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Review

Anti-HIV activity of southern African plants: Current developments, phytochemistry and future research

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Chemical compounds studied in this article: Chloroquine (PubChem CID: 2719)

genistein (PubChem CID: 5280961)

EGCG (PubChem CID: 65064) and stric tinin (PubChem CID: 73330)

fulvopumierin (PubChem CID: 7381541)

catechin (CID 73160)

epicatechin (PubChem CID: 72276)

melacine and 28-deacetylsendanin (PubChem CID: 73276)

narcicasline (PubChem CID: 73276)

pretazettine (PubChem CID: 73360)

varine (PubChem CID: CID 398937)

pretazettine (PubChem CID: 73360)

caffic acid (PubChem CID: 689043)

friedelin (PubChem CID: 91472)

B-sitosterol (PubChem CID: 222284)

eric acid (PubChem CID: 176920)

di-O-methyl-prodelphinidin B4 or 4'-O-methylgallocatechin (PubChem CID: 79412)

di-O-methylepigallocatechin (PubChem CID: 101601927)

punicalagin (PubChem CID: 16129869)

arjungulose 1 (PubChem CID: 14658050)

sericoside (PubChem CID: 76972524)

digalactosylin-3-O-glucoside (PubChem CID: 25200205)

4'-O-methylpygallatechin (ourateacatechin) (PubChem CID: 79412)

4'-O-methylpygallatechin (PubChem CID: 10087845)

4',4'-di-O-methylprodelphinidin B4 or 4'-O-methylpygallatechin (PubChem CID: 25200205)

3,4-dicaffeoylquinic acid (PubChem CID: 5281780)

3,5-dicaffeoylquinic acid (siscorhogenic acid A) (PubChem CID: 6474310)

tri-O-galloylquinic acid (PubChem CID: 452237)

galangin (PubChem CID: 73330)

bergenin (PubChem CID: 606650)

A B S T R A C T

Ethnopharmacological relevance: The African continent is home to a large number of higher plant species used over centuries for many applications, which include treating and managing diseases such as HIV. Due to the overwhelming prevalence and incidence rates of HIV, especially in sub-Saharan Africa, it is necessary to develop new and affordable treatments.

Aim of the study: The article provides an extensive overview of the status on investigation of plants from the southern African region with ethnobotanical use for treating HIV or HIV-related symptoms, or the management of HIV. The review also provide an account of the in vitro assays, anti-viral activity and phytochemistry of these plants.

Materials and methods: Peer-reviewed articles investigating plants with ethnobotanical information for the treatment or management of HIV or HIV-related symptoms from the southern African region were acquired from Science Direct, PubMed central and Google Scholar. The selection criteria was that (1) plants should have a record of traditional/popular use for infectious or viral diseases, HIV treatment or symptoms similar to HIV infection, (2) if not traditionally/popularly used, plants should be closely related to plants with popular use and HIV activity identified by means of in vitro assays, (3) plants should have been identified scientifically, (4) should be native to southern African region and (5) anti-HIV activity should be within acceptable ranges.

Results: Many plants in Africa and specifically the southern African region have been used for the treatment of HIV or HIV related symptoms and have been investigated suing various in vitro techniques. In vitro assays using HIV enzymes such as reverse transcriptase (RT), integrase (IN) and protease (PR), proteins or cell-based assays have been employed to validate the use of these plants with occasional indication of the selectivity index (SI) or therapeutic index (TI), with only one study, that progressed to in vivo testing. The compounds identified from plants from southern Africa is similar to compounds identified from other regions of the world, and the compounds have been divided into three groups namely (1) flavonoids and flavonoid glycosides, (2) terpenoids and terpenoid glycosides and (3) phenolic acids and their conjugated forms.

Conclusions: An investigation of the plants from southern Africa with ethnobotanical use for the treatment of HIV, management of HIV or HIV-related symptoms, therefore provide a very good analysis of the major assays employed and the anti-viral compounds and compound groups identified. The similarity in identified anti-viral compounds worldwide should support the progression from in vitro studies to in vivo testing in development of affordable and effective anti-HIV agents for countries with high infection and mortality rates due to HIV/AIDS.
1. Introduction

Southern Africa is remarkably rich in plant diversity with approximately 30 000 flowering plant species which equates to nearly 10% of the higher plants globally (van Wyk, 2001). Plants have been used medicinally for centuries and the medicinal plant trade is still prominent today. According to the World Health Organization (WHO), up to 80% of people living on the African continent, equating to more than a half billion people, use traditional medicines to meet their primary health care needs. Nonetheless, the industry is not yet...
exploited to its full capacity. In South Africa, for example, around 3 000 medicinal plant species are frequently used in plant-based medicines, however less than 40 indigenous species have been commercialized to some degree (van Wyk, 2008).

The statistics on HIV in the southern African region emphasizes its devastating effects. In 2015, there were 36.7 million people living with HIV. Worldwide, 2.1 million people became newly infected with HIV (UNAIDS, 2016). In 2012, sub-Saharan Africa accounted for 70% of all people newly infected with HIV and 71% of all people living with HIV (UNAIDS, 2013). Collectively eastern and southern Africa are home to 6% of the global population, but accounted for 52% of all people living with HIV and nearly half the approximated 2.3 million people who became infected with HIV in 2012 (UNAIDS, 2013).

Anti-retroviral therapy (ART) is an effective treatment for people living with HIV. The standard treatment seeks to suppress the HIV replication cycle and halt disease progression. Antiretroviral therapy is significant in improving the life of people living with HIV, however the drugs have many disadvantages, including resistance, toxicity, limited availability, and lack of curative effect (Chinsembo and Hedimenti, 2010a). The potential of HIV becoming resistant to anti-retroviral (ARV) treatment has become an increasing concern since it was first reported decades ago (De Clercq, 1995). As pathogens become drug resistant, the need for development of new medicines is being realized all over the world. These shortcomings open avenues for the use of natural products in the management of HIV/AIDS.

2. Methodology

Electronic searches of Science Direct, PubMed central and Google Scholar were undertaken with search terms “HIV”, “medicinal plants”, “Africa”, “anti-viral” and “southern Africa”. Initially publication titles were screened for suitability and plant species, active compounds and their mode of action were documented from primary literature sources. Ethnobotanical surveys in other African countries such as Ethiopia (Asres et al., 2001), Uganda (Lamorde et al., 2010), Cameroon (Mbaveng et al., 2011), Zimbabwe (Viol et al., 2016), Namibia (Chinsembo and Hedimenti, 2010) and Zambia (Chinsembo, 2016) also assisted in identifying plants traditionally used for management of HIV or HIV symptoms. The inclusion criteria were: (1) plants should have a record of traditional/popular use for infectious or viral diseases, HIV treatment or symptoms similar to HIV infection, (2) if not traditionally/popularly used, plants should be closely related to plants with popular use and HIV activity identified by means of in vitro assays, (3) plants should have been identified scientifically, (4) should be native to the southern African region and (5) anti-HIV activity should be within acceptable ranges. Clinical relevant concentrations have been defined as IC₅₀ of < 50 or < 100 µg/ml for extracts and at < 5 or < 25 µM for individual compounds and have been applied as a selection criterion in this study (Agarwal et al., 2014; Butterweck and Nahrstedt, 2012; Cos et al., 2006; Gertsch, 2009). Since traditional refers to plants with a long history of use, and HIV being a relatively new disease, the use of these plants are referred to as “popular” or “popularly used” against HIV.

3. Screening methods for anti-HIV activity in medicinal plants

Many plants have been traditionally used to treat viral infections and other ailments. Investigation of these claims led to the discovery of numerous plant derived anti-HIV compounds which are widely distributed in nature (Singh et al., 2011). Therefore, screening medicinal plants provides an opportunity for the discovery of HIV inhibitors with lower or no toxicity and/or side effects (Narayan et al., 2013). Biologically active substances harvested from plants, can be found in any organ of the plant, although leaf material is most traditionally used (Narayan et al., 2013).

Various laboratory based investigations have been conducted using plant extracts and isolated compounds employing a variety of assays. Most of the tests are performed on the enzymes reverse transcriptase (RT), integrase (IN) and protease (PR), proteins involved in activation of viral genes or cells that are infected with viruses or pseudoviruses, and the activity determined by an indicator such as MTT or luciferase activity. The MTT assays are based on the reduction of the yellow colour 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) by mitochondrial dehydrogenases of metabolically active cells. In metabolically active cells, blue formazan is produced which is measured spectrophotometrically to indicate cell viability in assays of cell proliferation and cytotoxicity (Cos et al., 2002; Shoemaker et al., 2004). These targets aim to determine the inhibition or reduction of viral infection on various levels, and present various advantages and disadvantages to be considered in evaluating anti-viral activity (Table 1).

RT converts the viral RNA genome to viral DNA using its polymerase domain (RNA dependent DNA polymerase activity), while the ribonuclease H (RNase H) domain degrades the RNA component from the intermediary RNA/DNA complex. The enzyme also has a DNA-dependent DNA polymerase function and most clinically available RT drugs, therefore target this enzyme. Numerous studies focused on the HIV-1 RT enzyme and various protocols have been employed measuring ethyl-3H thymidine triphosphate (3H TTP) by RT using polyadenylic acid-oligodeoxymyridic acid (polyA-oligodT) as template primer in the presence and absence of a test substance such as isolated compounds or plant extracts (Ali et al., 2002; Bessong et al., 2005). Various kits are used for determining the inhibition of enzymes and viral components such as the Capture ELISA kit (GenBio Health Science, India) (Chinnaiyan et al., 2013) and HIV-RT colourimetric enzyme-linked-immunosorbent serologic assay (ELISA) kit obtained from Roche Diagnostics, Mannheim, Germany (Chukwujekwu et al., 2014; Eldeen et al., 2011; Kapewango et al., 2013; Klos et al., 2009; Thuklalange et al., 2008a; Wang et al., 2014) or a purified recombinant HIV-1 RT enzyme (Merck, Darmstadt, Germany) (Kapewango et al., 2013). Several interactions and measures have been identified to optimise the assay conditions. It has been found that HIV-1 RT uses magnesium or manganese divalent ions as a co-factor (Bolton et al., 2002) and that palladium and iron might also affect the assay as they are responsible for irreversible inhibition of RT and subsequent reduction in virus proliferation (Filler and Lever, 1997). Since ions of various metals are accumulated by plants and therefore present in plant extracts, these ions may be present in and might affect the outcomes. Most methodologies describing the inhibition of HIV-1 RT by crude plant extracts do not take into consideration the effect of metal ions in regulating the activity of HIV-1 RT (Bessong and Obi, 2006). Consequently, it would be important to determine metal ions in plant extracts prior to screening in order to avoid false inhibitory observations at the screening stage.

IN, in conjunction with accessory viral proteins, is required for the integration of the synthesized viral double stranded DNA into the chromosome in the nucleus of the host cell. The HIV-1 integrase (HIV-1 IN) enzyme has also been employed on extracts and compounds such as catechins in various studies using the Xpress HIV-1 IN Assay Kit (Express Biotech International, USA) or in an in vitro model (Jiang et al., 2010). A recombinant HIV-1 IN of E. coli origin (Wang et al., 2014) and the evaluation against the 3’ processing activity of HIV-1 IN have been performed on extracts and compounds (Bessong et al., 2005). The unspecific binding of plant compounds to proteins is, however, mostly not considered.

PR cleaves viral polyproteins into structural and functional components which are assembled to form progeny virions (Bessong and Obi, 2006). The protease enzyme has also been investigated in various assays such as the fluorometric detection of HIV-PR activity using HIV-II PR HIV-FRET (fluorescence resonance energy transfer) (AnaSpec Inc., USA) and a recombinant HIV-1 protease solution (Bachem,
A summary of the most popular anti-HIV assays presenting the advantages and disadvantages of each assay.

| Assay | Target | Advantages | Disadvantages |
|-------|--------|------------|---------------|
| HIV-1 Reverse Transcriptase (RT) assay | Reverse Transcriptase is an enzyme that converts the viral RNA genome to viral DNA. | | |
| HIV-1 Integrate (IN) assay | Integrate enzyme is required for the integration of the retroviral viral double-stranded DNA into the chromosome in the nucleus. | | |
| HIV-1 Protease (PR) assay | Protease enzyme cleaves viral polyproteins into smaller units, which are assembled into mature virions. | | |
| HIV-1 p24 assay | p24 antigen is a marker of viral replication. | | |
| NF-κB activation assay | NF-κB is a transcription factor that regulates the expression of genes involved in immune response and inflammation. | | |
| HIV-1 Protease (PR) assay | The PR enzyme cleaves viral polyproteins into smaller units, which are assembled into mature virions. | | |
| HIV-1 Integrate (IN) assay | The IN enzyme integrates the retroviral viral double-stranded DNA into the chromosome in the nucleus. | | |
| HIV-1 Reverse Transcriptase (RT) assay | Reverse Transcriptase is an enzyme that converts the viral RNA genome to viral DNA. | | |
| HIV-1 p24 assay | p24 antigen is a marker of viral replication. | | |
| NF-κB activation assay | NF-κB is a transcription factor that regulates the expression of genes involved in immune response and inflammation. | | |

Table 1: Assays for testing antiviral compounds in vitro.
Switzerland). The glycohydrolase enzymes are found in the eukaryotic host cell’s Golgi apparatus and are responsible for glycosylation of proteins. Inhibition of the glycohydrolase enzymes decreases the infectivity of the HIV virion, as the HIV envelope proteins are highly glycosylated during the life cycle of the virus. Glucosidase was found to be partly responsible for the glycosylation of HIV gp120 (Harnett et al., 2005; Kapewangolo et al., 2013; Klos et al., 2009). Escherichia coli expressing recombinant HIV-1 PR has also been used to detect the inhibitory effects of samples on HIV-1 PR by observing the bacterial growth curve (Jiang et al., 2010; Wang et al., 2014).

An ELISA kit (another enzyme-linked immunosorbent assay) is also available to detect and quantify HIV-1 p24 core protein using the HIV-1 p24 Antigen Assay kit (Beckman Coulter, Miami, FL, USA) (Klos et al., 2009) and can be distinguished from the cell based assays. Cell based assays are commonly used with various different cell types and viruses. The CXCR4-tropic (NL4-3) or CCR5-tropic (NL-AD87) wild-type reference viruses (Louvel et al., 2013) and HIV-1c binding and entry assay on PBMCs have been described (Leteane et al., 2012). The utilised format “iFLGS” (Infection format of “Fusion-induced gene stimulation”) represents an in vitro infection system in human HeLa cells. Thereby, upon infection with HIV, the reporter gene will be induced in a quantifiable fashion as beta galactosidase allows quantification of inhibitory effects of compounds or extracts (Lubbe et al., 2012). HIV-1 pseudovirions and viruses has been used (Ngwira et al., 2015; Prinsloo et al., 2010; Wang et al., 2014) and Hela-Tat-Luc cells that are stably transfected with for instance a plasmid pcDNA3-TAT together with a reporter plasmid LTR-Luc indicates protein activation. Therefore the HIV-1 LTR is highly activated in this cell line as a consequence of high levels of intracellular Tat protein (Tshikalange et al., 2008b). Isolated HIV strains (strain HTLV-IIIB/LAI) obtained from the culture supernatant of a HIV-infected HUT-78 cell line have been tested and cell viability was evaluated using the MT assay (Cos et al., 2002). African green monkey kidney cells (Vero) have also been used (Dang et al., 2011) and linked to cytotoxicity assays on MT-4 cells (Maregesi et al., 2010a, 2010b).

Even though the MT assay is generally applied to determine cell viability in in vitro assays, very little or no consideration is given to the possibility of constituents with antioxidant potential that result in extremely high MTT readings, and might provide false positive results. The stabilization of the luciferase gene is also often not considered, even though many plant components might provide false positive results (Auld et al., 2008; Sotoca et al., 2010). The cytotoxicity of extracts and compounds are often also neglected and therefore the Selectivity Index (SI) which is achieved by dividing the cytotoxic concentration (CC50) by the effective inhibition concentration (EC50) or Therapeutic Index (TI) which is achieved by dividing the cytotoxic concentration (CC50) by the non-cytotoxic concentration that inhibits/protects 50% uninfected cells (ID50) are not reported. A value of more than 1 is indicative of an extract that is selective in inhibition and not only toxic to both the virus and the cells (Cos et al., 2002).

In vitro assays have an important role in determining anti-HIV activity, mindful of the pitfalls and false positives that might arise from compounds in plant crude extracts. The lack of absorption, distribution, metabolism and excretion (ADME) characteristics and the lack of direct correlation with in vivo/clinical doses, limit the scope of application of in vitro bioassays and add to the challenges faced by in vitro screening (Agarwal et al., 2014). It is often inaccurate to relate in vitro results from enzyme or protein inhibition assays to the in vivo situation, and these should be considered in screening of botanicals and botanical preparations. The hydrolysis and phase-II transformation of compounds within the in vivo system contribute to the incompatibility of in vitro results to the in vivo situation. Hydrolysis of flavonoids may result in formation of non-conjugated analogues able to induce a specific biological response to an even larger extent than the non-hydrolysed extract. Hydrolysis will also provide a site for conjugation which will result in excretion of the conjugate in the urine and bile (Day et al., 1998). The type of flavonoid, the position and nature of the sugar may also affect the metabolism in the intestine and passing to the large intestine for absorption there (Barrington et al., 2009; Hollman, 2004). Once the aglycone is absorbed it is quickly metabolised to form phase-II conjugates, mostly sulphates and glucuronides or O-methylation, which have a major impact on their activity as well as the ability of the body to excrete compounds (Barrington et al., 2009; Hollman, 2004). These phase-II conjugates, obviously are not representative of the compounds in the original plant extract or botanical preparation anymore, and challenge the results obtained from in vitro assays. Well-known flavonoids such as kaempferol, apigenin and galangin are only present in low concentrations in plasma as they are nearly exclusively present as conjugated glucuronides in the systemic circulation after phase-II biotransformation (Barrington et al., 2009; Chen et al., 2003; Hollman, 2004). Quercetin is often reported in antiviral assays and is known for its specific absorption and hydrolysis patterns. Quercetin glucoside is absorbed in the small intestine, whereas quercetin rutinoside is absorbed from the colon after deglycosylation (Hollman, 2004). Caffeic acid and ferulic acid, also well-known anti-viral compounds are examples of compounds subjected to transformation, both metabolised to glucuronides although not very effectively (Spencer et al., 1999). No glucuronides have however, been observed for chlorogenic acid and anthocyanidin glycosides which are rapidly absorbed and able to withstand deglycosylation reactions in humans (Hollman, 2004).

It is therefore important to consider the factors of transformation and conjugation of compounds in the intestines. Transformation of these compounds during absorption, or transformation by the liver in the human body affect the extrapolation of in vitro results to the in vivo situation. ADME characteristics for many compounds are not available, and therefore in vitro assays based on enzymes and cell based assays with protein targets are useful in screening and aims to link the

| Compound group | Example | Mode of action | Species/ Family | Reference |
|----------------|---------|----------------|----------------|-----------|
| Alkaloids      | Papaverine | Inhibits HIV replication in vitro and reduces HIV protein production | Papaver somniferum L. (Papaveraceae) | (Vliezinck et al., 1998) |
| Coumarins      | Sukodorfin | Inhibits HIV replication | Lomatium suksdorfin (S. Watson) J.M. Coult. & Rose (Apiaceae) | (Lee et al., 1994; Vliezinck et al., 1998) |
| Flavonoids     | Quercetin 3-O-(2-galloyl) α-L-arabinopyranose | Anti-HIV-1 integrase activity | Acer mono Maxim (Sapindaceae) | (Kim et al., 1998) |
| Saponins       | Escin | Moderate anti-HIV-1 protease activity | Aesculus chinensis Bunge (Sapindaceae) | (Kim et al., 1998) |
| Phenolics      | Gallic acid | Exhibits HIV integrase and reverse transcriptase activity | Terminalia chebula Retz. (Combretaceae) | (Yadav et al., 2009) |
| Quinones       | Conocurvene | Showed potent anti-HIV activity | Conopreum incurvum Lindl. (Proteaceae) | (Decostaer et al., 1993) |
| Lignans        | Demethoxyepiceaxisin | Good anti-HIV activity in vitro | Litsea verticillata Hance (Lauraceae) | (Hoang et al., 2002) |
The distribution of each species is presented and the traditional use of the medicinal plants to treat other viral infections, whereas the mode of action was scientifically reported in only 13 plants. The other 3 plants have been tested for anti-HIV activity based on similarity of plants with popular use for HIV or other viral infections, but not correctly identified or documented.

6. Phytochemistry of anti-viral components

By analyzing and comparing the information on plants from the southern African region popularly used for HIV treatment, or tested anti-HIV activity, several compounds and compound groups have been repeatedly reported, and by evaluation of these compounds and compound groups, been classified into three distinct groups. The three groups identified are:

- Flavonoids such as quercetin in *Vernonia amygdilana* and flavonoid glycosides in *Sutherlandia frutescens*.
- Terpenoid and terpenoid glycosides such as sericoside in *Combretum molle*, betulinic acid in *Peltophorum africanum* including the cardiac glycosides found in the two *Elaeodendron* species *E. croceum* and *E. schlechterum*.
- The phenolic acids such as gallic acid, rosmarinic acid and caffeine acid from *Alepidea amyatymbica* and their conjugated acids such as dicafeoylquinic acids (DCQA) from *Vernonia amygdilana*, di- and tricafeoylquinic acids (TCQA) from *Iscocoma cinnamomea* and *I. schlegeliana*.
An inventory of plants from the southern African region with anti-HIV activity, presenting their distribution, traditional uses, assays and results of the assays obtained.

| Plant species           | Distribution                                                                 | Traditional use                                                                 | Model/Control                                                                 | Active constituents                                                                                   | Mode of action                                                                                   | Pharmacological activity/Concentration ranges                                                                                     | Reference                                                                 |
|------------------------|-----------------------------------------------------------------------------|--------------------------------------------------------------------------------|--------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------|
| *Acacia brevispica*     | Widespread in Africa, found in Sudan, Ethiopia, Somalia, Kenya, Zaire, Angola and South Africa | Traditionally used to treat infectious diseases                               | Tetrazolium based colorimetric assay using HIV-1 (strain IIIb) and HIV-2 (strain ROD). AZT as positive control. | None tested, coumaric and ferulic acid, quercetin, kaempferol isoquercitrin and hyperin present. | None tested, coumaric and ferulic acid, quercetin, kaempferol isoquercitrin and hyperin present. | HIV-1, 80% methanol leaves extract IC₅₀ = 64.8 µg/ml and water extract IC₅₀ = 60.4 µg/ml. | (Maregesi et al., 2010a, 2010b; Mueller-Harvey et al., 1987) |
| *Acacia tortilis*       | Senegal, Nigeria, Sudan, Kenya, Tanzania, Israel, Jordan, Arabian Peninsula, Southern Africa and Namibia. | Traditionally used to treat infectious diseases, verminfuge, skin infections, edema and allergic dermatoses. | Tetrazolium based colorimetric assay using HIV-1 (strain IIIb) and HIV-2 (strain ROD). AZT as positive control. | None tested, coumaric and ferulic acid, quercetin, kaempferol isoquercitrin and hyperin present. | None tested, coumaric and ferulic acid, quercetin, kaempferol isoquercitrin and hyperin present. | HIV-2 Water extract of shoots IC₅₀ = 88.7 µg/ml. HIV-1 80% methanol stem bark extract IC₅₀ = 8.5 µg/ml and water extract IC₅₀ = 4.2 µg/ml. | (Maregesi et al., 2010a, 2010b) |
| *Adansonia digitata* L. | Endemic to Africa.                                                           | Antimicrobial, antimarial, diarrhoea, anaemia, asthma, antiviral, antioxidant and anti-inflammatory. | HIV-RT colorimetric ELISA assay, HIV-1 PR | Various flavonoid glycosides and proanthocyanidin compounds in the leaves and the epicatechin compounds in fruit pulp. | HIV-RT and a recombinant HIV-1 protease solution. Nevirapine as positive control. | HIV-1 RT | (Rahul et al., 2015; Sharma and Rangari, 2016) |
| *Alepidea amatymbica*   | Widely distributed in southern Africa from the eastern Cape northwards to eastern Zimbabwe | Colds, coughs, rheumatism, HIV, wounds and to wash divining bones. | A cell-based assay targeting the replication of prototypic CXCR4-tropic (NL4-3) or CCR5-tropic (NL-AD87) HIV-1 strains. Positive and negative controls included in the assay. HIV-1 (IBI) and HIV-2 (ROD) tested. Elavirenz (EFV) as control and Aspalathus linearis as negative drug. | Rosmarinic acid and caffeic acid. | Compounds bind to the catalytic core of purified HIV-1 integrase and blocks both activities of this enzyme. | EC₅₀ of aqueous extract at 22 µg/ml against the HIV-1 strain NL4-3 and 85 µg/ml against NL-AD87. | (Castro and Wyk, 1994; Louvel et al., 2013) |
| *Artemisia afra* Jacq. ex Willd. | Kenya, Tanzania, Uganda, north to Ethiopia and south to South Africa and Namibia. | Perfume, treat smallpox, infectious diseases and stomach ache. Anti-HIV for *A. annua*, but not *A. afra*. | Many volatile and non-volatile compounds. | Many volatile and non-volatile compounds. | Many volatile and non-volatile compounds. | Tea infusion IC₅₀ = 2.0 µg/ml. | (Asres et al., 2001; Liu et al., 2009; Lubbe et al., 2012) |
| *Aspilia pluriseta*     | DRC, Burundi, Rwanda, Uganda, Kenya, Tanzania, Malawi, Mozambique, Zambia, Zimbabwe | Traditionally used for infection and rheumatic diseases, fevers and malaria. | HIV (strain HTLV-III/HIV/LAI) evaluating cytotoxicity and viral cytopathic effect. | Thiarubrine-A, a dithiacyclohexadiene polyacetylen from the leaves | Target the interaction between the viral envelope glycoprotein gp120 and the CD4 receptor. The virus adsorbs to the cells, but also virus-induced syncytium. | Extract against HIV-1 and HIV-2 EC₅₀ of > 1213.5 µg/ml. Ethanol extract EG₅₀ = 16.3, SI > 12 with complete protection. | (Cos et al., 2002) |

(continued on next page)
| Plant species                  | Distribution                                                                 | Traditional use                                                                 | Model/Control                                                                 | Active constituents                                                                 | Mode of action                                                                 | Pharmacological activity/Concentration ranges                                                                                                                                                                                                 | Reference                                                                 |
|-------------------------------|------------------------------------------------------------------------------|---------------------------------------------------------------------------------|--------------------------------------------------------------------------------|------------------------------------------------------------------------------------|---------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------|
| *Bersama abyssinica* Fresen. (Melianthaceae) | From Guinea Bissau through the coastal countries of West Africa except Benin, east to Eritrea and Ethiopia and south to Angola, Zambia, Zimbabwe and Mozambique | A purgative to treat abdominal pain, colic, diarrhea, cholera, intestinal worms, amoebiasis, dysentery, rashes, syphilis, gonorrhea and malaria, cancer, rheumatism, as an aphrodisiac, diabetes mellitus, feverish pains, loss of appetite, debility, jaundice, leprosy, burns, ulcers, wounds, convulsions, snakebites, migraine, headache, colds, hemorrhoids and epilepsy. | HIV-RT colorimetric ELISA kit with Doxorubicin as positive control and the cytotoxicity and antiviral activity assays based on evaluating cell death caused by plant extract toxicity and inhibition of viral cytopathic effect with HIV-1 (IIIb) and HIV-2 (ROD). | The stem bark contain 2 bufadienolides, which are cardiac glycosides, steroids and mangiferin. | Methanol extracts of leaves, bark and root inhibition of 89.17%, 85.11% and 95.21% respectively at 200 µg/ml with IC50 of 11.95, 18.75 and 9.38 µg/ml. Methanol root bark 81 of 3.8 against HIV-1. (Asres et al., 2001; Mbaveng et al., 2011)         |                                                                                                                                                                                                 |                                                                                   |
| *Boerhavia coccinea* Mill. (Nyctaginaceae) | South-eastern United States, Africa including Namibia and South Africa, Asia, Australia, and South America. | Traditionally used to treat infectious diseases | Tetrazolium based colorimetric assay using HIV-1 (strain IIIb) and HIV-2 (strain ROD). AZT as positive control. | Boeravinone A, Boeravinone B, Boeravinone C, Coccineon B, Coccineon C, Coccineon D, Coccineon A | HIV-1, 80% methanol shoot extract IC50 = 54.8 µg/ml and water extract IC50 = 37.1 µg/ml. (Maregesi et al., 2010a, 2010b; Patil and Bhalse, 2016) |                                                                                                                                                                                                 |                                                                                   |
| *Boerhavia erecta* L. (Nyctaginaceae) | Native to the United States, Mexico, Central America and western South America, but now cosmopolitan in tropical and subtropical regions. In Africa its distribution extends from West Africa, eastwards to Somalia and down to South Africa. In Asia, it occurs in India, Java, Malaysia, the Philippines, China and the Ryukyu Islands. | Traditionally used to treat infectious diseases | HIV-1 IN assay | Procyanidin, catechin, epicatechin, quercetin, kaempferol and isorhamnetin glucosides present. | HIV-IN (Patil and Bhalse, 2016; Sántoz et al., 2004) |                                                                                                                                                                                                 |                                                                                   |
| *Bridelia micrantha* Native to most of | HIV, diarrhea, sore eyes, HIV-1 RT. Isolated compounds β-sitosterol and friedelin isolated | | | | Quercetin-3-O-rutinoside IC50 = 10 µg/ml and isorhamnetin-3-O-rutinoside IC50 = 22 µg/ml isolated from stem bark. Ethyl acetate fraction of roots (Bessong and Oh, 2011) |                                                                                   |                                                                                   |                                                                                   | (Bessong and Oh, 2011) |
| Plant species | Distribution | Traditional use | Model/Control | Active constituents | Mode of action | Pharmacological activity/ Concentration ranges | Reference |
|---------------|--------------|-----------------|---------------|--------------------|---------------|-----------------------------------------------|-----------|
| (Hochst.) Baill. (Euphorbiaceae) | sub-Saharan Africa except South Africa and Namibia. Distribution not well documented, occurs in South Africa. | stomach aches and abortifacient. | also evaluated on HIV-1 IN. from roots. | HIV-1 p24 antigen assay. | HIV-1 RT, positive control nevirapine. The HIV-1 PR assay, HIV-FRET and a recombinant HIV-1 protease solution. Positive control pepstatin and ritonavir. | IC$_{50}$ = 7.3 µg/ml. No effect on HIV-1 IN. | (Gail et al., 2015; Klos et al., 2009; Maroyi, 2014) |
| Bulbine alooides (L.) Willd. (Asphodeliaceae) | Burns, cracked lips, diarrhea, herpes simplex, itching, skin rash, ringworm, vomiting, HIV and wounds. | | | HIV-1c (M 5 A) p24 antigen assay with AZT as positive control. Therapeutic index determined by neutralization test to determine non-cytotoxic concentration (ID$_{50}$) that inhibits/protects 50% of the monolayer cells against destruction by the virus compared to uninfected cells using the Spearman-Karber formula and by the End point titration technique with acyclovir as positive control. | | | |
| Cassia abbreviata Oliv. (Caesalpiniaceae) | Backache, abdominal pains, diarrhea, constipation, tooth ache, fever, ulcers, STIs such as syphilis and gonorrhea. Root and bark used as a general blood cleanser, appetite enhancer and reducing HIV levels. | | | Cassia roots contains anthocyanin, anthra- noids, anthraquinones, polyphenols and tannins. | | Ethanol root extract EC$_{50}$ = 102.8 µg/ml. | (Leteane et al., 2012; Viol et al., 2016) |
| Centella asiatica (L.) Urb. (Apiaceae) | It is native to wetlands in Asia, but due to its invasive nature now found world-wide including southern Africa. | Used to treat various diseases, such as gonorrhea, syphilis, diabetes, fever, leprosy, wound healing, gastro-intestinal ailments, HIV, asthma and neurosis. | Contains various essential oils, asiatic acid, asiaticoside, madecassic acid, terminolic acid, quercetin, kaempferol and hederin. Also phenylpropane and acetate metabolites. | Immunomodulatory effect of aqueous or alcoholic extract. Asiatric acid and an anti-HIV agent with IC$_{50}$ of 36 and 8 µg/ml. | | (Brinkhaus et al., 2000; Lamorde et al., 2010; Yasurin et al., 2015) |
| Combretum adenogonium Steud. ex A.Rich (Combretaceae) | Traditionally used to treat infectious diseases. | Tetrazolium based colorimetric VSV T2 inhibition assay. AZT as positive control. | Flavonoids, tannins, saponins, phytoestrogens, sitosterol and stigmasterol. | HIV – 1 | 80% methanol leaf extract IC$_{50}$ = 2.7 µg/ml and water = 4.8 µg/ml. 80% methanol stem bark extract IC$_{50}$ = 4.4 µg/ml and water = 5.6 µg/ml. HIV-2 | (Jordaan et al., 2011; Maregesi et al., 2008) |

(continued on next page)
| Plant species                  | Traditional use                                                                 | Active constituents                                                                 | Mode of action                                                                                           | Pharmacological activity/Concentration ranges                                                                 |
|-------------------------------|----------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------|
| Combretum molle R.Br. ex G.Don (Combretaceae) | Wide distribution, from Saudi Arabia, Yemen and Ethiopia, to South Africa in the south and westwards to West Africa, DRC and Angola. | Tannin, ellagitannin, pentacyclic triterpene glucosides namely punicalagin, arjunglucoside and sericoside. | Inhibit RNA-dependent-DNA polymerase activity of HIV-1 RT IC\textsubscript{50} = 37.5 µg/ml (water) and 9.5 µg/ml (methanol) against RDDP and IC\textsubscript{50} of 13.7 µg/ml (water) and 9.7 µg/ml (methanol) against RNase H. | (Bessong et al., 2005; Jordaan et al., 2011) |
| Combretum paniculatum Vent. (Combretaceae) | West Africa, east to Ethiopia, south to Angola, Mozambique and South Africa.     | Pheophorbide a, pheophorbide, cardamonin, pinocembrin, quercetrin and kaempferol.     | Cytotoxicity and antiviral activity assays based on evaluating cell death caused by plant extract toxicity and inhibition of viral cytopathic effect with HIV-I (III\textsubscript{B}) and HIV-2 (ROD). | Acetone leaf extract SI of 6.4 and 32.0 for HIV-1 and HIV-2 and the methanol extract with SI of 4.7 for HIV-1. (Asres et al., 2001) |
| Dichrostachys cinerea (L.) Wight & Arn. (Leguminosae) | Native to Africa, Indian subcontinent and North Australia and introduced to the Caribbean and parts of Southeast Asia. | Friedelin, friedlan-3-ol, sitosterol and amyrin, octacosanol, hentricontanol, coumarins imperatorin, marmesin and aesculetin. | | Therapeutic index of 7.5 for leaves and 3.7 for roots methanol extract. (Viol et al., 2016) |
| Dodonaea angustifolia L.f. (Sapindaceae) | Southern Africa to Arabia, as well as in Australia and New Zealand.              | Cardiac glycosides and tannins namely 4'-O-methyl epigallocatechin dimethyl-1, 3, 8, 10-tetrahydroxy-9-methoxy-peltigynan, canophyllol, 30-hydroxylup-20(29)-en-3-one, 30-Hydroxylupeol, tingenin B, tingenone, galacticol, ouratea proanthocyanidin, ouratea proanthocyanidin-nona-O-acetate. | Recombinant HIV strain in an MT-2 VSV-pseudotyped recombinant virus assay. | HIV-1, 80% methanol stem bark extract IC\textsubscript{50} = 7.1 µg/ml and water extract IC\textsubscript{50} = 2.6 µg/ml. (Archer and Wyk, 1998; S.M. Maregesi et al., 2010) |

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Table 3 (continued)

| Plant species | Distribution | Traditional use | Model/Control | Active constituents | Mode of action | Pharmacological activity/Concentration ranges | Reference |
|---------------|--------------|-----------------|---------------|--------------------|----------------|-----------------------------------------------|-----------|
| *Elaeodendron transvaalense* (Burtt Davy) R.H.Archer (Celastraceae) | Widespread in southern Africa, including Namibia. | HIV, colds, skin rashes, fever, candidiasis, dysmenorrhea and stomach ache. | HIV-1 RT. Isolated compounds were additionally evaluated on HIV-1 IN and viral proteins (NF-κB and Tat). Mesuol as positive control. | 3-Oxo-28-hydroxybetuli-20(29)-ene and 3,28-dihydroxybetuli-20(29)-ene | Potent inhibitory activity in both the NF-κB and Tat assays with inhibitory activity of 76% and 75%. No activity of pure compounds. | IC<sub>50</sub> of 80 µg/ml (water) and 131 µg/ml (methanol) against RDDP and IC<sub>50</sub> of 31.2 µg/ml (water) and 30 µg/ml (methanol) against RNase H. Chloroform and ethyl acetate extracts 64% and 76% inhibition respectively (1 µg/ml) in the NF-κB assay. Chloroform and ethyl acetate extracts > 70% inhibition at 15 µg/ml. Methanol root extract therapeutic index of 1.9. (Archer and Wyk, 1998; Bessong et al., 2005; Mthethwa et al., 2014; Tshikalange et al., 2008a) |
| *Elephantorrhiza goetzei* (Harms) Harms (Leguminosea) | Wide distribution in south central Africa. | Pain, sores, sexually transmitted infections (STIs), gastrointestinal disorders, microbial infections and genito-urinary system disorders. | Neutralization test to determine non-cytotoxic concentration (ID<sub>50</sub>) that inhibits/protects 50% of the monolayer cells against destruction by the virus compared to uninfected cells with acyclovir as positive control. Determined the reduction factor (RF). | Phenolic compounds, coumarins, flavonoids, saponins, stilbenoids, tannins and triterpenoids from bark, leaves and roots. | | (Viol et al., 2016) |
| *Emilia coccinea* (Sims) G.Don (Compositae) | Native to DR Congo, Burundi, Sudan, Kenya, Uganda, Tanzania, Malawi, Zambia, Angola, Zimbabwe and Mozambique. | Traditionally used to treat infectious diseases, ulcers, craw-craw, ringworm, fever and convulsions in children. | | | | (Edeoga et al., 2005; Margetesi et al., 2010a, 2010b) |
| *Euphorbia hirta* L. (Euphorbiaceae) | Worldwide distribution. Very common in pantropic and partly | Traditionally used to treat infectious diseases, female disorders, respiratory ailments, cough, coryza, HIV-1 RT. The cytotoxic effect was measured by means of the colorimetric MTT assay. | Alkaloids, phenolics, flavonoids terpenoids and cardiac glycosides. | Inhibits HIV-1, 2 reverse transcriptase. | 80% methanol shoot extract IC<sub>50</sub> = 73.7 µg/ml and water = 27.9 µg/ml. | (Gyuris et al., 2009) |

(continued on next page)
| Plant species         | Distribution                                                                 | Traditional use                                                                 | Model/Control                                                                 | Active constituents                                                                                      | Mode of action                                                                                           | Pharmacological activity/Concentration ranges                                                                 | Reference |
|----------------------|------------------------------------------------------------------------------|---------------------------------------------------------------------------------|--------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------|-----------|
| subtropic areas      | including China, India, Philippines, Australia, Africa and Malaysia.          | bronchitis, asthma, worm infestations in children, dysentry, jaundice, pimples,   |                                                                                | Dose-dependent inhibition of RT on HIV-1, HIV-2 and SIVmac251 IC<sub>50</sub> = 38, 22 and 177 μg/ml respectively. | Aqueous and 50% methanolic extracts HIV-1,2 activity with IC<sub>50</sub> = 9 μg/ml and 5 μg/ml. HIV-1   | (Maregesi et al., 2010a, 2010b)                                                                          | (continued) |
| Euphorbia tirucalli L. (Euphorbiaceae) | Wide distribution in Africa, in northeastern, central and southern Africa. | Traditionally used to treat infectious diseases, cancer, tumors, warts asthma, cough, earache, neuralgia, rheumatism, toothache, | Tetrazolium based colorimetric assay using HIV-1 (strain III b) and HIV-2 (strain ROD). | Many compounds such as euphol, euphorbin and tirucallin                                                    |                                                                                                          |                                                          |           |
| Ficus sycomorus L. (Moraceae) | Native to Africa also grows naturally in Lebanon, Cyprus, Madagascar and naturalized in Israel and Egypt. | Traditionally used to treat infectious diseases.                                | Tetrazolium based colorimetric assay using HIV-1 (strain III b) and HIV-2 (strain ROD). |                                                                        |                                                                                                          | (Maregesi et al., 2010a, 2010b, 2008)                                                                      |           |
| Flacourtia indica (Burm. f.) Merr. (Salicaceae) | Tropical Africa south to northern South Africa, Swaziland, Madagascar, India, Sri Lanka, Indonesia and China | Snakebite, arthritis, STI, cough, pneumonia, and bacterial throat infection.     |                                                                                | Various volatile compounds                                                                                  |                                                                                                          | (Patro et al., 2013; Viol et al., 2016)                                                                   |           |
| Gymnosporia senegalensis (Lam.) Loes. (Celastraceae) | Sub-Saharan Africa from Senegal to Eritrea and south to northern Namibia, Botswana and South Africa. Also found in southern Spain, | Gastro-intestinal troubles, schistosomiasis mouthwash for toothache, tooth-     |                                                                                | Phenolic glycosides, lignin, β-sitosterol, polysaccharides, flavonoids, condensed tannins, alkaldoids,   |                                                                                                          | (Viol et al., 2016)                                                                                       |           |
|                      |                                                                              | alveases and mouth-infections, sores, eye-trouble, gastric ulcers,               |                                                                                | terpenoids, sugars, coumarin such as scoparone and aesculetin, also flavoaurin, pyroacetol, homaloside D, |                                                                                                          |                                                          |           |
|                      |                                                                              | terryphyllins, female                                                           |                                                                                | polioxyrhisoside, β-sitosterol, β-D-ghroopyranoside, ramanosiole & butynolactone lignan disaccharides. |                                                                                                          |                                                          |           |
|                      |                                                                              |                                                                                 |                                                                                | Methanol leaf and root extract therapeutic index of 0.9 and 1.3 respectively. Root RF of 10<sup>5</sup>. |                                                                                                          |                                                          |           |
|                      |                                                                              |                                                                                 |                                                                                | The leaves and branches contain ductile and tannins. The leaves contain sterol, flavonol,     |                                                                                                          |                                                          |           |
|                      |                                                                              |                                                                                 |                                                                                | slavonic glycoside, saponosides, flavone derivatives and tannins.                                        |                                                                                                          |                                                          |           |
|                      |                                                                              |                                                                                 |                                                                                | Methanol extracts of leaf, root and twig therapeutic index of 3.8, 3.8 and 1.2 respectively and RF of 10<sup>5</sup> for all extracts. | (Viol et al., 2016)                                                                                       |                                                          |           |
| Plant species | Distribution | Traditional use | Model/Control | Active constituents | Mode of action | Pharmacological activity/ Concentration ranges | Reference |
|---------------|--------------|-----------------|---------------|--------------------|---------------|-----------------------------------------------|-----------|
| Helichrysum spp. (30) investigated with the highest activity in Helichrysum populifolium | North Africa, Afghanistan to India, Aldabra and Madagascar. | complaints, leprosy and Blennorhea. | with acyclovir as positive control. Determined the reduction factor (RF). | Di- and tricaffeoylquinic acids; 3, 4 dicaffeoylquinic acid; 3, 5 dicaffeoylquinic acid and 4,5 dicaffeoylquinic acid. | Extracts tested at 25 and 2.5 µg/ml. | (Gail et al., 2015; Heyman et al., 2015) |
| Helichrysum populifolium DC (Asteraceae) | Most species occur in Africa, including Madagascar, South Africa and Namibia, also Southern Europe, south-west Asia, southern India, Sri Lanka and Australia. | Coughs, colds, fever, infection, headaches, menstrual pain, HIV and wound dressing. | The reporter cell line HeLa-SXR5, stably expresses the CD4 receptor and the CXCR4/CCR5 chemokine receptors. DeCIPhR method on a full virus model. | | | |
| Hosludia opposita Vahl (Lamiaceae) | Widespread in tropical Africa and south to South Africa. | Traditionally used to treat infectious diseases, gonorrea, cystitis, coughs, fever, wounds, convulsions, sores, mental disturbances, abdominal pains, snake bites and for the relief of swellings. | Efavirenz positive control and negative control PBV/5% DMSO. | Various naphthoquinones, iridoids, sterols, coumarins, flavonoids and alkaloids kigelin, β-sitosterol, 1,3-dimethylkigelin and ferulic acid have been isolated from the bark, quercetin. | Anti-HIV IC<sub>50</sub> = 12–21 µg/ml. | HIV-1 (Maregesi et al., 2010a, 2010b; Mujovo et al., 2008; Prakash and Staden, 2007) |
| Hypoxis hemerocallidea Fisch., C.A.Mey. & Avé-Lall. (Hypoxidaceae) | Native to southern Africa from South Africa as far north as Mozambique and Zimbabwe. | Headaches, stomach ailments, dysentery, dizziness, burns, cancer, HIV, symptoms of benign prostrate hypertrophy, diabetes, high blood pressure, pimples, wounds, skin rash, dermañsin, mental disorders and general tonic for good health. | HIV-1 RT assay and the therapeutic index determined by neutralization test to determine non-cyotoxic concentration (ID<sub>50</sub>) that inhibits/protects 50% of the monolayer cells against destruction by the virus compared to uninfected cells using the Spearman-Karber formula and by the End point titration technique with acyclovir as positive control. Determined the reduction factor (RF). | Remarkably stable CD lymphocyte counts concurrently with the decrease in serum p24 HIV antigen and expression of the HLA-DR CD8 lymphocyte activation marker on HIV patients. | Leaves extract (water) IC<sub>50</sub> = 14.8 µg/ml. | Inhibit HIV-1 RT. Methanol extract of tuber therapeutic index of 15 and RF of 10<sup>3</sup>. | (Gail et al., 2015; neube et al., 2013; Vish et al., 2016) |
| Hypoxis sobolifera Jacq. (Hypoxidaceae) | Endemic to South Africa | Traditionally used directly in HIV/AIDS or symptoms/ conditions closely associated with this disease. | HIV-1 RT and PR assay. | | | |
| Kigelia africana (Lam.) Benth. (Bignoniaceae) | Throughout tropical Africa and to the south in South Africa, Namibia and Swaziland. | Traditionally used to treat infectious diseases, HIV, fainting, anemia, sickle-cell anemia, epilepsy, respiratory ailments, | Tetrazolium based colorimetric assay using HIV-1 (strain III b) and HIV-2 (strain ROD). AZT as positive control. Neutralization test to | Various naphthoquinones, iridoids, sterols, oxasamin, flavonoids and alkaloids kigelin, β-sitosterol, 1,3-dimethylkigelin and ferulic acid have been isolated from the bark, quercetin. | ≥50% inhibition of HIV-1 RT and HIV-1 PR. Aquous and ethanolic extracts inhibition at 0.2 µg/ml against HIV-1 RT. | (Atawodi and Olowoniyi, 2015; Maregesi et al., 2010a, 2010b; Rubanga et al.) | (continued on next page)
| Plant species | Distribution | Traditional use | Model/Control | Active constituents | Mode of action | Pharmacological activity/Concentration ranges | Reference |
|---------------|--------------|-----------------|---------------|--------------------|---------------|-----------------------------------|-----------|
| *Lannea schweinfurthii* Engl. (Anacardiaceae) | Kenya, Uganda, Tanzania, Zanzibar, Malawi, Mozambique, Zambia, Zimbabwe, Swaziland and South Africa. | Treatment of infectious diseases. | Tetrazolium based colorimetric assay using HIV-1 (strain III b) and HIV-2 (strain ROD). AZT as positive control. | Stems bark water extract IC$_{50}$ = 83.2 µg/ml. Fruit 6.93% RT inhibition at 100 µg/ml and 5.21 at 50 µg/ml. Leaves 33% RT inhibition at 100 µg/ml and 11.3 at 50 µg/ml. Therapeutic index of methanol extract of bark and fruit of 1.2 for both and 10$^{-3}$ and 10$^{-4}$ RF values respectively. | Stem bark water extract IC$_{50}$ = 83.2 µg/ml. Fruit 6.93% RT inhibition at 100 µg/ml and 5.21 at 50 µg/ml. Leaves 33% RT inhibition at 100 µg/ml and 11.3 at 50 µg/ml. Therapeutic index of methanol extract of bark and fruit of 1.2 for both and 10$^{-3}$ and 10$^{-4}$ RF values respectively. | (Lamorde et al., 2010) |
| *Leonotis leonurus* (L.) R.Br. (Lamiaceae) | Native to southern Africa. | Fevers, headaches, coughs, dysentery, remedy for snake bite and charm to keep snakes away. | HIV-1 p24 antigen assay. | No anti-HIV compounds, contains sterols, diterpenes, triterpenoids, tannins, flavonoids, alkaloids, quinines and saponins. | HIV-1 RT. Positive control nevirapine. HIV-1 PR assay, HIV-FRET and a recombinant HIV-1 protease solution used. Positive control for HIV-1 PR acetyl pepstatin and ritonavir. | 80% methanol stem bark extract IC$_{50}$ = 7.1 µg/ml and water extract IC$_{50}$ = 51.2 µg/ml. HIV-2 80% methanol stem bark extract IC$_{50}$ = 9.9 µg/ml and water extract IC$_{50}$ = 89.4 µg/ml. Tannin-dereplicated ethanolic extracts HIV-1 PR inhibition IC$_{50}$ = 120.6 µg/ml. Ritonavir control IC$_{50}$ = 5.3 ng/ml | (Gail et al., 2015; Khos et al., 2009) |
| *Leonotis nepetifolia* (L.) R.Br. (Lamiaceae) | It is native to tropical Africa and southern India, Latin America and the West Indies. | Traditionally used to treat infectious diseases and HIV | Tetrazolium based colorimetric assay using HIV-1 (strain III b) and HIV-2 (strain ROD). AZT as positive control. | | | | |

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| Plant species                  | Distribution                          | Traditional use                                                                 | Active constituents                                                      | Mode of action                                                                                      | Pharmacological activity/Concentration ranges                                                                 | Reference |
|-------------------------------|---------------------------------------|---------------------------------------------------------------------------------|-------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------|-----------|
| Lobostemon trigonus         | Endemic to South Africa               | Decoction for wound healing, ulcers and ringworm or as an infusion for blood purification. | HIV-1 RT assay and HIV-II PR assay. The genus is known to contain naphthoquinone derivatives, pyrrolizidine alkaloids, cyclitols, phenolic acids, tannins and the ureide allantoin | HIV (strain HTLV-III/LAI) evaluating cytotoxicity and viral cytopathic effect. 80% methanol leaves extract IC₅₀ = 32.8 µg/ml and water extract IC₅₀ = 34.9 µg/ml. HIV-2 80% methanol leaves extract IC₅₀ = 119 µg/ml. Aqueous extract of leaves HIV-1 RT activity at IC₅₀ = 49 µg/ml. No HIV-II PR activity. | (Harrett et al., 2005)                                      |           |
| Myrothamnus glabellifolia   | South Africa, Angola, Botswana, Madagascar, Zimbabwe, Mozambique, Malawi, Tanzania, Namibia, Zambia and Kenya. | Treatment of chest complaints (smoke of burning leaves), wounds (ointments for topical application), and to treat cough, influenza, mastitis, headaches, kidney disorders, hemorrhoids, abdominal pains, scurvy, halitosis and gingivitis. | Polyphenolic compounds and 3, 4, 5 tri-O-galloylquinic acid. Pinocarveol, pinocarvone, and β-selinene are the most abundant volatiles, along with α-pinene, limonene, and a few other terpenoids. | HIV-1 attachment inhibitor with IC₅₀ for 3, 4, 5 tri-O-galloylquinic acid of 5 µM for M-MLV and 34 µM for HIV-1. Inhibition non-competitive, with IC₅₀ for 3, 4, 5 tri-O-galloylquinic acid of 5 µM for M-MLV and 34 µM for HIV-1. Interferes directly with viral infectivity and blocks the attachment of HIV-1 particles to target cells, protecting them from virus entry. | (Gechev et al., 2014; Gescher et al., 2011; Moore et al., 2007) |           |
| Ozoroa reticulata           | Widely distributed from southern Ethiopia, Zaire to southern Africa. | Traditionally used to treat infectious diseases, kidney and liver complaints, chest pain, diarrhea, chistosomiasis, ulcers and hernias, otitis, colic, dysentery, muscle pains, fever, hypertension and throat infections. | 6-pentadecylsalicylic acid, toxic to brine shrimp and anacardic acid and ginkgoic acid as cytotoxic components. | HIV-1 (Maregesi et al., 2010a, 2010b) | Leaves 80% methanol = 16.2 µg/ml and water = 81.4 µg/ml. Stem bark 80% methanol = 11.6 µg/ml and water = 15.8 µg/ml. Root bark 80% methanol = 20.6 µg/ml. HIV-1 attachment inhibitor with EC₅₀ = 8.13 µg/ml. EC₅₀ for galloacetate (4R)-4-galloylacetate 7.3 µg/ml. epigallocatechin-(4β)-8-gallocatechin 6.3 µg/ml. epigallocatechin 42.5 µg/ml and galloacetate 28.4 µg/ml. Galaktansin inhibited RDDP and RNAse H. RT IC₅₀ = 6.0 and 5.0 µM, respectively, and abolished the 3′-end processing activity of IN (100 µM). Bergenin no effect on IN (100 µM). | (Heller et al., 2014; Moyo and Van Staden, 2014) |           |
| Pelargonium sidoides        | Native to South Africa and Lesotho.   | Gonorrhea, diarrhea, dysentery, a prolapsed rectum and intisilia, colic, wounds, acute bronchitis, cold, acute rhino sinuitis, influenza and herpes virus. | Gallic acid, umckalin, catechin, oleic acid, linoleic acid and coumarins. | HIV-1 attachment inhibitor with IC₅₀ = 8.13 µg/ml. EC₅₀ for galloacetate (4R)-4-galloylacetate 7.3 µg/ml. epigallocatechin-(4β)-8-gallocatechin 6.3 µg/ml. epigallocatechin 42.5 µg/ml and galloacetate 28.4 µg/ml. Galaktansin inhibited RDDP and RNAse H. RT IC₅₀ = 6.0 and 5.0 µM, respectively, and abolished the 3′-end processing activity of IN (100 µM). Bergenin no effect on IN (100 µM). | (Bessong et al., 2005)                                      |           |
| Peltophorum africicum       | Native from southern DR Congo to South Africa and Swaziland. Cultivated in Kenya, Tanzania, Madagascar, Australia and the United States. | HIV, wounds, toothache, sore throat, cough, tuberculosis, abdominal disorders, diarrhea, dysentery, membranagia, infertility. | HIV-1 RT assay. Isolated compounds were additionally evaluated on HIV-1 IN. Contains flavonoids and C-galloylglycosides namely (+)-catechin, bergenin and bertlinic acid. | HIV-1 attachment inhibitor with IC₅₀ = 8.13 µg/ml. EC₅₀ for galloacetate (4R)-4-galloylacetate 7.3 µg/ml. epigallocatechin-(4β)-8-gallocatechin 6.3 µg/ml. epigallocatechin 42.5 µg/ml and galloacetate 28.4 µg/ml. Galaktansin inhibited RDDP and RNAse H. RT IC₅₀ = 6.0 and 5.0 µM, respectively, and abolished the 3′-end processing activity of IN (100 µM). Bergenin no effect on IN (100 µM). | (Bessong et al., 2005)                                      |           |

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### Table 3 (continued)

| Plant species | Distribution | Traditional use | Model/Control | Active constituents | Mode of action | Pharmacological activity/Concentration ranges | Reference |
|---------------|--------------|-----------------|---------------|---------------------|----------------|-----------------------------------------------|-----------|
| *Plectranthus barbatus* Andrews (Lamiaceae) | Worldwide, found in sub-Saharan Africa as an invasive species | Anti-spasmodic, gastric disturbances, malaria, candida, managing HIV/AIDS, and opportunistic infections, herpes simplex, herpes zoster and skin rashes. | HIV-1 PR assay. Acetyl pepstatin (AP) was used as a positive control. | Various compounds isolated including betulinic acid, caffeic acid, diterpenes and forskolin. | PR activity could be attributed to diterpenoids. | Catechin no effect on RT, moderate activity on HIV-1 IN. HIV-<sub>NL4</sub>–3: Betulinic acid = 0.04 µg/ml, HIV-1JRCSF Betulinic acid = 0.002 µg/ml. | (Alashahi and Melirig, 2010; Chinsembu and Hedimbi, 2010b; Kapewangolo et al., 2013; Kim et al., 2013) |
| *Prunus africana* (Hook. f.) Kalkman (Rosaceae) | Wide distribution in Africa, from central to South Africa and on the islands of Bisko, São-Tomé, and Grande Comore. | Fevers, malaria, wound dressing, arrow poison, stomach pain, purgative, HIV, kidney disease, appetite stimulant and gonorrhea. | HIV-1 RT assay | Ferulic acid, n-docosanol, lauric acid myristic acid, β-sitostenone and β-sitosterol | Stem bark ethanol extract %RT at 100 µg/ml = 99.2 and 50 µg/ml = 71.2 | Ethyl acetate extract 70% inhibition at 100 µg/ml (IC<sub>50</sub> = 62.07 µg/ml) Acetyl-pepstatin 97% at10 µg/ml and its IC<sub>50</sub> was 0.3 µg/ml. HIV-1 RT 50% inhibition. Doxorubicin, IC<sub>50</sub> = 25 µg/ml. | (Gail et al., 2015; Kadu et al., 2012; Rukunga et al., 2002) |
| *Rhus chirindensis* Baker f. (Anacardiaceae) | Widespread in African from Tanzania in the north to the Cape in the south. | Treating heart complaints, strengthen the body, stimulate circulation and treatment of rheumatism and mental disorders and sexually transmitted diseases. | Neutralization test to determine non-cytotoxic concentration (IC<sub>50</sub>) that inhibits/protects 50% of the monolayer cells against destruction by the virus compared to uninfected cells with acyclor as positive control. Determined the reduction factor (RF) | Flavonoids and triterpenoids. | Methanol root extract therapeutic index of 3.8. | (Viol et al., 2016) |
| *Rumex bequaertii* De Wild. (Polygonaceae) | Europe through Asia to China, Vietnam and Indonesia, Africa, mainly in the east from Eritrea and Somalia to South Africa. | Traditionally used for infection and rheumatic diseases, purgative, rheumatism, colic, stomach-ache and abdominal pains, abscesses, schistosomiasis and headaches. | HIV (strain HTLV-IIIB/LAI) evaluating cytotoxicity and viral cytopathic effect, examined spectrophotometrically by the MTT-method. | Emodin, chrysophanol, physcion, anthraquinones aloesin, rumensin, orientiakside, torachryson, nepodin, nepodin-8-O-β-D-glucopyranoside, torachryson and torachryson-8-O-β-D-glucopyranoside. | Target the interaction between the viral envelope glycoprotein gp120 and the CD4 receptor. The virus adsor to the cells, but also virus-induced syncytium (giant cell) formation is inhibited. Sulfated polysaccharides may also directly interfere with the binding of HIV particles to the heparin sulfate proteoglycan of the cell surface. | Ethanolic extract EC<sub>50</sub> = 17.69 with SI > 11% and 89% cell protection. | (Cos et al., 2002; Vassas et al., 2015) |
| *Scleroaorga birrea* A. | Widespread Hypertension, dysentery, Neutralization test to | Phenolic compounds, | | | Methanol extract of bark | (Viol et al., 2016) |
Table 3 (continued)

| Plant species | Distribution | Traditional use | Model/Control | Active constituents | Mode of action | Pharmacological activity/Concentration ranges | Reference |
|---------------|--------------|-----------------|---------------|--------------------|---------------|-----------------------------------------------|-----------|
| *Rich.) Hochst. (Anacardiaceae)* | throughout the semi-arid deciduous savannas of much of sub-Saharan Africa. | stomachache, gastroenteritis, anti-cough remedy, hypertension, anihyperglyceremic, diarrhea, dysentery, malaria, general tonic and sexually transmitted diseases. | determine non-cytotoxic concentration (ID$_{50}$) that inhibits/protects 50% of the monolayer cells against destruction by the virus compared to uninfected cells with acetal as positive control. Determined the reduction factor (RF). | proanthocyanidins, gallotannins and flavonoids such as quercetin 3-O-alpha-l-(5''-galloyl)-arabinofuranoside. | therapeutic index of 1.9 and RF of $10^3$. | | |
| *Securidaca longipedunculata Fresen. (Polygalaceae)* | Tropical and subtropical areas of Africa with protected status in South Africa. | Stomach complaints, tuberculosis, wound dressing, rheumatism, syphilis, cough, diarrhea, syphilis and typhus. | assessed in C8166 cells infected with HIV-1 III-B, acid and 4,5-di-O-caffeylquinic acid as well as Caffeic acid, rosmarinic acid and synapoic acid. | Caffeoylquinic acids: 3,4,5-tri-O-caffeylquinic acid and 4,5-di-O-caffeylquinic acid at 0.6 and 8 µg/ml, caffeic acid at 0.16 µg/ml and no activity and synapoic acid at 200 and >200 µg/ml. Rosmarinic acid 40 and 100 µg/ml. Methanol extract of root therapeutic index of 3.8 and RF of $10^3$. | | | |
| *Sutherlandia frutescens (L.) R.Br. (Fabaceae)* | Endemic to southern Africa | HIV, relieving cold, influenza, chicken pox, diabetes, varicose veins, piles, headache, rheumatism, physical and mental stress. | High levels of free amino acids, non-protein amino acids such as canavanine and GABA, cyclitol pinitol, flavonols and triterpenes including SU1. Canavanine is an inhibitor of nitric oxide synthase and has potential for the treatment of septic shock, a condition associated with advanced stages of AIDS. | ELISA kit with fluorometric detection of HIV-1 PR. Tetrazolium based colorimetric VSV T2 inhibition assay. AZT as positive control. | Leaves and flowers > 50% inhibition against HIV-1 RT. No HIV-1 PR activity (≥50%) when assayed at 0.2 mg/ml. | | |
| *Terminalia mollis M.A.Lawson (Combretaceae)* | Widespread in Africa occurring in West Africa, Angola, DR Congo, Uganda, Kenya, Tanzania, Zambia and Zimbabwe. | Treatment of infectious diseases. | Tannins and saponins. | 80% methanol stem bark extract IC$_{50} = 5.9$ µg/ml and | | |

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Table 3 (continued)

| Plant species | Distribution | Traditional use | Model/Control | Active constituents | Mode of action | Pharmacological activity/Concentration ranges | Reference |
|---------------|--------------|-----------------|---------------|--------------------|----------------|-----------------------------------------------|-----------|
| *Terminalia saroea* Burch. ex DC. (Combretaceae) | Endemic to Africa from Tanzania and the DR of Congo southwards to South Africa. | Diabetes, diarrhea and STL. | HIV-1 RT and viral proteins (NF-κB and Tat) assays. Reference drug Adriamycin. Mesuol as a reference inhibitor of NF-κB. Neutralization test to determine non-cytotoxic concentration (ID<sub>50</sub>) that inhibits/protects 50% of the monolayer cells against destruction by the virus compared to uninfected cells with acyclor as positive control. Determined the reduction factor (RF). | Triterpenoids, saponins, tannins and Anolignan B | Extracts tested at 50 µg/ml and the active extracts were further tested at 25, 15, 5 and 1 µg/ml. | HIV-1 RT | (Eldeen et al., 2011; Tshikalange et al., 2008a; Viol et al., 2016) |
| *Tithonia diversifolia* (Hemsl.) A.Gray (Asteraceae) | Native to Mexico, Central America and Cuba, naturalized in tropical parts of Asia, Africa and Pacific islands. | Traditionally used for infections, rheumatic diseases, ascariasis and diarrhea. | HIV (strain HTLV-III/LAI) evaluating cytotoxicity and viral cytopathic effect, examined spectrophotometrically by the MTT-method. | Sulfated polysaccharides, polyphenolic compounds such as hydrolysable tannins, diversifolin, diversifolin methyl ether and tirotundin. | Target the interaction between the viral envelope glycoprotein gp120 and the CD4 receptor. The virus adsorp to the cells, but also virus-induced syncytium (giant cell) formation is inhibited. In addition, sulfated polysaccharides may also directly interfere with the binding of HIV particles to the heparin sulfate proteoglycan of the cell surface. diversifolin, diversifolin methyl ether, tirotundin | EC<sub>50</sub> => 1.60 µg/ml with SI < 1. Water sub-fraction EC<sub>50</sub> = 0.04 µg/ml with SI > 461 with complete cell protection. | (Cos et al., 2002) |
| Plant species                | Distribution                                                                 | Traditional use                                                                 | Model/Control                                                                 | Active constituents                                                                 | Mode of action                                                                 | Pharmacological activity/Concentration ranges                                                                 | Reference                          |
|-----------------------------|------------------------------------------------------------------------------|---------------------------------------------------------------------------------|--------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|---------------------------------------------------------------------------------|---------------------------------------------------------------------------------|-------------------------------------|
| Vernonia amygdalina Delile  | Wild in most countries of tropical Africa, from Guinea east to Somalia and   | Traditionally used for infections, rheumatic diseases, ascariasis, diabetes,     | HIV (strain HTLV-III/LAI) evaluating cytotoxicity and viral cytopathic effect,   | Gallic acid, dihydroxyacetone, quercetin, vernolide, octahydrovernodalin, vernonium   | HIV-1 inhibition EC<sub>50</sub> = > 19.66 µg/ml.                              | (Cos et al., 2006; Lamorde et al., 2010; Toyang and Verpoorte, 2013)            |
| (Asteraceae)                | south to north-eastern South Africa, and in Yemen.                            | fever, STI, HIV, measles, skin problems and chicken pox.                         | examined spectrophotometrically by the MTT-method.                              | oxide A3, vernodalin, vernomenin, vernolide, vernodegin, vernolide, vernodalin and  |                                                                                                |                                    |
|                            |                                                                              |                                                                                  |                                                                                  | vernodalinol.                                                                        | HIV-1 inhibition EC<sub>50</sub> = > 19.66 µg/ml.                              | (Cos et al., 2006; Lamorde et al., 2010; Toyang and Verpoorte, 2013)            |
| Vernonia stipulacea Klatt   | Zambia, Tanzania, Malawi, Mozambique, Zimbabwe; widespread in tropical Africa,   | HIV, diarrhea, fever, flu and contraceptive.                                    | HIV-1 RT assay and HIV-II PR assay.                                             | Muzigadial, 9β-Octahydro-6, 6, 9β-Hydroxy-2H-pyran-1-(3H)-one (drimenin), 5, 10-Dihydro-6, | SI < 1. Weak HIV-1 RT activity at > 100 µg/ml.                                  | (Bossong et al., 2005)                                                         |
| (Asteraceae)                | extending to South Africa.                                                    |                                                                                  |                                                                                  | 7-dimethyl-4H-benzo[1, 2-b]furan, warburganal and polygodial.                      |                                                                                 |                                    |
|                            | Botswana, Namibia, Tanzania, Zambia, Mozambique, South Africa, Swaziland, Malawi and Zimbabwe. |                                                                                  |                                                                                  |                                                                                     | (Lamorde et al., 2010; Viol et al., 2016)                                      |                                    |
| Warburgia salutaris (G.Bertol.) Chiov. (Canellaceae) | Malaria, respiratory complaints such as cold and cough and sexually transmitted diseases and HIV. |                                                                                  |                                                                                  |                                                                                     |                                                                                 |                                    |
|                            | Botswana, Namibia, Tanzania, Zambia, Mozambique, South Africa, Swaziland, Malawi and Zimbabwe. |                                                                                  |                                                                                  |                                                                                     |                                                                                 |                                    |
|                            |                                                                                  |                                                                                  |                                                                                  |                                                                                     |                                                                                 |                                    |
| Zanthoxylum davyi Waterm. (Rutaceae) | Native to South Africa, western Swaziland and eastern Zimbabwe. | Traditionally used in treatment of sexually transmitted diseases, chest pains, wounds, toothache, cough, pleurisy, toothache, snakebites, heal sores, sore throat and aphrodisiac. | HIV-1 RT and viral proteins (NF-κB and Tat).                                      | Muzigadial, 9β-Octahydro-6, 6, 9β-Hydroxy-2H-pyran-1-(3H)-one (drimenin), 5, 10-Dihydro-6, 7-dimethyl-4H-benzo[1, 2-b]furan, warburganal and polygodial. | Therapeutic index of 1.2 and RF of 10<sup>3</sup>. | (Tarus et al., 2006; Tshikandle et al., 2008)                                   |
|                            |                                                                                  |                                                                                  |                                                                                  |                                                                                     |                                                                                 |                                    |
|                            |                                                                                  |                                                                                  |                                                                                  |                                                                                     |                                                                                 |                                    |
| Ximenia americana L. (Olacaceae) | Tropical and temperate regions.                                               | Contagious diseases, stomach complaints, placenta expulsion, internal parasitism and worm infestations. | HIV-1 (III) and HIV-2 (ROD) infected MT-4 cell lines. MTT colorimetric assay used for evaluation. | Benzo[c]phenanthidine alkaloids, chelerythrine, dihydrochelerythrine, boconoline, 6-hydroxydihydrochelerythrine and 6-methoxy-7,6-dimethyl-dihydrochelerythrine, together with 4-methoxy-1-methyl-2 (1H)-quinolone and the uncommon lignan meso-sesamin. | Inhibits HIV-1 replication saponin. | (Ares et al., 2001)                                                             |
|                            | Senegal to Ethiopia and south to South Africa.                                |                                                                                  |                                                                                  |                                                                                     |                                                                                 |                                    |
|                            |                                                                                  |                                                                                  |                                                                                  |                                                                                     |                                                                                 |                                    |

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is highly bioavailable as it is absorbed in the stomach and jejunum followed by absorption along the small intestine and also the large intestine (Farah et al., 2008). Other compounds such as 3-CQA, 5-CQA, 3,4-DCQA, 3,5-DCQA, and 4,5-DCQA are all present in the plasma with low concentrations of caffeic, ferulic, isoflavonic, and p-coumaric acids (Farah et al., 2008) and might not be realistically recorded in in vitro assays which are often reported at much higher concentrations.

Chlorogenic acids and other dihydroxycinnamic acids such as caffeic acid have been described previously as anti-oxidants and therefore beneficial compounds. A more possible explanation is however their pro-oxidant activity as they can be oxidized to form quinones when oxidized by peroxidase/H2O2 or tyrosinase/O2. These quinones can be very toxic in cells due to creating oxidative stress, but are kept in their unoxidised state by antioxidants such as glutathione or ascorbic acid (Moridani et al., 2011). Flavonoids act as pro-oxidants in producing a quinone which produce reactive oxygen species (ROS) which are very effective in the defense of herbivores and pathogenic attack. Of 0.2% caffeic acid or 0.2% 5-CQA to mice, resulted in a significantly increased level of Glutathione S-transferase (GST), probably due to their pro-oxidant activity and electrophile-responsive element (EpRE) activation. Similarly many cinnamic acids have been shown to be potent inducers of NAD(P)H:(quinone-acceptor) oxidoreductase (Clifford, 1999). The DCQA’s and DCTA’s are bis-catechols and do not appear to inhibit HIV-1 RT within the cell, but acting directly through inhibition of IN (McDougal et al., 1998).

The mechanism of how the phenolic acids act on viruses to inhibit their replication or infection is however not well researched. Compounds such as 5-CQA and related chlorogenic acids have been tested numerous, not only to confirm their anti-HIV activity, but also their activity on HSV-1, HSV-2 and Adenovirus-11 (Chiang et al., 2002; McDougall et al., 1998; Tamura et al., 2006; Thompson, 2006; Wang, 2006). Enough evidence has been accumulated in various studies to warrant further investigation into the active principles and chemical profile of anti-viral plants with specific focus on the three mentioned groups. A systematic review by Liu and Yang (2005) assessed the beneficial effects and risks of herbal medicines in patients with HIV infection and AIDS, and concluded that there is inadequate evidence to support the use of herbal medicines in HIV-infected individuals and AIDS patients. However, potential beneficial effects need to be confirmed in large, rigorous trials (Liu et al., 2005). Few southern African plants or plant compounds are currently in clinical trial studies. For example, S. frutescens, indigenous to Lesotho, South Africa, southern Namibia and southeastern Botswana has a wealth of pre-clinical data (van Wyk and Albrecht, 2008). A phase I study has shown

### Table 3 (continued)

| Plant species | Distribution | Active constituents | Model/Control | Pharmacological activity/ Concentration ranges | Mode of action |
|---------------|--------------|---------------------|---------------|-----------------------------------------------|---------------|
| *Ziziphus mucronata* Willd. and Zimbabwe (Rhamnaceae) | Botswana, Namibia-Caprivi, DR Congo, southern Tanzania, Mozambique, Zambia, southern Africa, Zimbabwe | 5-CQA = 77.5 µg/ml (water) and 81.5 µg/ml (methanol), H2O2 | HIV-1 RT IC50 > 100 µg/ml (water) and 75 µg/ml (methanol) | Antioxidant | 7. Discussion |

Poor nutrition, inaccessibility to health systems and overburdened health budgets and resources contribute to the spread and inadequate control and continued infection of HIV (Coovadia et al., 2009). Many studies have relied on the traditional uses of medicinal plants in treating viral infections and various accounts of very active plants have been documented (Bessong et al., 2005). Where anti-HIV activity could be linked to isolated compounds from southern African plants, it is often compounds previously identified for anti-HIV activity in plants from other regions of the world. It is therefore evident that similar compounds or compound groups have been identified as the active principles in plant preparations from various regions in the world and the repeated identification should be supported by progression into in vivo studies, especially in the southern African region where affordable and safe medicines are needed urgently.

Plants continue to provide drug leads and numerous plants and/or plant compounds have been advanced to clinical trials (Yang et al., 2011). Enough evidence has been accumulated in various studies to warrant further investigation into the active principles and chemical profile of anti-viral plants with specific focus on the three mentioned groups. A systematic review by Liu and Yang (2005) assessed the beneficial effects and risks of herbal medicines in patients with HIV infection and AIDS, and concluded that there is inadequate evidence to support the use of herbal medicines in HIV-infected individuals and AIDS patients. However, potential beneficial effects need to be confirmed in large, rigorous trials (Liu et al., 2005). Few southern African plants or plant compounds are currently in clinical trial studies. For example, *S. frutescens*, indigenous to Lesotho, South Africa, southern Namibia and southeastern Botswana has a wealth of pre-clinical data (van Wyk and Albrecht, 2008). A phase I study has shown
that *S. frutescens* is well tolerated and that it showed no significant side effects (Johnson et al., 2007). Recently the results of an adaptive two-stage randomized double-blind placebo controlled study were published. The study evaluated the safety of consuming dried *S. frutescens* by HIV seropositive adults with CD4 T-lymphocyte count of > 350 cells/μL. *Sutherlandia frutescens* did not change HIV viral load, and CD4 T-lymphocyte count and was similar in the two arms at 24 weeks; however, mean and total burden of infection was greater in the *S. frutescens* arm attributed to two tuberculosis cases in subjects taking isoniazid preventive therapy (IPT). The study concluded that possible interaction between *S. frutescens* and IPT needs further evaluation, although no other safety issues relating to consumption of *S. frutescens* were identified (Wilson et al., 2015). The equally good activity of some of the compounds such as the chlorogenic acids on other viruses such as HSV also responsible for antiviral activity from plants from other areas of the world, are repeatedly ed as anti-HIV agents. It is therefore argued that the presence of well-known and previously confirmed for their antiviral activity from plants from other areas of the world, are repeatedly identified as anti–HIV agents. It is therefore argued that the presence of well-known and well-researched plant compounds with anti–HIV activity from southern Africa should direct future focus in development of anti–viral agents for rapid development of affordable anti–HIV treatments. This should also be followed-up in in vivo studies as this information is lacking and anti–HIV activity is only dependent on the in vitro assay results currently available. In this review current information on southern African plants with traditional use against viral infections and specifically HIV treatment or HIV related diseases or symptoms is presented with the aim to develop treatments for people living with HIV/AIDS, as there is an urgent need to fast track in vivo testing and HIV/AIDS clinical trials of candidate drugs developed from compounds isolated from plants for effective and affordable alternatives to current treatment options.

Conflict of interest
The authors declare no conflict of interests.

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Archibald, S., Jacob, M., 2005. Anxiolytic and anticonvulsant effects (Johnson et al., 2007). Recently the results of an adaptive two-stage randomized double-blind placebo controlled study were published. The study evaluated the safety of consuming dried *S. frutescens* by HIV seropositive adults with CD4 T-lymphocyte count of > 350 cells/μL. *Sutherlandia frutescens* did not change HIV viral load, and CD4 T-lymphocyte count and was similar in the two arms at 24 weeks; however, mean and total burden of infection was greater in the *S. frutescens* arm attributed to two tuberculosis cases in subjects taking isoniazid preventive therapy (IPT). The study concluded that possible interaction between *S. frutescens* and IPT needs further evaluation, although no other safety issues relating to consumption of *S. frutescens* were identified (Wilson et al., 2015). The equally good activity of some of the compounds such as the chlorogenic acids on other viruses such as HSV also responsible for antiviral activity from plants from other areas of the world, are repeatedly ed as anti-HIV agents. It is therefore argued that the presence of well-known and previously confirmed for their antiviral activity from plants from other areas of the world, are repeatedly identified as anti–HIV agents. It is therefore argued that the presence of well-known and well-researched plant compounds with anti–HIV activity from southern Africa should direct future focus in development of anti–viral agents for rapid development of affordable anti–HIV treatments. This should also be followed-up in in vivo studies as this information is lacking and anti–HIV activity is only dependent on the in vitro assay results currently available. In this review current information on southern African plants with traditional use against viral infections and specifically HIV treatment or HIV related diseases or symptoms is presented with the aim to develop treatments for people living with HIV/AIDS, as there is an urgent need to fast track in vivo testing and HIV/AIDS clinical trials of candidate drugs developed from compounds isolated from plants for effective and affordable alternatives to current treatment options.

8. Conclusion
Throughout the paper, evidence is presented which shows that although southern Africa possesses a wealth of medicinal plants, most of the research on the screening and isolation of active compounds was carried out only in vitro on enzymes and viral proteins, with no follow-up research to validate the results in vivo. This could be attributed to the lack of long term funding and infrastructure and is supported by many plants tested in facilities not within the southern African region. From screening literature, it would therefore seem as if common compounds or compound groups from southern African plants, of which many are well-known and previously confirmed for their antiviral activity from plants from other areas of the world, are repeatedly identified as anti–HIV agents. It is therefore argued that the presence of well-known and well-researched plant compounds with anti–HIV activity from southern Africa should direct future focus in development of anti–viral agents for rapid development of affordable anti–HIV treatments. This should also be followed-up in in vivo studies as this information is lacking and anti–HIV activity is only dependent on the in vitro assay results currently available. In this review current information on southern African plants with traditional use against viral infections and specifically HIV treatment or HIV related diseases or symptoms is presented with the aim to develop treatments for people living with HIV/AIDS, as there is an urgent need to fast track in vivo testing and HIV/AIDS clinical trials of candidate drugs developed from compounds isolated from plants for effective and affordable alternatives to current treatment options.

Contribution of authors
Gerhard Prinsloo originated the work and led the discussions on topics, and managed the progress of the manuscript.
Cynthia Marokane is a postgraduate student which contributed significantly in collation of information and discussions on the manuscript.
Renée Street has extensive experience in medicinal plant research and an extensive background on HIV as part of the HIV unit at the MRC and contributed significantly to the content of the paper. She was also responsible for final editing and proof reading of the paper.

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