

**THE DEVELOPMENT AND PREPARATION OF A QUALITY
CONTROL DOSSIER FOR REGISTRATION OF *ARTEMISIA
AFRA* CAPSULES FOR THE TREATMENT OF CHRONIC
ASTHMA BY THE SOUTH AFRICAN HEALTH PRODUCTS
REGULATORY AUTHORITY**

Mabolaeng Sekhonyana-Khetsekile



A thesis submitted in fulfilment of the requirements for the degree of Magister Pharmaceuticiae in the School of Pharmacy at the University of the Western Cape, Bellville, South Africa.

Supervisor: Prof. James Syce

July 2018

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KEYWORDS

Artemisia afra

Freeze dried aqueous extract

Quality control

Herbal capsules

South African Medicines Control Council / South African Health Products
Regulatory Authority

Dossier

Investigational Drug Brochure

SUMMARY

The development and preparation of a quality control dossier for registration of *Artemisia afra* capsules for the treatment of chronic asthma by the South African Health Products Regulatory Authority

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Artemisia afra (*A. afra*) is a well-known traditional plant widely used for the management of respiratory illnesses such as asthma, in dosage forms such as teas or aqueous decoction. However, little information is available on the quality control of this dosage form and no product of *A. afra* has thus far been registered by the Medicines Control Council (MCC) or been clinically tested.

The aim of this study was to determine quality control specifications needed for a dossier and an investigator's brochure of *A. afra* capsules, which can be used to motivate the registration and clinical testing of *A. afra* capsules in chronic asthma. The specific objectives were: (1) to establish the minimum product quality requirements for registration of *A. afra* capsules, (2) to prepare and pharmaceutically characterize a capsule product of *A. afra* freeze dried aqueous extract (FDAE) suitable for registration, and (3) to identify pharmaceutical product quality aspects of an investigator's brochure (IB) that would be appropriate for use in motivating a clinical trial of *A. afra* capsules in chronic asthma.

To realise these objectives, herbal medicines quality guidelines from the following regulatory agencies Health Canada, the Therapeutic Goods Administration (TGA), the World Health Organization (WHO) and guidelines from the British Pharmacopoeia (BP) were reviewed. From these, a list of quality requirements appropriate for *A. afra* active pharmaceutical ingredient (API) and the encapsulated *A. afra* were obtained. Following preparation of the FDAE of dried *A. afra* leaves, various characteristics, viz: organoleptic features, particle size and shape, ash values, moisture content, total flavonoid content (TFC), total phenol content (TPC), and levels of

quality control marker compound (i.e. luteolin) of the extract were determined. Thereafter, the FDAE was encapsulated and the capsules also evaluated for organoleptic features, moisture content, weight and content uniformity as well as dissolution characteristics. Finally, to identify product quality aspects needed for an investigator's brochure (IB) of *A. afra* capsules to be used in a clinical trial, guidelines from agencies such as the European Medicine Agency (EMA), Health Canada, the TGA, and the International Conference on Harmonization (ICH) were reviewed. Based on the review, information required for an IB focussed on the quality control properties of *A. afra* capsules was identified.

The recommended product quality characteristics, specifications and test methods suggested by Health Canada, the TGA, the BP and the WHO as minimum quality requirements suitable for registration of *A. afra* capsules were divided into 2 groups, viz. properties required for the raw material (active pharmaceutical ingredient, API) and properties required for the finished herbal product (FHP). For the former (i.e. API), these properties include the organoleptic properties, particle size and shape, ash values, moisture content, microbial contamination, and chemical features such as TFC, TPC, as well as levels of quality control marker compound (luteolin). For the FHP, these properties include the organoleptic features, weight and content uniformity, and the dissolution profile. The analyses indicated that the *A. afra* FDAE was an irregularly shaped, coarse powder, light brown in colour, changing to dark brown on exposure to air, with an aromatic odour and bitter taste. It had a total ash value of 13.36 ± 0.939 %, acid insoluble ash of 0.679 ± 0.261 %, moisture content of 10.76 ± 0.56 %, TFC of 32.91 ± 0.46 mg QE/g, TPC of 101.75 ± 5.55 mg GAE/g, free luteolin content of 0.236 ± 0.036 µg/mg and total luteolin content of 0.945 ± 0.175 µg/mg. The capsules prepared were physically elegant, dark brown in colour and contained 105.05 ± 9.304 mg QE/g TFC and 335.47 ± 95.86 mg GAE/g TPC (n=10). They had average deviation in weight of 2.23 ± 1.879 %, showed immediate release of extract (i.e. dissolution of $> 70\%$ (Q) in 20 minutes) and *in vitro* bioavailability. The moisture content of the capsules was determined as 14.40 ± 0.727 % (required quality control specification $< 10\%$). Information required for the investigator's brochure (IB) was identified and included in the title page, confidential statement, summary of the product, introduction of the product, quality findings (e.g. physical, chemical as well as the pharmaceutical properties and formulations), and guidance for the investigator sections of the brochure.

Collectively, the results showed that capsules of *A. afra* could be prepared from *A. afra* FDAE. The extract and capsules met some of the quality control specifications set by various medicine regulatory agencies. However, the FDAE (API) was deemed too hygroscopic and not a suitable raw material for preparation of *A. afra* capsules. Since the IB only contained information on the product quality findings, the prepared IB for *A. afra* capsules could not be employed for motivating clinical trials, but it did conveyed a reasonable picture of the pharmaceutical product quality requirements of *A. afra* capsule. However, further information on clinical and non-clinical studies still needs to be researched in order to design an IB that can be used to motivate for a clinical trial of the product.



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DEDICATION

I dedicate this master's thesis to my loving husband Teboho Khetsekile, my children Lunga, Neo and Mpho, my mother 'Me' Masalemone and late father Khomo ea Mollo Morena Matsoso Sekhonyana for their love, affection, blessings, guidance, encouragement, support and sacrifices to my life.



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DECLARATION

I declare that the thesis **The development and preparation of a quality control dossier for registration of *Artemisia afra* capsules for the treatment of chronic asthma by the South African Health Products Regulatory Authority** is my own work, that has not been submitted before for any degree or examination in any other University and that all the sources I have used or quoted have been indicated and acknowledged by means of complete referencing.



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M. Sekhonyana-Khetsekile

July 2018

Signed

UWC, Bellville

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CONTENTS

TITLE.....	i
KEYWORDS	ii
SUMMARY	iii
DEDICATION	vi
DECLARATION	vii
ACKNOWLEDGEMENTS	viii
CONTENTS	ix
LIST OF FIGURES.....	xiv
LIST OF TABLES	xvi
LIST OF APPENDIX.....	xvii
CONFERENCE PRESENTATION.....	xvii
LIST OF ABBREVIATIONS	xviii
CHAPTER 1.....	1
Introduction	1
CHAPTER 2.....	4
Literature review	4
2.1 Introduction	4
2.2 Asthma.....	4
2.2.1 Definition of asthma.....	4
2.2.2 Aetiology of asthma	5
2.2.3 Treatment for asthma.....	5



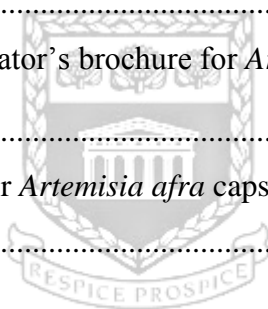
UNIVERSITY of the
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2.2.4	Challenges of asthma therapy.....	6
2.3	<i>Artemisia afra</i> – An important indigenous medicinal plant.....	6
2.3.1	Vernacular names.....	6
2.3.2	Geographical distribution of <i>Artemisia afra</i>	7
2.3.3	Botanical classification and morphology of <i>Artemisia afra</i>	8
2.3.4	Aspects of the uses, pharmacology and phytochemistry of <i>Artemisia afra</i>	9
2.3.5	Traditional dosage forms of <i>Artemisia afra</i>	13
2.3.6	Options for alternative dosage forms available for <i>Artemisia afra</i> and their advantages and disadvantages.....	15
2.4	Evaluation of herbal dosage forms.....	18
2.4.1	The general criteria for the evaluation of quality of herbal dosage forms.....	18
2.4.2	Characterization: quality of starting plant material(s) for herbal products.....	18
2.4.3	Design and development of quality control tests for herbal dosage forms.....	19
2.4.4	Quality control specifications for manufactured and finished products.....	19
2.5	Development and evaluation of herbal capsule dosage forms.....	20
2.5.1	Factors of relevance in the evaluation of <i>Artemisia afra</i> herbal raw material.....	20
2.5.2	Analytical techniques used in quality control of finished herbal capsules of <i>Artemisia afra</i>	25
2.6	Quality control dossier.....	28
2.7	Assessment of an investigational drug brochure.....	30

CHAPTER 3.....	32
WORK PLAN	32
3.1 Introduction	32
3.2 Objectives.....	32
3.2.1 Specific objectives.....	32
3.3 Hypothesis	33
3.4 Study approach.....	33
CHAPTER 4.....	35
Minimum quality control requirements for registration of <i>Artemisia afra</i> capsules.....	35
4.1 Introduction	35
4.2 Methods:.....	35
4.2.1 Establishment of the minimum quality control requirements needed for registration of herbal product such as <i>Artemisia afra</i> capsules.....	35
4.2.2 Establishment of quality characteristics, specifications and test methods suitable for pharmaceutical quality control of herbal capsule products such as <i>Artemisia afra</i> capsules	36
4.3 Results and Discussion:.....	37
4.3.1 Minimum quality requirements for registration of herbal product such as <i>Artemisia afra</i> capsules	37
4.3.2 Quality characteristics, tests and procedures and quality control specifications suitable for herbal solid dosage forms such as <i>Artemisia afra</i> capsules.	41
4.4 Conclusion:.....	47

CHAPTER 5.....	48
Quality characteristics of <i>Artemisia afra</i> capsules	48
5.1 Introduction	48
5.2 Equipment and material.....	48
5.3 Methods	49
5.3.1 Preparation and determination of selected quality characteristics of the <i>Artemisia afra</i> freeze dried aqueous extract.	49
5.3.1.1 Issues relating to preparation of the <i>A. afra</i> FDAE.....	49
5.3.1.2 Determination of the selected quality attributes of the <i>Artemisia afra</i> freeze dried aqueous extracts (API)	50
5.3.2 Preparation and determination of quality characteristics of the <i>Artemisia afra</i> freeze dried aqueous extracts (FDAE) capsules.....	58
5.3.2.1 Preparation of <i>Artemisia afra</i> capsule	58
5.3.2.2 Determination of quality attributes of <i>Artemisia afra</i> capsule:.....	58
5.4 Results and Discussion.....	60
5.4.1 Preparation and quality characteristics of <i>Artemisia afra</i> freeze dried aqueous extracts.	60
5.4.2 Preparation and quality characteristics of <i>Artemisia afra</i> freeze dried aqueous extracts (FDAE) capsules.....	70
5.4.2.1 Issues pertaining to preparation.....	70
5.4.2.2 Quality characteristics	70
5.4.2.2.1 Organoleptic features	70
5.4.2.2.2 Moisture content.....	71

5.4.2.2.3	Uniformity of weight.....	71
5.4.2.2.4	Uniformity of content.....	72
5.4.2.2.5	Dissolution profile.....	73
5.5	Conclusion.....	74
CHAPTER 6.....		77
Investigator’s brochure for <i>Artemisia afra</i> capsules		77
6.1	Introduction:	77
6.2	Methods:.....	77
6.2.1	Preparation of an investigator’s brochure for <i>Artemisia afra</i> capsules	77
6.3	Results and Discussion:.....	78
6.3.1	Investigator’s brochure for <i>Artemisia afra</i> capsules	78
6.4	Conclusion.....	80
CHAPTER 7.....		81
Conclusion and Recommendations		81
REFERENCES.....		85
APPENDIX.....		95



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LIST OF FIGURES

Figure 2. 1	Geographical distribution of <i>Artemisia afra</i> in South Africa (Hilliard 1977)	8
Figure 2. 2	<i>Artemisia afra</i>	8
Figure 2. 3	4- Oxo-flavonoid nucleus	11
Figure 2. 4	Quercetin (flavonol) 3,3',4',5,7-pentahydroxy flavone.....	12
Figure 2. 5	Kaempferol (flavone) 3,4',5,7-tetrahydroxy flavone	12
Figure 2. 6	Hesperetin (flavonone) 3',5,7-trihydroxy-4-methoxy flavone.....	12
Figure 2. 7	Apigenin (flavone) 4',5,7-trihydroxy flavone	13
Figure 2. 8	Luteolin (flavone) 3',4',5,7-tetrahydroxy flavone.....	13
Figure 2. 9	Hard capsules	16
Figure 2. 10	Comparison of manufacturing process of tablets and hard gelatin capsules	17
Figure 2. 11	The CTD triangle	29
Figure 5. 1	HR73 Halogen moisture analyser	53
Figure 5. 2	Manual capsule filling machine	58
Figure 5. 3	Particle size distribution of <i>Artemisia afra</i> FDAE (graded according to passing through different sieve sizes)	63
Figure 5. 4	Standard curve of quercetin concentration versus absorbance at 280 nm used for the quantification of the total flavonoid contents in <i>Artemisia afra</i> FDAE	65



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Figure 5. 5	Standard curve of gallic acid versus absorbance at 765 nm used for the quantification of the total phenolic contents of <i>Artemisia afra</i> FDAE.	66
Figure 5. 6	HPLC chromatogram of luteolin (inset is the UV-spectrum of the compound) with retention time of 28.25 minutes	67
Figure 5. 7	Standard curve for the luteolin.....	68
Figure 5. 8	Representative HPLC chromatogram of unhydrolysed <i>Artemisia afra</i> FDAE powder The retention time for luteolin was 28.25 minutes.....	68
Figure 5. 9	Representative HPLC chromatogram of hydrolysed <i>Artemisia afra</i> FDAE powder. The retention time for luteolin was 28.25 minutes.....	69
Figure 5. 10	The formulated <i>Artemisia afra</i> FDAE capsules	71
Figure 5. 11	Dissolution profile of <i>Artemisia afra</i> capsules (n=6).	74



UNIVERSITY of the
WESTERN CAPE

LIST OF TABLES

Table 4. 1	Summary of quality requirements for registration of herbal raw materials or API advocated by the TGA, Health Canada and WHO guidelines	38
Table 4. 2	Summary of quality requirements for registration of finished herbal product (FHP) advocated by the TGA, Health Canada and WHO guidelines	39
Table 4. 3	A summary of quality attributes, tests, test methods and specifications for quality control of herbal raw material	43
Table 4. 4	A summary of quality attributes tests, test methods and specifications for finished herbal product	44
Table 5.1	Organoleptic characteristics of <i>Artemisia afra</i> freeze dried aqueous extract powder	62
Table 5.2	Total flavonoid content (TFC) and total phenol content (TPC) of <i>Artemisia afra</i> capsules.....	72

UNIVERSITY of the
WESTERN CAPE

LIST OF APPENDIX

Appendix 5. 1	Yield of freeze-dried aqueous extract of <i>Artemisia afra</i>	95
Appendix 5. 2	Particle size and shape of <i>Artemisia afra</i> freeze dried aqueous extract powder	95
Appendix 5. 3	Total ash and acid-insoluble ash of <i>Artemisia afra</i> freeze dried aqueous extract powder	96
Appendix 5. 4	Moisture content of <i>Artemisia afra</i> freeze dried aqueous extract powder	96
Appendix 5. 5	Moisture content of <i>Artemisia afra</i> capsules.....	96
Appendix 5. 6	Uniformity of weight of <i>Artemisia afra</i> capsules.....	97
Appendix 5. 7	Dissolution profile of <i>Artemisia afra</i> capsules.....	97
Appendix 5. 8	Dissolution profile of total flavonoid content of <i>Artemisia afra</i> capsules	98
Appendix 5. 9	A compilation of quality control dossier for <i>Artemisia afra</i> capsules.....	99

CONFERENCE PRESENTATION

Poster Presentation 2016

“Preparation and characterisation of *Artemisia afra* capsules for compilation of quality control dossier” at the All Africa Congress on Pharmacology and Pharmacy in Johannesburg, Gauteng, South Africa on 4-8th October 2016.

LIST OF ABBREVIATIONS

<i>A. afra</i>	<i>Artemisia afra</i>
API	Active Pharmaceutical Ingredient
CTD	Common Technical Document
DDT	Dichlorodiphenyltrichloroethane
EMA	European Medicine Agency
FDAE	Freeze-dried aqueous extracts
FHP	Finished Herbal Product
FPP	Final Pharmaceutical Product
GAP	Good Agricultural Practice
GC	Gas Chromatography
GCP	Good Clinical Practice
GINA	Global Initiative for Asthma
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HPLC	High-performance liquid chromatography
IBD	Investigational Drug Brochure
ICH	International Conference of Harmonisation
IECs	Independent Ethics Committees
IRBs	Institutional Review Boards
MCC	Medicine Control Council
TLC	Thin Layer Chromatography
TGA	Therapeutic Goods Administration
TFC	Total Flavonoid Content
TPC	Total Phenolic Content
WHO	World Health Organization

µg	Microgram
µl	Microliter
°C	Degrees Celsius
kg	Kilogram
mg	Milligram
min	Minute
ml	Millilitre
PD	Percentage deviation
ppb	Parts per billion



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CHAPTER 1

Introduction

Asthma is a chronic inflammatory condition of the airways which results in hyper-secretion of mucus glands, bronchoconstriction, and increased hyper-responsiveness of the airways. It involves many cells of the immune system, in particular mast cells, eosinophils and T-lymphocytes. This chronic inflammation leads to recurrent episodes of breathlessness, reversible airflow obstruction, wheezing, chest tightness and coughing, particularly at night or early in the morning (GINA 2016). It is estimated to affect over 300 million individuals worldwide, with approximately 250,000 annual deaths (Bateman et al. 2008). South Africa is ranked 25th worldwide in the prevalence of asthma, estimated to affect about 8.1 % of the population. Shockingly, the country was also ranked fourth globally in highest asthma death rates among 5 to 35-year-olds in 2012 (Yakubovich et al. 2016; Bateman et al. 2008). Such statistics highlight the need for appropriate treatment.

Current asthma treatment involves the use of drugs such as corticosteroids, anti-inflammatories, β_2 -adrenoceptor agonists, anti-leukotrienes, and anti-cholinergics (Rang & Dale 2015). Despite the availability (and widespread use) of these anti-asthmatic drugs, the substantial economic load associated with disease control and morbidity continues to intensify. Moreover, the anti-asthmatic drugs may not always give the required therapeutic outcome, and sometimes do not appear to modify the course of the disease significantly (Barnes 2012). Asthma symptoms and inflammation rapidly recur when treatment is discontinued. All currently available inhaled corticosteroids are absorbed from the lungs and thus have the potential for systemic side effects (Tikiso 2015). There is thus potential for use of traditional therapies which have been shown to have effects in asthma, and may be used for treatment of asthma. One such traditional medicine is *Artemisia afra* (*A. afra*).

Artemisia afra is one of the oldest medicinal plants in South African traditional medicine (Afolayan 2012; Suliman 2011). Traditionally, it is used in the treatment of respiratory illnesses such as whooping cough, influenza and asthma (Afolayan 2012). Studies have also shown that *A. afra* has some potent effects on asthma (Tikiso 2015). Flavonoids are found in appreciable

quantities in *A. afra*, and are thought to be responsible for the bronchodilatory effect of the extracts (Mjiziza et al. 2013). This lends evidence for the use of *A. afra* as additional treatment in asthma. Attention however needs to be given to a suitable dosage form.

Generally, *A. afra* is used to treat respiratory diseases in a variety of dosage forms. The traditional process of treatment involves the inhalation of vapours arising from boiling of *A. afra* herb in water. Sometimes, it is also prepared as an aqueous decoction for oral consumption (Afolayan 2012). The traditional liquid dosage forms come with some disadvantages; they may be prone to physical, phytochemical and microbiological instability (Mjiziza et al. 2013; Komperlla 2004), the preparations are often bitter in taste and unpalatable, thus may result in poor patient compliance and the unit doses may produce storage difficulties (Komperlla 2004). In view of these disadvantages, solid dosage forms such as capsules may be a more appropriate dosage form for *A. afra*.

Capsule formulations of plant material should generally be more stable, easier to store, provide greater flexibility in dosing, and potentially improve patient compliance as capsules have the advantage of masking unpleasant taste (MA 2006; Aulton 2015). However, the quality of this dosage form has not been confirmed yet. Indeed such capsules must meet the quality requirements as indicated by the responsible medicine regulatory agencies or authorities (BP 2013). And the known quality requirements for capsules include them having consistent capsule appearance, weight and content uniformity, passing microbial testing and having a known dissolution profile (EMA 2011). The quality of a capsule product such as the *A. afra* envisaged must therefore also be ensured before its use.

To ensure the quality of the encapsulated product, it must comply with certain strict manufacture and pharmaceutical requirements. There are guidelines which ensure quality of the product; these include standards such as the Good Manufacturing Practice (GMP) and Good Laboratory Practice (GLP) requirements. Only when the product meets all the criteria for registration, including quality aspects, can it be considered for registration by a regulatory authority.

Product registration is attained through the submission and approval of a dossier and the latter is a documentation prepared or compiled in order to register a product. The dossier is usually submitted using the electronic Common Technical Document (eCTD) format and the registration

of medicines is done through recognised medicine authorities or agencies. In South Africa, medicines are registered through the South African Medicines Control Council (MCC) (changed to the South African Health Products Regulatory Authority (SAHPRA) in 2018). This council is a national medicine regulator which ensures that medicines registered are of the quality required. In order to register and clinically test a product such as *A. afra* capsule in terms of its quality, there is need for a quality control dossier and investigator's brochure (IB).

Currently in South Africa, a quality control dossier and an investigator's brochure for *A. afra* capsules do not exist. These documents are requirements for the registration and clinical testing of a product by the South African MCC. Nevertheless, a dossier of other herbal products such as Prospan™ exists. This can be used as a guide to prepare a dossier for *A. afra* capsules.

The proprietary medicine, Prospan™ contains dried ivy leaf extract. It is used for the treatment of respiratory diseases in various dosage forms. The ivy leaf extract is claimed to have bronchodilatory and anti-inflammatory effects, probably mediated through the actions of its main active ingredient, the saponins (Mendel et al. 2011). Prospan™ is mainly used in the treatment of productive cough. It is also reputed to assist with relief from symptoms of chronic inflammatory conditions such as bronchitis, asthma, emphysema and pneumonia (Zeil et al. 2014). The available dosage forms of Prospan™ include syrups, effervescent tablets, drops, and suppositories (Stauss-Grabo et al. 2011). Prospan™ seemed to have similar pharmacological effects as *A. afra* according to literature obtained on Prospan™. As a result, data and information from Prospan™ studies can be utilized to guide development of an IB which can be applied to motivate for a clinical trial of *A. afra* capsules in chronic asthma.

Given the above arguments, the overall aims of this study were to determine some of the product quality control data needed for a product registration dossier and to develop aspects of an clinical investigator's brochure of *A. afra* capsules for motivating its product registration and clinical testing in chronic asthma. The specific objectives were therefore to, firstly, establish (from various medicine regulatory authorities) the minimum product quality requirements for registration of *A. afra* capsules, secondly, prepare and pharmaceutically characterize a capsule product of *A. afra* FDAE for possible registration of such a herbal product and, thirdly, identify some pharmaceutical product quality aspects appropriate for use in an investigator's brochure (IB) motivating a clinical trial of *A. afra* capsules in chronic asthma.

CHAPTER 2

Literature review

2.1 Introduction

This chapter gives an overview of asthma and its current treatment with both allopathic and traditional medicines such as *A. afra* as the potential herbal medicine for asthma. A summary of *A. afra* is also given and includes the description, distribution, uses, pharmacology and phytochemistry of *A. afra*, traditional dosage forms, and options for an alternative dosage form. The quality characteristics and specifications of *A. afra* dosage form (capsule) with regard to the minimum quality requirements for registration with various medicine regulatory authorities and the investigator's brochure for *A. afra* capsules are also discussed.

2.2 Asthma

2.2.1 Definition of asthma

Asthma is defined based on its pathological, clinical and physiological characteristics, with emphasis placed on chronic inflammation and hyper-responsiveness (Tikiso 2015). The Global Initiative for Asthma (GINA) defined asthma as a chronic inflammatory condition of the airways which leads to hyper-secretion of the mucus glands, bronchoconstriction, and increased hyper-responsiveness of the airways, with involvement of many cells in particular mast cells, eosinophils and T-lymphocytes. This chronic inflammation leads to recurrent episodes of breathlessness, reversible airflow obstruction, wheezing, chest tightness and coughing, particularly at night or in the early morning (GINA 2016). The common risk factors for asthma include exposure to allergens (such as those from house dust mites, animals with fur, cockroaches, pollen and molds), occupational irritants, tobacco smoke, respiratory infection, emotions, chemicals, etc. (GINA 2016).

2.2.2 Aetiology of asthma

Asthma generally develops as a consequence of complex interactions between genetic susceptibility and environmental influences such as timing and dose of allergen and co-exposure to infection. This ends in an inappropriate inflammatory response from a wide variety of inflammatory cells and mediators (Bateman et al. 2008; Tikiso 2015). Asthmatic attacks have two phases, the immediate and the late (delayed) phase. The immediate phase of allergic asthma mainly results in bronchial spasms, mucous hyper-secretion and mucosal oedema. During this phase, re-exposure to an antigen leads to antigen-antibody interaction with antigen presenting cells (APC), dendritic cells, macrophages and mast cells on airway epithelial cells, resulting in release and synthesis of various mediators such as histamine, leukotrienes, prostaglandins D2 and tryptase (Tikiso 2015; Robinson 2012). The late phase occurs 2-8 hours after allergen inhalation and lasts for up to 24 hours. It is associated with cellular infiltration (predominantly eosinophils and lymphocytes) and the progressive interaction of these cells with local cells (e.g. mast cells, macrophages, epithelial cells, endothelial cells) creates a cascade of events that contributes to the chronic inflammation and clinical manifestations associated with asthma (Holgate 2011). Mediators produced by this interaction are cytokines characteristically produced by T helper lymphocytes type 2 (Th2) (particularly interleukins 4, 5, 9 and 13), eosinophils and the chemokine IL-8. In turn, these mediators attract other inflammatory cells, thereby contributing to further inflammation, sustained bronchoconstriction and mucus hyper-secretion (Vatrella et al. 2015). Asthma due to antigen challenge is very common, but in some instances bronchoconstriction can be caused by non-antigenic stimuli such as exercise, distilled water, cold water and sulphur dioxide. This is called non-specific bronchial hyper-reactivity to distinguish it from bronchial responsiveness to specific antigens (Tikiso 2015). To overcome all the above, there is usually need for treatment.

2.2.3 Treatment for asthma

The main aim of asthma therapy is to control current asthma impairment and reduce future exacerbations. Anti-asthmatic drugs are grouped into two categories, anti-inflammatories (inhibit and prevent the inflammatory process) and bronchodilators (reverse bronchoconstriction) (Barnes 2012). Within the current treatment guidelines for asthma, seven classes of drugs have been

adopted. These include β_2 -adrenoceptor agonists (sympathomimetics), corticosteroids, selective muscarinic receptor antagonists (anti-cholinergics), cromones (cromoglycate and nedocromil), xanthines (including theophylline, a precursor to the search for other phosphodiesterase (PDE) inhibitors, particularly of PDE4), cysteinyl leukotriene antagonists and the anti-IgE monoclonal antibody (Bateman et al., 2008). Drugs in common use are adrenoceptor agonists (used as relievers or bronchodilators) and inhaled corticosteroids (used as controllers or anti-inflammatory agents) (Barnes 2012; GINA 2016). Despite the available (and widespread use) of these anti-asthmatic drugs, there are some challenges.

2.2.4 Challenges of asthma therapy

Current management of asthma is aimed at controlling disease symptoms. For the majority of asthmatics, inhaled corticosteroids (ICS) with or without long-acting β_2 -agonists in combination are effective. However, even when taken regularly, ICS do not appear to modify the course of the disease significantly, and are not curative. Asthma symptoms and inflammation rapidly recur when the treatment is discontinued. All currently available ICS are absorbed from the lungs and thus have the potential for systemic side effects. This has led to a concerted effort to find safer ICS, with reduced oral bioavailability and reduced absorption from the lungs or inactivation in the systemic circulation. Concern has also been expressed about the long-term safety of long acting beta-adrenoceptor agonists (LABA), although when administered in combination with ICS, this does not seem to be a problem (Barnes 2012). This approach however requires lifetime therapy, and a subset of patients remains symptomatic despite optimal treatment. Furthermore, over 80 % of patients have poor adherence with regular inhaled therapy (Tikiso 2015). The same would not happen with herbal products such as *A. afra* hence there is a strong need for addition of such traditional therapies which have been shown to have beneficial effects in asthma and may possibly be used for treatment of asthma. .

2.3 *Artemisia afra* – An important indigenous medicinal plant

2.3.1 Vernacular names

Artemisia afra is one of the oldest South African traditional medicines still widely used by South African tribes such as the Zulu, Xhosa, Tswana and Sotho who all have different vernacular

names for it (Afolayan 2012; Patil et al. 2011; Van Wyk et al. 2008; Dube 2006). Early colonialists also made it popular because of its resemblance to the European worm wood (*Artemisia vulgaris*) (Komperlla 2004). The different names by which it is known among different peoples are:

English: African worm wood

Afrikaans: *Wilde als*

Xhoza: *Umhlonyane*

Zulu: *Mhlonyane*

Tswana: *Lenyana*

Sotho: *Lengana*

2.3.2 Geographical distribution of *Artemisia afra*

Artemisia afra is found in large quantities in the Eastern part of South Africa, including Swaziland and Lesotho. It is also found in Zimbabwe and Namibia, and further afield in the mountain regions of Uganda, Kenya and Tanzania (Afolayan 2012; Dube 2006; Komperlla 2004).

In South Africa, the plant can be found growing in regions from Stellenbosch in the Western Cape to Aliwal North and the Graaff Reinet mountains in the Eastern Cape. It is also widespread in Natal from the coast to the Drakensberg (Hilliard 1977). Its geographical distribution in South Africa is shown in Figure 2.1.

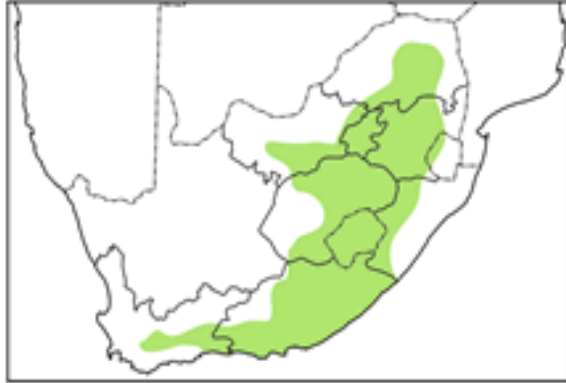


Figure 2. 1 Geographical distribution of *Artemisia afra* in South Africa (Hilliard 1977)

2.3.3 Botanical classification and morphology of *Artemisia afra*

Artemisia afra belongs to the:

- Division: Magnoliophyta
- Class: Magnoliopsida
- Sub class: Asteridae
- Order: Asterales
- Family: Asteraceae
- Genus: *Artemisia*
- Species: *A. afra*



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Figure 2. 2 *Artemisia afra*

Artemisia afra (figure 2.2) is a shrubby, woody, perennial plant which grows up to 2 m tall with a leafy and white hairy stem. It is easily identified by its characteristic aromatic odour. The fresh and dry leaves of *A. afra* plant grow up to 8 cm long and 4 cm wide. The leaf shape is narrowly ovate (egg-shape), feathery and finely divided (Afolayan 2012). The ultimate segments which are linear in shape with an acute tip and smooth or toothed margin grow up to 10 mm long and 2 mm wide. It has a pectinated midrib with similar lobes, a smooth or glandular-punctated upper surface and a canescent (greyish) lower surface. The petiole is up to 2 cm long, dilated at the base with a pair of simple or divided leaf-like stipules. The inflorescence is subglobose with nodding, leafy, terminal, racemose panicles up to 40 cm long. The fruits are about 1 mm long, somewhat 3-angled, slightly curved with a silvery-white coating (Afolayan 2012; Dube 2006, Hilliard 1977). The fresh and dry leaves of *A. afra* plant are the parts commonly used for medicinal purposes in tribal medicine.

2.3.4 Aspects of the uses, pharmacology and phytochemistry of *Artemisia afra*

Artemisia afra is a herb used for the treatment of several ailments (Tikiso 2015; Nkengla 2014; Afolayan 2012; Wyk 2008). It is a well-known treatment for coughs, colds, whooping cough, colic, heartburn, flatulence and gout (Tikiso 2015; Nkengla 2014). It is also commonly used for the treatment of asthma, acute bronchitis, hay fever, bladder and kidney disorders, convulsions, diabetes, fever, headache, inflammation, rheumatism, and stomach disorders caused by infestation with intestinal worm (Tikiso 2015; Van Wyk et al. 2008). A popular well-known use of the herb is as anti-protozoal for the treatment of malaria. In traditional Zulu medicinal use, leaf infusions are taken as teas or administered as enemas, and the steam from the infusions is commonly inhaled for the treatment of headaches and colds (Tikiso 2015; Nkengla 2014; Liu et al. 2009; Dube 2006). Enemas made from ground plants suspended in water or milk are administered for constipation or intestinal worms in children. Decoctions are also taken as blood purifiers for acne, boils, measles and smallpox (Afolayan 2012; Dube 2006; Komperlla 2004). In a survey of medicinal plant use in the Bredasdorp/ Elim region of the Southern Overberg in the Western Cape Province of South Africa, *Artemisia afra* was found to be the plant with the greatest use value amongst the respondents. In other words, *A. afra* was the plant mostly used

(more than any other traditional plant medicine) for treatment of various ailments among the people surveyed (Dube 2006).

Despite its wide use, there have been very few studies designed to scientifically verify the aforementioned uses or pharmacological actions of the plant, or to identify the active constituents responsible for its various pharmacological uses. In a few preliminary tests, Hutchings & Van Staden (1994) showed that *A. afra* may have analgesic and antihistaminic activity. In a recent report, aqueous extracts of *A. afra* were shown to have potent bronchodilatory activity on isolated guinea pig trachea (Tikiso 2015). The literature reveals that leaves of *A. afra* contain various essential oils such as 1, 8-cineole, α -thujone, β -thujone, camphor and borneol, which are the chief constituents of the volatile oil derived from the plant. These volatile oils are known to show definite anti-microbial and anti-oxidative properties (Liu et al. 2009; Wyk 2008; Hilliard 1977) and may thus be responsible for some of the activities of this plant.

Aqueous extracts of *A. afra* also contain flavonoids such as luteolin, kaempferol, apigenin, hesperetin and quercetin (Tikiso 2015; Nkengla 2014; Dube 2006; Komperlla 2004). This group of constituents has been linked to pharmacological activities apparently produced by other *Artemisia* species. For example, the flavonoids in *A. annua* exhibited anti-microbial activity and the flavonols present in the aqueous extracts of *A. abrotanum* are being clinically studied for use in allergic rhinitis (Komperlla 2004). As far as its pharmacological activity and uses are concerned, the flavonoids may thus be important constituents of *A. afra*.

Flavonoids are polyphenolic secondary metabolites found in plants. Structurally, the flavonoids consist of two benzene rings, A and B, which are connected by an oxygen-containing heterocyclic ring C (figure 2.3). Flavonoid compounds contain a fifteen-carbon atom basic nucleus (C6-C3- C6 configuration) (figure 2.3) with several phenolic groups and sometimes attached sugars to form glycosides. The flavonoid group thus consists of the flavonoid aglycones (i.e. flavonoids without attached sugars) and the flavonoid glycosides (i.e. flavonoids with attached sugars). Depending on the chemical bondage between aglycone and sugar moiety, the flavonoid glycosides may be classified into flavonoid *O*-glycosides and flavonoid *C*-glycosides. The flavonoid aglycone forms are known to exhibit pharmacological activities more than the glycoside forms (Komperlla 2004).

The flavonoids can be divided into 5 main groups *viz.* the flavonols, anthocyanidins, catechins, flavanones and flavones. These divisions are based on the molecular structure, degree of hydroxylation, presence of other substitutions, conjugations and degree of polymerization. The flavonoids that contain a pyran ring, i.e. a hydroxyl group in position C3 of the C ring are classified as 3-hydroxyflavonoids (i.e. the flavonols, anthocyanidins and catechins) and those lacking hydroxyl group in position C3 as 3-desoxyflavonoids (i.e. the flavanones and flavones). Further sub-classification within the flavanones and flavones is based on the presence of additional hydroxyl or methoxy groups at different positions in the molecule (Komperlla 2004).

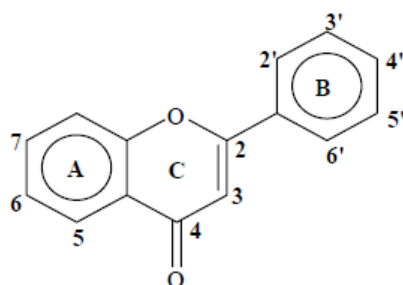


Figure 2.3 *4-oxo-flavonoid nucleus*

Quercetin (figure 2.4) and kaempferol (figure 2.5) are examples of flavonols that are present in plants in many different glycoside forms. Quercetin has been found as quercetin-3-glucoside, quercetin-4 glucoside, quercetin-3, 4-diglucoside and isorhamnetin-4 glucoside while kaempferol has been found as kaempferol-3-*O*-glucoside, kaempferol-7-*O*-glucoside, kaempferol-7 rhamnoside, kaempferol-3, 7-dirhamnoside, kaempferol-3-rahmnoside 7-glucoside, etc. (Komperlla 2004). Quercetin has been reported to have anti-oxidant (Hu et al. 2000; Sadik 2003), anti-inflammatory (Remberg et al. 2004; Adams et al. 2012; Bakasso et al. 2016) and anti-microbial (Ngueyem et al. 2009; Komperlla 2004) properties, while kaempferol was found to have anti-oxidant, anti-inflammatory and anti-clotting properties (Harborne & Williams 2000; Komperlla 2004). These and or other flavonoids are present in many food sources: fresh onions, red wine, tea, apples, berries, grapes and broccoli(Wang et al. 2009). The content of quercetin and its sugar moieties in these foods may not be constant, but may change depending on the plant variety, conditions and degree of ripeness and processing of the food (Komperlla 2004; Texier 1996).

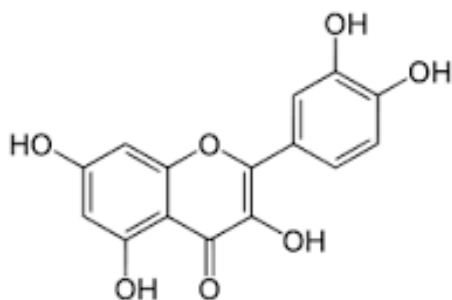


Figure 2. 4 Quercetin (flavonol) 3,3',4',5,7-pentahydroxy flavone

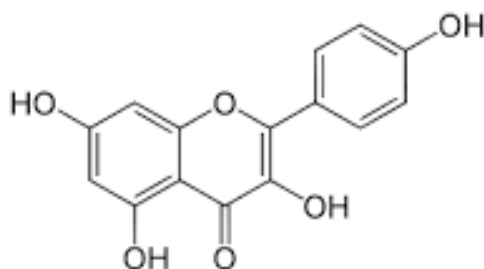


Figure 2. 5 Kaempferol (flavone) 3,4',5,7-tetrahydroxy flavone

Hesperetin (figure 2.6), naringenin, hesperidin, diosmin and didymin are important examples of flavanones that are most common and abundant in citrus fruits. Flavanones have anti-oxidant, anti-carcinogenic and anti-mutagenic properties (Komperlla 2004).

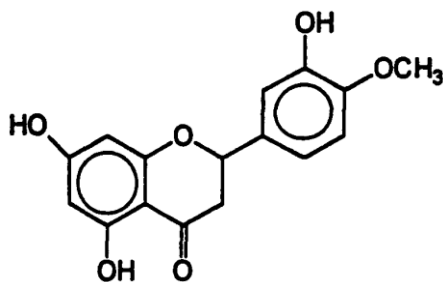


Figure 2. 6 Hesperetin (flavonone) 3',5,7-trihydroxy-4-methoxy flavone

Apigenin (figure 2.7) and luteolin (figure 2.8) are well known flavones generally found in aromatic herbs, red pepper, green chillies, winged beans, celery, chamomile tea, perilla leaf,

guava, and citrus fruits (Andarwulan et al. 2010; Mian & Mohamed 2001). Luteolin may be present in plants as luteolin-7-*O*-glucuronide, luteolin-5-*O*-glucuronide (Grayer et al. 2002) or luteolin-3-*O*-glucuronide forms (Heitz 2000), while apigenin may be present in plants as apigenin-7-*O*-glucuronide or 8-*C*-glucopyranoside, i.e. vitexin (Markham 1982). In terms of activity, luteolin has been reported to exhibit anti-inflammatory, anti-histaminic properties (Mukinda & Syce 2007), anti-oxidant and several other pharmacological properties (Sue et al. 2014). It was also found to have potent bronchodilatory activity (Tikiso 2015). Apigenin on the other hand has been reported to exhibit anti Type-1 allergic activity in mice (Makino & Fu 2001).

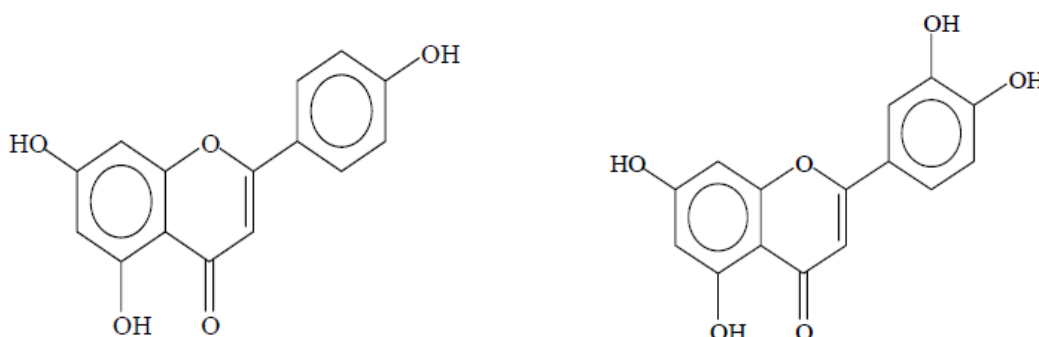


Figure 2.7 Apigenin (flavone)
4',5,7-trihydroxy flavone



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Figure 2.8 Luteolin (flavone)
3',4',5,7-tetrahydroxy flavone

According to literature, luteolin and quercetin are present in *A. afra* plant at considerable levels compared to apigenin, hesperetin and kaempferol. These two flavonoids are also stable on storage and can be assayed by simple extraction and HPLC analytical procedure (Komperlla 2004). The pharmacological activities of luteolin (anti-inflammatory-allergic effects) which are important in treating asthma thus make it a suitable marker compound and an important quality control parameter for *A. afra* dosage forms.

2.3.5 Traditional dosage forms of *Artemisia afra*

The South African traditional medicine, *A. afra*, is most commonly used in the form of an infusion or decoction and as *Wilde als* brandy, an alcoholic preparation (Hilliard 1977). The rolled fresh leaves of *A. afra* can also be inhaled. The infusions or decoctions are made with inconsistent quantities of fresh leaves and different forms of infusions are employed for treatment of diseases by different tribes (Nkengla 2014; Mulubwe 2007). The most common traditional method for making a decoction or infusion is by adding ¼ cup of fresh leaves to a cup of boiling water, and boiling for about 5-10 minutes. Since it has a bitter taste, honey is added to the filtrate to mask the taste (Dube 2006). A small amount of this decoction is sipped at a time and this is mainly used for cold and bronchial disorders, as well as a gargle for sore throat. Another form of infusion is made by taking a cup of fresh leaves combined with stems and adding two litres of boiling water. The mixture is allowed to stand for an hour before use; the filtrate is used as a wash for haemorrhoids, sores, bites, stings, and to relieve rash in measles (Komperlla 2004).

Yet another infusion known as strong brew is prepared from ½ cup of leaves with 1½ or 2 cups of boiling water. The filtrate is used as a mouthwash in mouth ulcers. Finally, there is *Wilde als* brandy, a popular preparation from the European tradition. It is made with brandy, *A. afra* leaves, thyme, mint leaves, sugar, ginger, and rosemary. This is mostly used for colds, coughs, chest ailments, indigestion, heartburn and stomach cramps (Nkengla 2014; Dube 2006).

In addition to the use of infusions, the vapours generated by boiling 2 or 3 cups of leaves in sufficient boiling water in a pot can be deeply inhaled while covering the pot with a towel tent. This is done until the mixture cools down. This method of therapy is used in bronchitis, nasal sinus congestion, tight chest and asthma (Komperlla 2004, Hilliard 1977).

The aforementioned traditional dosage forms of *A. afra* have some disadvantages. Firstly, the decoctions or infusions are generally deemed too smelly and awful tasting to justify their use. To address this shortcoming, honey is usually added to these to make them more palatable and to improve patient acceptability. These additives however, in turn, act as media for the growth of microorganisms like fungi, mould and bacteria, ultimately compromising the quality of the preparation. Secondly, the large volumes of preparation required may pose challenges for patients with respect to their consumption. Thirdly, the traditional dose measures of plant medicines used are not exact (e.g. ¼ cup or two teaspoonful, etc.). Such imprecise dosage

directions will have some effect on the uniformity of dosing among individual users. Most of these problems may however be remedied by the use of appropriate solid dosage forms.

2.3.6 Options for alternative dosage forms available for *Artemisia afra* and their advantages and disadvantages

There are some solid oral dosage forms of traditional medicines that are already available in place of liquid dosage forms. These include granules, powders, tablets and capsules (Joseph 2009; MA 2006). The term ‘powder’ is used for a dosage form in which a drug powder has been mixed with other powdered excipients to produce the final product. The function of the added excipients depends on the intended use of the product. Colouring, flavouring and sweetening agents may, for example, be added to powders for oral use (Aulton 2015).

Granules which are used as solid dosage form consist of powder particles that have been aggregated to form a larger particle, usually 2-4 mm in diameter. Granules as a dosage form are much larger than granules prepared as an intermediate for tablet manufacture (Aulton 2015).

Generally, powders and granules as solid preparations are chemically more stable than liquid preparations. They are convenient forms in which drugs are dispensed in large doses. Orally, administered powders and granules of soluble medicaments have a faster dissolution rate than tablets or capsules, but also have some disadvantages. Firstly, bulk powders or granules are far less convenient for the patient to carry than a small container of tablets or capsules, and are as inconvenient as liquid preparations. Secondly, the masking of unpleasant tastes may be a problem with bulk powder or granules. Thirdly, bulk powders or granules are not suitable for administering potent drugs used in low doses. Finally, the use of powders and granules is not suitable for the administration of drugs which are inactivated in, or cause damage to, the stomach (MA 2006). As a result, tablets or capsules may be considered as more suitable solid dosage forms for *A. afra* product.

Tablets are defined as ‘solid preparations each containing a single dose of one or more active ingredients, and are obtained by compressing uniform volumes of particles’ (Aulton 2015). The

tablet is one of the most popular oral dosage forms for several reasons. Firstly, when compared to liquid dosage forms, tablets have general advantages in terms of the chemical and physical stability of the dosage form. Secondly, the preparation procedure for tablets leads to accurate dose amounts of the drug in each tablet. Thirdly, tablets are convenient to handle and can be prepared in a way that is versatile with respect to their use and to the delivery of the drug. Fourthly, tablets can be mass-produced with robust and quality-controlled production procedures providing elegant preparations of consistent quality and, in relative terms, low price (Aulton & Alderborn 2015; MA 2006). Tablets seem to be a good choice for *A. afra* FDAE, but previous studies with *A. afra* (Komperlla, 2004) have indicated that the freeze-dried aqueous extracts of *A. afra* plant material are frequently very hygroscopic and not easily amenable for tableting. Capsules, as another popular solid dosage form, may however be an alternative option.

Capsules are the most versatile of all dosage forms. They are solid dosage forms in which one or more medicinal and inert ingredients are enclosed in a small shell or container usually made of gelatin. The soft gelatin capsule is also known as “one piece” and the hard capsule known as “two piece” as it consists of two pieces in the form of small cylinders closed at one end. The shorter piece of the hard capsule is called the “cap” which fits over the open end of the longer piece, called the “body” (Vihar et al. 2007; Stegemann & Bornem 2002) (figure 2.9). In addition, capsules have some benefits over other solid dosage forms.

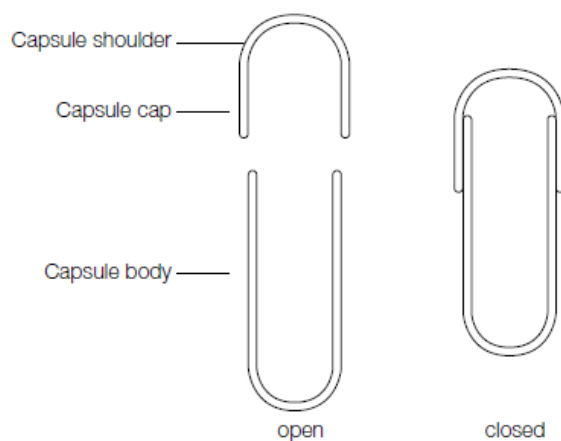


Figure 2.9 Hard capsules

Capsules offer advantages over other solid dosage forms such as tablets. Because of their elongated shape, they are easy to swallow thus increasing motivation for the manufacture of capsule-shaped tablets in the market today. Flexibility of formulation is another benefit of capsule dosage form. Since capsules are tasteless, they effectively mask any unpleasant taste or odour of their contents. They also offer rapid release characteristics due to the rapid dissolution rate of capsules compared to tablets. The manufacture of capsules as illustrated in figure 2.10 also involves a much shorter process compared to that of other modern solid dosage forms (e.g. tablet). Dry powder mixtures, granules, pellets and tablets can be filled into hard capsules. Moreover, a combination of two or three types of dosage forms (for instance, dry powder mixtures, tablets and pellets) can be filled into capsules. The biggest formulation advantage of capsules though is the lack of need for additional excipients.

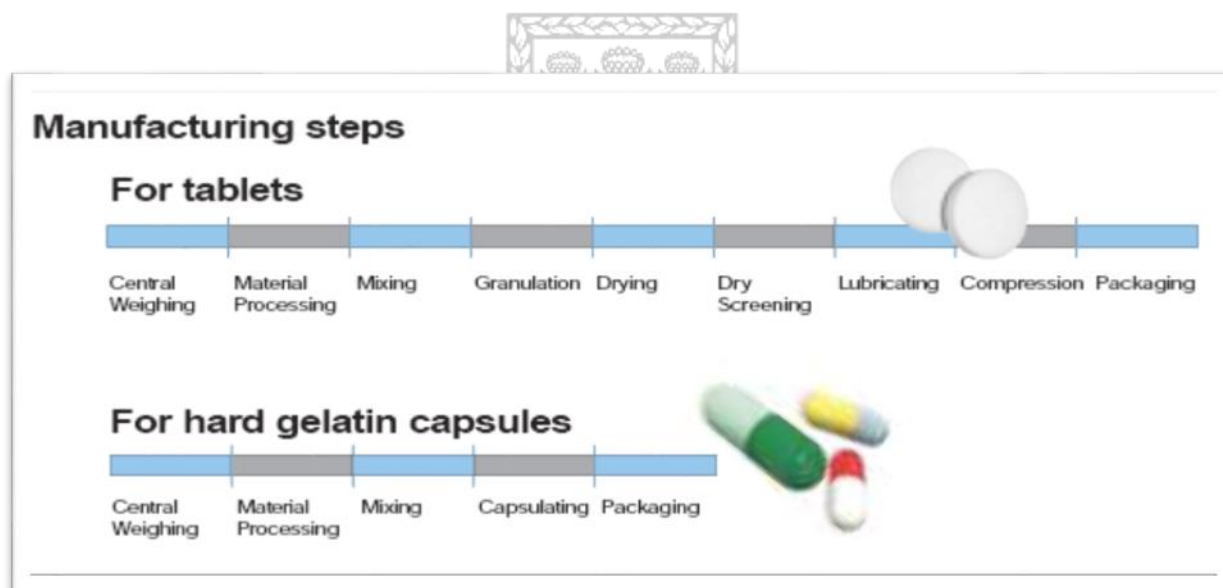


Figure 2. 10 Comparison of manufacturing process of tablets and hard gelatin capsules
(MA 2006)

In view of the above-mentioned advantages of capsules, this solid dosage form was deemed appropriate for *A. afra* plant medicine. As required for conventional pharmaceutical preparations, it is also important that herbal preparations are of the highest quality, highlighting the need for appropriate quality control and evaluation.

2.4 Evaluation of herbal dosage forms

2.4.1 The general criteria for the evaluation of quality of herbal dosage forms

Specifications of quality are important components of the quality assurance of any product. These specifications can be defined as tests or references to analytical and biological procedures, which can be stated in numerical limits, ranges, or other criteria and can be used to assure the quality of the product (EMA 2011). In the case of herbal dosage forms, the quality is determined by setting specifications for the quality of the starting plant material, the formulation development process, validation of the manufacturing process, consistency of production and quality assurance of the manufactured herbal product, as well as specifications for the shelf life during storage (Fong 2002).

2.4.2 Characterization: quality of starting plant material(s) for herbal products

The consistent quality of products of herbal origin can only be assured if the starting plant material is characterized in a detailed manner. Such characterization involves definition of the botanical source, plant part used and its state (i.e. whole, reduced, powdered, fresh or dry), as well as the geographical source and conditions under which the herbal drug or materials were obtained (Forte & Raman 2000).

To ascertain whether a plant material meets required quality criteria or specifications, it must be subjected to quality control tests (Liang et al. 2004). Detailed characterization of the plant material should be conducted in terms of its specific and unique organoleptic properties. The macroscopic and microscopic botanical characteristics of the plant material are taken into consideration. Biological, geographical, cultivation, harvesting, drying conditions and pre- or post-harvest chemical treatment variations are also considered in the characterization of plant material quality. Identification tests to discriminate between related species or potential adulterants/substitutes are also necessary for appropriate characterization of the plant materials.

2.4.3 Design and development of quality control tests for herbal dosage forms

The knowledge and data accumulated during development of the herbal dosage form is the primary information used to set specifications for the assurance of its quality. Certain specifications can be included or excluded based on the data obtained during development of the herbal dosage forms. For example, some limit tests for pesticide residues can be excluded from the regular battery of quality control tests if the herbal drug is grown under strict conditions of organic cultivation without pesticides. On the other hand, the inclusion of microbial contamination tests is recommended when the herbal drugs are hygroscopic, take up moisture into the product and are prone to harbor or encourage microbial growth. Such microbial limit tests can also be excluded for herbal extracts or tinctures depending on the alcohol content, and if justified by scientific evidence (Komperlla 2004).

2.4.4 Quality control specifications for manufactured and finished products

Currently, the quality control criteria used for herbal products are mainly those adapted and found in the specified Pharmacopoeias and WHO guidelines for quality control and evaluation of crude drugs (Sharad et al. 2011; Forte & Raman 2000). These include microscopic characteristics, chemical identification tests, total ash and hydrochloric acid insoluble ash levels, particle size, inorganic impurities and heavy metals limit tests, microbial contamination limit tests, mycotoxins-presence tests, and pesticide residue tests, etc.

For herbal products, the setting of quality control tests and specifications would generally pose challenges. This is partly due to the fact that for most herbs, the specific ingredient that causes a particular therapeutic effect is not known. Herbal medicines are complex mixtures, usually comprising at least 50 chemical constituents, although most contain many more (Liang et al. 2004). As such, reference material is important for evaluation of herbal medicines quality. A reference standard, or reference material, is a substance prepared for use as the standard in an assay, identification, or purity test (EMA 2011). The reference standard has a quality appropriate to its use. The composition of reference standards intended for use in assays should therefore be adequately controlled and the authenticity and/or purity of a standard should be determined by

validated procedures. In the case of herbal materials where the constituents responsible for the therapeutic activity are unknown, appropriate marker substances may be used (EMA 2011). For many herbal products, suitable marker compounds for assessment of product quality have not been established; however, such tests can be conducted using one or other constituent of the plant medicine as marker. Furthermore, appropriate statistical tests should be applied, when necessary, for analysis of the quantitative or and qualitative data obtained (Barnes 2003).

As test procedures for herbal materials can be adapted from that in the pharmacopoeias and WHO guidelines for crude drugs, the test procedures for dissolution, mass and content uniformity, and moisture content of herbal products can all also be adapted from specified procedures found in the pharmacopoeia. These can be used to assess the quality of herbal capsules in-process and/or finished products (EMA 2011).

2.5 Development and evaluation of herbal capsule dosage forms

2.5.1 Factors of relevance in the evaluation of *Artemisia afra* herbal raw material

Generally, the major constituent of herbal capsule product is the active ingredient, which is the powdered plant material (e.g. dried leaves, bark, roots and/or stems, aqueous or alcoholic extracts, etc.). In addition, various excipients may be added to the plant material to provide an appropriate powder mix, which is filled into the capsule. The nature of the powdered plant material in the powder mix to be incorporated into capsules is a crucial factor in the formulation development. Because the powdered plant material is the major constituent of the herbal capsule dosage form, it is essential that this powder is evaluated for its organoleptic features (e.g. colour, odour and taste), microscopic and macroscopic properties, physicochemical (i.e. ash values, moisture content, extraction matter etc.) and biological properties, and phytochemical parameters (assay for active constituents).

2.5.1.1 Organoleptic features

Organoleptic refers to any sensory properties of a product involving taste, colour, odour and feel or texture. Organoleptic testing involves inspection through visual examination, feeling, tasting and smelling of products (Nkengla 2014).

Colour is a vital means of identification for many pharmaceutical capsules, and is important for consumer acceptance. The eye cannot differentiate small differences in colour nor can it precisely define colour and as such, efforts have been made to quantitate colour evaluations (Sehume 2010). The colour of the product must be uniform within a capsule and from lot to lot. Non-uniformity of colour (referred to as mottling) not only lacks visual appeal but could also be associated with non-uniformity of content and poor product quality.

The smell of a product is another important determinant of consumer acceptance of the product. It can provide an indication of drug quality as the presence of an odour in a product batch could indicate a stability problem. An example is the characteristic odour of acetic acid in degrading aspirin tablets. The presence of an odour may however be characteristic of the drug (e.g. vitamins), added ingredients (e.g. flavouring agent) or the dosage form (e.g. film-coated tablets). Taste is also important for consumer acceptance of certain formulations, e.g. chewable tablets. Many companies utilize taste panels to assess preference for different flavours and flavour levels during product development. Taste preference is however subjective and the control of taste in product (for instance, chewable tablets) development is usually based on the presence or absence of a specified taste (Sehume 2010). Capsules have the advantage of masking taste which may enhance consumer patronage and compliance (Vihar et al. 2007).

2.5.1.2 Microscopic and macroscopic properties of powders

2.5.1.2.1 Macroscopic properties of powders

Sieving is one of the fundamental methods for classification of powders. It is the method of choice for determining size distribution of coarse powders (WHO 1998). Microscopic examination of the raw drug substance provides an indication of particle size and particle size range of the drug substance, as well as of its structure (Allen 2008). During some processing procedures, the solid drug powder must flow freely and not become entangled or agglomerated. Spherical and oval-shaped powders flow more easily than needle-shaped powders and may facilitate processing.

Particle size and its distribution affects certain physical and chemical properties of drug substances, such as drug dissolution rate, bioavailability, content uniformity, taste, texture, colour, and stability (Nkengla 2014; Dube 2006). They both play significant roles in flowability and other powder properties such as bulk density, angle of repose, and compressibility of bulk solids (Komperlla 2004). Flow characteristics and sedimentation rates (of suspensions) are also important factors related to particle size. It is essential to establish, as early as possible in the formulation process, how the particle size of a drug substance may affect formulation and product quality (Allen 2008). This is underscored by the fact that even a small change in particle size can cause significant alterations in flowability. A reduction in particle size tends to decrease flowability of a given granular material due to the increased surface area per unit mass (Allen et al. 2014).

2.5.1.2.2 Microscopic properties of powders

Quality control of herbal drugs has traditionally been based on appearance. Today, microscopic evaluation is crucial in the initial identification of herbs, as well as in identifying small fragments of crude or powdered herbs, and for detecting foreign matter and adulterants (Gautam 2010). A primary visual evaluation, which seldom requires more than a simple magnifying lens, can be used to ensure that the plant material is of the required species, and that the right part of the plant is being used. Sometimes microscopic analysis is also required to determine the correct species or to verify that the correct part of the species is present (Sehume 2010).

2.5.1.3 Water content

Assessment of water content is important for herbal drug preparations (especially extracts such as *A. afra*) which are known to be hygroscopic. One characteristic of hygroscopic powders is that they continuously change their physicochemical properties when exposed to relative humidity and temperature conditions that either favour the absorption or loss of moisture (Sehume 2010; Komperlla 2004). The change in physicochemical properties of the powder is reflected by changes in particle size and shape which, in turn, directly affects the flow properties of the powder. As such, the encapsulating properties (i.e. flowability) of powders directly depend on the hygroscopicity of the powder particles (Komperlla 2004).

The moisture acquired due to the hygroscopicity of the powder may lead to degradation of the powder when enzymes like glycosidase are activated. Water impurities generally include minerals, viruses, bacteria and other organic material (Sehume 2010) which may lead to microbial contamination. Such problem can be minimized or remedied by restricting capsule manufacturing to conditions where the relative humidity (RH) is below 40%.

2.5.1.4 Microbial contamination

Microbial contamination could be due to algae, bacteria and or fungi (Suliman et al. 2010; Kneifel et al. 2002). Inevitably, this microbiological background depends on several environmental factors, and ultimately exerts an important impact on the overall quality of herbal products and preparations (Breemen et al. 2008). Microbial contamination of medicinal plant parts could be the result of inappropriate harvesting and cleaning of raw plant material, unhygienic processing of plants and/or incorrect transport mechanisms. Plants intended for use in botanical dietary supplements should be cultivated using Good Agricultural Practice (GAP) guidelines. This approach provides quality assurance by helping to prevent microbial as well as heavy metal, herbicide, and pesticide contamination and by excluding weeds and insects. If wild plant specimens are collected or plant material is purchased from suppliers without GAP assurance, such material should be assayed for levels of pesticides, herbicides, heavy metals, and microbes (Breemen et al. 2008).

2.5.1.5 Level of heavy metal and pesticides

Heavy metals and pesticides are known contaminants or adulterants of many traditional remedies (Street 2012; Obi et al. 2006). A common misperception among the general population is that natural substances cannot be present in toxic concentrations in a variety of herbal preparations and dietary supplements (Ernest 1998). The study entitled “Heavy metal hazards of Nigerian herbal remedies” revealed that high levels of iron, nickel, calcium, copper, lead, selenium, and zinc in “herbal remedies” would cause adverse health effects when regularly taken as recommended (Obi et al. 2006). Failure to establish the true cause of exposure means that the

patient continues to take the metal-containing medication. Thus, the screening of traditional remedies for safety and efficacy has been recommended to protect the general public.

As heavy metal components can be dangerous in preparations, even in trace amounts, they have to be removed from medicinal products. Limit tests for these materials have been prescribed by the WHO (Parthik et al. 2011; WHO 1998). They have also established the maximum residue limit (MRL) for biocides in medicinal plant cultivation. The MRL is calculated after safety tests in human beings, which indicate toxicologically acceptable levels according to the reliable assay available. The MRL (in mg/kg) is calculated using the following formula:

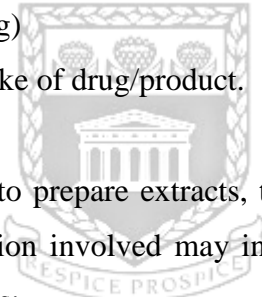
$$MRL = ADI \times W \times MDI \times [100 \times \text{safety factor}]$$

where ADI = acceptable daily intake (mg compounds/ kg body weight)

W = body weight (kg)

MDI = mean daily intake of drug/product.

When the herbal crude drug is used to prepare extracts, tinctures or other phytopharmaceutical formulations in which the manipulation involved may influence pesticide concentration of the final product, the MRL is calculated as:



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$$MRL = \frac{ADI \times W \times E}{MDI \times [100 \times \text{safety factor}]}$$

where E = the extraction coefficient of the pesticide which depends on the method of preparation and needs to be experimentally determined.

The WHO (2007) guidelines for assessing quality of herbal medicines with reference to contaminants and residues were used to assess the acceptability level of heavy metals in herbal medicines.

The need for inclusion of tests and acceptance criteria for inorganic impurities should be studied during product development. Such need and the appropriate tests should be based on knowledge of relevant plant species, as well as their cultivation and manufacturing processes. The

acceptability level of contaminants in herbal medicines can be adapted from the WHO guidelines for assessing herbal medicine quality. Acceptance criteria will ultimately depend on safety considerations. Where justified, procedures and acceptance criteria for contaminants should follow pharmacopoeial precedents or WHO guidelines, and other appropriate procedures where necessary (Govindaraghavan & Sucher 2015).

2.5.2 Analytical techniques used in quality control of finished herbal capsules of *Artemisia afra*

The general quality control specifications set for plant materials used in oral dosage forms as well as capsules manufactured from plant materials were explained in section 2.4.4. For many of these tests (e.g. content uniformity and dissolution test), a validated analytical procedure using a suitable marker compound is required. The question to answer however remains: “What is a suitable marker for evaluation of plant materials such as *Artemisia afra*?”

Artemisia afra is rich in chemicals that can be used as markers in analytical procedures. The presence of the flavonoids quercetin, luteolin, apigenin, hesperetin, and kaempferol in this plant has been documented (Tikiso 2015; Dube 2006; Komperlla 2004). At least two of these flavonoids, quercetin and luteolin, have, as mentioned in section 2.3.3, been documented as being pharmacologically active supporting the hypothesis that flavonoids may contribute to the biological activity of this plant, and could therefore be appropriate phytochemicals to monitor in quality control studies of *A. afra* herbal dosage forms. Thus, to study content uniformity of *A. afra* capsule dosage forms, quercetin and luteolin can serve as markers and their quantification by HPLC analysis used for evaluation of the capsule contents. Indeed, High Performance Liquid Chromatography (HPLC) and Mass spectrometry (MS) are the modern techniques used as tools for identification and quantitative analysis of flavonoids, and these have been successfully used for flavonoid identification and quantification in a number of studies (Tikiso 2015; Nkengla 2014; Dube 2006; Komperlla 2004; Harborne & Williams 2000).

The analysis of flavonoids is however a multi-step process. The first step in the quantitative analysis of flavonoids is acid-hydrolysis of the plant material. This is followed by organic phase

extraction to separate the free aglycones from the other flavonoids /flavonoid glycosides. Thereafter, HPLC is employed to further isolate individual compounds (Tura & Robards 2002). For HPLC analyses, the selection of suitable mobile phase, column, and absorption wavelength are important considerations in method development. The analytical procedure involves identification of component compounds, measurement of detector responses, development of calibration curves (detector response of standard samples *versus* concentration of standard sample), and validation of the assay. The validated HPLC assay can then be used to measure flavonoid content of the capsule or level of flavonoids released during dissolution test of the plant capsule.

Dissolution testing is a quality control method for evaluating physiological availability of products in an *in vitro* setting. It depends upon having the drug in a dissolved state (Sehume 2010). The dissolution test measures the percentage of the API that has been released from tablets or capsules and has dissolved in the dissolution medium during controlled testing conditions within a defined period. It is one of the most important and useful *in-vitro* tests for assuring product quality and batch to batch consistency (Sharma 2012; Darling 2012; WHO 2007a). *In vitro* dissolution often acts as a guide to the selection of prototype formulations. It also helps to determine optimum amounts of ingredients needed to achieve requisite drug release profiles. In addition, dissolution testing provides information on the impact of changes – in composition, process or site of manufacture – on drug release characteristics. This can help to identify potential problems with *in vivo* release and bioavailability following product administration (FDA 1997).

The dissolution of a pure substance follows the Noyes Whitney equation:

$$\frac{dc}{dt} = kS(C_s - C_t)$$

where

dc/dt	=	the rate of dissolution
k	=	dissolution rate constant
S	=	surface area of the dissolving solid
C_s	=	saturation concentration of the drug in the diffusion layer, and

C_t = concentration of the drug in dissolution medium (or the bulk)
(Sehume 2010)

To predict dissolution and bioavailability of drug products, a scientific framework – the Biopharmaceutics Classification System (BCS) – exists for classifying drug substances based on their aqueous solubility and intestinal permeability. The Biopharmaceutics Classification System (BCS) was originally developed for chemically defined synthetic drug substances; however, it may also help with Herbal Medicinal Products (HMPs) (Darling 2012; WHO 2007; FDA 1997). When combined with dissolution of the drug product, the BCS takes into account three major factors that govern the rate and extent of drug absorption from immediate-release solid oral dosage forms: dissolution, solubility, and intestinal permeability (FDA 1997). According to the BCS, drug substances are classified as follows:

Class 1: High Solubility, High Permeability

Class 2: Low Solubility, High Permeability

Class 3: High Solubility, Low Permeability

Class 4: Low Solubility, Low Permeability



In addition, solid oral dosage forms are categorized as having rapid or slow dissolutions. Within this framework, when certain criteria are met, the BCS can be used as a drug development tool to help medicine regulators justify requests for biowaivers (Hussain A 2002).

In conducting dissolution tests for herbal products, compendial methods for botanical products using the appropriate apparatus may be employed. Generally, dissolution testing of capsules is carried out using the basket (USP apparatus 1) method under mild agitation in aqueous buffer ranging in pH from 1.2 to 6.8. Data obtained is presented as cumulative percentage of dissolved material (BP 2013a).

Data from a product's quality control tests can be used to compile a quality control dossier for its registration with approved medicine regulatory authority. It can also be used to establish the product quality part of an Investigator's Brochure (IB) to be used to motivate clinical trials.

2.6 Quality control dossier

A dossier is a document submitted to an appropriate medicine regulatory authority, for consideration in view of granting approval, for the registration of a drug product. It is submitted in the form of a common technical document (CTD). The CTD is a harmonized format (template) for presenting product pre-registration data in countries that ascribe to the guidelines of the International Conference on Harmonization (ICH) (Swapna et al. 2014) (EMA 2013). Generally, the CTD is presented in an electronic Common Technical Document (eCTD) form. The eCTD is defined as an interface for industry to agency transfer of regulatory information while at the same time taking into consideration the facilitation of the creation, review, lifecycle management and archival of the electronic submission (Reddy 2011) (Medicine Control Council 2012).

The eCTD dossier is divided into five main modules (Figure 2.11) (Jordan 2014):

Module 1: Administrative information and prescribing information

Module 2: Overviews and summaries of modules 3–5,

Module 3: Quality (pharmaceutical documentation)

Module 4: Non-clinical reports (pharmacology/ toxicology)

Module 5: Clinical study reports (clinical trials)

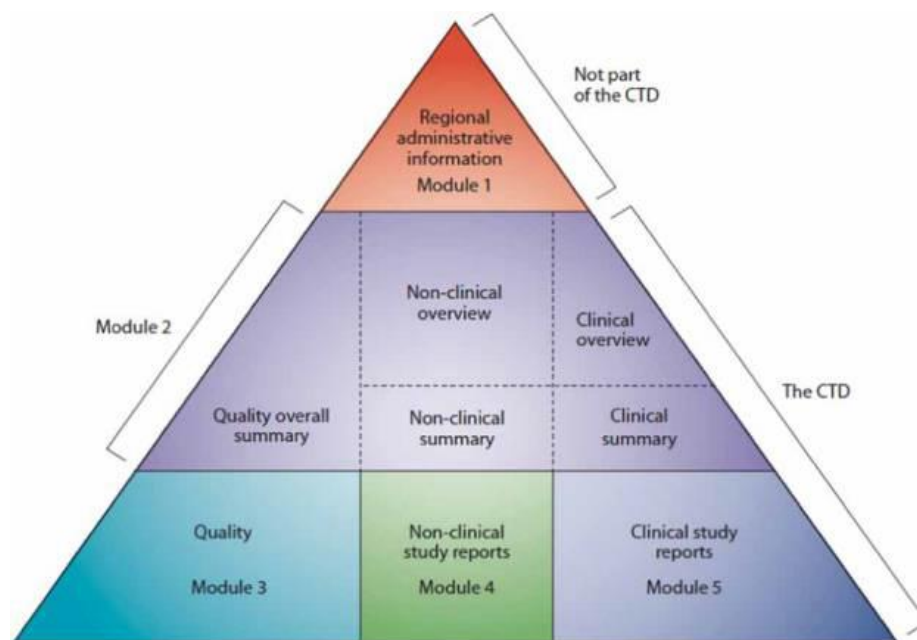


Figure 2. 11 The CTD triangle (Jordan 2014)

Module 1 is not strictly included in the eCTD since it contains regional administrative information, such as application forms or the proposed label. Modules 2–5 though are common to all regions, and these comprise the main body of the eCTD. Module 2 contains the eCTD overviews and summaries. It starts with a general introduction to the drug, including its pharmacological class, mode of action, and proposed clinical use. Module 2 then provides an overall summary of the ‘quality’ information (i.e. the pharmaceutical documentation) of the drug, as well as its non-clinical and clinical overviews, non-clinical written summaries and tabulated summaries, and the clinical summary. The information provided in Module 2 is based on the foundation material that is provided in module 3 for the quality information, module 4 for the non-clinical information, and module 5 for the clinical information (Swapna et al. 2014).

For this study, *A. afra* capsules were evaluated for quality control characteristics. Thereafter, the data was used to compile a quality control dossier which can be included in module 3 of the eCTD. A summary of the quality control dossier for *A. afra* capsule is given in appendix 5.10. The information gathered on the *A. afra* capsule can also be used to prepare the product quality part of an Investigational Drug Brochure (IDB) suitable for clinical trial of *A. afra* capsules in chronic asthma.

2.7 Assessment of an investigational drug brochure

An Investigator's Brochure (IB) is a compilation of clinical and nonclinical data on the investigational product(s) that are relevant to the study of the product(s) in human subjects (Fiebig 2014). Its purpose is to provide the investigators and others involved in the trial with information to facilitate their understanding of the rationale for, and compliance with many key features of the protocol such as the dose, dose frequency/interval, methods of administration, and safety monitoring procedures. The IB also provides insight to support the clinical management of the study subjects during the course of the clinical trial (Health Canada 2003). The information in the IB should be presented in a concise, simple, objective, balanced, and non-promotional form that enables a clinician, or potential investigator, to understand it and make his/her own unbiased risk-benefit assessment of the appropriateness of the proposed trial.

It is expected that the type and extent of information available will vary with the stage of development of the investigational product. If the investigational product is marketed and its pharmacology is widely understood by medical practitioners, an extensive IB may not be necessary. Where permitted by regulatory authorities, basic product information such as brochure, package leaflet, or labelling, may be an appropriate alternative, provided that it includes current, comprehensive, and detailed information on all aspects of the investigational product that might be of importance to the investigator. If a marketed product is being studied for new use (i.e., a new indication), an IB specific to that new use should be prepared. The IB should be reviewed at least annually, and revised as necessary, in compliance with a sponsor's written procedures. More frequent revision may be appropriate depending on the stage of development and the generation of relevant new information. However, in accordance with Good Clinical Practice (GCP) guidelines, relevant new information may be so important that it should be communicated to the investigators, and possibly to the Institutional Review Boards (IRBs)/Independent Ethics Committees (IECs) and/or regulatory authorities before it is included in a revised IB (ICH 2009; Health Canada 2003).

Generally, the trial sponsor is responsible for ensuring that an up-to-date IB is made available to the investigator(s) while the investigator(s) is/are responsible for providing the up-to-date IB to the responsible IRBs/IECs. In the case of an investigator-sponsored trial, the sponsor-investigator

should determine whether a brochure is available from the commercial manufacturer. If the investigational product is provided by the sponsor-investigator, then he or she should provide the necessary information to the trial personnel. In cases where preparation of a formal IB is impractical, the sponsor-investigator should provide as a substitute, an expanded background information section in the trial protocol that contains the minimum current information described in the guidance for IB (ICH 2009).

In the present project the intention was however just to focus on the product quality aspects of *A. afra* capsules that may need to be added to the IB.



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CHAPTER 3

WORK PLAN

3.1 Introduction

In this chapter, the specific objectives, hypothesis and study approach for this study are described.

3.2 Objectives

The overall aim of this study was to prepare a dossier and parts of an investigator's brochure (IB) for *A. afra* capsules suitable for motivating product registration and clinical testing in chronic asthma, using data obtained from quality control tests of the product.

3.2.1 Specific objectives

The specific objectives of the study were:

1. to establish (from various medicine regulatory authorities) the minimum product quality requirements for registration of *A. afra* capsules;
2. to prepare and pharmaceutically characterize a capsule product of *A. afra* FDAE using its product quality control data and
3. to identify the pharmaceutical product quality aspects of an investigator's brochure (IB) appropriate for use in motivating a clinical trial of *A. afra* capsules in asthma.

3.3 Hypothesis

It was hoped that the pharmaceutical monograph of a product such as Prospan™ may be used as a template to compile the product quality control component of a registration dossier and identify the product quality parts of an IB suitable for motivating registration and clinical testing of *A. afra* capsules in chronic asthma, respectively.

3.4 Study approach

To realize the above objectives, the following were done:

3.4.1 Establishment of minimum product quality control requirements for registration of *Artemisia afra* capsules

Information on the minimum product quality requirements for herbal medicine registration and the criteria that may be used to establish pharmaceutical quality of *A. afra* capsules was sourced from pharmacopoeias and the databases of various regulatory agencies such as the European Medicine Agency (EMA), Health Canada, the Therapeutic Goods Administration (TGA) of Australia, and the World Health organization (WHO). The information required by the British Pharmacopoeia (BP) was expected to be the similar to that of the other pharmacopoeias, such as the United States Pharmacopeia (USP), the Japanese Pharmacopeia (JP) and the German Pharmacopeia (GP), and hence only the BP was searched for this study. Among the regulatory agencies, the guidelines of the EMA were considered since most of the information provided by this agency typically relates to the strong conclusions reached by the EMA scientific committees following their procedures for evaluating the pharmaceutical quality of medicines (EMA 2011), while the regulatory guidelines set by the TGA and Health Canada were also considered mainly because the South African medicines regulator (MCC) frequently aligns itself with these best recognised medicine regulatory authorities (Vienings 2015). The WHO guidelines were also considered due to the global recognition of its guidelines on quality control of herbal medicines (Vienings 2015).

3.4.2 Preparation and characterization of *Artemisia afra* capsules

For this study, the freeze-dried aqueous extract (FDAE) of *A. afra* was used, as it closely resembled the traditional liquid dosage form of the plant used by consumers. In addition, the process of freeze-drying would produce a convenient form of the plant material. Capsule dosage forms were chosen as appropriate because they offered benefits such as flexibility of formulation and ease of transport. Consequently, a decoction of an *A. afra* leaves was prepared using distilled water as the solvent. The decoction was frozen in a dry ice-acetone bath and freeze-dried to afford an acceptable solid form of the plant extract. The FDAE and the capsules of *A. afra* were then evaluated for appropriate physicochemical properties. Data obtained from these quality control studies were used to compile quality control information for the dossier. The data was also utilised to identify pharmaceutical quality aspects of an investigator's brochure, which would be used for motivating registration and clinical testing of *A. afra* capsules in chronic asthma.

3.4.3 Identification of an Investigator's Brochure for *Artemisia afra* capsules

For design of the investigational drug brochure, information on the typical contents of investigator's brochures for herbal products was sourced online from various regulatory agencies' sites (e.g. EMA, Health Canada, TGA, and the International Conference on Harmonization (ICH) and from the Prospan™ dossier. Prospan™ is cough medication which is mainly used in the treatment of productive cough. It is also reputed to assist with relief from symptoms of chronic inflammatory conditions such as bronchitis, asthma, emphysema and pneumonia. Prospan™ contains dried ivy leaf extract and the ivy leaf extract is claimed to have bronchodilatory and anti-inflammatory effects, probably mediated through the actions of its main active ingredient, the saponins. This information was then used to identify pharmaceutical product quality aspects of an investigator's brochure (IB), which would be appropriate for use in motivating a clinical trial of *A. afra* capsules in chronic asthma.

CHAPTER 4

Minimum quality control requirements for registration of *Artemisia afra* capsules

4.1 Introduction

As elaborated in chapter 3, one very important objective of the study was to establish the minimum product quality requirements for registration of a herbal product such as *A. afra* capsules. For this, the minimum quality requirements for registration of the *A. afra* capsules as well as the quality attributes, specifications, and test methods for the pharmaceutical quality control of such *A. afra* capsules, were to be established. In this chapter, the methods used to achieve these objectives are described, and the results obtained and conclusions drawn from such results presented.

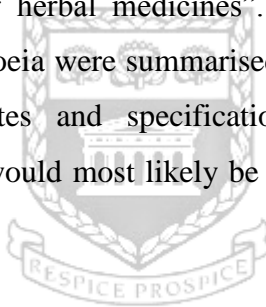
4.2 Methods:

4.2.1 Establishment of the minimum quality control requirements needed for registration of herbal product such as *Artemisia afra* capsules.

For this, internet searches were conducted from April to July 2015 for general information on the minimum quality control requirements needed for registration of herbal products. The websites of several regulatory authorities (www.ema.europa.eu, www.tga.gov.au, www.healthcanada-sc.gc.ac, www.who.int); the databases: PubMed and Science Direct; and the pharmacopoeias (e.g. British Pharmacopoeia (BP)) were searched. The search was conducted using the following key words and phrases: “registration of herbal products”, “quality control of herbal medicines” and “guidelines for registration of herbal medicines”. The information obtained from the various agencies was then compared and assessed for criteria that may be suitable for the establishment of minimum quality control requirements for registration of herbal capsule products such as *A. afra* capsules.

4.2.2 Establishment of quality characteristics, specifications and test methods suitable for pharmaceutical quality control of herbal capsule products such as *Artemisia afra* capsules

To determine information on the pharmaceutical quality attributes, specifications, and test methods for quality control that would be appropriate for *A. afra* capsules, an internet search of the websites and databases of the following medicine regulatory agencies: viz. the European Medicine Agency (EMA) (www.ema.europa.eu); the Australian agency or Therapeutic Goods Administration (TGA) (www.tga.gov.au); Health Canada (www.healthcanada-sc.gc.ac); the World Health Organization (www.who.int); Science Direct (www.sciencedirect.com); PubMed (www.pubmed.gov); and Pharmacopoeias (www.pharmacopoeia.com) such as the British Pharmacopoeia (BP); was conducted from July to September 2015. The following search terms were used: “guidelines for herbal medicines”, “quality control specifications for herbal medicines” and “quality control for herbal medicines”. The data obtained from the various medicines regulators and pharmacopoeia were summarised, compared and evaluated to establish the pharmaceutical quality attributes and specifications, as well as tests methods for pharmaceutical quality control that would most likely be applicable for herbal capsule products such as *A. afra* capsules.



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4.3 Results and Discussion:

4.3.1 Minimum quality requirements for registration of herbal product such as *Artemisia afra* capsules

The purpose of the chapter was to establish the minimum quality requirements for registration of herbal product such as *A. afra*. According to the TGA, Health Canada and WHO guidelines, quality requirements for herbal products were found to be classified into those quality requirements needed for raw materials (table 4.1) and those required for the finished herbal product (table 4.2). The requirements for herbal raw materials include information on: (1) identity of the plant, (2) parts and physical features of the plant used, (3) manufacturing details, (4) general identity tests, (5) molecular or structural formula of herbal ingredients, (6) purity tests, and (7) quality control specifications. For the finished herbal product, the quality requirements consist of the data on the following: (1) manufacturers of the product, (2) details on the qualitative and quantitative composition of the active components, (3) information on control of the excipients, (4) description and composition of the product, (5) stability data of the product, (6) packaging, (7) container closure system and labelling information, and (8) the certificate of analysis. There were some similarities and differences amongst the TGA, Health Canada and WHO guidelines in terms of the information required for the minimum quality requirements for registration of herbal medicinal product. However, the information provided by the three agencies were all important and ideal despite their differences. It was therefore concluded that the requirements advocated by the three would be suitable for registration of herbal product such as *A. afra* capsules.

Table 4.1 Summary of quality requirements for registration of herbal raw materials or API advocated by the TGA, Health Canada and WHO guidelines

Quality requirements for registration of herbal medicines	Australia (TGA)	Health Canada	WHO guidelines
Identity of the plant	Binomial scientific name of plant, synonyms mentioned in Pharmacopoeias, nomenclature of the plant material using the approved name in Australia, chemical name, compendial name.	Binomial, Latin, synonym, vernacular name of the plant material.	Definition (i.e. Latin name of the plant including Genus, species etc.), synonym, (i.e. legitimate Latin binomial synonyms for the plant), vernacular name, the geographical description of the plant and a brief description of the living plant including photographs and or drawings.
Parts and physical features of the plant used	The macroscopic features (e.g. shape, size) or organoleptic features (e.g. taste, smell, colour etc.), microscopic, physical form of the drug, structural formula of the ingredients.	The organoleptic features such as taste colour, smell etc., the morphology (macroscopic and microscopic features) and anatomical parts of the plant.	General appearance or organoleptic properties (e.g. taste, smell, colour etc.) of powdered plant material, macroscopic (e.g. shape, size) and microscopic features.
Manufacturing details	List of manufacturers, description of manufacturing process, controlled materials, process validation and evaluation of manufacturing and process development.	Process of validation, processes of manufacture, description of how materials were processed.	Description of the process of manufacture.
General identity tests	Physiochemical properties and other relevant properties.	Chemical identification procedures including methods such as infrared spectrometry.	Chemical, biological or physical assay.
Molecular structural formula of herbal ingredients	Structural formula including the relative and absolute stereochemistry for the ingredient, or components, e.g. herbal components.		
Purity tests	Acid insoluble ash, extract, loss on drying, pesticide residues, heavy metals.	Acid insoluble ash, water content, foreign matter, non-medicinal ingredients.	Foreign matter, water solute extractive, loss on drying, microbiological tests, etc.
Quality control specifications	Specifications providing a set of tests and limits, analytical procedures and validation, certificate of analysis, justification of specifications.	The pharmacopoeial monograph specifications should be considered as minimum specifications for testing of the medicinal ingredients. Isolates and synthetic duplicates of materials of origin, e.g. flavonoids such as rutin, and vitamins, should be identified at raw material stage by physical description and appropriate chemical identification tests such as infrared spectrometry.	Methods of analysis and validation.

Table 4.2 Summary of quality requirements for registration of finished herbal product (FHP) advocated by the TGA, Health Canada and WHO guidelines

Quality requirements for registration of herbal medicines	Australia (TGA)	Health Canada	WHO guidelines
Manufacturers of the product	Manufacturer's information, batch formula, description of the manufacturing process and process control, control of critical steps and intermediates.	Description of the test parameters, analytical procedures and tolerance limits of the finished herbal product.	Description of the manufacturing process for the herbal product.
Details on the qualitative and quantitative composition of the active components	Description of the constituents with known therapeutic activity or markers. If the marker is present, chemical identity tests should be conducted (e.g. comparison of chromatographic (HPLC) retention time).	Quantification by assay for the presence of a compound or excipient. Where a marker is present, chemical identity tests should be conducted (e.g. comparison of chromatographic (HPLC) retention time).	Quantification of the active ingredient; quantity and type of excipients.
Information on control of excipients	Specifications, analytical procedures, validation of analytical procedures, justification of specifications, excipients of human or animal origin, novel excipients.		
Description and composition of the product	Name, dosage form, e.g. modified release. Formulation of the medicine, e.g. tablets of the active ingredient and excipients. Total amount of residual solvents that may be present in the finished product.	Description of the final dosage form (physical features and tests for final product such as the appearance, name and dosage form).	Specifications of quality for the finished product - description of the dosage form, the designated name(s) of the active ingredient.
Stability data of the product	Stability data must be sufficient to demonstrate consistent quality of the herbal product.	Stability data produced must demonstrate that the product intended for the market will remain of the quality required throughout its stated shelf life.	Stability data produced must demonstrate that the product intended for the market will remain of the quality required throughout its stated shelf life.
Packaging, container closure system and labelling information	Description of the type and form of container in which the final product will be packaged and its closure system. A label should comply with all relevant legislation and advertising requirements and also be appropriate for the claimed therapeutic use in the targeted population group.	The type and form of container used for the final product. The label should contain the brand name or code of the natural health product, the expiry date, recommended storage conditions, the name and address of the manufacturer.	Packaging should be in accordance with national registration requirements. The label should have a quantitative list of the active ingredients, including the plant names, dosage form, manufacturing date, name of manufacturer or company with full address.
Certificate of analysis	Identity tests for presence of actives, microbiological count and tests for other toxins, uniformity of weight, dissolution profile, assay of actives if available.	Identification of references by chromatographic retention times (using HPLC and/or TLC), microbiological count and tests for other toxins (if the starting materials are not tested for these individually), uniformity of weight, loss on drying or water content, dissolution profile.	Microbiological count tests (if the starting materials are not tested for these individually), uniformity of weight and mass, loss on drying or water content, dissolution profile.

For this study, the focus was to determine the product quality requirements needed to fulfil the registration of *A. afra* capsules. Based on the findings, the TGA, Health Canada and WHO recommend that details for the minimum quality requirements must be provided on the raw materials. Among the three agencies, there were some differences and similarities on the minimum quality requirements for registration of herbal medicines. For instance, only the TGA required information on the molecular structure of the herbal ingredients which is important for identifying the plant material though it might be quite challenging to elucidate molecular and/or structural formula of herbal ingredients, given the potential for herbal products to contain up to a hundred or more ingredients /compounds. In terms of the manufacturing details, the TGA and Health Canada outlined in detail what should be provided (e.g. a list of manufacturers, description of manufacturing process as well as information on control of the excipients, validation processes, evaluation of manufacturing and process development) while the WHO only required the description of the manufacturing process. Another important feature was the details of the quality control specifications, where the TGA and Health Canada gave more information on what should be provided (e.g. specifications consisting of a set of all tests and limits, analytical procedures, validation of manufacturing and process development, etc.).

All the other minimum quality requirements for registration of herbal medicines were similar for the TGA, Health Canada and WHO, particularly with respect to information on the identity of the plant (which proves or ensures its correct identification). In addition, parts and physical properties of the plant used throughout the manufacturing process are required, as well as the general tests which further ensures the identity and quantification of references in plant materials as part of quality control measures.

As far as the finished herbal product (FHP) is concerned, similar information was required by all the three agencies particularly on the details of the manufacturers of the product, and information on quality assurance methods. Another similarity is in the details of the qualitative and quantitative composition of active components, as well as the identification and quantification of the active ingredient of the plant material. In addition, the description and composition of the product where the product should be clearly specified in terms of name, dosage form, and formulation should also be given. Furthermore, stability studies of the product which should

clearly state the appropriate storage conditions and support the claimed shelf-life are required. The packaging, container closure system and labelling, with packaging specifications in accordance with national regulatory requirements and the minimum mandatory information on the label (e.g. official name, dosage form, formulation, batch and expiry date etc.) should be given and adhered to. Lastly, the certificate of analysis containing all information on the minimum quality requirements, specification and results for registration should be provided.

While similarities are noted among the three agencies with right to the herbal requirements, details on the control of excipients were only required by the TGA.

Overall, after analysis, the minimum quality requirements for registration of herbal medicines advocated by the TGA, Health Canada and WHO seemed sufficient for use in registration of herbal product such as *A. afra* capsules. Therefore, it was concluded that the minimum quality requirements for registration of *A. afra* capsules, with respect to the raw materials, would consist of information on the following: (a) identity of the plant, (b) parts and physical features of the plant used, (c) details of manufacture, (d) general identity tests, (e) purity tests, (f) molecular structure formula of the herbal ingredient(s), and (g) quality control specifications for the raw materials. For the finished herbal product, the minimum quality requirements would entail information on (i) the manufacturer(s) of the product, (ii) the qualitative and quantitative composition of active component(s), (iii) control of excipients, (iv) description and composition of the product, (v) stability data of the product, (vi) packaging and container closure system and labelling, and (vii) certificate of analysis.

4.3.2 Quality characteristics, tests and procedures and quality control specifications suitable for herbal solid dosage forms such as *Artemisia afra* capsules.

The aim of the chapter was also to establish the quality characteristics, tests, procedures and quality control specifications that would be appropriate for herbal product such as *A. afra* capsules. It was found that such quality characteristics are categorised into those required for the raw material and those required for the finished herbal product. The characteristics required for the raw materials are shown in Table 4.3. These consist of information on the following: physical

properties (e.g. organoleptic, macro and microscopic features), chemical identity and quantity of plant actives (e.g. presence or level of marker compound), microbial, pesticides, mycotoxins and heavy metal contamination. For the finished product, required quality characteristics include: uniformity of dosage forms, dissolution profiles and stability studies (Table 4.4). The raw materials and the finished herbal product need to be assessed for quality using appropriate quality control tests. Such tests ensure that a product adheres to a defined set of quality criteria and specifications, and are used to assure the quality of the product. It was then concluded that the quality attributes required for raw materials and finished herbal product along with the set specifications and procedures as shown in table 4.3 and 4.4, respectively, would be appropriate for herbal product such as *A. afra* capsules.



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Table 4.3 A summary of quality attributes, tests, test methods and specifications for quality control of herbal raw material

Quality Attributes	Tests	Test methods	Specification (Tolerance limit)	
Physical properties	Organoleptic features (odour, colour & taste)	Visual, Olfactory	Consistency of odour, taste & colour	
	Macroscopic features (size and shape)	As per BP 2000 and WHO 1998	Must conform to herbarium reference material	
	Microscopic	As per BP 2000 and WHO 1998	Must conform to herbarium reference material	
Chemical identity	Chemical features – presence &/or level of marker compound(s)	TLC, HPLC or other internationally recognised methods	Presence and/or exact level of markers	
Quantity of plant active(s)	Level of marker compounds	Quantitative by GC*, HPLC, etc.	90-110% of label claim	
Microbial contamination	Presence & level of contaminating fungus (yeast and moulds)	Microbial test as per BP 2013, EMA 2006, WHO 2007	< 1 X 10 ⁴ CFU**/g or ml	
	Total Yeast Microbial Count	Microbial test as per BP 2013	10 ⁴ per gram of the product	
	Total Aerobic Count	Microbial test as per BP2013, EMA 2006, WHO 2007	< 1 X 10 ⁵ CFU**/g or ml	
	Total Anaerobic Microbial Count (TAMC)	BP 2013	10 ¹ CFU maximum acceptable count = 20	
	<i>Escherichia coli</i>	Microbial test as per BP2013, EMA2006, WHO 2007	Absent	
	<i>Salmonella</i> spp.	Microbial test as per BP2013, EMA2006, WHO 2007	Absent	
	<i>Staphylococcus aureus</i>	Microbial test as per BP2013, EMA2006, WHO 2007	Absent	
	<i>Pseudomonas aeruginosa</i>	Microbial test as per BP2013, EMA2006, WHO 2007	Absent	
	Heavy metal contamination	Level of Arsenic	Chemical test as per BP2013, EMA 2006, WHO 2007	< 0.14 µg/kg b.w ***/day
		Level of Cadmium	Chemical test as per BP2013, EMA 2006, WHO 2007	< 0.14 µg/kg b.w***/day
Level of Lead		Chemical test as per BP2013, EMA 2006, WHO 2007	< 0.09 µg/kg b.w***/day	
Level of total Mercury		Chemical test as per BP 2013, EMA 2006, WHO	< 0.29 µg/kg b.w***/day	
Pesticide contamination	Level and or presence of Pesticides (e.g. DDT)****	Chemical test as per BP 2013, EMA 2006, WHO 2007	Conforms to pharmacopoeial limits	
Mycotoxins contamination	Presence &/or level of Mycotoxins	Chemical test as per BP 2013, EMA 2006, WHO 2007	Aflatoxins < 20 ppb *****	

* GC Gas Chromatography
 ** CFU colony forming units
 *** b.w body weight

**** DDT dichlorodiphenyltrichloroethane
 ***** ppb parts per billion

Table 4. 4 A summary of quality attributes tests, test methods and specifications for finished herbal product

Quality Attributes	Tests	Test methods	Specification (Tolerance limit)
Uniformity of dosage form	Mass or content variation	As per BP 2013	The % average deviation in weight for capsules is $\pm 7.5\%$ (same as for conventional capsule formulation)
Dissolution profile	Percentage released in set time performance test	As per BP 2013.	75% of label claim to be released within 45 minutes of dissolution testing.
Stability	Shelf life; expiry date of the product; degradation half-life of chemical marker compounds, level (of active or impurity)	As per , EMA 2007 WHO 2013	

For the purpose of this study, the BP, EMA and WHO, will in subsequent discussions collectively be referred to as agencies. All three agencies seemed to advocate similar quality attributes, tests, methods and tolerance levels (i.e. specifications) for herbal medicines.

According to the requirements of the three agencies, a list of the physical attributes of the herbal raw material must be compiled based on its organoleptic, microscopic and macroscopic features. For this, the senses (i.e. visual, olfactory, etc.) and a variety of physical tests (e.g. using a BP method) can be used. Tolerance levels for these attributes must typically conform to that of the herbarium reference material, i.e. must be consistent in terms of organoleptic features (such as odour, taste and colour).

Another important characteristic for quality control is the chemical identity of the herbal material or product. This identifies the name of the compound or its structure. For this, an analytical test for the level or presence of specific chemical marker compound(s) found in the raw material can be conducted using internationally recognised methods such as thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). Some of the methods are preferred by analysts; for instance, HPLC offers advantages of speed, automation, improved accuracy, and precision over TLC. As such, the former is more

frequently employed in quality control studies (Sehume 2010). The specification, i.e. tolerance level for the chemical marker compounds of the herbal material typically should indicate the presence or exact level of a chemical marker(s).

Perhaps the most important quality control criterion for herbal products is the level of active constituents they contain. Various qualitative and quantitative chromatographic analytical methods (such as HPLC and gas chromatography, GC), can be used to identify and determine the levels of chemical marker compounds (that may or may not be the active pharmaceutical ingredient) in plant materials. For quality control, the tolerance limits for the quantity of active ingredients in normal/conventional pharmaceuticals is typically set at 80% to 120% (or some narrower range) of the label amount (especially if tested by HPLC). The same can be advocated for herbal products; and thus for these products to be of acceptable quality, the levels of target marker(s) or active constituent(s) should be well within a range set as the acceptance criteria for each marker (Sehume 2010).

Apart from the constituent chemicals of the herbal material, the presence or absence of extraneously introduced materials such as microorganisms, heavy metals toxins, pesticides and adulterants that could end up in the herbal materials is also an important determinant of herbal material quality. All three agencies recommended that herbal materials be tested for a list of microbial contaminants which include *Escherichia coli*, *Salmonella spp*, *Staphylococcus aureus*, fungi and molds using typical methods given in or by the BP, EMA or WHO. Typically, the quality control specification with respect to microbial contamination is based on the level of contaminants allowed to be present. For instance, the colony levels of yeasts and molds should be less than 1×10^4 CFU/g or ml and that for total aerobic counts $< 1 \times 10^5$ CFU/g or ml. There should also be a complete absence of some contaminants, e.g. *E. coli* and *Salmonella spp*.

For the identification and control of contamination by heavy metals, such as lead and mercury, the BP, EMA and WHO generally advocated the same tests, test methods and tolerance levels. In addition, there are tests for pesticides and mycotoxins (e.g. tests for the presence of DDT and aflatoxins) required to confirm the quality of herbal products. Specifically, the levels of mycotoxins (e.g. aflatoxins) should be below 20 ppb and the presence of pesticides must conform to specific pharmacopoeial limits.

As far as dosage forms of herbal products are concerned, the most important quality control criterion should perhaps be mass and content uniformity. These ensure that every dosage form contains equal amount of drug substance, i.e. active pharmaceutical ingredient (API), within a batch and is mainly used to test the consistency of powder filling into capsules. The test for mass uniformity is based on the use of a representative sample of 20 units that are individually weighed. The average mass of the 20 units is calculated, and the specifications require that not more than two of the individual masses deviate from the average mass by more than one percentage deviation (PD) and none deviates by more than twice that percentage. The limit on the acceptable deviation from average (in weight) for capsules is $\pm 7.5\%$ and the limit on the capsule content is 90 % to 110 % (BP 2013a; Ma 2006).

Another important criterion which may be advocated for quality of a herbal product, is the rate at which its marker compounds dissolve in solution, i.e. its dissolution profile. It is used to identify and prove the availability of active drug materials in their delivered form, and simulates the availability of active substance. It also allows prediction of the time for complete release of the material from the dosage form. Furthermore, it assesses batch-to-batch consistency of solid oral dosage forms such as capsules. The dissolution test and dissolution specification criterion is thus important for the quality control of herbal products. The test determines the percentage of the active constituent released into the dissolution medium over time, typically using a method and specification criterion given in the British Pharmacopoeia (BP 2013a). For instance, the tolerance limit set by the BP for a rapidly dissolving product specifies that 75 % of the label amount has to be released within 45 minutes. Once dissolution specifications are set, the pharmaceutical product or herbal product should, as a mark of continued quality, comply with such specifications throughout its shelf-life.

Lastly, the EMA and WHO recommended that a stability profile of the herbal product be provided. Appropriate stability test(s) should be used to determine the levels and degradation/shelf-life of chemical marker compounds (active or inactive), and thus the shelf life and expiry date of the product(s) calculated. Typically, the quantitative assay methods used to establish the dissolution profile and/or levels of active constituent(s) can be used in these stability tests. For stability studies, the limits that denote acceptable stability may be derived from the profile obtained when the herbal material was assessed in previous or pre-formulation studies. The tolerance limits of stability need to include upper limits for

individual and total impurities and degradation products. Normally, in the case of conventional pharmaceuticals, impurities and degradation products in concentrations higher than 0.1 % should be identified and the same could possibly apply for herbal products (Sehume 2010).

Overall, review of recommendations of the BP, EMA and WHO on the quality control of herbal products, as summarised in tables 4.3 and 4.4, provides similar and useful information on the quality attributes, test methods and specifications on which the quality profile of herbal products could be based. It was consequently concluded that quality characteristics such as: (a) physical features, (b) chemical identity, (c) quantity of plant active(s), (d) microbial and other contaminant levels, (e) content and mass uniformity, (f) dissolution and (g) stability profiles of marker compounds could provide the required information for the quality attributes of a herbal product such as *A. afra* capsules.

4.4 Conclusion:

The overall objective of this study was to establish the minimum pharmaceutical quality requirements, in terms of quality attributes, specifications and test methods that would be appropriate for the registration of an herbal product such as *A. afra* capsules.

Most of the agencies studied typically had similar requirements. It was concluded the minimum quality requirements for registration of *A. afra* capsules would, for the herbal raw material, include: information on (a) the identity of the plant, (b) parts and physical features of the plant used, (c) the manufacturing details, (d) general identity tests, (e) purity tests, (f) molecular structure formula of the herbal ingredient(s) and (g) quality control specifications; and that for the finished herbal product would consist of information on (i) the manufacturers of the product, (ii) the qualitative and quantitative composition of the active components, (iii) control of excipients, (iv) description and composition of the product, (v) stability of the product, (vi) packaging, container closure system and labelling and (vii) the certificate of analysis. In addition, the quality attributes, specifications and test methods supporting the pharmaceutical quality of *A. afra* raw materials would include information under the following headings: (a) physical features, (b) chemical identity, (c) quality of plant actives and (d) heavy metal, pesticides and mycotoxins contamination; whilst for finished herbal product (*A. afra* capsules), information on uniformity of dosage forms, dissolution profile and stability studies would be required.

CHAPTER 5

Quality characteristics of *Artemisia afra* capsules

5.1 Introduction

In chapter 4, the quality characteristics that would be required for registration of an *A. afra* capsule product were established. For this chapter, the main objective was to determine some of the identified quality attributes, based on the findings reported in Chapter 4, for the *A. afra* extract (i.e. active pharmaceutical ingredient (API)) that was to be used, and the finished herbal product (FHP) i.e. the *A. afra* capsule. The quality characteristics considered for the API were the organoleptic properties, ash values, moisture content and levels of active constituents while that for the FHP included organoleptic features, moisture content, weight and content uniformity, and the dissolution profile.

In this chapter, details of the preparation and characterization of the freeze dried aqueous extract (FDAE) of *A. afra* and the capsules are presented. In addition, the equipment, materials and procedures used to evaluate the pharmaceutical quality of the *A. afra* extract and capsules, as well as the results obtained, are also presented and discussed.

5.2 Equipment and material

The following materials were used in the preparation and characterization of the *Artemisia afra* extract and capsules:

Dried *A. afra* leaves (*Grassroot Group (Pty) Limited, Groenvlei Farm, South Africa*), **sodium carbonate (Na₂CO₃)** (*BDH Chemicals Ltd, 30121, England*), **gallic acid** (*Purum, Fluka, 48630, Switzerland*), **sodium nitrate**, **aluminum chloride**, **sodium hydroxide** and **quercetin dihydrate - HPLC grade** (*Sigma 085KO720, USA*), **carbon dioxide (CO₂)** (*99.95%, Afrox, 8007258270508, SA*), **acetonitrile** and **methanol** (*Burdick & Jackson, USA*), **ethanol** and **aluminium chloride** (*Merck, Darmstadt, Germany*), **formic acid** and **sodium acetate** (*BDH Chemicals Ltd, England*), **hydrochloric acid** (*Kimix, USA*), **distilled water**

(Millipore filtration system, *Milford, MA, USA*), **Folin-Ciocalteu Reagent** and **luteolin** (*Sigma Aldrich, Germany*), **Whatman No. 1 and Whatman No. 41 filter paper** (*Whatman, England*), **solvent filter paper** (47mm filter membrane, 0.45µm PVDF, *Millipore, USA*), **hard gelatine capsules** (size 0, *Afriplex (Pty) Ltd, Paarl, Cape Town*)

The equipment or instruments used for the preparation and characterization of the *Artemisia afra* extract capsules were:

Freeze drier (*Virtis®*, 125L New York, USA), **-80°C freezer** (*Lozone CFC, U855360, New Brunswick Scientific, USA*), **vortex machine** (*Vortex-2, G30 560E, Scientific Industries, Inc. Bohemia, N.Y. 11716 USA*), **HPLC system** (Agilent 1200 series consisting of an auto sampler (*G1329A, Germany*), quaternary pump (*G1311A, Germany*), thermostatted column compartment (*G1316A, Germany*), and a diode array detector (*G1315B, Germany*)), **C₁₈ reversed phase column** (250 x 4.60 mm, 4µm, *Phenomenex, USA*), **HPLC filter unit** (*Millipore Cameo 25 AS, DDA 02025 So MSI: Micro separation Inc., USA*), **filtration system (Supelco®)** connected to **vacuum pump** (*Medi-Pump Model 1132-2, Thomas Industries Inc., USA*), **finnpipette™** (*Thermo Electron T73676*), **dissolution tester** (*Sotax AT 7 Smart Art no.07100, dissolution testing unit Sotax AG Basel, USA*), **weighing machine** (*Ohaus® Corporation, Pioneer™, China*), **halogen moisture analyser** (*HR73 ©mettler Toledo GmbH 2001, CH-8606, Greifensee, Switzerland*), **capsule filler machine size 0** (*S.L. Sanderson and Co., Berry Creek, CA 95916*), **pH meter** (*Thermo Orion 2000, 074012, USA*), **desiccator** (*2atc, Gepruft, Germany*), **furnace** (*Naber L47T, Lilienthal/Bremen, West Germany*), **light microscope** (*Nikon, Japan*), **test sieve shaker** (*Endecott, E.F.L. IMK11, London, England*), **sieves** (*Incorporating Madison Test Sieves (Pty) Ltd., SA*), **hot plate** (*Kika-Werke GmbH & Co. Kg D-79219 Staufen, Germany*), **oven** (*Memmet 854, Schwabach, West Germany*).

5.3 Methods

5.3.1 Preparation and determination of selected quality characteristics of the *Artemisia afra* freeze dried aqueous extract.

5.3.1.1 Issues relating to preparation of the *Artemisia afra* FDAE

For this project, capsules containing an extract of *A. afra* were prepared. The procedure used was designed to, as far as possible, mimic the methods described in literature (see section 2.3.5) for preparation of the traditional dosage form. In traditional settings, a quarter cup quantity of *A. afra* leaves is usually added to a cup of boiling water and allowed to stand and seep for 10 minutes (Dube 2006). Therefore, the aqueous extract to be used in the capsules was prepared by boiling the dried leaves in water (a ratio of 1:20 leaves: solvent) (Mukinda & Syce 2007) for 30 minutes. The higher ratio of solvent in the preparation, compared to the ratio used in traditional settings, was to ensure maximum extraction of actives which may be hampered by solvent saturation if a smaller ratio of solvent (as obtained in traditional settings) is used. The mixture was then left to seep and draw for a further 10 minutes while cooling down. Thereafter, the mixture was filtered using Whatman no. 1 filter paper, the filtrate frozen at -80°C , and the frozen extract freeze-dried to obtain the FDAE. The latter was then weighed to calculate the percentage yield, placed in amber glass containers and stored in desiccators at 22°C until needed.

5.3.1.2 Determination of the selected quality attributes of the *Artemisia afra* freeze dried aqueous extracts (API)

Based on the information obtained with the review of quality requirements typically set for herbal products (see Chapter 4), characteristics of the *A. afra* API were determined according to the set requirements. The following section details the methods used to determine these characteristics.

5.3.1.2.1 Organoleptic features

5.3.1.2.1.1 Colour

To determine the colour of the *A. afra* FDAE, 0.5 g of the material was placed against a white background in diffuse daylight, viewed by eye and its colour described accordingly.

5.3.1.2.1.2 Odour

To determine the odour of the FDAE, 0.4 g of the material was placed in a 5 cm diameter watch glass, left for 15 minutes and thereafter the air above the sample was inhaled slowly and repeatedly. The strength of the odour was determined by classifying it as either non-existent, weak, distinct or strong and the odour sensation described as either aromatic, fruity, musky, mouldy or rancid (WHO, 2011).

5.3.1.2.1.3 Taste

For taste determination, the FDAE of *A. afra* was dispersed in distilled water at a concentration of 0.01 g/ml, stirred and allowed to stand for 10 minutes. The researcher then used the natural sense (i.e. smell and tongue) to determine the taste. The taste was described in terms of none, sweet, sour or bitter. Note: Although tasting by mouth is generally not allowed in the pharmaceutical industry where, because of fears of possible danger, an E-tongue “electronic sensory array technology” may be used, it was deemed appropriate in the present case since *Artemisia afra* is generally widely taken orally as the extract and or tea.

5.3.1.2.2 Particle size and shape

The sieve method was used to measure the particle size and microscopic procedures used to determine the shape of the *A. afra* FDAE extract.

Generally, the degree of fineness, i.e. particle size of a powder, may be expressed by reference to sieves through which they are passed (WHO 1998). In this way, powders can be classified or described as ‘coarse’, ‘moderately fine’, ‘fine’, or ‘very fine’ powders, and the degree of fineness of the powder expressed as a weight-to-weight percentage of the powder passing through different size sieves. In this study, sieves of sizes 335, 180, 125 and 90 μm were used for the *A. afra* FDAE. They were successively assembled in descending order, i.e. the 335 μm size sieve at the top and the 90 μm at the bottom. A receiving pan was closely fitted to the bottom sieve and the assembled set of sieves mounted on a sieve shaker. An accurately weighed amount (10 g) of *A. afra* FDAE was placed on the top-most sieve and the system agitated for 20 minutes. The retained powder on each sieve was collected, weighed and calculated as a percentage (w/w) of the total feed.

To determine the shape of the *A. afra* FDAE particles, the microscopic method was used (WHO 1998). Essentially, 3 mg of the prepared *A. afra* FDAE was sprinkled onto a microscopic slide and viewed using a Nikon monocular light microscope. The particles were micrographed and their shape observed and described as either irregular, round, cylindrical or rectangular (WHO 1998; Komperlla 2004).

5.3.1.2.3 Ash values

Ash values can be considered and used as quality parameters to indicate identity, purity or possible adulteration of herbal material (WHO 1998; Dube 2006). The total ash and acid-insoluble ash values were determined for the *A. afra* FDAE. To determine the total ash value, 2 g of *A. afra* FDAE powder was heated in a tarred crucible in a furnace (temperature 500 – 600°C) until the powder was white, which indicated the absence of carbon. The ash (i.e. remaining material) was allowed to cool in a desiccator for 30 minutes, and then weighed. Subsequently, the percentage of total ash of the air-dried material was calculated as follows:

$$\% \text{ Total ash} = \frac{\text{Weight of ash}}{\text{Weight of sample}} * 100$$

For the acid-insoluble ash value, 25 ml of 2M hydrochloric acid was added to the total ash of *A. afra* FDAE powder. The mixture was boiled for 5 minutes and the remaining insoluble matter collected on an ashless filter paper (Whatman No. 41). The residue retained on the filter paper was washed with hot water and finally ignited at temperature 500 – 600°C. The remaining acid insoluble ash was allowed to cool, then weighed and the percentage of acid-insoluble ash of the air-dried material calculated as follows:

$$\% \text{ Acid Insoluble Ash} = \frac{\text{Weight of the acid insoluble residue}}{\text{Weight of the Sample}} * 100$$

5.3.1.2.4 Moisture content

The moisture levels of the *A. afra* FDAE powder was determined by a “loss on drying method” using a halogen moisture analyzer (HR73, figure 5.1). The analyzer consisted of a halogen-heating unit, a sample plate and an electronic weighing balance, and worked on the thermogravimetric principle, i.e. measured changes in physicochemical properties of materials as a function of increasing temperature. Typically, the moisture analyzer determined the weight of the sample at the start, the integral halogen-heating module then quickly heated the sample and the moisture vaporized. During the drying process, the instrument continually measured the weight of the sample and displayed the reduction in moisture. To determine the moisture content of the *A. afra* FDAE, 0.3 g of extract was placed

on the balance plate, and the analyzer switched on. The apparatus then automatically calculated the weight of the moisture using **equation 1** and the moisture content, as a mass percentage, using **equation 2**. The whole procedure was repeated twice, with another 0.3g of extract each time, to obtain triplicate values.

$$\text{Moisture weight} = \text{Initial weight (Wet mass)} - \text{Final weight (Dry mass)} \dots\dots \text{Equation 1}$$

$$\text{Moisture content} = (\text{Moisture weight} / \text{Initial weight}) \times 100\% \dots\dots\dots \text{Equation 2}$$



Figure 5. 1 HR73 Halogen moisture analyser (Sehume 2010)

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5.3.1.2.5 Content uniformity

To assess content uniformity of the FDAE, the total flavonoid content, total phenol content and luteolin levels were measured.

5.3.1.2.5.1 Total flavonoid content

The total flavonoid content (TFC) of the plant material was determined using the aluminium chloride colorimetric method (Barku et al. 2013), with minor modifications. Quercetin was used as the flavonoid standard and to establish the flavonoid calibration curve. For the latter purpose, 5 mg of quercetin was dissolved in 5 ml of 80 % methanol in water (v/v) to obtain a flavonoid stock solution concentration of 1mg/ml. This was further diluted with 80 %

methanol to provide triplicate solutions of 0, 12.5, 25, 50, 80 and 100 µg/ml. To a series of test tubes, 1 ml of standard solution, 1.25 ml of distilled water and 0.075 ml of 5% sodium nitrate were added and the contents incubated at room temperature for 5 minutes. Thereafter, 0.15 ml of 10 % aluminium chloride was added and the mixture allowed to stand for 6 minutes at room temperature before 0.5 ml of 1M sodium hydroxide was added. Twenty microliter samples of the reaction mixture was injected into the Agilent HPLC system (with no HPLC column or guard column in place, i.e. the Agilent HPLC system was only used for its spectrophotometer component) using distilled water pumped at a flow rate of 1 ml/min as mobile phase, and peak readings at 280 nm recorded. As standard curve to be used in quantification of TFC, a plot of peak height *versus* concentration was then plotted.

To obtain the TFC of the FDAE, 5 mg of the FDAE was weighed and mixed with 5 ml of distilled water. A portion of this (0.25 ml of sample) was then placed in a screw-capped test tube, reacted with aluminium chloride, as described above, and twenty microliters of the mixture injected into the HPLC system. The TFC in the test samples were calculated from the calibration curve and expressed in terms of mg quercetin equivalence (QE)/g dry weight (D.W). All determinations were performed in triplicate.

5.3.1.2.5.2 Total phenol content

The total phenol content (TPC) of the *A. afra* FDAE was determined using the Folin Ciocalteu method (Barku et al. 2013), with minor modifications. Gallic acid was used as the standard to establish the calibration curve. A weighed quantity (5 mg) of gallic acid was dissolved in 5 ml of 20 % methanol in water (v/v) to prepare a stock solution of concentration 1mg/ml. From the stock solution, triplicate samples of 20, 40, 60, 80, 100 µg/ml standard solutions in 20 % methanol/water were prepared. In different test tubes, 1 ml of each standard solution, 5 ml of Folin Ciocalteu reagent (diluted tenfold with water) and 4 ml of Na₂CO₃ (75 g/l in water) were mixed and the mixture left to stand at 20°C for 30 minutes. Twenty microliter samples of the reaction mixture were injected into the Agilent HPLC system (as described for the TFC assay; section 5.3.1.2.5.1), and the peak absorbance at 765 nm was determined. The values were plotted as peak height size *versus* gallic acid concentration to establish the standard curve for quantification of TPC.

To determine the TPC of *A. afra* FDAE, 5 mg of the dried extract was weighed and mixed with 5 ml of distilled water. A measured amount (1 ml) of the extract solution was mixed with 5 ml of Folin-Ciocalteu reagent (diluted tenfold with water) and 4 ml of Na₂CO₃ (75 g/l). The mixture was allowed to stand at 20°C for 30 minutes. Thereafter, twenty microliter samples of the reaction mixture were injected into the Agilent HPLC system, the peak absorbance determined as described for the standard solutions and the TPC of the samples read from the calibration curve. The TPC was obtained and expressed as mg gallic acid equivalent (GAE)/g using the formula:

$$C = \frac{cV}{M}$$

where C = total content of phenolic compounds in mg GAE/g,

c = the concentration of gallic acid (mg/ml) established from the calibration curve,

V = volume of extract (ml) and

m = the weight of pure plant extract (g).

All determinations were performed in triplicate.

5.3.1.2.5.3 Quantification of luteolin

To measure the content of luteolin in the *A. afra* FDAE powder, a validated HPLC assay method was used. Briefly, separation of the marker compound was effected on a Phenomenex Luna® C₁₈ column (250 x 4.6 mm, 5 µm) maintained at temperature of 25°C. The mobile phase consisted of 0.1% v/v formic acid aqueous solution (solvent A) and 0.1% v/v formic acid in acetonitrile (solvent D) used. The elution gradient used was: 0-22 min: 85% A, 15% D; 22-27 min: 0% A, 100% D; and 27-35 min: 85% A, 15% D. Flow rate of the solvent was 0.8 ml/min, the sample injection volume was 20 µl and the luteolin peaks were detected at 350 nm.

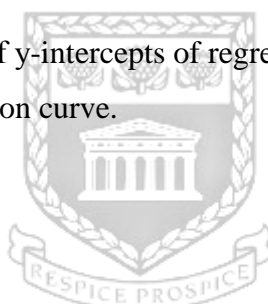
To validate the assay, the linear concentration range, assay specificity and precision, and limits of detection and quantification were determined. For this, a stock solution of luteolin, 165 µg/ml in 75 % methanol, was prepared. From this stock solution, standard solutions in the concentration range: 2.6 to 82.5 µg/ml in 75 % methanol, were used to plot the calibration curve.

The linearity of the calibration curve for the reference standards was assessed in triplicate over a six sample concentration range. The calibration curve was constructed as plots of peak area against concentration, was subjected to linear regression and the correlation coefficient (R^2) determined using GraphPad Prism 6. The detection and quantification limits (i.e. LOD and LOQ, respectively) were determined using the standard deviation of the response and the slope of the curve, according to the following equations (ICH, 2005)

$$LOD = \frac{3.3 \sigma}{S} \dots\dots\dots\text{Equation 3}$$

$$LOQ = \frac{10 \sigma}{S} \dots\dots\dots\text{Equation 4}$$

where σ = the standard deviation of y-intercepts of regression lines and
 S = the slope of the calibration curve.



To determine the specificity of the assay for luteolin in the plant extract, chromatograms of standard luteolin sample (165 $\mu\text{g/ml}$) and FDAE solution were compared for peak retention time and the peak in the chromatogram of the FDAE solution subjected to peak purity analysis using the Chemstation® software.

Lastly, to determine the precision of the assay, 3 replicates of the low, medium and high concentration standard solutions were assayed three times a day, on three consecutive days. On each occasion, the mean peak area, standard deviation and % relative standard deviation (RSD) were calculated.

In this study, *A. afra* was assayed in two ways, *viz* as hydrolysed and un-hydrolysed samples. From the former, the level of total flavonoid and for the latter, the level of free flavonoids in the plant extract, were determined.

Determination of free flavonoid levels

For this, 75 mg of *A. afra* FDAE was weighed and placed in centrifuge tubes, then 3 ml of aqueous methanol (50 %) was added and the solution vortex-mixed for 1 minute. Thereafter, 3 ml of ethyl acetate was added, the mixture vortex-mixed for 3 minutes and centrifuged at 3500 rpm for 15 minutes. Using a pipette, the ethyl acetate supernatant was transferred to a clean test tube and evaporated to dryness under a gentle stream of nitrogen gas. The residue was reconstituted in 1 ml of mobile phase solution (water: acetonitrile in a 50: 50 ratio), the tube gently vortexed for 20 seconds and the solution drawn up into a 2 ml syringe (without needle). The solution in the syringe was filtered through a 0.22 µm membrane syringe filter. Twenty microliters of each sample was injected onto the HPLC column for analysis.

Determination of total flavonoid levels

For this, 75 mg of *A. afra* FDAE was weighed and placed in a centrifuge tubes. To each tube, 3 ml of aqueous methanol (50%) was added and the solution vortex-mixed for 1 minute. To hydrolyse the samples, 3 ml of acidic aqueous methanol (MeOH: 2N HCl 50:50) was added to each tube and the mixture heated at 80°C for 40 minutes in a water bath. Thereafter, the tubes were removed and allowed to cool for 10 minutes. Subsequently, 3 ml of ethyl acetate was added, the samples vortex-mixed for 3 minutes and centrifuged at 3500 rpm for 15 minutes. The ethyl acetate top layer was transferred to a clean tube using a disposable plastic pipette and evaporated to dryness under a gentle stream of nitrogen. To the residue, 1 ml of mobile phase (acetonitrile: water 50:50) was added and the tube gently vortexed for 20 seconds. The solution was drawn into a 2 ml syringe (without needle) and filtered (through a 0.22 µm membrane syringe filter). Twenty microliters of each sample was injected onto the HPLC column for analysis using same procedure as for the determination of free flavonoid levels.

To identify luteolin in the plant samples, the retention time and UV-spectra of the possible luteolin peaks were identified and compared to the retention time and UV-spectrum obtained in the chromatograms of the standard reference luteolin sample. The area of the luteolin peak was noted and the levels of free or total luteolin in each sample determined from the standard curve regression equation. Three replicates of each preparation were assayed.

5.3.2 Preparation and determination of quality characteristics of the *Artemisia afra* freeze dried aqueous extracts (FDAE) capsules

5.3.2.1 Preparation of *Artemisia afra* capsule

The objective was to make capsules containing FDAE powder prepared from dried leaves of *A. afra*. For this study, the size 0 manual capsule filling machine shown in figure 5.2 was used. Empty size 0 capsules were placed into the holes by hand with the bodies of the capsule fitting snugly into the plate. The caps of the capsules were then removed. *Artemisia afra* FDAE was placed onto the surface of the body plate (plate containing the capsule bodies) and spread with a spatula so that it flowed into the empty capsule bodies. Once filled to the brim, the cap of each capsule was repositioned over the material-filled body of the capsule and the two capsule parts re-joined using gentle pressure. The prepared capsules were cleaned to remove any extract residues, their weights were determined and the acceptable capsules stored in a desiccator at a temperature of 22°C.

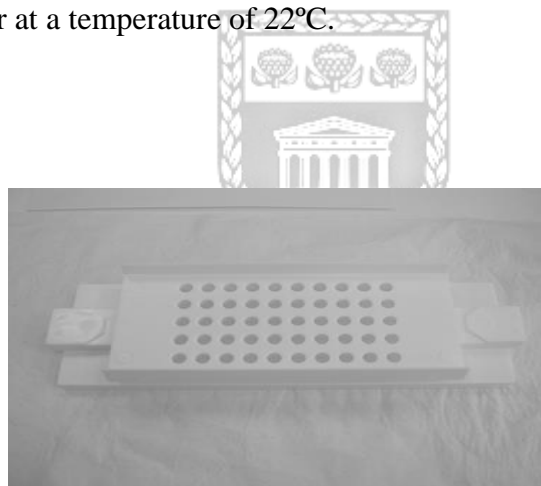


Figure 5. 2 Manual capsule filling machine

5.3.2.2 Determination of quality attributes of *Artemisia afra* capsule:

5.3.2.2.1 Organoleptic properties

For the organoleptic properties *A. afra* capsules, the human sensory evaluation (i.e. eyes) were used to determine the shape and colour of *A. afra* capsule

5.3.2.2.2 Moisture content

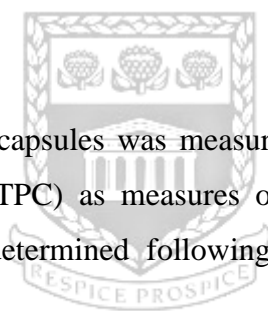
For this study, the shells of the capsules were removed and the moisture level of the capsule contents determined as described in section 5.3.1.2.4.

5.3.2.2.3 Weight uniformity

The method in the British Pharmacopoeia (BP 2013b) was used. Twenty capsules were randomly selected from the prepared batches. Each capsule was weighed and then completely emptied of its contents. The empty shells were brushed to remove any remaining particles, and the empty capsule reweighed. The mass of the capsule contents was then calculated by subtracting the mass of the empty capsule from the initial mass of the full capsule. The values for the 20 capsules were averaged, the percent deviation in weight from average was calculated and the results compared to the British Pharmacopoeia specifications.

5.3.2.2.4 Content uniformity

Uniformity of content for the ten capsules was measured using the total flavonoid content (TFC) and total phenol content (TPC) as measures of *A. afra* capsules content. Weight uniformity of ten capsules was determined following the same procedure as in section 5.3.2.2.3.



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To determine the TFC, 10 mg of the *A. afra* extract was weighed from each capsule and mixed with 10 ml of distilled water (1mg/ml) in a test tube. Then, 0.25 ml of the sample was placed in a screw-capped test tube and reacted with aluminium chloride to determine TFC in each capsule following the method previously described in section 5.3.1.2.5.1.

For the determination of TPC, 10 mg of the *A. afra* extract was weighed from each capsule and mixed with 10 ml of distilled water (1mg/ml). Then, 1 ml of the sample was reacted with 5 ml Folin Ciocalteu reagent (diluted tenfold with water) and the TPC was tested same way as previously reported in section 5.3.1.2.5.2.

5.3.2.2.5 Dissolution profile

The British Pharmacopoeia (BP 2013a) basket method was used to evaluate dissolution of the *A. afra* phytoconstituents (using *A. afra* extract and TFC) from the FDAE in 0.1N hydrochloric acid (pH 1.2, temperature $37 \pm 0.5^\circ\text{C}$) using AT7 Smart Dissolution Tester®.

Briefly, 500 ml of 0.1N hydrochloric acid was used as dissolution medium to simulate dissolution conditions in the stomach. For the actual test, two capsules (0.650g) were placed in each dry basket, the baskets lowered into position and the apparatus immediately started at a rotation speed of 100 rpm. At predetermined time points (*viz.* 5, 10, 15, 20, 30, 45, 60 and 90 minutes), 5 ml samples were withdrawn from a point halfway between the surface of the dissolution medium and the top of the rotating basket and not less than 10 mm from the wall of the vessel. This was immediately replaced with 5 ml of pre-warmed 0.1N hydrochloric acid solution. The sampling probes were fitted with in-line 0.45 μm filters so that filtered samples would be obtained.

To determine the absorbance of *A. afra* capsule, 1 ml of each sample was transferred to HPLC vials. From these vials, 20 μl aliquots were injected into the Agilent HPLC system (with no guard column or HPLC column in place) for ultraviolet (UV) spectroscopic analyses – to determine absorbance as described in section 5.3.1.2.5.1.

Finally, to determine the TFC in *A. afra* capsules, 0.25 ml of the dissolution sample was placed in a screw-capped test tube and reacted with aluminium chloride as described in section 5.3.1.2.5.1.

The sample with the highest absorbance reading at 280nm or the one at 90 minutes was taken as the fully dissolved sample. The percent material dissolved at each time point was calculated and the average percentage material dissolved for *A. afra* capsules at each time pointed plotted.

5.4 Results and Discussion

5.4.1 Preparation and quality characteristics of *Artemisia afra* freeze dried aqueous extracts.

5.4.1.1 Issues pertaining to preparation

The freeze dried aqueous extract of *A. afra* was prepared as presented in section 5.3.1.1. The average yield obtained was 22.74 ± 0.93 % (appendix 5.1). This result was similar to the average yield of 21.96% (Dube 2006) and 21.8 % (Nkengla 2014) reported by previous investigators.

5.4.1.2 Quality characteristics of *Artemisia afra* freeze dried aqueous extracts

5.4.1.2.1 Organoleptic features

Usually, an examination of organoleptic characteristics is the first step in establishing identity and degree of purity of plant materials. In terms of colour, consistency, odour or taste, if a plant material sample is found to be significantly different from the set specifications, it is considered as not fulfilling the quality requirements. *Artemisia afra* was found to be brittle, light brown in colour, changing to dark brown on prolonged exposure to air, with an aromatic odour and a bitter taste (table 5.1).

The *A. afra* extract had a characteristic aromatic odour which originated from its essential oils. Compounds such as α - and β -Thujone (52.1 - 39.8%), camphor (14.4 - 8.2%), 1, 8-cineole (21.8–13.1%) and borneol (7.8 - 2.7%), in the percentages enclosed in parentheses, are known major components responsible for the characteristic flavour of the species (Asekun, Grierson & Afolayan, 2007).

Colour can be used as an identifying characteristic of a particular substance. Several pharmacopoeias include the colour of a substance as part of the substances' monograph. The FDAE produced in the current study was light brown in colour but turned dark brown on extended exposure to air. The change in colour and appearance was most likely due to the hygroscopic nature of the FDAE, which was also reported in some previous studies (Nkengla 2014; Komperlla 2004; Dube 2006).

The *Artemisia afra* FDAE had a bitter taste, as determined by the researcher. Previous studies have also reported similar severity of bitterness associated with this species (Dube 2006; Nkengla 2014). Because this test is based on an individual's sensory functions, it may be rather subjective rather than objective, and is generally prone to large errors.

The bitter taste may present challenges when the extract needs to be consumed. There is thus a need to mask the taste. This served as one of the motivations for filling the extract into capsules.

Table 5.1 Organoleptic characteristics of *Artemisia afra* freeze dried aqueous extract powder

Organoleptic characteristics	<i>Artemisia afra</i> freeze dried aqueous extract powder
Physical appearance	Brittle, free flowing, small particulate powder, which clumps together to form a solid mass on prolonged exposure to air.
Colour of material	Light brown powder changing to dark brown on prolonged exposure to air.
Odour	Characteristic odour, aromatic.
Taste	Extremely bitter.

5.4.1.2.2 Particle size and shape

Particle size and shape can affect the physico-chemical properties of a dosage form, product performance, bioavailability and appearance of the end product. This can ultimately have an impact on the overall quality of the product. *Artemisia afra* FDAE appeared to be irregularly shaped. Based on the particle size distribution of the *A. afra* extract (figure 5.3 and appendix 5.2), it was classified as a coarse powder, in line with the WHO 1998 classification system. In the case of powders with large particle size (i.e. approximately 40 % > 335 µm) such as an *A. afra*, additional sieving and removal of bigger particles may be necessary before encapsulating.

Artemisia afra FDAE

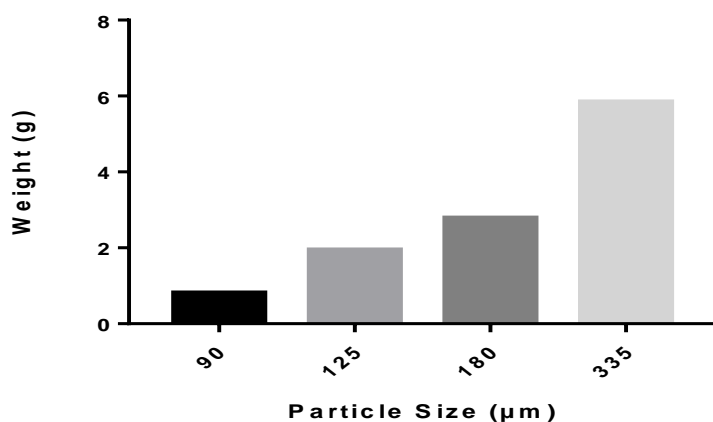


Figure 5.3 Particle size distribution of *Artemisia afra* FDAE (graded according to passing through different sieve sizes)

5.4.1.2.3 Ash values

The ash values were analysed to check for possible presence of any foreign matters like soil and sand. Determination of total ash values (of herbal drugs) is not always a reliable parameter for quality assessment; this is because there is risk of occurrence of non-physiological materials (Ahmad et al. 2015) in samples. Therefore, acid insoluble ash is also usually determined; and the same was done in this study. The average percentage values of total ash and acid insoluble ash were 13.3578 ± 0.9394 and 0.6794 ± 0.2605 , respectively (appendix 5.3).

There are no set specifications for ash values of *A. afra* FDAE powder. Previous investigation had reported ash values that were different: 17.4383 ± 0.054 and 2.52 ± 0.3134 % for total ash and acid insoluble ash, respectively (Komperlla, 2004). It was noted that the *A. afra* materials used in the previous investigation mentioned above was collected from a different source. It may therefore be inferred that *A. afra* plants growing under different conditions (climatic and soil conditions) possess different physiochemical properties.

5.4.1.2.4 Moisture content

Moisture content is one of the most important factors influencing quality and storability of plant materials. It is an especially important for herbal medicines which are hygroscopic. The

percent moisture content of *A. afra* FDAE powder was $10.76 \pm 0.56\%$ (appendix 5.4). This result was comparable to the $10.68 \pm 0.73\%$ (Nkengla 2014) yield found in a previous study. According to the BP 2013 recommendations, powders should contain less than 10 % w/w of water. As *A. afra* was found to contain more than this percentage of moisture, it has been described as hygroscopic.

The presence of moisture in plant extracts may promote hydrolysis of potential active ingredients (such as flavonoids, e.g. luteolin) in the extract. This may result to a poor quality product. Hygroscopic powders are also susceptible to microbial contamination. They may also cause sticking /adhesive problems in the final formulation process. For these reasons, *A. afra* should ideally be stored in desiccators, under controlled temperature and humidity conditions in order to minimize moisture absorption.

5.4.1.2.5 Content uniformity

For this, total flavonoid content, total phenol content and luteolin levels were used as measures of content.

5.4.1.2.5.1 Total flavonoid content (TFC)

Quercetin was used as the standard to establish the calibration plot (figure 5.4). The calibration plot was linear over the concentration range, 0 to 100 $\mu\text{g/ml}$ ($R^2 = 0.9995$). The TFC of the plant material was expressed as quercetin equivalent (QE) mg/g of extract. This value (TFC) was determined in the *A. afra* FDAE as 33.04 mg QE/g of the plant extract.

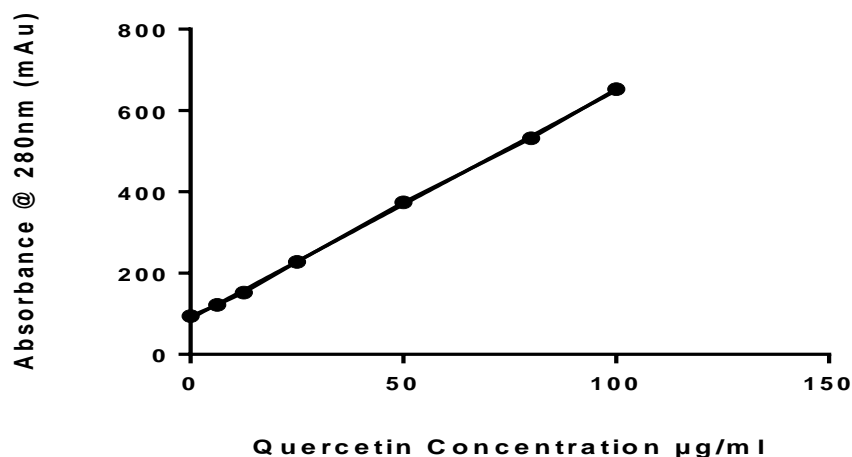


Figure 5.4 Standard curve of quercetin concentration versus absorbance at 280 nm used for the quantification of the total flavonoid contents in *Artemisia afra* FDAE ($R^2 = 0.9995$; slope = 5.609 ± 0.0576 ; y-intercept = 88.3 ± 3.057 , $Y = 5.609 * X + 88.3$).

Previous research reported the TFC of 29.68 mg QE/g of the plant extract (Sunmonu et al. 2012), which was close to the amount obtained in the present study. The results revealed the presence of flavonoids as literature indicated (Sunmonu et al. 2012). These flavonoids are found to be important anti-oxidants which also have anti-inflammatory and anti-microbial properties, which may be responsible for the protection they offer against harmful pathogenic microbes (Ahmad et al. 2015). Therefore, the determination of flavonoids is significant for identification and quality assessment of the API.

5.4.1.2.5.2 Total phenol content (TPC)

Primarily, gallic acid was the standard used to establish the calibration plot (figure 5.5). The calibration plot was linear over the concentration range, 20 to 100 µg/ml ($R^2 = 0.9962$). The TPC of the plant material was expressed as mg/g of gallic acid equivalent (QAE). Total phenol content in *A. afra* was determined as 101.75 ± 5.55 mg QAE/g of plant extract.

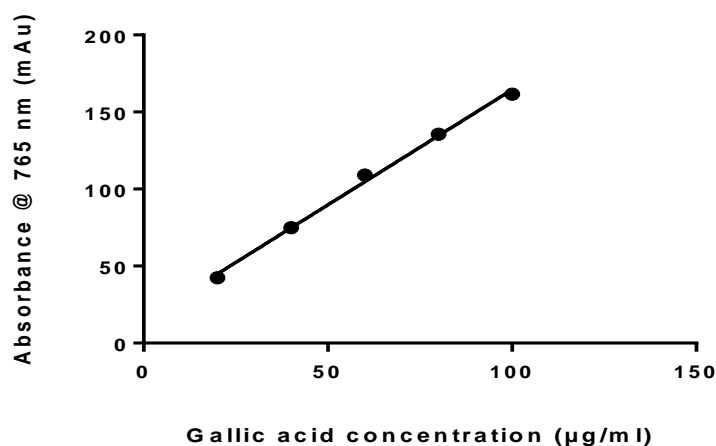


Figure 5. 5 Standard curve of gallic acid versus absorbance at 765 nm used for the quantification of the total phenolic contents of *Artemisia afra* FDAE ($r_2 = 0.9962$; slope = 1.495 ± 0.05338 ; y-intercept = 14.99 ± 3.541 , $Y = 1.495 * X + 14.99$).

The present study showed the presence of phenols in *A. afra* extract, in line with findings in literature (Mulubwe 2007). The phenol content in the extract represented pharmaceutical characteristics of the plant. This may also explain its medicinal, e.g. anti-inflammatory properties (Velicković et al. 2014). Generally, natural antioxidants come from plants in the form of phenolic compounds (Wickramaarachchi et al. 2016). As a result, it was essential to evaluate *A. afra* for the presence of possible active ingredients (such as phenols) as part of its quality control assessment.

The present study reported TPC values that differed from the 357.18 mg/g rutin equivalents (Mulubwe 2007) and 258.39 mg/g tannic acid equivalent (Sunmonu et al. 2012) found in previous studies. Different standards were used in these studies though, which might have resulted in the different TPC levels. It is also known from numerous studies that the contents of phenol compounds varied depending on the plant genotype, soil conditions, differences in plant ripening, environmental conditions and the contents of feeding material in the soil which could have an effect on the phenol metabolism (Velicković et al. 2014). Thus in turn could affect the quality of the product.

5.4.1.2.5.3 Levels of luteolin

To identify and quantitate the levels of luteolin in *A. afra* extract, the HPLC assay method was used.

Validation of the HPLC assay for luteolin

Under the HPLC conditions used in this study, a good symmetrical peak, with a retention time of 28.25 ± 0.255 minutes was found for luteolin (figure 5.6). The limit of detection was $0.03 \mu\text{g/ml}$ (0.61 ng on column) and the limit of quantification $0.09 \mu\text{g/ml}$ (1.84 ng on column).

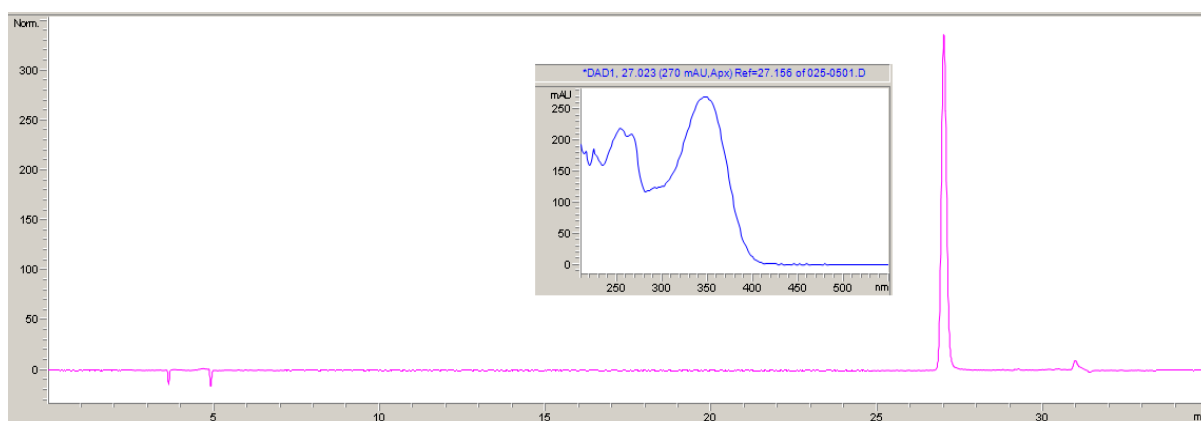


Figure 5. 6 HPLC chromatogram of luteolin (inset is the UV-spectrum of the compound) with retention time of 28.25 minutes

The standard curve of peak area *versus* concentration of luteolin was linear in the range, $1.29 - 82.5 \mu\text{g/ml}$ (i.e. $12.9 - 825 \text{ ng}$ of luteolin on column), with R^2 value of 0.999 (figure 5.7). It was described by the equation:

$$y = 90.01x + 13.86$$

Where y = peak height ratio

x = luteolin concentration in $\mu\text{g/ml}$.

In addition, the average intra-day and inter-day precision over the concentration range of the standard curve were $0.986 \pm 0.728 \%$ and $1.202 \pm 0.672 \%$, respectively.. The assay method was therefore deemed as precise since the intra-day and inter-day precision was less than 2% (FDA, 2000).

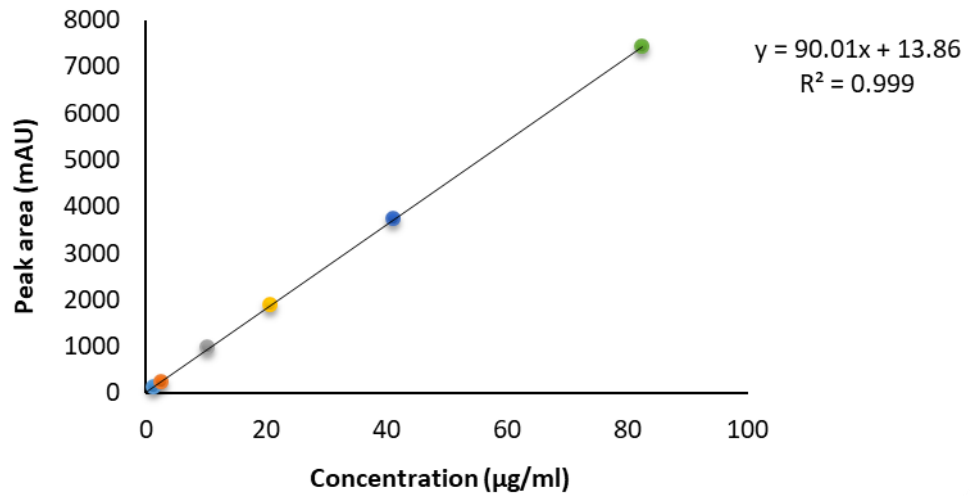


Figure 5.7 Standard curve for the luteolin

Identification and quantification of luteolin levels in *Artemisia afra* extract

The validated HPLC assay was used to identify and quantitate the luteolin levels in unhydrolysed and hydrolysed *A. afra* FDAE powder. The un-hydrolysed and hydrolysed samples were analysed and representative chromatograms obtained are given in figures 5.8 and 5.9, respectively.

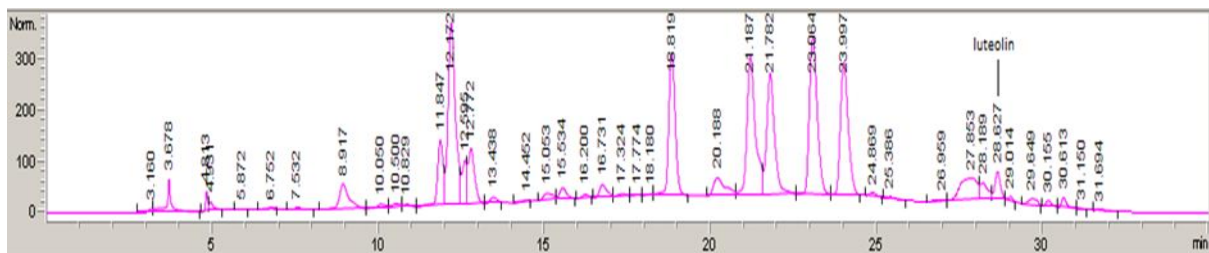


Figure 5.8 Representative HPLC chromatogram of unhydrolysed *Artemisia afra* FDAE powder. The retention time for luteolin was 28.25 minutes

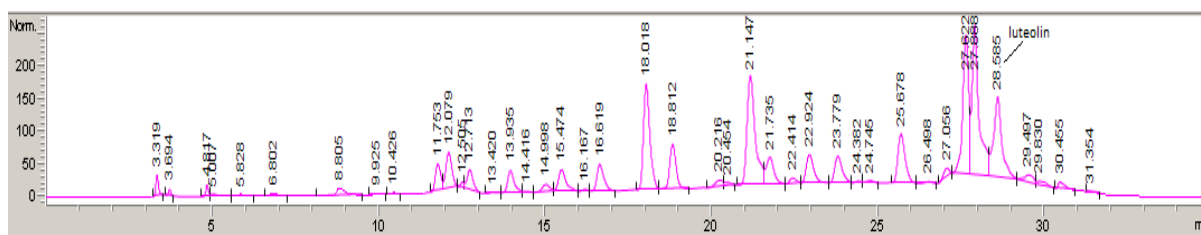


Figure 5.9 Representative HPLC chromatogram of hydrolysed *Artemisia afra* FDAE powder. The retention time for luteolin was 28.25 minutes

The chromatograms of the un-hydrolysed and hydrolysed *A. afra* showed peaks of several other compounds, most of which had UV/Vis spectra similar to that of flavonoids. The luteolin peak (retention time 28.25 ± 0.255 min) was identified by UV spectral analysis using the diode array detector.

The flavonoid profile of un-hydrolysed *A. afra* showed roughly 14 distinctive (above area of 60) peaks. About 10 of these peaks had UV/Vis spectra similar to that of flavonoids. Extra peaks were evident after acid hydrolysis. The luteolin peak area in the un-hydrolysed *A. afra* was 78.7 mAU, increasing to 148.3 mAU following hydrolysis. *Artemisia afra* contained 0.27 ± 0.014 $\mu\text{g}/\text{mg}$ free luteolin and 3.872 ± 0.296 $\mu\text{g}/\text{mg}$ total luteolin of FDAE. Acid hydrolysis presumably leads to increase in peak area of aglycones as a result of flavonoid glycoside hydrolysis to flavonoid aglycones (Baranowska & Magiera 2011). These results indicated the presence of luteolin in *A. afra*. It has been reported that luteolin may possess smooth muscle relaxant properties (Mjiqiza et al. 2013) and as such can serve as an active pharmaceutical ingredient (API) of *A. afra*. The determination of its level in the plant can thus be used for quality control.

In summary, the *A. afra* FDAE was an irregularly shaped, coarse and light brown powder. It had a bitter taste and aromatic smell, and its colour changed from light brown to dark brown on exposure to air. The extract contained measurable quantities of possible actives such as phenols and the quality control flavonoid marker compound (i.e. luteolin), which may be responsible for the plant's role /use in the treatment of respiratory and inflammatory disease conditions. However, the moisture content of the FDAE was more than the usual recommended specification of $< 10\%$ w/w moisture, which could lead to possible microbial contamination and formulation problems such as agglomeration of powder particles.

5.4.2 Preparation and quality characteristics of *Artemisia afra* freeze dried aqueous extracts (FDAE) capsules

5.4.2.1 Issues pertaining to preparation

The *Artemisia afra* capsules were prepared using a manual size zero capsule filling machine. During encapsulation, the *A. afra* powder was sticky, adhered to the parts of the filling machine and changed colour from light brown to dark brown. This was possibly due to the hygroscopicity of the FDAE powder. In order to minimize formulation errors as a result of these shortcomings any encapsulation should be done in an environment with controlled temperature and humidity.

5.4.2.2 Quality characteristics

5.4.2.2.1 Organoleptic features

The prepared *A. afra* capsule products were elegant, dark brown size 0 capsules with an oblong shape (figure 5.10). The dark brown colour of the capsule could have been due to the moisture absorbed by the extract during encapsulation. It could also be as a result of the capsules absorbing some moisture which then have affected the hygroscopic powder in the capsule. A third possible reason could have been that the powder was not completely dried or stored properly. In light of these, capsules of *A. afra* FDAE would need to be packed in an amber bottle and as a standard also packed with small packets of desiccant materials which will absorb excess moisture to prevent the product from degradation. This is a necessity as such degradation could affect the quality of the product. Vegetarian capsules (i.e. without gelatine) may also be a better choice for encapsulation of the moisture-sensitive *A. afra* FDAE.



Figure 5.10. The formulated *Artemisia afra* FDAE capsules

5.4.2.2.2 Moisture content

After the capsules were filled, the moisture level of its contents was again (within a week) tested to ascertain if there had been changes in moisture level during their preparation. The moisture level of the contents of *A. afra* capsules was 14.40 ± 0.727 % (appendix 5.5) while the moisture content for the *A. afra* FDAE extracts was 10.76 ± 0.562 . There thus appeared to have been an increase in the moisture level of *A. afra* FDAE after encapsulation. This suggests that the extract (i.e. API) absorbed some moisture during the filling procedure. Since the moisture absorbed may speed up degradation the humidity conditions during the manufacture and storage conditions of these capsules is a crucial factor and the capsules should preferably be prepared under more controlled humidity conditions and stored with a desiccant material.

5.4.2.2.3 Uniformity of weight

The data from the weight uniformity tests of *A. afra* capsules is given in appendix 5.6. The average percentage deviation in weight for the *A. afra* capsules was 2.23 ± 1.879 %. According to the British Pharmacopoeia (BP, 2013b), the limit on acceptable percentage deviation in weight from average for capsules is ± 7.5 . The afore-mentioned results thus indicated that the *A. afra* capsules met the British Pharmacopoeia specification for weight uniformity.

5.4.2.2.4 Uniformity of content

For this, total flavonoid and total phenol content were used as measures of content in *A. afra* capsules.

For TFC in the *A. afra* capsules, quercetin was used as the standard to establish the calibration plot (figure 5.4). The plot was linear over the 0 to 100 µg/ml concentration range ($R^2 = 0.9995$) and the equation was $Y = 5.609 * X + 88.3$). The TFC in the *A. afra* capsule was expressed as quercetin equivalent (QE) mg/g of the capsule, and was found to be 105.05 ± 9.304 mg QE/g of the *A. afra* capsules (n = 10) (Table 5.2).

For TPC in *A. afra* capsules, gallic acid was the standard used to establish the calibration plot (figure 5.5). The plot was linear over the 20 to 100 µg/ml concentration range ($R^2 = 0.9962$) and the equation was $Y = 1.495 * X + 14.99$). The TPC in *A. afra* capsules was expressed as gallic acid equivalent (GAE) mg/g of the capsule, and was found to be 335.47 ± 95.86 mg GAE/g of *A. afra* capsules (n = 10) (Table 5.2).

Table 5.2 Total flavonoid content (TFC) and total phenol content (TPC) of *Artemisia afra* capsules

Capsule No.	Weight of empty capsule (g)	Weight of capsule filled with extract (g)	Weight of extract (g)	* Deviation in weight average (%)	TFC (mg/g) in <i>A. afra</i> capsules	TPC (mg/g) in <i>A. afra</i> capsules
1	0,095	0,403	0,308	4,79	108,05	300,71
2	0,096	0,432	0,336	3,86	93,81	300,83
3	0,094	0,413	0,319	1,39	107,52	302,7
4	0,094	0,436	0,342	5,72	97,75	314,62
5	0,097	0,412	0,315	2,63	117,43	304,03
6	0,095	0,437	0,342	5,72	95,26	607,52
7	0,095	0,406	0,311	3,86	113,02	304,76
8	0,099	0,423	0,324	0,16	93,06	316,42
9	0,095	0,413	0,318	1,7	108,9	292,08
10	0,099	0,419	0,32	1,08	115,69	311
AVE	0,0959	0,4194	0,3235	3,091	105,049	335,467
SD	0,00185	0,0122	0,0123	1,988	9,304	95,861

Analyses of the capsules revealed the presence of flavonoids and phenols. Flavonoids are polyphenolic secondary metabolites found in plants (Komperlla 2004) and are believed to be important anti-oxidants. They also have antimicrobial, anti-allergic, and anti-inflammatory actions (Wickramaarachchi et al. 2016), and provide protection against harmful pathogenic microbes. For this reason, the quantitative analysis of such vital compounds is extremely significant to determine the quality of a dosage form (Ahmad et al. 2015). In addition, the content of active ingredients is a very important parameter in quality control because the therapeutic effectiveness of the dosage form is dependent on the amount of the active ingredient in the product (Awemu et al. 2015).

5.4.2.2.5 Dissolution profile

There were no pharmacopoeial specifications for the dissolution of *A. afra* preparations. Therefore, for this study, the acceptance criteria set was based on that given in the BP 2013a, viz. at least 75% of the plant contents is released within 45 minutes for immediate release dosage forms. The results of dissolution studies on the *A. afra* capsules are summarised in figure 5.11, and appendices 5.7 and 5.8.

For the dissolution profile of *A. afra* capsules contents, the data showed that >70% of the *A. afra* capsule contents was in the dissolution medium within 20 minutes and that > 75 % of the total flavonoids in the capsules were released in the dissolution medium within 30 minutes. Both the *A. afra* capsule contents and total flavonoids release were within the specification set in the British Pharmacopoeia (BP 2013a) and the results showed that the *A. afra* capsules was an immediate release solid oral dosage form with good *in vitro* bioavailability.

The dissolution profile is an essential tool used to assess the quality of solid dosage form herbal medicines intended for oral use. It is also an important quality control parameter for assuring batch to batch consistency of the product (Nair & Kanfer 2008). This parameter would be an important quality control specification for the *A. afra* capsules. It can also be used for the possible registration of such a product with recognised medicine regulatory authorities such as the MCC in South Africa.

Dissolution profile of *Artemisia afra* capsules

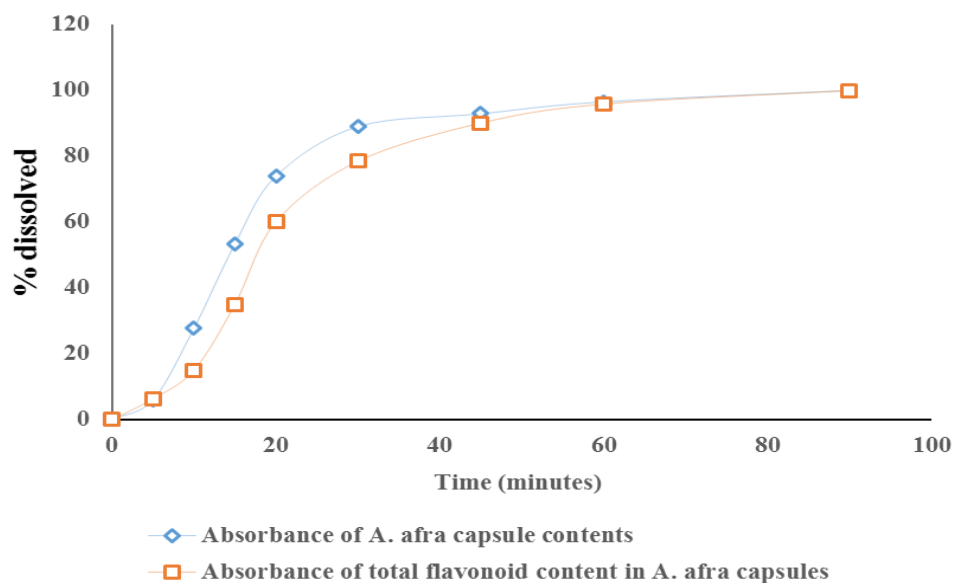


Figure 5. 11 Dissolution profile of *Artemisia afra* capsules (n=6). Dissolution conditions: basket method; 2 capsules (0.670g) in each vessel; 500ml pH 1.2 hydrochloric acid buffer; temp: $37\pm 0.5^{\circ}\text{C}$; samples quantitated by absorbance at 280 nm

In summary, the *A. afra* FDAE capsules produced were elegant, met the pharmacopoeial specifications of weight uniformity and immediate release with good *in vitro* bioavailability. They also showed the presence of possible active phytotherapeutic agents such as the flavonoids and phenols. However, the *A. afra* capsule content (i.e. *A. afra* FDAE) had a high moisture content which could lead to compromises in product quality.

5.5 Conclusion

For this study, *A. afra* FDAE (i.e. API) was prepared from the dried leaves of *A. afra* and thereafter, the FDAE encapsulated. Then the organoleptic properties, particle size and shape, ash values, moisture content, microbial contamination, TFC, TPC and levels of marker compound (i.e. luteolin) of the API; and the organoleptic features, moisture content, weight and content uniformity and the dissolution profile of *A. afra* capsules were determined. From the results obtained, the following conclusions could be drawn for *A. afra* FDAE (API) and the capsules.

For the *A. afra* FDAE (API):

Firstly, the FDAE was an irregularly shaped, coarse powder with a bitter taste which could be produced in a yield which was comparable to that obtained in previous studies.

Secondly, the FDAE had measurable amounts of flavonoids and phenols which are believed to be important anti-oxidants and also have antimicrobial, anti-allergic, and anti-inflammatory properties.

Thirdly, the extract contained substantial levels of quality control marker compound (i.e. luteolin) which is believed to possess smooth muscle relaxant properties and could easily be measured as total luteolin and free luteolin.

Lastly, the FDAE powder was hygroscopic, changed colour from light brown to dark brown on exposure to air and had a high moisture content of 10.76 % w/w (i.e. above the usually recommended ≤ 10 % w/w moisture content specification).

For the *A. afra* capsules:

Firstly, the manual capsule filling machine could be used to produce elegant capsules with uniform weight. The flavonoids and phenols in the *A. afra* capsules, i.e. the product's possible active phytotherapeutic agents, were easily and effectively quantitated and should be useful for product quality control purposes.

The capsules met the British Pharmacopoeia dissolution specification for immediate release solid oral dosage forms and had good *in vitro* bioavailability.

Finally, the capsule contents were hygroscopic and picked up more moisture during encapsulation and this could lead to compromised product quality.

In summary, many of the quality control parameters for the *A. afra* raw material and capsule final product could easily be determined. These included the organoleptic features, particle size and shape, ash values, moisture content, total flavonoid content and total phenol content, weight and content uniformity and dissolution profile. Some of the characteristics which were not assessed include density, flowability of the plant material, microbial contamination and stability. These could not be determined due to time constraints and logistics. However,

sufficient data was obtained, which were used to compile a preliminary quality control dossier for the *A. afra* capsules. Such a dossier could then be used to motivate for the registration of the *A. afra* capsules with a recognised medicine regulatory agency such as the South African Medicine Control Council.

A summary of the quality control data for the registration dossier for *A. afra* capsules is shown in appendix 5.9.



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CHAPTER 6

Investigator's brochure for *Artemisia afra* capsules

6.1 Introduction:

The previous chapter prepared and characterized *A. afra* capsules, for the purpose of compiling product quality control data. Such data can be used to prepare a medicine registration dossier and motivating for product registration. Evidence of clinical efficacy is also required for product registration and such evidence for clinical efficacy is usually obtained via clinical study. And, for the latter an Investigator's Brochure (IB), which amongst several other things also include information on the pharmaceutical quality of the product to be tested in the clinical study. The major objective of this chapter was to identify the pharmaceutical product quality aspects of the *A. afra* capsules that would be appropriate for use in motivating a clinical trial of *A. afra* capsules in chronic asthma.

In the following sections, the methods used to achieve this objective are described. Additionally, the results obtained and the conclusions drawn are also presented.

6.2 Methods:

6.2.1 Preparation of an investigator's brochure for *Artemisia afra* capsules

To identify the required "quality control" aspects of an investigator's brochure that would be used for clinical trial of *A. afra* capsule products, internet searches were conducted from July to October 2016. The websites searched were those of the following medicine regulatory agencies (www.ema.europa.eu, www.tga.gov.au, www.healthcanada-sc.gc.ac, www.who.int, www.ich.org, www.fda.gov), and the databases PubMed and Science Direct. Keywords and phrases such as "requirements for investigational drug brochure for herbal products", "investigator's brochure", "guidelines for investigator's brochure" and "guidelines for good clinical practice" were used. The information gathered from various medicine regulatory

agencies was assessed for data that would be needed to produce an investigator's brochure, suitable for use in clinical trial of a product such as *A. afra* capsules in chronic asthma.

6.3 Results and Discussion:

6.3.1 Investigator's brochure for *Artemisia afra* capsules

Generally, the purpose of an investigator's brochure is to provide the investigator and other people involved in a clinical trial with information on the study product and to facilitate their compliance with the study protocol. For this study the focus was mainly on the product quality aspects that would be required in the investigator's brochure (IB) of *A. afra* capsules to be tested in asthma and the guidelines of various medicine regulatory agencies such as Health Canada, the EMA and the International Conference on Harmonisation (ICH) were viewed.

From this search it emerged that the proposed IB for the *A. afra* capsules should contain information on the following pertinent to the quality control aspects of the trial drug: (i) Title page, (ii) Confidentiality statement, (iii) Introduction, (iv) Physical, chemical and pharmaceutical properties, as well as available formulations, and (v) Summary of data and guidance for the investigator.

The title page

The title page should provide the sponsor's name, the identity of each investigational product (i.e. research number, chemical or approved generic name, and trade name(s) where legally permissible and desired by the sponsor) and the release date. It was also suggested that an edition number be included, as well as a reference to the edition number and edition date it supersedes.

Confidentiality Statement

The sponsor may wish to include a statement instructing the investigator/recipients to treat the IB as a confidential document for the sole information and use of the investigator's team.

Summary

A brief summary (preferably not exceeding two pages) highlighting significant information on the product's physical, chemical, and pharmaceutical properties. It should also contain formulations of *A. afra* capsules that are available and relevant to the development of the investigational product.

Introduction

This is a brief introductory statement that contains the chemical name (and generic and trade name(s) when approved) of the investigational product(s), all active ingredients, and the rationale for performing research with the investigational product(s). The introductory statement should also provide information on the general approach to be followed in evaluating the investigational product.

Physical, chemical and pharmaceutical properties and formulation

The chemical and or structural formula, and a brief summary of the relevant physical, chemical, and pharmaceutical properties of the investigated product should be included. In addition, the formulation(s) to be used, including excipients (justified if clinically relevant), and the instructions for storage and handling of the dosage form, should be stated. Any structural similarities to other known compounds should be mentioned.

Summary of data and guidance for the investigator

This section should provide information, from various sources, on different aspects of the investigational product(s) wherever possible. In this way, the investigator can be provided with the most informative interpretation of available data and with an assessment of the implications of such information for future clinical trials.

It was therefore concluded that the IB for quality aspects of *A. afra* capsules would include the following: (1) Title page, (2) Confidentiality statement, (3) Summary of the product, (4) Introductory statement about the product, (5) Physical, chemical as well as pharmaceutical properties and available formulation of the product, and (6) Summary of data and guidelines for the investigator.

6.4 Conclusion

The overall objective of this study was to identify pharmaceutical product quality aspects of an investigator's brochure for *A. afra* capsules in chronic asthma management. The identified information on product quality aspects for an IB for *A. afra* include the: (1) Title page, (2) Confidentiality statement, (3) Summary of the product, (4) Introductory statement about the product, (5) Quality findings (e.g. physical, chemical and pharmaceutical properties as well as available formulations of the product), and (6) Guidance for the investigator.

However, this IB cannot be used to motivate clinical trials, as information on the clinical and non-clinical effects are not yet available. This IB only concentrated on quality aspects of the product.



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CHAPTER 7

Conclusion and Recommendations

The overall aim of this study was to prepare a quality control dossier and develop the product quality control aspects of an investigator's brochure of *A. afra* capsules for motivating its registration and clinical testing in chronic asthma. The specific objectives were to: (1) establish the minimum product quality requirements (from various medicine regulatory authorities) for registration of *A. afra* capsules, (2) to prepare and pharmaceutically characterize a capsule product of *A. afra* freeze dried aqueous extract (FDAE) for compiling quality data for a dossier suitable for *A. afra* registration, and (3) to identify pharmaceutical product quality aspects appropriate for use in an investigator's brochure (IB) motivating a clinical trial of *A. afra* capsules in chronic asthma.

The monograph for Prospan™ was used as a template to compile a quality control dossier and to prepare the relevant parts of the IB for *A. afra* capsules suitable for registration and clinical testing of such capsules.

From the results obtained, the following conclusions could be drawn;

- The minimum quality requirements that were suitable for registration of *A. afra* capsules could be classified into the quality requirements for the raw material and those required for the FHP. The minimum quality requirements for the raw materials would include: (1) information on identity of the plant, (2) parts and physical features of the plant (part) used, (3) manufacturing details, (4) general identity tests, (5) purity tests, (6) structural formula of the herbal ingredient, and (7) quality control specifications. The minimum quality requirements for the FHP would consist of: (i) information on the manufacturers of the product, (ii) details of the qualitative and quantitative composition of active components, (iii) information on control of excipients, (iv) description and composition of the product, (v) stability data of the product, (vi) packaging, container closure system and labelling information, and (vii) the certificate of analysis.

- The quality attributes, specifications and test methods for the pharmaceutical quality of *A. afra* capsules needed for the registration were separated into those quality characteristics for the raw materials and those for the finished herbal product (FHP). The quality characteristics of the raw materials included: (1) the organoleptic features, (2) chemical identity, (3) quality of plant actives, and (4) microbial, heavy metal, pesticides and mycotoxins contamination for the raw material. The quality characteristics of the FHP would consist of: (i) physical features, (ii) uniformity of dosage forms, (iii) dissolution profile, and (iv) stability studies.

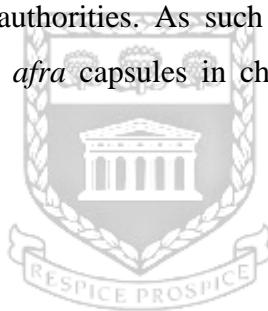
On the preparation and characterization of *A. afra* FDAE powder and capsules of *A. afra* FDAE it was found that:

- The FDAE was an irregularly shaped and coarse powder with a bitter taste. The taste is believed to have been masked by the use of capsules. It had measurable amount of flavonoids and phenols which are believed to act as important anti-oxidants. It also had measurable levels of the quality control marker compound (i.e. luteolin), which is thought to possess smooth muscle relaxant properties and may be responsible for its use in asthma. However, the FDAE was hygroscopic, changing colour from light brown to dark brown on exposure to air and it absorbed moisture to attain a moisture content of 10.76 % w/w (i.e. above the recommended limit of < 10 % w/w moisture content).
- The capsules were successfully prepared by manual encapsulation. They were elegant and uniform in weight and the *Artemisia afra* capsules showed presence of flavonoids and phenols. It also met the British Pharmacopoeia dissolution specification for immediate release solid oral dosage forms showing that it had good *in vitro* bioavailability. Analysis of the capsule content however revealed high moisture content of 14.40 % which could lead to a poor product quality.

The IB focused on the quality aspects for *A. afra* should cover the following: (a) Title page, (b) Confidentiality statement, (c) Summary of the product, (d) Introductory statement about the product, (e) Quality findings (e.g. physical, chemical, as well as pharmaceutical

properties and available formulations), and (f) Guidance for the investigator. The information gathered in this study was however insufficient to meaningfully complete the product quality aspects of the IB (e.g. lack of stability data to complete trial product storage conditions and shelf life, etc.).

Collectively, the results showed that the standards set by Health Canada, TGA, WHO and BP for the quality attributes, specifications and tests methods for the pharmaceutical quality of *A. afra* capsules with respect to the minimum quality requirements were established. Both the FDAE and the *A. afra* capsules met some of the quality control requirements, such as the chemical identity and weight uniformity, set by various drug regulatory authorities. However, the FDAE (i.e. raw material (API)) was hygroscopic and therefore not a suitable raw material for preparation of *A. afra* capsules. The quality aspects of the investigator's brochure for the *A. afra* capsules could not be prepared to fully comply with the present requirements set by the various medicine regulatory authorities. As such it was not yet suitable for use in motivating clinical trials of the *A. afra* capsules in chronic asthma and this needs further study.



Limitations of the study

Unfortunately, the microbial contamination tests were not performed on the *A. afra* FDAE. This was due to some logistics between the laboratory outside the university where the sample was supposed to be taken for testing and the university. The microbial contamination test is a crucial quality control parameter especially for herbal materials or products such as *A. afra* FDAE. The freeze-dried aqueous extracts are generally known to be hygroscopic, and are therefore prone to microbial contamination hence it was important to carry out the microbial contamination test.

The pharmaceutical monograph of herbal product such as Prospan™ which are already on the market was supposed to be used as a template to compile a quality control dossier and an IB for *A. afra* FDAE capsules. However, such a monograph was not accessible for use.

Recommendations

The hygroscopicity of the *A. afra* freeze-dried aqueous extract was probably the most important factor contributing to an unsuitable raw material for the capsules in this study. To overcome this problem, the following is recommended:

- Use of different types of solvent(s) such as methanol or methanol-water to prepare the extract. This may, of course, make the extract less similar to the traditional decoction forms of the plant.
- Control of the manufacturing environment, with special attention to the humidity level. Generally, pharmaceutical air conditioning is usually set below 50% RH. For very hygroscopic products however, particularly moisture sensitive ones as was the case in this study, humidity levels below 40% RH may be recommended for manufacturing and storage.
- Use of different capsule shells, e.g. vegetable capsules. Hard gelatin capsules are made of 86% bovine and/or pigs skin and 14% purified water while vegetable capsules are made of 92% methylcellulose and 8% purified water. The low moisture content of the vegetable capsule makes it more suitable (than the hard gelatine capsule) for moisture-sensitive raw materials such as the *A. afra* FDAE.
- The hygroscopic extract may be formulated with mannitol which is an inert excipient, and does not absorb significant amount of water or moisture.
- The beads of *A. afra* FDAE can be prepared by layering an aqueous extract onto non-pareil beads, these are uniform granules that are practically inert, tasteless and odourless. The beads can be followed by application of a moisture barrier film coating of Opadry®. Opadry® is a pigmented or white film coating system specifically developed for the coating of oral solid-dosage forms that need to be protected from environmental moisture.

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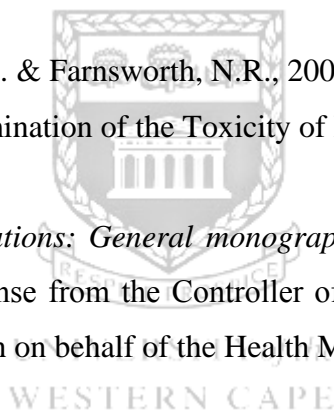
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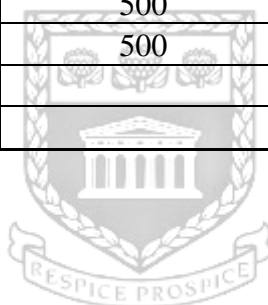
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APPENDIX

Appendix 5.1 Yield of freeze-dried aqueous extract of *Artemisia afra*

Sample	Weight of dry leaves (g)	Volume of water (ml)	Yield of aqueous extract	
			(g)	%
1	20	400	4,58	22,9
2	50	1000	12,32	24,64
3	50	1000	11,17	22,34
4	20	400	4,48	22,4
5	50	1000	11,1	22,2
6	50	1000	11,92	23,84
7	35	700	7,58	21,66
8	35	700	8,14	23,26
9	25	500	5,47	21,88
10	25	500	5,58	22,32
	AVE		8,23	22,74
	SD		3,16	0,93



Appendix 5.2 Particle size and shape of *Artemisia afra* freeze dried aqueous extract powder

<i>Artemisia afra</i> FDAE	Sieve No. (µm)	Weight of FDAE through sieve (g)	% weight of FDAE feed	Description of FDAE
FDAE of <i>Artemisia afra</i>	335	5.85	58.5	Coarse powder
	180	2.79	27.9	
	125	1.95	19.5	
	90	0.82	8.2	

Appendix 5.3 Total ash and acid-insoluble ash of *Artemisia afra* freeze dried aqueous extract powder

Sample no.	Mass of aqueous extract (g)	Mass of free carbon ash (g)	Percentage of total ash (%)	Mass of acid insoluble ash (g)	Percentage of acid insoluble ash (%)
1	2,0088	0,2607	12,9779	0,0101	0,5028
2	2,0055	0,2911	14,5151	0,0185	0,9225
3	2,0048	0,2541	12,6746	0,0095	0,4739
4	2,0017	0,2842	14,1979	0,0099	0,4946
5	2,0035	0,2489	12,4233	0,0201	1,0032
AVE	2,0049	0,2678	13,3578	0,0136	0,6794
SD	0,0026	0,0188	0,9394	0,0052	0,2605



Appendix 5.4 Moisture content of *Artemisia afra* freeze dried aqueous extract powder

Sample	Mass of aqueous extract of <i>Artemisia afra</i> (g)	Percentage of moisture (%)
1	0,3269	11,39
2	0,3175	10,31
3	0,3192	10,58
AVE	3212	10,76
SD	0,005	0,562

Appendix 5.5 Moisture content of *Artemisia afra* capsules

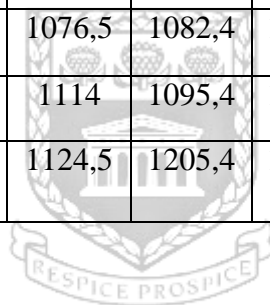
Sample	Mass of aqueous extract of <i>Artemisia afra</i> (g)	Percentage of moisture (%)
1	0.4952	13.64
2	0.5457	15.09
3	0.5284	14.47
AVE	0.5231	14.40
SD	± 0.0257	± 0.7275

Appendix 5.6 Uniformity of weight of *Artemisia afra* capsules

	weight of empty capsules (g)	weight of filled capsules (g)	weight of extract (g)	*Deviation in weight average (%)
1	0.095	0.393	0.298	6.88
2	0.096	0.427	0.331	3.44
3	0.094	0.406	0.321	0.31
4	0.094	0.425	0.331	3.44
5	0.096	0.416	0.320	0.00
6	0.097	0.424	0.327	2.19
7	0.094	0.426	0.332	3.75
8	0.097	0.418	0.321	0.31
9	0.095	0.412	0.317	0.94
10	0.095	0.414	0.319	0.31
11	0.099	0.426	0.327	2.19
12	0.092	0.397	0.305	4.67
13	0.095	0.415	0.320	0.00
14	0.100	0.431	0.331	3.44
15	0.099	0.420	0.321	0.31
16	0.098	0.421	0.323	0.94
17	0.096	0.424	0.328	2.50
18	0.095	0.401	0.306	4.34
19	0.096	0.418	0.322	0.63
20	0.098	0.402	0.304	5.00
AVE	0.096	0.416	0.320	2.23
SD	± 0.0020	± 0.011	± 0.0099	± 1.879

Appendix 5.7 Dissolution profile of *Artemisia afra* capsules

A. <i>afra</i> capsules									
Vessels									
Time	Red	Orange	Blue	Yellow	Green	Black	AVE	SD	% Dissolved
0	0	0	0	0	0	0	0	0	0
5	75,9	90,5	70,5	41,2	54,2	68,5	66,8	17,18	6
10	292,5	393,3	250,7	350,4	299,5	320,5	317,82	49,5	27,76
15	599,5	697,9	520,5	499,8	650,9	699,5	611,35	86,79	53,4
20	867,1	762,2	850,4	846,8	859,5	894,5	846,75	44,76	73,96
30	1044,5	927,1	995,8	1052,8	1041,5	1055,2	1019,48	50,18	89,05
45	1060,5	1061,4	1014	1076,5	1082,4	1092,4	1064,53	27,64	92,94
60	1082,9	1097,1	1073,3	1114	1095,4	1170,1	1105,47	34,54	96,56
90	1126,9	1104,5	1115,6	1124,5	1205,4	1192,1	1144,83	42,71	100



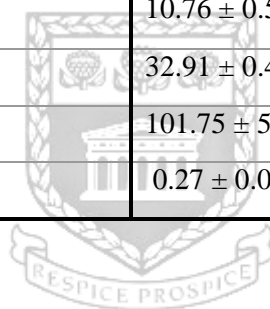
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Appendix 5.8 Dissolution profile of total flavonoid content of *Artemisia afra* capsules

Total flavonoid content in <i>Artemisia afra</i> capsules									
Vessel	Red	Orange	Blue	Yellow	Green	Black			
Time	TFC (mg/g)	TFC (mg/g)	TFC (mg/g)	TFC (mg/g)	TFC (mg/g)	TFC (mg/g)	AVE	SD	% dissolved
0	0	0	0	0	0	0	0	0	0
5	7,002	1,61	4,367	3,034	9,174	8,86	5,675	2,868	6,32
10	7,416	19,248	7,49	5,743	16,813	24,136	13,474	7,622	15,01
15	10,375	44,803	28,016	12,602	26,825	65,924	31,424	20,973	35,01
20	67,692	58,277	41,367	28,117	54,614	73,691	53,96	16,872	60,12
30	83,074	72,679	64,357	47,932	76,319	80,174	70,756	12,938	78,5
45	88,276	77,088	76,435	62,467	90,033	87,184	80,247	10,479	90,11
60	91,934	84,391	76,982	79,466	94,655	88,926	86,059	7,004	95,88
90	93,381	90,357	83,774	81,997	97,055	91,924	89,748	5,787	100

Appendix 5.9 A summary of select data for quality control dossier of *Artemisia afra* capsules

Artemisia afra capsules:	
Herbal raw material quality control tests and Specification	
Quality control tests	Specifications
Organoleptic properties (colour, odour, taste)	Light brown in colour changing to dark brown on exposure to air, aromatic odour and bitter
Particle size and shape	coarse powder and irregular
Total ash value (%)	13.36 ± 0.939
Acid insoluble ash (%)	0.679 ± 0.261
Moisture content (%)	10.76 ± 0.56
Total flavonoid content (mg QE/g)	32.91 ± 0.46
Total phenolic content (mg GAE/g)	101.75 ± 5.55
Free and total luteolin (µg/mg)	0.27 ± 0.014 and 3.872 ± 0.296



Artemisia afra capsules:	
Finished Herbal Product (Capsules) quality control tests and Specification	
Quality control tests	Specifications
Physical features	Elegant, dark brown size 0
Moisture content (%)	14.40 ± 0.727
Weight uniformity (% average deviation in weight)	2.23 ± 1.879
Content uniformity	
Total flavonoid content (mg QE/g)	105.05 ± 9.304
Total phenol content (mg GAE/g)	335.47 ± 95.86
Dissolution profile	Immediate release and good <i>in vitro</i> bioavailability (i.e. dissolution of > 70% (Q) in 20 minutes)



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