# A STUDY ON THE MEDICINAL POTENTIAL OF *ARTEMISIA AFRA*, A TRADITIONALLY USED HERB IN NAMIBIA

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**ABSTRACT**

The use of plants as medicines to relieve various symptoms, as well as to manage and cure ailments has been recorded since ancient times. Much interest in these ethnomedicinal plants stems from their long-standing use in traditional medicine. They are still regarded as making important contributions to health care even though great advances have been made in modern medicine. Traditionally, *Artemisia afra* ('Wilde als') is used to treat the common cold, flu and coughs, as well as nasal congestion. During the COVID-19 pandemic, communities in Namibia used 'Wilde als' as a treatment for the disease. Little is known about the efficacy and safety of 'Wilde als' found in Namibia. This study aimed to identify the bioactive classes of compounds for 'Wilde als'; as well as to determine the antioxidant, anti-inflammatory, and antiviral activities and the cytotoxic properties of the plant. Extracts of 'Wilde als' were prepared by aqueous and organic extraction methods, using water and a mixture of methanol and dichloromethane (1:1 v/v). The phytochemical profile was determined using thin-layer chromatography. The total flavonoid content within the extracts was also determined. The albumin denaturation assay was used for determining the *in vitro* anti-inflammatory response of the extracts of 'Wilde als' using egg albumin derived from a fresh hen's egg as the protein source. The DPPH antioxidant assay was used to determine the radical scavenging activity of the plant extracts using 2,2-diphenyl-1-picryl-hydrazyl-hydrate also known as DPPH, and the MTT cytotoxicity assay was used to determine the cellular reduction of the tetrazolium salt, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, also known as MTT. The Vero E6 cell line, a kidney epithelial cell line was used to determine cell proliferation and survival during the MTT assay. The *in vitro* antiviral effects of the plant extracts against a pseudovirus of SARS-CoV-2 were determined by calculating the reduction of the SAR-CoV-2 in

the Vero E6 cells. The extracts of the 'Wilde als' possessed flavonoids, coumarins, saponins, steroids and terpenoids, with TFC with the highest at 19.89 % for the organic extracts of the twigs. The extracts of *A. afra* exhibited significant antioxidant activities ranging from 0.1029 to 9.4197 µg/ml with the highest activity observed for the aqueous extract of the leaves. The extracts of *A. afra* also exhibited significant anti-inflammatory activity. The highest anti-inflammatory activity of the plant extracts was observed for the organic extract from the combination of the leaves and twigs (87.5 % at 100 µg/ml). The plant extracts displayed antiviral potential against the *beta*-strain of the SARS-CoV 2 virus, with the organic extracts exhibiting higher inhibition of the viral load with $EC_{50}$ values of 48.84 µg/ml (leaf extracts), 24.65 µg/ml (twig extracts) and 251.67 µg/ml (combination of the leaves and twigs extracts). The results of the MTT cytotoxicity assay indicated that the aqueous extracts showed little to no cytotoxicity; the organic extracts on the other hand showed high cytotoxicity with $IC_{50}$ values of 134.12 µg/ml (leaves and twigs), followed by 151.53 µg/ml (twigs) and 182.31 µg/ml (leaves). The findings of this study suggest that 'Wilde als' has the potential to be used as an antiviral agent with antioxidant and anti-inflammatory activities as the possible mechanisms of action, with low cytotoxic effects for the aqueous extracts. The organic extracts, however, were cytotoxic, but when used in the correct doses, they can have significant health benefits. This study also provides evidence that the use of 'Wilde als' an herbal remedy in the south of Namibia is rational and consistent with research from other regions of Africa.

Keywords: Medicinal plants, 'Wilde als', *Artemisia afra*, traditional medicine antioxidant, anti-inflammatory, antiviral, COVID-19, SARS-CoV-2, viral infections

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## LIST OF ABBREVIATIONS AND ACRONYMS

*ADMET:* Adsorption, Distribution, Metabolism, Excretion, and Toxicity

*AIKS:* African Indigenous Knowledge Systems

*ATL:* Adult T-cell Leukemia

*ATM:* African Traditional Medicine

*BDE:* Bond Dissociating Energy

*BSC:* Biosafety Cabinet

*DNA:* Deoxyribonucleic acid

*DMEM:* Dulbecco's Modified Eagle Medium

*DMEM-PS:* Dulbecco's modified eagle medium-penicillin streptomycin

*DPPH:* 2,2-diphenyl-1-picryl-hydrazyl-hydrate

*DUI:* Doing- using- interaction

*IKS:* Indigenous Knowledge Systems

*IS:* Innovation System

*MERS:* Middle East Respiratory Syndrome

*MTT:* 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide.

*NBRI:* National Botanical Research Institute

*NIAID:* National Institute of Allergy and Infectious Diseases

*PBS:* Phosphate Buffer Saline

*PEP:* Post-exposure Prophylaxis

*RLU:* Reactive Light Unit.

*RNA:* Ribonucleic acid

*ROS:* Reactive Oxygen Species

*TFC:* Total Flavonoid Content

*SARS*: Severe Acute Respiratory Syndrome

*STI:* Science, Technology and Innovation

*TGF-β:* Transforming Growth Factor-Beta

*WHO*: World Health Organization

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**DEDICATION**

This thesis is dedicated to my late father, whose guidance and support have helped me remain focused on my studies.

**DECLARATION**

I, Wennyth Farmer, declare hereby that this study is a true reflection of my own research, and that this work, or part thereof has not been submitted for a degree in any other institution of higher education.



W.L.Farmer…… Date October 2024……

Wennyth Luzaann Farmer

# CHAPTER ONE: INTRODUCTION

## 1.1 Background of the study

African traditional medicine refers to medicine which is based on traditional knowledge (Sifuna, 2022). Generally, this information is passed down within specific indigenous communities, usually along apprenticeships or familial lines, typically by word of mouth from generation to generation (Sifuna, 2022). The use of medicinal plants is reported to be one of the oldest of the world's medicinal systems and has existed far longer than the advent of modern medicine. Their use dates to the late 1600s as vital ingredients in medicines used in battling diseases and infections (Sifuna, 2022). What is interesting is that the African continent has a rich diversity of medicinal plants (Kuete, Karaosmanoglu and Sivas, 2017). In Africa, the earliest recorded use of traditional plants is the use of the sweet wormwood plant, also known as *Artemisia annua* (WHO, 2023), from the genus *Artemisia* which consists of approximately 500 diverse species (Liu *et al.*, 2010).

*Artemisia afra*, another species, is considered one of the most widely used herbal remedies in southern Africa and is regarded as a "cure-all" remedy (Kriel, 2010). Its reported uses are to treat or alleviate conditions such as coughs, influenza, colds, and malaria, among others (van Wyk, 2008). The leaves and stems of *A. afra*, either fresh or dried, are used to prepare tonics that are consumed in the form of teas, tinctures, and decoctions. In addition, the steam and fumes produced from brewing the plant material may be inhaled to clear asthma, hay fever, sinusitis, and headaches. In Namibia, *A. afra* is used by communities in the Hardap region to treat colds, flu, and more recently, COVID-19, especially during the pandemic.

The long-term use of *A. afra*, and its popularity has led to its successful commercialization in the pharmaceutical industry (Shahzadi *et al.,* 2020). The plant's application as a treatment for various ailments highlights its potential as an antiviral and immune-boosting agent. *In vivo* and *in vitro* studies have identified numerous phytochemicals including coumarins, terpenoids, sterols, caffeoylquinic acids, and flavonoids within numerous species of *Artemisia* with varied pharmacological activities, such as antimalarial, anti-inflammatory, and immune-modulating activities (Shahzadi *et al.,* 2020). Various studies have also indicated that the extracts from different Artemisia species are active against influenza, also known as the common flu (Gilmore *et al*., 2020; Nair *et al*., 2021). To our knowledge, little to no scientific evidence is available for *A. afra* ('Wilde als') found and used in Namibia.

During the COVID-19 pandemic, approximately 770,778,396 people died after contracting the SARS-CoV-2 virus, revealing the significant impact of viral infections on human health (WHO, 2022). Despite significant progress in understanding, preventing, and treating viral infectious diseases, major viral diseases such as HIV/AIDS, measles, rabies, yellow fever, dengue fever, and hepatitis continue to emerge (Armitage *et al*., 2021). According to Chauhan *et al.* (2020), Africa faces a high risk of animal- and vector-borne viral infections. This is caused by the tropical climate, severe poverty, limited resources, lack of knowledge, and traditional cultural practices (Armitage *et al.*, 2021).

Viral infections often trigger an inflammatory response in the body, which can be life-threatening. Individuals infected with the H5N1 virus showed increased levels of pro-

inflammatory cytokines including Interleukin-6 (IL-6), Tumor necrosis factor alpha (TNF-α), Interferon-gamma (IFN-γ), Interferon gamma-induced protein 10 (IP-10), and Monocyte chemoattractant protein-1 (MCP-1). These cytokines cause an imbalanced inflammatory response which in turn causes sickness/pathogenesis (Faist *et al.*. 2022); and may alleviate or aggravate disease or have no impact at all, depending on the patient (Casanova and Abel 2021). When viral-induced inflammation becomes harmful to the host, controlling it can reduce viral pathogenesis (Chakravarty, Chakravarti, and Chattopadhyay, 2023). Furthermore, an increase in oxidation in infected individuals was also reported. Reactive oxygen species (ROS) play vital roles in various cellular processes, however, increased to levels which cannot be neutralized by the defense mechanisms can damage biological molecules, alter their functions, and also act as signaling molecules thus generating a spectrum of pathologies (Ivanov, *et al.*, 2016). In COVID-infected patients, coronaviruses cause an imbalance between increased production of reactive oxygen species (ROS) and reduced antioxidant host responses, leading to increased redox stress (Gain *et al.*, 2023). This ultimately leads to decreased host defenses against viruses and a rise in virally-induced inflammation and apoptosis, which cause damage to cells and tissues and eventually end-organ disease (Gain *et al.*, 2023). Therefore, it is essential to regulate inflammatory and oxidative responses for controlling viral infections and associated diseases.

The use of conventional medicine for viral infections can cause side effects such as dizziness, headaches, and stomach aches (Tabish, 2008). Mothibe and Sibanda (2019) further outlined that allopathic medicines can cause allergic reactions such as hives and difficulty breathing; in extreme cases, these reactions may even be fatal (Caughey

*et al.*, 2017; Ferner, 2014). Furthermore, communities in rural areas may not have access to or are not able to afford conventional medicines (Vellingiri, 2020) and choose to use alternative methods such as traditional medicine to address their health issues (van Andel and Carvalheiro, 2013). The World Health Organization (WHO) encourages the use of traditional medicine despite its limitations and challenges (Sifuna, 2022). The plants are mostly used anecdotally. Many lack scientific evidence, which is primarily attributed to the fact that these medicines are founded on trial and error or superstition and mysticism. As a result, there is a lack of scientific evidence for traditional African remedies in developing countries (Kuete, Karaosmanoglu and Sivas, 2017; Logiel *et al*., 2021). The aim of this study, therefore, was to validate the traditional use of *A. afra* as a possible treatment for viral infections including COVID-19.

### 1.2 Statement of the problem

The impact of viral infections on health and the economy is significant, as seen during the COVID-19 pandemic. Moreover, conventional medicine has been reported to have more side effects compared to traditional medicines (Sifuna, 2022). These side effects range from dizziness to headaches, and stomach aches (Tabish, 2008), and may induce allergic reactions such as hives and difficulty breathing, and in extreme cases, these allergic reactions may lead to death. In COVID-infected patients, an increase redox stress has been observed (Gain *et al.*, 2023), which ultimately leads to decreased host defenses against viruses and a rise in virally induced inflammation and apoptosis, which cause damage to cells and tissues and eventually end-organ disease (Gain *et al.*, 2023). Therefore, it is essential to regulate inflammatory and oxidative responses for

controlling viral infections and associated diseases. According to Logiel *et al.* (2021) and the World Health Organization (2023), there are currently there are no effective vaccines for viral infections available and there is a shortage of research being which investigates the use of medicinal plants to combat viral infections and related symptoms. Moreover, scientific evidence on the safety and efficacy of the therapeutic applications of plants in Namibia, specifically *A. afra* ('Wilde als'), is not known.

### 1.3 Objectives of the study

The overall objective of the study was to determine the antiviral potential of *Artemisia afra* ('Wilde als') found in the Khomas region of Namibia. Hence, the specific objectives of the study were to:

1.3.1 To identify classes of phytochemical compounds with antiviral properties (i.e., flavonoids, terpenoids, and coumarins).

1.3.2 To identify the mechanism of action by screening the extracts for antioxidant properties.

1.3.3 To identify the mechanism of action by screening the extracts for anti-inflammatory activity.

1.3.4 To determine the antiviral activity using a simple rapid antiviral bioassay.

1.3.5 To determine the harmful effects of the plant extracts by screening them for cytotoxicity.

### 1.4 Hypothesis of the Study

The proposed hypothesis of this study was that *A. afra* can inhibit the growth of viruses such as the SARS-CoV 2 strand, due to its anti-inflammatory and antioxidant

properties as it is traditionally used to treat coughs, the flu, common cold, and sore throat. Additionally, it is hypothesized that *A. afra* is not toxic *in vitro*.

### 1.5 Significance of the Study

Overall, the finding of the study can create awareness of the use of *Artemisia afra* which can result in more people using it, and as a result, improving their health, boosting the immune system, and reducing morbidity and the mortality rate of viral-related diseases for the country. Additionally, further research on the medicinal value of *A. afra* can lead the pathway to drug discovery and eventually drug development. There is a potential to develop this plant on a large commercial scale which may create employment opportunities within these rural communities to aid in harvesting *A. afra*, drying the plant material, and packaging the final product.

### 1.6 Limitations of the Study

Environmental factors such as water availability and weather conditions may affect the presence of bioactive secondary metabolites in the plants. This may influence the antiviral and anti-inflammatory potential of the plant samples obtained. This plant was harvested in a sandy and dry environment in the Khomas region of Namibia where it grows naturally.

### 1.7 Delimitations of the Study

The *A. afra* was acquired from a single site to assess its potential as an antiviral. Plants growing in different geographical locations may produce varying secondary

metabolites and different levels of activity might be observed for the same species growing in different parts of the country. Additionally, only certain assays were performed, such as thin-layer chromatography (TLC) for determining the bioactive compounds, DPPH assay to determine the antioxidant activity, albumin denaturation assay to determine the anti-inflammatory activity, and an inactive SARS-CoV 2 beta-strain to determine the antiviral activity of the ‘Wilde als’ plant extracts. For future studies, it is important to use additional assays to ascertain the plant's therapeutic properties such as immune-modulating assays and techniques such as HPLC and or LCMS to identify the specific biologically active compounds.

# CHAPTER TWO: LITERATURE REVIEW

Viral infections are among the leading causes of morbidity and mortality worldwide. The recent COVID-19 pandemic is a very good example. During the pandemic, people turned to herbal remedies as there was no effective available treatment for the disease. Traditional medicine in the form of herbal remedies has been used for centuries, but it requires scientific evidence to be incorporated into healthcare systems and used on a large scale.

## 2.1 Cause of viral infections

Viruses are infective agents that cause viral infections. They typically consist of a nucleic acid molecule, either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) (Figure 1), which may be encapsulated within a protein coat or a protein shell (Taylor, 2014). Before the invention of the electron microscope, viruses were impossible to visualize due to their size as most viruses range from 20-400 nanometers in diameter (Taylor, 2014). The very first viruses that were visualized are regarded today as bacteriophages, which were described to have the appearance of a head and a tail-like structure, and it was observed that the nucleic acid entered bacterial cell walls through the tail of the bacteriophage (Taylor, 2014). In comparison, animal viruses which were later discovered were noted as either spherical or rod-shaped and these viruses were able to bind to the receptors on the cell wall and were absorbed by the cell (Raoult *et al.*, 2004).

Although all viruses have a unity of structure, they are regarded as diverse when it comes to the diseases they may inflict and the organs they attack (Raoult *et al.*, 2004). Raoult *et al.* (2004) define their mode of replication as non-binary as their replication

occurs as a bursting of thousands of viral particles from a single virus over a short period. Usually, for other organisms such as bacterial cells, replication is defined as a binary process as one cell generally splits into two cells, the two cells split into four cells, and so forth. Therefore, Taylor (2014) explains that this mode of replication is what makes viruses so unique. Another characteristic that makes viruses so unique is the presence of RNA or DNA as genetic material within the viral cell, however, viruses do not possess mitochondria, ribosomes, or any other cell-like organelles, and are therefore regarded as parasitic as they depend on a living host cell for their survival and replication (Raoult *et al.*, 2004).

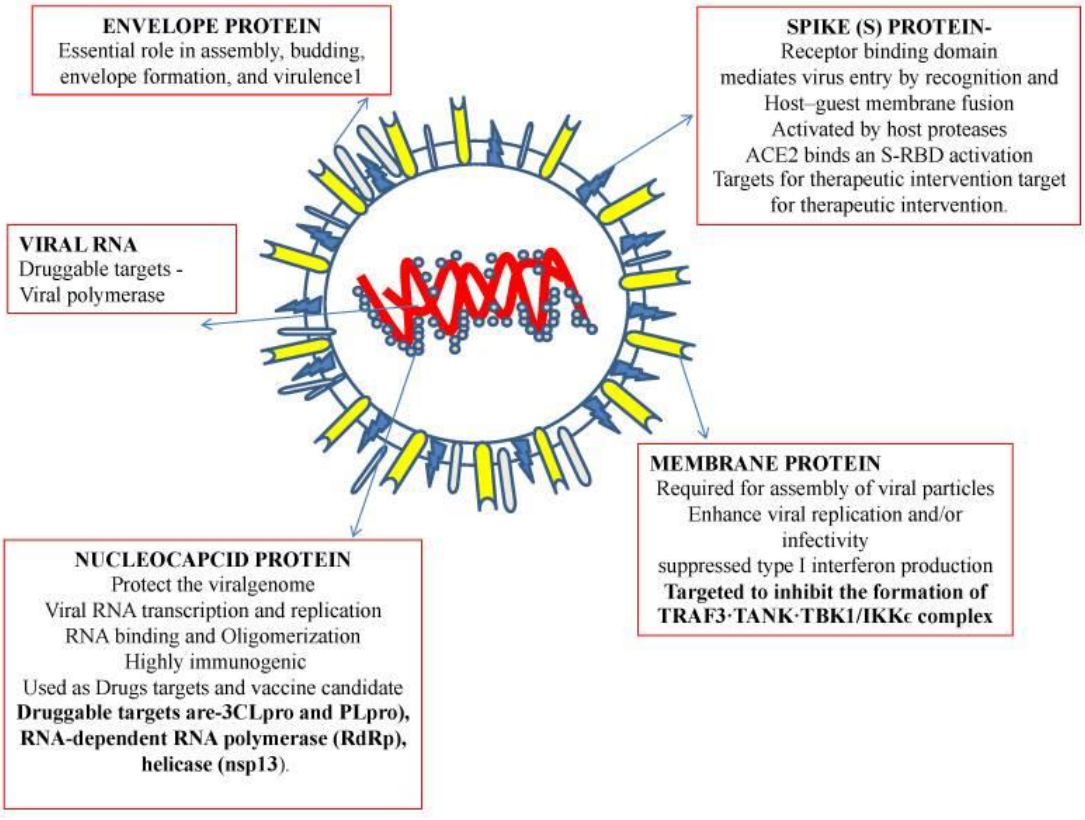


Figure 1: The structure of the coronavirus. Image downloaded from an article by Jamal (2022).

### 2.2 Symptoms of viral infections

Viruses may attack various organs and systems within the human body; thus, each viral infection and the resulting symptoms will differ. The symptoms that result from a viral infection often include chills, sore throat, fever, runny nose, nasal congestion, body aches and a persistent cough. These symptoms are short lasting and typically last for 3 days to a week according to Reta *et al.* (2020). There are several viruses which may cause different types of respiratory infections. According to Koonin, Krupovic and Agol (2021), the Baltimore Classification of viruses is a system that places viruses into one of seven groups depending on a combination of their nucleic acid (DNA or RNA), strand (single-stranded or double-stranded) and mode of replication. *Class I:* double-stranded DNA viruses consist of Adenoviruses, Herpesviruses, Poxviruses. Adenoviruses are 90- 100 nm in size and are non-enveloped icosahedral viruses. According to Houldcroft *et al.* (2018), adenoviruses are a collection of viruses which cause respiratory illnesses such as the common cold, croup, bronchitis, pneumonia and conjunctivitis.

Herpesviruses, also known as Herpes simplex virus (HSV), commonly known as herpes, is classified into two types, HSV-1 and HSV-2 and is primarily spread by skin-to-skin contact. According to Saleh, Yarrarapu and Sharma (2022) herpes is a liner dsDNA virus that belongs to the Alphaherpesviridae subfamily. HSV-1 spreads mostly via oral contact and may cause infections around or in the mouth area. HSV-2 is spread via sexual contact and is the main cause of genital herpes. Symptoms of herpes may include fever and swollen lymph nodes; the main indication of herpes is the presence of painful blisters or ulcers. As mentioned, another example of dsDNA viruses is Poxviruses. In a review by Efridi and Lappin (2022) Poxviruses were the first viruses

to be visualized using microscopy as the virus is made up by large and complex double-stranded DNA and can replicate and assemble itself within the cytoplasm of its host cell. Of the 28 known genera, 4 genera have been proven to infect humans, namely, *Orthopoxviridae, Parapoxviridae, Yatapoxviridae* and *Molluscipoxviridae.* The most widely known Poxvirus is the orthopox, a variola virus, which the main cause of smallpox. The main symptoms of smallpox are a sudden onset high fever, a widespread skin rash, vomiting, diarrhoea and severe headaches (Efridi and Lappin, 2022).

*Class II* single-stranded DNA viruses with (+) sense DNA, consist of Parvoviruses. Macri and Crane (2022) describe Parvoviruses as ssDNA liner viruses that generally contain two genes, one gene that encodes for a replication initiator protein known as NS1 and the other gene encodes for the protein the viral capsid comprised of Macri and Crane (2022) further state that parvovirus virions are one of the smallest virions in comparison to most viruses as they are 23-28 nm in diameter and mainly contain the genome encased in an icosahedral capsid which typically has a rugged surface. This virion group is the main cause of a variety of diseases in animals, such as canine parvoviral infection in dogs which is noted by loss of appetite, abdominal pain, fever, hypothermia and bloody diarrhoea, according to Macri and Crane (2022).

Whereas *Class III* double-stranded RNA viruses consist of Reoviruses. According to Kniert *et al.* (2022) is classified into the family Reoviridae and further divided into nine genera, four of which are known to cause infection in humans and animals, namely, Othoreovirus, Coltivirus, Rotavirus and Orbivirus. Structurally, Reoviruses measure 60-80 nm diameter with two concentric capsid shells, each of which has an

icosahedral shape. Internally, the RNA strands vary in size from 680 base pair to 3900 base pair. The virion core typically contains various enzymes that are needed for transcription and the capping of viral RNA (Kniert *et al.,* 2022). Reoviruses are responsible for causing pneumonia, meningitis and encephalitis according to Kniert *et al.* (2022).

*Class IV* positive single-stranded RNA with positive sense RNA, consists of Coronaviruses. Koonin, Krupovic and Agol (2021) describe Coronaviruses are a large family of virions which are the cause of mild to severe upper respiratory tract illnesses and fatal diseases in humans. Common diseases caused by this family are severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS). Symptoms of these infections are a cough, fever, runny nose, headaches and a sore throat. According to Koonin, Krupovic and Agol (2021) the coronavirus particles are organized by long RNA primers that are packed tightly into the center of the particle and is surrounded by a protective capsid. The capsid consists of a lattice of repeated protein molecules referred to as capsid or coat proteins, more specifically nucleocapsid.

*Class V* negative single-stranded RNA with negative sense RNA, consist of Rhabdoviruses. A review by Sheperd *et al.* (2023) noted that of the viral family *Rhabdoviridae* rep, the most known species is *Rabies lyssavirus,* the main cause of rabies. Rhabdoviruses display a characteristically bullet-shaped morphology and the virions range between 45-100 nm in diameter and 100-430 nm in length. Sheperd *et al.* (2023) illustrate that the nucleocapsid is coated with a lipid envelope that is derived

from the host's cell membrane. The envelope is studded with trimetric glycoprotein spikes which are responsible for facilitating the entry of the virion into the cell via endocytosis. Generally, rabies is passed onto humans via a bite mark from the host, common occurrence of infection is an infected canine bite. Once a human is infected with rabies symptoms include discomfort, itching or prickling at the site of the bite, as well as a fever, headache or weakness. If not treated, Shepard *et al.* (2023) explains that the rabies virus is fatal as the virions cause inflammation of the brain and spinal cord which results in paralysis and seizures.

*Class VI* single-stranded RNA containing reverse transcriptase viruses with positive sense RNA and DNA intermediate in life cycle consist of Retroviruses. *Retroviridae,* according to Zuma *et al.* (2022) is a family of viruses that characteristically carry their genetic blueprint in the form of RNA. What makes Retroviruses unique is the presence of an enzyme, known as reverse transcriptase (RT) which is known to transcribe RNA into DNA, a process that constitutes a reversal of the usual direction of cell transcription (Zuma *et al.,* 2022). This action of reverse transcriptase allows genetic material from the retrovirus to become permanently incorporated into the DNA of the infected host cell. Retroviruses can cause tumor growth as well as cancers in animals and, in humans they are responsible for causing human T-cell lymphotropic virus type 1 which is the causative agent of a form of cancer called adult T-cell leukemia (ATL). Retroviruses that most widely affect the human population are the human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS). Since the emergence of HIV and AIDS, approximately 20 million infected people have succumbed to opportunistic infections and have died (Zuma *et al.,* 2022).

Lastly, *Class VII* double-stranded DNA-RT viruses consist of Hepadnaviruses. A review by Seeger (2023) noted that Hepadnaviruses are unique viruses which are known to replicate their genomes using the process of reverse transcriptase via the formulation of covalently closed circular DNA and the reverse transcription of pregenomic RNA. Seeger (2023) further illustrated that this process leads to the synthesis of the relaxed DNA genome that is in a circular shape. According to Seeger (2023) Hepadnaviruses are known to cause chronic infections of liver cells and the most widely known member of the *Hepadnavir*idae family is hepatitis B virus (HBV). HBV is spread via blood, semen or other body fluids from an infected individual entering the bloodstream of an uninfected individual.

### 2.3 Health burden of viral infections

Viral infections have a huge impact on human health as evident during the COVID-19 pandemic when approximately 770,778,396 humans died after contracting the SARS-CoV 2 virus (WHO, 2022). According to the National Institute of Allergy and Infectious Diseases (NIAID), as cited by Chen *et al.* (2023), emerging infections are defined as the outbreaks of previously unknown diseases, diseases which are persistent and cannot be controlled, and finally, as known diseases which are rapidly increasing in incidence or geographic range in the last two decades. Trovato *et al.* (2020) state that since the start of the 21$^{st}$ century, the global community has faced multiple outbreaks due to the emergence of new or neglected pathogens from wildlife reservoirs that have spilt over into human populations and caused severe diseases. Trovato *et al.* (2020) continue to say that over the last two decades due to the outbreaks of these

emergent viral diseases, there were high threats to health security, the economy and biodefense worldwide. Armitage *et al.* (2021) add that despite the huge progress in the understanding, prevention and treatment of viral infectious diseases, there is currently still a burden on the global economy due to the emergence of major viral diseases, namely HIV/AIDS, measles, rabies, yellow fever, dengue fever and hepatitis.

According to Chauhan *et al.* (2020), Africa is a vast continent that is comprised of 54 countries and within the African continent there are various zoonotic and vector-borne viral infectious diseases. This is due to the tropical climate, rampant poverty, scarcity of resources and lack of knowledge coupled with traditional and cultural rituals have placed many African countries at risk of the outbreak of viral diseases (Armitage, 2021). It was reported by Chauhan *et al.* (2020) that one of the most challenging viruses, known as the human immunodeficiency virus (HIV), was transmitted to hunter-gatherers in the forests of Western Africa because of conflict with non-human primates during the act of hunting for meat. Over time, the HI virus has spread all over the continent and in southern Africa, an epicentre has formed (Satoh and Boyer, 2019). Zuma *et al.* (2022) state that since the global emergence of HIV, 39 million people were living with HIV in 2022, and 480,000-880,000 documented people have died from AIDS-related illnesses in 2022.

In a brief overview by Moyo *et al.* (2023) the emergence of human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) have greatly impacted the health and socioeconomic status of many countries worldwide. The main cause of this impact is due to the constraint of an infected individual's ability to work and earn

an income. In short, Moyo *et al.* (2023) noted that as the virus spread since its emergence and more individuals became infected, the overall workforce of the population was affected as more infected people are unable to work and when left untreated have progressed to AIDS. One of the implications of HIV is a weakened and ultimately non-existent immune system and over time, infected individuals become susceptible to opportunistic infections. As an individual becomes too ill to work, their employment status may be affected which can lead to unemployment (Moyo *et al.,* 2023).

### 2.4 COVID-19 as an emerging viral infection

COVID-19 (or 2019-nCoV) is caused by the virus β-coronavirus, scientifically known as severe acute respiratory syndrome virus 2 (SARS-CoV-2) (Jamal, 2022). The disease may be characterized by a series of clinical symptoms that are extremely complex. These symptoms include pneumonia, dry cough, fever, and shortness of breath. In July 2022, the World Health Organization announced approximately 570,005,017 confirmed cases globally and approximately 770,778,396 deaths (WHO, 2022). Despite the establishment of an immunization program which resulted in successful outcomes, the public health system was still challenged. Globally, the mortality rate of the human population increased as infection rates increased. COVID-19 also severely impacted the socio-economic conditions. According to Jamal (2022), the burden on the world and more specifically on Africa and Namibia had been devastating.

According to Logiel *et al.* (2021), most countries on the African continent are developing countries, consisting of rural communities and urbanized communities which are made up of 56 % of slums, suffered the most during the pandemic. Logiel *et al.* (2021) clarify that these communities were impacted the most due to their financial instability as they are often found in areas that are far from medical facilities. The same pattern is seen with the Namibian population. Communities in smaller towns and urban areas were greatly affected. It was found that only 34 % of the Namibian population has access to handwashing facilities, and weak health systems and the prevalence of various underlying health conditions such as malaria, tuberculosis, HIV/AIDS and malnutrition have rendered these communities more susceptible to contracting these viral infections, specifically COVID-19.

### 2.5 The role of inflammation and oxidation in viral infections

Viral infections often trigger an inflammatory response in the body, which can be life-threatening. Individuals infected with the H5N1 virus showed increased levels of pro-inflammatory cytokines including Interleukin-6 (IL-6), Tumor necrosis factor alpha (TNF-α), Interferon-gamma (IFN-γ), Interferon gamma-induced protein 10 (IP-10), and Monocyte chemoattractant protein-1 (MCP-1). These cytokines cause an imbalanced inflammatory response which in turn causes sickness/pathogenesis (Faist *et al*. 2022); and may alleviate or aggravate disease or have no impact at all, depending on the patient (Casanova and Abel 2021). Therefore, it is essential to regulate inflammatory responses for controlling viral infections and associated diseases. When viral-induced inflammation becomes harmful to the host, controlling it can reduce viral pathogenesis (Chakravarty, Chakravarti, and Chattopadhyay, 2023). Furthermore, an increase in oxidation in infected individuals was also reported.

Reactive oxygen species (ROS) play vital roles in various cellular processes, however, if levels become too high to be neutralized by defense mechanisms, they damage biological molecules, alter their functions, and act as signaling molecules, generating a range of pathologies (Ivanov, *et al.*, 2016). increased to levels which cannot be neutralized by the defense mechanisms, they damage biological molecules, alter their functions, and also act as signaling molecules thus generating a spectrum of pathologies (Ivanov, *et al.*, 2016). In COVID-infected patients, coronaviruses cause an imbalance between increased production of reactive oxygen species (ROS) and reduced antioxidant host responses, leading to increased redox stress (Gain, *et al.*, 2023). This ultimately leads to decreased host defenses against viruses and a rise in virally-induced inflammation and apoptosis, which cause damage to cells and tissues and eventually end-organ disease (Gain, *et al.*, 2023).

### 2.6 Treatment of viral infections

The first step in combating viral infections is to get a timely and accurate diagnosis once a person experiences symptoms related to viral infections (Reta *et al..*, 2020). The inability of the immune system to protect the body from viruses may be detrimental to humans and may lead to the development of life-threatening related viral diseases. Hence, it is important to implement methods to strengthen the immune system, although it is a complicated process as it is comprised of various cells, each responding differently to different microorganisms (Harvard Medical School, 2021). Harvard Medical School (2021) continues to explain that as we age, the capability of our immune system becomes reduced which leads to our immune systems becoming more susceptible to infections. Therefore, additional measures need to be implemented into our daily lives to boost our immune system. These methods include regular

exercise, a well-balanced diet, and a good night's rest (Wagner, Marcon and Caulfield, 2020). Another method to boost the immune system includes the intake of medicine that aids in modulating the immune system to protect the body from infections.

According to Akamine, Clark and Shandera (2023), possible treatments for viral infections may include three types of treatments namely, antiviral medication, Convalescent plasma and post-exposure prophylaxis. Antiviral medications are responsible for stopping the procedure where viruses replicate and make copies of themselves. These medications are used for managing chronic infections and for shortening the length of respiratory infections. Unfortunately, Akamine, Clark and Shandera (2023) explain that this type of medication is specific to one type of virus only and cannot be used for all viruses. According to Vardanyan and Hruby (2016), antivirals are available for combating the flu, hepatitis B (entecavir, formally known as Baraclude), hepatitis C (elbasvir/ grazoprevir, also known as Zepatier), HIV (various antiretroviral drugs, three mainly used ones are Ziagen, Emtriva and Epivir), smallpox (as of June 2021, Brincidofovir is most commonly used) and COVID-19 (such as nirmatrelvir plus ritonavir, commonly known as Paxlovid).

Convalescent plasma is the procedure whereby someone who survived and recovered from an infection resulting from a specific virus donates their blood. This is because the plasma contains antibodies which can help the recipient of the blood transfusion to fight the infection. Lastly, Akamine, Clark and Shandera (2023) explain that post-exposure prophylaxis (PEP) is a form of treatment taken to prevent a viral infection as soon as possible after exposure. For example, someone who has been exposed to HIV

will be taken PEP within 72 hours after a possible exposure to prevent HIV infection. According to Akamine, Clark and Shadera (2023), an example of a PEP is the combination of the three drugs tenofovir, emtricitabine and the third drug which could be either raltegravir or dolutegravir. These drugs must be taken once or twice a day for 28 days, depending on what is indicated by the medical practitioner, (Akamine, Clark and Shadera, 2021).

Unfortunately, Caughey *et al.* (2017) explain that the use of conventional medicines may potentially have adverse side effects that can harm patients. Ferner (2014) lists these possible adverse effects as dizziness, headaches, constipation, heart problems, liver failure, immune response suppression, blood thinning, allergic reactions such as hives, itching, skin rashes, the narrowing of the throat, difficulty of breathing, and shortness of breath. Another problem with conventional medicines is their inaccessibility and unaffordability in rural areas. Vellingiri (2020) continues by explaining that the socioeconomic issues within rural areas have an impact on the health care of the rural population. Due to poverty and distance from hospitals and clinics, people living in rural areas have limited access and options when it comes to medicine. Therefore, they opt to use traditional or alternative methods to treat their ailments, according to van Andel and Carvalheiro (2013). One of these methods is the use of plants that possess medicinal properties.

## 2.7 Ethnomedicinal plants

A commentary paper by Sifuna (2022) defined the use of traditional medicine as a broad term that incorporates the various systems and forms of indigenous medicine. The World Health Organization (WHO) defined the term by referring to the totality of health practices, knowledge, beliefs, and approaches that incorporate mineral, plant and animal-based medicines which are applied singularly or in combination to maintain well-being or to treat, diagnose and prevent illnesses (Sifuna, 2022). Sifuna (2022) emphasized that the use of ethno-medicine is one of the oldest forms of medical practice in the world and that this knowledge has been passed down from generation to generation. This indigenous knowledge was usually transferred along familial lines within a specific community. Mothibe and Sibanda (2019) noted that the counterparts of traditional medicine, commonly known as conventional or allopathic medicine, are mainly biopharmaceuticals which are manufactured via industrial processes using chemicals that often contain high toxicity. Mothibe and Sibanda (2019) further cited that the use of ethnomedicines presents fewer side effects that are usually linked to the use of allopathic medicines.

As stated by the World Health Organization (2023), also known as WHO, a medicinal plant is a plant that contains certain chemicals, known as phytochemicals, that may be used for therapeutic purposes. Rao *et al.* (2012) noted that medicinal plants which are highly undervalued have a vital role in modern medicine, owing to the mass of dynamic principles that nature has provided the plants with through eons of evolution. The traditional use of these plants is knowledge built on thousands of years of experience and experimentation that has been passed on to each generation. In Africa, specifically Southern Africa, the use of traditional medicines is widely used and

practiced (Siddique *et al.,* 2021) thus these ethnomedicinal plants play a significant role in health and overall well-being.

The indigenous vegetation found within Southern Africa is notable for its richness and a high degree of endemicity, wherein an estimated 23,404 plant species have been recorded according to Siddique *et al.* (2021). The ethnomedicinal value of many of these plants has been described by Laldingliani *et al.* (2022) as an ancient tradition, dating back to the arrival of the San people to the southern regions of Africa more than 20,000 years ago. Therefore, the World Health Organization has encouraged the sensible use of traditional medicines by member countries of the World Health Assembly in the WHO's pursuit to provide easily accessible, affordable, and culturally accepted health care to the global populace (WHO, 2023). For the process to be facilitated, guidelines that assess whether the herbal medicines are safe and high quality have been drawn up, according to Laldingliani *et al.* (2022). Guidelines, and pharmaceutical monographs for approximately 90 widely utilized medicinal plants have been complied with by the World Health Organization (WHO, 2022). However, from these monographs, very few are related to African traditional medicines, thus, there is very little information currently available in the scientific literature that is concerned with the safety, efficacy, and quality of the plant species that are used as traditional medicines in Southern Africa, WHO (WHO, 2022) claims.

Namibia has a very strong history with the use of ethnomedicinal plants and continues to emphasize that scientific studies on many of these medicinal plants have not occurred (Du Preez *et al.,* 2020). There are approximately 2400 vascular plant species in Namibia and thus numerous plant species are being used for the treatment of various

illnesses (Cheikhyoussef *et al.*, 2011). Examples of such plants include *Gomphocarpus fruticosus, Cryptolepis decidua* and *Acanthosicyos naudinianus.* Maguraushe (2017) states that the leaves of *G. fruticosus* may be used as a sedative for the treatment of headaches and can be inhaled to treat tuberculosis, while the roots of this plant are used to relieve stomach aches and general body pains. A review by Osafo, Mensah and Yeboah (2017) noted that a decoction made by the roots of *C. decidua* may treat constipation while the raw root can be chewed and can treat menstrual pain, stomach-ache, and tuberculosis and may stimulate milk production when given to cattle. Lastly, a study by Du Preez *et al.* (2020) noted that *A. naudinianus* may be used for treating skin rashes, pain, fever and other inflammatory disorders. The immunomodulatory effects of these three plants were studied by Du Preez *et al.* (2020), and they yielded positive results.

### 2.7.1 Therapeutic properties of ethnomedicinal plants

Secondary metabolites, also known as phytochemicals or chemical compounds, are biologically active and have beneficial effects and provide plants with protection from various bacteria, insects, viruses, and certain herbivores (Rao *et al.*, 2012). These phytochemicals work alone or in combination with other phytochemicals to influence multiple pathways simultaneously to manufacture the desired pharmacological outcome (Barkat *et al.*, 2020). The wide variety of active phytochemicals including flavonoids, terpenoids, sulphides, polyphenolics, saponins, coumarins, alkaloids, and thiophenes can be attributed to the plants' potential as novel medicines (Jassim and Naji, 2003). Many medicinal plants and herbs are revered by ancient medical traditions, for example, medicinal plants played vital roles in Chinese medicine and

Native American tribes due to their healing benefits. Furthermore, about 40 % of modern medicines are derived from plants (Barkat *et al.*, 2020).

The development of antiviral and anti-infectious agents presents as a major focus in modern medicinal research. Unfortunately, most of the pharmacopoeia of phytochemical compounds in medicinal plants with antiviral potential are still unknown given the limited classes of compounds that have been investigated. Jassim and Naji (2003) revealed that several of these classes of phytochemical components have overlapping and complementary mechanisms of action, which include antiviral activity by inhibiting the formation of viral DNA or RNA and/or inhibiting the activity of viral reproduction.

### 2.7.2 Challenges and limitations of ethnomedicine

An article by the New Era (2020) noted that indigenous knowledge in Africa and more so in Namibia has been forgotten and relegated due to the political and social despondency because of the implementation of the Western education system, as well as the introduction of Western medical practices. The New Era argues that (2020) the Western education system and medical practices have assisted in the erosion of IKS to the extent that it has been regarded as almost extinct. Lastly, the New Era (2020) noted that when considering the background of the education system and medical care in Namibia, the Namibian government was tasked with providing access to the previously disadvantaged Black population whose education and medical care was ignored during the missionary settler's era as well as during the colonial period (1885 – 1989) of the country. Therefore, these periods resulted in the condemnation and marginalization of Namibian indigenous knowledge.

Additionally, a review by Jauhiainen and Hooli (2017) conducted from the 1990s until early 2016 analyzed the development trajectory of indigenous knowledge systems in Namibia and highlighted the main challenges Namibia faced post-colonialism and independence. Jauhiainen and Hooli (2017) noted that while conducting their review, they discovered that many African theorists believed there are grave dangers and risks about the negligence of preserving IKS. The review by Jauhiainen and Hooli (2017) also emphasized the spatiotemporal challenges of creating an innovation system (IS) as Namibia has limited science, technology, and innovation (STI) resources in addition to the potential mismatching of related policies, practices, and strategies. Edquist (2005) as cited by Jauhiainen and Hooli (2017) stated the concept of the application of an IS in a developing country, like Namibia, is based on the cooperation and collaboration of all economic, political, institutional, organizational, and social factors, as well as other factors that can influence the development and application of innovations. Therefore, the focus of the review by Jauhiainen and Hooli (2017) was to discuss IKS in Namibia and its influence on the development of IS.

Subba Rao (2006) defined IKS as locally embedded knowledge which is context-specific has accumulated over time and is unique to a given culture, local community, or society. Subba Roa (2006) continued by noting that IKS is often based on oral transmission or through demonstration and imitation and the practices are learned by repetition, giving IKS its characteristic doing-using-interaction (DUI) mode of development. Lundvall *et al.* (2009) noted that IKS is the main strategic direction of the innovation policies in multiple sub-Saharan African countries. Hardly any previous research exists on the role that IKS plays in innovation policies and in turn the

developing innovation systems (Lundvall *et al.,* 2009). Lundvall *et al,* (2009) noted that indigenous knowledge can provide a competitive advantage towards the fostering of content and impact of innovation systems and create the needed varieties of innovation systems. To add, Adebowale *et al.* (2014) claimed that the tailored specialization of IS in a developing country will primarily contribute to socioeconomic sustainability and the alleviation of poverty, instead of competing with more developed and advanced countries.

Lastly, Adebowale *et al.* (2014) noted that the implementation of an innovation system may alleviate poverty if the system is designed to include and involve local communities. For instance, the development of a medicine from an ethnomedicinal plant will involve local communities as the plant would have to be grown on a farm, harvested, dried and packaged and transported to a laboratory for testing as well as drug formulation. By involving a local community where the plant grows wildly, the innovation system will employ farm workers from that local community, thus lightening the burden for the families of the selected farm workers.

A commentary review by Sifuna (2022) noted that one of the main challenges related to the use of ethnomedicinal plants is that currently, ethnomedicine is predominantly informal and therefore traditional practitioners do not require prior professional or formal training. This leads to the two parallel systems that have developed in African traditional medicine (ATM), Sifuna (2022) further stated. The parallels are informally regarded by Mothibe and Sibanda (2019) as cited by Sifuna (2022) as ‘genuine medical practice’ and ‘deceptive medical practice’. Mothibe and Sibanda (2019) noted the

latter is often based on the deception and trickery of practitioners to exploit and fleece the public. Sifuna (2022) further mentioned that another disadvantage of the informal practice of ethnomedicines is the proneness to inaccuracies in diagnosis and further explained that a major problem with treatment and diagnosis is that practitioners depend on guesswork and generalization to treat various illnesses. Sifuna (2022) explained that the reason for the inaccuracies concerning diagnosis is due to two factors, namely, ethnomedicine focuses on disease symptoms and their cure, instead of focusing on primary health. Therefore, Sifuna (2022) highlighted the importance of encouraging researchers to study and provide scientific evidence for the use of African traditional medicine as this will result in the education of traditional medical practitioners and reduce the risk of inaccuracies with diagnoses and treatments.

There is a need to preserve indigenous knowledge systems that were established decades and centuries ago, as one of the gaps identified by this study is the possibility of the loss of IKS in Namibia, which further highlights the importance of the preservation and protection of indigenous knowledge. Ludvall *et al.* (2009) claimed that the preservation and protection of IKS will aid in the development of tailored innovation systems that will provide strategies and policies to guide the Namibian Bioeconomy and promote socioeconomic sustainability. In addition, to adhere to the guidelines provided by the Chauhan Organization (2022), there is a need to provide scientific evidence which will strengthen the use of ethnomedicinal plants as well as the drug formulation and drug development of these ethnomedicinal plants. One of the gaps identified by Sifuna (2022) was the lack of education on ethnomedicine and ethnomedical practices due to limited or no scientific data that supports the utilization of ethnomedicine and other branches of indigenous knowledge.

### 2.7.3 The importance of ethnomedicinal plants as antivirals

According to Dhama *et al.* (2018), there has recently been a noteworthy development within the field of antiviral herbal treatment owing to growing concern about the advancement of drug resistance and limited improvements in the field of antiviral drug discovery. Dhama *et al.* (2018) state that in many countries if not all, medicinal plants have been widely used throughout history for the treatment of ailments, infections, and diseases. These plants were used as traditional healing remedies due to the broad therapeutic spectrum and minimal side effects that they possessed. Parasuraman, Thing, and Dhanaraj (2014) further explain that as synthetic antiviral drugs are not available to work against a hefty quantity of viral agents, hence possible efforts should be dedicated to discovering new drugs and alternative medicines from diverse herbal formulations. Moreover, studies conducted by Nie *et al.* (2021) have discovered that traditional medicines based on herbal extracts have shown potential as affordable treatments for patients suffering from the coronavirus disease in the year 2019 (COVID-19) that results from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

## 2.8 *Artemisia afra* ('Wilde als')

The plant considered for this study is *Artemisia afra* (Figure 2), and according to Mukinda (2007), it is very diverse and has garnered many common names over the years. This is due to the widespread use of the plant in various ethnic groups. In Xhosa the plant is referred to as "Umhlonyane", in English "African wormwood", in Afrikaans "Wilde als", and in Zulu, the plant is called "Mhlonyane". The plant is

classified as follows according to du Toit and can der Kooy (2019) as Kingdom: Plantae, Division: Magnoliophyta, Class: Magnoliopsida, Sub-class: Asteridae, Order: Asterales, Family: Asteraceae, Genus: *Artemisia* and Species: *Artemisia afra*.

It is defined as one of the vast genera of the Asteraceae or Compositae family which is regarded as one of the biggest flowering plant family. A review by du Toit and van der Kooy (2019) explains that the genus name *Artemisia* is derived from the name of a Greek goddess, Artemis, the goddess of hunting. This plant genus is comprised of an estimated 600 taxa and subspecific and specific levels. This plant genus is known to grow and be present in all continents, besides Antarctica, and is the highest distributed in the Northern Hemisphere according to du Toit and van der Kooy (2019). du Toit and van der Kooy (2019) also stated that no more than 25 taxa are found in the Southern Hemisphere.

The *Artemisia* genus has displayed a high level of ecological plasticity. Pigliucci, Murren and Schlichting (2006) describe plasticity in ecology as individual genotypes having the ability to produce various phenotypes when they are exposed to diverse conditions within their environment. This is notable as this genus may be located and can grow in sea-level areas to high mountains and from wetlands to arid and barren zones according to Shinyuy *et al.* (2023). It may also be regarded as cosmopolitan, which means that the range of this taxon extends across most or in some cases all over the world (Shinyuy *et al.*, 2023). Many species from this genus *Artemisia* have great importance in terms of their use at both folk and industrial levels, and it is noted by Kriel (2010) that many of these plants are widely cultivated and submitted to multiple breeding programs as crops.

*Figure 2:* The *Artemisia afra* (‘Wilde als’) in its natural habitat, image obtained from van der Beer (2019).

### 2.8.1 Geographical distribution of *Artemisia afra*

*Artemisia afra* has been identified as an indigenous plant that grows in Eastern and Southern Africa, usually at altitudes that range between 1500 and 3000 m. According to van Wyk (2011) in Southern Africa it normally grows in very mountainous areas and along the margins of forests and streams. *Artemisia afra* has been noted to grow in Kenya, Ethiopia, South Africa, Uganda, Tanzania and Namibia. In Namibia, this plant has been found in the northern regions of Namibia such as in the Oshikoto,

Kunene and Khomas regions (Figure 3). However, this plant can also be commercially grown in the Khomas and Hardap regions.

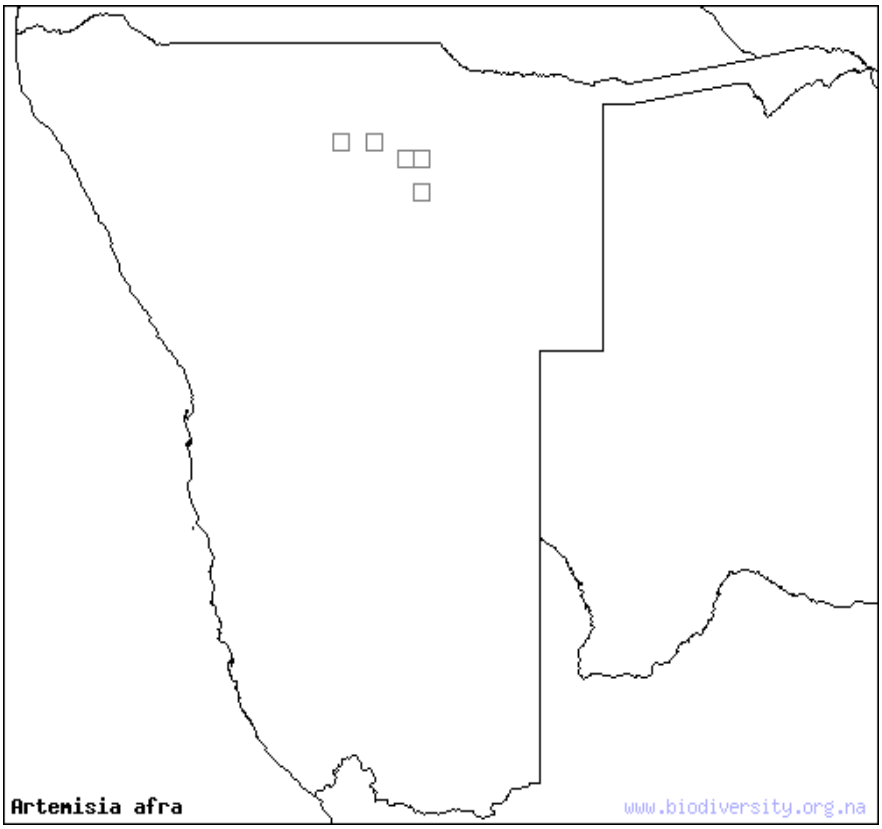


*Figure 3:* A map depicting the geological location where the *Artemisia afra* species naturally grow in Namibia (Irish, 2022).

### 2.8.2 Traditional uses of *Artemisia afra*

The plant is typically used for treating various ailments such as headaches, colds, coughs, loss of appetite, bladder and kidney disorders, chills, malaria, whooping cough, croup, asthma, gout, influenza, gastric issues, convulsions, heart inflammation, rheumatism, and dyspepsia (indigestion), as well as diabetes according to Mukinda (2007). The plant is also used to treat sore throats, stomach ailments, measles, wounds, and asthma, heartburn, colic, earache, constipation, haemorrhoids, flatulence, and gout

are also ailments for this plant is used as a cure (Mukinda, 2007). According to Kriel (2010) *A. afra* is commonly used as an anthelminthic and analgesic Therefore, *A. afra* is regarded as a cure-all remedy by Mukinda (2007).

Kriel (2010) states that the most common method to prepare tea made from the *A. afra* is to add a quarter of a cup of fresh leaves to one cup of boiling water. The leaves must be steeped for five minutes and strained before the tea is drunk. Alternatively, Kriel (2010) continues, one tablespoon of dried leaves may be added to one cup of boiling water and steeped for twenty minutes. Additionally, from the leaves, a decoction may be prepared by pouring two litres of boiling water over a cup of fresh leaves and stems. This mixture is then allowed to soak for an hour before it is strained. The water extract is then used as a wash to treat haemorrhoids and in a bath to treat measles and fevers, as a wound wash for rashes, bites, stings, and sores. The decoction may also be diluted to form an eyewash to soothe irritated, red eyes (van Wyk, 2008). Furthermore, Mukinda (2007) as stated by Kriel (2010) explains that once the dried leaves are ground into a powder-like form and are soaked in hot water an enema is prepared and given to children who suffer from intestinal worms as well as aiding in constipation in adults. The plant may also be used as a mouthwash that is used to treat oral ulcers and gumboils. For inhalation, three cups of leaves are placed in a pot with enough water to cover the leaves. The pot is then put on a boil and the patient must hover above the boiling pot with a towel draped over their head. As the leaves boil, the steam is deeply inhaled. Traditionally, this method is used to cure and treat bronchitis, asthma, and a tightened chest, to expel phlegm, a blocked nose, and chest colds (Mukinda, 2007). The fresh leaves of the plant is rolled and inserted into the nostril to kill inhaled bacteria (Mukinda, 2007).

Another popular preparation of the plant is known as the “Wilde als brandy” which is regarded as an old remedy for coughs, chest pains, colds, chest ailments, stomach cramps, and indigestion (Kriel, 2010). The method to prepare this is mixing one bottle of brandy with one cup of *A. afra* leaves. A quarter cup of thyme, half a cup of mint leaves, a thumb-length piece of ginger, a quarter cup of rosemary, and one cup of sugar are added to the mixture for flavoring purposes. The ingredients are pushed into the brandy bottle and shaken every day for a month, afterwards, it is strained or allowed to age with the addition of more herbs. From this “Wilde als brandy,” one tablespoon is mixed with water and ingested as needed.

In the southern regions of Namibia, more specifically in the Hardap region, the plant is popularly used as a tonic to treat colds and the common flu. The leaves and stems are dried and steeped in hot water to form tea. However, steam and fumes may be inhaled to clear headaches, congestion, hay fever, sinusitis, and asthma according to research done by van Wyk (2011). The application of this plant as a treatment for such many ailments may be seen as an indication that the *A. afra* plant may possess antiviral, antibacterial, and anti-inflammatory activities (Kriel, 2010).

### 2.8.3 Previous studies on *Artemisia afra*

*Artemisia afra* is of high value and importance as a potential antiviral and immune-boosting agent. Shi *et al.,* (2015) illustrate that the reason why *Artemisia* species are so widely sought after is due to the presence of the phytochemical compound classes flavonoids, terpenoids and coumarins.

Flavonoids display a wide range of biological activities as they contribute to the plant's ability to be antioxidant, anti-inflammatory, anti-allergenic, anti-microbial, immune-modulating, and antiviral. Due to the presence of flavonoids, the *Artemisia* species were found by Boudreau *et al.* (2020) to be effective in the treatment of inflammatory conditions that include rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus, and various allergic disorders (Boudreau *et al.*, 2020).

A study by Tholl (2015) noted that terpenoids, also regarded as isoprenoids, represent the most diverse and largest class of phytochemicals in plants. Terpenoid metabolites are employed by plants for various basic growth and development functions, however, terpenoids play a major role in specialized chemical interactions and protection of the plant in abiotic and biotic environment (Yimam and Desalew, 2022). These interactions have provided terpenoids with a range of pharmacological activities such as antiviral, antimalarial, antibacterial, anti-inflammatory, hypoglycemic potential and may be used as signal molecules to attract insects for pollination (Singh and Sharma, 2015).

Coumarins as noted by Wu *et al.* (2009) constitute as an important class of pharmacological agents that possess a wide range of physiological potentials such as anti-inflammation, antiviral, comparative and analgesic immune modulation, anticancer and antioxidant. However, a recent study by Zhu et al. (2013) noted that coumarin compounds found in *Artemisia* species have an ineffective influence on the antiviral and antioxidant activity of *Artemisia afra.* The study determined that the more effective compounds for immune modulation, antiviral, antioxidant and anti-inflammatory activity were flavonoids and terpenoids.

The anti-inflammatory mechanisms of the *Artemisia* species include the inhibition of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) pathways, the suppression of extracellular signal-regulated kinases (ERK) and nuclear factor kappa B (NF-κB) signaling, the inhibition of pathogenic T-cell activation, suppressing B-cell activation and the production of antibodies and finally the inhibition of protein kinase B (PKB or Akt) phosphorylation and inhibitory kappa B degradation through the phosphatidylinositol-3 kinase (PI3K)/Akt signaling pathway downstream of tumour necrosis factor α (TNF-α) (Cheng *et al.*, 2011). Thus, Cheng *et al.* (2011) state that the various mechanisms through which these phytochemical compounds produced by *Artemisia* exhibit anti-inflammatory effects warrant investigation into their role as therapeutic candidates for autoimmune disorders and inflammatory conditions.

*Artemisia afra* has also exhibited a significant antiviral ability according to Qin *et al.* (2021) as serums manufactured from the plant material have demonstrated the potential to inhibit the central regulatory processes of virally infected calls, also known as the NF-κB pathways. Ultimately, Kshirsagar and Rao (2021) explain that this blocks the host cell type, and the metabolic requirements needed for viral replication. Thus, owing to their potential anti-inflammatory, immune-regulatory, and antiviral characteristics, *A. afra* plants are actively pursued for their activity against SARS-CoV-2 infection. Studies conducted by Sharma *et al.* (2020) used in silico tactics to examine whether artemisinin, a compound found within *A. afra,* or its derivatives could be physically bound to any of the COVID-19 target proteins including SAR-CoV-2 spike glycoprotein, main protease of the virus (MPro), spike ectodomain structural protein or spike receptor-binding domain. By binding the artemisinin to

these target proteins, the SARS-CoV-2 is prevented from binding to the host receptor ACE-2 (Rolta *et al.*, 2020).

Several investigations by Nair *et al.* (2021) have shown that extracts from various species of *Artemisia* are active against influenza, also known as the common flu. This is attributable to the mechanism of antiviral activity via the induction of cellular reactive oxygen species (ROS), blunting the PI3K/Akt/P70s6K signaling pathway, and binding to the NF-κB/Sp1 or even inducing an endocytosis inhibitory mechanism. These mechanisms lead to the inhibition of viral replication and viral cell growth (Gendrot *et al.*, 2020). Chen (2020) explains that the Transforming Growth Factor-beta (TGF-β) plays an imperative role in the modulation of the immune system and in addition, it is known to display various activities on different types of immune cells. Therefore, studies conducted by Yao *et al.* (2018) have discovered that artemisinin as well as its derivatives, can suppress TGF-β in several models of inflammatory diseases. Hence, Kshirsagar and Rao (2021) deduce that the inhibition of TGF-β signaling by artemisinin and its derivatives may be regarded as a promising therapeutic strategy, making them excellent candidates as drugs against SARS-CoV-2 infection.

Cao *et al.* (2020) analyzed the adsorption, distribution, metabolism, excretion, and toxicity, also known as ADMET of artemisinin to present that the plant metabolites were non-cytotoxic, possessed excellent aqueous solubility and adequate permeability through the blood-brain barrier with a promising therapeutic potential. Molecular docking studies conducted by Tomic *et al.* (2020) revealed that artemisinin bound to all four proteins and in certain instances, the artemisinin displayed improved binding modes than hydroxychloroquine. Therefore, Tomic *et al.* (2020) reveal that artemisinin

and its derivatives could serve as the best leads for further drug development process for SARS-CoV-2 infection.

# CHAPTER THREE: MATERIALS AND METHODS

## 3.1 Plant material

The whole plant, i.e., the leaves and twigs of *Artemisia afra* was obtained from a farm in Groot Aub, which is located within the Khomas region (22°56'0" S, 17°11'0" E). A voucher specimen (BRL2021-0925) was prepared and deposited with the National Herbarium at the National Botanical Research Institute (NBRI) of Namibia, where the plant's identity was verified. It was confirmed that the plant species was *Artemisia afra*, also known as 'Wilde als'.

## 3.2 Preparation of plant extracts

Plant material was air-dried in the shade for approximately 6 weeks at room temperature and ground into a powder-like form using a blender. The coarse powder was subjected to aqueous and organic extraction, using distilled water and methanol-dichloromethane (MeOH-DCM (1:1, v/v), respectively.

### 3.2.1 Aqueous extraction

This procedure followed the method described by Nagappan (2012) in which 2.5 g of plant material was transferred into a centrifuge tube and 25 ml of de-ionized water was added to the plant material. The plant material-water mixture was then placed in a 60 ˚C water bath for 2 hours. The centrifuge tube was vortexed every 30 minutes. The tubes were then centrifuged for 10 minutes at 10,000 rpm and filtered using the gravity filtration method, using 110 mm Grade 1 filter paper.

### 3.2.2 Organic extraction

This procedure followed a method explained by Njateng *et al.* (2017) where 10 g of the ground plant material was weighed and transferred into Erlenmeyer flasks. A

solvent mixture consisting of methanol and dichloromethane in a one-to-one ratio, measuring at 100 ml each was mixed and added to the flasks containing the plant material. The mouth of the flasks was covered with two layers of parafilm. The flasks were placed on an orbital shaker at room temperature in a dark room for 48 hours at a speed of 150 rpm. Using 110 mm Grade 1 filter paper and the gravity filtration method, the plant material was filtered.

To remove the solvent for both aqueous and organic extracts, a rotary evaporator was used. The water bath of the rotary evaporator was set at 40 ˚C and the pressure at 64 mbar for the organic solvent, and 60 °C at 72 mbar for the aqueous mixture, respectively. The flasks were then placed in the -80 °C freezer for 24 hours. Thereafter, the plant extracts were freeze-dried by attaching the round-bottom flasks to a freeze-dryer for 72 hours and were scraped out of the round-bottom flasks with clean spatulas. The plant extracts were then weighed using an analytical balance and transferred to Eppendorf 1.5 ml centrifuge tubes. To ensure that the quality of the plant extracts did not deteriorate after the extraction process, the Eppendorf tubes a stored at -20 ˚C.

## 3.3 Thin-Layer Chromatography (TLC)

The outline for this protocol was adjusted according to the study conducted by Harbone (1998).

### 3.3.1 Preparation of extracts and reference compounds

To prepare the extracts, 1 mg of each plant extract was weighed in 1.5 ml Eppendorf tubes. One hundred microliters of solvent were then added (water for aqueous extracts and MeOH-DCM (1:1) for organic extracts). The Eppendorf tubes were then vortexed for 1 minute. To prepare the reference compounds, also known as the positive controls

(Table 1), 4 mg of each compound was weighed in 1.5 ml Eppendorf tubes. One hundred microliters of solvent were added to each reference compound. The Eppendorf tubes were then vortexed for 1 minute.

### 3.3.2 Preparation of solvent system and spray reagents

Refer to Table 1 for the preparation of the spray reagents and solvent systems.

### 3.3.3 Preparation of TLC plates

The silica gel plates were cut into rectangles (20 cm x 10 cm). Using a ruler, a 1 cm line from the top and bottom of each gel plate was drawn horizontally. The compounds were then spotted on the TLC plate on the horizontal bottom line which was previously drawn. A distance of 1.5 cm was measured between each spot. About 10 µl of each reference compound and plant extract was spotted onto the plate and allowed to dry. The first spot on each plate represented the reference compound, also known as the positive control, while each spot that followed the control represented the negative control. The negative control comprised either de-ionized water or the dichloromethane-methanol (1:1) mixture. Following the positive and negative controls, the plant extracts were spotted as follows: *Artemisia afra* leaves (organic) – *Artemisia afra* leaves (aqueous) – *Artemisia afra* twigs (organic) – *Artemisia afra* twigs (aqueous) – *Artemisia afra* leaves + twigs (organic) – *Artemisia afra* leaves + twigs (aqueous). The desired developing solvent (Table 1) was poured into the TLC tank until the solvent reached a depth of 5 mm. The TLC plates were placed in the respective tanks and the chambers were covered. The plates remained in the tanks until the solvent had reached the solvent front line that was 1 cm from the top of the plate. The plates were then viewed under UV light and or sprayed to visualize the colour changes.

Table 1. Guide to preparing the reference compounds, spray reagents and solvent systems for TLC.

| Phytochemical class of compounds | Reference compound | Solvent system | Spray reagent | Colour change |
|---|---|---|---|---|
| Alkaloids | 4 mg of Quinine hydrochloride (Sigma Aldrich, Missouri, USA) in 1 ml of methanol | 1.5 ml of ammonium hydroxide dissolved in 98.5 ml methanol | Dragendorff reagent | Orange |
| Anthraquinones | 4 mg of Alizarin (Merck KGaA, Darmstadt, Germany) in 1 ml of chloroform | A mixture consisting of 77 ml ethyl acetate, 13 ml methanol, and 10 ml water | 10 ml potassium hydroxide dissolved in 90 ml of ethanol | Pink-violet |
| Flavonoids | 4 mg of Quercetin (Sigma Aldrich, Missouri, USA) in 1 ml of ethanol | A mixture consisting of 95 ml chloroform and 5 ml methanol | 1 ml of aluminum chloride dissolved in 99 ml of ethanol | Yellow Fluorescence in UV light at 366 nm |
| Coumarins | 4 mg of Coumarin (Sigma Aldrich, Missouri, USA) in 1 ml of diethyl ether | A mixture consisting of 10 ml acetic acid in 90 ml of de-ionized water | Benedict's reagent | Blue-green fluorescence in UV light at 366 nm |
| Steroids | 4 mg of β-sitosterol (Sigma Aldrich, Missouri, USA) in 1 ml of chloroform + 4 mg of σ-sitosterol in 1 ml of chloroform | A mixture consisting of 54 ml chloroform, 29 ml acetic acid, 10 ml methanol, and 7 ml water | Freshly prepared 1 ml of ρ-anisaldehyde in 100 ml of glacial acetic acid and 2 ml sulfuric acid | Heat at 100-105 ˚C until maximal visualization occurs |

| | | | | |
|---|---|---|---|---|
| Terpenoids | 4 mg of β-sitosterol (Sigma Aldrich, Missouri, USA) in 1 ml of chloroform + 4 mg of σ-sitosterol in 1 ml of chloroform | A mixture consisting of 50 ml hexane and 50 ml ethyl acetate | Freshly prepared 1 ml of ρ-anisaldehyde in 100 ml of glacial acetic acid and 2 ml sulfuric acid | Heat at 100-105 ˚C until maximal visualization occurs |
| Saponins | 4 mg of Saponin (Merck KGaA, Darmstadt, Germany) in 1 ml of ethanol | A mixture consisting of 64 ml of chloroform, 27 ml methanol, and 9 ml of water | 7.5 g vanillin dissolved in 125 ml of ethanol and 1.25 ml sulfuric acid | Blue fluorescence in UV light at 366 nm |

### 3.4 Total flavonoid content

The total flavonoid content was determined by using the aluminum chloride, $AlCl_3$, (Sigma Aldrich, Missouri, USA) colorimetric assay outlined by Fattahi *et al.* (2014). Various concentrations of the standard, quercetin (Sigma Aldrich, Missouri, USA) (2.0, 1.0, 0.5, and 0.25 mg/ mL) were prepared in methanol by serial dilution. One milliliter of the quercetin solutions for each of the concentrations was added to 10 mL centrifuge tubes containing 4 mL deionized water. At the starting time, 0.3 mL 5% sodium nitrite was added, after 5 minutes 0.3 mL of 10% $AlCl_3$ was added, and at 6 minutes 2 mL of 1 M sodium hydroxide was added to the mixture. The addition of 2.4 mL of deionized water made the total volume of the mixture 10 mL. The absorbance of the pink color mixture was determined at 510 nm versus a blank containing all reagents except quercetin. The average absorbance values obtained at different concentrations of quercetin were used to plot the bar graph with error bars.

### 3.5 Antioxidant Assay

This assay was conducted according to the protocol outlined by Compaore *et al.* (2016). The positive controls, ascorbic acid (Merck KGaA, Darmstadt, Germany) and quercetin (Sigma Aldrich, Missouri, USA) were prepared by dissolving 10 mg of each in 1 ml of ethanol. Ten milligrams of the plant extracts were weighed and dissolved in 1 ml ethanol. Each extract was serially diluted in a 96-well plate (dilution plate). The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) solution was prepared by weighing out 2 mg of DPPH and dissolving it in 100 ml ethanol. Two hundred microliters of the DPPH solution were added to each well of a new 96-well plate. From the dilution plate, 100 µl of the extract-methanol solution was added to each well. The blank consisted of 300 µl of ethanol and the negative control consisted of 100 µl ethanol and 200 µl DPPH. The plates were then incubated in the dark for 10 minutes and read using a UV/VIS plate reader to measure the absorbance of the extracts at 517 nm. The antioxidant activity (%) was calculated according to the following formula:

$$AA\% = 100 - \left[\frac{Abs_{sample} - Abs_{blank}}{Abs_{control}}\right] \times 100$$

### 3.6 Albumin denaturation assay

The Albumin denaturation assay was done using the protocol as outlined by Gunathilake, Ranaweera and Rupasinghe (2018). To attain the following concentrations, 100, 50, 25, 12.5, 6.25 µg/ml serial dilutions of the extracts were added to centrifuge tubes containing 0.1 ml of 2 % Bovine Albumin solution and 2.3 ml Phosphate buffer saline. The mixtures were then mixed thoroughly using a vortex and incubated at 37 °C for 15 minutes. The centrifuge tubes were then heated in a water bath set at 70 °C for 5 minutes. The centrifuge tubes were allowed to cool for 10

minutes before 2 ml of the mixtures were transferred to clear cuvettes and the absorbance was measured at 660 nm using a UV/VIS spectrophotometer. The reaction and control mixtures were prepared in 15 ml centrifuge tubes according to Table 2 below. The percentage inhibition of protein was determined using the following equation:

$$Inhibition\ (100\%) = \left(\frac{Absorbance_{control} - Absorbance_{test}}{Absorbance_{control}}\right) x\ 100$$

Table 2. A guide to preparing the reaction and control mixtures for the albumin denaturation assay.

| Treatment mixtures | Albumin serum (ml) | Phosphate buffered saline (ml) | Sample (ml) | Total volume (ml) |
|---|---|---|---|---|
| Reaction mixture of plant extracts | 0.1 | 2.3 | 0.1 | 2.5 |
| Positive control (Diclofenac potassium) | 0.1 | 2.3 | 0.1 | 2.5 |
| Negative control | 0.1 | 2.4 | 0 | 2.5 |

### 3.7 MTT cytotoxicity assay

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) assay was used to determine the inhibition or slowing down of the cell growth induced by the extracts of 'Wilde als' (Houghton *et al.,* 2007). The exposure of the extracts to the cells may result in a reduction of the proliferation of cells towards the end of the assay in comparison to the control wells. In the control wells, no cytotoxic substance was

added. The $IC_{50}$ value was determined under these specific conditions which are known as the ‘exposure’ dose.

### 3.7.1 Maintenance of Vero-h/SLAM cells

Vero/hSLAM cells were used for the cytotoxicity assay. The cells were maintained within a Class-II Biological Safety Cabinet (BSC) in T-25 and T-75 flasks. Dulbecco’s Modified Eagle Medium (DMEM) which contains 4500 mg/L D-glucose with L-glutamine, and 10 % fetal bovine serum (FBS) was used for the maintenance of the Vero cells. When the cell layer became confluent, the Vero/hSLAM cells were passaged via the process of trypsinization. The cell monolayer within the T-25 flask was washed using warm phosphate-buffered saline (PBS) for approximately 30 seconds to 1 minute to remove and wash off the excess PBS. The wash media was then discarded into a beaker that contained a hypochlorite solution. To the washed T-25 flask, 5 ml pre-warmed trypsin solution was added to the cells. The flask was then incubated at room temperature and pressure on the bench top for 3 minutes. Approximately 4 ml of the trypsin was removed from the flasks to allow the monolayer to remain wet or moist. The flask was placed at 37 °C for approximately 3 minutes. The flask was observed every few minutes to prevent over-trypsinizing of the cells. This means that the cells should only dislodge from the flask after the flask is tapped firmly by the palm of the hand. The cells then were gently mixed with a 2 ml pipette to break apart any cell clumps that could have formed. The cells were re-suspended in 5 ml of Dulbecco’s modified eagle medium-penicillin streptomycin (DMEM-PS) with 10 % FBS. A 1:2 split was performed to allow the cells to produce mono layers that have sufficient density for viral isolation after 24 hours of incubation.

### 3.7.2 Screening of cytotoxicity

The MTT colorimetric assay is a staining method adopted by Mosmann (1983) with minor adjustments. Firstly, after an incubation period of 24 hours, a 3-fold serial dilution of the samples with a starting concentration of 0.333 mg/ml was performed and 100 µl was transferred to the plate containing cells, except in the cell control wells. The cells were then incubated for 48 hours. Following the 48 hours incubation media was removed and replaced with 25 µl of 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent (5 mg/ml) (Sigma-Aldrich, St. Louis, MO). This was followed by 3 hours of incubation at 37 ℃ to allow the formazan product to form from viable cells. After incubation, the MTT was removed and 100 µl DMSO was added and incubated for 15 minutes at room temperature. Afterwards, the absorbance was read at a wavelength of 620 nm using the Tecan Infinite F500 luminometer. Determining the percentage viability of the cells is calculated according to the following equation:

$$\%\, Viability = \frac{Total\ number\ of\ viable\ cells}{Total\ number\ of\ viable\ and\ non-viable\ (dead) cells}\ x\ 100$$

## 3.8 Antiviral assay

### 3.8.1 Generation of SARS-CoV-2 pseudoviruses

HIV-1 SARS-CoV-2 pseudovirus was generated by co-transfection of the Envelope containing plasmid, with a plasmid carrying the luciferase reporter gene (Wei *et al.*., 2003), into $2x10^6$ 293T cells/10 ml of growth media using the X-tremeGENE transfection reagent (Sigma Aldrich, Missouri, US). The $TCID_{50}$ of the virus stock was quantified by infecting 293T-ACE cells with a serial 4-fold dilution of the supernatant in quadruplicate in the presence of DEAE dextran (37.5 µg/mL) (Sigma-Aldrich, St.

Louis, MO). The Bright Glo™ Reagent (Promega, Madison, WI) was used to measure infection after 48 h of tissue culture, according to the manufacturer's instructions. Luminescence was measured in the luminometer Tecan Infinite F500.

### 3.8.2 Inhibition of SARS-CoV-2 infection assay

This assay was set up to investigate the ability of the plant extracts to inhibit the SARS-CoV-2 infection of 293T-ACE cells. The experiment was performed in triplicates in a 96-well flat bottom plate. To all test wells, 100 µl of growth media was added except in the cell control wells where 150 µl of media was added. The starting concentration for the extracts was 0.133 µg/ml from which a 3-fold serial dilution was performed. After dilution of the plant extracts, 50 µl of the SARS-CoV-2 pseudovirus was added and incubated for 1 hour at 37 ℃ to allow the extracts to interact with the pseudovirus. This was followed by the addition of 10,000 cells/well/100 µl of 293T-ACE cells and incubation for 48 hours at 37 ℃ and 5 % $CO_2$. After 48 hours of incubation, 150 µl of media was removed from the plates followed by the addition of 100 µl Bright-Glo Luciferase substrate and incubation for 2 minutes. Thereafter, 150 µl was transferred to 96-well black plates luminescence was read using the Tecan Infinity F500 and infection was recorded as Relative Light Unit (RLU). The equation to determine the rate of infection of the SARS-CoV 2 cells against their interaction with the plant extracts is shown below:

$$\%\ infection\ inhibition = \frac{Absorbance\ of\ viable\ cells - background\ absorbance}{Absorbance\ of\ non - viable\ cells - background\ absorbance}\ x\ 100$$

### 3.9 Statistical analysis

The data on biological studies were reported as means ± standard deviation. For determining the statistical significance and standard error, the Kruskal- Wallis non-parametric test at 5 % level significance was employed using the statistical program SPSS. For determining the median effective concentration ($EC_{50}$) of each sample, a non-linear regression model was performed using SPSS, the same statistical program. The samples were also subjected to an additional Dunn's test for multiple comparisons to determine whether there was a significant difference between the controls and the concentration of the extracts. P-values $< 0.05$ were considered significant. Each assay conducted was performed in triplicates.

# CHAPTER FOUR: RESULTS

## 4.1 Yield of the plant extracts

In comparison to the aqueous extracts, the organic extracts of the plant species exhibited a higher percentage yield (i.e. weight of extract/weight of dried plant material) (Table 3). The percentage yield of the organic extract of the twigs had the highest yield (12.76 %) compared to the aqueous extract of the twigs which was 6.21 %. The same trend can be seen with the leaves of the organic and aqueous extracts, where the percentage yield for the organic extract is 8.54 % and the percentage yield for the aqueous extract is 6.37 %. The combination of the leaves and twigs for the organic extract was 10.87 % while the aqueous extract displayed a yield of 4.10 %, which was the lowest overall percentage yield overall.

Table 3. The percentage extract yield of the organic (MeOH-DCM) and aqueous extracts of the *Artemisia afra* plant species.

| | Organic extract | | | Aqueous extract | | |
|---|---|---|---|---|---|---|
| Plant part | Twigs | Leaves | Twigs + Leaves | Twigs | Leaves | Twigs + Leaves |
| Yield (%) | 12.76 | 8.54 | 10.87 | 6.21 | 6.37 | 4.10 |

### 4.2 Preliminary phytochemical analysis of the extracts

The following class of compounds were present in all the organic extracts of the leaves, twigs, and a combination mixture of the leaves and twigs for *Artemisia afra*: flavonoids, coumarins, steroids, terpenoids, and saponins (Table 4). The aqueous extracts contained the following compounds, namely, coumarins, steroids, terpenoids, flavonoids as well as saponins. However, for the aqueous extracts, only the twig extracts had flavonoids, coumarins, steroids, terpenoids, and saponins present. The leaf extracts did not possess any terpenoids and the combination mixture of the leaves and twigs did not have steroids and terpenoids present. Alkaloids and anthraquinones were absent in both the organic and the aqueous extracts.

Table 4. Phytochemical classes of compounds in the aqueous and organic extracts of *Artemisia afra* species using thin-layer chromatography.

| Class of compound | Organic extracts | | | Aqueous extracts | | |
|---|---|---|---|---|---|---|
| | Leaves | Twigs | Leaves + Twigs | Leaves | Twigs | Leaves + Twigs |
| Alkaloids | - | - | - | - | - | - |
| Anthraquinones | - | - | - | - | - | - |
| Flavonoids | + | + | + | + | + | + |
| Coumarins | + | + | + | + | + | + |
| Steroids | + | + | + | + | + | + |
| Terpenoids | + | + | + | - | + | + |
| Saponins | + | + | + | + | + | + |

+ = Present, and - = Absent

### 4.3 Total flavonoid content of the extracts

As shown in Figures 4, 5 and 6, the total flavonoid content (TFC) of the plant extracts was concentration-dependent; as the concentration of the plant extract increased, the concentration of the flavonoid content also increased. Overall, the highest TFC was observed in the organic extracts of *A. afra.* The highest TFC of the organic extracts was the twigs with a total flavonoid content of 19.89 %. This was followed by the organic extract of the leaves at 18.52 % and the organic extract of the combination of the leaves and twigs at 16.45 %.

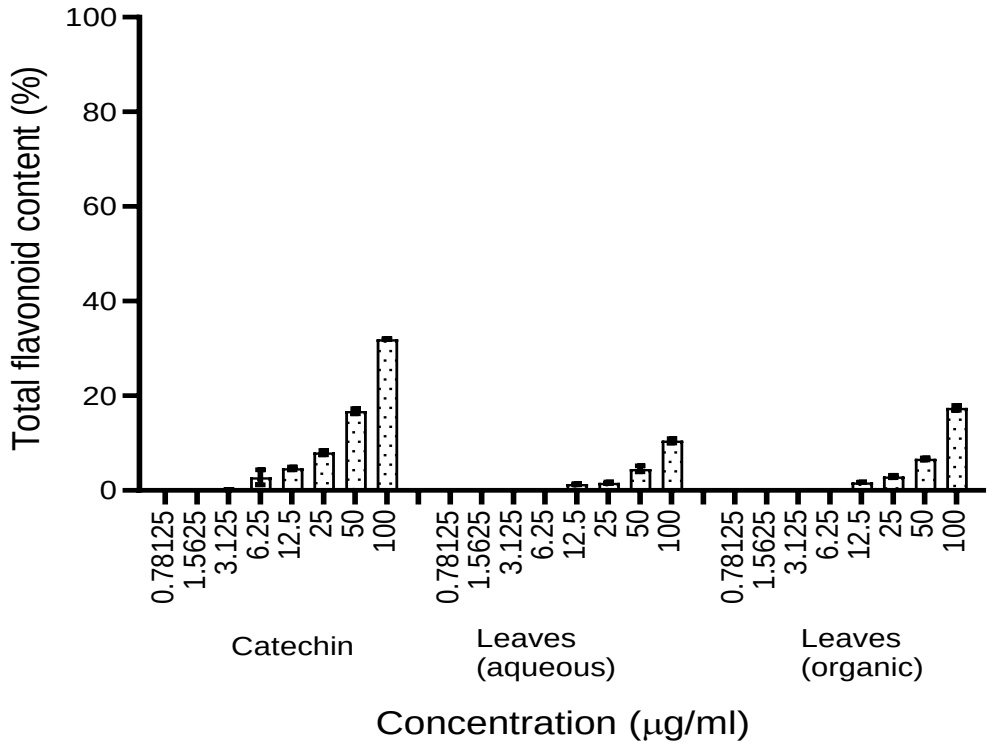


*Figure 4*: The total flavonoid content for the aqueous and organic extracts of the leaves of *Artemisia afra*. The highest TFC was observed for the organic extract of the leaves with a total flavonoid content of 18.52 %. There, however, was no significant difference in the TFC between the aqueous extract and organic extract of the leaves (P = 0.2761).

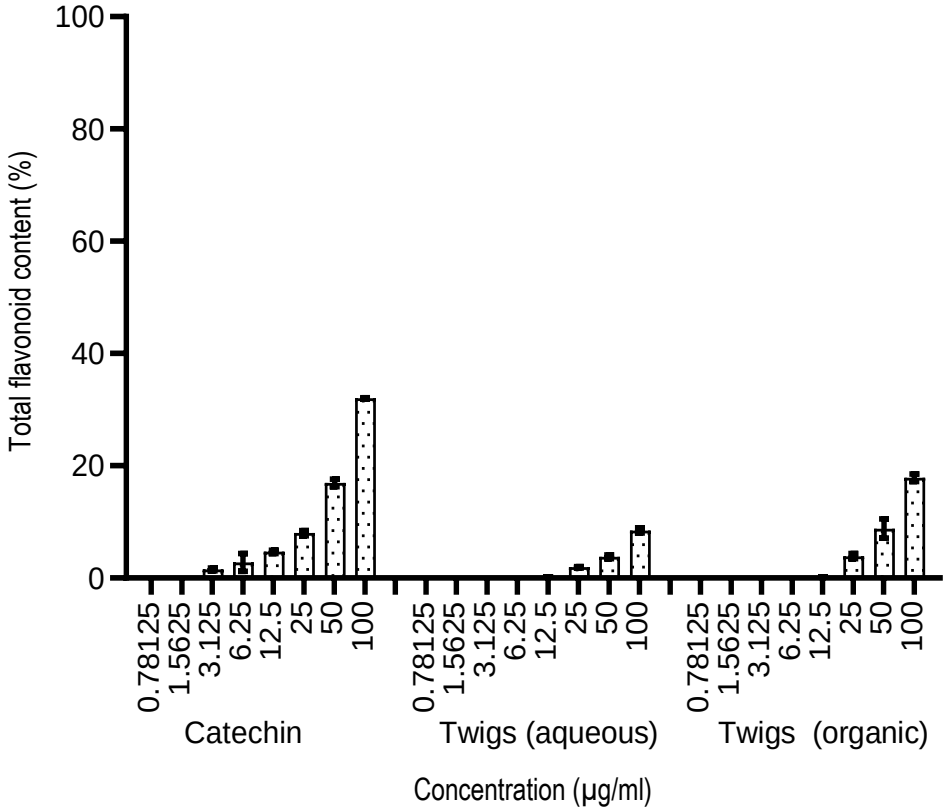


*Figure 5*: The total flavonoid content for the aqueous and organic extracts of the twigs of *A. afra*. The highest TFC was observed for the organic extract of the leaves with a total flavonoid content of 19.89 %. There, however, was no significant difference in the TFC between the aqueous extract and organic extract of the twigs (P = 0.2469).

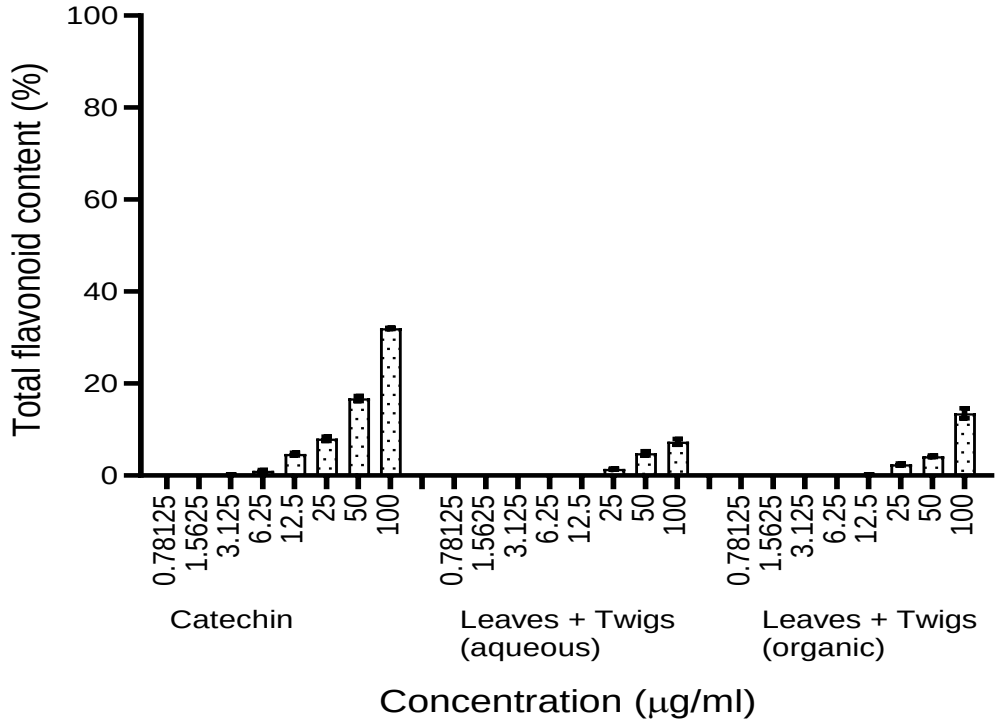


*Figure 6*: The total flavonoid content for the aqueous and organic extracts for the combination of leaves and twigs of *Artemisia afra*. The highest TFC was observed for the organic extract of the combination of leaves and twigs with a total flavonoid content of 16.45 %. There, however, was no significant difference in the TFC between the aqueous extract and organic extract of the twigs (P = 0.1737).

### 4.4 Antioxidant activity of the extracts

The aqueous and organic extracts of the *Artemisia afra* plant species exhibited high antioxidant activity (Figures 7, 8 and 9) compared to the positive control ascorbic acid which exhibited antioxidant activity of 98.69 % at the concentration of 100 µg/ml, and the positive control quercetin which displayed antioxidant activity of 92.31 % at the concentration of 200 µg/ml. The activity was concentration dependent. When considering the $EC_{50}$ values shown in Table 5, the organic extracts exhibited higher

antioxidant activity in comparison to the aqueous extracts – organic (8.233 µg/1L) and aqueous (12.455 µg/mL) twig extract; and organic (5.9118 µg/ml) and aqueous (9.4197 µg/ml) *combo* extract. However, the aqueous leaf extracts (0.1029 µg/ml) had higher antioxidant activity than the organic leaf extract (4.4344 µg/mL). The plant extract and plant part with the highest antioxidant activity *was* the aqueous extract of Artemisia afra leaves with an $EC_{50}$ value of 0.1029 µg/ml.

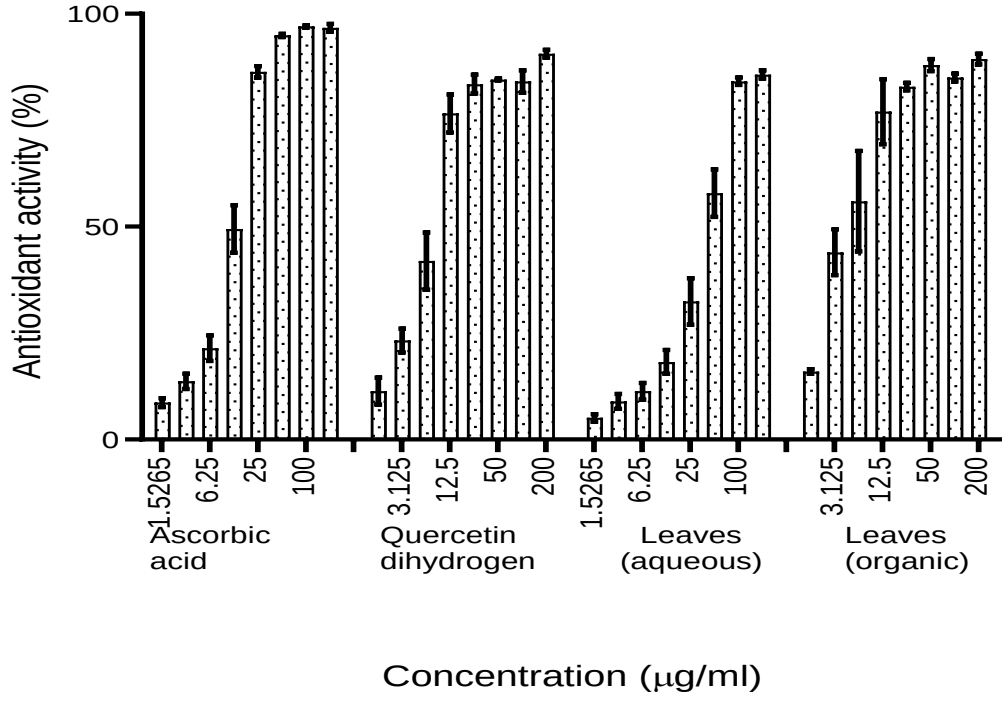


Figure 7: The antioxidant activity of the aqueous and organic extracts of the leaves of *A.* afra. For the leaves, the highest antioxidant activity was observed for the aqueous extract which is 84.49 % at a concentration of 200 µg/ml. There, however, were no significant differences between the means of the aqueous and organic extracts (P = 0.327).

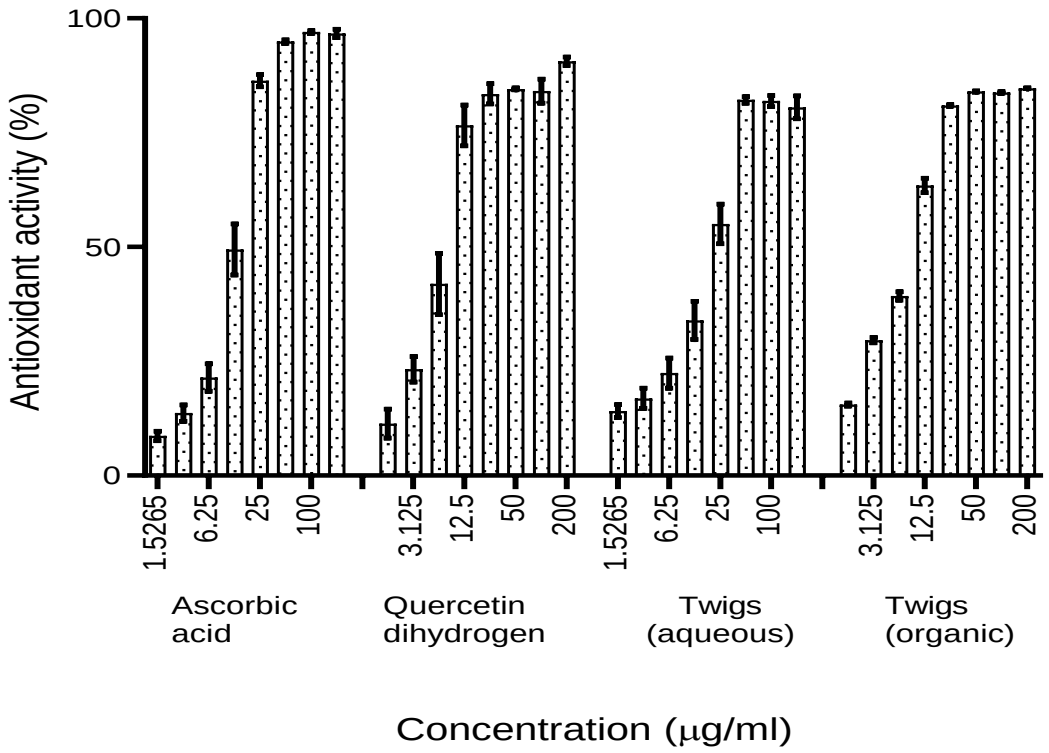


Figure 8: The antioxidant activity of the aqueous and organic extracts of the twigs of A. afra. For the twigs, the highest antioxidant activity was observed for the organic extract which is 86.52 % at a concentration of 200 μg/ml. There, however, were no significant differences between the means of the aqueous and organic extracts (P = 0.8404).

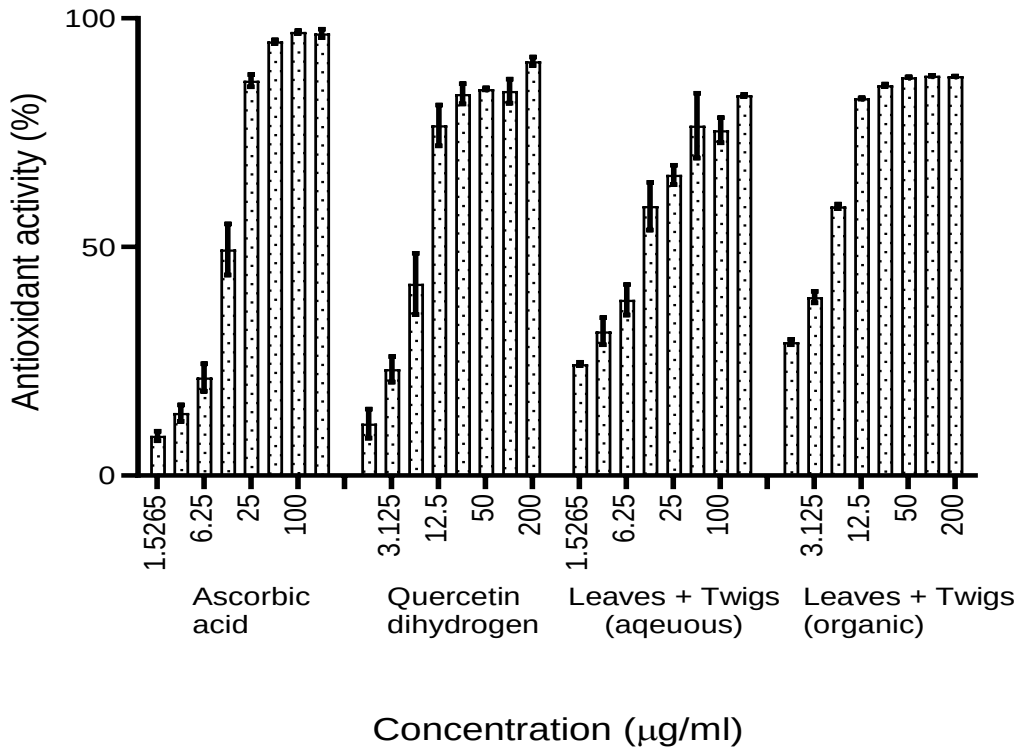


*Figure 9*: The antioxidant activity of the aqueous and organic extracts of the cocktail mixture of the leaves and twigs *of A. afra*. For the combination of leaves and twigs, the highest antioxidant activity was observed for the organic extract which is 85.81 % at a concentration of 200 µg/ml. There, however, were no significant differences between the means of the aqueous and organic extracts (P = 0.8370).

Table 5. $EC_{50}$ of the antioxidant activity of *Artemisia afra* and the positive controls ascorbic acid and quercetin.

| | $EC_{50}$ value (µM) | |
|---|---|---|
| Treatment | Aqueous extracts | Organic extracts |
| Ascorbic acid | 0.2649 | 2.7565 |
| Quercetin | 3.2269 | 4.5849 |
| *Artemisia afra* (Leaves) | 10.291 | 4.4344 |
| *Artemisia afra* (Twigs) | 12.455 | 8.2331 |
| *Artemisia afra (Leaves + Twigs)* | 9.4197 | 5.9118 |

### 4.5 Anti-inflammatory activity of the extracts

The aqueous and organic extracts exhibited anti-inflammatory activity compared to the positive control (Figures 10, 11 and 12). The organic extracts had higher activity, which was concentration dependent. The combination of the leaves and twigs exhibited the highest anti-inflammatory activity of 87.5 % at the highest concentration (100 µg/ml).

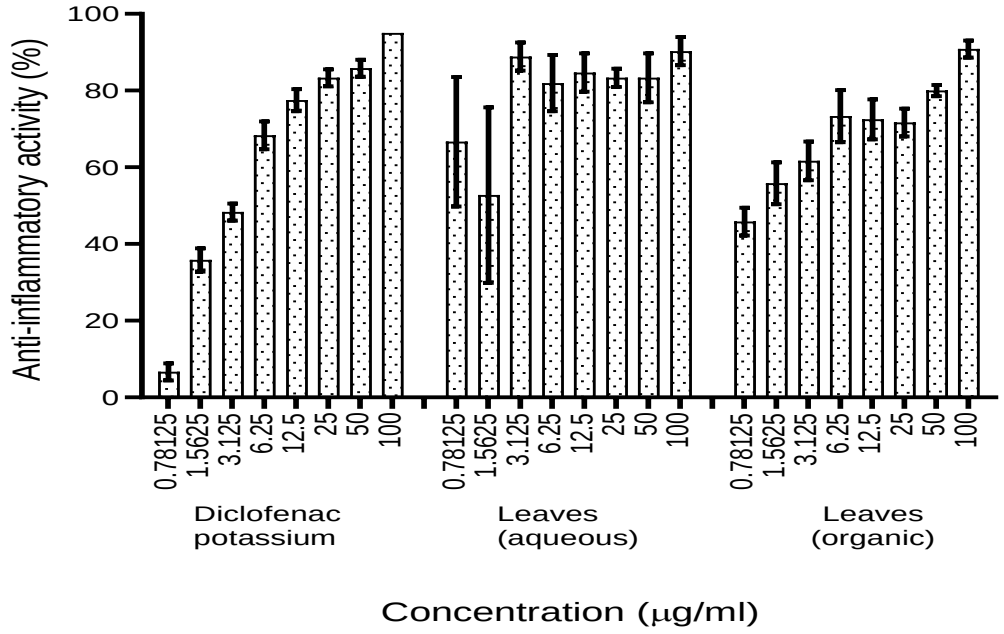


*Figure 10*: The anti-inflammatory activity of the *Artemisia afra* aqueous and organic extracts of the leaves of *A. afra.* Diclofenac potassium was used as the positive control. The highest anti-inflammatory activity is displayed by the organic extract which is 81.92 % at a concentration of 100 µg/ml. There were no significant differences between the means of the aqueous and the organic extracts (P = 0.2952).

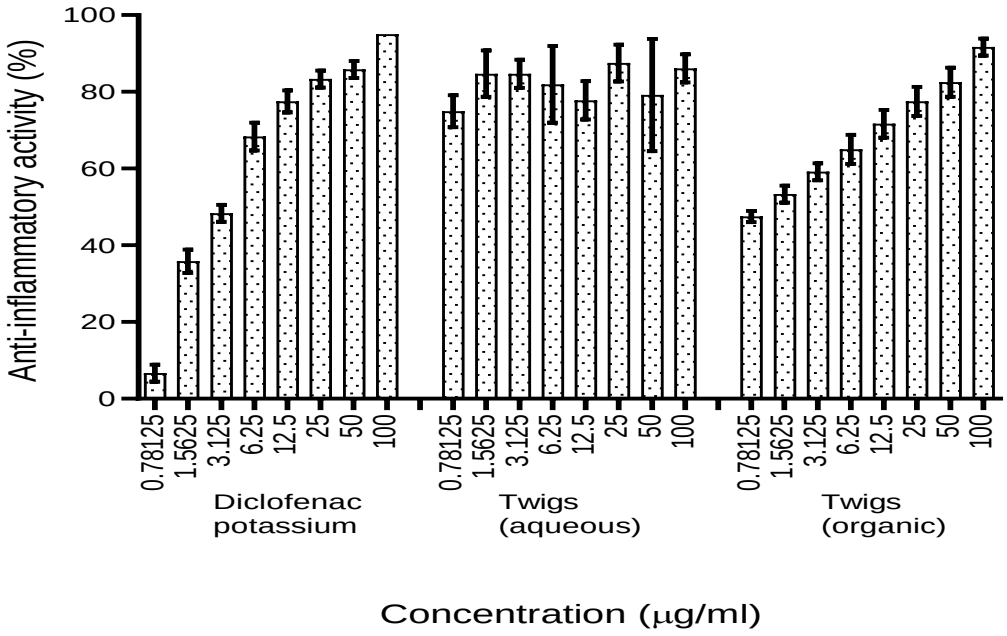


*Figure 11*: The anti-inflammatory activity of the *Artemisia afra* aqueous and organic extracts of the twigs of *A. afra.* Diclofenac potassium was used as the positive control. The highest anti-inflammatory activity is displayed by the organic extract which is 84.61 % at a concentration of 100 µg/ml. There were no significant differences between the means of the aqueous and the organic extracts (P = 0.0496).

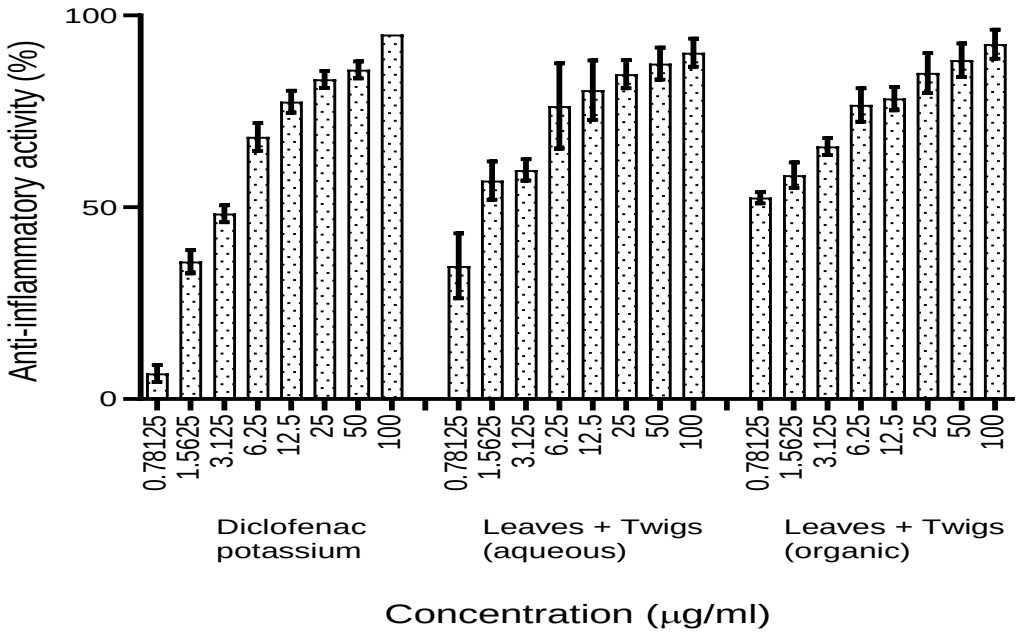


*Figure 12*: The anti-inflammatory activity of the *Artemisia afra* aqueous and organic extracts of the combination of the leaves and twigs of *A. afra.* Diclofenac potassium was used as the positive control. The highest anti-inflammatory activity is displayed by the organic extract which is 87.50 % at a concentration of 100 µg/ml. There were no significant differences between the means of the aqueous and the organic extracts (P =0.5422).

### 4.6 Cytotoxicity of the extracts

The aqueous and organic extracts inhibited the growth of the Vero/hSLAM cells (Figures 13, 14 and 15). The organic extract exhibited higher inhibition than the aqueous extracts and was comparable to the positive control (a poly(vinylchloride) receptor stabilized with organo-tin). The aqueous extracts of each plant part displayed low cytotoxicity of 7.14, 22.82, and 29.2 % at the highest concentrations (333.0 µg/ml) for the leaves, twigs, and combination of leaves and twigs, respectively. At the same concentration, the organic extracts exhibited cytotoxicity of 91.71, 88.49 and 92.59 % for the leaves, twigs, and combination of leaves and twigs, respectively. According to the $IC_{50}$ values (Table 6), the extracts of *A. afra* exhibited significant cytotoxicity. The percentage cytotoxicity obtained for the aqueous extracts was too low to determine the $IC_{50}$ values, thus, only the values for the organic extracts are presented in Table 6. The organic extract of the combination of leaves and twigs was the highest with an $IC_{50}$ value of 134.12 µg/ml, followed by the twigs (151.53 µg/ml), and lastly the leaves (182.31 µg/ml).

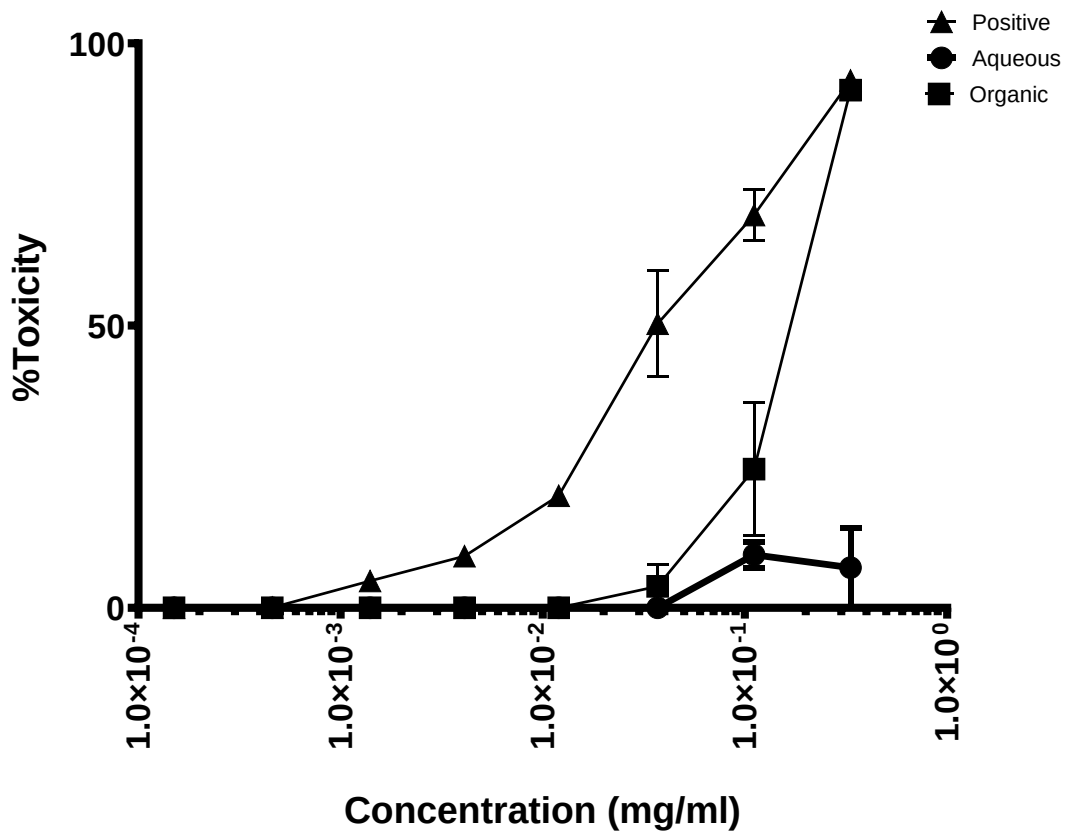


*Figure 13*: The cytotoxicity of the *Artemisia afra* aqueous and organic extracts for the leaves. The highest inhibitory activity is displayed by the organic extract which is 91.71 % at a concentration of 1 mg/ml, while the aqueous extract had an inhibitory activity of 7.14 % at the same concentration. There was a significant difference between the extracts at this concentration ($P < 0.05$). A poly (vinyl chloride) receptor stabilized with organo-tin was used as the positive control.

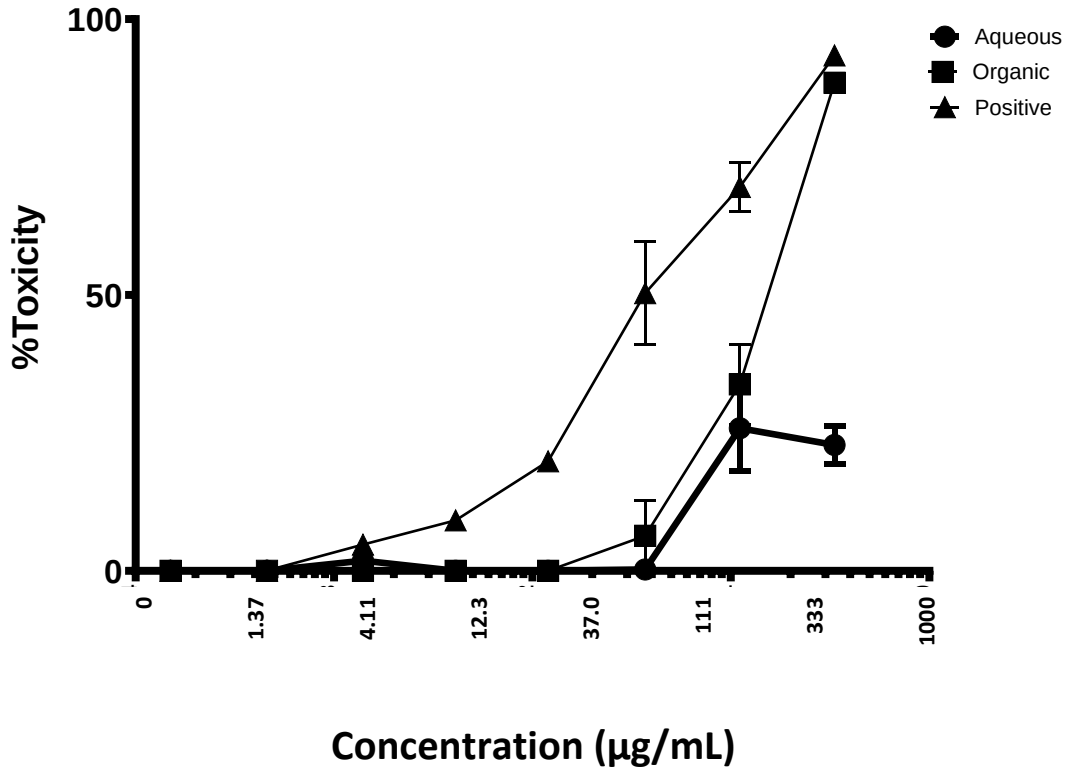


*Figure 14*: The cytotoxicity of the *Artemisia afra* aqueous and organic extracts for the twigs. The highest inhibitory activity is displayed by the organic extract which is 88.49 % at a concentration of 1 mg/ml, while the aqueous extract had an inhibitory activity of 22.82 % at the same concentration. There was a significant difference between the extracts at this concentration ($P < 0.05$). A poly (vinyl chloride) receptor stabilized with organo-tin was used as the positive control.

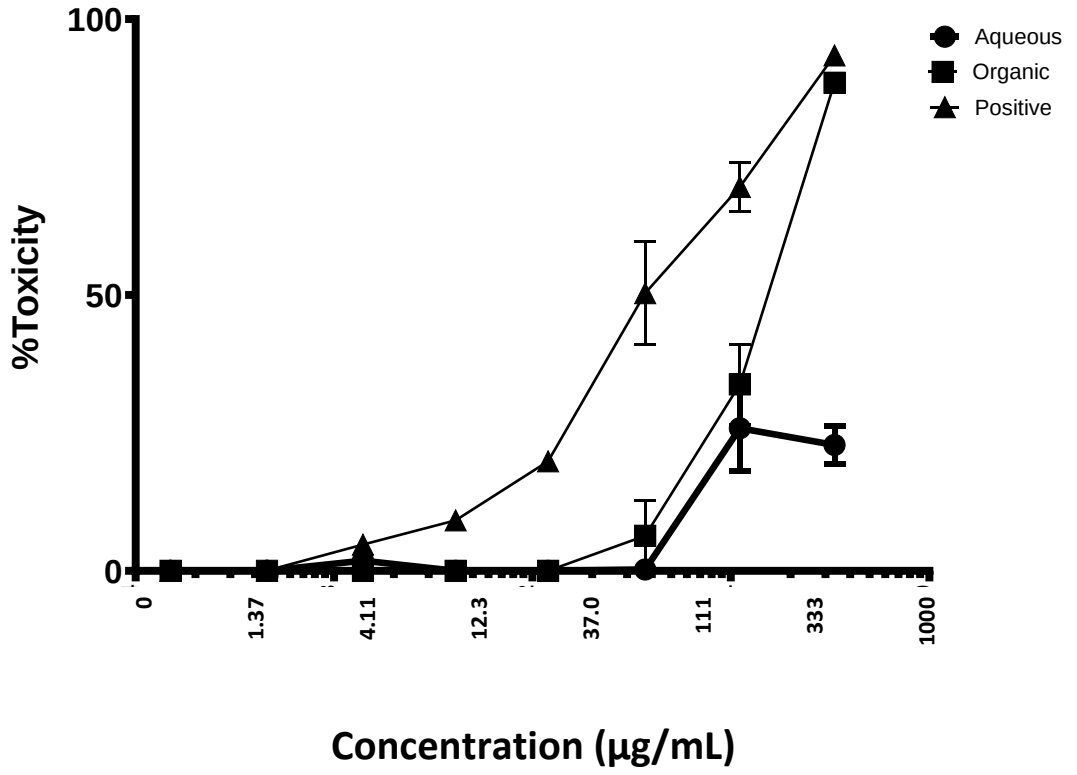


*Figure 15*: The cytotoxicity of the *Artemisia afra* aqueous and organic extracts, for the combination mixture of the leaves and twigs. The highest inhibitory activity is displayed by the organic extract which is 92.59 % at a concentration of 1 mg/ml, while the aqueous extract had an inhibitory activity of 29.20 % at the same concentration. There was a significant difference between the extracts at this concentration ($P < 0.05$). A poly (vinyl chloride) receptor stabilized with organo-tin was used as the positive control.

Table 6. The $IC_{50}$ values for the different plant parts of the organic extracts of the *A. afra* plant using MTT cytotoxicity assay.

| Organic plant extract of *A. afra* | $IC_{50}$ value (µg/ml) |
|---|---|
| Leaves | 182.31 |
| Twigs | 151.53 |
| Cocktail | 134.12 |

### 4.7 Antiviral activity of the extracts

Inhibition of the SARS-CoV-2 infection of 293T-ACE cells was observed for the aqueous and organic extracts of *A. afra* (Figures 16, 17 and 18). The observed antiviral activity increased as the concentration of the extracts also increased. The organic extracts displayed higher activity than the aqueous extracts for the leaves and twigs displaying inhibition of 97.65 and 89.75 %, respectively. The inhibition for the aqueous extracts was 56.55 and 52.50 %, respectively. However, for the combination of the leaves + twigs, the aqueous extract (62.70%) had higher activity than the organic extract (50.45%). According to Table 7, the organic extracts of the twigs displayed the highest activity with an $EC_{50}$ value of 24.65µg/ml, followed by the organic extract of the leaves 48.85 µg/ml. The aqueous extract of the twigs had the lowest antiviral activity with an $EC_{50}$ value of 352.12 µg/ml.

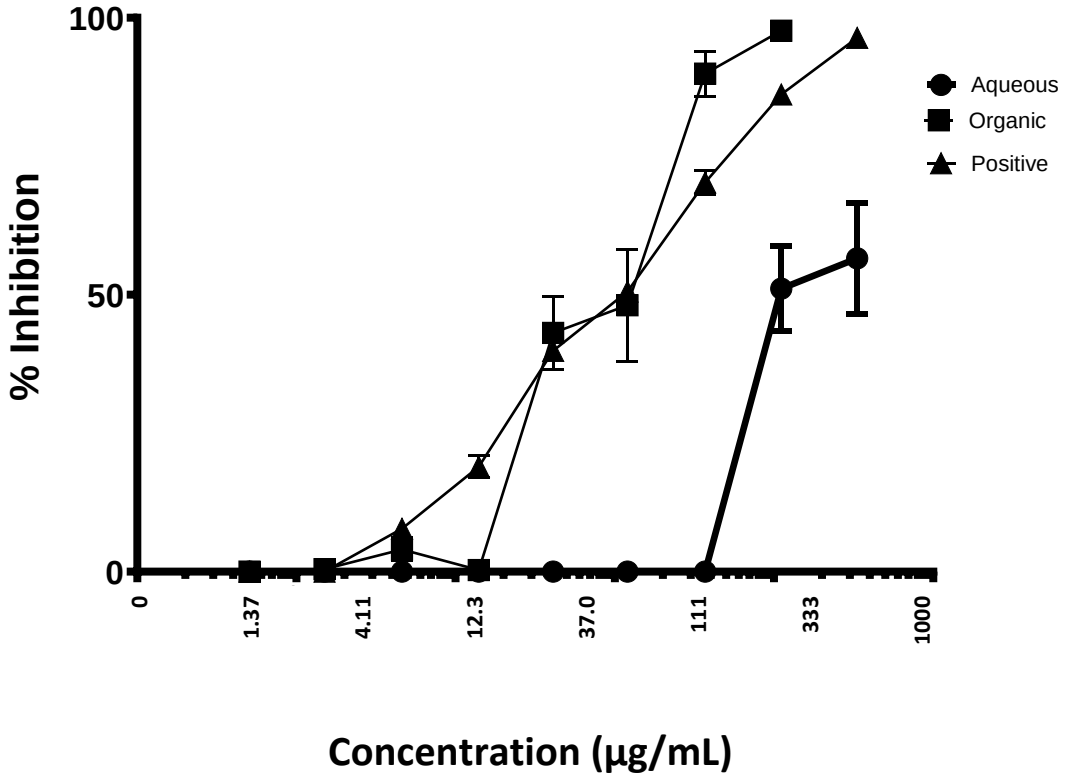


*Figure 16*: The percentage inhibition of the SARS-CoV-2 infection in 293T-ACE cells by the organic and aqueous extracts of the leaves of *A. afra*. The highest inhibitory activity is displayed by the organic extract which was 97.65 % at a concentration of 1 mg/ml, while the aqueous extract displayed an inhibition of 56.55 %. There was a significant difference between the extracts at this concentration ($P < 0.05$). A mixture of DMEM, luciferase substrate and untreated SARS-Cov 2 infected cells was used as the positive control.

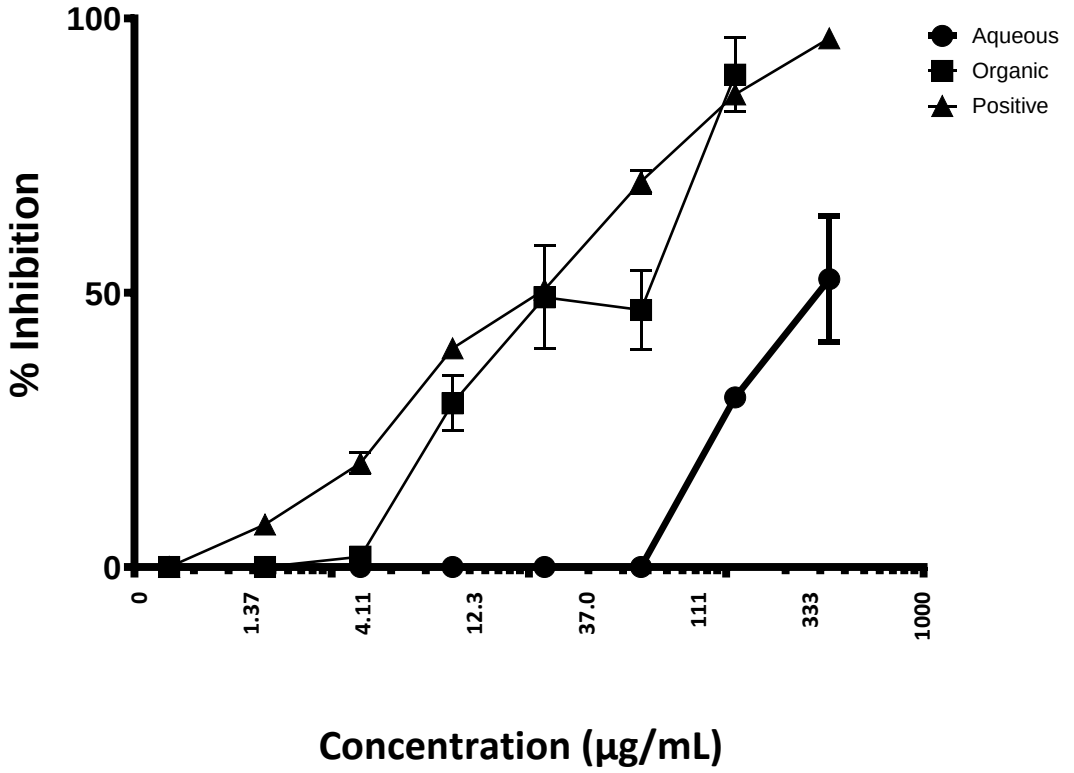


*Figure 17*: The percentage inhibition of SARS-CoV-2 infection in 293T-ACE cells by the organic and aqueous extracts of the twigs of *A. afra*. The highest inhibitory activity is displayed by the organic extract which was 89.75 % at a concentration of 1 mg/ml, while the aqueous extract displayed an inhibition of 52.50 %. There was a significant difference between the extracts at this concentration ($P < 0.05$). A mixture of DMEM, luciferase substrate and untreated SARS-Cov 2 infected cells was used as the positive control.

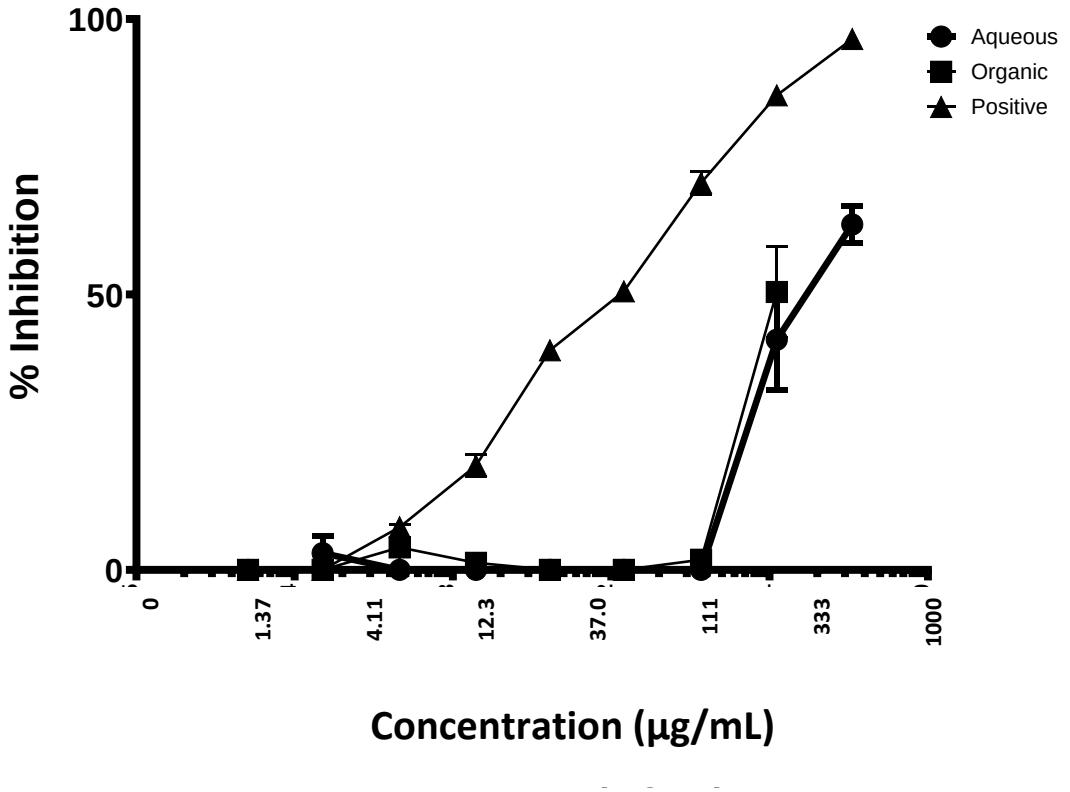


*Figure 18*: The percentage inhibition of SARS-CoV-2 infection in 293T-ACE cells by the organic and aqueous extracts of the combination of the leaves and twigs of *A. afra*. The highest inhibitory activity is displayed by the organic extract which was 50.45 % at a concentration of 1 mg/ml, while the aqueous extract displayed an inhibition of 62.70%. There was no significant difference between the extracts at this concentration ($P > 0.05$). A mixture of DMEM, luciferase substrate and untreated SARS-Cov 2 infected cells was used as the positive control.

Table 7. The half-maximal effective concentration ($EC_{50}$) of the *Artemisia afra* plant shows the inhibition of the SARS-CoV-2 infecting 293T-ACE cells.

| Plant extract of *A. afra* | $EC_{50}$ value (µg/mL) |
|---|---|
| Leaves (aqueous) | 222.07 |
| Leaves (organic) | 48.84 |
| Twigs (aqueous) | 352.11 |
| Twigs (organic) | 24.65 |
| Leaves + Twigs (aqueous) | 336.96 |
| Leaves + Twigs (organic) | 251.67 |

## CHAPTER FIVE: DISCUSSION

To explore the potential of 'Wilde als' *(A. afra)* as an antiviral agent, the organic and aqueous extracts of *Artemisia afra* were screened for flavonoid content, antioxidant, anti-inflammatory and antiviral activities, as well as cytotoxic properties. Thin-layer chromatography was also used to determine classes of biologically active compounds. In this study, the organic extracts of the plant species exhibited a higher percentage extraction yield. The cell polarity of the *Artemisia afra* may have affected the yield of the aqueous and organic extracts. A study by Dettmer and Friml (2011) noted that a vital feature of cell polarity is the distribution of proteins in or surrounding the plasma membrane in an asymmetrical manner. An investigation by Yang *et al.* (2020) defined cell polarity as the reason for significantly low yields for aqueous extractions. This is due to the reactions that occur between the polar water solvent and the polar plant cells. For future research purposes, Cheng *et al.* (2021) encouraged the use of subcritical water for water extraction processes. Subcritical water is defined by Cheng *et al.* (2021) as water in a high-temperature and high-pressure form – the reason for this is to dramatically decrease the polarity of water so that it may act like nonpolar solvents such as hexane.

The purpose of thin-layer chromatography was to provide a preliminary screening of the various phytochemical compounds within plant extracts with anti-inflammatory, antiviral, antimalarial, antioxidant and antimicrobial activity. The findings of this study indicated that all the plant parts of the aqueous and organic extracts contained flavonoids, coumarins, steroids, terpenoids and saponins. According to Kane *et al.* (2019), a previous study attributed the known antioxidant and antiviral properties of

*A. afra* to flavonoids. Flavonoids are a group of natural substances with variable phenolic structures and are regarded as natural antioxidant agents; because of their chemical structure, flavonoids can prevent oxidative and reductive stress on the cells by binding to free radicals that may be present. Panche, Diwan and Chandra (2016) further illustrated flavonoids can modulate cellular enzyme function. They are chemically classified or subdivided depending on the carbon of the C ring on which the B ring is attached, secondly, the classification depends on the degree of unsaturation and oxidation of the C ring. Isoflavones are flavonoids in which the B ring is linked in position 3 of the C ring; while flavonoids that have a B ring attached to position 3 of the C ring are called isoflavones (Panche, Diwan and Chandra, 2016). Lastly, flavonoids where the B ring is linked in position 4 of the C ring are classified as neoflavonoids; while flavonoid compounds in which the B ring is linked in position 2 are further subdivided into various subgroups based on the structural features of the C ring (Panche, Diwan and Chandra, 2016). The subgroups are notably: flavones, chalcones, flavanonols, flavanones, flavonols, flavanols, catechins and anthocyanins.

Significant antioxidant activity was found in both the aqueous and organic extracts of *A. afra*. The total flavonoid content was analyzed to confirm this. The results indicated that the aqueous extract of the twigs of *A. afra* had the highest flavonoid content of 19.89 % which is similar in comparison to the study conducted by Kane *et al.* (2019) where an ethanolic extract of the twigs contained 21.2% flavonoids. Both these solvents that were used have high polarity indices. The antioxidant activity *of A. afra* extracts in this study is consistent with a study by Sunmonu and Afolayan (2012), demonstrating strong antioxidant activity in the plant's aerial components., which according to literature is true for all plants (Sunmonu and Afolayan, 2012). A review

by Lee, Jung and Hong (2021) noted that the reason for the high antioxidant activity of the aerial parts of plants is attributed to the presence of chloroplasts and mitochondria as they are the active sites of ROS generation within plant cells during the respiration process (Kasote *et al..,* (2015). Since the leaves are the main site of respiration in plants, Lee, Jung and Hong (2021) noted the high levels of reactive oxygen species which were the resulting by-products of aerobic pathways of the metabolic processes.

To add, a study by Codorniu-Hernandez, Rolo-Naranjoa, and Montero-Cabrera (2007) investigated the antioxidative mechanism of flavonoids and determined that the radical scavenging activity of flavonoids is attributed to the presence of phenolic hydroxyl groups. The transfer of the hydrogen atom or single electron is followed by the transfer of a proton (Trung *et al.,* 2022). This bond dissociation energy (BDE) of the hydroxyl moiety is a key factor in the comparison and explanation of the antioxidant properties (Trung *et al.,* 2022). Simply put, BDE is the energy required during an endothermic reaction to break the bond and form two molecular or atomic fragments. Each fragment has one electron of the original shared pair. Vo *et al.* (2019) rationalized that the BDE of flavonoids is significantly large – thus attributing to the flavonoid's ability to act as an antioxidant.

A study by Chaiya *et al.* (2022) focused on *in vitro* anti-inflammatory activity of medicinal plant extracts and determined the link between the thermally inhibiting protein denaturation of egg albumin and the process of inflammation. Protein denaturation is a process where external factors such as heat, an organic solvent, a strong base or acid, or an inorganic salt could cause the protein to denature which

means that the tertiary structure and ultimately the secondary structure of the protein becomes disoriented (Liu *et al..*, 2013). As this process occurs, enzymes will lose their activity since the substrates are no longer able to attach to the active site of the enzyme (Liu *et al..*, 2013). According to Dharmadeva *et al.* (2018), the denaturation of the protein process is based on an unpredictable mechanism that includes the modification of electrostatic hydrogen, as well as hydrophobic and disulfide bonding. The link between inhibition of protein denaturation and inflammation is the production of autoantigens due to the denaturation of proteins which may result in inflammatory conditions such as diabetes, cancer and rheumatic arthritis (Dharmaveda *et al..*, 2018). Therefore, the inhibition of protein denaturation may inhibit inflammatory activity. The extracts of *A. afra* in this study had anti-inflammatory activity, indicating their ability to inhibit the denaturing process of proteins.

Chaiya *et al.* (2022) defined inflammation as a defense mechanism responsible for enabling the body to protect itself from burns, toxic chemical allergens, infections, or any other harmful stimuli; Chaiya *et al..* (2022) continued by noting that inflammation is a substantial reaction to any damage, destruction or disease that may be portrayed by redness, heat, swelling and any disturbed physiological functions. The role of inflammation is to repair any possible which does not result from the inflammation itself but from the overactivation or deviation of underlying physiological processes (Silvestrini and Silvestrini, 2022). Various mechanisms have been suggested to explain the anti-inflammatory potential of the *A. afra* plants including the inhibition of 15- lipoxygenase (LOX). The lipoxygenase group of enzymes, such as 5, 8, 12 and 15 play a role in multiple inflammatory disorders. Chidea and Jisaka (2013) explain that

15-lipoxygenase is a key isomeric enzyme that is involved in the synthesis of leukotrienes from arachidonic acids.

Chidea and Jisaka (2013) further explain that biologically active leukotrienes are the main mediators of multiple pro-inflammatory and allergic reactions, thus the inhibition of the synthesis of leukotrienes by 15-LOX is regarded as one of the therapeutic strategies in managing inflammatory conditions. Another mechanism which explains the anti-inflammatory potential of *A. afra* is the inhibition of NOS. NOS, also referred to as nitric oxide by Steyn *et al.* (2002) has reported that plant flavonoids inhibit the production of NO, thereby the downregulation of the expression of iNOS. Lastly, amino-substituted flavones and flavones have been reported to inhibit NO production (Steyn *et al.*, 2002).

The observed cytotoxic effects of the organic extracts of *A. afra* were higher than the aqueous extracts, which may be explained by the presence of terpenoids. Terpenoids are organic compounds that are derived from isoprene – a five-carbon ring structure that has various cytotoxic activities based on the assembly and modification of the isoprene unit (Perez-Soto, 2019). Erasto and Viljoen (2008) as cited by Perez-Soto *et al.* (2019) noted that the main mechanism of most terpenoids is the inhibition of the posttranslational isoprenylation of the cell-growth regulatory proteins like Ras, which induces cell death. Malko and Wroblewska (2016) pointed out that the terpenoids in the review demonstrated the ability to decrease cell viability in a dose-dependent manner by the induction of apoptotic cell death. The induction of apoptotic cell death led to the activation of caspase-3 and caspase-9, PARP cleavage and Bax protein. The

induction of apoptotic cell death also activated the cytosolic release of cytochrome C from the mitochondria and the attenuation of the expression of the Bcl-2 protein. Malko and Wroblewska (2016) note that the mechanism of induction of apoptotic cell death suggests that terpenoids could induce apoptosis via the mitochondrial pathway via the suppression of the PI3K/AKT pathway (He *et al..*, 2021).

In the SARS-CoV 2 inhibition assay, the organic extracts exhibited higher antiviral potential which is in line with the cytotoxic activities observed in this study, and the antiviral activities obtained by Nie *et al.* (2021) which notes that the antiviral activity of the *Artemisia afra* plant may be attributed to its ability to inhibit ACE-2 protein activity which leads to the loss of a viral receptor of the host cell. Efferth *et al.* (2008) as cited by Nie *et al.* (2021) indicated that the antiviral compound found in *Artemisia* species, known as artemisinin, is the active pharmaceutical agent that is responsible for the antiviral potential of the *Artemisia* species.

## CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

The findings of this study suggest that ‘Wilde als’ has the potential to be used as an antiviral with antioxidant and anti-inflammatory activities as the possible mechanisms of action, with low cytotoxic effects for the aqueous extracts. The organic extracts, however, were cytotoxic; but when used in the correct doses, they can have significant health benefits. This study also provides evidence that the utilization of Wilde as an herbal remedy in the southern region of Namibia is logical and aligns with findings from other areas of Africa. Validating ‘Wilde als’ as an antiviral agent may open the door to further studies and for the integration of it into mainstream health care as complementary or alternative medicine for colds, flu and other viral infections including COVID-19, and viral infections that exist or may emerge. This study also showed that *A. afra* contains flavonoids, which may contribute to the observed biological activities. To our knowledge, this is the first study on *A. afra* found in Namibia as no previous research has been done. To conclude, further research on medicinal and traditionally used plants is necessary as a high percentage of traditional healers and indigenous communities across the African continent have vital information on these medicinal plants which have not been properly researched y*et al.*

For future studies, it is important to conduct additional assays to ascertain the plant's therapeutic properties such as immune-modulating assays to determine whether *A. afra* can modulate and maintain the immune system and activities related to the immune system, and techniques such as HPLC and or LCMS to identify the specific biologically active compounds. Other studies can also include such as the redox titration antioxidant assay using an iodine solution assay to determine the

concentration of vitamin C or ascorbic acid within the aqueous and organic extracts. Another recommendation would be to conduct the SRB cytotoxicity assay to confirm the safety of the extracts using more cell lines including Vero cells, as well as

## REFERENCES


Adebowale, B., Diyamett, B., Lema, R. and Oyelaran-Oyeyinka, O. (2014). Innovation research and economic development in Africa. *Introduction of African Journal of Science, Technology, Innovation and Development, 6,* 5-11.

Akamine, C., Clark, E and Shandera, W. X. (2023). *Adenovirus Infections.* McGraw-Hill Publishers.

Armitage, C. J., Keyworth, C., Leather, J. Z., Byrne- Davis, L. and Epton, T. (2021). Identifying targets for interventions to support public adherence to government instructions to reduce transmission of SARS-CoV-2. *BMC Public Health, 21,* 522.

Barkat, M.A., Goyal, A., Barkat, H.A., Salauddin, M., Pottoo, F.H. and Anwer, E.T. (2020) Herbal medicine: Clinical perspective and regulatory status. *Combination Chemistry*.

Boudreau, A., Burke, S. J., Collier, J. J., Richard, A. J., Ribnicky, D. M. andStephens, J. M. (2020) Mechanisms of *Artemisia scoparia*'s anti-inflammatory activity in cultured adipocytes, macrophages, and pancreatic β-cells. *Obesity, 28,* 1726–1735.

Cao, R., Hu, H., Li, Y., Wang, X., Xu, M., Liu, J., Zhang, H., Yan, Y., Zhao, L., and Li, W., *et al.*. (2020). Anti-SARS-CoV-2 potential of artemisinins in vitro. *ACS Infectious Diseases, 6,* 2524–2531

Casanova, J. and Abel, L. (2021). Mechanisms of viral inflammation and disease in humans. *Science, 374*(6571), 1080 – 1086.

Caughey, G. E., Tait, K., Vitry, A. I., and Shakib, S. (2017). Influence of medication risks and benefits on treatment preferences in older patients with multimorbidity. *Patient Preference Adherence, 11,* 131-140.

Chaiya, P., Senarat, S., Phaechamud, T. and Narakornwit, W. (2022). *In vitro* anti-inflammatory activity using thermally inhibiting protein denaturation of egg albumin and antimicrobial activities of some organic solvents. *Materials Today: Proceedings, 65*(4), 2290-2295.

Chauhan, A., Walton, M., Manias, E., Walpola, R. L., Seale, H., Latanik, M., Leone, D., Mears, S. and Harrison, R. (2020). The safety of health care for ethnic minority patients: a systematic review. *International Journal for Equity in Health, 19,* 118.

Cheikhyoussef, A., Shapi, M., Matengu, K. and Ashekele, H. M. (2011). Ethnobotanical study of indigenous knowledge on medicinal plant use by traditional healers in Oshikoto region, Namibia. *Journal of Ethnobiology and Ethnomedicine. 7.*

Chen, W. (2020). A potential treatment of COVID-19 with TGF-beta blockade. *International Journal of Biological Sciences, 16,* 1954-1955.

Chen, B., Julg, B., Mohandas, S. and Bradfute, S. B. (2023). Viral persistence, reactivation and mechanisms of long COVID. *ELife, 12,* e86015.

Cheng, C., Ho, W. E., Goh, F. Y., Guan, S. P., Kong, L. R., Lai, W. Q., Leung, B. P., and Wong, W. S. (2011). Anti-malarial drug artesunate attenuates experimental allergic asthma via inhibition of the phosphoinositide 3-kinase/Akt pathway. *PLoS ONE.*

Cheng, Y., Xue, F., Yu, S., Du, S. and Yang, Y. (2021). Subcritical water extraction of natural products. *Molecules, 26*(13), 4004.

Chidea, V. S. and Jisaka, M. (2013). Lipoxygenase and carotenoids: a co-oxidation story. *Academic Journals, 12*(20), 2786-2791.

Codorniu-Hernández, E., Rolo-Naranjoa, A. and Montero-Cabrera, L. A. (2007). Theoretical affinity order among flavonoids and amino acid residues: an approach to understanding flavonoid–protein interactions. *Journal of Molecular Structures, 819,* 121–129

Compaore, M., Meda, R. N., Bakasso, S., Vlase, L. and Kiendrebeogo, M. (2016). Antioxidative, anti-inflammatory potentials and phytochemical profile of *Commiphora Africana* (A. Rich.) Engle. (Burseraceae) and *Loeseneriella africana* (Willd.) (Celastraceae) stem leaves extracts. *Asian Pacific Journal of Tropical Biomedicine, 6*(8), 665-670.

Dettmer, J. and Friml, J. (2011). Cell polarity in plants: when two do the same, it is not the same… *Current Opinion in Cell Biology, 23*(6), 686-696.

Dhama, K., Karthik, K., Khandia, R., Munjal, A., Tiwari, R., Rana, R., Khurana, S. K., Ullah, S., Khan, R. U., Alagawany, M., Farag, M. R., Dadar, M., and Joshi, S. K. (2018). Medicinal and therapeutic potential of herbs and plant metabolites/ extracts countering viral pathogens- current knowledge and future prospects. *Current Drug Metabolism, 19*(3), 236-263.

Dharmadeva, S., Galgamuwa, L. S., Prasadinie, C. and Kumarasinghe, N. (2018). *In vitro* anti-inflammatory activity of *Ficus racemosa* L. bark using albumin denaturation method. *Ayu Journal, 39*(4), 239-242.

Du Preez, C. I., Grundermann, C., Reinhardt, J. K., Mumbengegwi, D. R. and Huber, R. (2020). Immunomodulatory effects of some Namibian plants traditionally used for treating inflammatory diseases. *Journal of Ethnopharmacology*, 254.

Du Toit, A. and van der Kooy, F. (2019). *Artemisia afra*, a controversial herbal remedy or a treasure trove of new drugs? *Journal of Ethnopharmacology, 244,* 112127.

Ebu, V., Anoh, R., Offiong, R., *et al.*. (2021). Survey of medicinal plants used in the treatment of 'Ailments of utmost native importance' in cross river state, Nigeria. *Open Journal of Forestry, 11,* 330-339.

Edquist, C. (2005). Systems of innovation: perspectives and challenges. *The Oxford Handbook of Innovation.* Oxford University Press, UK, 181-208.

Efferth, T., Romero, M. R., Wolf, D. G., Stamminger, T., Marin, J. J. G. and Marschall, M. (2008). The antiviral activities of artemisinin and artesunate. *Clinical Infectious Diseases. 47*(6), 804-811.

Efridi, W. and Lappin, S. L. (2023). Poxviruses. *StatPearls.*

Elesela, S. and Lukacs, N. W. (2021). Role of mitochondria in viral infections. *Life, 11*, 232 – 247.

Erasto, P. and Viljoen, A. (2008). Limonene – A review: Biosynthetic, ecological and pharmacological relevance. *Natural Product Communications, 3*(7), 1193-1199.

Faist A, Janowski J, Kumar S, Hinse S, Çalışkan DM, Lange J, *et al.*. Virus Infection and Systemic Inflammation: Lessons Learnt from COVID-19 and Beyond. Cells. 2022 Jul 14;11(14):2198.

Fattahi, S., Zabihi, E., Abedian, Z., Pourbagher, R., Ardekani, A. M., Mostafazadeh, A. and Akhavan-Niaki, H. (2014). Total phenolic and flavonoid contents of aqueous extract of stinging nettle and *in vitro* antiproliferative effect on Hela and BT – 474 cell lines. *International Journal of Molecular and Cellular Medicine. 3*(2), 102-107.

Ferner, R. E. (2014). Harms from medicines: inevitable, in error or intentional. *British Journal of Clinical Pharmacology, 77*(3), 403-409.

Gain, C., Song, S., Angtuaco, T., Satta, S. and Kelesidis, T. (2022). The role of oxidative stress in the pathogenesis of infections with coronaviruses. *Frontiers in Microbiology, 13*, 1111930.

Gendrot, M., Duflot, I., Boxberger, M., Delandre, O., Jardot, P., Le Bideau, M., Andreani, J., Fonta, I., Mosnier, J. and Rolland, C. (2020). Antimalarial artemisinin-based combination therapies (ACT) and COVID-19 in Africa: in vitro inhibition of SARS-CoV-2 replication by mefloquine-artesunate. *International Journal of Infectious Diseases, 99*, 437–440.

Gilmore, K., Zhou, Y., Ramirez, S., Pham, L. V., Fahnøe, U., Feng, S., Offersgaard, A., Trimpert, J., Bukh, J., and Osterrieder K., *et al.*. (2020). In vitro efficacy of artemisinin-based treatments against SARS-CoV-2. *Scientific Reports, 11*, 14571.

Gunathilake, K. D. P. P., Ranaweera, K. K. D. S. and Rupasinghe, H. P. V. (2018). *In vitro* anti-inflammatory properties of selected green leafy vegetables. *Biomedicines. 6*(4), 107.

Harbone, A. J. (1998). *Phytochemical methods a guide to modern techniques of plant analysis.* (3rd ed.). Springer Science and Business.

Harvard Medical School. (2021, 27 April). *How to boost your immune system.* Harvard Health Publishing. https://www.health.harvard.edu/staying-healthy/how-to-boost-your-immune-system

He, Y., Sun, M. M., Zhang, G. G., Yang, J., Chen, K. S., Xu, W. W. and Li, B. (2021). Targeting PI3K/AKT signal transduction for cancer therapy. *Signal Transduction and Targeted Therapy, 6*(425).

Houghton, P., Fang, R., Techatanawat, I., Steventon, G., Hylands, P. J. and Lee, C. C. (2007). The sulphorhodamine (SRB) assay and other approaches to testing plant extracts and compounds for activities related to reputed anticancer activity. *Methods, 42*, 377-387.

Houldcroft, C. J., Roy, S., Morfopoulou, S., Margetts, B. K., Depledge, D. P., Cudini, J., Shah, D., Brown, J. R., Roy, E. Y., Williams, R., Cloutman-Green, E., Rao, K., Standing, J. F., Hartley, J. C. and Breuer, J. (2018). Use of whole-genome sequencing of Adenovirus in immunocompromised pediatric petients to identify Nosocomial transmission and mixed-genotype infection. *The Journal of Infectious Diseases, 218*(8), 1261-1271.

Irish, J. (2022). *Artemisia afra. Tree Atlas of Namibia*. https://treeatlas.biodiversity.org.na/viewspec.php?nr=66

Ivanov AV, Valuev-Elliston VT, Ivanova ON, Kochetkov SN, Starodubova ES, Bartosch B, *et al.*. Oxidative Stress during HIV Infection: Mechanisms and Consequences. Oxid Med Cell Longev. 2016;2016:8910396.

Jamal, Q. M. S. (2022). Antiviral potential of plants against COVID-19 during outbreaks- an update. *International Journal of Molecular Sciences, 23*(21), 13564.

Jassim, S. A. A., and Naji, M. A. (2023). Novel antiviral agents: a medicinal plant perspective. *Medicina, 57*(3), 217.

Jauhiainen, J. and Hooli, L. J. (2017). Indigenous knowledge and developing countries'innovation systems: the case of Namibia. *International Journal of Innovation Studies, 1*(1), 89 - 106.

Kane, N. F., Kyama, M. C., Nganga, J. K., Hassanali, A., Diallo, M. and Kimani, F. T. (2019). Comparison of phytochemical profiles and antimalarial activities of *Artemisia afra* plant collected from five countries in Africa. *South African Journal of Botany, 125,* 126-133.

Kasote, D. M., Katyare, S. S., Hedge, M. V. and Bae, H. (2015). Significance of antioxidant potential of plants and its relevance to therapeutic applications. *International Journal of Biological Sciences. 11*(8), 982 – 991.

Koonin, E. V., Krupovic, M. and Agol, V. I. (2021). The Baltimore Classification of viruses 50 years later: how does it stand in the light of virus evolution. *Microbiology and Molecular Biology Reviews, 85*(3), e00053-21.

Kniert, J., dos Santos, T., Eatin, H. E., Cho, W. J., Plummer, G. and Shmulevitz, M. (2022). Reovirus uses temporospatial compartmentalization to orchestrate core versues outercapsid assembly. *Plos Pathogens.*

Kriel, Y. (2010). The immune-modulating activity of *Artemisia afra*. Magister Scientiae- MSc Thesis. Cape Town: The University of the Western Cape.

Kshirsagar, S. G., and Rao, R. V. (2021). Antiviral and immunomodulating effects of *Artemisia. Medicina, 57*(3), 217.

Kuete, V., Karaosmanoğlu, O., and Sivas, H. (2017). Chapter 10 - Anticancer Activities of African Medicinal Spices and Vegetables. *Medicinal Spices and Vegetables from Africa, Academic Press,* 271-297.

Laldingliani, T. B. C., Thangjam, N. M., Zomuanawma, R., Bawitlung, L., Pal, A. and Kumar, A. (2022). Ethnomedicinal study of medicinal plants used by Mizo tribes in Champhai district of Mizoram, India. *Journal of Ethnobiology and Ethnomedicine. 18*(22).

Lee, N. H., Jung, D. S. and Hong, J. (2021). Antioxidant properties and protective effects of aerial parts from *Cnidium officinal* makino on oxidative stress-induced neuronal cell death. *Preventative Nutrition and Food Science. 26*(2), 200 – 208.

Liu, D., Ahmet, A., Ward, L., Krishnamoorthy. P. and Mandelcorn, E.D. (2013) . A practical guide to the monitoring and management of the complications of systemic corticosteroid therapy. *Canadian Society of Allergy and Clinical Immunology, 9*, 30

Liu, N. Q., Choi, Y. H., Verpoorte, R. and van der Kooy, F. (2010). Comparative quantitative analysis of artemisinin by chromatography and qNMR. *Phytochemical Analysis*, *21*(5), 451-456.

Logiel, A., Jors, E., Akugizibwe, P., and Ahnfeldt-Mollerup, P. (2021). Prevalence and socio-economic factors affecting the use of traditional medicine among 159 adults of Katikekile Subcounty, Moroto District, Uganda. *African Health Sciences, 21*(3), 1410-1417.

Lundvall, B., Joseph, K., Chaminde, C. and Vang, J. (2009). *Handbook of Innovation and Developing Countries: Building Domestic Capabilities in a Global Setting*, Edward Elgar, UK.

Lwoff, A. (1957). The concept of virus. *Journal of General Microbiology*, *17*(2), 239-253.

Macri, A. and Crane, J. S. (2022). Parvoviruses. *StatPearls.*

Malko, M. W. and Wroblewska, A. (2016). The importance of R- (+)-limonene as the raw material for organic syntheses and for organic industry. *Chemik, 70*(4), 193-202.

Maguraushe, K. A. (2017). Phytochemical analysis, radical scavenging and the anti-microbial activity of the leaves of *Gomphocarpus fruticosus. UNAM Repository.*

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods. 65*(1-2), 55-63.

Mothibe, M. E. and Sibanda, M. (2019). African traditional medicine: South African perspective

Moyo, E., Mhango, M., Moyo, P., Dzinamarira, T., Chitungo, I. and Murewanhema, G. (2023). Emerging infectious diseases outbreaks in Sub-Saharan Africa: learning from the past and present to be better prepared for future outbreaks. *Frontiers Public Health, 11*.

Mukinda, J. T. (2007). Acute and chronic toxicity of the flavonoid- containing plant, *Artemisia afra* in rodents. Magister Scientiae- MSc Thesis. Cape Town: The University of the Western Cape.

Nagappan, R. (2012). Evaluation of aqueous and ethanol extract of bioactive medicinal plant, Cassia didymobotrya (Fresenius) Irwin and Barneby against immature stages of filarial vector, Culex quinquefasciatus Say (Diptera: Culicidae). *Asian Pacific Journal of Tropical Biomedicine*, *2*(9),707-11

Nair, M. S., Huang, Y., Fidock, D. A., Polyak, S. J., Wagoner, J., Towler, M. J., and Weathers, P. J. (2021). *Artemisia annua* L. extracts inhibit the *in vitro* replication of SARS-CoV-2 and two of its variants. *Journal of Ethnopharmacology, 274,* 114016.

National Institute of Allergy and Infectious Diseases. (2023. 25 April). *Types of primary immune deficiency diseases.* https://www.niaid.nih.gov/diseases-conditions/types-pidds

New Era. (2020, March 02). *Reclaiming indigenous knowledge in Namibia's post colonial curriculum.* https://neweralive.na/posts/reclaiming-indigenous-knowledge-in-namibias-post-colonial-curriculum

Nie, X., Dickison, V., Singh, M., De Koeyer, D., Xu, H., Bai, Y. and Hawkins, G. (2021). Potato tuber necrosis induced by Alfalfa mosaic virus depends on potato cultivar rather than on virus strain. *Plant Disease. 104*, 340-347.

Njateng, G. S. S., Du, Z., Gatsing, D., Mouokeu, R. S., Liu, Y., Zang, H., Gu, J., Luo, X. and Kuiate, J. (2017). Antibacterial and antioxidant properties of crude extract, fractions and compounds from the stem bark of *Polyscias fulva* hiern (Araliaceae). *BMC Complementary Alternative Medicine and Therapies.* 17, 99.

Osafo, N., Mensah, K. B. and Yeboah, O. K. (2017). Phytochemical and pharmacological review of *Cryptolepis sanguinoleta* (Lindl.) Schlecter. *Advances in Pharmacological Sciences.* 3026370.

Paciolla, C., Fortunato, S., Dipierro, N., Paradiso, A., De Leonardis, S., Mastropaspua, L. and Concetta de Pinto, M. (2019). Vitamin C in plants: from functions to biofortification. *Antioxidants, 8*(11), 519.

Panche, A. N., Diwan, A. D. and Chandra, S. R. (2016). Flavonoids: an overview. *Journal of Nutritional Science. 5,* e47.

Parasuraman S., Thing G.S. and Dhanaraj S.A. (2014) Polyherbal formulation: Concept of ayurveda. *Pharmacognitive. Review, 8,* 73–80.

Perez-Soto, E., Estanislao-Gomez, C. C., Perez-Ishiwara, D. G., Ramirez-Celis, C. and del Consuelo Gomez-Garcia, M. (2019). Cytotoxic effect and mechanisms from plant-derived compounds in breast cancer. *Chapter Metric Overview,* 105772.

Pigliucci, M. Murren, C. J. and Schlichting, C. D. (2006). Phenotypic plasticity and evolution by genetic assimilation. *Journal of Experimental Biology. 209*(12), 2362-2367.

Qin, D., Li, T., Shao, J., He, X., Shi, D., Wang, Z., Xiao, W., Yao, X. and Li, H. (2021). Arteannoides U-Z: six undescribed sesquiterpenoids with anti-inflammatory activities from the aerial parts of *Artemisia annua* (Qinghao). *Fitoterapia. 154*, 105002.

Rao, R. V., Descamps, O., John, V. and Bredesen, D. E. (2012). Ayurvedic medicinal plants for Alzheimer's disease: A review. *Alzheimers Research. Therapy.*

Raoult, D., Audic, S., Robert, C., Abergel, C., Renesto, P., Ogata, H., La Scola, B., Suzan, M. and Claverie, J. M. (2004). The 1.2- megabase genome sequence of Mimivirus. *Science*, 306 (5700), 1344-1350.

Reta, D. H., Tessema, T. S., Ashenef, A. S., Desta,- A. F., Labisso, W. L., Gizaw, S. T., Abay, S. M., Melka, D. S. and Reta, F. A. (2020). Molecular and immunological diagnostic techniques of medical viruses. *International Journal of Microbiology, 2020,* 8832728.

Rolta, R., Salaria, D., Kumar, V., Sourirajan, A. and Dev, K. (2020). Phytocompounds of *Rheum emodi*, *Thymus serpyllum* and *Artemisia annua* inhibit COVID-19 binding to ACE2 receptor: In silico approach. *Research Square.*

Saleh, D., Yarrarapu, S. N. S. and Sharma, S. (2022). Herpes Simplex type 1. *StatPearls.*

Satoh, S. and Boyer, E. (2019). HIV in South Africa. *Lancet, 394*(10197), 467.

Seeger, C. (2023). A CRISPR-based system to investigate HBV cccDNA biology. *Journal of Virology, 97*(10).

Shahzadi, I., Abdullah, F. M., Ali, Z., Ahmed, I. and Mirza, B. (2020). Chloroplast genome sequences of Artemisia maritima and Artemisia absinthium: Comparative analyses, mutational hotspots in genus Artemisia and phylogeny in family Asteraceae. *Genomics*, 112 (2), 1454-1463.

Sharma, S., and Deep, S. (2020). In-silico drug repurposing for targeting SARS-CoV-2 Mpro. *Journal of Biomolecules, Structure and Dynamics.*

Sheperd, J. G., Davis, C., Streicker, D. G. and Thomson, E. C. (2023). Emerging Rhabdoviruses and human infection. *Biology*, *12*(6), 878.

Shi, C., Li, H., Yang, Y., and Hou, L. (2015). Anti-inflammatory and immunoregulatory functions of artemisinin and its derivatives. *Mediators Inflammation, 2015,* 435713.

Shinyuy, L. M., Loe, G. E., Jansen, O., Mamede, L., Ledoux, A., Noukimi, S. F., Abenwie, S. N., Ghogomu, S. M., Souopgui, J., Robert, A., Demeyer, K. and Frederich, M. (2023). Secondary metabolites isolated from *Artemisia afra* and *Artemisia annua* and their anti-malarial, anti-inflammatory and immunomodulating properties – pharmacokinetics and pharmacodynamics: a review. *Metabolites. 13*(5), 613.

Siddique, H., Pendry, B., Rashid, M. A. and Rahman, M. M. (2021). Medicinal plants used to treat infectious diseases in the central part and northern district of Bangladesh-an ethnopharmacological perception. *Journal of Herbal Medicine. 29*, 100484.

Sifuna, N. (2022). African traditional medicine: its potential, limitations and challenges. *Journal of Healthcare, 2689.*

Silvestrini, B. and Silvestrini, M. (2022). Medical implications of the relationships among protein denaturation, necrosis and inflammation: an intriguing story. *Chapter Metrics Overview.* 108018.

Steyn, W. J., Wand, S. J. E., Holcroft, D. M. and Jacobs, G. (2002). Anthocyanins in vegetative tissues: a proposed unified function in photoprotection. *The New Phytologist, 155*(3), 349-361.

Subba Rao, S. (2006). Indigenous knowledge organization: an Indian scenario. *International Journal of Information Management, 26*(2006), 224-233.

Sunmonu, T. O. and Afolayan, A. J. (2012). Protective role of *Artemisia afra* aqueous extract on tissue antioxidant defense systems in streptozotocin-induced diabetic rats. *African Journal of Traditional, Complementary and Alternative Medicines, 10*(1), 15-20.

Tabish, S. A. (2008). Complementary and alternative healthcare: is it evidence-based. *International Journal of Health Sciences, 2*(1), 5-9.

Taylor, M. W. (2014). What is a virus? *Viruses and Man: A History of Interactions,* 23-40.

Tomic, N., Pojskic, L., Kalajdzic, A., Ramic, J., Kadric, N. L., and Ikanovic, T. (2020). Screening of preferential binding affinity of selected natural compounds to SARS-CoV-2 proteins using in silico methods. *Eurasian Journal of Medicine and Oncology, 4,* 319–323.

Trovato, M., Sartorius, R., D'Apice, L., Manco, R. and De Berardinis, P. (2020). Viral emerging diseases: challenges in developing vaccination strategies. *Frontiers in Immunology. 11,* 2130.

Trung, N. Q., Mechler, A., Hoa, N. T. and Vo, V. (2022). Calculating bond dissociation energies of X-H (X=C, N, O, S) bonds of aromatic systems via density functional theory: a detailed comparison of methods. *Royal Society Open Science.* 10.1098.

Vardanyan, R. and Hruby, V. (2016). Antiviral drugs. *Synthesis of Best-Seller Drugs, 1016,* 687 – 736.

van Andel, T., and Carvalheiro, L. G. (2013). Whyurban citizens in developing countries use traditional medicines: the case of Suriname. *Evidence-Basedd Complementary and Alternative Medicine, 2013,*13.

van der Beer, L. (2004). *Artemisia afra Jacq. Ex Willd.* [Photograph]. PlantZAfrica. https://pza.sanbi.org/artemisia-afra

van Wyk, B. E. (2008). A broad review of commercially important southern African medicinal plants. *Journal of Ethnopharmacology,* 119, 342-355.

van Wyk, B. E. (2011). The potential of South African plants in the development of new medicinal products. *South African Journal of Botany, 77*(4), 812-829.

Vellingiri, B., Jayaramayya, K., Iyer, M., Narayanasamy, A., Govindasamy, V., Giridharan, B., Ganesan, S., Venugopal, A., Venkatesan, D., and Ganesan, H., *et al.*. (2020). COVID-19: A promising cure for the global panic. *Science and the Total Environment, 725,* 138277.

Vo, Q. V., Nam, P. C., Thong, N. M., Trung, N. T., Phan, C. D. and Mechler, A. (2019). Antioxidant motifs in flavonoids: O-H versus C-H bond dissociation. *ACS Omega. 4*(5), 8935-8942.

Wagner, D. N., Marcon, A. R., and Caulfield, T. (2020). “Immune Boosting” in the time of COVID: selling immunity on Instagram. *Allergy, Asthma* and *Clinical Immunology, 16,* 76.

Wei, X., Decker, J. M., Wang, S., Hui, H., Kappes, J. C., Wu, X., Salazar-Gonzales, J. F., Salazr, M. G., Kilby, J. M., Saag, M. S., Komarova, N. L., Nowak, M. A., Hahn, B. H., Kwong, P. D. and Shaw, G. M. (2003). Antibody neutralization and escape by HIV-1. *Nature*, *422*(6929), 307-312.

WHO (2023): The Promotion and Development of Traditional Medicine. WHO Technical Report 622. WHO, Geneva.

WHO (2022): Research Guidelines for Evaluating the Safety and Efficacy of Herbal Medicines. WHO, Geneva.

WHO (2022): Quality Control Methods for Medicinal Plant Materials. WHO, Geneva.

WHO (2023): WHO Monographs on Selected Medicinal Plants, Vol. 5. WHO, Geneva.

WHO (2022): WHO Monographs on Selected Medicinal Plants, Vol. 2. WHO, Geneva

Yang, K., Wang, L., Le, J. and Dong, J. (2020). Cell polarity: regulators and mechanisms in plants. *Journal of Integrative Plant Biology, 62*(1), 132-147.

Yao Y., Guo Q., Cao Y., Qiu Y., Tan R., Yu Z., Zhou Y. and Lu N. (2018). Artemisinin derivatives inactivate cancer-associated fibroblasts through suppressing TGF-beta signaling in breast cancer. *Journal of Experimental* and *Clinical Cancer Research*, 20.

Zhou Y, Gilmore K, Ramirez S, Settels E, Gammeltoft KA, Pham LV, *et al.*. In vitro efficacy of artemisinin-based treatments against SARS-CoV-2. Sci Rep. 2021 Jul 16;11(1):14571.

Zuma, K., Simbayi, L., Zungu, N., Moyo, S., Marinda, E., *et al.*, and Ramlagan, S. (2022). The HIV epidemic in South Africa: key findings from 2017 national population-based study. *International Journal of Environmental Research and Public Health*, *19*(13), 8125.

# APPENDICES

## Annex A: Ethical clearance certificate

UNAM
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ETHICAL CLEARANCE CERTIFICATE

**Ethical Clearance Reference Number: SOS-0076 Date: 02 June 2022**

This Ethical Clearance Certificate is issued by the University of Namibia Ethics Committee (REC) in accordance with the University of Namibia's Research Ethics Policy and Guidelines. Ethical approval is given in respect of undertakings contained in the Research Project outlined below. This Certificate is issued on the recommendations of the ethical evaluation done by the ethics committee.

**Title of Project:** EVALUATION OF THE ANTI-VIRAL AND IMMUNE-BOOSTING ACTIVITIES OF THE ARTEMISIA AFRA (WILDE ALS) PLANT

**Student:** WENNYTH FARMER

**Student Number:** 201501901

**Supervisor(s):** DR. IWANETTE DU PREEZ;
PROF. DAVIS MUMBENGEGWI

**Centre for Research Services**

Take note of the following:

1. Any significant changes in the conditions or undertakings outlined in the approved Proposal must be communicated to the ethics committee. An application to make amendments may be necessary.
2. Any breaches of ethical undertakings or practices that have an impact on ethical conduct of the research must be reported to the ethics committee
3. The Principal Researcher must report issues of ethical compliance to the ethics committee (through the Chairperson) at the end of the Project or as may be requested by the ethics committee
4. The ethics committee retains the right to:

i) Withdraw or amend this Ethical Clearance if any unethical practices (as outlined in the Research Ethics Policy) have been detected or suspected,

ii) Request for an ethical compliance report at any point during the course of the research.

The ethics committee wishes you the best in your research.

Dr. Zivayi Chiguvare (Chairperson Ethics Committee)

Prof. Davis Mumbengegwi (Head, Multidisciplinary Research)

**Annex B: One-way ANOVA data sets**

### 7.1 One-way ANOVA test: Antioxidant assay

#### 7.1.1 One-way ANOVA test for Leaves

| Table Analyzed | Leaves |
|---|---|
| One-way analysis of variance | |
| P value | 0.3278 |
| P value summary | ns |
| Are means signif. different? (P < 0.05) | No |
| Number of groups | 4 |
| F | 1.200 |
| R square | 0.1140 |
| Bartlett's test for equal variances | |
| Bartlett's statistic (corrected) | 1.106 |
| P value | 0.7757 |
| P value summary | ns |
| Do the variances differ signif. (P < 0.05) | No |

| ANOVA Table | SS | df | MS |
|---|---|---|---|
| Treatment (between columns) | 3947 | 3 | 1316 |
| Residual (within columns) | 30687 | 28 | 1096 |
| Total | 34634 | 31 | |

#### 7.1.2 One-way ANOVA test for Twigs

| Table Analyzed | Twigs |
|---|---|
| One-way analysis of variance | |
| P value | 0.8407 |

| | | | |
|---|---|---|---|
| P value summary | ns | | |
| Are means signif. different? (P < 0.05) | No | | |
| Number of groups | 4 | | |
| F | 0.2782 | | |
| R square | 0.02894 | | |
| Bartlett's test for equal variances | | | |
| Bartlett's statistic (corrected) | 0.9285 | | |
| P value | 0.8185 | | |
| P value summary | ns | | |
| Do the variances differ signif. (P < 0.05) | No | | |
| ANOVA Table | SS | df | MS |
| Treatment (between columns) | 890.4 | 3 | 296.8 |
| Residual (within columns) | 29877 | 28 | 1067 |
| Total | 30768 | 31 | |

### 7.1.3 One-way ANOVA test for Combination (Leaves and Twigs)

| | |
|---|---|
| Table Analyzed | Cocktail |
| One-way analysis of variance | |
| P value | 0.8370 |
| P value summary | ns |
| Are means signif. different? (P < 0.05) | No |
| Number of groups | 4 |
| F | 0.2834 |
| R square | 0.02947 |
| Bartlett's test for equal variances | |
| Bartlett's statistic (corrected) | 2.757 |
| P value | 0.4306 |
| P value summary | ns |

| | | | |
|---|---|---|---|
| Do the variances differ signif. (P < 0.05) | No | | |
| ANOVA Table | SS | df | MS |
| Treatment (between columns) | 775.2 | 3 | 258.4 |
| Residual (within columns) | 25533 | 28 | 911.9 |
| Total | 26308 | 31 | |

## 7.2 One-way test: Anti-inflammatory

### 7.2.1 One-way test Leaves:

| | |
|---|---|
| Table Analyzed | Leaves |
| One-way analysis of variance | |
| P value | 0.2952 |
| P value summary | ns |
| Are means signif. different? (P < 0.05) | No |
| Number of groups | 3 |
| F | 1.294 |
| R square | 0.1097 |

| | | | |
|---|---|---|---|
| Bartlett's test for equal variances | | | |
| Table Analyzed | Twigs | | |
| Repeated Measures ANOVA | | | |
| P value | 0.0496 | | |
| P value summary | * | | |
| Are means signif. different? (P < 0.05) | Yes | | |
| Number of groups | 3 | | |
| F | 3.752 | | |
| R square | 0.349 | | |
| Was the pairing significantly effective? | | | |

| | | | | | |
|---|---|---|---|---|---|
| R square | 0.5248 | | | | |
| F | 3.393 | | | | |
| P value | 0.0247 | | | | |
| P value summary | * | | | | |
| Is there significant matching? (P < 0.05) | Yes | | | | |
| ANOVA Table | SS | df | MS | | |
| Treatment (between columns) | 1601 | 2 | 800.5 | | |
| Individual (between rows) | 5067 | 7 | 723.8 | | |
| Residual (random) | 2987 | 14 | 213.3 | | |
| Total | 9655 | 23 | | | |
| Dunnett's Multiple Comparison Test | Mean Diff. | q | Significant? P < 0.05? | Summary | 95% CI of diff |
| Diclofenac potassium vs Twigs (aqueous) | -19.51 | 2.672 | Yes | * | -37.46 to -1.570 |
| Diclofenac potassium vs Twigs (organic) | -5.937 | 0.813 | No | ns | -23.88 to 12.01 |

**7.2.2 One-way test Twigs:**

| Table Analyzed | Twigs |
|---|---|
| | |
| Repeated Measures ANOVA | |
| P value | 0.0496 |
| P value summary | * |
| Are means signif. different? (P < 0.05) | Yes |
| Number of groups | 3 |
| F | 3.752 |
| R square | 0.349 |

| | |
|---|---|
| | |
| Was the pairing significantly effective? | |
| R square | 0.5248 |
| F | 3.393 |
| P value | 0.0247 |
| P value summary | * |
| Is there significant matching? (P < 0.05) | Yes |

| ANOVA Table | SS | df | MS | | |
|---|---|---|---|---|---|
| Treatment (between columns) | 1601 | 2 | 800.5 | | |
| Individual (between rows) | 5067 | 7 | 723.8 | | |
| Residual (random) | 2987 | 14 | 213.3 | | |
| Total | 9655 | 23 | | | |
| | | | | | |
| Dunnett's Multiple Comparison Test | Mean Diff. | q | Significant? P < 0.05? | Summary | 95% CI of diff |
| Diclofenac potassium vs Twigs (aqueous) | -19.51 | 2.672 | Yes | * | -37.46 to -1.570 |
| Diclofenac potassium vs Twigs (organic) | -5.937 | 0.813 | No | ns | -23.88 to 12.01 |

**7.2.3 One-way test Combination (Leaves and Twigs):**

| Table Analyzed | Leaves and Twigs |
|---|---|
| | |
| One-way analysis of variance | |
| P value | 0.5422 |
| P value summary | ns |

| | |
|---|---|
| Are means signif. different? (P < 0.05) | No |
| Number of groups | 3 |
| F | 0.6303 |
| R square | 0.05663 |
| | |
| Bartlett's test for equal variances | |
| Bartlett's statistic (corrected) | 3.589 |
| P value | 0.1662 |
| P value summary | ns |
| Do the variances differ signif. (P < 0.05) | No |

| ANOVA Table | SS | df | MS | | |
|---|---|---|---|---|---|
| Treatment (between columns) | 623.1 | 2 | 311.6 | | |
| Residual (within columns) | 10380 | 21 | 494.3 | | |
| Total | 11004 | 23 | | | |
| Dunnett's Multiple Comparison Test | Mean Diff. | q | Significant? P < 0.05? | Summary | 95% CI of diff |
| Diclofenac potassium vs Cocktail (aqueous) | -8.75 | 0.7871 | No | ns | -35.11 to 17.61 |
| Diclofenac potassium vs Cocktail (organic) | -12.08 | 1.087 | No | ns | -38.45 to 14.28 |

### 7.3 One- way test: Total flavonoid content

#### 7.3.1 Leaves

| Table Analyzed | Leaves |
|---|---|
| | |
| One-way analysis of variance | |
| P value | 0.0514 |
| P value summary | ns |
| Are means signif. different? (P < 0.05) | No |
| Number of groups | 3 |
| F | 3.431 |
| R square | 0.2463 |
| | |
| Bartlett's test for equal variances | |
| Bartlett's statistic (corrected) | 39.49 |
| P value | < 0.0001 |
| P value summary | **** |
| Do the variances differ signif. (P < 0.05) | Yes |

| ANOVA Table | SS | df | MS | | |
|---|---|---|---|---|---|
| Treatment (between columns) | 0.02788 | 2 | 0.01394 | | |
| Residual (within columns) | 0.08531 | 21 | 0.00406 | | |
| Total | 0.1132 | 23 | | | |
| Dunnett's Multiple Comparison Test | Mean Diff. | q | Significant? P < 0.05? | Summary | 95% CI of diff |
| Catechin vs Leaves (aqueous) | 0.07075 | 2.22 | No | ns | -0.004827 to 0.1463 |

| | | | | | |
|---|---|---|---|---|---|
| Catechin vs Leaves (organic) | 0.07375 | 2.314 | No | ns | -0.001827 to 0.1493 |

7.3.2 Twigs

| Table Analyzed | Twigs |
|---|---|
| | |
| One-way analysis of variance | |
| P value | 0.2469 |
| P value summary | Ns |
| Are means signif. different? (P < 0.05) | No |
| Number of groups | 3 |
| F | 1.496 |
| R square | 0.1247 |
| | |
| Bartlett's test for equal variances | |
| Bartlett's statistic (corrected) | 9.833 |
| P value | 0.0073 |
| P value summary | ** |
| Do the variances differ signif. (P < 0.05) | Yes |
| | |

| ANOVA Table | SS | df | MS |
|---|---|---|---|
| Treatment (between columns) | 0.01653 | 2 | 0.008267 |
| Residual (within columns) | 0.1160 | 21 | 0.005525 |
| Total | 0.1326 | 23 | |

| Dunnett's Multiple Comparison Test | Mean Diff. | q | Significant? P < 0.05? | Summary | 95% CI of diff |
|---|---|---|---|---|---|
| Catechin vs Twigs (aqueous) | 0.06354 | 1.710 | No | ns | -0.02460 to 0.1517 |

| | | | | | |
|---|---|---|---|---|---|
| Catechin vs Twigs (organic) | 0.04025 | 1.083 | No | ns | -0.04789 to 0.1284 |

### 7.3.3 Combination (Leaves and Twigs)

| Table Analyzed | Leaves and Twigs |
|---|---|
| | |
| One-way analysis of variance | |
| P value | 0.1737 |
| P value summary | Ns |
| Are means signif. different? (P < 0.05) | No |
| Number of groups | 3 |
| F | 1.904 |
| R square | 0.1535 |
| | |
| Bartlett's test for equal variances | |
| Bartlett's statistic (corrected) | 12.95 |
| P value | 0.0015 |
| P value summary | ** |
| Do the variances differ signif. (P < 0.05) | Yes |

| ANOVA Table | SS | df | MS | | |
|---|---|---|---|---|---|
| Treatment (between columns) | 0.01851 | 2 | 0.00926 | | |
| Residual (within columns) | 0.1021 | 21 | 0.00486 | | |
| Total | 0.1206 | 23 | | | |

| Dunnett's Multiple Comparison Test | Mean Diff. | q | Significant? P < 0.05? | Summary | 95% CI of diff |
|---|---|---|---|---|---|
| Catechin vs Cocktail (aqueous) | 0.06363 | 1.825 | No | ns | -0.01904 to 0.1463 |
| Catechin vs Cocktail (organic) | 0.05267 | 1.511 | No | ns | -0.03000 to 0.1353 |

### 7.4 Mann-Whitney U tests: Cytotoxicity assay

#### 7.4.1 Leaves:

| Table Analyzed | Leaves |
|---|---|
| Column A | Leaves (aqueous) |
| vs | vs |
| Column B | Leaves (organic) |
| Mann Whitney test | |
| P value | 0.5656 |
| Exact or approximate P value? | Gaussian Approximation |
| P value summary | ns |
| Are medians signif. different? (P < 0.05) | No |
| One- or two-tailed P value? | Two-tailed |
| Sum of ranks in column A,B | 63 , 73 |
| Mann-Whitney U | 27.00 |

7.4.2 Twigs:

| Table Analyzed | Twigs |
|---|---|
| Column A | Twigs (aqueous) |

| | |
|---|---|
| vs | vs |
| Column B | Twigs (organic) |
| Mann Whitney test | |
| P value | 0.9539 |
| Exact or approximate P value? | Gaussian Approximation |
| P value summary | ns |
| Are medians signif. different? (P < 0.05) | No |
| One- or two-tailed P value? | Two-tailed |
| Sum of ranks in column A,B | 68 , 68 |
| Mann-Whitney U | 32.00 |

7.4.3 Combination (Leaves and Twigs):

| | |
|---|---|
| Table Analyzed | Leaves and Twigs |
| Column A | Cocktail (aqueous) |
| vs | vs |
| Column B | Cocktail (organic) |
| Mann Whitney test | |
| P value | 0.8360 |
| Exact or approximate P value? | Gaussian Approximation |
| P value summary | ns |
| Are medians signif. different? (P < 0.05) | No |
| One- or two-tailed P value? | Two-tailed |
| Sum of ranks in column A,B | 66 , 70 |
| Mann-Whitney U | 30.00 |

**7.5 One- way test: SARS-CoV 2 Inhibition Assay**

**7.5.1 Leaves**

| | |
|---|---|
| Table Analyzed | Leaves |
| | |

| | |
|---|---|
| One-way analysis of variance | |
| P value | 0.1884 |
| P value summary | ns |
| Are means signif. different? (P < 0.05) | No |
| Number of groups | 3 |
| F | 1.797 |
| R square | 0.1351 |
| | |
| Bartlett's test for equal variances | |
| Bartlett's statistic (corrected) | 2.186 |
| P value | 0.3352 |
| P value summary | ns |
| Do the variances differ signif. (P < 0.05) | No |

| ANOVA Table | SS | df | MS | F (DFn, DFd) | P value |
|---|---|---|---|---|---|
| Treatment (between columns) | 3987 | 2 | 1994 | F (2, 24) = 2.040 | P=0.1539 |
| Residual (within columns) | 21500 | 22 | 977.3 | | |
| Total | 25487 | 24 | | | |

**7.5.2 Twigs:**

| Table Analyzed | Twigs |
|---|---|
| | |
| One-way analysis of variance | |
| P value | 0.1539 |
| P value summary | ns |
| Are means signif. different? (P < 0.05) | No |

| | |
|---|---|
| Number of groups | 3 |
| F | 2.040 |
| R square | 0.1564 |
| | |
| Bartlett's test for equal variances | |
| Bartlett's statistic (corrected) | 2.476 |
| P value | 0.2990 |
| P value summary | ns |
| Do the variances differ signif. (P < 0.05) | No |

| ANOVA Table | SS | df | MS | F (DFn, DFd) | P value |
|---|---|---|---|---|---|
| Treatment (between columns) | 4263 | 2 | 2131 | F (2, 23) = 1.797 | P=0.1884 |
| Residual (within columns) | 27285 | 23 | 1186 | | |
| Total | 31547 | 25 | | | |

### 7.5.3 Combination of Leaves and Twigs:

| Table Analyzed | Leaves and Twigs |
|---|---|
| | |
| One-way analysis of variance | |
| P value | 0.0462 |
| P value summary | Sd |
| Are means signif. different? (P < 0.05) | Yes |
| Number of groups | 3 |
| F | 3.548 |
| R square | 0.2439 |
| | |

| | |
|---|---|
| Bartlett's test for equal variances | |
| Bartlett's statistic (corrected) | 3.820 |
| P value | 0.1481 |
| P value summary | Ns |
| Do the variances differ signif. (P < 0.05) | No |

| ANOVA Table | SS | df | MS | F (DFn, DFd) | P value |
|---|---|---|---|---|---|
| Treatment (between columns) | 5612 | 2 | 2806 | F (2, 22) = 3.548 | P=0.0462 |
| Residual (within columns) | 17400 | 22 | 790.9 | | |
| Total | 23012 | 24 | | | |