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Old plants newly discovered: *Cassia sieberiana* D.C. and *Cassia abbreviata* Oliv. Oliv. root extracts inhibit in vitro HIV-1c replication in peripheral blood mononuclear cells (PBMCs) by different modes of action

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ABSTRACT

Ethnopharmacological relevance: Despite advances in anti-retroviral therapy which has transformed HIV/AIDS from a fatal to a manageable chronic disease, increasing viral drug resistance, side effects and uneven access to anti-retroviral drugs remain considerable therapeutic challenges. Partly as a consequence of these shortcomings and partly based on the fact that HIV/AIDS gives rise to opportunistic infections whose symptoms have been managed in Africa in an HIV/AIDS-independent context by traditional healers for centuries, many HIV/AIDS patients use herbal medicines. The aim of this study was to screen selected medicinal plants from Botswana, used by traditional healers to treat/manage HIV/AIDS, for inhibitory activities on HIV replication.

Materials and methods: Based on an ethnomedical survey, ethanolic tannin-containing and tannin-free extracts from 10 medicinal plants were tested for inhibitory properties against a clone of HIV-1c (MJ_4) measuring cytopathic effect protection and levels of viral p24 antigen in infected PBMCs.

Results: Cassia sieberiana D.C., *Cassia abbreviata* Oliv. Oliv. and *Plumbago zeylanica* L. extracts showed significant inhibition of HIV-1c (MJ_4) replication. The inhibitory activity of the *Plumbago zeylanica* extract could be attributed to its tannin content. Anti-HIV activity of *Cassia sieberiana* root and bark extracts, and *Cassia abbreviata* root extracts occurred in a concentration-dependent manner with an effective concentration (EC_{50}) of 65.1 μg/ml, 85.3 μg/ml and 102.8 μg/ml, respectively. Experiments to elucidate possible mechanism(s) of action revealed that *Cassia sieberiana* root and bark extracts blocked HIV replication at its binding- (EC_{50} = 70.2 μg/ml and 90.8 μg/ml, respectively) and entry stage (EC_{50} = 88.9 μg/ml and 100.5 μg/ml, respectively) while *Cassia abbreviata* extracts did not.

Conclusions: We report here for the first time a direct inhibitory effect on HIV-1c replication of extracts from two extremely popular medicinal plants, *Cassia sieberiana* and *Cassia abbreviata*. Considering the traditional uses of both *Cassia* species, our findings strongly suggest pilot clinical observational studies involving traditional healers to further evaluate the therapeutic potential of the *Cassia* extracts.

1. Introduction

More than 25 years after the discovery of the human immunodeficiency virus (HIV) there is still a lack of effective preventive measures and curative therapeutics to combat the global AIDS epidemic. Despite significant breakthroughs with the availability of anti-retroviral drugs (ARVs) as constituents of highly active anti-retroviral therapy (HAART) which transform HIV/AIDS from a fatal into a manageable chronic disease, there remain considerable

Abbreviations: AIDS, Acquired Immunodeficiency Syndrome; ARVs, anti-retroviral drugs; AZT, azidothymidine; HIV, human immunodeficiency virus; STI, sexually transmitted infections.

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challenges. HAART as a chronic suppressive therapy to contain viral reservoirs is limited by its cost, the requirement of life long adherence, side effects and the presently unknown effects of long-term treatment (Richman et al., 2009). Therapeutic choices for HIV/AIDS treatment are even more compromised in developing countries due to a persistent lack of access to ARVs, limited health care capacities and other factors that counteract the benefits of HAART, such as malnutrition and poverty. In 2008, an estimated 1.9 million people living in sub-Saharan Africa became newly infected with HIV, bringing the total number of people living with HIV to 22.4 million (UNAids, 2009). Even though the provision of ARVs in sub-Saharan Africa has expanded dramatically, the sheer scale of need in the region means that only 44% of people living with HIV/AIDS are receiving it (UNAids, 2009). The acquisition of the drugs remains very expensive (UNDP, 2001) which raises concerns about the sustainability of HAART in regions of highest need. Additionally, the increased use of ARVs has been associated with the emergence of drug-resistant viral strains which adds to the extraordinary variability of HIV (Montagnier, 2010) and which makes it impossible to rely only on a few standard drug regimens. There is, therefore, a perpetual need for new and more affordable anti-HIV therapeutics. One strategy is to develop them from natural products. Natural products as lead anti-HIV agents from plants (Cos et al., 2004; Singh et al., 2005; Yu et al., 2007; Mukhtar et al., 2008) and marine organisms (Gochfeld et al., 2003; Singh et al., 2005) have received more attention in recent years due to their general structural diversity and structural uniqueness (Gochfeld et al., 2003), their new modes of action (Yu et al., 2007), their ability to counteract side effects of ARVs (Zha et al., 2010) and their interactions with multiple targets (Notka et al., 2003; Hupfeld and Efferth, 2009) which renders natural products as potentially attractive candidates to overcome drug resistances of HIV (Hupfeld and Efferth, 2009). Particularly in developing countries medicinal plants embedded in holistic traditional medical knowledge systems have sustained people for generations. According to the World Health Organisation (WHO) about 80% of the world population depends on traditional medicine, which is 90% plant-based, for their health needs (Kasilo, 2000; African Health Monitor, 2003). This scenario has not changed in the context of the HIV/AIDS pandemic. Traditional medicine use in Africa is common amongst individuals with moderate and advanced HIV infection (Babb et al., 2007; Peltzer et al., 2008), which raises important issues, such as in vitro and clinical validation of efficacy of medicinal plant preparations. However, concerns about validation often tend to underestimate valuable patient observations by traditional healers themselves. The rationale of this study was to characterize anti-HIV activities of selected medicinal plants used by traditional healers in Botswana. In a rather innovative way, we included a traditional healer and his assistant in the research team, whose observations with HIV/AIDS patients guided us to investigate the medicinal plant extracts which displayed the anti-HIV activities reported in this paper.

2. Materials and methods

2.1. Plant species collection

Eleven traditional healers in the North-West District of Botswana were interviewed between December 2007 and December 2008 on types of medicinal plants they used to treat sexually transmitted infections (STIs), HIV/AIDS or potential HIV/AIDS-related opportunistic infections with symptoms which form part of diagnostic criteria of HIV/AIDS. These included persisting cough, diarrhoea, fever, skin rashes, thrush and weight loss (CDC, 1993). A plant list documenting information on their uses, plant parts and their local and scientific names was generated. The consistency of given information on plant uses was probed additionally to the interviews in two workshops in 2007 and in 2009 in Maun, Ngamiland, as well as in a number of follow-up visits to traditional healer's consultancies and telephone conversations over the entire research period. Supporting evidence for mentioned medicinal plant uses was compiled from the available literature on medicinal plants in Botswana and neighbouring countries. Plant samples were collected with the help of traditional healers at different times during the research period and taxonomically authenticated by the University of Botswana Herbarium curator Mr. M. Muzila. Voucher specimens were deposited in the University of Botswana Herbarium (UCBG) and voucher numbers are displayed in Table 1.

2.2. Extract preparation

Respective plant parts were dried at room temperature and ground to a fine powder using a grinding machine (Grinder A10, IKA Labortechnik, Germany). One gram of the pulverised material was extracted with 10 ml of absolute ethanol for 10 min at room temperature using a Vortex® mixer (E10 Vortex 2 Genie, LASEC, South-Africa). The suspension was centrifuged for 10 min at room temperature and 10 000 rpm (Biofuge stratus, Heraeus, Germany) and the supernatant was transferred into a pre-weighed glass vial. The extract was freed from solvent under a stream of air and weighed. Extracted material was either subjected to the removal of tannin (see Section 2.3) or a stock solution of 10 mg/ml in ethanol was prepared and sterile-filtered using a 0.2 μm filter (Sterile Millex-FG, Millipore, Bedford, MA, USA). We chose ethanol as extraction medium because its polarity is closest to water, which is the main solvent for traditional medicinal preparations, but lesser prone to antimicrobial contamination during extract preparation. Plant extract dilutions for respective antiviral assays were prepared by diluting the stock solution with RPMI-1640 medium.

2.3. Tannin removal

Tannins are present in many plants and have a high affinity for proteins and other biomolecules which affects many biochemical reactions. Often, crude plant extracts exhibit anti-HIV effects as a consequence of the presence of tannins in the plant material and it was recommended to remove these compounds in order to avoid non-specific interference with HIV replication (Harnett et al., 2005; Klos et al., 2009). In this study plant extracts were subjected to removal of tannins using a chloroform:methanol (4:1) mixture/water interphase as previously described (Houghton and Raman, 1998; Klos et al., 2009). The organic phase, containing tannin-free extract material, was then air-dried at room temperature and assayed for anti-HIV activity. Dried extracts were reconstituted in dimethylsulphoxide (DMSO) at 10 mg/ml. For thin-layer chromatographic (TLC) profiling quantities were upscaled to 60 g powdered plant material, which was first exhaustively extracted with ethanol using a Soxhlet apparatus and then subjected to methanol/chloroform extraction (100 ml ethanol/400 ml chloroform).

2.4. Cells and virus clone

Peripheral blood mononuclear cells (PBMCs) were obtained from HIV-1 seronegative donors (kind gifts of the Botswana/Harvard Partnership Laboratory (BHPL), Princess Marina Hospital, Gaborone, Botswana). PBMCs are available to BHPL on a routine basis for which approval by the relevant Institutional Review Boards and Ethics Committees and consent has been obtained. Cells were cultured in sterile 96-well plates (Costar) at 37 °C and 5% CO_2 in RPMI-1640 medium (Highveld