International Scholars Journals

African Journal of Virology Research ISSN 3421-7347 Vol. 13 (7), pp. 001-007, July, 2019. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Review

Ethnopharmacology of human immunodeficiency virus in South Africa–a minireview

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Accepted 09 April, 2019

Infection with the human immunodeficiency virus (HIV), the etiologic agent of acquired immune deficiency syndrome (AIDS), continues to pose an unprecedented public health problem of enormous proportions worldwide. Current treatment options for HIV/AIDS have not been satisfactory and the quest for effective curative or preventive therapies goes on. Plants are increasingly seen as an alternative source for the discovery of novel anti-HIV molecules. Africa, particularly southern Africa, endowed with a rich diversity of medicinal plants, represents the region of the world worse hit by HIV. Anecdotal evidence of the therapeutic benefits to AIDS patients of some plant-derived preparations abound. This mini-review takes a look at the evaluations of South African medicinal plants to determine their effects on HIV/AIDS, and the initial attempts at the isolation and characterization of putative anti-HIV molecules.

Key words: Human immunodeficiency virus, Medicinal plants, South Africa.

INTRODUCTION

Human immunodeficiency virus (HIV) is currently the most significant infectious pathogen with devastating consequences. Since the description of HIV as the causative agent of acquired immune deficiency syndrome (AIDS), HIV has produced a worldwide pandemic. In 2005, UNAIDS estimated that 40 million people were infected worldwide, with 25 million in sub-Saharan Africa, while approximately 2.4 million deaths were attributed to AIDS in 2005. Southern Africa represents one of the subregions hardest hit by HIV. Base on antenatal seroprevalence surveys, infection levels surpass 30 percent in Botswana, Lesotho, Malawi, South Africa and Swaziland; with Angola, Mozambique, Namibia, Zambia, and Zimbabwe having 5, 15, 18, 16 and 25 percent (UNAIDS, 2005). Recent data for South Africa suggests that prevalences are increasing in all age groups, and about 5 million people are infected with the virus. The high endemicity of HIV in Southern Africa is already taking its toll on the socio-economic aspects of societies (Rosen et al., 2004). National programmes for the delivery of antiretrovirals are still in their infancy, unfortunately in the countries where they are most needed, due to the high costs of drug procurement, and limited human and structural resources.

One characteristic feature of HIV is the high degree of variability of its genome among independent virus isola-tes. This feature impacts many aspects of the biology of HIV, including tissue and target cell specificity, clinical spectrum and pathogenesis, geographic and temporal distribution of virus, susceptibility to antiretrovirals, and prospects of developing an effective cross-reactive vac-cine. Secondly, infection with HIV sooner or later, creates a window for infections with other microbes, which were hitherto contained by the immune system, to become progressive. Added to this is the concern that weakened human immune systems may become favourable envi-ronments for the adaptation of traditionally non-human infectious agents. Most importantly, there has been a fervent and continuous search for preventive and curative therapeutics in an effort to stem the spread of the pandemic (McMichael and Hanke, 2003; Pomerantz and Horn, 2003).

The efficacies of clinically available HIV enzymes and fusion inhibitors have been limited by the selection and development of resistant variants, appreciable levels of toxicity and the absence of a curative effect. As a cones-

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quence, the search for better anti-HIV agents continues. Alongside, the endeavours in designing HIV antagonists based on the structure, catalytic properties of its enzymes, and surface receptors, alternative approaches such as the search for secondary metabolites from plant species capable of arresting viral replication are being pursued.

PLANTS AS PROSPECTIVE SOURCES OF ANTI-HIV COMPOUNDS

Several studies have demonstrated the inhibitory properties of a variety of crude plant extracts, as well as chemically characterized phytomolecules against differ-rent stages of the life cycle of HIV. Some of these studies focused on plant parts used traditionally in specific geographic locales in the treatment of various forms of infectious diseases. Interestingly, a few plant derived compounds such as papavarine, glycyrrhizin and tricho-santhin were seen to have promise and have been evaluated in AIDS patients. These developments show that useful anti-HIV agents could be obtained from plants sources (Vlietinck et al., 1998; De Clercq, 2000; Kong et al., 2003).

Specific African-based studies have also indicated the potential of local medicinal plants for anti-HIV activity. Asres and colleagues (Asres et al., 2001) investigated the effect of 71 polar and nonpolar extracts from 21 Ethiopian medicinal plants comprising 14 plant families on HIV-1 and HIV-2 replication. They found that the acetone fraction of the leaves of *Combretum paniculatum* (Vent.) and the methanol fraction of the leaves of *Dodonaea angustifolia* L.f. strongly inhibited HIV-1 replication with selective indices of 6.4 and 4.9 respectively. These extracts also prevented virus induced cytopathic effect by almost 100% using MT-4 cells as targets. The greatest degree of antiviral activity against HIV-2 was achieved with the acetone extract of *C. paniculatum* (EC_{50}=: 3 µg/ml), and a selective index of 32 was noted for this extract. Polar extracts that were obtained by extraction with hydroalcohol, methanol or acetone exhibited inhibition of viral growth at subtoxic concentrations. In a Sudanese study, forty-eight metha-nol and aqueous extracts from Sudanese plants were screened for their inhibitory activity on viral replication. Nineteen extracts showed inhibitory effects on HIV-induced cytopathic effects (CPE) on MT-4 cells. Screen-ing the extracts against HIV-1 protease (PR) by an HPLC assay, the methanol extracts of the bark and pods of *Acacia nilotica*, the leaves of *Euphorbia granulata*, and the stem-bark of *Maytenus senegalensis*, together with the aqueous extracts of the pods of *A. nilotica* and the stem-barks of *M. senegalensis* showed considerable inhibitory effects against HIV-1 PR (Hussein et al., 1999). Extract from the leaves of *Combretum hartmannianum* used in Sudan in the treatment of malaria and other tropi-cal diseases was shown to totally inhibit HIV-1 reverse transcriptase (HIV-1 RT) at a concentration of 66 µg/ml (Ali et al., 2002).

Central Africa, particularly Cameroon, represents the region of the world with the greatest diversity of HIV variants. Indeed, all the genetic subtypes of HIV-1 major group M (subtypes A-D, F-H, J and K), the outliers (group O), and the non-M-non-O variants (group N), have been described in Cameroon. Coupled to these is the presence of circulating recombinant forms CRF01_AG, CRF02_AE, and CRF09_cpx (Simon et al., 1998; Zhong et al., 2003; Yamaguchi et al., 2004). Within a decade of the knowledge of the huge public health problem posed by HIV, the atropisomeric naphthylisoquinoline alkaloid dimers, michellamines A, B, and C, were isolated from *Ancistrocladus korupensis* from the Korup rainforest in the South West Province of Cameroon. Michellamine B was shown to inhibit HIV-induced cell killing and viral replication in a variety of human cell lines, and in cultures of human peripheral blood leukocytes and monocytes. Futhermore, Michellamine B was inhibitory to several laboratory and clinical strains of HIV-1, including the AZT-resistant strain G910-6 and the pyridinone-resistant strain A17; as well as strains of HIV-2. However, the high toxicity of this compound to several human cell lines prevented its further evaluation (Boyd et al., 1994). Cos et al. (2002) in an *in vitro* investigation of a selection of Rwandan plants used in traditional medicine for the treatment of infections and/or rheumatoid diseases reported that an aqueous fraction from the leaves of *Tithonia diversifolia* had a strong anti-replicative property but with a high selective index of 461. El-Mekkawy and colleagues (1995), studying Egyptian plants used in folklore medicine for activity against HIV-1, through a bioassay-guided fractionation of the methanol extract of the fruit of *Phylanthus emblica* isolated Putranjivain A with a potent inhibitory effect on HIV-1 RT (IC50 = 3.9 µM). The mode of action of Putranjivain A was seen to be non-competitive with respect to the substrate, but competitive with respect to the template-primer.

SOUTH AFRICAN MEDICINAL PLANTS INHIBITORY TO HIV

Laboratory based investigations of South African medicinal plants against HIV have sought to seek inhibitory properties of water and organic extracts, and isolated compounds against the essential enzymes RT, integrase (IN) and PR. 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 polymer-rase function. Integrase in conjunction with accessory viral proteins, namely the matrix and viral protein U and nuclear import signals is required for the transportation of the synthesized viral double stranded DNA to the nucleus

and catalyzes its integration into the chromosome of the host cell. Protease cleaves viral polyproteins into structural and functional components which are assembled to form progeny virions. The selection of plants for anti-HIV evaluation has been mainly done through an ethnobotanical survey, in which plants whose decoctions have been reported by traditional healers to be of therapeutic value are selected for antiviral evaluation.

In one of our *in vitro* studies to directly assess the inhibitory effects of South African medicinal plants on HIV, using a radioactive cell free assay a strong inhibition (IC_{50} = 7.5 µg/ml) by the methanol extract of the leaves of *Terminalia sericea* (Combretaceae) against the ability of RT to form a complementary DNA strand was observed (Bessong et al., 2004). This extract also inhibited the RNase H activity by a similar magnitude (IC_{50} = 8.1 µg/ml). Harnett et al. (2005) described a significant reduction in the activity of HIV RT by the water extracts of the leaves of *Lebostomon trigonus* (Boraginaceae) with a 50% inhibitory concentration (IC_{50}) of 49.0 µg/ml. A minimal inhibition was also noted for *Sutherlandia frutescens* L. Br. (Fabaceae). No activity against HIV-2 protease was observed. In our own studies, following a bioassay guided fractionation, we determined that a partially characterized red coloured gallotannin isolated from an active fraction of the methanol extract of the stem-bark and roots of *Peltophorum africanum* Sond. (Fabaceae), strongly inhibited RT functions, and the 3'-end processing activity of IN (Bessong et al., 2005). Specific and non-specific binding of polyphenols to receptors have been described (Wall et al., 1996; Zhu et al., 1997), so whether the activity of this gallotannin is non-specific or of broad spectrum remains to be determined. We also showed that catechin equally derived from the stem-bark of *P. africanum* inhibited the 3'-end processing activity of HIV-1 IN by as much as 65% at 100 µM, but had no activity on HIV-1 RT. Details of methodologies and outcomes of studies in which South African plants have been investigated for anti-HIV replicative properties are presented in Table 1.

SUGGESTIONS FOR EXPERIMENTAL APPROACHES

HIV-1 RT uses magnesium or manganese divalent ions as a co-factor, but activity is optimal with magnesium ions. A marginal increase in the concentration of manganese ions retards HIV-1 RT activity (Bolton et al., 2002). Furthermore, Filler and coworkers (Filler and Lever, 1997) have showed the irreversible inhibition of RT by palladium and iron, and the subsequent reduction in virus proliferation. Ions of manganese, palladium and iron may be found in soils and subsequently in plant sap. Most methodologies describing the inhibition of HIV RT by crude plant extracts do not take into consideration the effect of metal ions in regulating the activity of HIV-1 RT. 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.

Most clinically available RT drugs target the polymerase function of the enzyme. However, it has been shown that point mutations in the RNase H domain of RT significantly reduce viral proliferation (Tarrago-Litvak et al., 2002). Hence, it would be interesting to seek plant-derived molecules which have activity against either the RNase H or the polymerase domain. Although a few drugs minimally interact with the RNase H function, the identification of an extract against the RNase H but not the polymerase domain or vice versa may signify a unique molecule of significant specificity. In addition, despite the high toxicity ascribed to some identified anti integrase molecules, this enzyme should remain an interesting target to arrest retroviral replication, since no cellular homologue exists. Investigations on the biological activity of plant extracts against the strand transfer, 3'-end processing and concerted integration functions of IN, crucial steps in the establishment of infection and viable therapeutic targets, would widen the scope of the search for plant-based anti-HIV molecules.

SOUTH AFRICAN PLANTS AND AIDS

South Africa, one of the countries with a huge HIV/AIDS crisis, has a rich diversity of medicinal plants and members of different indigenous communities have tapped the medical benefits of plants for centuries. Evidently, a good proportion of South African HIV/AIDS patients, for traditional and financial reasons, seek treatment from traditional healers who administer different preparations from a variety of plants. In some quarters, the usefulness of these preparations receives applause, while to others its remains an open question and sometimes controversial.

One of the earliest investigations on a South African plant deemed to be useful to HIV/AIDS patients was the cytotoxicity tests on *Sutherlandia frutescens* subspecies *microphylla* (Fabaceae). This plant is widely used for its reported ability to increase CD4 counts and decrease viral load in AIDS patients (www.sutherlandia.org/aids). The *in vivo* studies carried in 2000 by the Indigenous Knowledge Systems Unit of the South African Medical Research Council (MRC) in velvet monkeys noted that when the monkeys were fed with powdered leaves of *S. frutescens* for a period of three months there were no nefarious outcomes when a battery of hematological, physiological and biochemical parameters were analyzed. Although the use of a particular plant for decades may point to its pharmacological safety, the MRC results were very significant and encouraging considering the large number of people who take *S. frutescens* for relief. In a study to clinically assess the efficacy of South African traditional medicine on viral load and CD4 counts,

Table 1. South African medicinal plants studied for anti-HIV properties; assays used and main findings.

Plants investigated	Type of extracts/compounds investigated	Test system used and viral target	Significant outcomes	Reference
Terminalia sericea Burch. Ex Dc (Combretaceae), *Combretum molle* R. Br. Ex G. Don (Combretaceae), *Bridelia micrantha* Baill.(Euphorbiaceae).	Water and methanol extracts of the leaves and stem-bark	Radioactive, cell-free systems using HIV-1RT expressed in *Saccharomyces cerevisiae.* Inhibition of polymerase and RNase H functions of RT were studied.	Methanol extract of *T. sericea* inhibited the polymerase and RNase functions with IC_{50} of 7.2 and 8.1 µg/ml respectively.	Bessong et al., 2004
Lobostemon trigonus (Boraginacea), *Sutherlandia frutescens* L. Ex Br. (Fabaceae),	Water, methanol, acetone, ethanol and methylene dichloride extracts of the leaves and flowers of *S. frutescens* and leaves of *L. trigonus*	ELISA and fluorescence resonance energy transfer assays. RT and PR activities were investigated.	Aqueous extract of *L. trigonus* inhibited HIV-1 RT with an IC_{50} of 49 µg/ml.	Harnett et al., 2005
Peltophorum africanum Sond. (Fabaceae), *Sutherlandia frutescens* subspecies *micropylla* L.. R.Br.ex W.T. Aiton (Fabaceae), *Vernonia stipulacea* Klatt. (Asteraceae), *Ziziphus mucronata* Willd. (Rhamnaceae), *Ricinus communis* L. (Euphorbiaceae), *Elaendendron tranvaalensis* Jacq. (Celasteraceae), *Mucuna coriacea* Baker (Fabaceae), *Bridelia micrantha* Baill. (Euphorbiaceae), *Combretum molle* R.Br.ex G. Don (Combretaceae)	Water and methanol extracts of roots, leaves and stem-barks. A partially characterized gallotannin; bergenin and catechin isolated from the stem-bark of *P. africanum*.	Radioactive, cell-free system, using HIV-1RT and IN expressed in *Saccharomyces cerevisiae*. Inhibition of polymerase and RNase H functions of RT, and 3'-end processing activity of IN was studied.	Methanol extract of the stem-bark of *P. africanum* inhibited the polymerase and RNase H functions of RT with IC_{50} of 3.5 µg/ml and 5.0 µg/ml respectively. The gallotannin inhibited the polymerase and RNase activities with IC_{50} of 6.0 µM and 5.0 µM respectively, and abolished the 3'-end processing activity of IN at 100 µM. Catechin reduced IN activity by 65% at 100 µM. The aqueous and methanol extracts were non-toxic to a HeLaP4 cell line at a concentration of 400 µg/ml.	Bessong et al., 2005
Sutherlandia frutescens subspecies *micropylla* L.. R.Br.ex W.T. Aiton (Fabaceae), *Vernonia stipulacea* Klatt. (Asteraceae), *Ricinus communis* L. (Euphorbiaceae), *Elaendendron tranvaalensis* Jacq. (Celasteraceae), *Mucuna coriacea* Baker (Fabaceae), *Bridelia micrantha* Baill. (Euphorbiaceae).	Butanol and acetyl acetate fractions of the methanol extracts of roots. ß-sistosterol and friedelin isolated from the roots of *B. micrantha*	Radioactive cell free systems employing recombinant HIV-1 RT and HIV-1 IN	ß-sistosterol and friedelin had no effect on HIV-1 RT and 3'-processong activity of HIV-1 IN. The ethyl acetate fraction of the roots of *B. micrantha* showed an IC_{50} of 7.3 µg/ml on RDDP activity of RT, but had no effect on 3'-processing activity of HIV-1 IN	Bessong et al., 2006

Tshibangu et al. (2004) described an improvement in the immune system and general well-being of patients, due to increases in CD4+ T cell and decrease in viral load, when these markers were monitored for 12 months in HIV/AIDS participants.

AIDS is a syndrome comprising a dysfunction of the immune system, compounded by opportunistic infections of bacterial, fungal, protozoan or viral aetiology. The beneficial effect of a plant-derived extract administered to HIV/AIDS patients could be due to one or a combination of several factors: a direct inhibition of HIV replication, boosting of the immune system, or having inhibitory properties against one or several opportunistic infections. The stories of 'successful' herbal therapy against HIV/AIDS have components of cures relating to skin, respiratory, intestinal disease manifestations, and re-establishment of vitality. These are pathologies closely tied to fungal, bacterial and other viral infections. Many investigations have documented the biological activity of South African plants against bacteria, fungi, viruses, protozoans and non-infectious conditions. In these studies, the use of plants in traditional medicine was validated by the demonstration of antimicrobial action of crude plant extracts against potential pathogens, and in some cases molecules with specific bioactivity were identified (Meyer et al., 1997; Fennel et al., 2004; Tshikalange et al., 2005). Of interest are those organisms which are common opportunistic infections in AIDS-related complex and AIDS. Diospyrin isolated from *Euclea natalensis* (Ebenaceae) and its amino acetate derivative is active against sensitive and resistant strains of *Mycobacterium tuberculosis*, the cause of tuberculosis, and a case defining illness in AIDS (Lall and Meyer, 2001; Lall et al., 2003). In our own studies (Obi et al., 2002; Obi et al., 2003), we reported the antibacterial activity of aqueous and methanol extracts of Venda medicinal plants against enteropathogens such as *Campylobacter* responsible for Campylobacter-associated diarrhea in HIV/AIDS patients with substantial morbidity and mortality (Quinn, 1997; Coker et al., 2002). Motsie and colleagues (2003) reported the antifungal effect of extracts from *Polygala myrtifolia* (Polygalaceae) and *Glycyrrhiza glabra* (Fabaceae) against *Candida albicans*, responsible in the most part for oral and oesophagal thrush common in AIDS patients (Lattif et al., 2004). Methanol and aqueous extracts of *Dombeya rotondifolia* (Hochst.), *Terminalia sericea* and *Gunnera perpensa* are bactericidal against *Staphylococcus aureus*, implicated in skin infections (Miller et al., 2003), and that this activity is mediated by fatty acids in the extracts (Reid et al., 2005). In addition, many plant species are rich in sterols and sterolines which have been shown to have immuno-modulatory effects and boost the vitality of AIDS patients (Bouic et al., 2001; Bouic, 2002). An on-going bioassay-guided fractionation of the roots of *Bridelia micrantha* Baill. (Euphorbaceae), ethnobotanically selected to study its effects on HIV enzymes, has yielded the phytosterols β-sistosterol and fredelin (Bessong et al., 2006). Whether β-sistosterol and fredelin contribute to the well-being of patients and as such give the plant a spot in the HIV/AIDS pharmacopoeia of some Venda traditional healers remains to be determined.

There is therefore good reason to expect an appreciable clinical management of an AIDS patient presenting with a fungal infection such as candiadiasis, diarrhea of bacterial etiology and fever, and treated with plant extracts known to kill fungi, reduce stool output and are antipyretic. Even though, the extracts have no direct virucidal effect, minimizing the opportunistic infections improves the well-being of the individual. However, for purposes of empiric evidence, and as a means of identifying specific anti-HIV/AIDS properties of different plants preparations, the performance of concomitant investigations of the antibacterial, antifungal and immunostimulatory effects when plants are evaluated for anti-HIV properties will provide a vast array of valuable data from which the antimicrobial properties of a particular plant in the HIV/AIDS condition could be assessed.

CONCLUSION

Once AIDS was defined and HIV identified as the underlying cause, the determination of its modes of transmission and development of effective diagnostics were relatively rapid. Even the first antiretroviral for HIV (zidovudine) was already 'at hand'. These early successes highlight the perennial importance of basic research in the generation of a pool of knowledge and information easily harnessed to answer new questions.

Due to the rampant administration of plant-based decoctions and concoction to HIV/AIDS individuals and the numerous anecdotes of the benefits derived from plant-based preparations, research on the anti-HIV replicative properties of South African medicinal plants is on the increase. Huge financial and human capital are required to pursue the search of novel anti-HIV molecules through systematic screening, isolating, identifying active ingredients, verifying their mode of action, specificity, toxicity evaluations and interactions with antiretrovirals. The deteriorating AIDS situation in South Africa and Southern Africa as a whole calls for such a commitment. It is only through these investments that outcomes of high value to the efforts geared at identifying molecules that could serve as templates in obtaining better anti-HIV molecules would be achieved. In addition, concrete answers are required from the scientific community on what specific benefits could be derived from the use of medicinal plants by HIV/AIDS patients.

The HIV situation in North America and Europe is stabilizing and decreasing in some populations. It is plausible that in several years to come HIV will no longer

be a concern for nations of these parts of the world. The implication is that research efforts in the development of therapeutics will dwindle, reminiscent of the tuberculosis and malaria situations. The World Health organization estimates that about 80% of the population of developing countries makes use of plant based traditional medicine for their primary health care needs. Coupled with the challenge of widespread antimicrobial resistance, herbal medicine and traditional healers are receiving more attention. Invigorating medicinal plant research on HIV with defined goals has broad expected outcomes in terms of human capacity and biotechnology development, and above all service to the community.

ACKNOWLEDGEMENTS

POB acknowledges support from Centre National de la Recherche Scientifique (France) and the National Research Foundation (South Africa).

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