.222	0.032	0.959
G1-07-08	GAN1	2	0.493	0.378	0.391	0.225	0.032	0.962
G1-07-08	GAN1	3	0.504	0.383	0.403	0.231	0.032	0.987
G1-07-09	GAN1	1	0.341	0.714	0.581	0.362	0.034	1.056
G1-07-09	GAN1	2	0.351	0.712	0.582	0.347	0.035	1.091
G1-07-09	GAN1	3	0.33	0.701	0.558	0.34	0.034	1.034
G1-07-10	GAN1	1	0.326	0.501	0.666	0.529	0.038	0.813
G1-07-10	GAN1	2	0.337	0.513	0.693	0.549	0.038	0.821
G1-07-10	GAN1	3	0.328	0.498	0.681	0.54	0.038	0.813
G1-07-11	GAN1	1	0.124	0.604	1.781	0.687	0.022	1.452
G1-07-11	GAN1	2	0.29	0.626	1.941	0.734	0.021	1.488
G1-07-11	GAN1	3	0.284	0.614	1.891	0.715	0.021	1.488
G1-07-12	GAN1	1	0.371	0.415	1.239	0.567	0.027	1.175
G1-07-12	GAN1	2	0.368	0.407	1.266	0.564	0.027	1.192
G1-07-12	GAN1	3	0.378	0.412	1.31	0.586	0.027	1.193
G1-07-13	GAN1	1	0.161	0.187	0.55	0.432	0.044	2.016
G1-07-13	GAN1	2	0.157	0.18	0.724	0.429	0.043	1.996
G1-07-13	GAN1	3	0.153	0.181	0.545	0.429	0.043	1.966
G1-07-14	GAN1	1	0.229	0.354	0.704	0.48	0.04	1.369
G1-07-14	GAN1	2	0.231	0.353	0.715	0.486	0.039	1.374
G1-07-14	GAN1	3	0.229	0.353	0.696	0.477	0.039	1.366
G1-07-15	GAN1	1	0.196	0.373	0.724	0.364	0.027	0.943
G1-07-15	GAN1	2	0.196	0.386	0.745	0.372	0.027	0.934
G1-07-15	GAN1	3	0.203	0.404	0.78	0.38	0.027	0.946
G1-07-16	GAN1	1	0.554	0.305	0.496	0.302	0.053	4.454
G1-07-16	GAN1	2	0.559	0.308	0.5	0.304	0.053	4.395
G1-07-17	GAN1	1	0.441	0.416	2.088	0.616	0.047	0.959
G1-07-17	GAN1	2	0.431	0.418	2.03	0.609	0.048	0.952
G1-07-17	GAN1	3	0.438	0.422	2.1	0.647	0.048	0.956
G1-07-18	GAN1	1	0.331	0.67	1.181	2.012	0.058	1.621
G1-07-18	GAN1	2	0.337	0.677	1.202	2.053	0.058	1.671
G1-07-18	GAN1	3	0.328	0.67	1.155	1.996	0.057	1.585
G1-07-19	GAN1	1	0.369	0.185	0.809	0.333	0.053	1.368

G1-07-19	GAN1	2	0.393	0.188	0.834	0.343	0.054	1.43
G1-07-19	GAN1	3	0.367	0.184	0.783	0.325	0.052	1.349
G1-07-20	GAN1	1	0.174	0.41	0.884	0.543	0.035	1.571
G1-07-20	GAN1	2	0.181	0.413	0.889	0.544	0.036	1.621
G1-07-20	GAN1	3	0.175	0.411	0.884	0.539	0.035	1.561
G1-07-21	GAN1	1	0.46	0.665	0.689	0.483	0.032	1.204
G1-07-21	GAN1	2	0.456	0.667	0.688	0.481	0.032	1.203
G1-07-21	GAN1	3	0.464	0.672	0.696	0.488	0.033	1.223
G1-07-22	GAN1	1	0.826	0.32	1.381	0.941	0.036	0.922
G1-07-22	GAN1	2	0.819	0.316	1.374	0.934	0.036	0.903
G1-07-22	GAN1	3	0.821	0.322	1.367	0.921	0.036	0.908
G1-07-23	GAN1	1	0.538	1.151	1.969	1.199	0.048	1.06
G1-07-23	GAN1	2	0.543	1.186	1.975	1.175	0.048	1.033
G1-07-23	GAN1	3	0.545	1.21	1.994	1.195	0.049	1.024
G1-07-24	GAN1	1	0.288	0.432	0.691	0.476	0.041	1.105
G1-07-24	GAN1	2	0.299	0.446	0.718	0.501	0.042	1.165
G1-07-24	GAN1	3	0.28	0.413	0.684	0.486	0.041	1.112
G1-07-25	GAN1	1	0.228	0.301	0.741	0.361	0.045	1.04
G1-07-25	GAN1	2	0.224	0.312	0.779	0.369	0.046	1.026
G1-07-25	GAN1	3	0.225	0.337	0.808	0.374	0.045	1.01

Ganyesa 2 data

Plant name	Locality	Rep.	Harpagid(%)	Verbasc.(%)	Isoverbasc.(%)	6-Acetylac.(%)	Coumaroy.(%)	Harpagos(%)
G2-07-01	GAN2	1	0.552	0.835	0.763	0.396	0.011	1.273
G2-07-01	GAN2	2	0.553	0.829	0.73	0.388	0.011	1.251
G2-07-01	GAN2	3	0.555	0.818	0.719	0.388	0.011	1.221
G2-07-02	GAN2	1	0.686	0.541	0.928	0.419	0.007	0.9
G2-07-02	GAN2	2	0.717	0.549	0.974	0.422	0.006	0.819
G2-07-02	GAN2	3	0.722	0.568	0.947	0.423	0.007	0.838
G2-07-03	GAN2	1	0.809	1.195	2.535	1.04	0.019	2.216
G2-07-03	GAN2	2	0.835	1.232	2.558	1.057	0.02	2.243
G2-07-03	GAN2	3	0.916	1.368	2.827	1.162	0.021	2.386
G2-07-04	GAN2	1	0.685	2.224	2.341	0.913	0.016	2.703
G2-07-04	GAN2	2	0.708	2.232	2.421	0.942	0.016	2.811
G2-07-04	GAN2	3	0.724	2.292	2.468	0.965	0.016	2.878
G2-07-05	GAN2	1	0.263	0.48	0.847	0.56	0.009	1.324
G2-07-05	GAN2	2	0.271	0.488	0.871	0.58	0.009	1.383
G2-07-05	GAN2	3	0.268	0.496	0.875	0.572	0.009	1.342
G2-07-06	GAN2	1	0.353	1.169	2.039	1.213	0.018	1.551
G2-07-06	GAN2	2	0.349	1.181	2.064	1.237	0.018	1.58
G2-07-06	GAN2	3	0.35	1.182	2.049	1.224	0.017	1.567
G2-07-07	GAN2	1	0.291	0.485	1.074	0.479	0.006	0.805
G2-07-07	GAN2	2	0.297	0.495	1.124	0.488	0.006	0.801
G2-07-07	GAN2	3	0.309	0.526	1.151	0.461	0.007	0.812
G2-07-08	GAN2	1	4.013	0.252	0.909	0.39	0.006	0.471
G2-07-08	GAN2	2	3.94	0.258	0.919	0.395	0.006	0.469
G2-07-08	GAN2	3	3.909	0.254	0.915	0.389	0.006	0.464

G2-07-09	GAN2	1	0.375	0.782	0.924	0.509	0.008	1.322
G2-07-09	GAN2	2	0.37	0.77	0.906	0.492	0.008	1.308
G2-07-09	GAN2	3	0.37	0.779	0.943	0.508	0.008	1.321
G2-07-10	GAN2	1	0.285	0.306	0.874	0.483	0.005	1.022
G2-07-10	GAN2	2	0.292	0.322	0.928	0.506	0.005	1.032
G2-07-10	GAN2	3	0.29	0.322	0.91	0.508	0.005	1.02
G2-07-11	GAN2	1	0.3	0.435	1.007	1.679	0.02	1.78
G2-07-11	GAN2	2	0.311	0.444	1.063	1.766	0.021	1.817
G2-07-11	GAN2	3	0.3	0.431	1.019	1.71	0.02	1.791
G2-07-12	GAN2	1	0.489	0.464	0.612	0.341	0.01	1.39
G2-07-12	GAN2	2	0.514	0.484	0.627	0.348	0.01	1.415
G2-07-12	GAN2	3	0.499	0.472	0.628	0.343	0.01	1.375
G2-07-13	GAN2	1	0.733	0.665	1.536	1.443	0.013	0.78
G2-07-13	GAN2	2	0.676	0.62	1.458	1.395	0.012	0.741
G2-07-13	GAN2	3	0.681	0.599	1.444	1.379	0.012	0.726
G2-07-14	GAN2	1	0.392	0.274	1.055	0.475	0.005	1.235
G2-07-14	GAN2	2	0.387	0.279	1.079	0.49	0.005	1.232
G2-07-14	GAN2	3	0.384	0.277	1.092	0.982	0.005	1.225
G2-07-15	GAN2	1	0.247	0.609	1.674	0.528	0.011	1.184
G2-07-15	GAN2	2	0.247	0.604	1.677	0.53	0.011	1.195
G2-07-15	GAN2	3	0.247	0.576	1.615	0.505	0.011	1.159
G2-07-16	GAN2	1	0.274	1.079	0.719	0.661	0.013	1.044
G2-07-16	GAN2	2	0.263	1.053	0.72	0.658	0.013	1.02
G2-07-16	GAN2	3	0.267	1.075	0.717	0.664	0.013	1.047
G2-07-18	GAN2	1	0.236	0.74	1.05	0.504	0.008	1.466
G2-07-18	GAN2	2	0.218	0.585	0.881	0.456	0.008	1.412
G2-07-18	GAN2	3	0.21	0.561	0.876	0.445	0.007	1.351
G2-07-19	GAN2	1	0.496	0.935	1.134	0.514	0.01	1.122
G2-07-19	GAN2	2	0.499	0.955	1.153	0.518	0.01	1.119
G2-07-19	GAN2	3	0.492	0.939	1.143	0.515	0.01	1.146
G2-07-20	GAN2	1	0.4	0.79	0.849	0.601	0.007	1.002
G2-07-20	GAN2	2	0.393	0.761	0.818	0.583	0.006	1.001
G2-07-20	GAN2	3	0.394	0.779	0.821	0.59	0.006	1.003
G2-07-21	GAN2	1	0.621	0.42	0.935	0.424	0.015	2.19
G2-07-21	GAN2	2	0.594	0.404	0.905	0.41	0.015	2.115
G2-07-21	GAN2	3	0.594	0.408	0.908	0.412	0.015	2.108
G2-07-22	GAN2	1	0.551	1.086	1.545	0.66	0.007	0.673
G2-07-22	GAN2	2	0.533	1.069	1.479	0.659	0.006	0.665
G2-07-22	GAN2	3	0.527	1.043	1.501	0.661	0.006	0.654
G2-07-23	GAN2	1	0.56	0.784	2.067	0.603	0.009	1.028
G2-07-23	GAN2	2	0.584	0.821	2.166	0.65	0.009	1.092
G2-07-23	GAN2	3	0.566	0.799	2.075	0.606	0.009	1.061
G2-07-24	GAN2	1	0.206	0.749	1.287	0.141	0.01	1.295
G2-07-24	GAN2	2	0.211	0.787	1.356	0.141	0.01	1.346
G2-07-24	GAN2	3	0.226	0.861	1.464	0.147	0.011	1.412
G2-07-25	GAN2	1	0.393	0.699	1.15	0.856	0.013	1.286
G2-07-25	GAN2	2	0.403	0.711	1.167	0.835	0.013	1.331
G2-07-25	GAN2	3	0.388	0.683	1.134	0.824	0.013	1.291

Appendix

Ganyesa 3 data

Plant name	Locality	Rep.	Harpagid(%)	Verbasc.(%)	Isoverbasc.(%)	6-Acetylac.(%)	Coumaroy.(%)	Harpagos(%)
G3-07-01	GAN3	1	0.063	0.085	0.149	0.114	0.003	0.456
G3-07-01	GAN3	2	0.065	0.084	0.147	0.105	0.002	0.467
G3-07-01	GAN3	3	0.061	0.083	0.147	0.113	0.002	0.446
G3-07-03	GAN3	1	0.607	4.353	6.403	2.77	0.002	0.449
G3-07-03	GAN3	2	0.618	4.358	6.408	2.839	0.012	0.455
G3-07-03	GAN3	3	0.606	4.343	6.411	2.759	0.011	0.448
G3-07-04	GAN3	1	0.824	0.246	1.208	0.539	0.008	0.774
G3-07-04	GAN3	2	0.833	0.242	1.197	0.53	0.008	0.772
G3-07-04	GAN3	3	0.837	0.242	1.182	0.526	0.008	0.78
G3-07-06	GAN3	1	0.732	0.881	1.007	0.659	0.006	0.499
G3-07-06	GAN3	2	0.755	0.907	1.028	0.646	0.006	0.519
G3-07-06	GAN3	3	0.719	0.856	0.983	0.613	0.006	0.506
G3-07-07	GAN3	1	0.404	0.14	0.365	0.185	0.007	0.337
G3-07-07	GAN3	2	0.414	0.135	0.36	0.182	0.006	0.317
G3-07-07	GAN3	3	0.417	0.135	0.353	0.179	0.006	0.33
G3-07-08	GAN3	1	0.592	0.822	1.265	0.906	0.006	1.098
G3-07-08	GAN3	2	0.599	0.852	1.29	0.925	0.006	1.099
G3-07-08	GAN3	3	0.586	0.855	1.265	0.909	0.006	1.096
G3-07-09	GAN3	1	0.329	0.052	0.111	0.094	0.003	-0.009
G3-07-09	GAN3	2	0.407	0.038	0.111	0.096	0.002	-0.012
G3-07-09	GAN3	3	0.395	0.038	0.111	0.095	0.002	-0.013
G3-07-10	GAN3	1	0.606	0.395	0.893	0.525	0.014	1.096
G3-07-10	GAN3	2	0.602	0.392	0.866	0.513	0.014	1.093
G3-07-10	GAN3	3	0.621	0.394	0.9	0.527	0.014	1.113
G3-07-11	GAN3	1	0.984	0.473	0.745	0.311	0.007	0.302
G3-07-11	GAN3	2	0.966	0.484	0.723	0.304	0.007	0.306
G3-07-11	GAN3	3	0.85	0.496	0.747	0.314	0.007	0.315
G3-07-12	GAN3	1	0.869	0.297	1.257	0.443	0.008	1.245
G3-07-12	GAN3	2	0.888	0.282	1.281	0.45	0.008	1.26
G3-07-12	GAN3	3	0.872	0.3	1.257	0.449	0.008	1.294
G3-07-13	GAN3	1	1.527	0.452	2.811	0.798	0.007	1.29
G3-07-13	GAN3	2	1.55	0.453	2.865	0.807	0.007	1.29
G3-07-13	GAN3	3	1.512	0.437	2.785	0.782	0.007	1.294
G3-07-14	GAN3	1	0.787	0.257	1.701	1.379	0.012	1.797
G3-07-14	GAN3	2	0.953	0.316	2.126	1.722	0.014	2.205
G3-07-14	GAN3	3	0.942	0.303	2.045	1.659	0.014	2.18
G3-07-15	GAN3	1	0.708	0.244	0.675	0.39	0.01	1.25
G3-07-15	GAN3	2	0.643	0.239	0.647	0.373	0.01	1.228
G3-07-15	GAN3	3	0.653	0.241	0.653	0.374	0.01	1.234

G3-07-16	GAN3	1	0.58	0.465	0.769	0.42	0.008	1.844
G3-07-16	GAN3	2	0.576	0.463	0.779	0.416	0.008	1.792
G3-07-16	GAN3	3	0.561	0.449	0.752	0.406	0.008	1.789
G3-07-17	GAN3	1	0.644	0.353	0.778	0.782	0.011	2.02
G3-07-17	GAN3	2	0.643	0.35	0.784	0.783	0.011	1.99
G3-07-17	GAN3	3	0.638	0.343	0.77	0.766	0.011	1.997
G3-07-18	GAN3	1	0.31	0.039	0.31	0.206	0.009	0.58
G3-07-18	GAN3	2	0.312	0.039	0.305	0.202	0.009	0.581
G3-07-18	GAN3	3	0.302	0.039	0.304	0.2	0.009	0.572
G3-07-19	GAN3	1	0.745	0.284	0.676	0.285	0.011	1.209
G3-07-19	GAN3	2	0.77	0.362	0.736	0.296	0.011	1.236
G3-07-19	GAN3	3	0.77	0.359	0.735	0.3	0.011	1.242
G3-07-20	GAN3	1	0.358	0.187	0.462	0.291	0.008	1.405
G3-07-20	GAN3	2	0.348	0.18	0.443	0.281	0.007	1.367
G3-07-20	GAN3	3	0.355	0.177	0.447	0.283	0.008	1.391
G3-07-22	GAN3	1	0.553	0.222	0.534	0.271	0.004	0.773
G3-07-22	GAN3	2	0.515	0.214	0.5	0.264	0.004	0.726
G3-07-22	GAN3	3	0.546	0.219	0.513	0.27	0.004	0.746
G3-07-23	GAN3	1	0.71	0.518	1.276	0.674	0.008	0.729
G3-07-23	GAN3	2	0.676	0.497	1.208	0.639	0.007	0.712
G3-07-23	GAN3	3	0.677	0.493	1.204	0.635	0.007	0.703
G3-07-25	GAN3	1	0.476	0.38	0.549	0.321	0.009	1.186
G3-07-25	GAN3	2	0.47	0.374	0.536	0.313	0.009	1.184
G3-07-25	GAN3	3	0.468	0.372	0.539	0.314	0.009	1.162

Zimbabwe data

Plant name	Locality	Rep.	Harpagid(%)	Verbasc.(%)	Isoverbasc.(%)	6-Acetylac.(%)	Coumaroy.(%)	Harpagos(%)
Z1	ZIM	1	3.322	0.537	3.526	0.481	0.145	4.34
Z1	ZIM	2	3.423	0.562	3.655	0.465	0.149	4.479
Z1	ZIM	3	3.581	0.591	3.867	0.442	0.156	4.751
Z2	ZIM	1	4.036	0.354	2.656	0.648	0.115	4.149
Z2	ZIM	2	4.221	0.367	2.808	0.685	0.123	4.349
Z2	ZIM	3	4.063	0.352	2.669	0.669	0.117	4.175
Z3	ZIM	1	2.607	1.234	4.712	0.219	0.108	2.843
Z3	ZIM	2	2.562	1.203	4.644	0.217	0.107	2.798
Z3	ZIM	3	2.636	1.25	4.798	0.219	0.11	2.886
Z4	ZIM	1	4.646	0.413	4.711	0.789	0.232	5.341
Z4	ZIM	2	4.874	0.45	5.059	0.853	0.243	5.535
Z4	ZIM	3	4.784	0.432	4.918	0.844	0.237	5.393
Z5	ZIM	1	2.577	0.281	2.23	0.261	0.07	2.993
Z5	ZIM	2	2.584	0.282	2.232	0.258	0.07	3.017
Z5	ZIM	3	2.609	0.282	2.233	0.254	0.07	3.038
Z6	ZIM	1	1.695	0.255	2.081	0.000	0.08	2.741

Z6	ZIM	2	1.796	0.268	2.175	0.000	0.082	2.847
Z6	ZIM	3	1.709	0.253	2.039	0.000	0.078	2.705
Z7	ZIM	1	1.274	0.291	1.853	0.000	0.061	1.745
Z7	ZIM	2	1.285	0.311	1.931	0.000	0.062	1.796
Z7	ZIM	3	1.237	0.297	1.862	0.000	0.06	1.732
Z8	ZIM	1	1.332	0.318	1.931	0.000	0.079	2.498
Z8	ZIM	2	1.343	0.324	1.862	0.000	0.081	2.899
Z8	ZIM	3	1.334	0.324	1.947	0.000	0.079	2.951
Z9	ZIM	1	1.379	0.656	2.002	0.000	0.064	3.084
Z9	ZIM	2	1.401	0.707	1.946	0.000	0.065	3.096
Z9	ZIM	3	1.428	0.67	2.638	0.000	0.065	3.266
Z10	ZIM	1	1.578	0.237	2.671	0.000	0.101	3.084
Z10	ZIM	2	1.535	0.236	2.7	0.000	0.1	3.096
Z10	ZIM	3	1.581	0.25	2.228	0.000	0.104	3.266
Z11	ZIM	1	1.565	0.203	2.224	0.000	0.06	1.615
Z11	ZIM	2	1.567	0.211	2.307	0.000	0.059	1.626
Z11	ZIM	3	1.617	0.21	1.595	0.000	0.063	1.731
Z12	ZIM	1	1.264	0.408	1.605	0.000	0.09	2.431
Z12	ZIM	2	1.269	0.418	1.676	0.000	0.089	2.238
Z12	ZIM	3	1.348	0.445	2.309	0.000	0.095	2.408

Namibia data

Plant name	Locality	Rep.	Harpagid(%)	Verbasc.(%)	Isoverbasc.(%)	6-Acetylac.(%)	Coumaroy.(%)	Harpagos(%)
N1	Namibia	1	1.672	0.32	2.35	0.502	0.028	2.553
N1	Namibia	2	1.674	0.325	2.391	0.506	0.028	2.551
N1	Namibia	3	1.72	0.336	2.492	0.522	0.029	2.636
N2	Namibia	1	3.183	0.331	2.146	1.107	0.026	1.665
N2	Namibia	2	3.25	0.346	2.268	1.16	0.027	1.695
N2	Namibia	3	3.4	0.362	2.391	1.205	0.028	1.82
N3	Namibia	1	0.677	0.594	2.816	1.645	0.039	4.361
N3	Namibia	2	0.704	0.614	2.907	1.704	0.04	4.506
N3	Namibia	3	0.664	0.589	2.787	1.623	0.038	4.277
N4	Namibia	1	1.965	0.361	1.973	1.69	0.032	3.125
N4	Namibia	2	1.999	0.387	2.126	1.782	0.033	3.188
N4	Namibia	3	1.975	0.39	2.15	1.773	0.033	3.196
N5	Namibia	1	3.654	0.938	5.57	3.071	0.166	5.608
N5	Namibia	2	3.773	1.015	6.042	3.283	0.178	6.009
N5	Namibia	3	3.724	1.011	5.994	3.267	0.173	5.894

Moswana 1 data

Plant name	Locality	Rep.	Harpagid(%)	Verbasc.(%)	Isoverbasc.(%)	6-Acetylac.(%)	Coumaroy.(%)	Harpagos(%)
M1-07-02	MOS1	1	0.299	0.542	0.752	0.529	0.011	1.735
M1-07-02	MOS1	2	0.287	0.531	0.745	0.528	0.011	1.735

M1-07-03	MOS1	1	0.347	0.149	0.984	0.258	0.01	1.05
M1-07-03	MOS1	2	0.422	0.153	1.019	0.255	0.01	1.056
M1-07-03	MOS1	3	0.418	0.154	1.036	0.254	0.01	1.038
M1-07-04	MOS1	1	0.482	0.255	2.121	0.537	0.006	0.252
M1-07-04	MOS1	2	0.491	0.153	2.082	0.526	0.006	0.25
M1-07-04	MOS1	3	0.47	0.154	2.053	0.519	0.006	0.254
M1-07-05	MOS1	1	0.373	0.255	0.182	0.154	0.007	1.356
M1-07-05	MOS1	2	0.371	0.152	0.178	0.152	0.007	1.36
M1-07-05	MOS1	3	0.341	0.138	0.169	0.145	0.006	1.271
M1-07-06	MOS1	1	1.044	0.322	0.849	1.665	0.007	6.196
M1-07-06	MOS1	2	1.18	0.361	0.983	2.142	0.025	7.062
M1-07-06	MOS1	3	1.229	0.138	0.631	2.264	0.025	7.428
M1-07-07	MOS1	1	0.852	0.322	1.166	0.913	0.017	5.618
M1-07-07	MOS1	2	0.838	0.757	1.189	0.887	0.025	7.062
M1-07-07	MOS1	3	0.802	0.727	0.631	0.852	0.025	7.428
M1-07-08	MOS1	1	0.34	0.274	1.166	0.453	0.017	0.983
M1-07-08	MOS1	2	0.365	0.286	1.189	0.462	0.017	1.049
M1-07-08	MOS1	3	0.368	0.282	1.163	0.462	0.018	1.085
M1-07-09	MOS1	1	0.312	0.2	0.574	0.271	0.015	1.768
M1-07-09	MOS1	2	0.32	0.207	0.597	0.275	0.015	1.795
M1-07-09	MOS1	3	0.315	0.196	0.576	0.27	0.015	1.79
M1-07-11	MOS1	1	0.342	0.234	0.31	0.102	0.005	2.603
M1-07-11	MOS1	2	0.3	0.234	0.322	0.099	0.004	2.623
M1-07-11	MOS1	3	0.298	0.231	0.319	0.097	0.004	2.658
M1-07-12	MOS1	1	0.721	0.238	0.767	0.492	0.01	1.475
M1-07-12	MOS1	2	0.722	0.245	0.796	0.512	0.01	1.509
M1-07-12	MOS1	3	0.722	0.235	0.749	0.47	0.01	1.422
M1-07-13	MOS1	1	0.246	0.287	0.207	0.101	0.008	2.597
M1-07-14	MOS1	1	0.586	0.378	0.385	0.483	0.008	1.887
M1-07-14	MOS1	2	0.576	0.375	0.381	0.501	0.008	1.822
M1-07-14	MOS1	3	0.576	0.368	0.376	0.497	0.008	1.788
M1-07-15	MOS1	1	0.281	0.098	0.327	0.173	0.003	2.155
M1-07-15	MOS1	2	0.295	0.096	0.33	0.171	0.003	2.219
M1-07-15	MOS1	3	0.32	0.098	0.357	0.174	0.003	2.205
M1-07-17	MOS1	1	0.563	0.197	0.377	0.415	0.005	1.79
M1-07-17	MOS1	2	0.566	0.197	0.378	0.417	0.005	1.801
M1-07-17	MOS1	3	0.575	0.197	0.374	0.409	0.005	1.791
M1-07-21	MOS1	1	1.31	0.922	1.489	1.013	0.009	1.484
M1-07-21	MOS1	2	1.232	0.924	1.48	1.002	0.009	1.487
M1-07-22	MOS1	1	0.826	0.678	0.88	0.185	0.007	1.784
M1-07-22	MOS1	2	0.834	0.66	0.871	0.182	0.006	1.785
M1-07-22	MOS1	3	0.856	0.661	1.48	0.181	0.007	1.793
M1-07-23	MOS1	1	0.443	0.074	0.88	0.205	0.018	1.707
M1-07-23	MOS1	2	0.468	0.077	0.871	0.218	0.019	1.737
M1-07-23	MOS1	3	0.404	0.07	0.871	0.196	0.016	1.509
M1-07-24	MOS1	1	1.312	0.134	0.384	0.239	0.009	1.398
M1-07-24	MOS1	2	1.233	0.124	0.418	0.226	0.009	1.346
M1-07-24	MOS1	3	1.278	0.127	0.367	0.227	0.009	1.334

M1-07-25	MOS1	1	0.426	0.188	1.09	0.346	0.008	1.379
M1-07-25	MOS1	2	0.421	0.184	0.34	0.34	0.008	1.354
M1-07-25	MOS1	3	0.426	0.185	0.342	0.343	0.008	1.386

Moswana 2

Plant name	Locality	Rep.	Harpagid(%)	Verbasc.(%)	Isoverbasc.(%)	6-Acetylac.(%)	Coumaroy.(%)	Harpagos(%)
M2-07-01	MOS2	1	0.697	0.624	1.035	1.689	0.009	1.737
M2-07-01	MOS2	2	0.71	0.627	1.056	1.744	0.009	1.737
M2-07-02	MOS2	1	0.353	1.559	0.975	1.554	0.017	0.368
M2-07-02	MOS2	2	0.357	1.558	0.983	1.559	0.017	0.404
M2-07-02	MOS2	3	0.36	1.59	1.017	1.603	0.018	0.404
M2-07-03	MOS2	1	0.412	0.764	0.732	0.726	0.007	0.705
M2-07-03	MOS2	2	0.429	0.799	0.782	0.742	0.007	0.769
M2-07-03	MOS2	3	0.438	0.788	0.787	0.734	0.007	0.771
M2-07-04	MOS2	1	0.282	0.424	0.545	0.76	0.015	1.331
M2-07-04	MOS2	2	0.284	0.429	0.546	0.773	0.015	1.327
M2-07-04	MOS2	3	0.285	0.426	0.548	0.771	0.015	1.314
M2-07-05	MOS2	1	0.358	1.537	0.745	0.963	0.009	0.552
M2-07-05	MOS2	2	0.351	1.514	0.722	0.935	0.009	0.535
M2-07-05	MOS2	3	0.373	1.652	0.812	1.03	0.01	0.589
M2-07-06	MOS2	1	0.464	1.246	1.944	1.569	0.011	1.124
M2-07-06	MOS2	2	0.458	1.259	1.971	1.578	0.011	1.092
M2-07-06	MOS2	3	0.448	1.218	1.921	1.552	0.011	1.078
M2-07-07	MOS2	1	0.348	0.684	1.119	1.208	0.007	1.15
M2-07-07	MOS2	2	0.354	0.7	1.135	1.224	0.007	1.163
M2-07-07	MOS2	3	0.363	0.701	1.148	1.236	0.007	1.18
M2-07-08	MOS2	1	0.341	0.5	0.945	1.314	0.019	2.62
M2-07-08	MOS2	2	0.344	0.497	0.938	1.309	0.019	2.649
M2-07-08	MOS2	3	0.345	0.497	0.941	1.324	0.02	2.665
M2-07-09	MOS2	1	0.48	3.846	5.175	1.409	0.013	1.594
M2-07-09	MOS2	2	0.47	3.794	5.081	1.379	0.012	1.561
M2-07-09	MOS2	3	0.471	3.817	5.085	1.388	0.013	1.58
M2-07-10	MOS2	1	0.764	1.543	1.365	1.014	0.022	1.19
M2-07-10	MOS2	2	0.78	1.561	1.367	1.02	0.022	1.19
M2-07-10	MOS2	3	0.773	1.559	1.362	1.023	0.022	1.197
M2-07-11	MOS2	1	0.419	0.666	1.184	0.651	0.005	0.609
M2-07-11	MOS2	2	0.375	0.618	1.088	0.573	0.005	0.558
M2-07-11	MOS2	3	0.395	0.619	1.151	0.609	0.005	0.576
M2-07-12	MOS2	1	0.461	0.947	1.25	1.456	0.015	0.952
M2-07-12	MOS2	2	0.457	0.937	1.26	1.471	0.015	0.888
M2-07-12	MOS2	3	0.457	0.946	1.254	1.462	0.015	0.886
M2-07-13	MOS2	1	0.407	1.412	1.98	1.793	0.009	1.314
M2-07-13	MOS2	2	0.443	1.416	2.016	1.875	0.009	1.385
M2-07-13	MOS2	3	0.457	1.445	2.053	1.873	0.009	1.391
M2-07-14	MOS2	1	0.577	0.998	0.786	1.352	0.008	1.691
M2-07-14	MOS2	2	0.605	1.021	0.81	1.407	0.009	1.746
M2-07-14	MOS2	3	0.602	0.999	0.811	1.407	0.009	1.749

M2-07-15	MOS2	1	0.429	0.812	2.142	1.48	0.012	0.625
M2-07-15	MOS2	2	0.439	0.838	2.203	1.52	0.012	0.633
M2-07-15	MOS2	3	0.434	0.822	2.257	1.526	0.012	0.617
M2-07-16	MOS2	1	0.218	0.53	1.504	1.435	0.01	1.925
M2-07-16	MOS2	2	0.208	0.509	1.452	1.391	0.01	1.848
M2-07-16	MOS2	3	0.218	0.533	1.512	1.45	0.01	1.848
M2-07-17	MOS2	1	0.401	0.788	1.361	1.535	0.011	1.777
M2-07-17	MOS2	2	0.421	0.814	1.414	1.604	0.012	1.833
M2-07-17	MOS2	3	0.415	0.802	1.397	1.575	0.012	1.819
M2-07-18	MOS2	1	0.496	0.93	1.135	1.522	0.007	0.993
M2-07-18	MOS2	2	0.518	0.961	1.17	1.552	0.007	1.016
M2-07-18	MOS2	3	0.483	0.913	1.131	1.52	0.007	0.954
M2-07-19	MOS2	1	0.338	0.563	0.84	0.697	0.009	1.564
M2-07-19	MOS2	2	0.354	0.581	0.873	0.719	0.009	1.638
M2-07-19	MOS2	3	0.353	0.584	0.867	0.716	0.009	1.612
M2-07-20	MOS2	1	0.237	1.584	2.18	1.253	0.006	1.81
M2-07-20	MOS2	2	0.189	1.579	2.183	1.251	0.006	1.825
M2-07-21	MOS2	1	0.369	1.26	1.993	1.978	0.01	0.903
M2-07-21	MOS2	2	0.37	1.246	1.992	1.994	0.01	0.91
M2-07-21	MOS2	3	0.366	1.25	1.987	1.983	0.01	0.912
M2-07-22	MOS2	1	0.175	0.383	1.079	0.914	0.012	1.257
M2-07-22	MOS2	2	0.182	0.398	1.114	0.937	0.013	1.299
M2-07-22	MOS2	3	0.181	0.397	1.114	0.938	0.013	1.301
M2-07-23	MOS2	1	0.477	1.661	3.603	2.811	0.011	1.071
M2-07-23	MOS2	2	0.478	1.619	3.542	2.775	0.011	1.06
M2-07-23	MOS2	3	0.483	1.677	3.674	2.875	0.011	1.092
M2-07-24	MOS2	1	0.536	1.382	1.457	1.182	0.012	0.845
M2-07-24	MOS2	2	0.557	1.426	1.476	1.208	0.012	0.875
M2-07-24	MOS2	3	0.575	1.457	1.49	1.224	0.013	0.902
M2-07-25	MOS2	1	0.214	1.538	1.15	0.555	0.008	1.446
M2-07-25	MOS2	2	0.213	1.498	1.129	0.544	0.008	1.442
M2-07-25	MOS2	3	0.206	1.486	1.122	0.539	0.008	1.406

Moswana 3

Plant name	Locality	Rep.	Harpagid(%)	Verbasc.(%)	Isoverbasc.(%)	6-Acetylac.(%)	Coumaroy.(%)	Harpagos(%)
M3-07-01	MOS3	1	1.365	0.506	1.735	0.928	0.012	1.774
M3-07-01	MOS3	2	1.392	0.46	1.692	0.912	0.012	1.575
M3-07-01	MOS3	3	1.399	0.477	1.761	0.942	0.012	1.614
M3-07-02	MOS3	1	0.304	0.197	0.448	0.331	0.009	3.848
M3-07-02	MOS3	2	0.297	0.195	0.446	0.331	0.009	3.902
M3-07-02	MOS3	3	0.313	0.21	0.469	0.346	0.009	4.011
M3-07-03	MOS3	1	0.6	0.581	1.035	0.938	0.006	2.243
M3-07-03	MOS3	2	0.598	0.586	1.045	0.942	0.006	2.263
M3-07-03	MOS3	3	0.367	0.585	1.047	0.946	0.006	2.25
M3-07-04	MOS3	1	0.931	0.68	2.973	1.459	0.014	1.891
M3-07-04	MOS3	2	0.938	0.713	3.05	1.472	0.015	1.869
M3-07-04	MOS3	3	0.913	0.689	2.943	1.445	0.015	1.857

M3-07-05	MOS3	1	0.534	0.644	1.038	0.96	0.011	2.042
M3-07-05	MOS3	2	0.54	0.672	1.105	1.013	0.011	2.059
M3-07-05	MOS3	3	0.588	0.718	1.161	1.072	0.011	2.239
M3-07-06	MOS3	1	0.672	0.665	1.039	1.209	0.014	4.658
M3-07-06	MOS3	2	0.66	0.646	1.045	1.233	0.014	4.567
M3-07-06	MOS3	3	0.675	0.656	1.076	1.272	0.014	4.673
M3-07-07	MOS3	1	0.185	0.844	1.336	2.971	0.014	3.274
M3-07-07	MOS3	2	0.194	0.884	1.39	3.041	0.014	3.307
M3-07-07	MOS3	3	0.189	0.863	1.336	2.99	0.014	3.345
M3-07-08	MOS3	1	2.034	0.526	4.369	2.36	0.025	2.471
M3-07-08	MOS3	2	2.098	0.534	4.535	2.436	0.026	2.582
M3-07-08	MOS3	3	2.051	0.523	4.415	2.36	0.025	2.481
M3-07-09	MOS3	1	0.535	0.25	0.521	0.447	0.007	2.343
M3-07-09	MOS3	2	0.551	0.23	0.519	0.444	0.007	2.37
M3-07-09	MOS3	3	0.549	0.255	0.53	0.457	0.007	2.38
M3-07-10	MOS3	1	0.516	1.069	1.633	1.612	0.017	4.38
M3-07-10	MOS3	2	0.519	1.087	1.825	1.729	0.017	4.314
M3-07-10	MOS3	3	0.521	1.114	1.809	1.724	0.018	4.359
M3-07-11	MOS3	1	0.157	0.344	1.017	0.574	0.01	2.201
M3-07-11	MOS3	2	0.162	0.358	1.047	0.588	0.01	2.279
M3-07-11	MOS3	3	0.155	0.349	1.015	0.568	0.01	2.237
M3-07-12	MOS3	1	0.257	0.637	2.249	1.514	0.011	2.927
M3-07-12	MOS3	2	0.26	0.631	2.264	1.524	0.011	2.891
M3-07-12	MOS3	3	0.287	0.681	2.521	1.655	0.011	2.951
M3-07-13	MOS3	1	0.411	0.55	1.088	0.864	0.014	2.578
M3-07-13	MOS3	2	0.411	0.547	1.089	0.902	0.014	2.758
M3-07-13	MOS3	3	0.411	0.544	1.081	0.892	0.014	2.726
M3-07-14	MOS3	1	0.94	2.734	10.415	3.215	0.019	2.937
M3-07-14	MOS3	2	0.966	2.815	10.603	3.235	0.019	2.949
M3-07-14	MOS3	3	0.945	2.744	10.417	3.136	0.019	2.888
M3-07-15	MOS3	1	0.443	0.539	1.536	1.601	0.013	1.72
M3-07-15	MOS3	2	0.441	0.537	1.544	1.61	0.013	1.727
M3-07-15	MOS3	3	0.451	0.553	1.557	1.637	0.013	1.752
M3-07-16	MOS3	1	0.262	1.771	1.544	2.293	0.044	3.459
M3-07-16	MOS3	2	0.264	1.821	1.597	2.288	0.044	3.472
M3-07-16	MOS3	3	0.276	1.882	1.586	2.346	0.045	3.55
M3-07-17	MOS3	1	0.672	1.137	1.778	1.02	0.023	2.366
M3-07-17	MOS3	2	0.686	1.155	1.794	1.046	0.024	2.392
M3-07-17	MOS3	3	0.684	1.147	1.832	1.015	0.024	2.444
M3-07-18	MOS3	1	0.325	1.681	2.34	2.585	0.014	1.915
M3-07-18	MOS3	2	0.334	1.729	2.377	2.634	0.014	1.91
M3-07-18	MOS3	3	0.344	1.699	2.331	2.579	0.014	1.935
M3-07-19	MOS3	1	0.165	0.475	1.595	1.009	0.013	3.513
M3-07-19	MOS3	2	0.158	0.448	1.542	0.988	0.013	3.428
M3-07-19	MOS3	3	0.161	0.458	1.56	0.996	0.013	3.507
M3-07-20	MOS3	1	0.612	1.029	2.012	1.416	0.023	3.393
M3-07-20	MOS3	2	0.626	1.03	2.054	1.459	0.024	3.452
M3-07-20	MOS3	3	0.654	1.079	2.165	1.469	0.025	3.571

M3-07-21	MOS3	1	0.583	0.613	1.104	1.763	0.019	2.297
M3-07-21	MOS3	2	0.616	0.627	1.122	1.828	0.02	2.379
M3-07-21	MOS3	3	0.637	0.659	1.16	1.904	0.02	2.463
M3-07-22	MOS3	1	0.778	0.613	2.209	1.838	0.017	3.223
M3-07-22	MOS3	2	0.802	0.586	2.235	1.832	0.017	3.289
M3-07-22	MOS3	3	0.773	0.582	2.232	1.867	0.017	3.248
M3-07-23	MOS3	1	0.665	0.526	2.703	0.927	0.014	2.787
M3-07-23	MOS3	2	0.677	0.535	2.833	0.965	0.014	2.905
M3-07-23	MOS3	3	0.653	0.516	2.761	0.947	0.014	2.814
M3-07-24	MOS3	1	0.68	0.661	1.606	2.412	0.018	4.045
M3-07-24	MOS3	2	0.699	0.677	1.642	2.47	0.019	4.134
M3-07-24	MOS3	3	0.706	0.686	1.659	2.498	0.019	4.203
M3-07-25	MOS3	1	0.237	0.741	2.252	1.205	0.025	2.969
M3-07-25	MOS3	2	0.309	0.748	2.279	1.242	0.024	2.878
M3-07-25	MOS3	3	0.318	0.77	2.32	1.25	0.025	2.936

Molopo Nature Reserve data

Plant name	Locality	Rep.	Harpagid(%)	Verbasc.(%)	Isoverbasc.(%)	6-Acetylac.(%)	Coumaroy.(%)	Harpagos(%)
MNR-07-01	M-NR	1	1.057	0.168	1.176	0.492	0.014	1.274
MNR-07-01	M-NR	2	1.161	0.191	1.354	0.547	0.015	1.299
MNR-07-01	M-NR	3	1.147	0.19	1.346	0.536	0.015	1.268
MNR-07-02	M-NR	1	0.559	0.094	0.425	0.134	0.008	0.881
MNR-07-02	M-NR	2	0.555	0.091	0.406	0.13	0.007	0.86
MNR-07-02	M-NR	3	0.547	0.09	0.4	0.133	0.007	0.848
MNR-07-03	M-NR	1	0.2	0.066	0.196	0.114	0.005	0.879
MNR-07-03	M-NR	2	0.205	0.065	0.195	0.113	0.005	0.889
MNR-07-03	M-NR	3	0.195	0.063	0.192	0.113	0.005	0.864
MNR-07-04	M-NR	1	1.018	0.105	0.647	0.179	0.013	1.228
MNR-07-04	M-NR	2	1.041	0.108	0.664	0.182	0.013	1.229
MNR-07-04	M-NR	3	1.009	0.108	0.647	0.178	0.013	1.212
MNR-07-05	M-NR	1	0.451	0.071	0.24	0.107	0.008	1.336
MNR-07-05	M-NR	2	0.402	0.082	0.252	0.115	0.008	1.37
MNR-07-05	M-NR	3	0.392	0.084	0.256	0.159	0.008	1.315
MNR-07-06	M-NR	1	0.965	0.215	1.228	0.614	0.018	1.008
MNR-07-06	M-NR	2	0.981	0.222	1.294	0.627	0.017	1.007
MNR-07-06	M-NR	3	0.986	0.226	1.295	0.638	0.018	1.04
MNR-07-07	M-NR	1	0.139	0.127	0.283	0.203	0.009	1.926
MNR-07-07	M-NR	2	0.137	0.124	0.275	0.2	0.009	1.955
MNR-07-07	M-NR	3	0.209	0.13	0.289	0.206	0.009	1.994
MNR-07-08	M-NR	1	0.493	0.269	0.426	0.209	0.011	1.473
MNR-07-08	M-NR	2	0.492	0.271	0.437	0.212	0.011	1.466
MNR-07-08	M-NR	3	0.502	0.279	0.448	0.217	0.011	1.51
MNR-07-09	M-NR	1	0.283	0.106	0.172	0.128	0.005	1.181
MNR-07-09	M-NR	2	0.281	0.104	0.169	0.127	0.005	1.195
MNR-07-09	M-NR	3	0.277	0.102	0.168	0.136	0.005	1.171
MNR-07-10	M-NR	1	0.386	0.218	0.952	0.397	0.009	1.795

MNR-07-10	M-NR	2	0.37	0.215	0.913	0.381	0.009	1.718
MNR-07-10	M-NR	3	0.383	0.222	0.95	0.399	0.009	1.761
MNR-07-11	M-NR	1	0.358	0.273	0.634	0.359	0.01	1.065
MNR-07-11	M-NR	2	0.353	0.268	0.63	0.355	0.01	1.049
MNR-07-11	M-NR	3	0.355	0.278	0.633	0.357	0.01	1.045
MNR-07-12	M-NR	1	0.436	0.31	1.238	0.389	0.015	1.527
MNR-07-12	M-NR	2	0.443	0.313	1.254	0.391	0.015	1.562
MNR-07-12	M-NR	3	0.437	0.312	1.251	0.395	0.015	1.543
MNR-07-13	M-NR	1	0.825	0.272	1.655	0.518	0.019	1.874
MNR-07-13	M-NR	2	0.815	0.278	1.661	0.525	0.019	1.872
MNR-07-13	M-NR	3	0.815	0.283	1.66	0.524	0.019	1.855
MNR-07-14	M-NR	1	0.326	0.19	0.329	0.2	0.009	1.349
MNR-07-14	M-NR	2	0.318	0.191	0.329	0.2	0.009	1.35
MNR-07-14	M-NR	3	0.307	0.187	0.316	0.194	0.009	1.313
MNR-07-15	M-NR	1	0.83	0.299	2.285	0.747	0.02	1.802
MNR-07-15	M-NR	2	0.846	0.311	2.363	0.774	0.02	1.859
MNR-07-15	M-NR	3	0.837	0.314	2.372	0.776	0.02	1.818
MNR-07-16	M-NR	1	0.629	0.266	1.453	0.688	0.019	1.852
MNR-07-16	M-NR	2	0.607	0.258	1.411	0.67	0.018	1.791
MNR-07-16	M-NR	3	0.585	0.249	1.369	0.654	0.018	1.726
MNR-07-17	M-NR	1	0.506	0.19	0.741	0.355	0.019	1.82
MNR-07-17	M-NR	2	0.514	0.244	0.839	0.363	0.019	1.848
MNR-07-17	M-NR	3	0.512	0.24	0.832	0.362	0.019	1.854
MNR-07-18	M-NR	1	0.222	0.162	0.406	0.255	0.014	2.11
MNR-07-18	M-NR	2	0.211	0.152	0.379	0.237	0.013	1.988
MNR-07-18	M-NR	3	0.208	0.149	0.377	0.236	0.013	2.052
MNR-07-19	M-NR	1	0.172	0.057	0.217	0.129	0.009	1.677
MNR-07-19	M-NR	2	0.184	0.058	0.223	0.131	0.009	1.745
MNR-07-19	M-NR	3	0.175	0.058	0.222	0.131	0.009	1.726
MNR-07-20	M-NR	1	0.443	0.083	0.4	0.18	0.016	1.437
MNR-07-20	M-NR	2	0.431	0.083	0.397	0.18	0.015	1.398
MNR-07-20	M-NR	3	0.464	0.087	0.415	0.188	0.016	1.497
MNR-07-21	M-NR	1	0.435	0.373	1.667	0.3	0.01	1.474
MNR-07-21	M-NR	2	0.422	0.393	1.614	0.297	0.01	1.392
MNR-07-21	M-NR	3	0.429	0.423	1.679	0.306	0.01	1.431
MNR-07-22	M-NR	1	0.23	0.118	0.208	0.13	0.006	0.53
MNR-07-22	M-NR	2	0.227	0.118	0.2	0.129	0.006	0.514
MNR-07-22	M-NR	3	0.247	0.113	0.221	0.138	0.006	0.56
MNR-07-24	M-NR	1	0.335	0.165	0.7	0.444	0.01	1.549
MNR-07-24	M-NR	2	0.343	0.185	0.717	0.456	0.01	1.581
MNR-07-24	M-NR	3	0.345	0.186	0.725	0.464	0.01	1.562
MNR-07-25	M-NR	1	0.407	0.345	0.922	1.072	0.012	1.078
MNR-07-25	M-NR	2	0.404	0.34	0.896	1.046	0.012	1.056
MNR-07-25	M-NR	3	0.397	0.343	0.897	1.043	0.012	1.048

Appendix

Lafras data

Plant name	Locality	Rep.	Harpagid(%)	Verbasc.(%)	Isoverbasc.(%)	6-Acetylac.(%)	Coumaroy.(%)	Harpagos(%)
L-07-02	LAF	1	0.39	1.357	0.447	0.768	0.017	2.049
L-07-02	LAF	2	0.39	1.388	0.454	0.779	0.017	2.003
L-07-02	LAF	3	0.382	1.382	0.462	0.786	0.018	1.956
L-07-03	LAF	1	0.473	0.676	0.845	0.452	0.015	1.3
L-07-03	LAF	2	0.482	0.694	0.854	0.462	0.015	1.313
L-07-03	LAF	3	0.472	0.675	0.841	0.447	0.014	1.318
L-07-04	LAF	1	0.539	3.518	3.067	1.324	0.019	1.801
L-07-04	LAF	2	0.534	3.54	3.112	1.349	0.019	1.796
L-07-04	LAF	3	0.523	3.522	3.102	1.328	0.019	1.731
L-07-05	LAF	1	0.714	1.836	0.984	0.296	0.016	1.092
L-07-05	LAF	2	0.709	1.839	0.98	0.297	0.016	1.069
L-07-05	LAF	3	0.724	1.885	0.995	0.303	0.016	1.086
L-07-06	LAF	1	0.668	0.89	1.537	0.109	0.013	1.478
L-07-06	LAF	2	0.647	0.874	1.489	0.111	0.013	1.44
L-07-06	LAF	3	0.677	0.91	1.594	0.101	0.014	1.515
L-07-07	LAF	1	0.417	1.056	1.175	0.926	0.015	1.623
L-07-07	LAF	2	0.427	1.02	1.125	0.999	0.015	1.651
L-07-07	LAF	3	0.425	1.01	1.135	0.955	0.015	1.654
L-07-08	LAF	1	0.522	3.453	0.958	0.981	0.022	1.104
L-07-08	LAF	2	0.51	3.401	0.941	0.955	0.021	1.083
L-07-08	LAF	3	0.527	3.502	0.983	0.993	0.022	1.122
L-07-10	LAF	1	0.502	0.763	1.647	1.071	0.013	1.359
L-07-10	LAF	2	0.515	0.779	1.691	1.101	0.013	1.386
L-07-10	LAF	3	0.528	0.814	1.769	1.144	0.014	1.421

Terra Firma data

Plant name	Locality	Rep.	Harpagid(%)	Verbasc.(%)	Isoverbasc.(%)	6-Acetylac.(%)	Coumaroy.(%)	Harpagos(%)
TF-07-01&02	Terra F	1	0.233	0.468	0.654	0.283	0.01	0.549
TF-07-01&02	Terra F	2	0.219	0.475	0.681	0.283	0.01	0.536
TF-07-01&02	Terra F	3	0.226	0.485	0.678	0.29	0.01	0.544
TF-07-03&04	Terra F	1	0.411	0.537	1.203	0.789	0.01	0.785
TF-07-03&04	Terra F	2	0.391	0.532	1.166	0.766	0.009	0.748
TF-07-03&04	Terra F	3	0.417	0.544	1.226	0.797	0.01	0.784
TF-07-05&06	Terra F	1	0.408	0.669	1.174	0.998	0.011	0.71
TF-07-05&06	Terra F	2	0.348	0.591	1.077	1.018	0.01	0.62
TF-07-05&06	Terra F	3	0.373	0.612	1.103	1.05	0.01	0.655
TF-07-07&08	Terra F	1	0.008	0.441	0.743	0.71	0.008	0.674
TF-07-07&08	Terra F	2	0.277	0.338	0.727	0.674	0.008	0.54
TF-07-07&08	Terra F	3	0.299	0.366	0.806	0.745	0.008	0.594
TF-07-09&10	Terra F	1	0.403	0.818	1.205	1.298	0.014	1.037
TF-07-09&10	Terra F	2	0.401	0.82	1.212	1.294	0.014	1.036
TF-07-09&10	Terra F	3	0.396	0.828	1.192	1.265	0.014	0.942
TF-07-11&12	Terra F	1	0.416	0.812	2.051	1.415	0.011	0.773
TF-07-11&12	Terra F	2	0.387	0.735	1.895	1.308	0.01	0.715

TF-07-11&12	Terra F	3	0.375	0.739	1.866	1.29	0.01	0.712
TF-07-13&14&15	Terra F	1	0.331	0.547	1.527	0.932	0.012	0.749
TF-07-13&14&15	Terra F	2	0.342	0.567	1.565	1.018	0.013	0.766
TF-07-13&14&15	Terra F	3	0.348	0.574	1.571	1.021	0.013	0.771
TF-07-22	Terra F	1	0.31	0.392	1.645	0.961	0.008	0.725
TF-07-22	Terra F	2	0.326	0.408	1.714	1.004	0.008	0.756
TF-07-22	Terra F	3	0.3	0.38	1.589	0.932	0.008	0.702
TF-07-29	Terra F	1	0.315	0.396	1.652	0.959	0.008	0.723
TF-07-29	Terra F	2	0.314	0.394	1.653	0.964	0.008	0.728
TF-07-29	Terra F	3	0.311	0.395	1.651	0.969	0.008	0.729
TF-07-36	Terra F	1	0.498	0.327	1.266	0.934	0.01	1.087
TF-07-36	Terra F	2	0.49	0.319	1.251	0.924	0.01	1.067
TF-07-36	Terra F	3	0.5	0.326	1.278	0.939	0.01	1.096
TF-07-38	Terra F	1	0.295	0.247	0.684	0.551	0.009	1.831
TF-07-38	Terra F	2	0.285	0.24	0.661	0.534	0.009	1.787
TF-07-38	Terra F	3	0.29	0.245	0.681	0.55	0.009	1.827
TF-07-50	Terra F	1	0.367	0.903	0.592	0.652	0.008	0.562
TF-07-50	Terra F	2	0.368	0.901	0.593	0.654	0.008	0.566
TF-07-50	Terra F	3	0.374	0.923	0.595	0.666	0.008	0.572
TF-07-101	Terra F	1	0.518	0.663	0.549	0.585	0.008	0.429
TF-07-101	Terra F	2	0.519	0.661	0.562	0.595	0.008	0.431
TF-07-101	Terra F	3	0.487	0.556	0.523	0.565	0.007	0.426
TF-07-102	Terra F	1	0.256	0.646	1.136	0.922	0.007	0.469
TF-07-102	Terra F	2	0.238	0.639	1.062	0.909	0.007	0.424
TF-07-102	Terra F	3	0.23	0.622	1.008	0.88	0.006	0.394
TF-07-103	Terra F	1	0.263	0.81	0.686	0.74	0.006	0.415
TF-07-103	Terra F	2	0.241	0.752	0.658	0.698	0.006	0.39
TF-07-103	Terra F	3	0.248	0.788	0.694	0.727	0.006	0.399
TF-07-104	Terra F	1	0.295	0.494	0.213	0.419	0.005	0.392
TF-07-104	Terra F	2	0.304	0.51	0.214	0.427	0.005	0.406
TF-07-104	Terra F	3	0.306	0.512	0.214	0.433	0.005	0.412
TF-07-105	Terra F	1	0.218	0.79	1.065	0.335	0.007	0.713
TF-07-105	Terra F	2	0.221	0.818	1.127	0.347	0.007	0.746
TF-07-105	Terra F	3	0.2	0.718	0.967	0.329	0.006	0.711
TF-07-106	Terra F	1	0.19	0.242	0.343	0.469	0.008	0.728
TF-07-106	Terra F	2	0.205	0.26	0.349	0.47	0.008	0.751
TF-07-106	Terra F	3	0.197	0.25	0.346	0.469	0.008	0.738
TF-07-107	Terra F	1	0.358	0.419	0.447	0.451	0.005	0.395
TF-07-107	Terra F	2	0.355	0.419	0.436	0.454	0.005	0.425
TF-07-107	Terra F	3	0.351	0.411	0.442	0.452	0.005	0.025
TF-07-108	Terra F	1	0.285	0.585	0.323	0.177	0.005	0.295
TF-07-108	Terra F	2	0.289	0.603	0.323	0.176	0.005	0.302
TF-07-108	Terra F	3	0.293	0.611	0.328	0.176	0.005	0.304
TF-07-109	Terra F	1	0.416	0.765	1.694	1.836	0.013	0.841
TF-07-109	Terra F	2	0.364	0.65	1.566	1.728	0.012	0.726
TF-07-109	Terra F	3	0.357	0.632	1.563	1.73	0.012	0.704
TF-07-110	Terra F	1	0.3	0.786	1.017	0.313	0.006	0.413
TF-07-110	Terra F	2	0.314	0.824	1.085	0.324	0.006	0.427

TF-07-110	Terra F	3	0.308	0.783	1.079	0.323	0.006	0.412
TF-07-113	Terra F	1	0.448	0.465	0.486	0.415	0.006	0.512
TF-07-113	Terra F	2	0.429	0.448	0.462	0.395	0.005	0.494
TF-07-113	Terra F	3	0.402	0.387	0.416	0.349	0.005	0.447
TF-07-114	Terra F	1	0.505	0.525	0.553	0.474	0.008	0.845
TF-07-114	Terra F	2	0.442	0.478	0.528	0.473	0.008	0.803
TF-07-114	Terra F	3	0.533	0.537	0.584	0.542	0.009	0.806
TF-07-115	Terra F	1	0.393	0.683	1.36	1.252	0.008	0.73
TF-07-115	Terra F	2	0.396	0.69	1.368	1.267	0.008	0.749
TF-07-115	Terra F	3	0.396	0.69	1.333	1.233	0.008	0.734
TF-07-116	Terra F	1	0.204	0.393	0.493	0.565	0.007	0.768
TF-07-116	Terra F	2	0.212	0.406	0.504	0.591	0.007	0.789
TF-07-116	Terra F	3	0.219	0.425	0.518	0.591	0.007	0.806
TF-07-117	Terra F	1	0.118	0.377	0.794	1.192	0.008	0.672
TF-07-117	Terra F	2	0.126	0.409	0.848	1.277	0.009	0.707
TF-07-117	Terra F	3	0.127	0.411	0.847	1.285	0.009	0.722
TF-07-118	Terra F	1	0.416	0.938	1.045	0.613	0.013	0.63
TF-07-118	Terra F	2	0.419	0.934	1.034	0.591	0.013	0.622
TF-07-118	Terra F	3	0.448	1.004	1.114	0.634	0.013	0.67
TF-07-119	Terra F	1	0.432	0.976	0.833	0.602	0.01	0.63
TF-07-119	Terra F	2	0.441	0.979	0.841	0.643	0.01	0.618
TF-07-119	Terra F	3	0.435	0.963	0.832	0.613	0.01	0.637
TF-07-120	Terra F	1	0.34	0.486	2.281	1.398	0.014	1.04
TF-07-120	Terra F	2	0.356	0.507	2.441	1.513	0.015	1.096
TF-07-120	Terra F	3	0.35	0.502	2.39	1.488	0.014	1.074

Caprivi data

Plant name	Locality	Rep.	Harpagid(%)	Verbasc.(%)	Isoverbasc.(%)	6-Acetylac.(%)	Coumaroy.(%)	Harpagos(%)
CAP1	CAP	1	0.605	0.16	0.54	0.174	0.46	0.598
CAP1	CAP	2	0.606	0.163	0.556	0.17	0.456	0.6
CAP2	CAP	1	0.604	0.203	0.77	0.206	0.525	0.659
CAP2	CAP	2	0.601	0.199	0.771	0.208	0.541	0.678
CAP3	CAP	1	0.807	0.242	1.04	0.301	0.658	0.744
CAP3	CAP	2	0.876	0.261	1.144	0.337	0.729	0.808
CAP4	CAP	1	0.487	0.168	0.689	0.178	0.415	0.67
CAP4	CAP	2	0.494	0.181	0.71	0.192	0.435	0.681
CAP5	CAP	1	0.308	0.865	2.012	0.304	0.175	0.851
CAP5	CAP	2	0.308	0.877	1.985	0.288	0.18	0.868
CAP6	CAP	1	0.266	0.36	1.393	0.169	0.192	0.934
CAP6	CAP	2	0.255	0.335	1.314	0.155	0.173	0.886

Ferrolands data

Plant name	Locality	Rep.	Harpagid(%)	Verbasc.(%)	Isoverbasc.(%)	6-Acetylac.(%)	Coumaroy.(%)	Harpagos(%)
F1	FERRO	1	0.835	0.054	0.158	0.048	0.012	0.051
F1	FERRO	2	0.883	0.059	0.169	0.048	0.012	0.055
F2	FERRO	1	0.536	0.043	0.084	0.053	0.013	0.000
F2	FERRO	2	0.565	0.044	0.085	0.054	0.013	0.000
F3	FERRO	1	0.567	0.037	0.078	0.045	0.015	0.000

F3	FERRO	2	0.649	0.044	0.086	0.046	0.015	0.000
F4	FERRO	1	2.372	0.018	0.055	0.041	0.012	0.000
F4	FERRO	2	2.517	0.018	0.055	0.041	0.012	0.000
F5	FERRO	1	0.276	0.041	0.071	0.049	0.012	0.010
F5	FERRO	2	0.287	0.042	0.072	0.05	0.012	0.012
F6	FERRO	1	0.803	0.047	0.072	0.047	0.016	0.000
F6	FERRO	2	0.857	0.051	0.074	0.048	0.016	0.000
F7	FERRO	1	1.945	0.118	0.221	0.155	0.013	0.005
F7	FERRO	2	1.969	0.121	0.222	0.164	0.013	0.005
F8	FERRO	1	2.884	0.018	0.055	0.041	0.012	0.000
F8	FERRO	2	2.958	0.018	0.055	0.041	0.012	0.000
F9	FERRO	1	0.818	0.131	0.122	0.047	0.012	0.007
F9	FERRO	2	0.848	0.139	0.127	0.05	0.012	0.011
F10	FERRO	1	0.889	0.06	0.134	0.045	0.012	0.000
F10	FERRO	2	0.852	0.058	0.134	0.045	0.012	0.000
F11	FERRO	1	0.646	0.029	0.059	0.041	0.014	0.000
F11	FERRO	2	0.665	0.03	0.059	0.041	0.014	0.000
F12	FERRO	1	1.152	0.046	0.083	0.041	0.012	0.024
F12	FERRO	2	1.227	0.051	0.086	0.041	0.012	0.026
F12-UV	FERRO	1	0.984	0.04	0.105	0.041	0.012	0.041
F12-UV	FERRO	2	1.022	0.042	0.108	0.041	0.012	0.043
F13	FERRO	1	0.974	0.094	0.283	0.041	0.012	0.030
F13	FERRO	2	1.034	0.101	0.267	0.041	0.012	0.041
F13-UV	FERRO	1	0.909	0.088	0.235	0.041	0.012	0.037
F13-UV	FERRO	2	0.951	0.093	0.28	0.041	0.012	0.036
F14	FERRO	1	0.778	0.036	0.155	0.044	0.012	0.000
F14	FERRO	2	0.776	0.038	0.153	0.044	0.012	0.000
F15	FERRO	1	0.566	0.116	0.086	0.06	0.012	0.042
F15	FERRO	2	0.524	0.116	0.083	0.057	0.012	0.036
F16	FERRO	1	0.478	0.063	0.181	0.061	0.012	0.018
F16	FERRO	2	0.434	0.06	0.168	0.06	0.012	0.016
F17	FERRO	1	0.382	0.059	0.07	0.058	0.012	0.003
F17	FERRO	2	0.364	0.061	0.07	0.058	0.012	0.003
F18	FERRO	1	0.659	0.103	0.169	0.048	0.012	0.088
F18	FERRO	2	0.634	0.102	0.167	0.048	0.012	0.085
F19	FERRO	1	0.623	0.025	0.06	0.058	0.012	0.000
F19	FERRO	2	0.619	0.026	0.06	0.058	0.012	0.000
F20	FERRO	1	0.467	0.028	0.073	0.075	0.012	0.000
F20	FERRO	2	0.465	0.027	0.073	0.074	0.012	0.000
F21	FERRO	1	0.514	0.046	0.074	0.062	0.012	0.000
F21	FERRO	2	0.527	0.05	0.076	0.063	0.012	0.000
F22	FERRO	1	1.131	0.083	0.157	0.059	0.012	0.000
F22	FERRO	2	1.092	0.081	0.155	0.058	0.012	0.000
F23	FERRO	1	0.446	0.048	0.131	0.057	0.012	0.005
F23	FERRO	2	0.425	0.048	0.12	0.057	0.012	0.006
F24	FERRO	1	0.635	0.032	0.109	0.059	0.013	0.055
F24	FERRO	2	0.587	0.032	0.106	0.058	0.013	0.050
F25	FERRO	1	0.686	0.028	0.081	0.058	0.012	0.000

F25	FERRO	2	0.689	0.027	0.076	0.056	0.012	0.000

Springbokfontein data

S1	SPRING	1	0.656	0.104	0.144	0.156	0.012	0.000
S1	SPRING	2	0.695	0.112	0.152	0.166	0.012	0.000
S1-UV	SPRING	1	0.668	0.053	0.149	0.164	0.012	0.000
S1-UV	SPRING	2	0.728	0.058	0.164	0.182	0.012	0.000
S2	SPRING	1	0.522	0.082	0.146	0.178	0.012	0.000
S2	SPRING	2	0.508	0.083	0.142	0.175	0.012	0.000
S3	SPRING	1	0.824	0.136	0.235	0.317	0.012	0.000
S3	SPRING	2	0.93	0.159	0.274	0.364	0.012	0.000
S4	SPRING	1	0.439	0.198	0.193	0.415	0.014	0.000
S4	SPRING	2	0.533	0.256	0.236	0.522	0.014	0.000
S5	SPRING	1	0.514	0.674	0.201	0.328	0.012	0.000
S5	SPRING	2	0.596	0.816	0.239	0.407	0.012	0.000
S6	SPRING	1	0.877	0.542	0.435	1.707	0.015	0.000
S6	SPRING	2	1.031	0.665	0.525	2.079	0.016	0.000
S7	SPRING	1	0.604	0.077	0.168	0.137	0.012	0.000
S7	SPRING	2	0.615	0.08	0.176	0.139	0.012	0.000
S7-UV	SPRING	1	0.579	0.096	0.191	0.152	0.012	0.000
S7-UV	SPRING	2	0.57	0.095	0.188	0.15	0.012	0.000
S8	SPRING	1	0.559	0.049	0.17	0.17	0.012	0.000
S8	SPRING	2	0.647	0.054	0.191	0.193	0.012	0.000
S10	SPRING	1	0.578	0.389	0.298	0.48	0.012	0.000
S10	SPRING	2	0.653	0.447	0.337	0.551	0.012	0.000
S11	SPRING	1	0.538	0.055	0.122	0.219	0.012	0.000
S11	SPRING	2	0.531	0.056	0.121	0.214	0.012	0.000
S12	SPRING	1	0.613	0.048	0.14	0.159	0.012	0.000
S12	SPRING	2	0.597	0.048	0.138	0.157	0.012	0.000
S13	SPRING	1	0.319	0.031	0.082	0.074	0.012	0.000
S13	SPRING	2	0.31	0.031	0.082	0.074	0.012	0.000
S14	SPRING	1	0.403	0.054	0.099	0.116	0.012	0.000
S14	SPRING	2	0.375	0.051	0.093	0.106	0.012	0.000
S15	SPRING	1	0.262	0.092	0.08	0.126	0.012	0.000
S15	SPRING	2	0.244	0.086	0.078	0.119	0.012	0.000
S16	SPRING	1	0.467	0.076	0.099	0.12	0.012	0.000
S16	SPRING	2	0.462	0.08	0.1	0.121	0.012	0.000
S17	SPRING	1	0.521	0.323	0.223	0.198	0.012	0.000
S17	SPRING	2	0.516	0.316	0.21	0.192	0.012	0.000
S18	SPRING	1	1.488	0.075	0.154	0.446	0.012	0.000
S18	SPRING	2	1.644	0.08	0.168	0.509	0.012	0.000
S19	SPRING	1	0.353	0.075	0.1	0.113	0.012	0.000
S19	SPRING	2	0.319	0.07	0.096	0.104	0.012	0.000
S20	SPRING	1	0.345	0.033	0.065	0.067	0.012	0.000
S20	SPRING	2	0.345	0.034	0.066	0.068	0.012	0.000
S22	SPRING	1	0.217	0.046	0.071	0.065	0.012	0.000
S22	SPRING	2	0.212	0.044	0.07	0.064	0.012	0.000
S23	SPRING	1	0.113	0.04	0.061	0.055	0.012	0.000

S23	SPRING	2	0.115	0.041	0.062	0.056	0.012	0.000
S24	SPRING	1	0.438	0.039	0.235	0.156	0.012	0.000
S24	SPRING	2	0.465	0.04	0.243	0.162	0.012	0.000
S25	SPRING	1	0.529	0.057	0.135	0.113	0.012	0.000
S25	SPRING	2	0.535	0.06	0.138	0.112	0.012	0.000

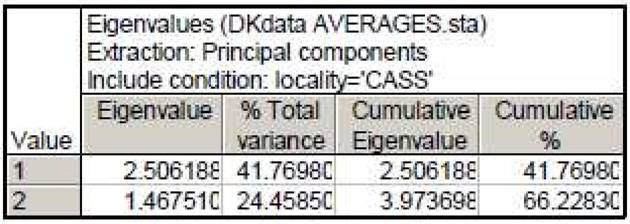
Makgabeng data

M1	MAKGA	1	0.901	0.085	0.111	0.117	0.012	0.000
M1	MAKGA	2	0.9	0.086	0.112	0.118	0.012	0.000
M1-UV	MAKGA	1	0.979	0.04	0.096	0.105	0.012	0.000
M1-UV	MAKGA	2	0.996	0.042	0.098	0.107	0.012	0.000
M2	MAKGA	1	0.64	0.049	0.161	0.159	0.012	0.000
M2	MAKGA	2	0.648	0.049	0.162	0.161	0.012	0.000
M2-UV	MAKGA	1	0.74	0.042	0.172	0.15	0.012	0.000
M2-UV	MAKGA	2	0.747	0.042	0.171	0.149	0.012	0.000
M3	MAKGA	1	0.681	0.042	0.127	0.105	0.012	0.000
M3	MAKGA	2	0.662	0.045	0.132	0.114	0.012	0.000
M3-UV	MAKGA	1	0.662	0.029	0.107	0.094	0.012	0.000
M3-UV	MAKGA	2	0.635	0.029	0.104	0.092	0.012	0.000
M4	MAKGA	1	0.451	0.054	0.086	0.07	0.012	0.000
M4	MAKGA	2	0.419	0.052	0.081	0.066	0.012	0.000
M5	MAKGA	1	0.441	0.06	0.084	0.085	0.012	0.000
M5	MAKGA	2	0.426	0.058	0.082	0.082	0.012	0.000
M5-UV	MAKGA	1	0.74	0.046	0.115	0.119	0.012	0.000
M5-UV	MAKGA	2	0.769	0.047	0.118	0.121	0.012	0.000
M6	MAKGA	1	0.559	0.104	0.147	0.119	0.012	0.000
M6	MAKGA	2	0.537	0.101	0.145	0.116	0.012	0.000
M6-UV	MAKGA	1	0.861	0.123	0.154	0.112	0.012	0.000
M6-UV	MAKGA	2	0.912	0.126	0.161	0.116	0.012	0.000
M7	MAKGA	1	0.576	0.097	0.08	0.068	0.012	0.000
M7	MAKGA	2	0.601	0.103	0.082	0.07	0.012	0.000
M8	MAKGA	1	0.702	0.063	0.095	0.09	0.012	0.000
M8	MAKGA	2	0.71	0.062	0.096	0.091	0.012	0.000
M8-UV	MAKGA	1	0.624	0.038	0.091	0.077	0.012	0.000
M8-UV	MAKGA	2	0.609	0.037	0.089	0.074	0.012	0.000
M9	MAKGA	1	0.418	0.047	0.127	0.123	0.012	0.000
M9	MAKGA	2	0.429	0.048	0.133	0.129	0.012	0.000
M10	MAKGA	1	0.708	0.037	0.087	0.091	0.012	0.000
M10	MAKGA	2	0.726	0.038	0.089	0.093	0.012	0.000
M10-UV	MAKGA	1	0.561	0.029	0.083	0.088	0.012	0.000
M10-UV	MAKGA	2	0.585	0.03	0.085	0.091	0.012	0.000
M11	MAKGA	1	0.393	0.074	0.116	0.106	0.012	0.000
M11	MAKGA	2	0.375	0.066	0.108	0.097	0.012	0.000
M12	MAKGA	1	0.446	0.057	0.107	0.116	0.012	0.000
M12	MAKGA	2	0.424	0.053	0.105	0.112	0.012	0.000
M12-UV	MAKGA	1	0.364	0.049	0.091	0.09	0.012	0.000
M12-UV	MAKGA	2	0.371	0.049	0.091	0.089	0.012	0.000

M13	MAKGA	1	1.65	0.053	0.166	0.147	0.013	0.000
M13	MAKGA	2	1.629	0.054	0.161	0.146	0.013	0.000
M13-UV	MAKGA	1	1.227	0.045	0.174	0.136	0.012	0.000
M13-UV	MAKGA	2	1.427	0.052	0.198	0.157	0.012	0.000
M14	MAKGA	1	0.673	0.08	0.104	0.112	0.013	0.000
M14	MAKGA	2	0.737	0.088	0.11	0.12	0.014	0.000
M15	MAKGA	1	0.813	0.146	0.19	0.149	0.012	0.000
M15	MAKGA	2	0.959	0.17	0.226	0.175	0.012	0.000
M16	MAKGA	1	0.41	0.053	0.156	0.208	0.012	0.000
M16	MAKGA	2	0.394	0.052	0.151	0.194	0.012	0.000
M17	MAKGA	1	1.337	0.078	0.203	0.222	0.012	0.000
M17	MAKGA	2	1.296	0.078	0.198	0.214	0.012	0.000
M17-UV	MAKGA	1	1.219	0.042	0.173	0.169	0.012	0.000
M17-UV	MAKGA	2	1.111	0.039	0.158	0.15	0.012	0.000
M18	MAKGA	1	0.708	0.046	0.161	0.156	0.012	0.000
M18	MAKGA	2	0.748	0.047	0.172	0.166	0.012	0.000
M19	MAKGA	1	0.441	0.046	0.113	0.103	0.012	0.000
M19	MAKGA	2	0.459	0.05	0.117	0.105	0.012	0.000
M19-UV	MAKGA	1	0.965	0.034	0.103	0.106	0.012	0.000
M19-UV	MAKGA	2	0.956	0.036	0.107	0.11	0.012	0.000
M20	MAKGA	1	0.776	0.049	0.192	0.145	0.012	0.000
M20	MAKGA	2	0.777	0.051	0.21	0.145	0.012	0.000
M20-UV	MAKGA	1	0.58	0.04	0.131	0.098	0.012	0.000
M20-UV	MAKGA	2	0.58	0.04	0.132	0.099	0.012	0.000
M21	MAKGA	1	0.612	0.048	0.089	0.089	0.012	0.000
M21	MAKGA	2	0.618	0.05	0.088	0.091	0.012	0.000
M22	MAKGA	1	0.795	0.042	0.149	0.148	0.012	0.000
M22	MAKGA	2	0.809	0.043	0.156	0.152	0.012	0.000
M22-UV	MAKGA	1	0.664	0.037	0.169	0.155	0.012	0.000
M22-UV	MAKGA	2	0.662	0.038	0.177	0.157	0.012	0.000
M23	MAKGA	1	0.672	0.068	0.345	0.304	0.012	0.000
M23	MAKGA	2	0.698	0.073	0.373	0.324	0.012	0.000
M23-UV	MAKGA	1	0.815	0.068	0.444	0.374	0.012	0.000
M23-UV	MAKGA	2	0.857	0.073	0.462	0.389	0.012	0.000
M24	MAKGA	1	0.194	0.026	0.067	0.059	0.012	0.000
M24	MAKGA	2	0.196	0.026	0.067	0.059	0.012	0.000
M24-UV	MAKGA	1	0.42	0.034	0.073	0.063	0.012	0.000
M24-UV	MAKGA	2	0.413	0.033	0.073	0.063	0.012	0.000
M25	MAKGA	1	0.708	0.052	0.205	0.203	0.014	0.000
M25	MAKGA	2	0.727	0.051	0.204	0.203	0.014	0.000
M25-UV	MAKGA	1	0.758	0.042	0.177	0.17	0.015	0.000
M25-UV	MAKGA	2	0.78	0.044	0.183	0.178	0.015	0.000

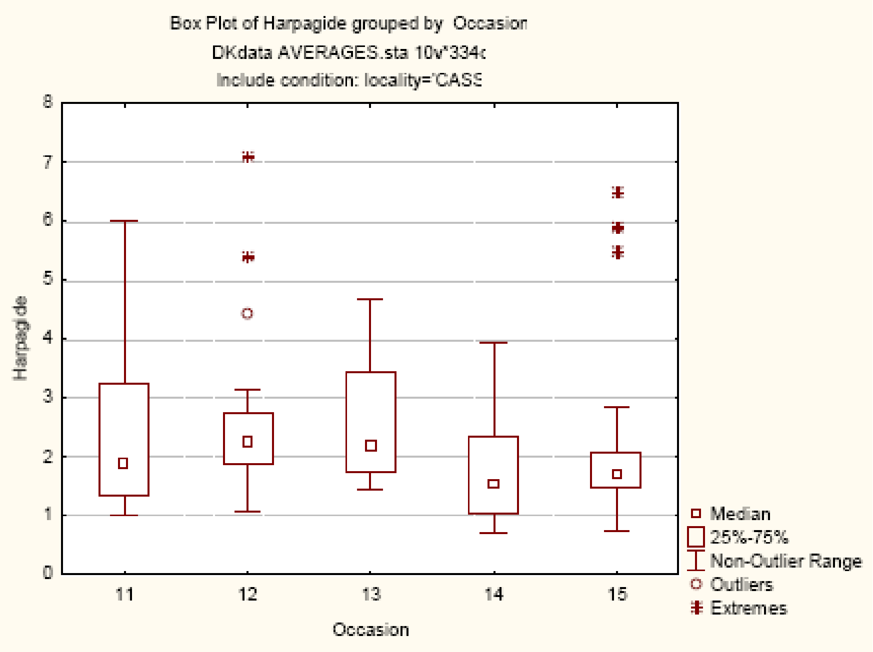
Appendix B: Additional Statistical analysis for Chapter 4

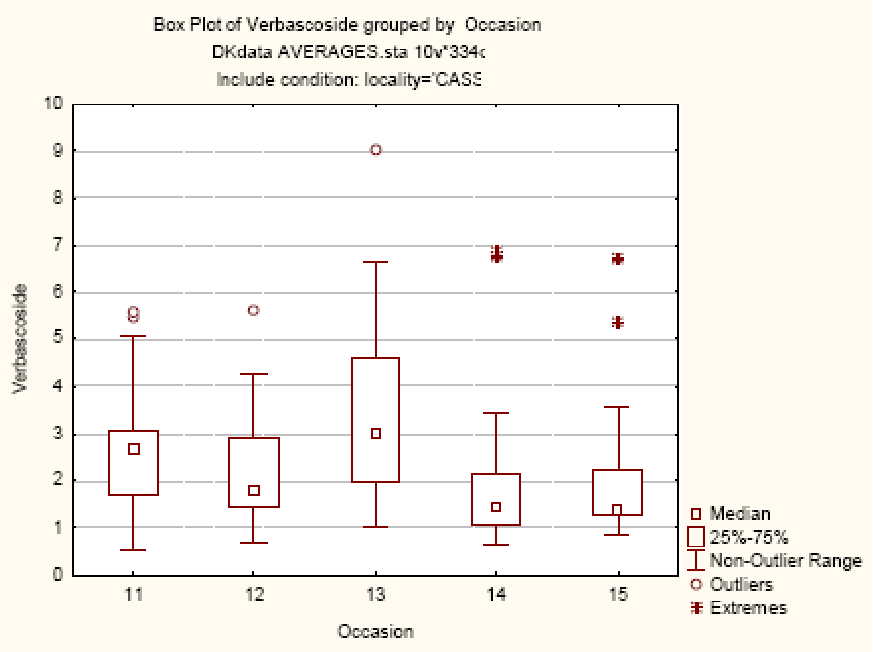
Two factors:

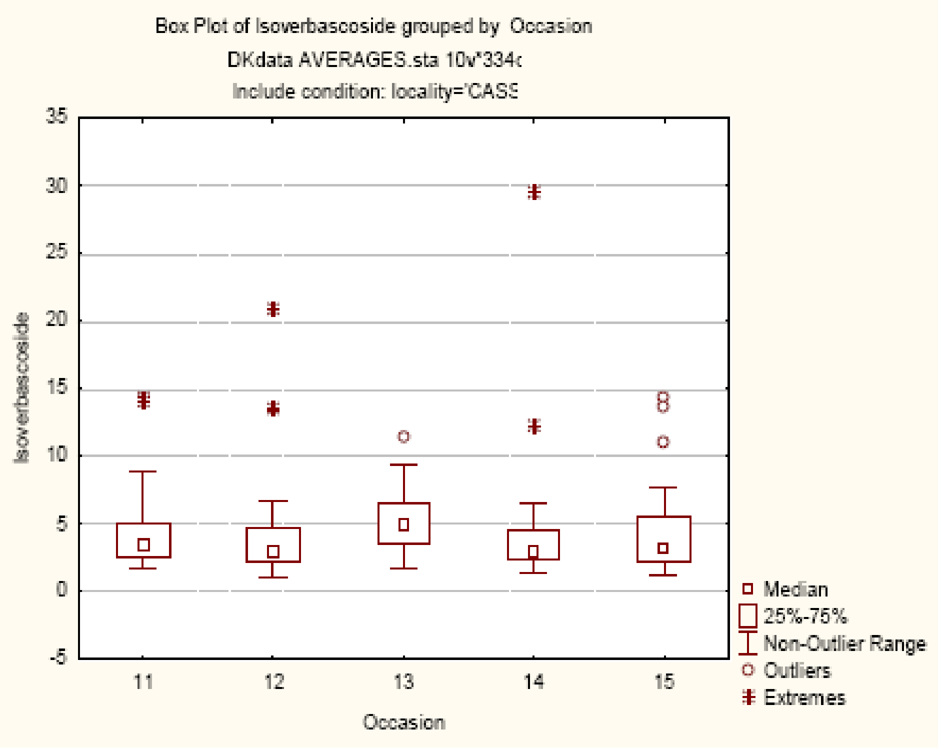


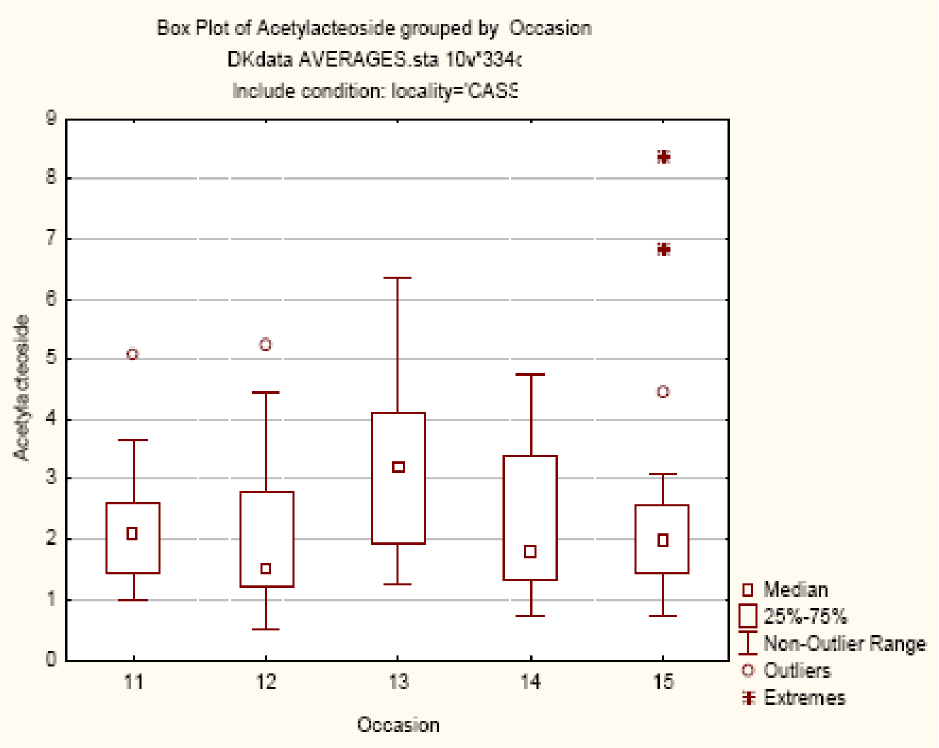
Eigenvalues (DKdata AVERAGES.sta)
Extraction: Principal components
Include condition: locality='CASS'

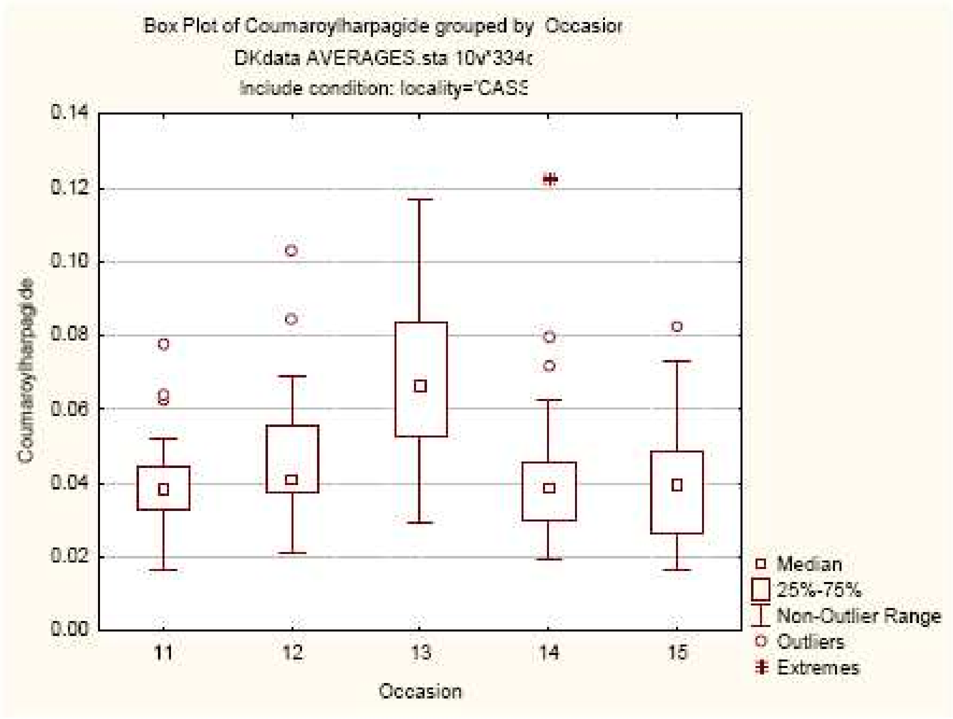
Value	Eigenvalue	% Total variance	Cumulative Eigenvalue	Cumulative %
1	2.506188	41.76980	2.506188	41.76980
2	1.467510	24.45850	3.973698	66.22830

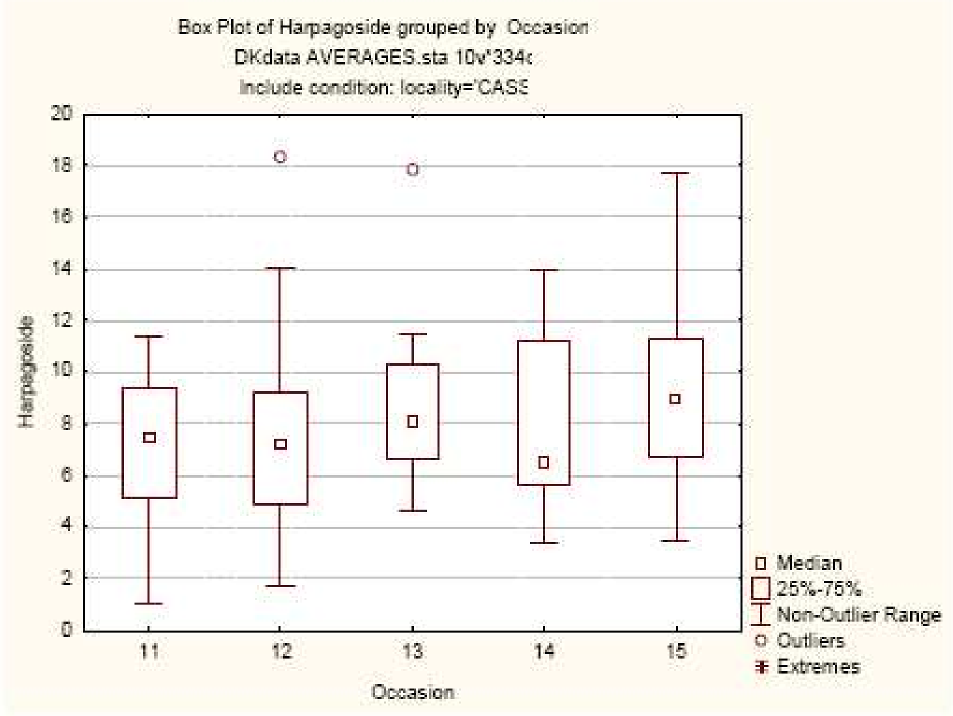

Box Plot of Harpagide grouped by Occasion
DKdata AVERAGES.sta 10v*334c
Include condition: locality='CASS
Harpagide
0
1
2
3
4
5
6
7
8
11
12
13
14
15
Occasion
Median
25%-75%
Non-Outlier Range
Outliers
Extremes


Box Plot of Verbascoside grouped by Occasion
DKdata AVERAGES.sta 10v*334c
Include condition: locality='CASS
Verbascoside
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
Occasion
Median
25%-75%
Non-Outlier Range
Outliers
Extremes


Box Plot of Isoverbascoside grouped by Occasion
DKdata AVERAGES.sta 10v*334c
Include condition: locality='CASS
Isoverbascoside
35
30
25
20
15
10
5
0
-5
11
12
13
14
15
Occasion
Median
25%-75%
Non-Outlier Range
Outliers
Extremes


Box Plot of Acetylacteoside grouped by Occasion
DKdata AVERAGES.sta 10v*334c
Include condition: locality='CASS
Acetylacteoside
9
8
7
6
5
4
3
2
1
0
11
12
13
14
15
Occasion
Median
25%-75%
Non-Outlier Range
Outliers
Extremes

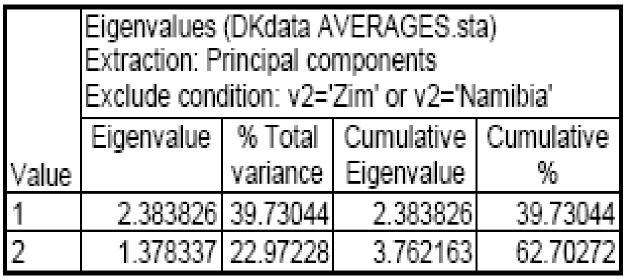

Box Plot of Coumaroylharpagide grouped by Occasior
DKdata AVERAGES.sta 10v*334c
Include condition: locality='CASS
Coumaroylharpagide
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
11
12
13
14
15
Occasion
Median
25%-75%
Non-Outlier Range
Outliers
Extremes


Box Plot of Harpagoside grouped by Occasion
DKdata AVERAGES.sta 10v*334c
Include condition: locality='CASS
Harpagoside
20
18
16
14
12
10
8
6
4
2
0
11
12
13
14
15
Occasion
Median
25%-75%
Non-Outlier Range
Outliers
Extremes

Appendix C: Additional Statistical analysis for Chapter 5.

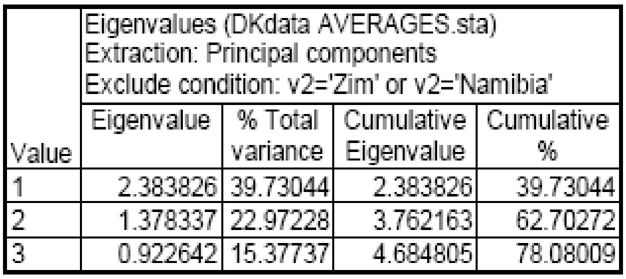
1. The eigen values from factor analysis.

Two factors:



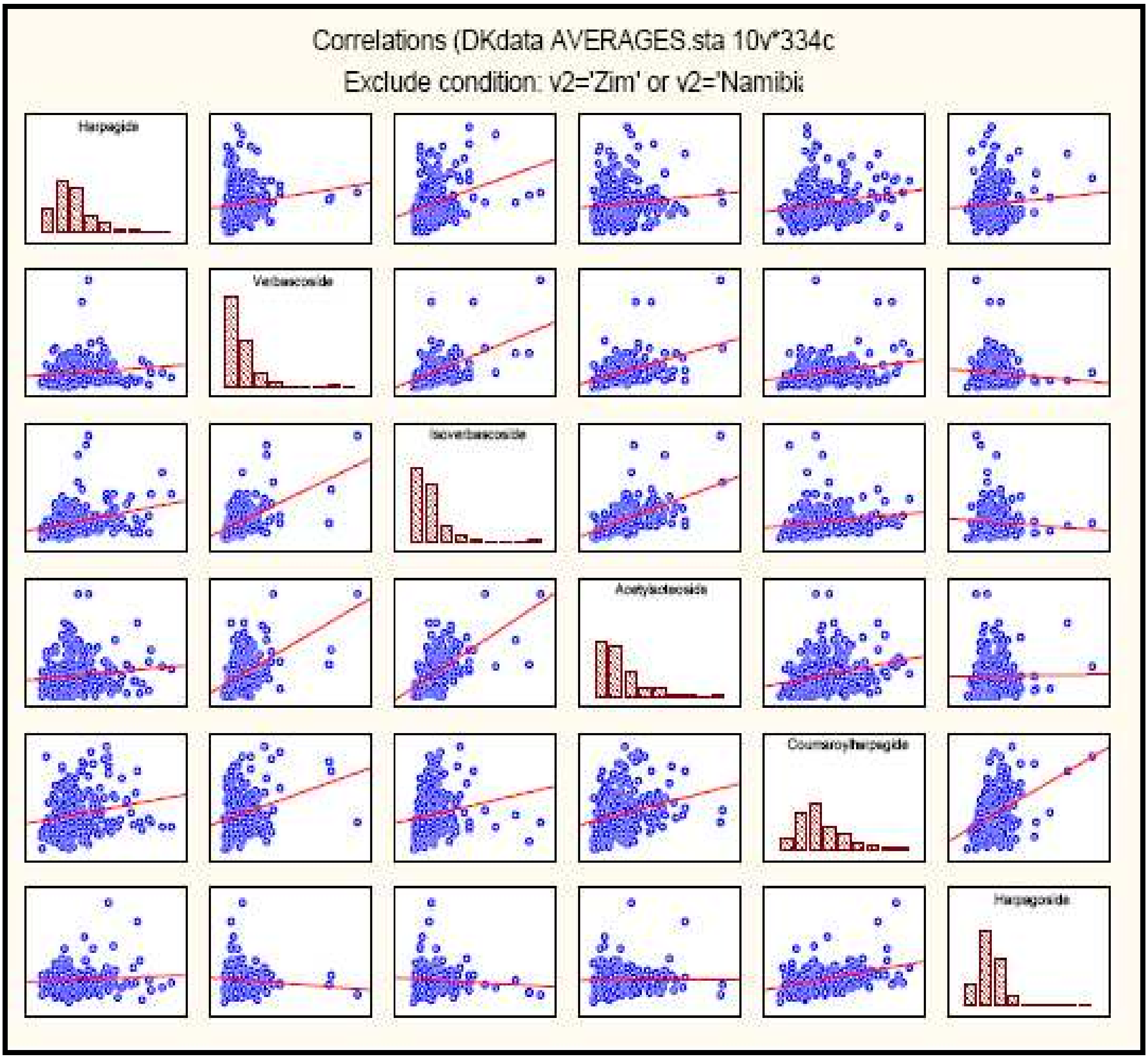
	Eigenvalues (DKdata AVERAGES.sta) Extraction: Principal components Exclude condition: v2='Zim' or v2='Namibia'			
Value	Eigenvalue	% Total variance	Cumulative Eigenvalue	Cumulative %
1	2.383826	39.73044	2.383826	39.73044
2	1.378337	22.97228	3.762163	62.70272

Three factors

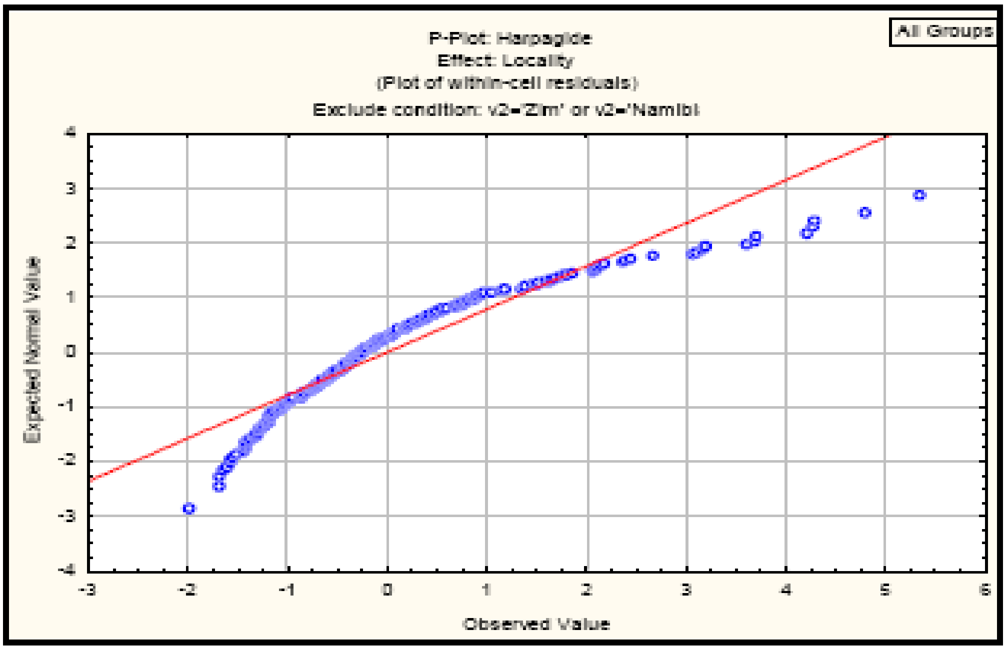


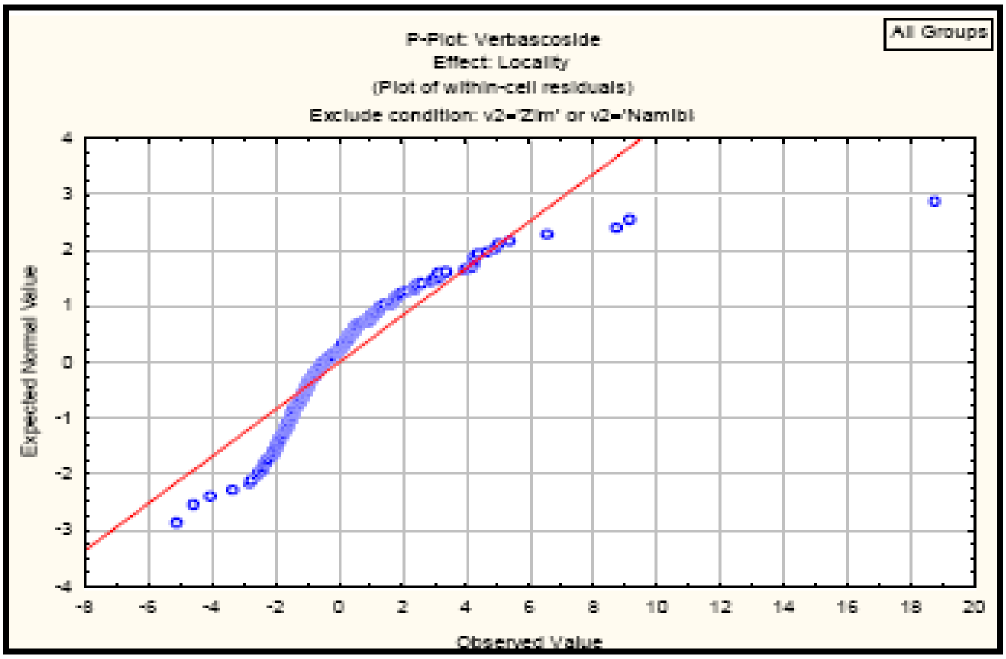
	Eigenvalues (DKdata AVERAGES.sta) Extraction: Principal components Exclude condition: v2='Zim' or v2='Namibia'			
Value	Eigenvalue	% Total variance	Cumulative Eigenvalue	Cumulative %
1	2.383826	39.73044	2.383826	39.73044
2	1.378337	22.97228	3.762163	62.70272
3	0.922642	15.37737	4.684805	78.08009

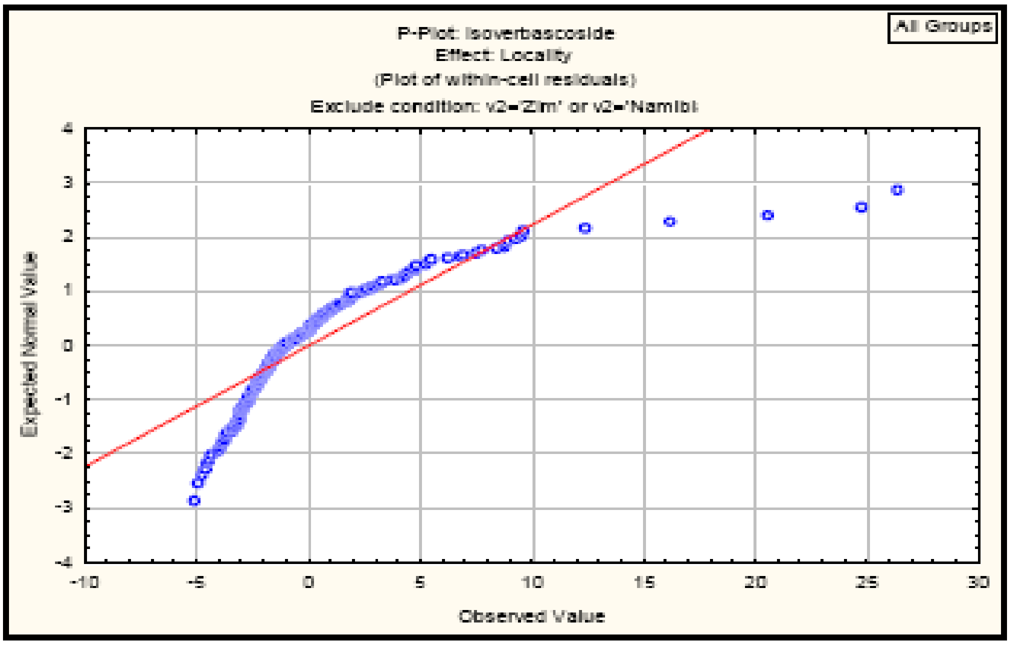
2. Matrix plots show distribution of six variables and the scatter among them

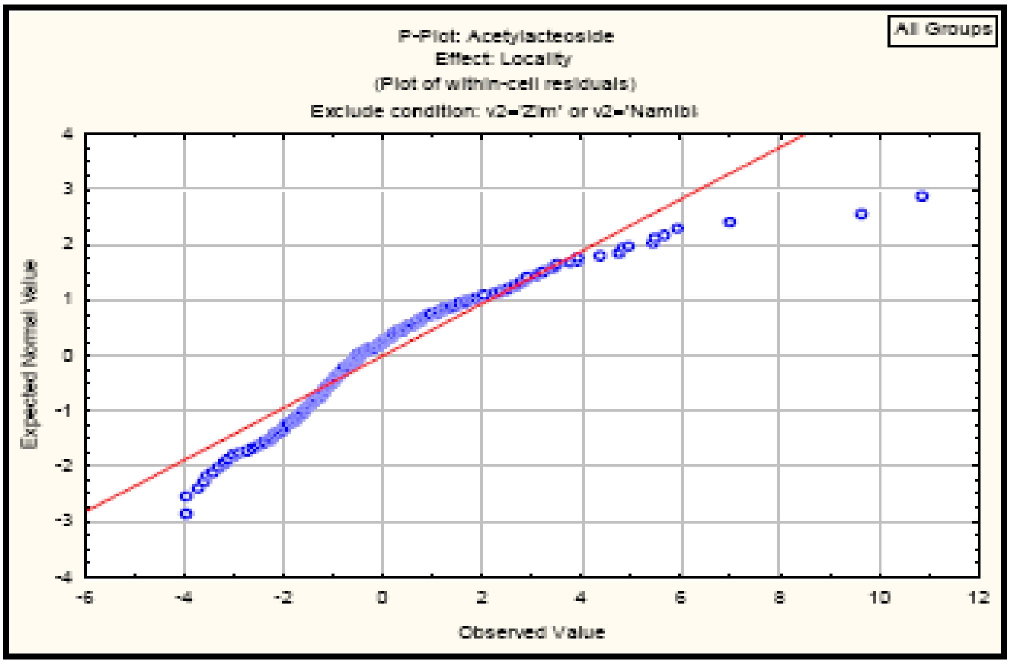


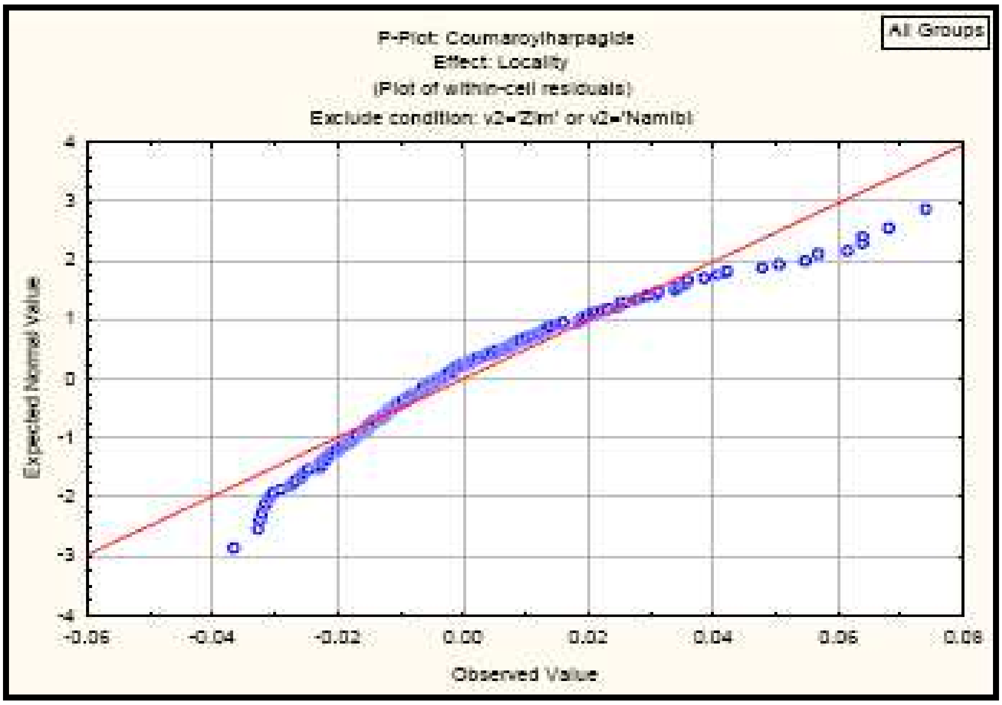
3. Normal probability plots: data not normal

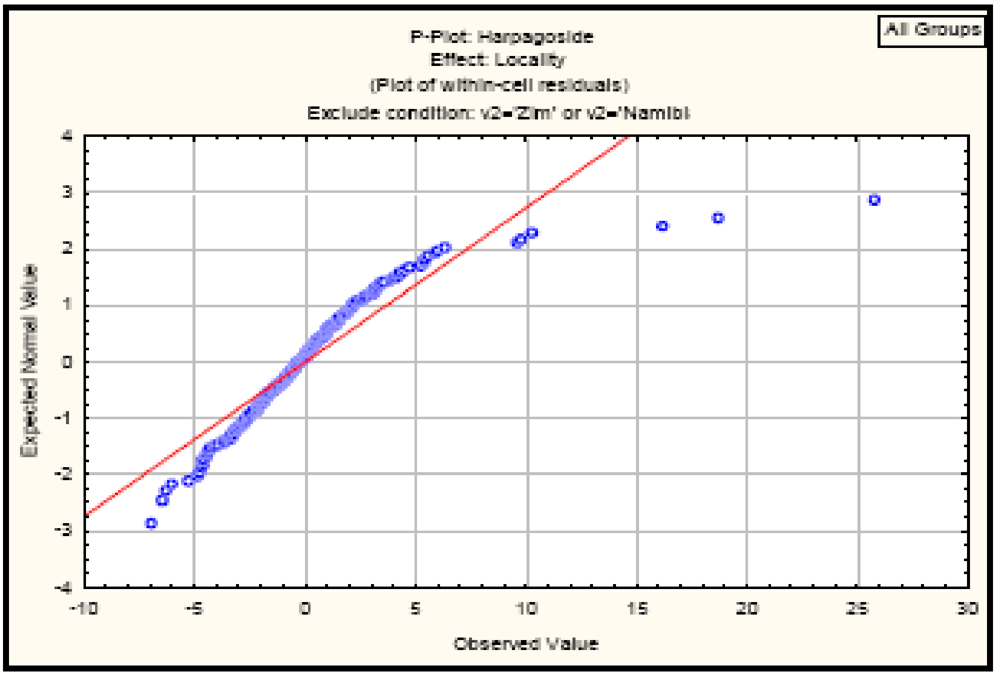

P-Plot: Harpagide
Effect: Locality
(Plot of within-cell residuals)
Exclude condition: v2='Zim' or v2='Namibi
All Groups
Expected Normal Value
Observed Value
4
3
2
1
0
-1
-2
-3
-4
-3
-2
-1
0
1
2
3
4
5
6


P-Plot: Verbascoside
Effect: Locality
(Plot of within-cell residuals)
Exclude condition: v2='Zim' or v2='Namibi
All Groups
Expected Normal Value
Observed Value
4
3
2
1
0
-1
-2
-3
-4
-8
-6
-4
-2
0
2
4
6
8
10
12
14
16
18
20

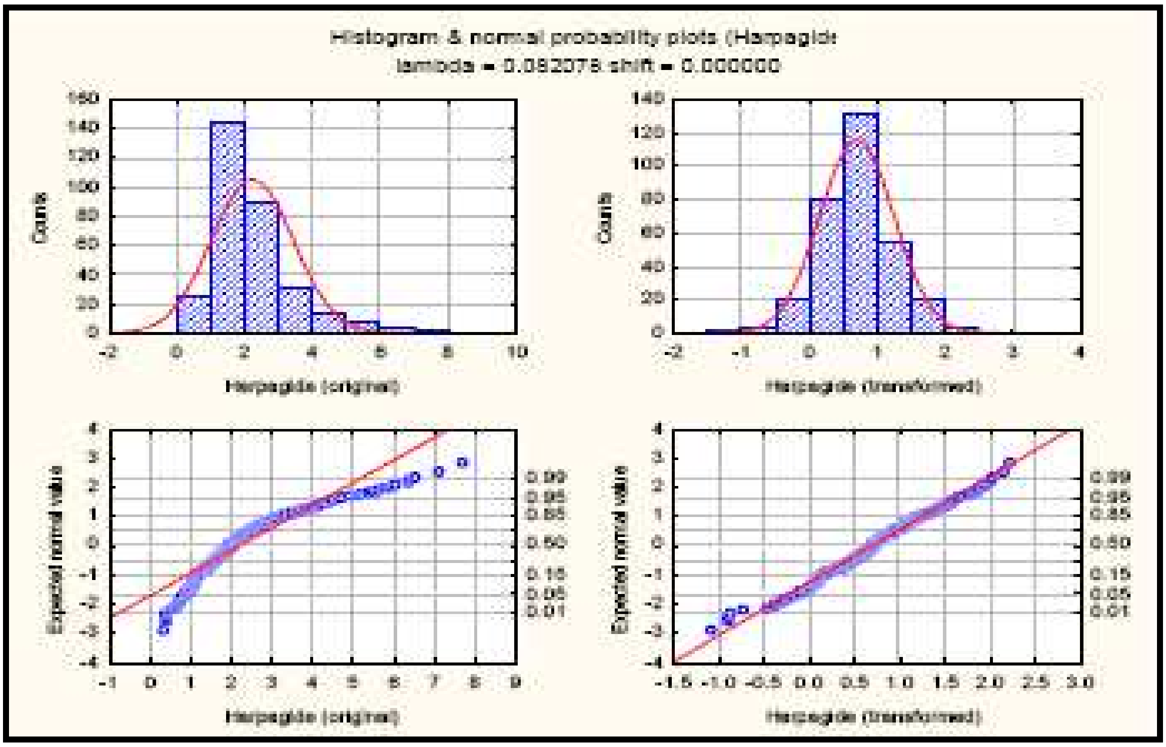

P-Plot: Isoverbascoside
Effect: Locality
(Plot of within-cell residuals)
Exclude condition: v2='Zim' or v2='Namibi
All Groups
Expected Normal Value
Observed Value
4
3
2
1
0
-1
-2
-3
-4
-10
-5
0
5
10
15
20
25
30

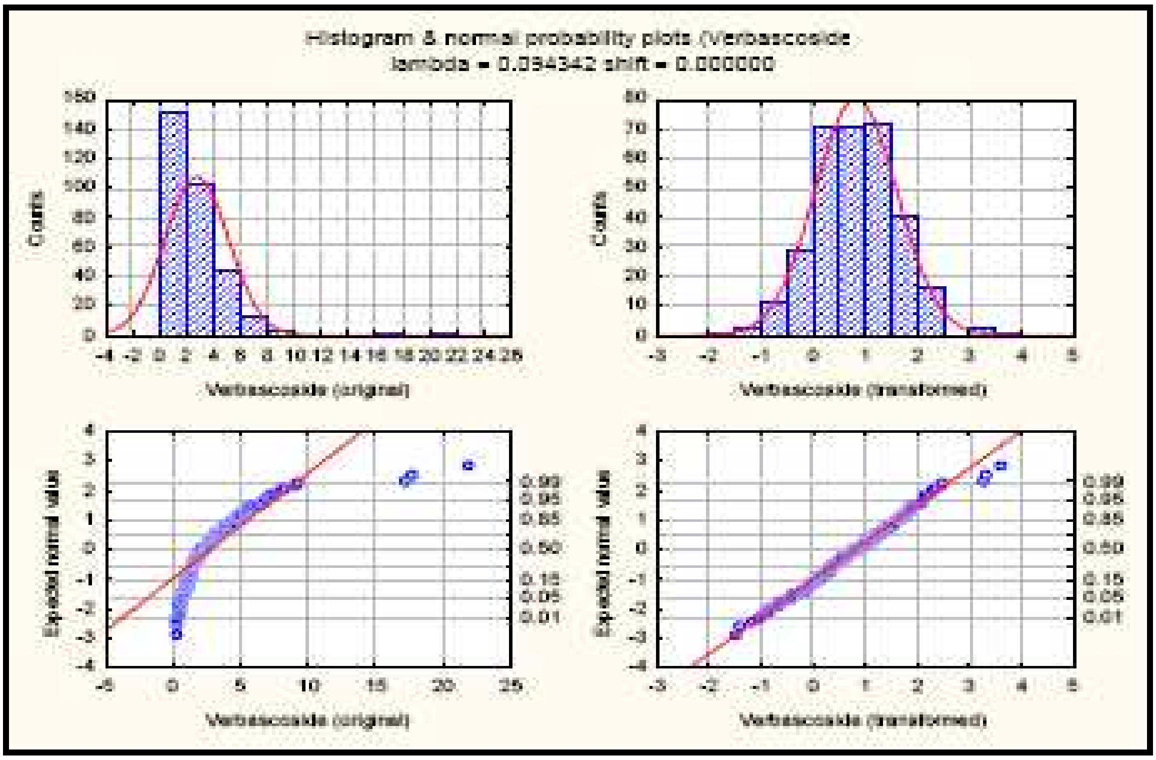

P-Plot: Acetylacteoside
Effect: Locality
(Plot of within-cell residuals)
Exclude condition: v2='Zim' or v2='Namibi
All Groups
Expected Normal Value
Observed Value
4
3
2
1
0
-1
-2
-3
-4
-6
-4
-2
0
2
4
6
8
10
12

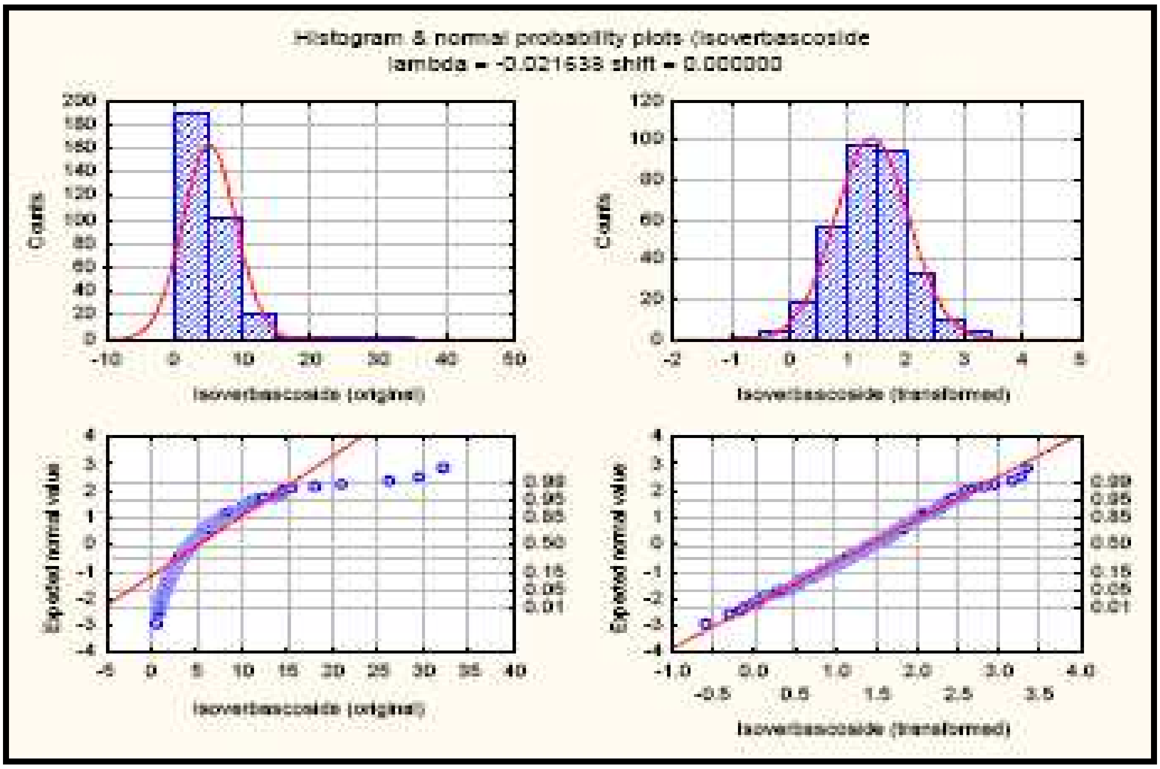


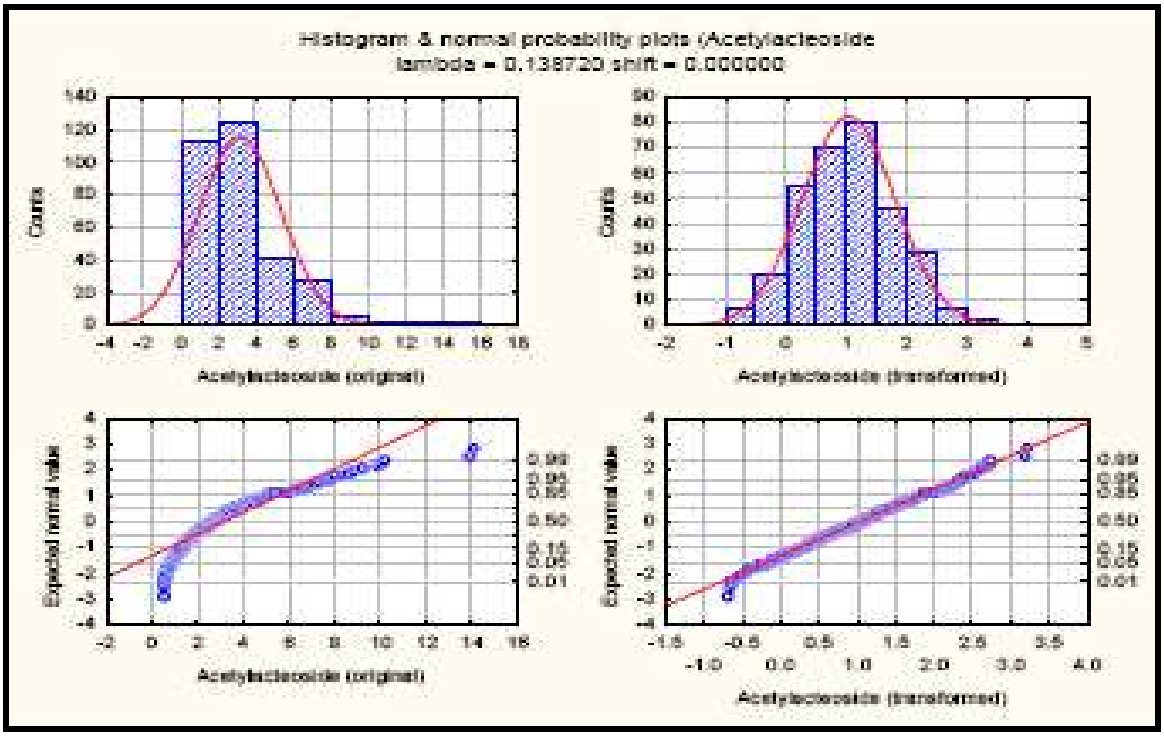


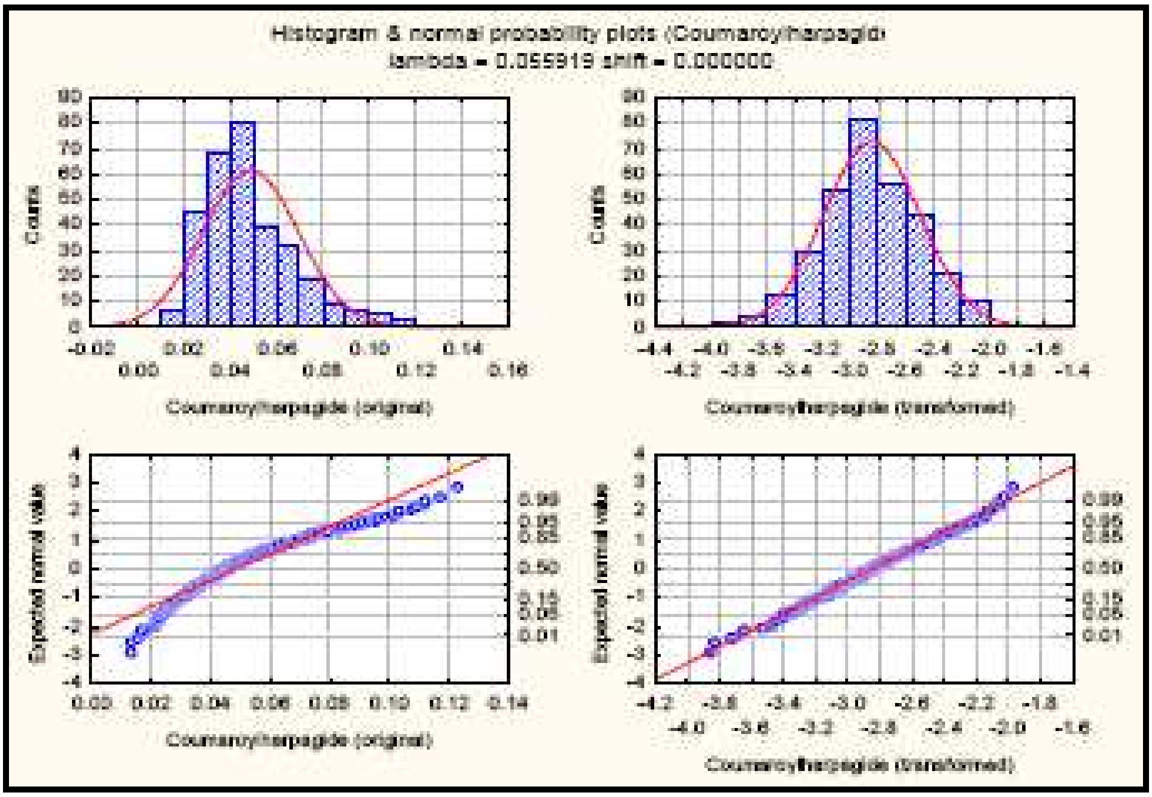
3. Selection of appropriate Box-Cox transformations to normalize data

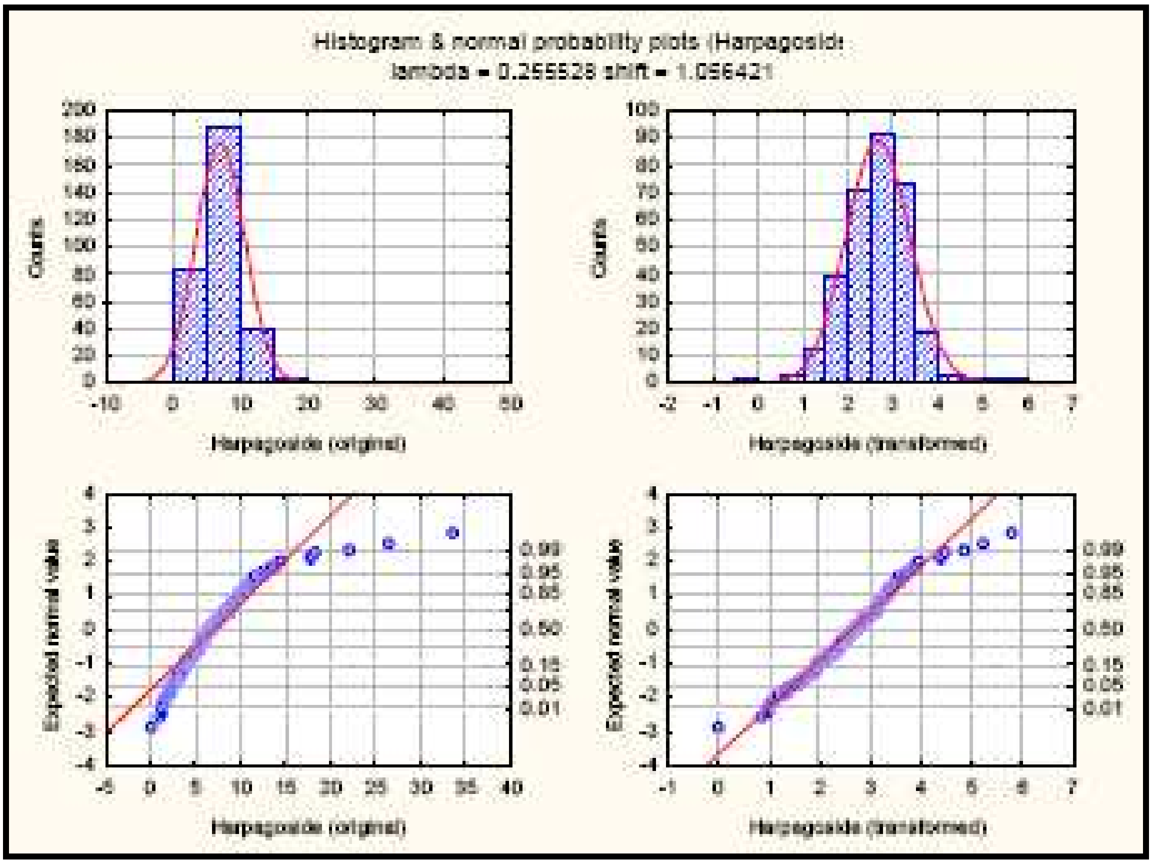

Histogram & normal probability plots (Harpagide
lambda = 0.082078 shift = 0.000000
Counts
Harpagide (original)
Harpagide (transformed)
Expected normal value
0.99
0.95
0.85
0.50
0.15
0.05
0.01


Histogram & normal probability plots (Verbascoside
lambda = 0.094342 shift = 0.000000
Counts
Verbascoside (original)
Verbascoside (transformed)
Expected normal value
0.99
0.95
0.85
0.50
0.15
0.05
0.01


Histogram & normal probability plots (Isoverbascoside
lambda = -0.021638 shift = 0.000000
Counts
Isoverbascoside (original)
Isoverbascoside (transformed)
Expected normal value
0.99
0.95
0.85
0.50
0.15
0.05
0.01


Histogram & normal probability plots (Acetylacteoside
lambda = 0.138720 shift = 0.000000
Counts
Acetylacteoside (original)
Acetylacteoside (transformed)
Expected normal value
0.99
0.95
0.85
0.50
0.15
0.05
0.01

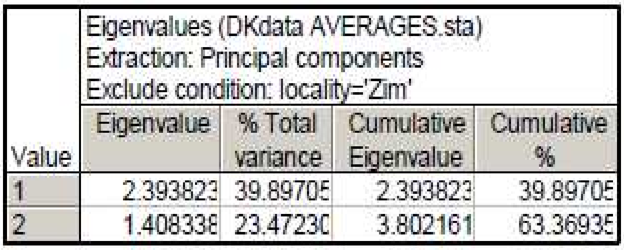

Histogram & normal probability plots (Coumaroylharpagid
lambda = 0.055919 shift = 0.000000
Counts
Coumaroylharpagide (original)
Coumaroylharpagide (transformed)
Expected normal value
0.99
0.95
0.85
0.50
0.15
0.05
0.01


Histogram & normal probability plots (Harpagoside
lambda = 0.255528 shift = 1.066421
Counts
Harpagoside (original)
Harpagoside (transformed)
Expected normal value
0.99
0.95
0.85
0.50
0.15
0.05
0.01

Appendix D: Additional Statistical analysis for Chapter 6.

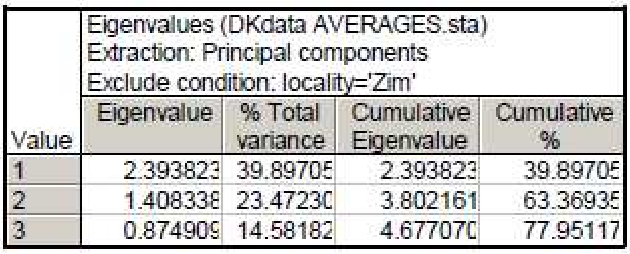
1. The eigen values from factor analysis.

Two factors:



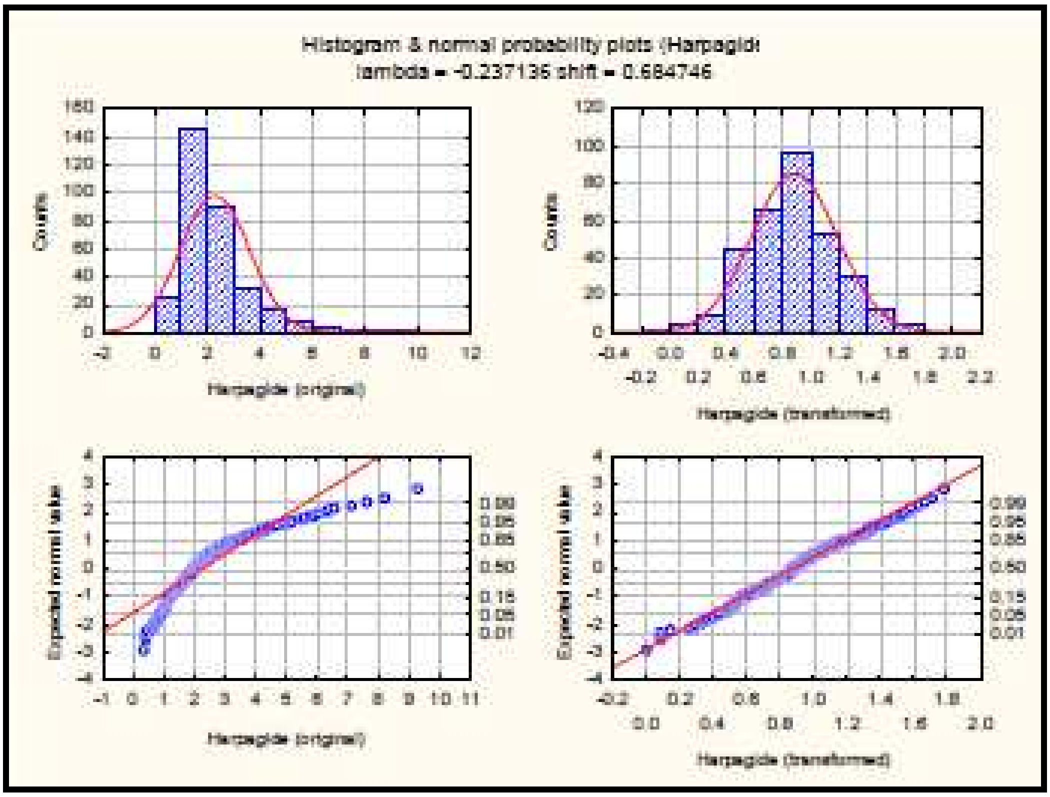
Value	Eigenvalues (DKdata AVERAGES.sta) Extraction: Principal components Exclude condition: locality='Zim' Eigenvalue	% Total variance	Cumulative Eigenvalue	Cumulative %
1	2.393823	39.89705	2.393823	39.89705
2	1.408338	23.47230	3.802161	63.36935

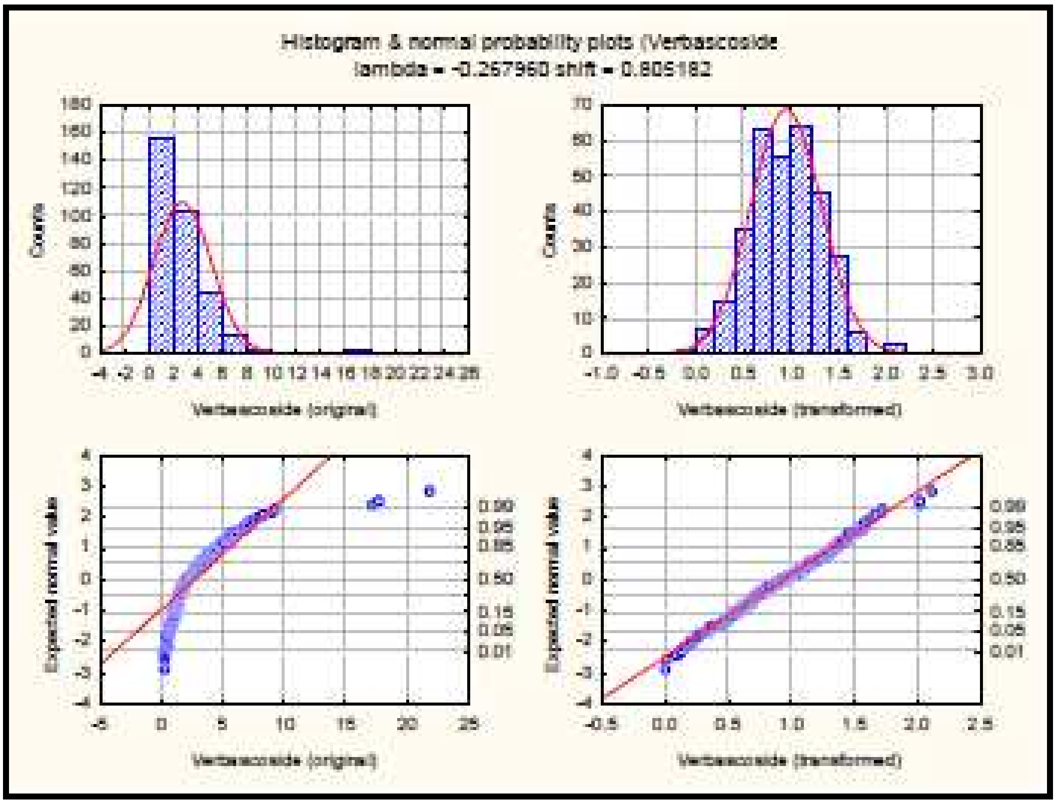
Three factors

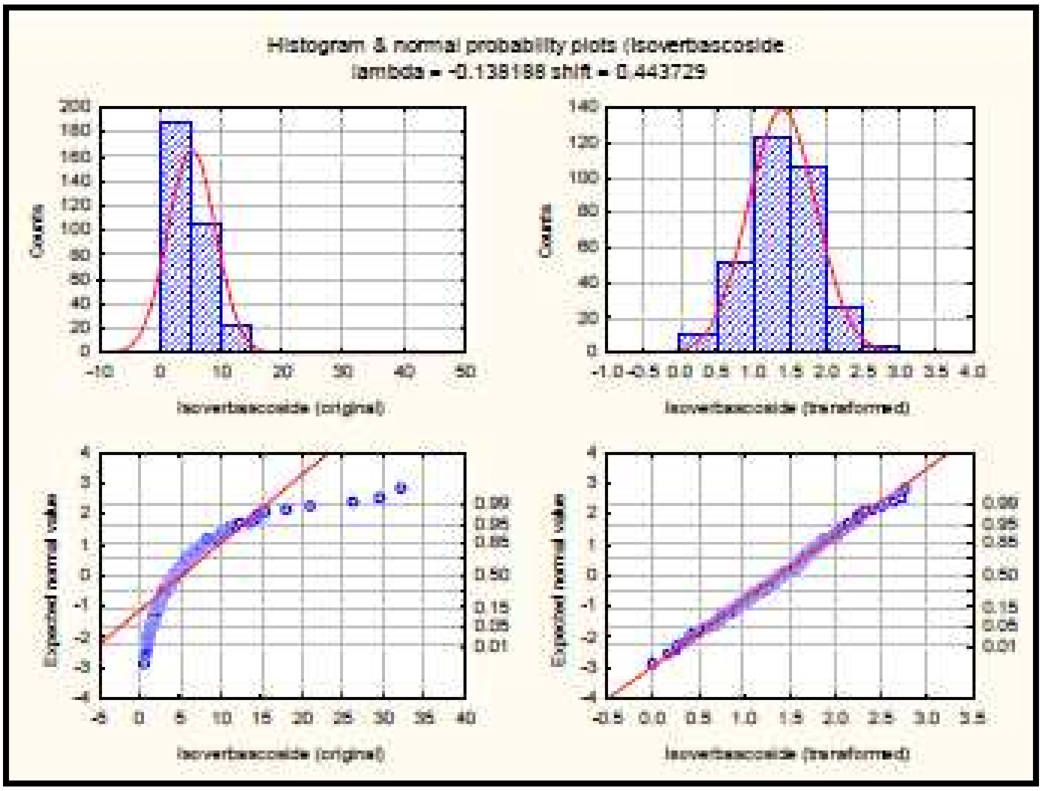


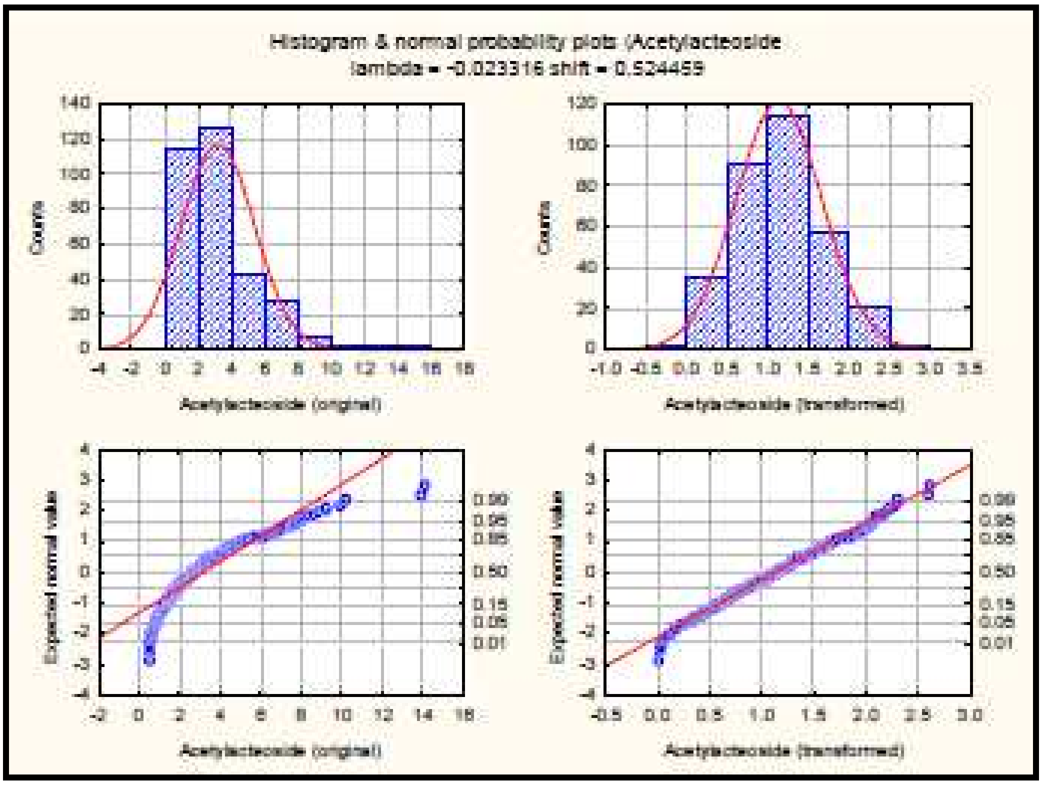
Value	Eigenvalues (DKdata AVERAGES.sta) Extraction: Principal components Exclude condition: locality='Zim' Eigenvalue	% Total variance	Cumulative Eigenvalue	Cumulative %
1	2.393823	39.89705	2.393823	39.89705
2	1.408338	23.47230	3.802161	63.36935
3	0.874909	14.58182	4.677070	77.95117

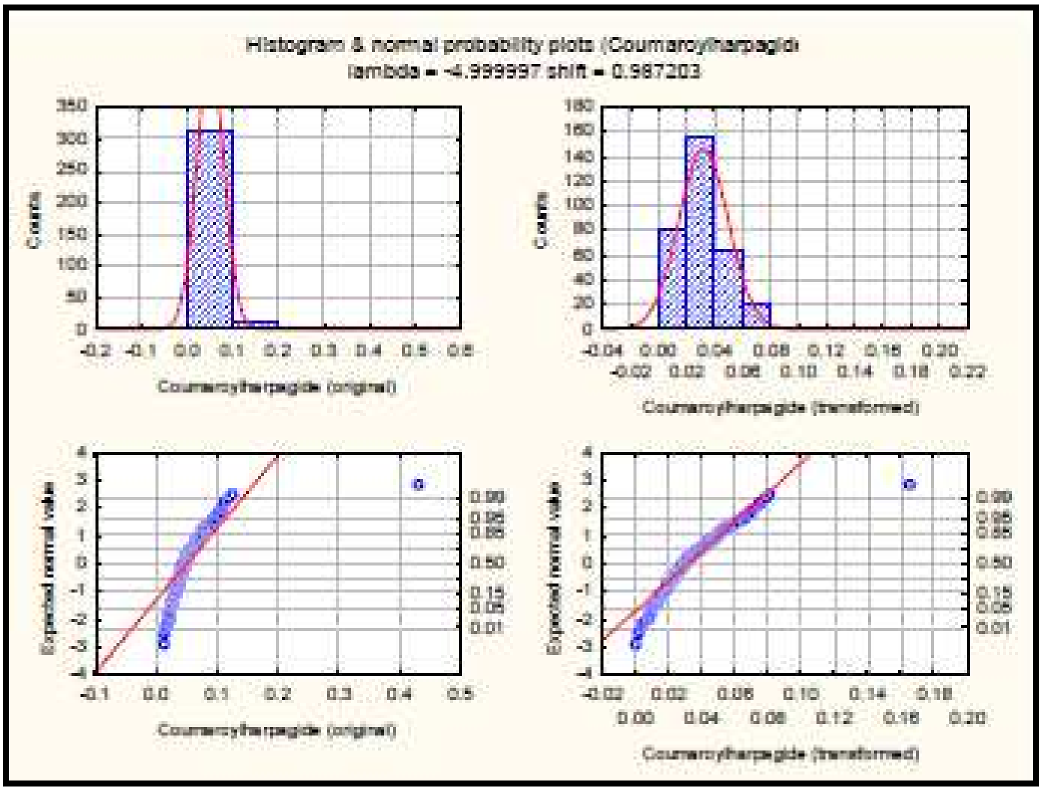
2. Selection of appropriate Box-Cox transformations to normalize data

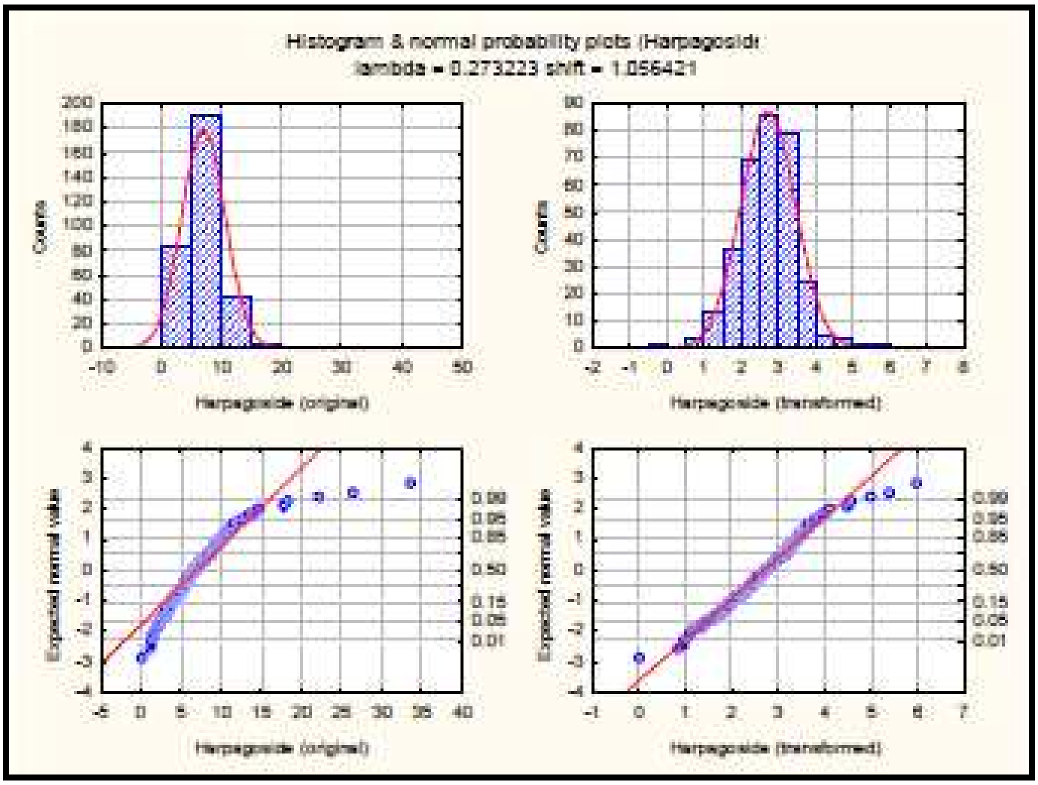



Histogram & normal probability plots (Verbascoside
lambda = -0.267960 shift = 0.805182
Counts
Verbascoside (original)
Verbascoside (transformed)
Expected normal value
0.99
0.95
0.85
0.50
0.15
0.05
0.01


Histogram & normal probability plots (Isoverbascoside
lambda = -0.138188 shift = 0.443729
Counts
Isoverbascoside (original)
Isoverbascoside (transformed)
Expected normal value
0.99
0.95
0.85
0.50
0.15
0.05
0.01


Histogram & normal probability plots (Acetylacteoside
lambda = -0.023316 shift = 0.524459
Counts
Acetylacteoside (original)
Acetylacteoside (transformed)
Expected normal value
0.99
0.95
0.85
0.50
0.15
0.05
0.01

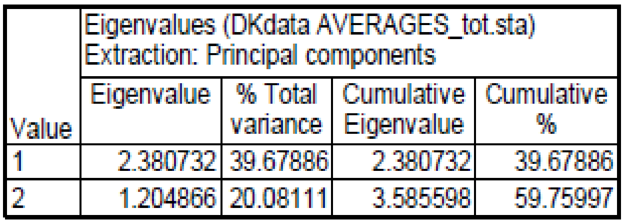

Histogram & normal probability plots (Coumaroylharpagid
lambda = -4.999997 shift = 0.987203
Counts
Coumaroylharpagide (original)
Coumaroylharpagide (transformed)
Expected normal value
0.99
0.95
0.85
0.50
0.15
0.05
0.01



Appendix E: Additional Statistical analysis for Chapter 7

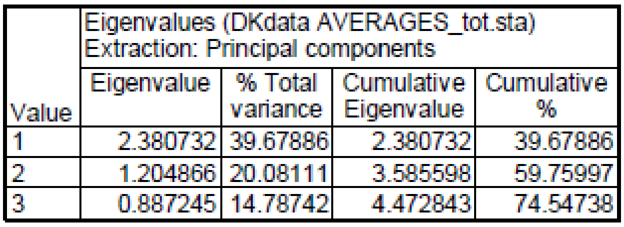
1. Eigenvalues

Two factors:



Value	Eigenvalues (DKdata AVERAGES_tot.sta) Extraction: Principal components			
	Eigenvalue	% Total variance	Cumulative Eigenvalue	Cumulative %
1	2.380732	39.67886	2.380732	39.67886
2	1.204866	20.08111	3.585598	59.75997

Three factors:

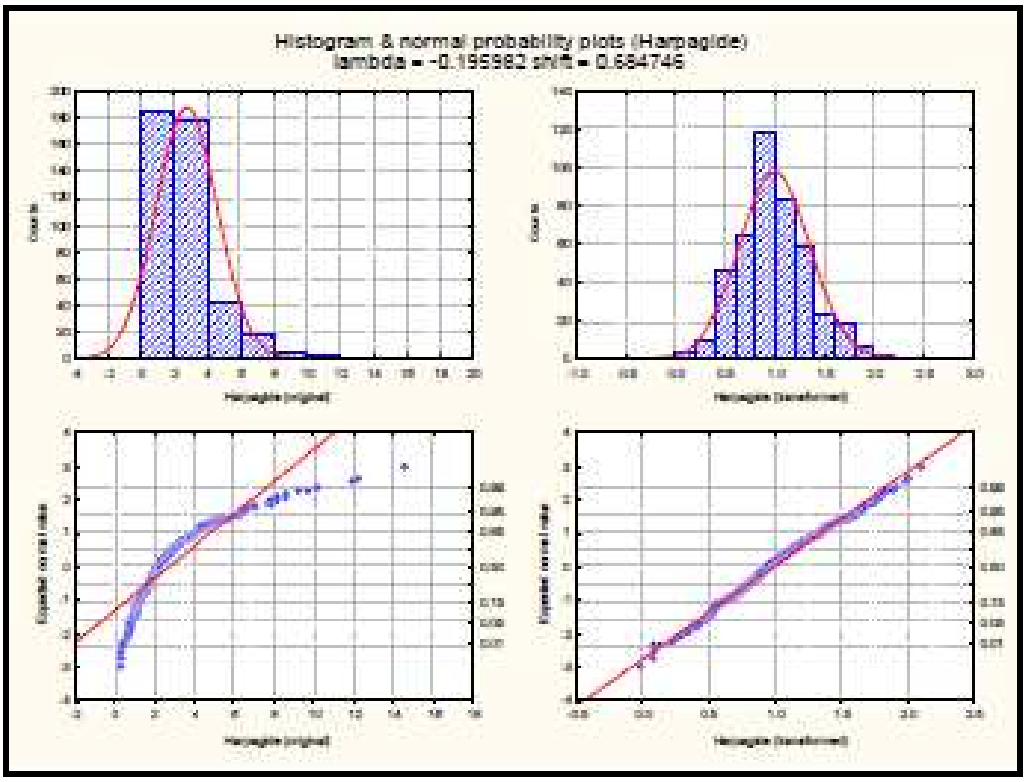


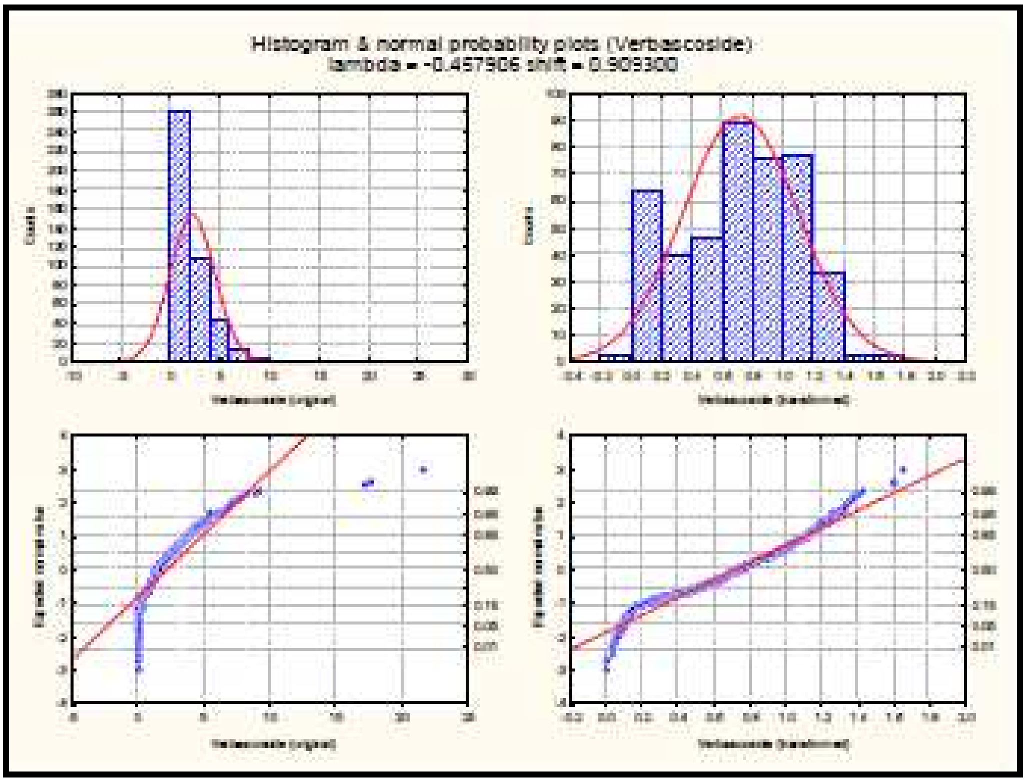
Value	Eigenvalues (DKdata AVERAGES_tot.sta) Extraction: Principal components			
	Eigenvalue	% Total variance	Cumulative Eigenvalue	Cumulative %
1	2.380732	39.67886	2.380732	39.67886
2	1.204866	20.08111	3.585598	59.75997
3	0.887245	14.78742	4.472843	74.54738

Four factors

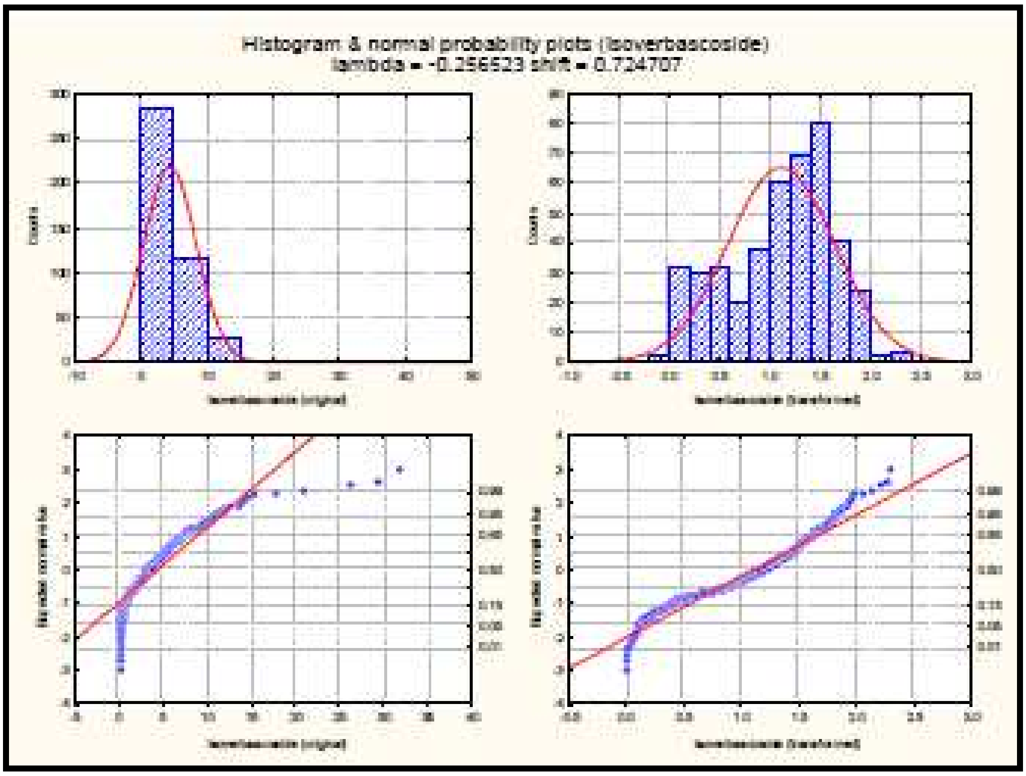
Value	Eigenvalues (DKdata AVERAGES_tot.sta) Extraction: Principal components Eigenvalue	% Total variance	Cumulative Eigenvalue	Cumulative %
1	2.380732	39.67886	2.380732	39.67886
2	1.204866	20.08111	3.585598	59.75997
3	0.887245	14.78742	4.472843	74.54738
4	0.833975	13.89959	5.306818	88.44697

2. Box-cox transformations on each variable:

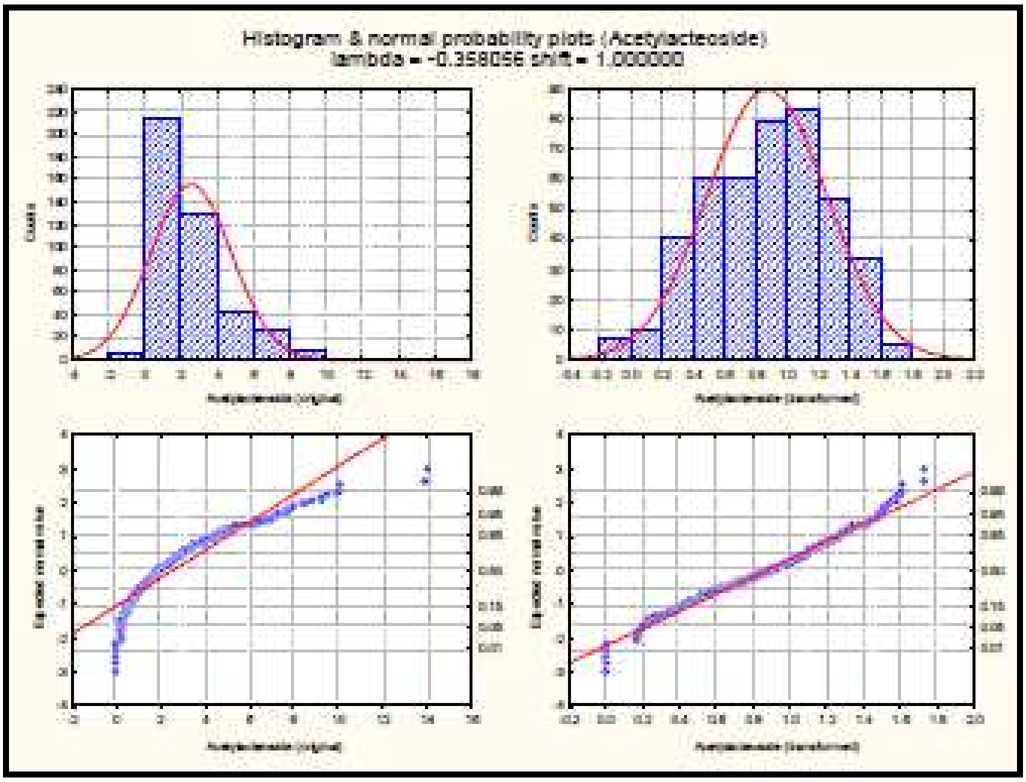


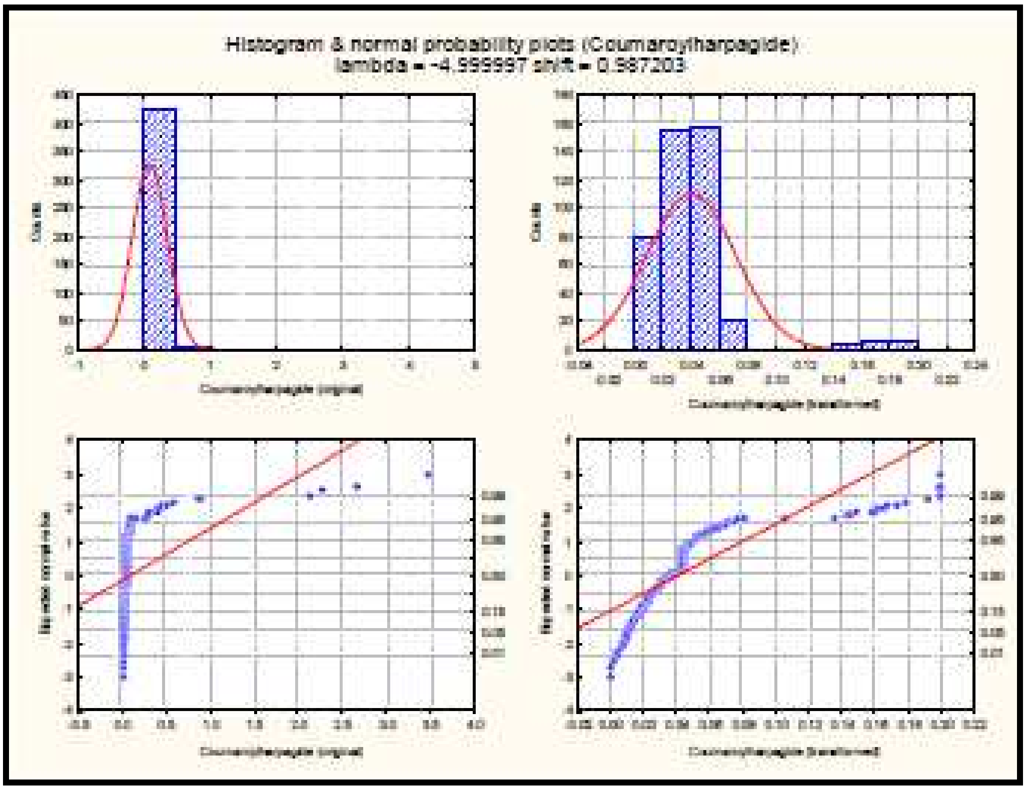


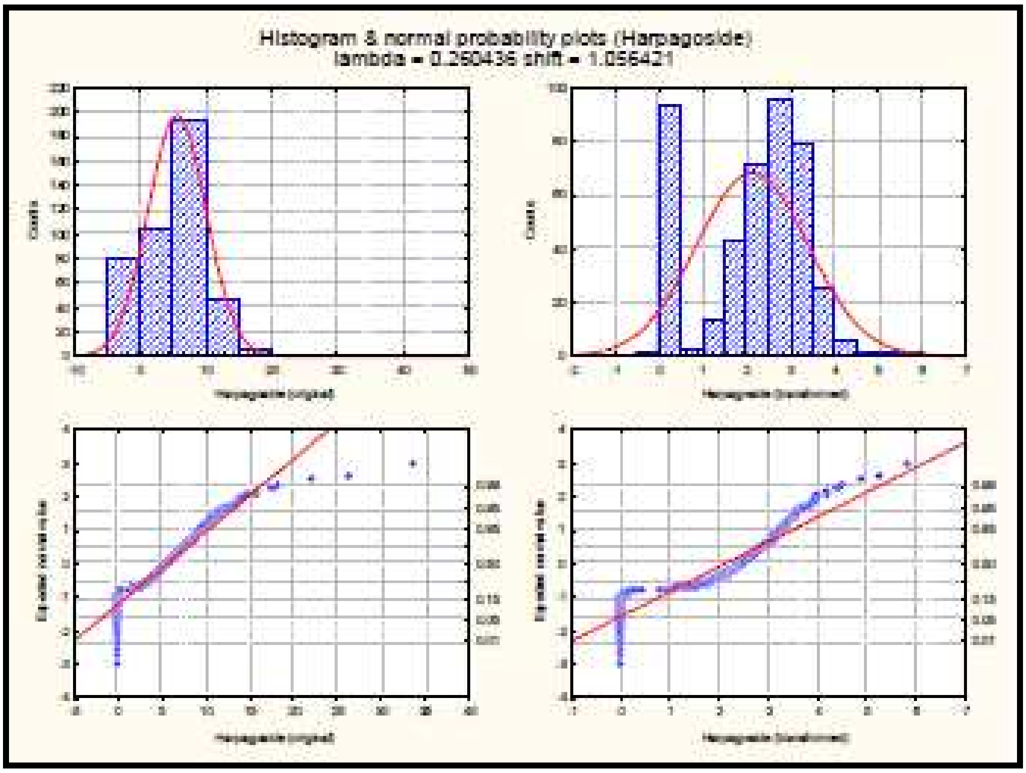
Histogram & normal probability plots (Verbascoside)
lambda = -0.457906 shift = 0.909300



Histogram & normal probability plots (Isoverbascoside)
lambda = -0.256523 shift = 0.724707


Histogram & normal probability plots (Acetylacteoside)
lambda = -0.358056 shift = 1.000000
Counts
Acetylacteoside (original)
Acetylacteoside (transformed)
Expected normal value


Histogram & normal probability plots (Coumaroylharpagide)
lambda = -4.999997 shift = 0.987203
Counts
Coumaroylharpagide (original)
Coumaroylharpagide (transformed)
Expected normal value


Histogram & normal probability plots (Harpagoside)
lambda = 0.260436 shift = 1.056421
Counts
0
20
40
60
80
100
120
140
160
180
200
220
-10
0
10
20
30
40
50
Harpagoside (original)
Counts
0
20
40
60
80
100
-2
-1
0
1
2
3
4
5
6
7
Harpagoside (transformed)
Expected normal value
-4
-3
-2
-1
0
1
2
3
4
-5
0
5
10
15
20
25
30
35
40
0.99
0.95
0.85
0.50
0.15
0.05
0.01
Harpagoside (original)
Expected normal value
-1
0
1
2
3
4
5
6
7
0.99
0.95
0.85
0.50
0.15
0.05
0.01
Harpagoside (transformed)