MEDICINAL PROPERTIES OF TRIPLE-COMBINATION PLANTS USED BY THE *KHOISAN* COMMUNITY FOR COMMON COLD AND INFLUENZA-LIKE ILLNESS

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DECLARATION

I Teboho Moses Taaka declare that this research study is my own original work. It is submitted in fulfilment for the degree of Master of Science at North West University Mafikeng campus not to any other university.

This research study is the part of Seboka NRF project
Project: Indigenous knowledge system (IKS)
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Signature:

SEBOKA DECLARATION

SEBOKA

The potency of a lifelong initiative

This research project is a sub-project of the Seboka research Team. The African academic is firstly the child of mother Africa and secondly the creator of knowledge in the primary context of Africa and secondarily in the global sphere. The configuration of an African scholar's identity necessarily entails accepting a bundle of responsibilities shaped by mother Africa's potent imperatives. Etymologically defined, 'Seboka' denotes a 'group,' a 'team,' a 'community' and a phenomenal 'coming together' of sorts. The term of necessity subsumes one's ephemeral individuality under the value-generating ethos of 'communitarian' solidarity. A signifier of the shared benefits of synergy, the Seboka emblem - depicting a pride of lions on a mission under the supreme guidance of collective vision - is a celebration of the invaluable wealth of sharing and reciprocal engagement which lies at the heart of Africa's philosophy. As such, the Seboka concept was born out of respect for the imperatives of mother Africa, whose breast has availed the milk of human kindness moulding the African children into a team of valiant warriors in legitimate defence of their priceless heritage.

The Seboka logo summons to memory the telling axiom, 'A lion that goes on a hunt by itself, without co-existing in a pride, will always fail to catch even a limping deer.' In the same communitarian spirit, Seboka uses the claypot as a key emblem, symbolising sharing and communal solidarity. The Seboka team perceptively unpacks this definitive element of African life and essence, the profound *Ubuntu* philosophy, potently encapsulated in the dictum 'I am, because we are,' hence placing community and group care above the focus of the self. This Seboka team is a rich confluence of various tributaries, but the Community is their first consideration.

The hallmark of Seboka's invaluable research output has been the endeavour to strike signature partnerships with the community, the very custodians of the forests, mountains and rivers which are the abode of nature's healing essence and strength. Quite enlightening is the Khoi-chief's statement made recently in an open platform, *'The veld is our chemist'* (Kok V, 2013). The wisdom enshrined in this statement is a telling testimony of how conventional medical practice has always tapped into the resourcefulness of medicinal plants and other curative phenomena in Africa's rich forests. Notwithstanding the research on medicinal plants, the Seboka team predominantly re-engineer the broader practices of the African child

Seboka Greeting

SALUTATIONS

I would like to salute my mother Mateboho, my sister Kedu, my bother Abel and my ‘twin’ sister Neo for their endless motivational support. Ladies and gentleman you are special. Thank you.
To my extended family and friends, if I did not have time to refresh and regenerate strength in your presence, this study would have been the heavy load on my shoulder. Thank you.

My supervisor Prof A.J Pienaar and co-supervisor Prof O. Ruzvidzo, thank you very much, I would not have made it if you did not play your role.

Dr F. K. Treurnicht at National Institute for Communicable Diseases Division of National Health Laboratory Services. Thank for making time to capacitate the researcher on the NA-XTD neuraminidase assay.

The indigenous *KhoiSan* Campbell community. Thank you for giving the researcher the legacy, this research study would not succeed if you did not participate fully.

Seboka Indigenous Research project team, as we say “A solitary lion is incapable of even catching a limping deer. Thank for your support.

DEDICATIONS

To beloved Mom, sister, the extended family, the friends, *KhoiSan* Campbell community and the Seboka Indigenous research project**.**

LIST OF ABREVIATIONS

APA	American Psychological Association
ICHPC	Indigenous Community Health Practitioner Committee
VC	Virus Control
WHO	World Health Organisation

ABSTRACT

Key-words: medicinal plants, medicinal properties, common cold, influenza, mono-therapy decocotion and triple-combination concoction, KhoiSan Community

The *KhoiSan* society in the last 2000 years depended on nature for their basic needs, such food, clothing and shelter, and of the most significant for the research study, the management and the treatment of ailments by the use of the medicinal plants. The decoction of *Artemisia afra, Ruta graveolens and Sutherlandia frutescens* and the triple-combination therapy concoction of these medicinal plants are used to treat upper respiratory track disease such as common cold and influenza-like (flu) illness. The medicine is indigenously harvested and prepared by the *KhoiSan* community for medicinal use. This prompted the researchers to explore how medicinal plants are harvested, prepared, stored and conserved by the *KhoiSan* community for the treatment of common cold and influenza-like illness. This research deployed two phases.

In the first phase of the research a clinical ethnography method was used to explore this phenomenon with specific emphasis on respectful engagement with the *KhoiSan* Community's medicine men and women to guide the process. This research employed a purposive sampling method. Data were collected using clinical ethnography and analyzed thematically. The results yield inexpensive preparation, time-saving and easily manageable method that is unique and acceptable to this indigenous community.

In the second phase, an In vitro study by making use of NA-XTD influenza neuraminidase assay kit was conducted to test the antiviral properties of this medicine, and to determine the 50% reduction of the neuraminidase activity and the inhibitory concentration (IC_{50}) of each medicinal decoction and triple-combination concoction. Conclusively, the findings confirms with the community medicinal utilization of these medicinal plants for common cold and influenza-like illness. This medicine has antiviral properties as reflected by the reported results of the research study.

Operational Definitions

Medicinal plant: plants found in the community-gardens and the veld used for healing purposes

Medicinal properties: the therapeutic, healing constituents in medicinal plants

Common cold/Influenza: According to Khoisan community's explanation, common cold or influenza is coughing, fever and painful body that is accompanied by lack of appetite as well as tiredness

Mono-therapy decoction: Medicinal plants singulary prepared

Triple-combination therapy concoction: The mixture of all three medicinal plants added together in synergy

KhoiSan Community: The indigenous Griqua community from the Northern-Cape

OPSOMMING

Sleutelwoorde:medisinale plante, medisinale gebruikswaarde, verkoue, griep, enkel-en drie-kombinasie afkooksel, KhoiSan community

Die KhoiSan gemeenskap het in die laaste 2000 jaar op die natuur staatgemaak vir basiese behoeftes, soos voedsel, klere en behuising, asook wat mees belangrik is vir hierdie navorsing, die hantering en behandeling van siekte deur medisinale plante te gebruik. Die mono-terapeutiese gebruik van wildeals, wynruit en kankerbos asook die drie-kombinasie-afkooksel van die genoemde medisinale plante word gebruik om boonste lugweginfeksies soos verkoue en koors te behandel. Die medisyne word inheems ge-oes en voorberei deur die KhoiSan gemeenskap vir medisinale gebruik. Laasgenoemde het die navorser geprikkel om te verken hoe hierdie medisinale plante ge-oes, voorberei, gestoor en bewaar word in die KhoiSan gemeenskap vir die jarelange behandeling van verkoue en griep. Die navorsing gebruik twee fases.

In die eerste fase van die navorsing is ‘n kliniese etnografiese metode gebruik om die fenomeen te verken met ‘n spesifieke fokus op ‘n respekvolle tussentrede met die KhoiSan gemeenskap se medisyne mans en -vroue om die proses te begelei. Die navorser het gebruik gemaak van ‘n doelgerigte steekproefneming. Inligting is ingesamel deur genruik te maak van semi-gestruktureerde vraelys en die data was tematies geanaliseer. Die uitkoms van die fase wys op ‘n kosste-effektiewe voorbereiding, wat tyd bespaar en maklik hanteerbaar is in die gemeenskap. Die gebruik is aanvaarbaar en uniek in diè gemeenskap.

In die tweede fase is ‘n in-vitro metode wat gebruik gemaak het van die NA-XTD griep neuraminidase plaatjie-toets. Hierdie toets is toegepas om die antivirale effek die die plantmedisyne asook die 50%-reduksie van die neuraminidase aktiwiteit insluitende die inhiberende konsentrasie (IC_{50}) van elke momo-afkooksel asook die drie-kombinasie afkooksel te bepaal. Opsommend het die bevindinge die gemeenskap se gebruik van die medisinale plante vir verkoue en griep bevestig. Die plantmedisyne het dus antivirale effekte soos bevestig deur die bevindinge van die studie.

Operational Definitions

Medisinale plante: plante in gemeenskapstuine en die veld wat vir heling gebruik word

Medisinale gebruikswaarde: medisinale en terapeutiese waarde in medisinale plante gevind

Verkoue/Griep: Volgens die KhoiSan gemeenskap; hoeserigheid, koors en pynlike liggaam

Enkel-terapie afkooksel: Enkel-voorbereiding van medisinale plant

Drie-kombinasie afkooksel: Mengsel waar al dire medisinale plante bymekaar gevoeg word

KhoiSan Gemmenskap: Die inheemse Griekwa-gemeenskap van die Noord-kaap

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1.1 TITLE

Medicinal properties of triple-combination plants used by the KhoiSan community for common cold and influenza-like illness

1.2 OVERVIEW OF THE RESEARCH

This research consists of phases, namely; the qualitative phase and quantitative phase. The researcher used an article format for the research. In the overview, the introduction and background, preliminary literature study, problem statement, the aim and objectives of the research study, research methodology, trustworthiness of the qualitative phase are discussed. Subsequently, the quantitative phase in which the following are also discussed: investigation of the antiviral properties, introduction, antiviral activity against influenza virus, the influenza (flu) virus, preparation of medicinal plants extracts, research design, in vitro assay, the half maximal inhibitory concentration (IC_{50}).

1.3 INTRODUCTION AND RATIONALE FOR THE RESEARCH

The utilization of medicinal plants dates back to the origin of human civilization on earth and continues unabatedly Mukhtar *et al*. (2008:112). Cragg and Newman, (2013:3670) concur that throughout the ages, humans have relied on nature for their basic needs, and of the most interest to the study is the use of medicinal plants for the treatment of a wide range of diseases. Gurib-Fakim (2006:3) is of the opinion that the use of medicinal plants is a primordial custom as the medicinal plants are the foundation of the indigenous medicine systems that have been in existence for thousands of years and continue to provide mankind with new remedies for every ailment. Mukhtar *et al*. (2008:112) also mentioned that medicinal plants have been used throughout the world. However, their wide usage had been limited to China, India, Japan, Pakistan, Sri Lanka, Thailand and a number of African countries. Furthermore, Scott and Hewett (2008:340) agree that the use of medicinal plants is an ancient custom, dating ages back from the arrival of *KhoiSan* community in Southern Africa.

Deducing from this literature evidence, the commanding statement asserted by the Chief of the *KhoiSan* in the Northern Cape as stated below, harmonizes with the previous authors:

> "The veld is my chemist, is the source of the natural medicinal plants for the treatment and management of all kinds of unhealthy conditions that perturb our bodies. From the ages of our grandparents until today, the medicinal plants have been our natural medicine that we use freely without or with less harmful effects as compared to Western medicine with very unbearable side effects."

The evidence and the statement of the Chief illuminate the indication by Scott and Hewett (2008:340), that the primeval humanity, the San and the *KhoiSan* are the indigenous people in Southern Africa. Therefore, the knowledge of the medicinal properties of plants has always been not only a matter of value, but of healing for this community. Hence, for the matter of indigenous confirmation and western scientific validation, Mphuthi (2015:257), endeavours to explore and explain one of the *KhoiSan* legacies which are still been practiced in this community currently, namely; the management of common cold or influenza-like illness by the use of the triple-combination therapy *of Artemisia afra, Ruta graveolens and Sutherlandia frutescens*. In support of the use of combination therapy, Zonyane *et al.* (2013: 144) state that combination of plants to treat sicknesses has been practiced for centuries in indigenous medicine systems with great success. Similarly and Williamson (2001:401) posit that combination therapy is not only limited to indigenous medicine, but is also a practice in conventional medicinal therapy for ailments such as cancer chemotherapy, HIV management, infectious diseases and life style diseases such as hypertension and diabetes to mention few.

It is also noted that several plants have been used to treat microbial infections (Mukhtar *et al.,* 2008:112). Sequel to the previous assertion; Balunas and Kinghorn (2005:431) agree that medicinal plants were used as medicine for thousands of years and added that these medicines were utilized in its crude forms like teas, powders and other formulations. Zonyane *et al.* (2013: 144) concur with Balunas' statement and adds that the combination of medicinal plants to manage illness have been practiced for centuries.

According to Gathirwa *et al.* (2008:224), in indigenous medicine, different plants are combined for the treatment of a disease, and this combination has occurred for decades in cultural medicinal plants systems. The medicinal plants used are often not related in any way or may be used singularly for the treatment of that ailment as (Sibandze 2010:507). In his study of synergy research, Wanger (2011:36) asserts that the combination therapy regime for antimicrobials is known to prevent antibiotic resistance Furthermore; Gathirwa *et al.* (2008:229) concurs that drug combinations also help reduce danger of resistance development. In addition, Gathirwa *et al.* (2008:225) maintain that it is evident that the combination of medicinal plants to treat ailments has been proficient for years in

indigenous medicine systems with immense victory. Furthermore, as mentioned by Lim *et al.* (2008:2898), there is a belief that antimicrobial combination therapy also increases the efficacy spectrum of activity and potentially reduces side effects. Moreover, there is a belief that indigenous antimicrobial combination therapy also increases the spectrum of activity as seen in Van Vuuren and Viljoen (2008:701) study. Also, the better therapeutic effect in combination therapy is credited to synergistic interactions between the different bioactive plant constituents (Wagner & Ulrich-Merzenich, 2009:98).

Additionally, the status quo remains for natural scientists to investigate medicinal plants just to find the single constituent accountable for the therapeutic effect (Rates 2001:604). When considering that the biological activity may be the result of the combination of several compounds, the isolation process may lead to its loss or reduction of the therapeutic effect (Carmona & Pereira 2013:379-380). Some authors have mentioned that sometimes the whole mixture of the medicinal plants have greater effects than isolated compounds. Moreover, Wanger (2011:34) highlighted in his study that sometimes diseases possess a multi-causal etiology and a complex pathophysiology, which can be treated more effectively with well-chosen medicinal combination than with a single isolated constituent.

Currently, the irresistible interest in medicinal plants is due to several reasons, namely; conventional medicine is becoming inefficient; for example, side effects and ineffective therapy, abusive and or incorrect use of synthetic drugs results in side effects and drug resistance. A large percentage of the world's population does not have access to conventional pharmacological treatment, and indigenous medicine and ecological awareness suggest that natural products are harmless (Rates 2001:604). According to World Health Organisation (WHO), indigenous medicinal system is a care that is close to homes, accessible and affordable. It is also culturally acceptable and trusted by large numbers of people. The affordability of most indigenous medicines makes them all the more attractive at a time of soaring health-care costs (WHO, 2013:17). Plant-based medicine is especially suitable for long-term treatment of chronic diseases, in geriatric and convalescent patients, for follow-up treatment, and in the prophylaxis of infectious, degenerative and metabolic diseases (Cravotto *et al.*, 2010:11). Adding to the latter, combination treatment was linked to the belief that antimicrobial combinations increases the spectrum of activity and reduces the potential of side-effects (Zonyane, *et al.,* 2013: 145). Therefore, this research focuses on a triple combination plant medicinal therapy used for common cold and influenza-like ailments in a *KhoiSan* community.

1.4 A PRELIMINARY LITERATURE STUDY

Medicinal plants were the first and only medicines available to mankind since time immemorial. The whole of the crude extracts remain the primary healthcare for a majority of the world's population (Ganesan 2008: 306). In the same vein, Cragg and Newman (2013:3670) concur that plant-based medicine continues to play an essential role in healthcare, and their usage by different cultures has been comprehensively documented. The above authors agree with WHO (2013:17) which maintains that for many millions of people, medicinal plants, indigenous treatments, and indigenous practitioners are the main source of health care, and sometimes the only source of care. In addition, WHO also mentions that, in many developing countries, indigenous medicine plays a significant role in meeting the primary health care needs of the population, and it has been used for a long time (WHO 2013:29).

The two examples of medicine combinations with clinically confirmed synergy effects are as follows: First, the medicinal plants preparation Iberogast® consists of nine plant extracts and is used for the treatment of functional dyspepsia and motility related disorders. The preparation exhibits therapeutic equivalence when compared with the synthetic drugs such as cisapride and metoclopromide, with the additional advantage that this medicinal plants combination preparation exhibited fewer or no side effects (Wanger 2011:36). Secondly, Ayurveda medicine, which uses many fixed combination formulae, includes *"Trikatu."* This mixture contains black pepper, *Piperlongum*, and ginger, *Zingi berofficinalis* and although an ancient recipe, it is only recently that this combination has been investigated scientifically, and the study revealed that the pepper in the combination contains the alkaloid piperine, which is known to increase the bioavailability of a number of drugs such as vasicine (also known as peganine), an antiasthmatic alkaloid from *Adhatodavesical* (Williamson 2001:402).

According to Williamson (2001:401), the total medicinal plant extract shows a better therapeutic effect than an equivalent dose of an isolated active compound. Noteworthy, Ncube *et al.* (2012: 81-82) is in agreement, that the total contents of a medicinal plant product has a significant better effect than an equivalent dose of a single isolated active ingredient, or a single constituent medicinal plant. In elaboration, many phytomedicines on the market today such as *Ginkgo biloba* and *Echinaceae purpurea* are sold as whole extracts and it is understood that the synergistic interactions between the constituents are responsible for the therapeutic efficacy (Van Vuuren & Viljoen 2008:700). Van Wyk (2011:814) states that number of medicinal plants are regularly sold as crude, unprocessed drugs in markets in various parts of South Africa.

The indigenous medicines remain a source of viable mainstream for affordable healthcare, especially in developing countries. In South Africa, an estimated 70% of the population depend on medicinal plants that can be used as mono or combination therapy (Mjiqiza *et al.,* 2013:648). Most popular used of the medicinal plants include plants such as *Artemisia afra, Ruta graveolens* and *Sutherlandia*

frutescens (Suliman *et al*., 2010:655). The plants that this research focus on for the combination therapy used in the *KhoiSan* community are *Artemesia afra, Ruta graveolens* and *Sutherlandia frutescens*. Hence, the researcher focuses on the medicinal use of these plants below:

Artemisia afra also known as (African wormwood or *Wilde-als*), is a multi-stemmed aromatic perennial shrub, resembling the A. vulgaris (English wormwood) with greyish-green leaves (Mjiqiza *et al.,* 2013:648; Viljoen *et al.,* 2006:19). The African wormwood is commonly found in most parts of the Southern Africa. In South Africa, *Artemisia afra* is well used as a medicinal plant by diverse indigenous communities however it is locally locally known under different names. In *Sotho* and *Tswana* it is called "*Lengana*", in *Xhosa* and *Zulu* it is known as "*Umhlonyane*" and "*Mhlonyane*" respectively, and in *Afrikaans* "*Wilde-als*" (Liu, Van der Kooy, & Verpoorte, 2009:186, Viljoen *et al.,* 2006:19).

Adding to that, *Artemisia afra* is one of the most ancient- and well-known of all the indigenous medicinal plants in Southern Africa. Mukinda and Syce (2007:138) and van Wyk (2008:347) concur with one another on this fact. It is usually used for treating a variation of ailments such as coughs, colds, headaches, chills, dyspepsia, gastric conditions, colic, croup, whooping-cough, gout, asthma, malaria, diabetes, bladder and kidney disorders, influenza, convulsions, and fever (Viljoen *et al.,* (2006:19), Ntutela, (2009:S34), Mukinda & Syce 2007:138). Apart from this, *Artemisia afra* also exhibits potent pharmacological activities inclusive of antimicrobial, antioxidant, CNS-effects (sedative, antidepressant), cardiovascular, and spasmolytic activity which has been well documented and reviewed (Suliman *et al.,* 2010:655).

Not withstanding the above however; the predominant use of *Artemisia afra* is for the treatment of respiratory disorders such as coughs, colds, bronchitis, blocked sinuses and tight-chest or asthma in the form of a steam inhalation of the leaves extracts (Mjiqiza *et al., .*2013:648). Moreover, it is normally used as mono- or combination therapy in conjunction with other medicinal plants to manage of upper and lower respiratory tract infections (Suliman *et al.,* 2010:655). The aromatic leaves of this plant exhibit various pharmacological effects including relaxation (bronchodilatory effects) of respiratory smooth muscles (Mjiqiza *et al.,* 2013:648).

In further elaboration of the aforementioned, its indigenous use as an anti-infectious therapy is supported by the scientific confirmation studies which validate its antimicrobial and antifungal properties. The essential oils of *Artemisia afra* exhibit inhibitory activity against some Gram-positive and Gram-negative bacteria, fungi, as well as protozoa (Suliman *et al.,* (2010:655) and *Aspergillus*

ochraceus, *Artemisia niger, Artemisia parasiticus, Candida albicans, Alternaria alternata* and *Geotrichum candidum* (Ntutela, *et al.,* 2009:S34). These scientific confirmation studies which confirm that *A. afra* exhibits an extensive range of biological and pharmacological activities thus validating and substantiating the therapeutic use of these medicinal plants by the indigenous community in South Africa (Mukinda & Syce 2007:138).

Apart from that, in South Africa *Ruta graveolens* is also known as (*wynruit*), and together with *Artemisia afra* (*wildeals*) are the most popular medicinal plants used. The infusion of their leaves is used for the treatment of cold (Van Wyk *et al.,* 2008:701), and as to strengthen the medicinal uses of *Ruta graveolens*, De Beer and Van Wyk (2011:748) said that the Rue infusion are taken for colds, headache and influenza, and Van Wyk (2008:338) mentioned that Rue infusion is for inflammation, rheumatism, fever, chest ailments, diabetes and high blood pressure.

Adding to that, Harish Kumar *et al.* (2014:9) mention that medicinal plants are valuable source of natural active constituents that are used to maintain human health and also used for the treatment of many human diseases. According to Ahmadi Jalali Moghadam *et al.* (2012:4542), *Ruta graveolens* is a well-known medical plant in primordial civilizations and is used for treating many sicknesses such as: Seizure, cough, hypertension, and for wound repair by Asian and European scientist, and added to say *Ruta graveolens* has antimicroibial activity on fungi, protozoa, worms and bacteria. *Ruta graveolens* is used as a natural source of antibacterial compounds active against susceptible bacteria such as *Bacillus cereus* and *Staphylococcus aureus* which are used in the *pharmaceutical industry as indicators during the development of conventional antimicrobial drugs* (França Orlanda & Nascimento, 2015:103). Ahmad (2010:597) also reported that volatile *Ruta graveolens* oil possesses antibacterial activity against *Micrococcus pyogenes var aureus* and *Escheriachia coli*. *Ruta graveolens* also has antipyretic properties and antihistamine activity (Gutiérrez-Pajares, 2003: 667; Asgarpanah & Khoshkam, 2012:3943).

Sutherlandia frutescens (also known as the cancer bush or *kankerbos*) is a well-known plant in the indigenous medicine (Skerman, Joubert & Cronjé 2011:1250), it is indigenous to South Africa, Lesotho, Southern Namibia and South-eastern Botswana. In South Africa, it is widespread in the drier areas of the South Western and Northern Cape Provinces (Chinkwo 2005:163). The plant belongs to the Legume family which is the third largest family of flowering plants. It is a shrub with roughly 1.2m in height with the compound pinnate leaves and with slightly hairy leaflets, the flowers are bright scarlet (Afolayan & Sunmonu 2010:103).

Sutherlandia frutescens has a long history of medicinal use by a number of indigenous groups such as the *Zulu, Xhosa*, *Sotho* and *KhoiSan* to treat ailments such as fever, cough, colds (Skerman *et al.*, 2011:1250). In addition to Skeman and other's findings, it was mentioned that *Sutherlandia frutescence* is used for many ailments such as cancer, peptic ulcers and diabetes (Chinkwo, 2005:163). The plant was used in South Africa as reported by the British botanists by the indigenous *Zulu* traditional healers to treat patients during the 1918 influenza pandemic which claimed nearly 20 million lives (Shaik *et al.,* 2010:180).

Following the literature engagement regarding the mentioned medicinal plants, it is evident that the three medicinal plants are all used for common cold and influenza-like illness. Therefore, as it has been already deduced from the literature, it becomes clear that the *KhoiSan* indigenous community, through their own indigenous science system, combine the correct combination to manage common cold and flu-like ailments. However, the researcher still endeavours to engage in a robust scientific process to convince the health and science community about the competence and value that medicinal plants have in the *KhoiSan* community.

1.5 PROBLEM STATEMENT OF THE RESEARCH

Combination therapy is an unabated practice in both indigenous - and in conventional- / synthetic medicine. The triple-combination therapy consisting of a mixture of *Artemisia afra* (wildeals), *Ruta graveolens* (wynruit) and *Sutherlandia frutescens* (willekeur) is well known therapy in the Northern Cape Province of South Africa for its usage as treatment and management of common cold and influenza-like illnesses. Although, the community of Griqualand West (*KhoiSan*) is utilizing this triple-combination therapy since they can remember, centuries back, the ratification and validation of this combination therapy for its medicinal properties has neither been explored, nor tested. Furthermore, the antiviral properties of this triple-combination decoction have also not been investigated. Therefore, the researcher endeavours to explore and present these indigenous ways of knowing the use of the triple-combination therapy for common cold and influenza-like illnesses, and subsequently test the same decoction to confirm its medicinal properties as well as validating it through an in vitro process in the western context.

This research uses an article format; therefore the aims as well as the objectives will be presented as per article. The article titles, aims and objectives are illuminated below:

1.5.1 Article One:

Exploration of harvesting and conservation of triple-combination therapy by the *KhoiSan*-Community for common cold and influenza-like illnesses

The aim of this article was to explore the harvesting and conservation of medicinal plants that are used for triple-combination therapy by indigenous *KhoiSan* for the treatment of common cold and influenza-like illness. Based on the aim, the objectives of this research were as follows:

- Explore the harvesting and conservation practices of the *KhoiSan* community with regards to harvesting and sustenance of triple-combination therapy for the treatment of common cold; and
- Contribute to the learning of African indigenous knowledge systems through including the findings of this research in the re-vitalization of indigenous health knowledge systems of the main research project.

1.5.2 Article Two

Description of the preparation, storage and usage of medicinal plants used as triple-combination therapy by the *KhoiSan*-Community for common cold and influenza-like illnesses

The aim of this article was the description of medicinal plants prepared, stored, and used as triple-combination therapy by the *KhoiSan* community for the treatment of common cold and influenza-like illnesses. In order to reach this aim, the following objectives were set:

- Describe the *KhoiSan* indigenous health practice with regard to the preparation, storage, and use of triple-combination therapy for the treatment of common cold and influenza-like illness; and
- Contribute to the learning of African indigenous knowledge systems by including the findings of this research in the re-vitalization of indigenous health knowledge systems of the main research project, that is, the Seboka indigenous research project that is engaging with this community on the indigenous health practice.

1.5.3 Article Three

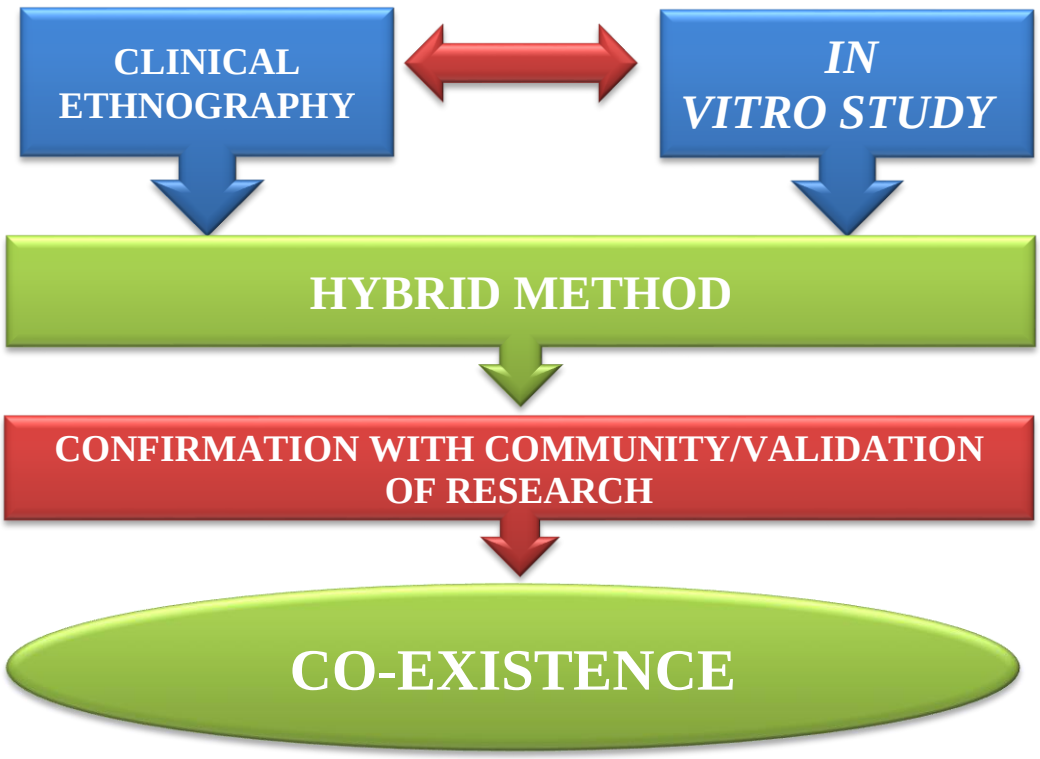
Investigation of the medicinal properties of triple-combination therapy of *Artemisia afra, Ruta graveolens and Sutherlandia frutescens in vitro*, using of *NA-XTD™* Influenza Neuraminidase Assay Kit

The aim of this article was to investigate the medicinal properties of the triple-combination therapy concoction of *Artemisia afra, Ruta graveolens* and *Sutherlandia frutescens in vitro.* The article seeks to achieve the following objectives:

- Test the antiviral properties of *Artemisia afra, Ruta graveolens* and *Sutherlandia frutescens* decoction singularly;
- Test the antiviral properties of the triple-combination therapy concoction; and
- Determine the 50% reduction of the neuraminidase activity and the inhibitory concentration (IC_{50}) of each medicinal plants decoction and of the triple-combination concoction.

1.6 RESEARCH METHODOLOGY

(Figure 1.1: Diagram of the collective research methodology followed in this research)



The researcher follows two phases in the methodology thus making it a hybrid method (Pienaar, 2016). In this framework for African Indigenous health research, the author explains the process when authentic African indigenous research methodology as practiced by the community as well as western research methodology as practiced by western scientist are used distinctly in the same research, with phase one being followed by phase two. In this research, the qualitative phase and the quantitative phase complimented each other in the strength of the outcome. The communities' century long epistemology concurred with the western epistemology in the distinct contexts. Therefore, this research illuminates the facts that support the co-existence of African indigenous - and western health practices in South Africa and the African continent. The phases that this research followed are discussed below and the articles, stating the outcomes, are in Section two of this research.The researcher follows two phases in this research.

1.6.1 Phase one: **Clinical Ethnography** (Two articles generated)

Table 1.1 Clinical ethnography

Research approach	Qualitative
Research Design	Clinical Ethnography
Population	KhoiSan Community of the Northern Cape
Sampling	Purposive
Sampling Size	Data saturation
Data-collection	Semi-structures interview schedule (Appendix)
Data-analysis	Qualitative data analysis (Pienaar, 2016)

Ethnographic research methodology refers to any qualitative method that involves participant observation, non-participant observation, or qualitative interviewing (Khoo *et al*., 2012: 84).

The research followed participative participant observation method, qualitative interviewing according to a semi-structured schedule, as the researcher identified clinical ethnography to be the suitable approach for the study taken in this indigenous community. According to Stahler and Cohen (2000:1), observation involves either participation or non-participation by the researcher within the context that

is being studied. Similarly, (Varjas *et al.*, 2014:157) mention that participant observational research method involves observing and interacting with participants, and represents a combination of observing and informal interviewing. Evidently as stated by the last authors, the researcher based the participative participant observation on these principles.

In addition, Small *et al.* (2014:157) highlight that participant observational research method allows the researcher to learn about a group's beliefs as well as behaviours, through social participation and personal observation within the community. Except for the mentioned process, interviews and discussion with individual members of the group in the community also informs the research. In support of the deeper information gained from the community, Lopez-dicastillo and Belintxon, (2014:523) said that the researcher who uses an ethnographic approach carries out three fundamental tasks such as observing people's behaviour; studying what people say they do, believe and think; and interpreting what they actually do, believe and think. Therefore, this participative participant observational research study will lead and guide the researcher on how the community harvests the medicinal plants; measurements for the preparation of the synergic decoction and how the medicinal decoction is prepared from the three medicinal plants.

1.6.1.1 Research Method

In order to illuminate this method, the process of clinical ethnography is described and supported by the schedule that will lead the participative participant observation and qualitative interviewing. The three main areas covered by the schedule will include 'the harvesting of the medicinal plants; the measurements used by the community and the process of preparation and use of the synergic decoction.

1.6.1.2 Clinical ethnography

The ethnographic research historically focused on the investigation on human culture, interaction and experiences (Cashin *et al*., 2009:3).Following that, the ethnographic research method adapted in this study is clinical ethnography. Clinical ethnography is proposed to examine the human experience of illness or care-giving in an indigenous context of the Griqua community. It counts on fieldwork and observation in the indigenous culture, relations and experiences of indigenous healthcare system (Dean & Major, 2008:1089).

Moreover, Mangula (2010:32) supported the previous authors by postulating that clinical ethnography is intended scrutinize the human experience of illness or care-giving in an interpersonal context.

Hence, the researcher utilizes participative observation and qualitative interviewing according to semi-structured approach to conduct the study. In this case, the healthcare system that was researched is the one that the indigenous community healer giving indigenous healthcare to the community members for the management of the different diseases that the community encounters in their daily living. Dean and Major's (2008:1088) findings are corroborated by Mangula and Pienaar (2013). Hence, the researcher follows the following process of executing the clinical ethnography the study of culture that goes all-out to attain an in-depth understanding of culture, norms, values, beliefs and concepts in which the researcher gain emic perspective of the members of the culture (Vargas *et al.,* 2005: 244). In addition, traditionally, the researcher gained a deeper understanding of a certain civilisation by engaging in the world of the subjects over a lengthy period of time.

1.6.1.3 Population

According to Burns and Grove (2007:324), a population is described as a total set of people eligible to participate in a research study, while De Vos *et al*., (2008:193) define the population as the participants or the objects that have characteristics from which the sample of the research will be deduced. The population of the research was elderly of the community who are the knowledge holders of the indigenous medicinal plants use, and it included the medicinal man of the community and indigenous community healer who are the health care givers in the community. It was envisaged that the adult community members of the Griqualand will provide the relevant and rich information which is experience-based on the use of the medicinal plants as the well-known practice in the KhoiSan community.

1.6.1.4 Sample

According to De Vos *et al*. (2008:193), research sample is the portion of the overall population that represents the entire population of the study. According to Burns and Grove (2007:324), a sample is a portion of a population selected for a study. The sampling strategy that was used for the selection of the sample is convenience sampling which is one of the purposive sampling methods. Stahler and Cohen (2000:3) explain convenience sampling as the strategy where the informants are selected by convenience. Therefore, the sample of the study was the indigenous community healers and the medicinal man of the community. According to the community's definition, the medicinal man was either male or the female member of the community who is the knowledge holder of the indigenous medicinal plants use, and who are the healthcare giver of the community at large.

1.6.1.5 Data collection

Data collection is an organised process of gathering important information for the research study according to Burns & Grove (2007:41), and as defined by Grove *et al.* (2007:523) is the process of selecting participants and gathering evidence from these participants. According to Bassett (2004:118-120) during the data collection, the researcher established a trusting relationship with the participants in order to acquire more and rich information from the participants.

In benefit of the study, the needed information focused on how the medicinal plants are harvested, the measurement and the preparation of the triple combination medicinal plants for the medicinal decoction used by the indigenous community healer to manage common cold. In this research study, data were collected in two separated approaches of the research method. Firstly, during participant observational approach, in which data collection was conducted through the written fields notes during and after each period of observation, and by the use of the videotaping and sound recording. Secondly, data was collected during semi-structured interview and informal interviewing. The data-collection method for phase one is discussed below:

1.6.1.6 Participative participant observation

According to Varjas *et al*. (2014:157) participant observational research method involves observing and interacting with participants, and it represents a recipe of observing and informal interviewing; hence the researcher utilized this research method. During the participative observation, the researcher functioned fully and became the active member of the community. Furthermore, the researcher observed the community as it harvested the medicinal plants. The measurements of the medicinal plants were used by the indigenous community healer for the management of the common cold and the preparation of the medicinal plants decoction. The participative observation took place in the cultural context of the community in which the researcher wrote fields notes during and after each period of observation, and the videotaping and the use of the sound record will be of utmost important for the keeping of the records for the data analysis.

1.6.1.7 Semi-structured interviews

According to Stahler and Cohen, (2000:2), qualitative interview in ethnographic research is an in-depth semi-structured interview to acquire rich and detailed information. Therefore, the researcher used semi-structured interview as another approach for the clinical ethnography design. The semi-structured interview with conversational or other informal interview conducted as a part of field work, and contextual interview as the harvesting of the medicinal plants, the measurements used of the medicinal plants for the preparation of the medicinal plants decoction is concern. There were three components of structured data collection and consisted of observations, questionnaires and semi-structured interviews, and the interviews were tape recorded and transcribed for analysis.

The approach of the semi-structured interview and the questionnaires were done in consultation with the medicinal man of the community, the indigenous community healer and other knowledge holders of the indigenous medicine use in the community. The semi-structured interview and the questionnaires on the harvesting, the measurements of the used medicinal plants, and the preparation of the medicinal decoction out of the triple combination of the medicinal plants used for the management of common cold.

1.6.1.8 Data analysis

Data analysis is described as conduction to reduce, organize, and give a meaning to the collected data (Burns & Grove 2007:41). The notes from the observations were transcribed and their transcriptions, together with the interviews, were studied carefully. The researcher reduced, organized and gave meaning to the data collected during the observational and interview approaches. The analysis of the information assisted the researcher to acquire sensible information from the emic perspective point view of the indigenous community on the use of the medicinal plants. The following four steps as stated by Pienaar (2016) followed in the analysis:

Level one: Basic concept from the spoken word from the KhoiSan community. The researcher explored the audio and video recordings. Concepts converged from the spoken words.

Level two: The researcher joined the concepts of level one or group of similar concepts to form a theme or pattern.

Level three: This is an intuitive deduction, convergence or discovery of new themes or clusters normally called an insight or discovery (this is formulated with close collaboration of the community)

Level Four: The building of a narrative or design to form a framework to guide research.

1.6.1.9 Rigour

The rigour of this research was secured in phase one by the trustworthiness, and phase two by reliability and validity.

1.6.1.10 Trustworthiness

The trustworthiness of the research is determined by the ability to inspire belief or trust in the indigenous community with which the researcher partnered (the believability or credibility), able to trust and to depended on (dependability) and to verify the truth or the validity (confirmability) of the general research study taken, (De Vos *et al.,* 2008:346). According to Creswell and Plano Clark (2011:14-15), in certifying trustworthiness, it is significant for the researcher to know the vital concerns of persuasiveness in qualitative research such as credibility, dependability and confirmability, which the researcher will take note of as the research trustworthiness is concerned. (See article one and two in section (2) of this work for an in-depth explanation.)

The semi-structured interview schedule was also translated to Afrikaans to accommodate the communities' language.

The researcher followed two phases in the methodology that corroborate with the hybrid method as stated by Pienaar (2016). In this framework for African Indigenous health research, the author explains the process when authentic African indigenous research methodology as practiced by the community. Furthermore western research methodology as practiced by western scientist is used distinctly in the same research, with phase one being followed by phase two. In this research, the qualitative phase and the quantitative phase complimented each other in the strength of the outcome. The communities' century long epistemology concurred with the western epistemology in this distinct context. Therefore, this research illuminates the facts that support the co-existence of African indigenous - and western health practices in South Africa and the African continent. The phases that this research followed are discussed below and the articles, whereas the outcomes are in Section two of this research.

PHASE TWO: QUANTITATIVE (**ONE ARTICLE)**

Investigation of the antiviral properties of triple-combination therapy of *Artemisia afra, Ruta graveolens and Sutherlandia frutescens* in vitro by the use of NA-XTD™ Influenza Neuraminidase Assay Kit

Table 1.2 Quantitative phase of the research

Research approach	Quantitative
Research Design	Laboratory analysis
Research method	*In vitro* -Antiviral activity against influenza virus
Investigation Process	NA-XTD™ Influenza Neuraminidase Assay Kit.
Data-analysis	50% reduction of neuraminidase activity- half maximal inhibitory concentration (IC_{50})

1.6.2 RESEARCH DESIGN

1.6.2.1 In vitro assay

According to Gallo (2002:2), *in vitro* assay refers to the laboratory test used to measure the activity of the sample to investigate the medicinal properties or the testing of the sensitivity of the organism towards the medicine being investigated.

In this research study, the *in vitro* assay was carried out by the use of NA-XTD™ Influenza Neuraminidase Assay kit to determine the susceptibility of the influenza virus against the decoction of *Artemisia afra*, *Ruta graveolens* and *Sutherlandia frutescens* singularly and as the concoction of the triple-combination medicinal plants against the influenza virus. The decoction and the triple-combination therapy concoction prepared by the indigenous KhoiSan descendants are used for the treatment of the common cold and influenza-like illness as their indigenous medicine.

1.6.2.2 Research Method: Antiviral activity against influenza virus

1.6.2.2.1 The half maximal inhibitory concentration (IC_{50})

Ikematsu *et al*. (2015: 634), refer to the IC_{50} in this study as the half maximal medicinal plants concentration inhibiting the influenza neuraminidase enzyme activity. It indicates how much of the medicinal plants decoction or the concoction is needed to suppress the influenza neuraminidase enzyme activity by 50% of the virus control. The 50% reduction in neuraminidase activity is calculated as half the relative luminescence units achieved with the virus control (VC) in no medicinal plants added.

According to the Health Protection Agency (2012), the IC_{50} calculation can be achieved by different method. The curve-fitting, during which the statistical software; such as graph pad prism can be used or the use of graffiti, in which the sigmoidal dose-response curve is produced are both examples of such methods employed.

The IC_{50} can be also be calculated by a point-to-point calculation method. The point-to-point method simply works as it is self-explanatory; i.e. the straight line is fitted between each data point. This method does not produce the sigmoidal curve. Only the virus control is used to calculate the IC_{50} value. In this study, the point-to-point method was used to calculate the IC_{50}. The results are graphically represented after the luminescence data was measured in the luminometer and by the use of Microsoft Excel the IC_{50} of the medicinal plants automatically calculated.

1.6.2.3 Preparation of medicinal plant extracts

The medicinal plants tested in this study were as precisely how the indigenous community prepared the medicine for the treatment of common cold and influenza-like illness. The *Artemisia afra, Ruta graveolens* and *Sutherlandia frutescens* are medicinal plants the KhoiSan community uses individually and as the triple-combination therapy. The decoction of *Artemisia afra*, *Ruta graveolens* and *Sutherlandia frutescens* and the concoction of the triple-combination were tested. The results are reported in section two of this research study in the article entitled: Investigation of the antiviral properties of triple-combination therapy of *Artemisia afra, Ruta graveolens and Sutherlandia frutescens in vitro by the use of* NA-XTD™ Influenza Neuraminidase Assay Kit.

1.6.2.4 Data collection and analysis

The influenza neuraminidase inhibitory assay was conducted at National Health Laboratory Service under the National Institute for Communicable Diseases. The three medicinal plants decoction of the *Artemisia afra, Ruta graveolens* and *Sutherlandia frutescens* and the concoction of these three medicinal plants were tested against the influenza neuraminidase enzyme activity. The medicinal plants concentration inhibiting 50% of the influenza neuraminidase enzyme activity was determined and IC_{50} results reported in Excel spreadsheet and graphed for visual display of results. The positive control of the neuraminidase inhibitors oseltamivir and zanamivir used to compare the inhibitory activity of the medicinal plants with these on market neuraminidase inhibitors.

1.6.2.5 Reliability and Validity

1.6.2.5.1 Reliability

The reliability was strengthened by following the process of testing the microbe consistently according to the description of consistency (Burns & Grove 2007:365).

Applicable in this research, the process of testing is meticulously outlined for researchers to follow the same pathway and to get a similar outcome. This is how consistency is reliably upheld. The guidance and support of an independent laboratory manager, who is a qualified and practising virologist, was used to confirm the reliability of the test outcomes (*see Annexure G*). The analysis of this section was independently executed by the researcher and the virologist and a consensus was confirmed.

1.6.2.5.2 Validity

The validity of the research study depends on the degree or the extent to which the instruments used for measuring are successful at measuring accurately what are supposed to measure (De Vos *et al.*, 2008:182). The researcher followed the guidelines set for *in vitro* as described.

1.6.2.6 Ethical and moral considerations

The researcher will be guided by the following ethical principles as coded by the American Psychological Association APA, 2010: 2-3). The Principle of Beneficence and Non-maleficence that underlines that there should be no harm to the participants and their welfare and rights are taken into consideration. Although no overt harm was anticipated, the researcher ensured that there are no participants exposed to any form of harm. Hence, the researcher signed the code of conduct of Seboka that guides the principles of ethical research under this project.

The second ethical Principle is Fidelity and Responsibility that advocates that trust relationship with the community should be of utmost importance during the research study. The researcher has already developed trust relationship with community since 2010, where the research study was conducted as the researcher has been working with this community for almost five years in indigenous knowledge health system project. Apart from that, the researcher upheld good professional standards during the conduct of the research study and consulted the community in every stage of the research.

Thirdly, the Principle of Integrity was observed. This ethical principle highlights that during the research process, the researcher should seek to promote accuracy, honesty and truthfulness. The researcher was accurate, honest and truthful with the collection, analysis of the data and with the finding of the research study, supported by sciences as well as the guidance of two competent supervisors.

The fourth Principle is of Justice. The principle denotes that fairness and justice prevail to all people. Therefore, the researcher practiced justice by ensuring that the community has access and benefit from the contribution the research will contribute and to ensure equity through information sharing and respect.

Lastly, Principle of Respect for people's rights and dignity was adhered to. The principle highlights the respect of people's dignity, the rights of individuals to privacy, confidentiality and self-determination (autonomy). In order to augment this discussed framework, the researcher also perused the following principles and is applying it in this research:

1.6.2.6.1 Right to privacy

According to De Vos *et al*. (2008:61), privacy is defined as that which is not intended for others to observe or analyse. In addition, it is the individual's right to decide when, where, to whom and to what extent his or her attitudes, beliefs and behaviour would be revealed. The privacy to information provided by the participants was handled confidentially and shall only be used for research purposes.

1.6.2.6.2 Right to confidentiality

According to Burns and Grove (2007:212), confidentiality is the protection and management of the information by the researcher that is provided by participants, and this information should be protected and handled confidentially at all times and be used solely for research purposes. The researcher practiced this principle accordingly during the research study.

1.6.2.6.3 Right to self-determination or autonomy

Autonomy is viewed as the capability of a person to control his or her destiny, and to have the freedom to conduct his or her life, without any force, or control (Burns & Grove 2007:204). The researcher provided participants with the necessary information as to empower the participants to make informed self-decision with regard to their participation during the research.

Ethics clearance for conducting the study was obtained from the Ethics Committee of the North-West University in Mafikeng Campus. Following that, the permission to conduct the study was also requested from the Chief in the Griqualand West community in the Northern Cape Province, who already pledged collaboration in the project. Informed consent was obtained from the participants following a comprehensive explanation of the purpose of the study.

1.7 RESEARCH OUTLAY

The research in the article format consists of the following sections:

Section One: Overview of the research

Section Two: **Three** Articles (See section 2)

Section Three: Contribution, recommendations and the conclusion of the research

1.8 CONCLUSION

This section provides a brief overview of this research. The following section contains the three articles from the research, because the researcher worked within an article format research. Two of the research articles were submitted and corrections were done. The two articles are final review for publication process. The third article will be submitted for publication soon. Following section two is the last section: Contribution, recommendations and conclusion of this research.

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SECTION 2

MANUSCRIPT

Medicinal properties of triple-combination plants used by KhoiSan community for common cold and influenza-like illness.

ARTICLE 1

Learning bush-medicine from the Bushman descendants: Exploration of harvesting and conservation of triple-combination therapy by the *KhoiSan*-Community for common cold and influenza-like illness

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ABSTRACT

The harvesting and conservation of medicinal plants dates back millions of years in Africa. Despite this, the exploration of this practice by Africans for Africans only became apparent after 1994, post-apartheid, in South Africa. The marginalized *KhoiSan* Community of the Northern Cape Province is currently the trendsetters regarding the harvesting and conservation of medicinal plants in order to avoid the extinction of these plants. Hence, the *KhoiSan* Chief of the Northern Cape states unapologetically that “The veld is our chemist”. This prompted the researchers to explore how medicinal plants are harvested and conserved by the *KhoiSan* community for the treatment of common cold and influenza-like illness. A clinical ethnography method was used to explore this phenomenon with specific emphasis on respectful engagement with the *KhoiSan* Community’s medicine men and women to guide the process. This research employed a purposive sampling method. Data were collected using clinical ethnography and analyzed thematically. The results yielded sustainable harvesting and conservation methods within an indigenous context.

Keywords: medicinal plants; combination therapy; triple-combination therapy; common cold; influenza-like illness; indigenous medicine

INTRODUCTION

The usage of plant medicine continues unabated in the face of fears illuminated regarding sustainable harvesting. This statement is supported by Pondani, Bhatt, Negi, Kothyari, Bhatt and Maikhuri (2016) in their assertion that more than 85% of individuals in general continue to use plant medicine in spite of the concern regarding the over-harvesting of these medicinal plants. In addition, Mondal, Mondal and Mondal (1999) found that as a result of this, sustainable conservation is hampered at this point in time. Van On, Quen, DinhBich, Jones, Wunder, Russel-Smith (2001) corroborate these findings and indicate that overharvesting is a threat for cultivation and harvesting. Perusing literature, it becomes evident that in the last 15 years, various authors presented their concerns regarding overharvesting and the possible threat thereof for natural cultivation as well as sustainable harvesting. Van On *et al.* (2001) are also of the opinion that research regarding cultivation and harvesting of medicinal plants seems very inadequate.

Fuelling the mentioned threats stated on cultivation and harvesting, Ghimire, McKey and Thomas (2005) further contend that sustainable harvesting of medicinal plants in the Himalayas are under threat and that the exploitation of local plants are the prevailing custom. The KhoiSan community verbalised the same fear. Furthermore, Van Andel, Croft, Van Loon, Quiros, Towns and Raes (2015) state that millions of Africans rely on medicinal plants, but the trade of the same medicinal plants became a lucrative business in Africa, thereby potentiating the threats around sustainable cultivation and harvesting. Kala's (2000) findings also support the economic exploitation of the medicinal plants. Moyo, Aremu, Gruz, Šubrtová, Szüčová, Doležal, and Van Staden (2013) illuminate the cold reality of the higher demand on natural plant medicines in contrast with the extinction of the same medicinal plants due to unsustainable cultivation and harvesting.

Important to note, is that these plants were used centuries before the pharmaceutical industry started the professional exploitation thereof (Barata, Rocha, Lopez & Carvalho 2016). And yet, limited research exists specifically on the exploration of sustainable cultivation and harvesting from indigenous communities' perspective even though they are the fundamental knowledge holders in this area. Contrary to western-educated scholars' expectations, Pondani *et al.* (2016) opine that indigenous communities have the insight for using these medicinal plants for healing.

Furthermore, indigenous medicines remain a source of viable mainstream for affordable healthcare, especially in developing countries. In South Africa, an estimated 70% of the population depend on medicinal plants that can be used as mono or combination therapy (Mjiqiza, Syce & Obikeze, 2013), of which the most popular used of the medicinal plants include *Artemisia afra, Ruta graveolens* and *Sutherlandia frutescens* (Suliman, Van Vuuren & Viljoen, 2010).

The conservation of the environment, cosmos, and ecosystem is not a new or recent phenomenon to the African indigenous community (Adu-Gyamfi, 2011). Mawere (2013) indicates that pre-colonially, indigenous people used indigenous strategies to conserve the ecosystem. These strategies included cultural taboos, totemism and folktales to promote the sustainable use of the natural resources which did not omit the medicinal plants. Following from that, Adu-Gyamfi (2011) states that taboos are unwritten social rules that indigenous governance utilized not only to control human social behaviour but also to regulate the ethical interdependence between humans and the ecosystem that limits the overexploitation of man of the vulnerable natural resources. From literature, however, it is evident that there is an overwhelming interest in the use of natural plant medicine.

Currently, the overwhelming interest in medicinal plants is due to several reasons. They include the following: Conventional medicine is becoming inefficient, for example, side effects, ineffective therapy, and abusive or incorrect use of synthetic drugs, results in side effects and drug resistance. A large percentage of the world's population does not have access to conventional pharmacological treatment and indigenous medicine and ecological awareness suggest that natural products are harmless (Rates 2001). As highlighted by the World Health Organization (WHO 2013), the indigenous medicinal system is a form of care that is close to homes, accessible, and affordable. Furthermore, it is perceived as culturally acceptable and trusted by large numbers of people and the affordability of most indigenous medicines makes them all the more attractive at a time of soaring health-care costs (WHO 2013). Plant-based medicine is especially suitable for the long-term treatment of chronic diseases in geriatric and convalescent patients, for follow-up treatment, and in the prophylaxis of infectious-, degenerative-, and metabolic diseases (Cravotto, Boffa, Genzini & Garella, 2010). Adding to the latter, combination treatment is linked to the belief that antimicrobial combinations increases the spectrum of activity and reduces the potential of side-effects (Zonyane, Van Vuuren & Makunga, 2013).

Adu-Gyamfi (2011) points out that indigenous authorities control the harvesting of medicinal plants during forbidden seasons and sacred days. This is linked to the teaching from the elder of KhoiSan community who said it is "a taboo for the menstruating lady and any person with morally unacceptable behaviour to touch and harvest medicinal plants as according to community indigenous religion system as the medicinal plants will not prosper in the next season". Based on this, the researchers are of the opinion that the same indigenous communities have the knowledge and insight to sustain these plants through local cultivation and harvesting. Therefore, this research depicts the wisdom of a rural KhoiSan community regarding sustainable cultivation and harvesting. Deduced from the literature, medicinal plants have been used for many years as the mainstream of health care in majority of African indigenous communities. Furthermore, developing countries still use medicinal plants in their healing practices and developed countries use medicinal plants via pharmaceutical

companies, which can be seen as exploitative. As a result, this increase in usage can lead to extinction and depletion of medicinal plants. Subsequently as stated in the discussed literature, the biggest threat for this extinction is the irresponsible harvesting and conservation of medicinal plants. However, the Griqua community of KhoiSan had been harvesting and conserving medicinal plants in their daily health care practice since time immemorial. Therefore, the researcher undertook to explore their ways of medicinal plant harvesting and conservation to contribute to the body of knowledge of indigenous knowledge systems. The aim of this research was to explore the harvesting and conservation of medicinal plants that are used for triple-combination therapy by indigenous KhoiSan for the treatment of common cold and influenza-like illness. Based on the aim, the objectives of this research were to:

- Explore the harvesting and conservation practices of the KhoiSan community with regards to harvesting and sustenance of triple-combination therapy for the treatment of common cold; and
- Contribute to the learning of African indigenous knowledge systems through including the findings of this research in the re-vitalization of indigenous health knowledge systems of the main research project.

METHODOLOGY

The methodology provides information pertaining to the research design, sampling, data collection, data analysis, trustworthiness and ethical considerations.

Research Design

This research followed a qualitative, clinical ethnography research design. Ethnographic research methodology refers to a qualitative method that involves participant observation, non-participant observation, or qualitative interviewing (Khoo, Rozaklis & Hall, 2012).

Population and Sampling

The population for this study included the KhoiSan community in the Northern Cape Province of South Africa. The medicine men and women and elders of this community were sampled through the use of a purposive sampling method. Inclusion criteria adhered to the following: indigenous healers, indigenous medicine man and woman (indigenous pharmacists) and elders knowledgeable of medicine plants.

Data Collection

Data collection is an organised process of gathering important information for the research study (Burns & Grove, 2007). As defined by Grove, Burns and Gray (2013), data collection is the process of selecting participants and gathering evidence from these participants. According to Bassett (2004), during the data collection, the researcher has to establish a trusting relationship with the participants in order to acquire in-depth and rich information from the participants. In benefit of the study, the needed information focused on how the medicinal plants used for the triple combination therapy by the KhoiSan community for common cold and influenza-like illness is harvested and conserved.
The data collection took place in the cultural environment of the indigenous community, where clinical ethnography participative observational data collection method took place. During participative observational approach, the field notes were taken and the videotaping as well as the audio-recordings was done. Secondly, the questionnaire, the semi-structured interview and informal interviewing took place, and all materials of the questionnaire and semi-structured interview together with tape record transcribed for analysis.

A participative participant observation method and qualitative interviewing with the use of a semi-structured interview schedule were used to collect data. According to Stahler and Cohen (2000), observation involves either participation or non-participation by the researcher/s within the context that is being studied. Therefore, the researchers embarked on participative participant observation since the qualitative interviewing followed the observation. Similarly, Varjas, Nastasi, Moore and Jayasena (2014) mention that participant observational research method involves observing and interacting with participants and represent a combination of observing and informal interviewing. The researchers based the participative participant observation on these principles.

Adding to the previous discussion, Small, Maher and Kerr (2014) highlight that participant observational research method allows the researcher/s to learn about a group's beliefs and behaviours through social participation and personal observation within the community. Except for the mentioned process, interviews and discussion with individual members of the group in the community also informed the research. In support of the deeper information gained from the community, Lopez-Dicastillo and Belintxon (2014) state that the researcher who uses an ethnographic approach carries out three fundamental tasks, namely; observing people's behaviour; studying what people say they do, believe, and think; and interpreting what they actually do, believe, and think.

The utilization of this participative participant observational research approach informed the researchers on how the community harvests the medicinal plants and carefully applies certain principles to ensure that the plants do not become extinct. This methodology was applied by the researchers by meticulously observing and asking clarity-seeking questions regarding the preparations the KhoiSan community make for harvesting, how they harvest, and what principles they apply to sustain these medicinal plants. Therefore, the process that unfolded depicts the harvesting and sustenance of the medicinal plants used for triple combination therapy decoction that the KhoiSan community uses for common cold and influenza-like illness. Clinical ethnographic research can lead to the change of clinical practice in health. Against this background, the researchers plan to use the outcome of this research to enhance the education of undergraduate and postgraduate indigenous knowledge systems and natural science students' learning in order to improve sustenance of medicinal plants in South Africa.

Data Analysis

Data analysis is described as a process aimed to reduce, organize and giving a meaning to the collected data (Burns & Grove, 2007). The notes from the observations were transcribed and their transcriptions, together with the interviews, studied carefully. The researchers then reduced, organized and gave meaning to the data collected during the observational and interview approaches.

The following four steps as stated by Pienaar (2015) were followed in the analysis:

- Level one: Basic concept from the spoken word. The researcher explored the audio and video recordings regarding harvesting and conservation. Concepts were derived from the spoken words.
- Level Two: Joining or grouping of similar concepts to form a theme or cluster.
- Level three: This is an intuitive deduction, convergence or discovery of new themes or clusters normally called an insight or discovery (with close collaboration of the community) followed by:
- Level four: The building of a storyline or pattern to form a framework to guide research (please see conclusion for this level).

Trustworthiness

The trustworthiness of the research was determined by the ability to inspire belief or trust (the believability or credibility), ability to trust and to depend on (dependability), and the verification of the truth or the validity (confirmability) of the general research study taken (De Vos et al., 2008).

The credibility of this research was ensured by the trust relationship and the prolonged community engagement the Seboka indigenous research project have with the community where the research took place. The researcher is the member of this project. Therefore, the data the community provided was dependable. The in-depth discussion of the methodology and the application of this methodology provided consistency and valid grounding should this research be repeated in the same context. This process contributed to the confirmability of the process. According to Creswell and Plano Clark (2011), in certifying trustworthiness, it is significant for the researcher to know the vital concerns of persuasiveness in qualitative research, such as credibility, dependability and confirmability which the researchers took note of where the research trustworthiness is concerned.

Ethical Considerations

The research received institutional approval and was guided by the ethical principles as coded by the American Psychological Association (APA). These principles focus on the protection of the community's rights in research.

RESULTS AND DISCUSSION

The following sections provide an inclusive discussion combining the data of the medicinal plants, namely; *Artemisia afar, Ruta graveolens and Sutherlandia fructescens* as the result were similar. The communities' voices are illuminated in this discussion. Therefore, literature is attended to in the introduction. Table 2.1 provides an overview of the findings on the three levels identified in the data analysis section and the findings are discussed in more detail below:

Table 2.1 Overview of the findings

LEVEL ONE: Basic concept from the spoken words	**LEVEL TWO: Joining similar concepts to form a theme or cluster**	**LEVEL THREE: Intuitive deduction, convergence or discovery of new themes or clusters**
Part of the plant used : Leaves, Branches and Aerials. Reason-: the medicinal property is more located on these parts. Also to conserve the plant	Harvesting the parts to be used for plant medicine Rationale for Medicinal properties	Conservation for sustainability, because indigenous use mostly the parts that will not 'kill' the plant. Therefore most medicinal plants are conserved
Harvesting method Tools used: Sharp animal horn. Hands to pluck needed parts without destroying the plant. Avoid plant damage Reason: Harvest sustainably	Prescriptions for the person harvesting Ways of harvesting Preservation Sustainability Extinction	Using hand and horn t is more effective to contain original medicinal properties, because these 'tools' does not having metal contaminants that will chemically influence the medicinal properties of the plant Avoid plant extinction
Harvesting time When the leaves are green		The ideal harvesting time (morning)

In the morning time of the day <u>Caution</u>: avoid human shadow to fall on the plant to be harvested. The harvesting person should be in the peaceful mood, communicating with the plants in a humility manner. After harvesting of the plant put the coin or the button as the way of thanking the plant or the nature for providing with that medicinal plant. The person should have a respect to cosmos, nature and humans. Adults can harvest medicinal plants, if not, the young person with respectable behaviour selected by the community indigenous medicinal practitioner can.	Communication with the plants Respecting of the cosmos, nature and humans. Thanking the nature and cosmos Respectable behaviour	Harvesting in the morning after the last day photosynthesis process completed as to obtain rich medicinal components of the plant. The African belief (meaning of shadow) Equating the plants with humans (personification) Appreciation of the medicinal plant (putting of the coin or the button after harvesting the plant)
<u>Area of plant collection</u> Around community gardens (domestic) The veld	Context of plant collection	The veld is seen as the best area to collect plants “The veld is our chemist”

The various parts of the medicinal plants such as bark, rhizome, bulb, roots and leaf material are harvested, processed and used as medicine to health issues in this community. Therefore, acquiring full information regarding harvesting is valuable and important for the preparation of indigenous African medicine. This knowledge about the medicinal plants used, when to harvest, how to harvest such plants, and what equipment to use to harvest the plant, is imperative for the conservation and the sustainability of the medicinal plants. Following this argument, for these three medicinal plants, the community uses the leaves and aerials to prepare the indigenous medicinal decoction.

Harvesting Technique that Conserves

The harvesting method for indigenous medicine is essential for the preservation thereof. Moreover, the preservation and sustainability of the medicinal plants is of utmost importance to avoid extinction, which is a crucial problem in the conservation of the indigenous medicine system. During the harvesting of these medicinal plants, the community is cautiously avoiding the unnecessary damage of the plants that might destroy these precious resources that bring health to their life. The re-growing of the medicinal plants is vital to them.

Apart from that, the equipment used and when to harvest (e.g. the best time for collection, for example, the season, date or the time of the day) play an important role in the harvesting of medicinal plants. The community takes special care with the leaves and flowers which are more vulnerable to deterioration. They use their hands to pluck leaves carefully avoiding the disruption of the medicinal plants. They pluck individual leaves instead of leaf striping in order to ensure sustainable harvesting of leaves. Secondly, they use the knife-shaped animal horn to cut the needed parts of the plants and after each harvest they clean the used equipment. It was also mentioned that they avoid using the top leaves with seeds and flowers and would rather harvest the bottom leaves of the medicinal plant. This consideration contributes to conservation of the medicinal plants.

The Community's Power Regarding Their Belief and World-View on African Indigenous Healing

The community's belief and world-view is that the person harvesting medicinal plants should be a person who respects and appreciates nature, the cosmos, and humanity. This person should be free from pollutants and taboos as according to them, that reflects 'cleanness' to prepare medicine. As an example of this, the community holds the belief that if the shadow of a polluted person comes in contact with medicinal plants, it will disturb or destroy the healing power embedded in that particular medicinal plant.

Medicinal plants are preferably harvested by an indigenous adult community member. In cases where an adult cannot do so, a responsible young person who is nominated by the Indigenous Community Health Practitioner Committee (ICHPC) can harvest medicinal plants. However, the young person who is considered not 'clean' or free from pollutants and taboos cannot harvest medicinal plants or prepare indigenous medicine. Furthermore, the menstruating, sexually active young woman and sexually active young man are considered not 'clean'. So, they are not allowed to prepare indigenous medicine.

The usage of medicinal plants in the indigenous (traditional) health system is a key pillar of the broader health system of indigenous persons. During this time of unaffordable Western health services, the indigenous (traditional) health service still remains the outstanding, easily accessible health service in this community. The community collects the medicinal plants from their back yard gardens and from the veld to prepare this medicinal decoction for the management of common cold.

CONCLUSION

The harvesting method used by this community is ideal for conservation and sustainability of the medicinal plants. The harvesting techniques such as the use of hands to pluck the leaves and the knife-shaped horn needed to cut parts of medicinal plants is essential to avoid contamination of the metal traces that might chemically react with medicinal components thus lowering the quality of medicinal constituents in the medicinal plants. During harvesting, unnecessary damage of the medicinal plants that might ultimately destroy them (medicinal plants) is avoided and the re-growing of the medicinal plants is essential to avoid plants' extinction.

African belief and world-view on the healing is treasurable to this community. This is seen by how the community respectfully communicate with and handle the medicinal plants. The community appreciates and are thankful for nature, which they perceive to provide them with precious natural medicinal plants. After harvesting of the medicinal plants, it is their belief that they should thank nature. They place precious ornaments, such as coins or buttons near the medicinal plants as their form of thanksgiving to the cosmos and nature.

Further opportunity for research after this phase is possible to describe the preparation, usage, and storage of the medicinal decoction and to test the medicinal properties of the triple-combination from a Western perspective (in-vitro) in order to compare and contrast the data found during the indigenous phase of the research. This will subsequently lead to the development of a natural solution to combat common cold in the community as well as creating opportunity for income generation.

The community was willing to share deeper practices in medicine. However, the scope of this research focused on three medicinal plants, thus not including the broader plants knowledge of the community. A second limitation was the time frame of allocated to for the research.

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ARTICLE 2

Learning bush-medicine from the Bushman descendants: Description of the preparation, storage and usage of medicinal plants used as triple-combination therapy by the KhoiSan-Community for common cold and influenza-like illness

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Abstract

The usage of medicinal plants among the KhoiSan community has been in existence since time immemorial. Despite this extensive history, there is no contextual evidence regarding the indigenous scientific processes used for preparation, storage, and usage of these medicinal plants for the formulation of triple-combination therapy decoctions. In giving feedback to confirm research findings, the KhoiSan community indicated that they use a triple-combination decoction for common cold and influenza-like illnesses. A natural question that arises is: How does the community prepare, store, and use the triple-combination decoction? Therefore, this article focuses on the preparation, storage, and usage of this triple-combination decoction and the medicinal indications as presented by the KhoiSan community. This research used purposive sampling and data were collected using clinical ethnography and analyzed thematically. The results yield inexpensive preparation, time-saving and easily manageable method that is unique and acceptable to this indigenous community.

Keywords: combination therapy, common cold, indigenous medicine, medicinal plants, triple-combination therapy.

INTRODUCTION

The utilization of medicinal plants dates back to the origin of human civilization on earth and continues unabatedly (Mukhtar, Arshad, Ahmad, Pomerantz, Wigdahl & Pareen, 2008). Cragg and Newman (2013) concurs that throughout the ages, humans have relied on nature for their basic needs. Of most interest to this study is the use of medicinal plants for the treatment of a wide range of diseases. Similarly, Gurib-Fakim (2006) opines that the use of medicinal plants is a primordial custom as these plants are the foundation of the indigenous medicine systems that have been in existence for thousands of years and continue to provide mankind with new remedies for every ailment. Mukhtar et al. (2008) also mention that the practice of medicinal plants is common throughout the world. However, their wide usage has been limited to China, India, Japan, Pakistan, Sri Lanka, Thailand, and a number of African countries. In support of this, Scott and Hewett (2008) agree that the use of medicinal plants is an ancient custom, dating ages back from the arrival of the *KhoiSan* in Southern Africa.

In the last 2000 years, the *KhoiSan* people have relied on nature for their basic needs, including food, clothing, and shelter for their living and, most importantly, for the management and the treatment of ailments using medicinal plants. Deduced from this literature evidence, the commanding statement asserted by the KhoiSan Chief in the Northern Cape as stated below harmonizes with the previous authors when he postulates:

> "The veld is my chemist. It is the source of the medicinal plants for the treatment and management of all kinds of unhealthy conditions that perturb our bodies. From the ages of our grandparents until today, the medicinal plants have been our natural medicine that we use freely without or with less harmful effects as compared to Western medicine with very unbearable side effects."

This statement by the Chief reinforces Scott and Hewett's (2008) finding that the primeval people the KhoiSan are the indigenous people in Southern Africa. Therefore, the knowledge of the medicinal properties of plants has always been not only a matter of value, but of healing for this community. Hence, for the matter of indigenous confirmation and Western scientific validation, the researchers endeavoured to explore and describe one of the KhoiSan legacies that are still being practiced in the KhoiSan Community, namely; the management of common cold using triple-combination therapy (Mphuthi 2015).

In support of the use of combination therapy, Zonyane, Van Vuuren, and Makunga (2013) argue that the combination of plants to treat sicknesses has been practiced for centuries in indigenous medicine systems with great success. Williamson (2001) further explains that combination therapy is not only limited to indigenous medicine, but is also a practice in conventional medicinal therapy for cancer chemotherapy, HIV management, infectious diseases, and lifestyle diseases such as hypertension and diabetes.

It is also noted that several plants have been used to treat microbial infections (Mukhtar *et al.,* 2008). Balunas and Kinghorn (2005) also found that medicinal plants were used as medicine for thousands of years and added that these medicines were utilized in crude forms like teas, powders, and other formulations. Zonyane *et al.* (2013) concur with this statement and add that the combination of medicinal plants to manage illness has been practiced for centuries.

According to Gathirwa, Rukunga, Njagi, Omar, Mwitari, Guantai, Tolo, Kimani, Muthaura, Kirira, Ndunda, Amalemba and Mungai (2008), in indigenous medicine, different plants are combined for the treatment of a disease. The triple-combination therapy of common cold has existed for decades in indigenous medicinal plants systems. The medicinal plants used are often not related in any way or may be used singularly for the treatment of that ailment (Sibandze, Van Zyl, & Van Vuuren 2010). In his study of synergy research, Wagner (2011) observed that combination therapy regime for antimicrobial is known to prevent antibiotic resistance. While Gathirwa *et al.* (2008) found in their study that drug combinations are important for drug synergistic properties.

In echoing the sentiments of research cited above, Gathirwa *et al.* (2008) also state that the combination of medicinal plants to treat ailments has been proficient for years in indigenous medicine systems with enormous success. Furthermore, Lim, Ledesma, Chang, Hou, Kwa, Nikolaou, Quinn, Prince and Tam (2008) point out that there is a belief that antimicrobial combination therapy also increases the efficacy spectrum of activity and potentially reduces side effects. Moreover, there is a belief that indigenous antimicrobial combination therapy also increases the spectrum of activity (Van Vuuren & Viljoen's 2008). The latter study found that the better therapeutic effect in combination therapy is also credited to synergistic interactions between the different bioactive plant constituents (Wagner & Ulrich-Merzenich 2009).

Additionally, it is still a practice for natural scientists to investigate medicinal plants in order to determine the constituent accountable for the therapeutic effect (Rates 2001). Considering that the medicinal properties activity of the medicinal plant may be the result of the combination of several compounds, the isolation process may lead to its loss or reduction of the therapeutic effect (Carmona & Pereira 2013). Some researchers indicate that the whole mixture of the medicinal plants can

sometimes have greater effects than isolated compounds. Wagner (2011) highlights in his study that sometimes diseases possess a multi-causal etiology and a complex pathophysiology, which can be treated more effectively with a well-chosen medicinal combination than with a single isolated constituent.

Today, the interest in medicinal plants is due to several reasons, including that: Conventional medicine can be inefficient, for example, side effects, ineffective therapy, abusive or incorrect use of synthetic drugs, can result in side effects and other problems. Secondly, a large percentage of the world's population does not have access to conventional pharmacological treatment (Rates 2001). Lastly, the World Health Organization (WHO 2013) maintains that the indigenous medicinal system is a care that is located close to homes and that is both accessible and affordable.
The affordability of most indigenous medicines makes them all the more attractive at a time of soaring healthcare costs (WHO 2013). Indigenous medicinal plants are especially suitable for the long-term treatment of chronic diseases in geriatric and convalescent patients, for follow-up treatment, and in the prophylaxis of infectious, degenerative, and metabolic diseases (Cravotto, Boffa, Genzini, & Garella 2010). Adding to that, combination treatment is linked to the belief that antimicrobial combinations increase the spectrum of activity and reduces the potential side-effects (Zonyane *et al.,* 2013).

It is also clear that medicinal plants were the first, and for a long time, the only medicines available to mankind. Today, the whole of the crude extracts remain the primary form of healthcare for the majority of the world's population (Ganesan 2008). In addition, Ganesan (2008) and Cragg and Newman (2013) concur that indigenous medicinal plants continue to play an essential role in healthcare and that their usage by different cultures has been comprehensively documented. WHO (2013) observes that for many millions of people, medicinal plants, indigenous treatments, and indigenous practitioners are the main source of healthcare and sometimes the only source of care. Adding to that, WHO (2013) indicates that in many developing countries, indigenous medicine plays a significant role in meeting the primary healthcare needs of the population and it has been used in this manner for a long time. The two examples of medicine combinations with clinically confirmed synergy effects are as follows: First, the medicinal plants preparation Iberogast® which consists of nine plant extracts and is used for the treatment of functional dyspepsia and motility-related disorders. The preparation exhibits therapeutic equivalence when compared with the synthetic drugs *Cisapride* and *Metoclopromide*, but with the additional advantage that this medicinal plant combination preparation exhibited fewer or no side effects (Wagner 2011). Secondly, is *Ayurveda*, which uses many fixed combination formulae, including *Trikatu*, a mixture containing black pepper, Piperlongum,

and ginger; *Zingiber officinale* which as an ancient recipe, has only recently been investigated scientifically as a combination. The study revealed that the pepper in the combination contains the alkaloid piperine, which is known to increase the bioavailability of a number of drugs such as vasicine (also known as peganine), an antiasthma alkaloid from *Adhatodavesical* (Williamson 2001).

According to Williamson (Ibid), the total medicinal plant extract shows a better therapeutic effect than an equivalent dose of an isolated active compound. Ncube, Finnie and Van Staden (2011) report that the whole contents of a medicinal plant product has a significantly better effect than an equivalent dose of a single isolated active ingredient or a single constituent medicinal plant. For instance, many phytomedicines on the market today, such as *Ginkgo biloba* and *Echinaceae purpurea,* are sold as whole extracts and it is understood that the synergistic interactions between the constituents are responsible for their therapeutic efficacy (Van Vuuren & Viljoen 2008). In support of this, Van Wyk (2011) states that a number of medicinal plants are regularly sold as crude, unprocessed drugs in markets in various parts of South Africa.

The indigenous medicines remain a source of viable mainstream for affordable healthcare, especially in developing countries. In South Africa, an estimated 70% of the population depend on medicinal plants that can be used as mono or combination therapy (Mjiqiza, Syce & Obikeze 2013) and the most popularly used of the medicinal plants include plants such as *Artemisia afra, Ruta graveolens* and *Sutherlandia frutescens* (Suliman, Van Vuuren & Viljoen 2010).

The *Artemisia afra* is one of the ancient and well-known plants of the indigenous medicinal plants in Southern Africa (Mukinda & Syce 2007). It is usually used for treating a variation of ailments such as coughs, colds, headaches, chills, dyspepsia, gastric conditions, colic, croup, whooping-cough, gout, asthma, malaria, diabetes, bladder and kidney disorders, influenza, convulsions, and fever (Mukinda & Syce, 2007; Ntutela, Smith, Matika, Mukinda, Arendse, NasiemaAllie, Esteef, Mabuselae, Folba, Steyn, Johnson, Folk, Syce & Jacobs 2009; Viljoen, Van Vuuren, Gwebu, Demirci, Husu & Baser 2006). Apart from that, *Artemisia afra* also exhibits potent pharmacological activities, including antimicrobial, antioxidant, CNS-effects (sedative, antidepressant), cardiovascular, and spasmolytic activity, which has been well documented and reviewed recently (Suliman *et al.,* 2010).

However, the predominant use of *Artemisia afra* is for the treatment of respiratory disorders such as coughs, colds, bronchitis, blocked sinuses, and tight-chest or asthma in the form of a steam inhalation of the leaf extracts (Mjiqiza *et al.,* 2013). It is normally used as mono or combinations therapy with other medicinal plants for the management of upper and lower respiratory tract infections (Suliman *et al.*, 2010). The aromatic leaves of this plant exhibit various pharmacological effects including relaxation (bronchodilatory effects) of respiratory smooth muscles (Mjiqiza *et al.,* 2013).

In South Africa, *Ruta graveolens* (also known as wynruit) together with *Artemisia afra* (wildeals) are the most popular medicinal plants used with the infusion of their leaves being used for the treatment of cold (Van Wyk 2008). De Beer and Van Wyk (2011) point out that the leaf infusions are taken for colds, headache, and influenza. Furthermore, Van Wyk (2008) indicates that Rue infusion is for inflammation, rheumatism, fever, chest ailments, diabetes and high blood pressure.

Adding to that, Harish Kumar, Shanmugavadivu, Rajamania, and Kuppsamy (2014) report that medicinal plants are a valuable source of natural active constituents that are used to maintain human health, and for the treatment of many human diseases. *Ruta graveolens* is used as a natural source for antibacterial compounds for the most susceptible bacterium, such as Bacillus cereus and Staphylococcus aureus, as used in the pharmaceutical industry for the development of conventional antimicrobial drugs (França Orlanda & Nascimento 2015). Ahmad (2010) found that the volatile *Ruta graveolens* oil possesses antibacterial activity against *Micrococcus pyogenes varaureus* and *Escheriachia coli*. Furthermore, *Ruta graveolens* has antipyretic properties (Gutiérrez-Pajares 2003) and antihistaminic activity (Asgarpanah & Khoshkam 2012).

Skerman, Joubert, and Cronjé (2011) argue that *Sutherlandia frutescens* has a long history of medicinal use by a number of indigenous groups such as the *Zulu, Xhosa, Sotho* and *KhoiSan* as their medicine plant in order to treat ailments such as fever, cough, and cold. Adding to Skerman et al.'s (2011) findings, Chinkwo (2005) observes that *Sutherlandia frutescence* is used for many ailments such as cancer, peptic ulcers, and diabetes. According to Shaik, Dewir, Singh, and Nicholas (2010), the plant is used by indigenous *Zulu* traditional healers for the treatment of influenza pandemic, which claimed nearly 20 million lives in the early 1900's.

From the above information, it is clear that the use of combination therapy is a well-practiced therapy in both indigenous- and in conventional- or synthetic medicine. The triple-combination therapy of *Artemisia afra* (wildeals), *Ruta graveolens* (wynruit) and *Sutherlandia frutescens* (willekeur) is a well-known form of therapy in the Northern Cape Province of South Africa for its usage as the therapy for the treatment or the management of common cold and influenza-like illness. Although the community of Griqualand West (*KhoiSan* community) is utilizing this triple-combination therapy, the ratification and validation of this combination therapy for its medicinal properties has not been explored with specific reference to preparation, storage, and usage. Therefore, the researchers undertaken to explore and describe this indigenous way of knowing the preparation, storage, and use of the triple-combination therapy for common cold and influenza-like illness. The aim of this research was to describe how medicinal plants are prepared, stored, and used as triple-combination therapy by the *KhoiSan* community for the treatment of common cold and influenza-like illness.

In order to reach this aim, the following objectives were set:

- Describe the *KhoiSan* indigenous health practice with regard to the preparation, storage, and use of triple-combination therapy for the treatment of common cold and influenza-like illness; and
- Contribute to the learning of African indigenous knowledge systems by including the findings of this research in the re-vitalization of indigenous health knowledge systems of the main research project of the Seboka indigenous research project that is engaging with this community on the indigenous health practice.

METHODOLOGY

The methodology provides information pertaining to the research design, population and sampling, data collection, data analysis, and rigour.

Research Design

The researchers utilized a qualitative, clinical ethnography research design (Dean & Major 2008; Mangula & Pienaar, 2013) to explore and describe the plants used as triple-combination therapy by indigenous Africans for the treatment of common cold and influenza-like illness. The clinical ethnography design was chosen as it implements fieldwork and observation in the indigenous culture, relations, and experiences of an indigenous healthcare system (Dean & Major 2008).

Population and Sampling

The population included the elderly of the community who are the knowledge holders of indigenous medicinal plant use and included the medicinal man of the community and indigenous community healer who are the health caregivers in the community (Burns & Grove 2007; De Vos, Strydom, Fouché, & Delport 2008). The notion was that the adult community members would provide the relevant and rich information that is experience-based on the use of the medicinal plants as the well-known practice in the *KhoiSan* Community. A purposive sampling technique was used to select the participants and the participants included the indigenous community healers and the medicinal man of the community. According to the community, the definition of the medicinal man could be either male or the female member of the community who are the knowledge holders of the indigenous medicinal plant use and who are the health caregivers of the community at large (Stahler & Cohen 2000).

Data Collection

Data collection is an organised process of gathering important information for the research study (Burns & Grove, 2007; Bassett 2004). During the data collection, the researcher established a trusting relationship with the participants in order to acquire more and rich information from the participants.

In benefit of the study, the needed information focused on how the medicinal plants were measured, prepared, stored, and used for the triple-combination therapy medicinal decoction used by the indigenous community healer to manage common cold and influenza-like illness. Data were collected in two separate approaches of the research method. Firstly, through the implementation of the participant observational approach, data were collected through written field notes during and after each period of observation and use of video- and sound recording. Secondly, data were collected with the use of semi-structured interviews and informal interviews.

According to Varjas, Nastasi, Moore and Jayasena (2014), the participant observational research method involves observing and interacting with participants. Moreover, it represents a recipe of observing and informal interviewing; hence the researchers utilized this research method. During the participative observation, the researcher became a participative member of the community as the researcher was observing the community, took the measurements of the medicinal plants used by the indigenous community healer for the management of the common cold and influenza-like illness and the preparation of the medicinal plants decoction as well as the storage. The participative observation took place in the cultural context of the community in which the researchers wrote field notes during and after each period of observation. The use of video- and sound recording was of the utmost importance in order to keep proper and detailed records for the data analysis.

The qualitative interview, as indicated by Stahler and Cohen (2000) in ethnographic research, is an in-depth semi-structured interview used to acquire rich and detailed information. Semi-structured interviews were conducted with the medicinal man of the community, the indigenous community healer, and other knowledge holders of how the indigenous medicine was measured, prepared, used, and stored in the community.

Data Analysis

The researcher reduced, organized, and gave meaning to the data collected during the observational and interview approaches. The analysis of the information assisted the researcher to acquire sensible information from the emic perspective viewpoint of the indigenous community on the use of the medicinal plants. The following four steps as stated by Pienaar (2015) were followed in the analysis:

- Level one: Basic concept from the spoken word on the measurement, preparation, storage, and usage of the triple-combination therapy medicinal plant decoction. The researchers explored the audio- and video recordings. Concepts were derived from the spoken words;
- Level two: Joining or grouping of similar concepts to form a theme or cluster;
- Level three: This is an intuitive deduction, convergence or discovery of new themes or clusters normally called an insight or discovery (with close collaboration of the community);
- Level four: The building of a storyline or pattern to form a framework to guide research (please see the conclusion for this level).

Rigour

The rigour of this research was secured by the trustworthiness as it is the expectation in qualitative research. The trustworthiness of the research was determined by the ability to inspire belief or trust (the believability or credibility), ability to trust and to depend on (dependability) and to verify the truth or the validity (confirmability) of the general research study undertaken (De Vos *et al.,* 2008; Creswell & Plano Clark 2011). The credibility of this research was ensured by the trust relationship and the prolonged community engagement the Seboka indigenous research project have with the community where the research took place which the researcher is the member of this project. Therefore, the data the community provided were dependable. The in-depth discussion of the methodology and the application of this methodology provided consistency and valid grounding should this research be repeated in the same context. This process contributed to the confirmability of the process. The researcher used this framework to ensure trustworthiness in the community during research.

The researcher was further guided by the following ethical principles as coded by the American Psychological Association (APA 2010). The principle of beneficence and non-maleficence that underlines that there should be no harm to the participants and that their welfare and rights are taken into consideration. Although there is no overt harm in this research, the researchers further avoided any aspect that might indirectly harm the *KhoiSan* community and for this reason, consultation and respect was maintained throughout the study. The second ethical principle is fidelity and responsibility that advocates a trust relationship with the community should be of the utmost importance during a research study. The researchers had already developed a trust relationship with the community since 2010. Thirdly, the principle of integrity, which highlights that during the research process, the researcher should seek to promote accuracy, honesty and truthfulness was upheld by being honest and truthful with the collection and analysis of the data and with the finding of the research study. The fourth principle of justice denotes that fairness and justice prevail to all people.

Therefore, the researchers practiced justice by making sure that the community had access, benefitted from the contribution of the research, and ensured equity through information sharing and respect. Lastly, the principle of respect for people's rights and dignity was upheld by considering the rights of the *KhoiSan* participants to privacy, confidentiality, and self-determination.

RESULTS AND DISCUSSION

The results provide an inclusive discussion combining the data of the three plants, namely; *Artimisia afra, Ruta graveolens* and *Sutherlandia fructescens*. The discussion for all three plants is done under the three headings presented in the different tables below. Where there is a difference, the researchers illuminate the diversity. In this section, the researcher embarked on the discussion and the literature integration will be attended to after the in-vitro experiment. Table 2.2 provides an overview of the results regarding the measurement of the medicinal plants used. Unlike the traditional way of reporting qualitative findings, the researchers chose to illuminate the voice of this community by not integrating literature, because most of the data was unique to this community, however, relevant literature were included in the introduction section of this article.

Table 2.2 Measurement of the medicinal plants used

LEVEL ONE: Basic concept from the spoken words	**LEVEL TWO: Joining similar concepts to form a theme or cluster**	• **LEVEL THREE: This is an intuitive deduction, convergence or discovery of new themes or clusters normally called an insight or discovery (with close collaboration of the community);**
<u>Measurement of medicinal plant used</u> Different (Natural) standard unit or the fixed scale to measurement the leaves, branches and aerials used. The amount of wildeals and wynruit leaves used is equal as scaled on the bases of the eye look and length of the fingers. The leaves of the kankerbos is the quarter measurement of the wildeals and wynruit	Ways of measurement	Specific way of measurement, using the hands or fingers of the indigenous health care user (e.g. length of the index finger-ten leaves)
<u>Dosage measurement of the prepared decoction.</u> Take medicine of the cup scale 2 times a day for the mild flu. 3 time a day of cup medicine for severe flu.	Unique natural manner of dosage	Natural measurement process of dosage that fits the role and activity of the health care user

The triple combination medicinal decoction is the combination of three medicinal plants (wildeals, wynruit and kankerbos) that the community uses for the management of common cold. The measurement of these medicinal plants (leaves and aerials) is on the scale of looking at the quantity of the leaves and aerials. There is no special equipment used to measure the amount of the leaves

and aerials for medicinal preparation, except the fingers and hands. The community uses equal amount of *Artemisia afra* (wildeals) and *Ruta graveolens* (wyruint) leaves while the amount of *Sutherlandia frutescens* (kankerbos) is a quarter quantity of the leaves due to its potency. The community uses a unique, natural self-determined way of measuring that does not require extra resources.

Unique natural dosage measurement of the prepared medicinal decoction is determined by the severity of the common cold. For the mild common cold, a cup of medicine two times a day is orally administered while for the severe common cold, a cup of medicine three times a day is administered. People use the length of their own hand (bottom of palm to index finger) to measure the dosage. The dosage measurement of the medicine is straightforward and the instructions are easily remembered. Table 2.3 provides an overview of the results regarding the preparation and storage of the medicinal triple-combination decoction.

Table 2.3 Preparation and storage of the medicinal triple-combination decoction

LEVEL ONE: Basic concept from the spoken words	**LEVEL TWO: Joining similar concepts to form a theme or cluster**	**LEVEL THREE: An intuitive deduction, convergence or discovery of new themes or clusters**
Preparation of medicinal decoction Medicinal plant's leaves are boiled together. The pot used for boiling the medicinal decoction is not closed. The medicine is prepared per intake intervals. The longer the boiling time the more concentrated medicine become and the stronger the indigenous medicine. Stored in a cool space in the house- under an elevated bed.	Process of medicinal preparation and storage	Preparation is inexpensive, time-saving and easily manageable. Storage in low temperature

The leaves of these medicinal plants are boiled together and the preparation of the medicine is per intake intervals. The community utilizes easily affordable apparatus to prepare the medicine. They use cooking pots to boil the medicine and the pot remains not closed until the medicine is ready. The longer it takes for the medicine to boil the more concentrated the medicine becomes and the stronger it is. The readiness is noticed by the colour change to a deep, dark colour. The preparation is inexpensive, time-saving and easily manageable and the indigenous medicine is stored after it has cooled down to a low temperature.

CONCLUSIONS

African belief and world-view on healing is treasurable to this community. During the process of indigenous medicine preparation, the community considers their belief and world-view practices, which add the meaning to the indigenous medicine preparation. They believe that the person preparing the indigenous medicine should be 'clean', be free from pollutants and taboos. Apart from

that, they also equate medicinal plants with human beings (personification). This is evident through how the community communicates with, and handle the medicinal plants in a respectful manner.

The measurement of medicinal plants is a unique, natural self-determined way of measurement that indigenous (traditional) health practitioners use and does not require any extra resources. The dosing method and administration instructions are straightforward and easily remembered while the preparation of the complete indigenous medicine is inexpensive, time saving and manageable.

It is clear that for all three plants, the processes are the same, except for the measurement of willekeur. A second step of the process consists of *cost-effective and time-saving medicinal preparation* wherein the community use easy measurements and low-cost preparation as discussed in level three. The last step of the process is the *triple-combination medicinal decoction* for common cold and influenza-like illness, which is the product of these community-based processes. Following prolonged usage, the community experiences this decoction as effective. The processes that the community use are ordered, logical, and systematic and agree with the definition of science. Therefore, the researchers advocate for the acceptance of African Indigenous Knowledge as scientific**.**

Further opportunity for research after this phase is possible to test the medicinal properties of the triple-combination from a Western perspective (in-vitro) to compare and contrast the data found during the indigenous phase of the research. This will subsequently lead to the development of a natural solution to combat common cold in the community as well as creating opportunity for income generation.

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ARTICLE 3

Investigation of the medicinal properties of triple-combination therapy of *Artemisia afra, Ruta graveolens and Sutherlandia frutescens in vitro, using of NA-XTD™ Influenza Neuraminidase Assay Kit*

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Abstract

The *KhoiSan* society in the last 2000 years depended on nature for their basic needs, such food, clothing and shelter, and of the most significant for the research study, the management and the treatment of ailments by the use of the medicinal plants. The decoction of *Artemisia afra, Ruta graveolens* and *Sutherlandia frutescens* and the triple-combination therapy concoction of these medicinal plants are used to treat upper respiratory track disease such as common cold and influenza (flu) virus. The medicine was indigenously prepared by the *Khoisan* community. Null hypothetically the medicine has not medicinal properties, as such, the research endeavours to investigate the medicinal properties of this medicine as used by the community for the treatment of common cold and influenza-like illness.

In vitro study by making use of NA-XTD influenza neuraminidase assay kit was conducted to test the antiviral properties of this medicine, and to determine the 50% reduction of the neuraminidase activity and the inhibitory concentration (IC_{50}) of each medicinal decoction and triple-combination concoction. Conclusively, the findings confirms with the community medicinal utilization of these medicinal plants for common cold and influenza-like illness. This medicine has antiviral properties as reflected by the reported results of the research study.

Keywords*:* common cold; indigenous medicine; influenza-like illness; influenza (flu)virus; *in vitro* study medicinal plants; triple-combination therapy.

INTRODUCTION

Medicinal plants have been and are still of great benefit to the indigenous and modern Africans. The plants used as indigenous medicine are not only important for the treatment and the management illness, but also for cultural, spiritual and economic benefit (WHO 2013; Dahlberg & Trygger 2009; 80). These indigenous medicinal plants remedies are particularly vital in the primal – and primary health care of people in rural areas (Appidi *et al.,* 2008; 1962). Similarly, the use of medicinal plants as medicine by indigenous people in Southern Africa is approximately 80% of the population and this high usage can be attributed to very high cost and unaffordable by most of the people, particularly those living in rural areas (Mander *et al*., 2007:54).

Following from that, according to Gathirwa *et al.* (2008:224), in indigenous medicinal system, different plants are combined for the treatment of a disease. This combination has existed for decades in ethno-medicinal plants systems. The medicinal plants used are often not related in any way or may be used singularly for the treatment of an ailment (Sibandze *et al.,* 2010:507). In his study of synergy research, Wanger (2011:36) reveals that the combination therapy regime for antimicrobials is known to prevent antibiotic resistance. It is further supported by Gathirwa *et al.* (2008:229) in their studies where they found that drug combinations also help reduce danger of resistance development. It is evident that the combination of medicinal plants to treat ailments has been proficient for years in indigenous medicine systems with immense victory.

Lim *et al.* (2008:2898) postulate that there is a belief that antimicrobial combination therapy also increases the efficacy spectrum of activity and potentially reduces side effects. Moreover, there is a belief that indigenous antimicrobial combination therapy also increases the spectrum of activity as seen in Van Vuuren and Viljoen (2008:701) study. Also, the better therapeutic effect in combination therapy is credited to synergistic interactions between the different bioactive plant constituents (Wagner & Ulrich-Merzenich 2009:98). It is also noted that several plants have been used to treat microbial infections (Mukhtar *et al.,* 2008:112). Therefore, in this study, influenza (flu) is the infectious condition concerned. The section below discusses Influenza in more detail.

Influenza (flu) virus

The Society for General Microbiology (2011) describes influenza (flu) as the upper respiratory track disease caused by influenza virus, while WHO (2013) stipulates that influenza (flu) is a seasonal viral infection that causes severe illness and even death. Charyasriwong *et al.* (2015: 8) further assert that influenza viruses are classified under *Orthromyxoviridae* family and virus A and B are responsible for

influenza in humans. Supporting the mentioned authors, Burnham *et al*. (2013: 521) opine that many people throughout the world are infected by the influenza virus and develop and acute respiratory infection. Burnham and the colleagues argue that, if the restorative measures are delayed to treat the influenza (flu), the pulmonary complications such as viral pneumonia, secondary bacterial or fungal pneumonia may occur. It is clear that this assertion aligns with the first statements quoted from the Society for General Microbiology. A spontaneous question arises: How does this influenza virus operate?

The influenza virus has two surface antigens, namely; the hemagglutinin (HA) and neuraminidase (NA). HA identifies and binds to the sialic acid residue on a host cell surface to facilitate viral infection and infusion. NA hydrolyses host sialic acid to allow the release of the progeny virus and blowout of the infection. The pharmacological management of influenza infection include the prevention/vaccination, prophylaxis and the treatment with antiviral medication (Burnham *et al.,* 2013:523). Following from that, Charyasriwong *et al.* (2015:8-9) mention that the two main pharmacological classes of anti-influenza virus are the old class, the amantadine and rimantadine being the first drugs for flu treatment which are no more used recently due to drug resistance. The other group of the anti-influenza is the neuraminidase inhibitors such as *ganamivir, oseltamivir* and *laninanivir* (Goodman & Gilman 2011).

Linking to the previous discussion, Ikematsu *et al.* (2015:634) stated that the antiviral resistance is the major concern not only about the antiviral drugs but of the antimicrobial therapy in general. This argument is further supported by Cheryasriwong *et al.* (2015:9) who contend that drug resistance of influenza virus is a serious public problem. Against this background, the researcher investigated the new anti-influenza virus combination therapy originating from the three medicinal plants used by the Khoisan community to overcome the serious day-to-day escalating drug resistance with regard to influenza virus. This study also endeavours to confirm the indigenous health practice of the community on the usage of the this triple-combination therapy for treatment of common cold and influenza-like illness as well as the comparison and contrasting of the findings with the current Western health practices for validation purposes. The discussion of the indigenous health practice follows henceforth.

Preparation of medicinal plant extracts

The medicinal plants tested in this study were intended to find out how the indigenous community prepared the medicine for the treatment of common cold and influenza-like illness. The *Artemisia afra, Ruta graveolens* and *Sutherlandia frutescens* are medicinal plants the *KhoiSan* community uses individually and as the triple-combination therapy. The decoction of *Artemisia afra*, *Ruta graveolens* and *Sutherlandia frutescens* were tested separately. Furthermore, the concoction of the triple-combination medicinal plants was also investigated against the influenza virus.

Chinyama (2009:34) reports that the indigenous health care practitioners had relied on the medicinal plants to manage and treat any certain illness that the community faces as part of their day-to-day living, and that, they acquired skills and knowledge of the medicinal plants preparation. Chinyama (2009) stated that the most common medicinal preparation is the boiling of the medicinal plant. Mukinda and Syce (2007:138) supported Chinyama (2009) by concurring that indigenous medicinal plant preparations are in different forms and the preparation methods are not the same. The medicinal plants preparations usually are decoction which is the extraction process by boiling the medicinal plant, and the infusion, the process of soaking medicinal plants in boiled or cold water for some time or soaking it in any liquid that is preferred for the effective extraction process.

The *KhoiSan* descendants where the study took place also are part of the indigenous community that utilizes medicinal plants. This community acquired the knowledge of preparing medicine out of the medicinal plants. The preparation of the triple-combination concoction of the *Artemisia afra, Ruta graveolens* and *Sutherlandia frutescens* is a unique medicinal plants combination that the community uses to manage or treat common cold and influenza-like illness. Most importantly, the *KhoiSan* community follows very low cost effective preparation method.

Firstly, the harvesting of the medicinal plants which are located within the community gardens or in the natural veld setting seems to be part of their dietary plants. The harvesting is done as detailed in the results of the qualitative first phase results of the medicinal plants harvesting. The decoction of each medicinal plant is prepared by boiling the medicinal plants separately until the colour change satisfactory according to their long time experience in using and preparing these medicinal plants. Each medicinal plant decoction is transferred to pre-sterilized bottle containers separately. Secondly, the preparation of the concoction of these three medicinal plants, the leaves and the aerials as the parts of these plants used for medicinal preparation were boiled together as decoction, then was transferred to its pre-sterilized bottle container.

Null hypothetically, the medicinal plant concoction of a triple-combination therapy of *Artemisia afra (Wildeals), Ruta graveolens (Wynruit)* and *Sutherlandia frutescens (Willekeur)* (WWW) as used by the indigenous Khoisan community has no influence on the treatment of common cold and influenza-

like illness symptoms. Hence the researcher aimed to investigate the medicinal properties of the triple-combination therapy concoction of *Artemisia afra, Ruta graveolens* and *Sutherlandia frutescens* in vitro being directed by the following objectives, to:

- Test the antiviral properties of *Artemisia afra, Ruta graveolens* and *Sutherlandia frutescens* decoction singularly;
- Test the antiviral properties of the triple-combination therapy concoction; and
- Determine the 50% reduction of the neuraminidase activity, and the inhibitory concentration (IC_{50}) of each medicinal plants decoction and of the triple-combination concoction.

NA-XTD™ Influenza Neuraminidase Assay kit and protocol as described by (Applied Biosystems by life technologies, 2010)

This is a next-generation chemiluminescence-based assay kit that provides long signal readout as compared to the first-generation NA-star® Influenza Neuraminidase Inhibitor Resistance Detector Kit. NA-XTD™ Influenza Neuraminidase Assay kit provides necessary assay reagents such as chemiluminescence neuraminidase substrate which provides longer-lasting chemiluminescence signal with high detection sensitivity. The assay buffer as diluent for virus samples, neuraminidase inhibitors and NA-XTD substrate.

Adding to the list, NA sample Prep Buffer which is for Triton® X-100 detergent addition to virus preparations which increase NA activity. The NA-XTD™ Accelerator solution to trigger high intensity and light emission from the NA-XTD reaction products. Lastly, the NA- Detection Microplates, 96-well solid white assay microplates, of the optimum assay performance and high signal intensity. Furthermore, Okomo-Adhiambo *et al.* (2012: 46) mentioned that next-generation chemiluminescence-based assay provides the easy measurement reading of the neuraminidase activity of the influenza virus and gives out reliable and consistent results through standardised reagents and protocols.

Medicinal plants' neuraminidase inhibition activity assay procedure

The medicinal plants tested in this study as precisely how the indigenous community prepared the medicine for the treatment of common cold and influenza-like illness. The *Artemisia afra, Ruta graveolens* and *Sutherlandia frutescens* are medicinal plants the *KhoiSan* community uses individually and as the triple-combination therapy.

The following procedure was followed to investigate these medicinal plants neuraminidase inhibition activity against the Washington virus, which was a deactivated prior assay procedure. The neuraminidase inhibition activity investigation was against each individual medicinal plant as the decoction and triple-combination as the concoction.

First procedure: medicinal plants extract in 10 well reagents reservoirs *(please see table 2.4)*

Step 1: The undiluted 400µl of each medicinal plant decoction and triple-combination concoction was added in the column 1 of the 10 well reagents reservoir.

Step 2: 200µl assay buffer was added from column 2 up to column 10 by the use of the multichannel pipette.

Step 3: 200µl of each undiluted medicinal plants decoction and of the triple-combination concoction was transferred from column 1 to column 2 and serial diluting 200µl up to column 10 by the use of multichannel pipette.

First three steps table: Medicinal plants extract dilution preparation. Column 1 was 400µl undiluted medicinal plant. From column 2, 200µl assay buffer added up to column 10 for the serial dilution.200µl of undiluted medicinal plants from column 1 serially diluted until column 10. The medicinal plants dilution was prepared in duplicates as presented in the table 2.4.

Table 2.4 Medicinal plants extract dilution preparation

DILUTIONS	UNDILUTED	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512
MEDICINAL PLANTS	1	2	3	4	5	6	7	8	9	10
Artemisia afra decoction										
Artemisia afra decoction										
Sutherlandia frutescens decoction										
Sutherlandia frutescens decoction										
Ruta graveolens decoction										
Ruta graveolens decoction										
Triple-combination concoction										
Triple-combination concoction										

After the first three steps were completed, the medicinal plants dilutions were equivalently transferred to the 96 well plates then followed step 4 up to step 13.

Please *see (table 2.5) linked* to the process below

Step 4: by the use of the multichannel pipette, 25µl of each medicinal plants dilution were equivalently transferred to the 96 well plates starting from column 10 ending at column 1. And 200µl assay buffer was added in column 12 only.
Step 5: the prepared virus dilution was added from column 1 up to column 11 of the 96 well plates.
Step 6: the virus and medicinal plants dilution were mixed gently by tapping the covered plate.
Step 7: the 96 well plates was incubated for 30 minutes at the room temperature.
Step 8: 1/1000 dilution of NA-XTD substrate was prepared in assay buffer (4µl substrate plus 4ml assay buffer per 96 well plates.
Step 9: 25µl diluted NA-XTD substrate was added starting from column 12 ending at column 1 using a multichannel pipette.
Step 10: the 96 well plates covered with lid, wrapped in foil, tapped lightly and incubated for 30 minutes at the room temperature in dark.
Step 11: 60µl accelerator was added to each well starting from column 12 ending column 1
Step 12: the 96 well plates covered, wrapped in foil, tapped to mix and incubated for 5 minutes before reading in the luminometer.
Step 13: the 96 well plates was placed in luminometer to measure luminescence.

Table 2.5 in the following section presents the medicinal plants plate. Each medicinal plant was done in duplicate.

Table 2.5 Medicinal plants plate

DILUTIONS	UNDILUTED	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	Virus only	Buffer only
MEDICINAL PLANTS	1	2	3	4	5	6	7	8	9	10	11	12
Artemisia afra decoction												
Artemisia afra decoction												
Sutherlandia frutescens decoction												
Sutherlandia frutescens decoction												
Ruta graveolens decoction												
Ruta graveolens decoction												
Triple-combination concoction												
Triple-combination concoction												

Neuraminidase inhibitor resistance (IC_{50}) assay procedure *(see table 2.6)*

The on market neuraminidase inhibitor, Oseltamivir and Zanamivir were used as the positive control in this study.

Step 1: 980µl assay buffer was added to column 1 of the 10 well reagents reservoir.
Step 2: 540µl assay buffer was added from column 2 up to column 10 of the reagents reservoir.
Step 3: 20µl 100µM oseltamivir was added to column 1 and mixed by pipetting up and down.
Step 4: By use of multichannel pipette 250µl was transferred from column 1 to column 2, mixed and 250µl was transferred to column 3, and serial diluting 250µl up to column 10.

Table 2.6 presents the *Oseltamivir* and *Zanamivir* reagents reservoir plate which served as the positive control.

DILUTION CONCENTRATION	1000	317	100	31.7	10	3.17	1.01	0.317	0.1	0.032
DRUG	1	2	3	5	6	7	8	9	10	
Oseltamivir										
Oseltamivir										
Zanamivir										
Zanamivir										
x	x	x	x	x	x	x	x	x	x	x
x	x	x	x	x	x	x	x	x	x	x
x	x	x	x	x	x	x	x	x	x	x
x	x	x	x	x	x	x	x	x	x	x

Please see table 2.7 linked to the process below

Step 5: By the use of the multichannel pipette, 25µl of each drug dilution were equivalently transferred to the 96 well plates starting from column 10 ending at column 1. And 540µl assay buffer was added in column 12 only.

Step 6: The prepared virus dilution was added from column 1 up to column 11 of the 96 well plates.

Step 7: The virus and medicinal plants dilution were mixed gently by tapping the covered plate.

Step 8: The 96 well plates was incubated for 30 minutes at the room temperature.

Step 9: 1/1000 dilution of NA-XTD substrate was prepared in assay buffer (4µl substrate plus 4ml assay buffer per 96 well plates.

Step 10: 25µl diluted NA-XTD substrate was added starting from column 12 ending at column 1 using a multichannel pipette.

Step 11: The 96 well plates covered with lid, wrapped in foil, tapped lightly and incubated for 30 minutes at the room temperature in dark.

Step 12: 60µl accelerator was added to each well starting from column 12 ending column 1

Step 13: The 96 well plates covered, wrapped in foil, tapped to mix and incubated for 5 minutes before reading in the luminometer.

Step 14: The 96 well plates was placed in luminometer to measure luminescence

Table 2.7 Oseltamivir 96 well plate, standard control

DILUTIONS	1000	317	100	31.7	10	3.17	1.01	0.317	0.1	0.032	Virus only	Buffer only
DRUG	1	2	3	4	5	6	7	8	9	10	11	12
Oseltamivir												
Oseltamivir												
Zanamivir												
Zanamivir												
x	x	x	x	x	x	x	x	x	x	x	x	x
x	x	x	x	x	x	x	x	x	x	x	x	x
x	x	x	x	x	x	x	x	x	x	x	x	x
X	x	x	x	x	x	x	x	x	x	x	x	x

Furthermore the results of the previously discussed processes will be elaborated henceforth. This elaboration will include (*Tables 2.8, 2.9, 2.10) and (Graph 2.1 & 2.2).*

Table 2.8 Luminescence results of the 96 well plates measured from the luminometer

MEDICINE	undiluted	2	4	8,00	16,0000	32,0000	64,0000	128,0000	256,0000	512,0000	VC	NEG
Artemisia afra	1262	2949	3262	10406	16174	18116	21345	26988	21539	20971	27143	273
Sutherlandia frutescens	4002	5936	8460	13848	19017	18633	23284	26768	21577	20873	28854	311
Ruta graveolens	2645	3013	10833	16193	19816	20511	23164	23739	21731	19772	28332	320
Triple-combination	990	4221	7619	12110	15727	18779	23114	23333	21659	20523	29029	341
Oseltamivir	352	344	358	292	552	420	382	548	364	504	40206	318
Oseltamivir	360	470	392	364	594	322	366	566	380	534	37305	302
Zanamivir	366	356	370	358	434	388	360	514	364	484	35498	308
Zanamivir	312	288	334	268	288	254	326	350	354	340	29686	266
										NEG MEAN		304,88

This (*table 2.8*) was formed after the procedures of medicinal plants and of the positive controls. As the luminescence points of the corresponding medicine are joined by the line (point to point), they form dose-response curve as reflected on the graph 8 and 9. VC= Virus control, and in this column no medicine added only the virus. NEG/Negative, in this column no virus, no medicine investigated added, only the buffer, NA-XTD substrate and the accelerator. The dilution factor used for medicine is 2.

Table 2.9 50% reduction of neuraminidase activity (50% of the virus control)

Log(Concentration)	#VALUE!	0,30103	0,60206	0,90309	1,20412	1,50515	1,80618	2,10721	2,40824	2,70927	VC	50%CUT
***Artemisia afra* dose-response curve**	957,13	2644,125	2956,625	10101,13	15869,13	17810,63	21039,63	26682,63	21234,13	20666,13	26837,63	13418,81
***Sutherlandia frutescens* dose-response curve**	3697,13	5631,13	8155,13	13543,13	18712,13	18328,13	22978,63	26463,13	21271,63	20568,13	28549,13	14274,56
***Ruta graveolens* dose-response curve**	2340,13	2707,63	10528,13	15887,63	19511,13	20205,63	22858,63	23433,63	21425,63	19467,13	28027,13	14013,56
Triple-combination dose-response curve	685,13	3915,63	7314,13	11804,63	15422,13	18474,13	22809,13	23028,13	21354,13	20217,63	28724,13	14362,06
Oseltamivir dose-response curve	47,13	39,13	53,13	-12,88	247,13	115,13	77,13	243,13	59,13	199,13	39901,13	19950,56
Oseltamivir dose-response curve	55,13	165,13	87,13	59,13	289,13	17,13	61,13	261,13	75,13	229,13	37000,13	18500,06
Zanamivir dose-response curve	61,13	51,13	65,13	53,13	129,13	83,13	55,13	209,13	59,13	179,13	35193,13	17596,56
Zanamivir dose-response curve	7,13	-16,88	29,13	-36,88	-16,88	-50,88	21,13	45,13	49,13	35,13	29381,13	14690,56

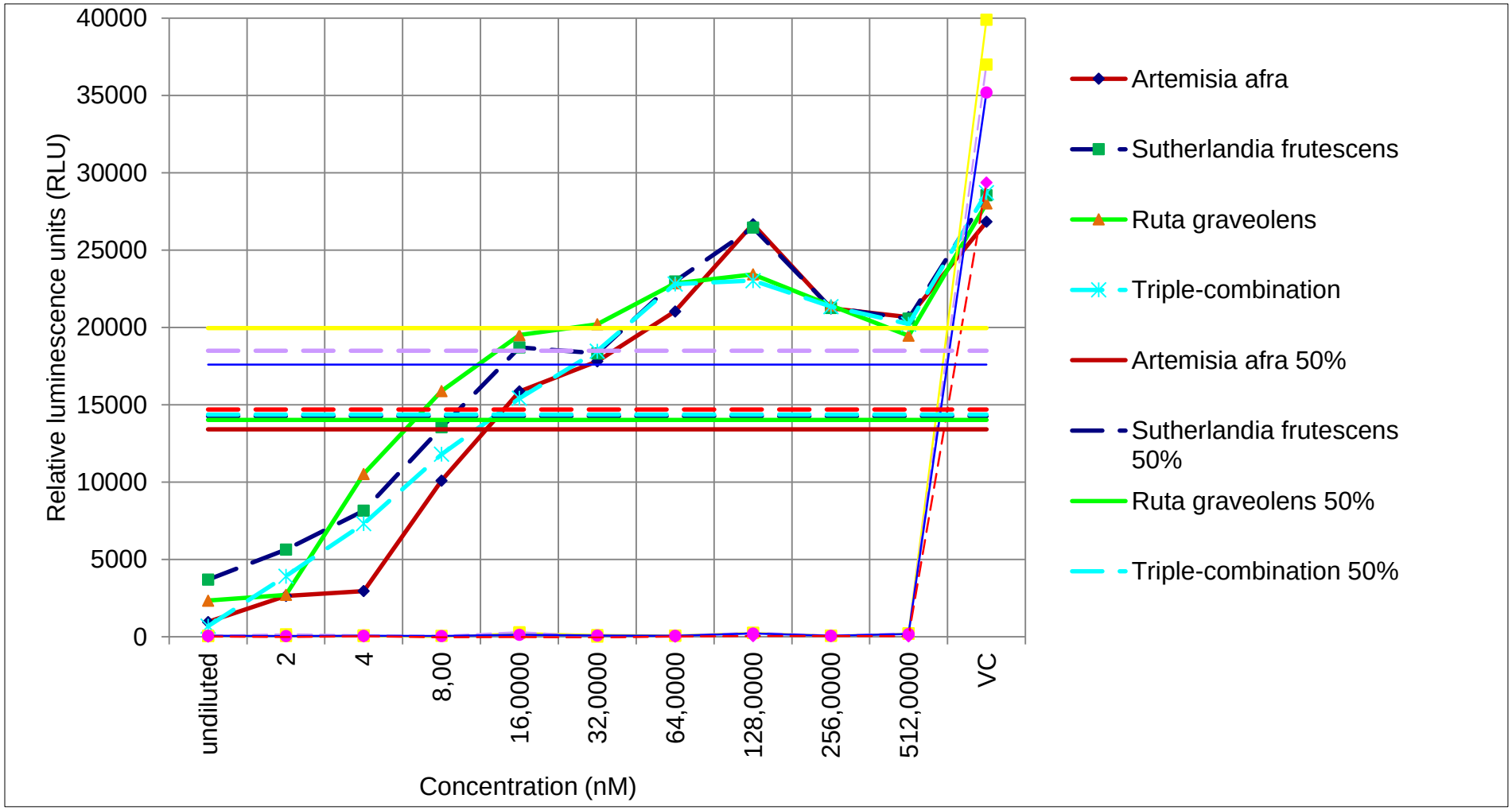
On this (*table 2.9)*, the important values are the **50% CUT.** The 50% reduction of neuraminidase activity is calculated as half the relative luminescence units achieved with the virus control (VC) in no medicinal plants were added. For each tested medicine, the 50% neuraminidase reduction (**50%CUT**) is presented by the horizontal lines on the graph 8 and 9. Their significance is to calculate the IC_{50} of each medicine investigated at the point where they cross over the dose-response curve of the related medicine.

Table 2.10 Inhibitory concentration (IC50) of each medicinal decoction and triple-combination concoction

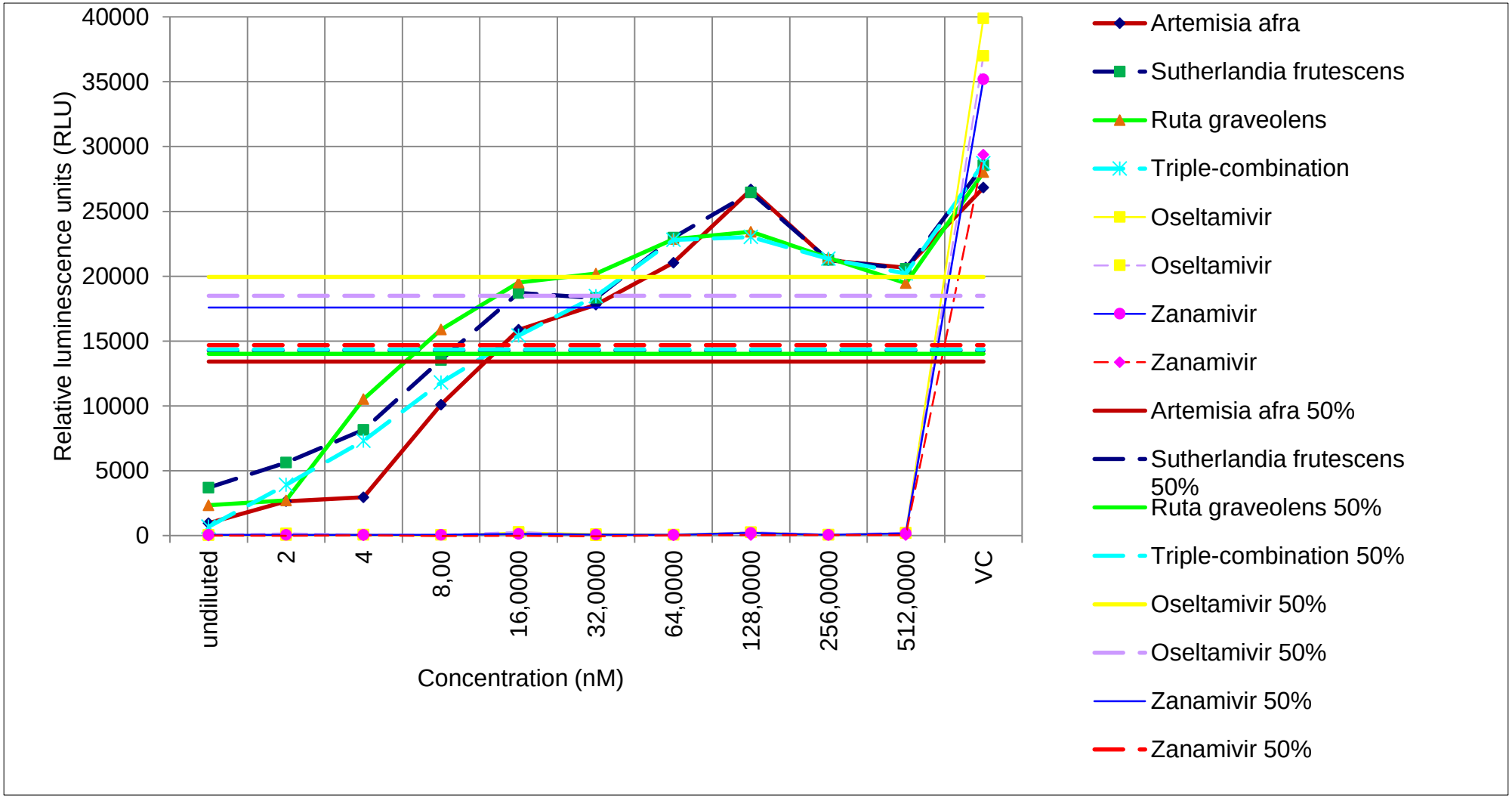
FINDIC50	#VALUE!	0,30103	0,60206	0,90309	1,20412	1,50515	1,80618	2,10721	2,40824	2,70927	Res	IC50
Artemisia afra **dose-response curve**				1,076239							1,076239	11,91898
Sutherlandia frutescens **dose-response curve**				0,945687							0,945687	8,824439
Ruta graveolens **dose-response curve**			0,797828								0,797828	6,278104
Triple-combination dose-response curve				1,115907							1,115907	13,05891
Oseltamivir dose-response curve										-9999	-9999	<
Oseltamivir dose-response curve										-9999	-9999	<
Zanamivir dose-response curve										-9999	-9999	<
Zanamivir dose-response curve										-9999	-9999	<

On this table *(Table 2.10),* the important values are the IC_{50} that indicates how much of the medicinal plants decoction or the concoction needed to suppress the influenza neuraminidase enzyme activity by 50% of the virus control. They are automatically calculated as the luminescence data are entered in the excel spread sheet and by the use of the formula. The IC_{50} can be also estimated from the graph *(Graphs 2.1 & 2.2)* as the dose-response curve and the 50% neuraminidase reduction (**50%CUT**) as presented by the horizontal lines cross each other.

Graph 2.1 Medicinal plants dose-response curves and the 50% neuraminidase reduction activity (horizontal) lines



Graph 2.2 Medicinal plants dose-response curves and their 50% neuraminidase activity reduction and the positive controls dose-response curves and the horizontal 50% neuraminidase activity reduction line.



DISCUSSION *(see table 2.9 and 2.10 as well as graph 2.1 and 2.2)*

Artemisia afra

The 50% NA activity reduction line and the *Artemisia afra* dose-response curve cross at the **dilution 1/16** producing IC_{50} of **11.9nM** as estimated from the *(Graph 2.1 & 2.2).* In terms dilution, this medicinal plant is effective to treat common cold and influenza-like illness as undiluted (just as the community prepared it) and it is still effective until dilution 1/16. If compared with *Sutherlandia frutescens* with **IC_{50} 8.8nM** and with Ruta graveolens **of 6.3nM**, *Sutherlandia frutescens* and *Ruta graveolens a*re more effective to reduce 50% of NA activity.

Sutherlandia frutescence

The 50% reduction in NA activity line and the dose-response curve cross within the dilution 1/8 and produce **8.8nM IC_{50}** as estimated from *(Graph 2.1 & 2.2).* According to the results, as the indigenous medicinal plant, *Sutherlandia frutescens* is effective to reduce the 50% NA activity of the virus control within the range of the undiluted to the **dilution 1/8** to treat common cold and the influenza-like illness singularly.

Ruta graveolens

The 50% NA activity reduction line and the dose-response curve cross at the **dilution 1/8** and produce the **IC50 of 6.3nM** as estimated from the *(Graph 2.1 & 2.2).* Comparing this medicinal plant with the *Artemisia afra* and *sutherlandia frutescens. Ruta graveolens* is more potent in inhibiting the 50% NA activity of the virus control. Furthermore, the results reflect that it is more effective when used singularly to inhibit 50% of the NA activity.

Triple-combination concoction

The 50% NA activity reduction line and the dose-response curve cross at the **dilution 1/16** as estimated from the *(Graph 2.1 & 2.2)* produce the **IC50 of 13.3nM**. As the triple-combination compared to single medicinal plants, the synergistic effect takes place as the results reveal that the effect of the triple-combination is more than the sum of the three medicinal plants. Furthermore, from the undiluted to the dilution 1/16 the, the medicinal plants reduced the NA activity by 50% of the virus control, which means within this range, the medicinal plant is effective to treat common cold and influenza-like

illness. As the dilution stretches beyond dilution 1/16 to dilution 1/512, the medicinal plant loses its effectiveness subsequently.

CONCLUSION

In conclusion, the aim of the research study has been achieved successfully. The study investigated the medicinal properties of the triple-combination therapy concoction of *Artemisia afra, Ruta graveolens* and *Sutherlandia frutescens* in vitro. More specifically, study sought to achieve the following objectives, which were subsequently achieved:

- Testing of the antiviral properties of *Artemisia afra, Ruta graveolens* and *Sutherlandia frutescens* decoction singularly;
- Testing of the antiviral properties of the triple-combination therapy concoction and determination of the 50% reduction of the neuraminidase activity; and
- The inhibitory concentration (IC_{50}) of each medicinal plants decoction and of the triple-combination concoction.

Adding to that, the results of the in vitro assay study affirms the medicinal knowledge of the indigenous *KhoiSan* community who were using medicinal plants for different conditions in more than thousand years. They used them for several purposes, including the infectious illnesses. Therefore, the results of the study are validation to the Western health system that African indigenous health practices is also scientific knowledge that should be respected and acknowledged, and most importantly, be integrated with the Western health system to research some of the existing incurable diseases.

Finally, the findings of the in vitro assay confirmed these medicinal plants the indigenous *KhoiSan* community use as the triple-combination therapy have antiviral properties against the influenza (flu) virus. It has been confirmed that these medicinal plants are capable of inhibiting 50% and more of the neuraminidase activity, both singularly as decoction and in triple-combination concoction and as the IC_{50} of these medicinal plants decoction and the triple-combination concoction are reported.

The three objectives for this research were successfully accomplished and the null hypothesis is refuted as depicted by the process of discussion. Furthermore, the outcome of the research concluded that the triple combination indeed have antiviral properties as proved in the in vitro part of this research. This is a breakthrough for the indigenous community because this is a confirmation for what they believed for the past centuries and used it unabatedly. On the other hand, the outcome of this research validates the results for the Western science community convincing them to gain more respect for the indigenous sciences in a western context. This further proves the fact that science

applied in context has a probability of truth, whether it is indigenous or western. This illuminates the need for co-existence of indigenous and western health care practices.

Concurrent to the outcome is the ground-breaking realization that by closer examination of the IC_{50}, the value of the triple-combination therapy is substantially lower than the value of the sum of the mono therapy of the different medicinal plants combined. Therefore, the synergy of the triple combination is higher. This emphasizes a gestalt effect in the western health context, but above it all the holism in an African health context, which personifies *Ubuntu* in medicine. Plants are seen as equal with animals and human beings and therefore viewing it from that perspective, the one plant in synergy with the other is because of the other plants in health.

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SECTION 3
CONTRIBUTION, RECOMMENDATIONS AND CONCLUSION OF THIS RESEARCH

3.1 INTRODUCTION

This section discusses the contributions that this research has made to the body of knowledge in health sciences with regards to indigenous medicinal plants use, especially the integration of the African indigenous health practice and the Western health practice. As the research composed of two phases (one and two), the contributions are tabled in these phases: the qualitative phase one and quantitative phase two. This section further outlines the recommendations and the conclusion about this research study.

3.2 CONTRIBUTIONS FROM THE QUALITATIVE PHASE ONE.

3.2.1 Contribution in harvesting and conservation

The community practice sterility (cleanliness) to promote health and the following is considered:

3.2.1.1 Who is allowed to harvest?

- The person harvesting medicinal plants should be a person who gives respect and appreciation to the nature, cosmos and humanity. Therefore, spiritual cleanliness is observed.
- This person should be free from pollutants and taboos (e.g. women who menstruates, young men who are involved in irresponsible intercourse) as that reflects ‘cleanness’ to prepare medicine. For example, it is of the community belief and world-view that if the shadow of polluted person comes in contact with medicinal plants that will disturb the healing power embedded in that particular medicinal plant.
- Medicinal plants are preferably harvested by an indigenous adult community member.
- In case where an adult cannot, a responsible young person who is nominated by the Indigenous Community Health Practitioner Committee (ICHPC) can harvest medicinal plant.
- The harvesting person should be in the peaceful mood, communicating with the plants in a humility and respectful manner, that is, equating the medicinal plants with humans (personification).

3.2.1.2 Who is not allowed to harvest?

- The menstruating women are not supposed to harvest medicinal plants as they are considered impure on cultural belief.
- The menstruating girls and who are not married but sexually active are considered not ‘clean’; so, they are not allowed to prepare the indigenous medicine.
- The sexually active young man not married is considered not ‘clean’; so, they are not allowed to prepare the indigenous medicine.
- The young person who is considered not ‘clean’ or free from pollutants and taboos cannot harvest the medicinal plant or even to prepare the indigenous medicine.

3.2.1.3 Gratitude after harvesting

- After harvesting of the plant put the coin or the button as the way of thanking the plant or the nature for providing with that medicinal plant.

3.2.2 Protocol of harvesting

3.2.2.1 The season and time of the medicinal plants harvest

- The medicinal plants are harvested during the optimal season to ensure the production of the medicinal plants materials and medicinal plants components/constituents of the best possible quality.
- The medicinal plants are affected by the time, the day and by the changes that take place throughout the month in the season.
- The aboveground medicinal plants parts that are used for medicinal purpose in this research study are best harvested in spring and summer before or during flowering.
- The ideal time of the day for harvesting is in the morning before the sun has wilted the medicinal plants.
- Harvesting in the morning after the last day photosynthesis process completed as to obtain rich medicinal components of the plant.

3.2.2.2 The Indigenous Practice of conservation

- During the harvesting of these medicinal plants, the community is cautiously avoiding the unnecessary damage of the plants that might destroy these precious species that bring health to their life. The re-growing the medicinal plants is vital to them.
- It was also mentioned that they avoid using the top leaves with seeds and flowers and would rather harvest the bottom leaves of the medicinal plant. This consideration contributes to conservation of the medicinal plants.

- They plucked individual leaves instead of leaf striping to ensure sustainable harvesting of leaves.
- They use the knife-shaped animal horn to cut the needed plants parts of the plants, and after each harvest they clean the used equipment.
- Apart from that, the equipment used and when to harvest; for example, the best time for collection, that is, the season, date or the time of the day play a spiritual role in medicinal plants harvesting. The community took special care with the leaves and flowers which are more vulnerable to deterioration.
- The community appreciates and are thankful to nature which provides them with precious natural being medicinal plants.
- After harvesting of the medicinal plants, it is their belief that they should thank nature. They show gratitude and appreciation to the plants by placing precious ornaments near by the medicinal plants such as a coin or the button.

3.2.3 Measurement, Preparation and Storage

3.2.3.1 Measurement Protocol

- Different (Natural) standard unit or the fixed scale to measure the leaves, branches and aerials used.
- The amount of *Artemisia afra* (wildeals) and *Ruta graveolens* (wynruit) leaves used is equal as scaled on the bases of the eye look and length of the fingers.
- The leaves of the *Sutherlandia frutescens* (kankerbos) is the quarter measurement of the *Artemisia afra (*wildeals) and *Ruta graveolens* (wynruit)
- Specific way of measurement, using the hands or fingers of the indigenous health care user (for example, length of the index finger- ten leaves)

3.2.3.2 Dosage measurement of the prepared decoction.

- Take medicine of the cup scale 2 times a day for the mild flu.
- 3 times a day of cup medicine for severe flu.

3.2.3.3 Preparation of the medicinal plants

- The preparation of the triple-combination concoction of the *Artemisia afra, Ruta graveolens* and *Sutherlandia frutescens* as a unique medicinal plants combination that the community uses to manage or treat common cold and influenza-like illness follows very low cost effective preparation method.

- The preparation of the concoction of these three medicinal plants, the leaves and the aerials as the parts of these plants used for medicinal preparation were boiled together as decoction, then was transferred to its pre-sterilized bottle container. The longer the boiling time the more concentrated medicine become and the more strong medicine.

- The pot used for boiling the medicinal decoction is not closed.
- The medicine is prepared per intake intervals.
- Preparation is inexpensive, time-saving and easily manageable.
- Medicinal plants' leaves are boiled together.
- The longer the boiling time the more concentrated medicine become and the more strong medicine.
- Preparation is inexpensive, time-saving and easily manageable.

3.2.3.4 The storage of the medicinal plants

- Stored in a cool space in the house.
- Storage in low temperature

3.3 CONTRIBUTIONS FROM THE QUANTITATIVE PHASE TWO

In this part the medicinal properties of *Artemisia afra, sutherlandia frutescens, Ruta graveolens* as decoctions and the triple-combination of these medicinal plants as the concoction were tested for antiviral properties, using NA-XTD^{TM} Influenza Neuraminidase Assay Kit*.*

The medicinal neuraminidase inhibition activity assay was conducted to validate the usage of these medicinal plants by the indigenous *KhoiSan* community for the treatment of common cold and influenza-like illness. The following table *(Table 3.1)* represents the findings of this assay as the contribution of this research study to the knowledge and the utilization of the medicinal plants by the African indigenous health practices.

Table 3.1 IC50, the 50% reduction of the neuraminidase activity and the dilution of the medicinal plants investigated

MEDICINAL PLANTS	VIRUS CONTROL (VC) (RLU)	50% REDUCTION OF NA ACTIVITY (RLU)	IC_{50} (nM)	DILUTION IN WHICH 50% REDUCTION OF NA ACTIVITY OCCURRED
Artemisia afra decoction	26837.63	13418.81	11.9	1/16
Sutherlandia frutescens decoction	28549.13	14274.56	8.8	1/8
Ruta graveolens decoction	28027.13	14013.56	6.3	1/8
Triple-combination concoction	28724.13	14362.06	13.1	1/16

3.4 RECOMMENDATIONS

The recommendations are given to the primary health care providers, the health science practitioners and to the indigenous community researchers.

3.4.1 Recommendations to the primary health care providers

- The probability of efficacy of the combination medicinal plant has been proven both through African indigenous and Western sciences in this research. Therefore, primary health care providers where the indigenous health practices plays the vital role, should acknowledge and respect the indigenous health practices as it has been proven to be effective;
- Furthermore, this research confirms that primary health care providers need to familiarize themselves with the indigenous health practices within the community they serve as this is important for the benefit of the people who would like to use both health systems;

- Considering the indigenous methods, corroborated by the in vitro study results of this research, the researcher recommends co-existence of the African indigenous health practices and the Western health practices on the primary health care level; and
- The primary health care providers should refrain from a discouraging attitude to the indigenous health practices as the results of this research validated the effectiveness of a combination therapy against influenza virus.

3.4.2 Recommendations to the health science education practitioners

- The researcher recommends that the African indigenous health practices be included in the curriculum of the health science practitioners as this will help them to understand African indigenous health practices better; and
- The researcher recommends that the health science practitioners not only study the conventional medicine but to study also African medicinal plants system as both of these medicinal systems exist and play a major role in facilitating a healthy community.

3.4.3 Recommendations to the indigenous community researchers

- The researcher recommends that the indigenous community based researchers build the rapport with community in which the research is taking place as this is the best approach to acquire more information from the community;
- The indigenous community based researcher should work hand in hand with community throughout the research study and give feedback to the community; and
- The indigenous community based researcher should conduct the research for the benefit of the community.
- The researcher further recommends that the community should be assisted to develop natural plants medicine for the public health and broader beneficiancy, e.g. usage and income generation.

3.4.4 Recommendations to Primal (indigenous) health care practitioners

- Primal health care practitioners should form partnerships with Western health practitioners and Western science practitioners to promote their practice;
- These practitioners should form lobby groups to advocate for this health care practice to be revitalized and acknowledged;
- Primal health care practitioners and other indigenous healing practitioners should form a community of practice to inform other health practitioners about their practice and also to stay abreast and competent in their own practices

3.5 CONCLUSIONS FOR THE RESEARCH

In conclusion, the research objectives of the study are reiterated:

- Exploration of indigenous medicinal plants formulation (harvesting, preparation) and the indigenous health practice of the *KhoiSan* indigenous health practitioners/healers with regard to the use of triple-combination therapy for the treatment of common cold and influenza-like illness;
- The investigation of antiviral properties of decoctions and concoction prepared from the medicinal plants used as the triple-combination therapy for the treatment of common cold and influenza-like illness *in vitro*; and
- Confirm with the community the findings of the second objective and validate these findings in the western health context and promote the co-existence of African indigenous knowledge system and the Western health system through the findings of this research study by making recommendations in learning and teaching of the health science practitioners have been achieved successfully.

Furthermore, the findings of the *in vitro* assay confirmed that the medicinal plants the indigenous *KhoiSan* community using as the triple-combination therapy do have antiviral properties against the influenza (flu) virus as these medicinal plants singularly as decoction and in triple-combination concoction are capable of inhibiting 50% and more of the neuraminidase activity, and as the IC_{50} of these medicinal plants decoction and the triple-combination concoction are reported.

ANNEXURE A

THE GRIQUA ROYAL HOUSE
DIE GRIEKWA KONINGLIKE-HUIS

Administration Office: c/o 26th & Baardman Str. Faith Mission Centre Leonsdale Elsies River 7490 PO Box 316 Elsies River 7480
Tel: 021 932 9901 Fax: 021 931 9400 Email: adamkokv@polka.co.za

MEMORANDUM OF UNDERSTANDING

This memorandum serves as an agreement reached between the Seboka Team under the leadership of Prof. Abel J. Pienaar and the Griqua Royal House. An appointed Seboka member will be expected to abide and respect the values and norms of the community while conducting research.

The following are the terms and conditions of agreement.

- The Seboka team is given the permission to conduct the research within the Griqua community, on a mutual capacity principle;
- The conducted research will be based on the Indigenous Knowledge Systems, of which the community will lead and guide the proceedings;
- The team (Seboka) will conduct the research using multiple research methodologies but the primary approach will be conducting "makgotla" with the assistance of a nominated member of the Griqua Royal House;
- The Seboka team will always respect the community and make sure that all the information is treated confidential;
- The research will be conducted by the appointed Seboka researcher, who will in turn sign the consent;
- Both parties also agree that the shared information remains the property of the Griqua Royal House unless otherwise stated;

This memorandum will be used as a global consent for conducting the research in the community. The participants' rights will also be taken into consideration and be respected while conducting the research. These rights are but not limited to the following:

- Autonomy and self-identification
- Privacy
- Confidentiality
- Justice
- Non-maleficence
- Voluntary participation
- Freedom of speech and movement as it will be an open forum

These conditions were discussed and agreed upon by the two parties (Community leader and Seboka Team leader). The terms and conditions discussed above are legal and bonding to the parties.

Signed at Campbell on 10th of January 20.15

Seboka team leader : .....

Griqua Royal House : .....

Researcher : .....

Copies to: Griqua Royal House, Seboka office and researcher

His Majesty King Adam Kok v
His Excellency High Commissioner A.M.W Messeliar

ANNEXURE B

"Ditau di senang seboka di siiwa ke none e tlhotsa"(A lion that goes on a hunt by itself, without co-existing in a pride, will always fail to catch even a limping deer)

CODE OF CONDUCT FOR SEBOKA MEMBERS

1. Purpose of the code of conduct

The code of conduct is a set of values, principles and practices that forms the foundation of the SEBOKA PROJECT. These principles depict the professional integrity, relationships with others, as well as the responsibility and accountability of Seboka Team Members towards each other and the communities they serve. The code of conduct is underpinned by the principle of UBUNTU.

The Code of Conduct governs how Seboka members behave in public or in private, or whenever the project will be judged by the actions of its members. The Seboka code of conduct is expected to be honored by everyone who represents the project officially or informally, claims affiliation with the project, or participates directly in the project.

2. Definitions

Professional Integrity- The Seboka member will act with care and diligence and make decisions that are honest, factual, fair, impartial and timely, after considering all relevant information (Harvard online dictionary, 2013) and (Online Dictionary, 2013).

Relationship with others- Means to treat other people with respect, courtesy and sensitivity, whilst recognizing their beliefs, interest, rights, safety and welfare.

Respect- is defined as consideration for self and for others. Respect includes consideration for other people's privacy, their physical space and belongings; as well as respect for different viewpoints, philosophies, physical ability, beliefs and personality (Balovich, 2006).

Accountability- This is to use information and resources in a responsible and accountable mannerthat ensures the efficient, effective and appropriate use (Online Dictionary, 2013).

Ubuntu-A quality that includes the essential human virtues; compassion and humanity (Online Dictionary, 2013).

3. Code of Conduct for Individuals:

The Seboka team expects that members will be truthful and forthright, and not engage in behavior that has serious ramifications for the welfare, academic well-being or professional obligations, of Seboka or others.

Be considerate

Decisions taken by any student or supervisor in Seboka might affect fellow members, therefore any decision taken within Seboka should be dealt with in consideration of how it

1

"Ditau di senang seboka di siiwa ke none e tlhotsa"(A lion that goes on a hunt by itself, without co-existing in a pride, will always fail to catch even a limping deer)

would affect supervisors and students, and we should consider them when making decisions.

Be respectful

Disagreement is no excuse for acting in a disrespectful manner. Seboka members will work together to resolve conflict, act in good faith and do their best to act in an empathic fashion. Seboka members will not allow frustration to turn into a personal attack on any other team member.

Take responsibility for our words and our actions

To err is human; when we do, we take responsibility for our mistakes. If someone has been harmed or offended, we listen carefully and respectfully, and work to right the wrong.

Be collaborative

What Seboka will produce is a complex whole made of many parts, it is the sum of many dreams. Collaboration between members who each have their own goal and vision is essential; for the whole to be more than the sum of its parts, each Seboka member must make an effort to understand the whole.

Collaboration improves the quality of our work. Internally and externally, we celebrate good collaboration.

Value decisiveness, clarity and consensus

Disagreements are normal, but we do not allow them to persist and fester, resulting inleaving Seboka members uncertain of the agreed direction.

Seboka members will aim to resolve disagreements constructively. When they cannot, the matter the matter will be escalated to Seboka leaders to arbitrate and provide clarity and direction.

Ask for help when unsure

Nobody is expected to be perfect in this community. Asking questions early avoids many problems later, so questions are encouraged, though they may be directed to the appropriate forum. Those who are asked should be responsive and helpful.

Step down considerately

When somebody leaves or disengages from the Seboka project, it is expected that they do so in a way that minimizes disruption to the project.

Conflicts of interest

It is expected that Leaders and Students in Seboka be aware when they are conflicted due to employment or other projects they are involved in, and abstain or delegate decisions that

2

may be seen to be self-interested. It is expect that everyone who participates in the Seboka project does so with the goal of making life better for its users and the community, whom Seboka serves.

When in doubt, ask for a second opinion. Perceived conflicts of interest are important to address; as a leader.

Credit

Individual Seboka members do not seek the limelight, but celebrates team members for the work they do, with specific consideration of the community.

4. Code of Conduct for Leadership/supervisors and authority

Responsibility for the project starts with the project leader, who may delegate specific responsibilities to Seboka members. The Project leader may delegate decision making, governance and leadership from senior bodies to the most able and engaged candidates in Seboka.

A leader's foremost goal is the success of the team.

Leaders may be more visible than members of the team, leaders will use that visibility to highlight the great work of Seboka as a team.

Leaders must act to ensure that decisions are credible even if they must occasionally be unpopular, difficult or favourable to the interests of the Seboka project over an individual member.

Supervisors will guide students without prejudice, and will respect the input of the student.

Feedback from supervisors to students will be given within a timeframe agreed upon between student and supervisor.

Feedback given will be dealt with in a respectful manner to enhance the academic growth of the student, and to empower the student.

5. Conduct in service to communities

Seboka will foster collaboration between groups with very different needs, interests and skills.

Seboka will strive to ensure that diverse groups collaborate to mutual advantage and benefits.

Seboka will challenge prejudice that could jeopardise the participation of any person in the project or during the engagement between any members and the communities which Seboka serves.

A.M

"Ditau di senang seboka di siiwa ke none e tlhotsa"(A lion that goes on a hunt by itself, without co-existing in a pride, will always fail to catch even a limping deer)

No Seboka member will engage in any activity which is not to the benefit of the communities which Seboka serves.

6. **Code of Academic conduct for Seboka members**

Absolute integrity is expected of every Seboka member in all academic undertakings.

Integrity entails a firm adherence to a set of values, and the values most essential to an academic community such as Seboka are grounded on the concept of honesty with respect to the intellectual efforts of oneself and others.

Academic integrity is expected not only in formal academic situations, but in all relationships and interactions connected to the educational process, including the use of Intellectual property and knowledge of the communities we serve.

All knowledge from the communities and fellow Seboka members should be acknowledged, and the Seboka members will truthfully report it at all times.

Intellectual property generated in Seboka remains the property of Seboka.

The Seboka logo and all trademarks associated with Seboka remains the property of Seboka, and may not be used without the permission of the Project leader and Senior project managers, without the necessary approval.

This Code is not exhaustive or complete. It is not a rulebook; it serves to distill our common understanding of a collaborative, shared environment and goals. The Seboka team expects it to be followed in spirit as much as in the letter.

I, TEBOHO MOSES TAAKA ..... (Full Name and Surname) endeavours to uphold the above mentioned values, principles and practices as a member of the Seboka team.

.....

Signature (Student)

Date: 10 JAN 2015

.....

Project leader/Supervisor

Date: 10 Jan 2015

A.M 4 T

ANNEXURE C

NORTH-WEST UNIVERSITY
YUNIBESITI YA BOKONE-BOPHIRIMA
NOORDWES-UNIVERSITEIT

Private Bag X6001, Potchefstroom
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Institutional Research Ethics Regulatory Committee
Tel +27 18 299 4849
Email Ethics@nwu.ac.za

ETHICS APPROVAL CERTIFICATE OF PROJECT

Based on approval by the **Health Science Ethics Committee (FAST)**, the North-West University Institutional Research Ethics Regulatory Committee (NWU-IRERC) hereby approves your project as indicated below. This implies that the NWU-IRERC grants its permission that, provided the special conditions specified below are met and pending any other authorisation that may be necessary, the project may be initiated, using the ethics number below.

Project title: Medical properties of plants used as triple-combination therapy by indigenous Africans for common cold Nursing Sciences.

Project Leader: Prof AJ Pienaar
Student: TM Taaka

Ethics number: N W U - 0 0 4 8 6 - 1 5 - A 9
(Institution | Project Number | Year | Status)

Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation

Approval date: 2016-04-01 **Expiry date**: 2018-03-10 **Category** N/A

Special conditions of the approval (if any): None

General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, please note the following:

- *The project leader (principle investigator) must report in the prescribed format to the NWU-IRERC:*
  - *annually (or as otherwise requested) on the progress of the project,*
  - *without any delay in case of any adverse event (or any matter that interrupts sound ethical principles) during the course of the project.*
- *The approval applies strictly to the protocol as stipulated in the application form. Would any changes to the protocol be deemed necessary during the course of the project, the project leader must apply for approval of these changes at the NWU-RERC. Would there be deviated from the project protocol without the necessary approval of such changes, the ethics approval is immediately and automatically forfeited.*
- *The date of approval indicates the first date that the project may be started. Would the project have to continue after the expiry date, a new application must be made to the NWU-IRERC and new approval received before or on the expiry date.*
- *In the interest of ethical responsibility the NWU-IRERC retains the right to:*
  - *request access to any information or data at any time during the course or after completion of the project;*
  - *withdraw or postpone approval if:*
    - *any unethical principles or practices of the project are revealed or suspected,*
    - *it becomes apparent that any relevant information was withheld from the NWU-IRERC or that information has been false or misrepresented,*
    - *the required annual report and reporting of adverse events was not done timely and accurately,*
    - *new institutional rules, national legislation or international conventions deem it necessary.*

The IRERC would like to remain at your service as scientist and researcher, and wishes you well with your project. Please do not hesitate to contact the IRERC for any further enquiries or requests for assistance.

Yours sincerely

Prof LA Du Plessis

Digitally signed by Prof LA Du Plessis
DN: cn=Prof LA Du Plessis, o=North-West University, ou=Campus Rector, email=Linda.DuPlessis@nwu.ac.za, c=ZA
Date: 2016.03.04 08:46:59 +02'00'

Prof Linda du Plessis
Chair NWU Institutional Research Ethics Regulatory Committee (IRERC)

ANNEXURE D

INFORMED CONSENT TO PARTICIPATE IN THE RESEARCH STUDY

Title of the research project:

Medicinal properties of the plants used as triple-combination therapy by indigenous African for common cold.

The medicinal men of the community as nominated according to the community protocol are invited to participate in this research study. The researcher is interested in asking the medicinal men of the community about how they harvest, measure and prepare medicinal decoction out of these three medicinal plants (wildeal, wynruit and kankerbos/willekeur) for the management of common cold condition.

The meeting with community medicinal men will follow the community meetings protocol.

Purpose of the study

The purpose is to investigate how the community prepare their medicine for the management of ailments, how the community use their own indigenous health approaches or practices to manage common cold. This is to:

Confirm with community about the medicinal properties of this triple combination therapy after the in vitro test of this decoction.

Validate the findings after in vitro study results to the Western health practices why the community use these medicinal plants to manage common cold.

I read the foregoing information, or it was read to me. I had the opportunity to ask questions about it and any questions that I had were answered to my satisfaction. I consent voluntarily to participate as a participant in this research.

Print name of participant: _____

Signature of participant: _____ Date: _____

ANNEXURE E

(ENGLISH AND AFRIKAANS)

CLINICAL ETHNOGRAPHY- SEMI-STRUCTURED INTERVIEW SCHEDULE

ARTEMISIA AFRA (WILDEALS)

SEMI-STRUCTURED QUESTIONS/ SEMI-GESTRUKTUREERDE VRAE

A. HARVESTING METHOD

1. What part of the wildeals medicinal plant used in the combination for the management of common cold? Wattergedeelte van die wildeals plant gebruik u in die medisynemengselvir die hantering van verkoue?

……
……

i. Why? Hoekom?

……
……

2. How is the wildeals medicinal plant harvested? Hoe oes/pluk u die wildeals plant?

……
……

ii. Why? Hoekom?

……
……

3. What time of the year? Wattertydvan diejaar?

……
……

iii. Why? Hoekom?

……
……

4. What time of the day? Watter tyd van die dag?

……
……

iv. Why? Hoekom?

……
……

5. What kind of harvesting tool is used? Watter tipe oes-aparaat word gebruik?

……
……

v. Why? Hoekom?

……
……

6. Where is this medicinal plant harvested (from the veld or from the community medicinal gardens)? Waaroes u die plant? (in die veld of in die gemeenskaps-medisynetuin)

……
……

vi. Why? Hoekom?

……
……

RUTA GRAVEOLENS (WYNRUIT)

SEMI-STRUCTURED QUESTIONS/ SEMI GESTRUKTUREERDE VRAE

A. HARVESTING METHOD

7. What part of the wynruit medicinal plant used in the combination for the management of common cold? Wattergedeelte van die wynruit plant gebruik u in die medisynemengselvir die hantering van verkoue?

……
……

vii. Why? Hoekom?

……
……

8. How is the wynruit medicinal plant harvested? Hoe oes/pluk u die wynruit plant?

……
……

viii. Why? Hoekom?

……
……

9. What time of the year? Wattertydvan diejaar?

……
……

ix. Why? Hoekom?

……
……

10. What time of the day? Wattertydvan die dag?

……
……

x. Why? Hoekom?

……
……

11. What kind of harvesting tool is used? Wattertipeoes-aparaat word gebruik?

……
……

xi. Why? Hoekom?

……
……

12. Where is this medicinal plant harvested (from the veld or from the community medicinal gardens)? Waaroes u die plant? (in die veld of in die gemeenskaps-medisynetuin)

……
……

xii. Why? Hoekom?

……
……

SUTHERLANDIA FRUTESCENS (KANKERBOS) CANCER BUSH

QUESTIONS

A. HARVESTING METHOD

13. What part of the cancerbush medicinal plant used in the combination for the management of common cold? Wattergedeelte van die kankerbos plant gebruik u in die medisynemengselvir die hantering van verkoue?

……
……

xiii. Why? Hoekom?

……
……

14. How is the cancerbush medicinal plant harvested? Hoe oes/pluk u die kankerbos plant?

……
……

xiv. Why? Hoekom?

……
……

15. What time of the year? Wattertydvan diejaar?

……
……

xv. Why? Hoekom?

……
……

16. What time of the day? Wattertydvan die dag?

……
……

xvi. Why? Hoekom?

……
……

17. What kind of harvesting tool is used? Wattertipeoes-aparaat word gebruik?

……
……

xvii. Why? Hoekom?

……
……

18. Where is this medicinal plant harvested (from the veld or from the community medicinal gardens)? Waarroes u die plant? (in die veld of in die gemeenskaps-medisynetuin)

……
……

xviii. Why? Hoekom?

……
……

B. MEASUREMENT OF THIS MEDICINAL PLANT AS USED IN COMBINATION FOR THE MANAGEMENT OF COMMON COLD.

ARTEMISIA AFRA (WILDEALS)

QUESTIONS

1. What is the tool used to measure this medicinal plant for the preparation of medicinal decoction? Watterapparaatgebruik u om die hoeveelheid van die plant vir die voorbereiding van medisynetebepaal?

……
……

a. Why? Hoekom?

……
……

RUTA GRAVEOLENS (WYNRUIT)

1. What is the tool used to measure this medicinal plant for the preparation of medicinal decoction? Watterapparaatgebruik u om die hoeveelheid van die plant vir die voorbereiding van medisynetebepaal?

……
……

i. Why? Hoekom?

……
……

SUTHERLANDIA FRUTESCENS (KANKERBOS)

1. What is the tool used to measure this medicinal plant for the preparation of medicinal decoction? Watterapparaatgebruik u om die hoeveelheid van die plant vir die voorbereiding van medisynetebepaal?

……
……

i. Why? Hoekom?

……
……

C. PREPARATION OF TRIPLE COMBINATION OF THESE MEDICINAL PLANTS DECOCTION

QUESTIONS

1. How is this medicinal plants decoction prepared? Hoe berei u die medisynemengselvoor?

……
……

2. Do these medicinal plants used as fresh or first dried up? Gebruik u die plant vars of gedroogd?

……
……

3. If the medicinal plants are dried first, what is the drying method applied? As u die medisyneplantedroog, watter proses gebruik u vir die droging?

……
……

a) Why? Hoekom?

……
……

D. THE STORAGE OF THE COMPLETE PREPARED MEDICINAL DECOCTION.

QUESTIONS

1. After the preparation is ready- Where does the medicine be stored and why? Na dievoorbereiding van die mengsel- Waarstoor u die medisyne en hoekom?

……
……

2. How long can the medicine be stored and still in good condition to be still used? Hoe lank kan u die medisynemengselstoor en ditstil in ‘n goeietoestandvirgebruik is?

……
……

E. ADMINISTRATION OF THE MEDICINE

QUESTIONS

1. How is the medicine taken and how times per day? Hoe neem u die medisynemengsel en hoeveelkeer per dag?

……
……

ANNEXURE F

EDITING AND PROOFREADING CERTIFICATE

7542 Galangal Street

Lotus Gardens

Pretoria

0008

05 December 2016

TO WHOM IT MAY CONCERN

This letter serves to confirm that I have edited and proofread Mr T.M. Taaka's dissertation entitled: **"MEDICINAL PROPERTIES OF TRIPLE-COMBINATION PLANTS USED BY INDIGENOUS KHOISAN COMMUNITY FOR COMMON COLD AND INFLUENZA-LIKE ILLNESS."**

I found the work easy and enjoyable to read. Much of my editing basically dealt with obstructionist technical aspects of language which could have otherwise compromised smooth reading as well as the sense of the information being conveyed. I hope that the work will be found to be of an acceptable standard. I am a member of Professional Editors Guild and also a Language Editor at Bureau of Market Research at the University of South Africa.

Hereunder are my particulars:

Jack Chokwe (Mr)

Bureau of Market Research (Unisa)

Contact numbers: 072 214 5489 / 012 429 3327

jmb@executivemail.co.za

Professional EDITORS Guild

ANNEXURE G

MEMO

Centre for Respiratory Diseases and Meningitis (CRDM)
1 Modderfontein Road, Sandringham

To: University of North West

Date: **07 October 2016**

From: **Dr Florette K Treurnicht**

RE: Mr Taaka's training

Dear Sir/ Madam,

Herewith I would like to confirm that Mr Teboho Taaka spend time from the 03 October 2016 to 07 October 2016 at the National Institute for Communicable Diseases where he was trained on the NA-XTD neuraminidase assay (from Applied Biosystems). He also used this assay to determine if various water extracts of medicinal plants can inhibit submitted neuraminidase activity of inactivated influenza A viruses. He learned quickly and the last 2 days had worked independently when performing the assays.

It was a pleasure having him in our laboratory and any further questions you may have may be addressed to me at any time.

Kind regards,

Florette K Treurnicht (PhD)
Senior Medical Scientist
Director for the National Influenza Centre

Cell. 0732875708