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Extracts of *Moringa oleifera Lam*. showing inhibitory activity against early steps in the infectivity of HIV-1 lentiviral particles in a viral vector-based screening

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Moringa oleifera **Lam. (Moringaceae) is one of the many medicinal plants employed by herbalist to treat or manage people living with HIV/AIDS (PLWHA) in African Traditional Medicine (ATM) and there are many claims to the fact that it improves quality of life and reverses the course of the HIV/AIDS disease progression. This practice and the claims of efficacy spurred the present study in which the inhibitory activities of three different extracts of *M. oleifera* on lentiviral vector infectivity were studied on HeLA cells by measuring the expression of green fluorescent protein (GFP) transgene in the vector using flow cytometry. An infectious VSV-G-pseudotyped, human immunodeficiency virus type 1-based, self-inactivating lentiviral vector particles were generated by transient co-transfection of the vector plasmid (pHIV-1 CSCG) with packaging plasmids encoding tat, rev, gag-pol (pCMVΔR8.2), a VSV-G expression plasmid (pHIT-G), a secretory alkaline phosphate expression plasmid (pSEAP) which are all necessary for viral infectivity. The extracts studied were obtained by solvent extraction of the leaf powder of *M. oleifera* with ethyl ether (EM), methanol (MM) and water (AM). All the extracts (EM, MM, and AM) were active against the HIV-1 lentiviral vector and inhibited the early events of the viral replication cycle on HeLa cells in a concentration-dependent manner with IC_{50} of 7.59 µg EM/ml, 7.72 µg MM/ml and 7.17 µg AM/ml, respectively. Cytotoxicity of the extracts evaluated in parallel on HeLA cells by the MTT assay method showed TC_{50} values of 32.33 µg EM/ml, 38.88 µg MM/ml and 41.58 µg AM/ml with selectivity indices (SI) of 4.26, 5.04 and 5.8, respectively. In this study, *M. oleifera* leaf extracts showed potent and selective inhibition of early steps in HIV-1 infectivity and could serve as source of antiretroviral lead molecules. The outcome of this investigation could partly explain the benefits and improvement in quality of life claimed by PLWHA in the use of this medicinal plant as supplement.**

Key words: Antiviral activity, antiviral screening, lentiviral vector particles, *Moringa oleifera,* HIV-1, viral vector-based assay.

INTRODUCTION

Medicinal plants have been widely used to treat a variety of infectious and non-infectious ailments. The existence of the humankind has been under constant threat by emerging infectious disease; prominent among these are

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infections associated with the human immunodeficiency virus. HIV/AIDS does not have a curative therapy yet and currently available options are management therapies. Although, a lot of progress has been made in the recent times, the accessibility and affordability of anti-retroviral medications in middle and low-income countries with high prevalence rate of HIV/AIDS infections are still knotty issues (UNAIDS, 2012) and heavily depend on the benevolence of international donor agencies. Although, the adoption of highly active antiretroviral therapy (HAART) regimen has been largely successful in reducing viral load, increasing quality of life (QoL) and life expectancy and in decreasing HIV-related complications; this has however come at a high price (Harrington and Carpenter, 2000). HIV rapidly evolves resistance to these antiretroviral agents, unless taken precisely on schedule; even short lapses in taking the pills can lead to resistance (Richman, 2004; Cane, 2009). Additionally, these drugs cause a variety of minor and serious adverse effects.

Due to the unsatisfactory efficacy, high cost, non-availability and intolerable adverse effects of conventional antiretroviral medicines, People Living with HIV/AIDS (PLWHA) often seek alternative and complementary therapies including herbal medicines. It has been reported that due to the chronic course and the effect of the HIV-related diseases on quality of life and the possibility of severe complications and death, people with HIV/AIDS are very likely to seek herbal therapies (Ernst, 1997; Ozsoy and Ernst, 1999; Faragon et al., 2002), particularly in poor countries and in rural communities with limited access to the highly active anti-retroviral therapy (HAART). Even in developed countries like the United States, some surveys have found that HIV-infected people use herbal remedies and made substantially high expenses on complementary and alternative therapies (CAM) and have also reported much improvement with these treatments (Fairfield et al., 1998; Wootton and Sparber, 2001). The increased patronage of CAM could have been informed by the growing body of knowledge and the accumulation of anecdotal scientific evidences which establishes their safety and effectiveness in reducing HIV load, strengthening immune system functions, and tackling the many and diverse immunodeficiency-related opportunistic infections.

The efficacy and the benefits claimed by CAM providers as well as patients who use these herbal remedies are too remarkable to ignore. Investigation into these herbal remedies could hold the key to some potent and safe alternative therapies or could uncover lead molecules for further development. Investigation into local herbal remedies for anti-retroviral activities has been the focus of some of our recent investigations (Esimone et al., 2009, 2010; Okoye et al., 2012). In the present study, the activity of different extracts of *Moringa oleifera* Lam (Monringaceae) against HIV-1 lentiviral vector infectivity in a viral vector-based assay was undertaken. *M. oleifera* (commonly called drumstick tree or horseradish tree) is a very versatile plant with many valuable medicinal and nutritional uses and found widely in tropical and subtropical regions of the world. Many bioactive phytoconstituents have been reported in different parts of the plant, such as beta-carotene, proteins, vitamins and variety of phenolics (Siddhuraju and Becker, 2003; Anwar et al., 2007). *M. oleifera* is rich in zeatin, quercetin, beta-sitosterol, caffeoylquinic acid and kaempferol, a rare combination of important bioactive compounds (Siddhuraju and Becker, 2003; Anwar et al., 2007). Various parts of this plant such as the leaves, roots, seed, bark, fruit, flowers and immature pods have been reported to act as cardiac and circulatory stimulants (Duke, 2001; Nandave et al., 2009), have antitumor (Guevaraa et al., 1999; Parvathy and Umamaheshwari, 2007), antipyretic (Hukkeri et al., 2006), anti-inflammatory (Muangnoi et al., 2012), antiulcer (Rujjanawate et al., 2004; Debnath and Guha, 2007), antispasmodic (Gilani et al., 1994), diuretic (Morton, 1991), antihypertensive (Gilani et al., 1994), cholesterol lowering (Ghasi et al., 2000), antioxidant (Singh et al., 2009), antidiabetic (Ndong et al., 2007), hepatoprotective (Pari and Kumar, 2002) and antibacterial and antifungal activities (Chuang et al., 2007; Saadabi and Abu Zaid, 2011) and for these reasons have been employed in folk and Ayurvedic traditional medicine of Africa and South Asia for a variety of medicinal purposes. In African Traditional Medicine (ATM), *M. oleifera* is one of the many medicinal plants employed by herbalist to treat or manage PLWHA and there are so many claims on its effectiveness in improving quality of life and in reversing the course of the HIV/AIDS disease progression. For this reason, our present study has been devoted to studying different solvent extracts of *M. oleifera* for antiretroviral activity using a viral vector-based assay technique.

Research on anti-viral medicinal plants has been limited by the shortcomings of the available screening techniques. Traditional methods of evaluating antiviral agents have involved one or more of the following *in vitro* techniques: plaque inhibition assay, plaque reduction assay, inhibition of virus-induced cytopathic effect, virus yield reduction assay, end point titre determination assay, reduction or inhibition of the synthesis of virus-specific polypeptides, immunological assays detecting viral antigens and viral enzyme inhibition-based assays (Vlietinck and Vanden Berghe, 1991; Cowan, 1999). While these methods have successfully been employed to screen many and diverse antiviral agents, limitations and shortcomings relating to safety, long assay period, cumbersomeness, experimental-biased read-out, high cost and the inability to distinguish between mere toxic effects of agents on host cells and selective antiviral effects associated with some of these methods has limited their application in high throughput assays of the vast and yet to be screened medicinal plants with potential antiviral activities (Esimone et al., 2005, 2007;

Okoye et al., 2012).

In this study, we have used a modification of a rapid and reproducible vector-based antiviral screening technique as earlier described (Esimone et al., 2005, 2010) to screen three different solvent extracts of the plant *M. oleifera* (Moringaceae) for anti-retroviral activity. This technique is novel and a high throughput approach for the screening of medicinal plant extracts for possible antiviral activities (Esimone et al., 2005).

MATERIALS AND METHODS

Collection and extraction of plant materials

Fresh leaves of *M. oleifera* plants were collected from Nibo in Awka-south L.G.A, Anambra State, Nigeria. The identity was authenticated by Prof. C. C. Okeke of the Department of Botany, Nnamdi Azikiwe University, Awka. The plant leaves were washed, dried and ground to powder using a mechanical grinder. The methanol extract (MM) and petroleum ether extracts (EM) were obtained by macerating 40 g portions of the plant powder in 200 ml of either methanol or petroleum ether for 48 h at room temperature with intermittent agitation. The solutions were filtered using Whatman No 1 filter paper and concentrated to dryness *in vacuo* at 40°C. The aqueous extract (AM) was also obtained by macerating a 40 g portion of the plant powder in 400 ml of distilled water at room temperature for 24 h. The resulting solution was concentrated by evaporation at 40°C and the remaining solvent was removed by freeze drying.

Phytochemical analysis of plant extracts

The extracts (EM, MM and AM) was tested for the presence of alkaloids, flavonoids, tannins, saponins, glycosides, protein, carbohydrate, terpenoids, resins, fats and oil, steroids and reducing sugar using standard phytochemical methods (Evans, 1989; Harborne, 1998).

Cell lines

Human embryonic kidney cells expressing SV40 Large T-antigen (293 T) and Henrietta Lacks cells were propagated in D-10 medium, consisting of Dulbecco's modified Eagle's medium (DMEM) with high glucose, 2 mM $_{L}$-glutamine and supplemented with 10% heat-inactivated foetal bovine serum (FBS), 100 U/ml penicillin and 100 µg/ml streptomycin. Tissue culture medium and supplements were purchased from Invitrogen (Karlsruhe, Germany). The cell cultures were maintained in a humidified 5% CO_2 atmosphere at 37°C.

Generation of HIV-1 viral vectors transferring GFP genes

Infectious HIV lentiviral particles transferring the Green Fluorescent Protein (GFP) gene were generated by transient co-transfection of GFP-expressing vector constructs based on HIV-1 (pHIV-CSCG, 5 µg), *Vesicular stomatitis* virus- G (VSV-G) protein expression plasmid (pHIT-G, 2 µg), a secretory alkaline phosphate expression plasmid (pSEAP, 0.1 µg) and the HIV-1 codon-optimised gag-pol, tat, rev expression plasmids pCMVΔR8.2 using polyethyleneimine (PEI) transfection protocol (Wagner et al., 2000) in 293T cells. The cells were sub-cultured and transfected during the log growth phase in a T-75 cell culture flask using a 1:1 ratio of DNA/PEI. Supernatant containing the viral particles were harvested after 48 h of transfection.

Vector-based antiviral assay

The effect of the test extracts on HIV-1 vector infectivity was determined according to a previously described technique (Wagner et al., 2000; Esimone et al., 2005). HeLa cells were plated in triplicates into 96-well plates at a density of 6000 cells/well and incubated overnight. Various concentrations of the test extracts (at twice the final concentrations indicated) were prepared in DMEM (without FBS) such that the final DMSO concentration does not exceed 0.5%. These various concentrations were pre-incubated with equal volumes of the HIV-1 vector supernatant (100 µl) for 30 min at 37°C. Culture medium from the HeLa cell monolayers was replaced with 50 µl of the above vector/drug mixtures in triplicates and incubated for 2 h at 37°C + 5% CO_2. Fresh D-10 medium (50 µl) containing the drugs at the final concentrations stated were then added and the culture was incubated for two days. Control wells (containing vector alone but without drug) received D-10 medium containing 0.5% DMSO instead. The number of infected cells expressing the green fluorescent protein (GFP) was determined by FACS acquisition (FACScalibur^{TM} flow cytometer, Becton Dickinson). The FACS data was analysed with FCS Express V3 software®.

Cytotoxicity assay

The cytotoxicity assay was performed in parallel to the antiviral screening using the MTT assay method as previously described (Esimone et al., 2005) on HeLa cells. In the MTT assay, cells were seeded onto a 96-well plate at a concentration of 10^4 cells/well and a volume of 100 µl per well. A volume of 100 µl of the different concentrations of the test extracts (5, 25 and 62.5 µg/ml) were added to culture wells in triplicate. Culture medium without any drug was used as the "no-drug" control. After incubation at 37°C under 5% CO_2 for 2 days, a solution of MTT (3 mg/ml, 50 µl per well) was added to each well and further incubated at 37°C + 5% CO_2 for 4 h to allow formazan formation. After this time, the medium was removed and 150 µl of DMSO was used to dissolve the resulting blue formazan crystals in living cells. The optical density was determined at 550 nm using a multi-well microtitre plate reader (Tecan, Austria). Each single value of the triplicates was expressed as percentage of the mean of triplicates of the "no-drug" control cultures and the mean and standard deviation of the percent values were calculated for each triplicate. The concentration of 50% cellular toxicity (TC_{50}) of the test extracts was calculated by non-linear regression.

RESULTS

Phytochemical analysis

The phytochemical analysis of the different solvent extracts of *M. oleifera* showed the presence of saponins, alkaloids, glycosides, tannins, carbohydrates, flavonoids, resins, acidic compounds and proteins. Ether extract of *M. oleifera* contained only alkaloids and flavonoids while the aqueous extract contained saponins, alkaloids, glycosides, tannins, carbohydrates, acidic compound, and protein. The methanol extract of *M. oleifera* had

Table 1. Relative abundance of different phytochemical constituents of *M. oleifera extracts*.

Phytoconstituent	Extract		
	EM	AM	MM
Saponins	_	+++	++
Alkaloids	++++	+++	++
Glycosides	-	+++	+++
Tannins	-	++++	+++
Carbohydrates	-	++	+
Reducing sugar	-	-	-
Flavonoids	++++	-	++
Resins	+	-	++
Steroids	-	-	-
Terponoids	-	-	-
Fats and oil	-	-	-
Acidic compounds	-	+++	-
Proteins	-	+	++

-, Not present; +, present in small concentration; ++, present in moderately high concentration; +++, present in very high concentration; ++++, abundantly present.

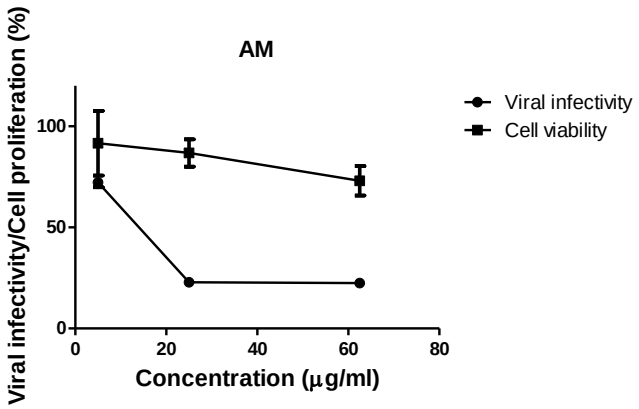

Figure 1. Inhibitory effect of aqueous extract of *M. oleifera* against HIV-1 viral vector.

relatively higher phytochemicals constituents as compared to other solvent extracts (Table 1).

Viral vector infectivity inhibitory studies and cytotoxicity

Flow cytometry and FACS analysis of viral infectivity rate showed that the aqueous extract of *M. oleifera* (AM) was active against the HIV-1 lentiviral vector and inhibited the early events of the viral replication cycle on HeLa cells in a concentration-dependent manner (5 to 62.5 µg/ml) (Figure 1). Inhibition of viral vector infectivity of HeLa cells was about 77.51% as compared to the untreated control cells at 62.5 µg AM/ml. A concentration of 7.17 µg AM/ml inhibited 50% of the viral vector infectivity (IC_{50}).

Table 2. Antiviral selectivity indices of *M. oleifera* extracts against HIV-1 lentiviral vector.

Parameter	AM	MM	EM
TC_{50} (µg/ml)	41.58	38.88	32.33
IC_{50} (µg/ml)	7.17	7.72	7.59
Selectivity index (TC_{50}/IC_{50})	5.80	5.04	4.26

AM, Aqueous extract of *M. oleifera;* EM, ether extract of *M. oleifera;* MM, methanolic extract of *M. oleifera*; IC_{50}, concentration of extract that inhibited viral infectivity (cytopathic effect) by 50%; $TC_{50,}$ concentration of extract that is cytotoxic to 50% of the cells.

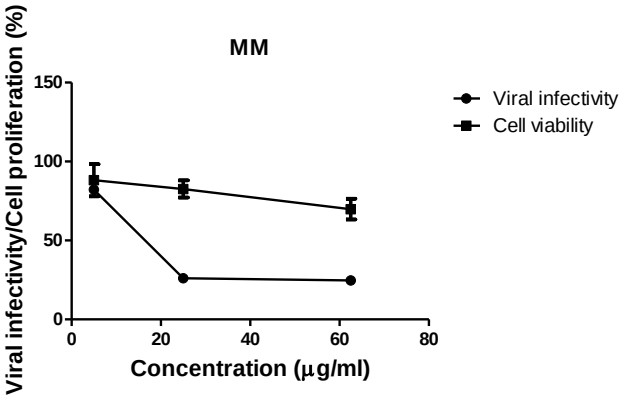

Figure 2. Inhibitory effect of methanol extract of *M.oleifera* against HIV-1 viral vector.

The 50% cytotoxic concentration (TC_{50}) was 41.58 µg AM/ml in the assay performed by MTT assay in parallel with the study on viral vector infectivity inhibition. This implies an inhibition selectivity index (SI) of 5.80 for the aqueous extract of *M. oleifera* (Table 2).

The methanol extract of *M. oleifera* (MM) showed inhibition of 50% of viral infectivity (IC_{50}) at a concentration of 7.72 µg MM/ml while cytotoxicity of 50% of the cells (TC_{50}) was at 38.88 µg MM/ml thus giving a selectivity index of 5.0 (Table 2). Inhibition of viral vector infectivity of HeLa cells was about 75.33% as compared to the untreated control cells at 62.5 µg MM/ml and the cells were still up to 70% viable at this concentration (Figure 2).

The ether extract of *M. oleifera* (EM) inhibited 50% of viral infectivity (IC_{50}) of HeLA cells at a concentration of 7.59 µg EG/ml while 32.33 µg EM/ml was cytotoxic to 50% of the cells. This implies a viral inhibition selectivity index of 4.26 (Table 2). The percentage inhibitions of viral infectivity of EM (5, 25 and 62.5 µg/ml) were 28.2, 75.6 and 76.6%, respectively. The cells were still viable by as much as 55.9% for the 62.5 µg/ml of EM (Figure 3).

DISCUSSION

Despite unrelenting research efforts and huge spending, HIV/AIDS is still a daunting challenge and burden to glo-

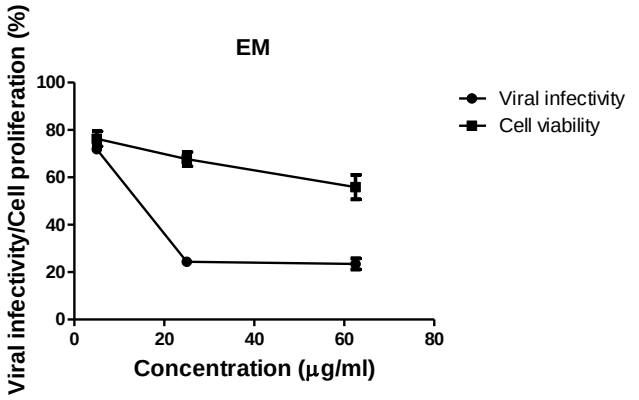

Figure 3. Inhibitory effect of ether extract of *M. oleifera* against HIV-1 viral vector.

bal public health and there are still no cure or effective vaccines against the disease. Presently, the use of prevention strategies and highly active antiretroviral therapy (HAART) are the best options available to decrease morbidity and mortality due to HIV/AIDS. Several classes of antiretroviral drugs have been developed and used mostly in combinations to target viral proteins at different stages of the HIV life cycle or host factors. The HAART cocktail uses a nucleoside reverse transcriptase inhibitor (NRTI) and/or a nonnucleoside reverse transcriptase inhibitor in combination with either a protease inhibitor (PI), fusion inhibitor (FI), CCR5 antagonist or an integrase inhibitor (II) to treat PLWHA (Henkel, 1999; Bartlett, 2004; USDHHS, 2012). Some of the major limitations of current antiretroviral drugs are the constant emergence of multi-resistant strains of HIV, intolerable adverse side effects, and the unavailability/affordability of these drugs in poor countries; especially in sub-Saharan Africa where HIV/AIDS is rampaging. Therefore, developing cost-effective, safe, highly specific drugs that will be less susceptible to development of resistance by HIV is an urgent unmet need.

It has long been recognised that natural products, especially those derived from plants, are excellent sources of new anti-HIV-1 drugs (De Clercq, 2000, 2005; Esimone et al., 2009, 2010, 2012). Some of these plants-derived antiretroviral therapies have been shown to have inhibitory activities against several HIV-1 processes, including viral entry, reverse transcription, replication, integration, virus maturation and virion budding. Some of these compounds have been clinically tested, with favourable results (Asres et al., 2005; Park et al., 2009; Vo and Kim, 2010; Jiang et al., 2010; Filho et al., 2010; Sing and Bodiwala, 2010).

In the present study, we used a novel vector-based assay technique to screen lipophilic and polar solvent extracts of the plant, *M. oleifera* for inhibitory activities against infectivity of HIV-1 lentiviral vector particles. A VSV-G-pseudotyped, human immunodeficiency virus type 1-based, self-inactivating lentivirus vector expressing green fluorescent protein (GFP) under the control of Cytomegalovirus (CMV) promoter was constructed and used as viral infective particles on HeLa cells. The infectious vector particles were generated by transient co-transfection of the vector plasmid (pHIV-1 CSCG) with packaging plasmids encoding tat, rev, gag-pol (pCMVΔR8.2), a VSV-G expression plasmid (pHIT-G), a secretory alkaline phosphate expression plasmid (pSEAP) all necessary for viral infectivity. Transient supply of the packaging plasmids and the self-inactivation of the vector by deletion of the U3 region in the 3^1-LTR to make sure that the resulting lentiviral vector is only capable of a single round of replication, makes the viral vectors comparatively safer than their parent wild retroviral virus. The infectious vector particles additionally differ from the wild-type HIV-1 virus in that they lacked some of the HIV-1 accessory genes such as nef, vif, vpu and vpr. Because the transgene (in this case, Green fluorescent protein) is integrated into the genome of target cells in the process of infection, vector infectivity in the presence or absence of various concentrations of the extracts is easily determined by flow cytometrically as a function of the amount of GFP expression. In this system, GFP expression is driven by an internal CMV promoter and occurs after integration. The implication is that inhibitors of late stages in viral replication cycle such as inhibitors of new viral assemblage and inhibitors of budding are not detected by the screening assay used in the study. However, this cell-based assay is one of the high-throughput screening techniques used to identify new inhibitors that target different steps in the HIV-1 life cycle. Single-cycle infectious pseudotyping of HIV-1 using the envelope glycoprotein of the vesicular stomatitis virus (VSV-G) can mimic some activities of the wild-type viruses (Aiken, 1997).

For the pilot screening of potential anti-HIV medicinal plants, this vector based assay used in this study offers multiple advantages. Firstly, it reduces the risk of working with the wild type HIV-1 while retaining the sensitivity and reliability of the assay. Fear of the risks associated with working with wild type HIV-1 viruses has deterred many investigators from evaluating potential anti-HIV medicinal plant extracts, especially in less developed countries with limited resources and ill-equipped laboratories. Secondly, pseudo-typing of the vector with the VSV-G envelope ensures a wider cell tropism. Therefore, antiviral screening is not only confined to cell lines which express CD4 and/or CCR5 receptors (Esimone et al., 2007). The viral vector can infect a range of other cell lines. Thirdly, the assay is very rapid. Read out is obtained in about two days as opposed to 5 to 10 days for many conventional anti-HIV screening techniques. Fourthly, read out is simple and highly reproducible as opposed to the cumbersome procedure of enumerating HIV-induced cytopathic effects. In this study, a flow cytometric estima-

tion of viral infectivity is simple and devoid of experimental bias and therefore reproducible. Fifthly, it is possible to make a clear distinction between direct antiviral effect of plant extract and the mere cytotoxic effects of the extract to target cell lines.

Results of the study show that the different solvent extracts of *M. oleifera* inhibit early events in the viral replication cycle. The selectivity index of the *M. oleifera* extracts (4.26to 5.80) against the HIV-1 lentiviral vector is sufficiently high and demonstrates that the antiviral activities observed are not due to the cytotoxicity of the extracts on HeLa cells used in the study. Previously, the structure of a bioactive thiocarbamate, niaziminin, extracted from the leaf of *M. oleifera* was shown to be enough in inhibiting the activation of a tumour promoting virus, EpsteinBarr virus (EBV) (Murakami et al., 1998). It has also been reported in earlier studies that *M. oleifera* leaf may be applicable as a prophylactic or therapeutic anti-HSV (Herpes simplex virus type 1) medicine and may be effective against the acyclovir-resistant variant of the virus (Lipipun et al., 2003). In another recent study, hydroalcoholic extract of *M. oleifera* fruits showed anti-hepatitis B virus (HBV) activity (Waiyaput et al., 2012).

In this investigation, preliminary phytochemical analysis of the extracts of *M. oleifera* showed, generally, the presence of saponins, alkaloids, glycosides, tannins, carbohydrates, flavonoids, resins, acidic compounds and proteins. Previously, *M. oleifera* has been shown to be rich in compounds containing the simple sugar, rhamnose and a fairly unique group of compounds called glucosinolates and isothiocyanates (Fahey et al., 2001; Bennett et al., 2003). Two alkaloids moringine and moringinine (Kerharo, 1969), vanillin, β-sitosterol, β-sitostenone, 4-hydroxymellin and octacosanoic acid have been isolated from the stem of *M. oleifera* (Faizi et al., 1994). Although, the specific contribution of these phytochemical constituents is yet to be investigated, the antiretrovial activities of *M. oleifera* could be attributed to some of these phytoconstituents in the extracts. Tannins which were found present in *M. oleifera* have been shown in earlier studies to prevent a variety of viral infections (Serafini et al., 1994; Nonaka et al., 1990). Tannins have also been demonstrated to inhibit viral reverse transcriptase (Nonaka et al., 1990). Similarly, flavonoids have been shown to show inhibitory effects against viruses including HIV and respiratory syncytial virus (Li et al., 2000). Plant-derived flavonoids have been reported to inhibit critical steps in the life cycle of HIV infectivity such as viral entry (Liu et al., 2011), reverse transcriptase (Li et al., 2011), integrase (Lee et al., 2003; Tewtrakul et al., 2003), viral transcriptional activities of HIV tat (Mehla et al., 2011) and protease inhibitory activities (Lee et al., 2009) and other activities. *M. oleifera* is also rich in saponins which have been reported to inhibit HIV infectivity, *in vitro* (Konoshima et al., 1995; Yang et al., 1999; Li et al., 2012).

In this study, the extracts of *M. oleifera* potently inhibited HIV-1 lentiviral vector infectivity and cytotoxicity studies conducted in parallel show that the inhibition of viral vector infectivity of these extracts are not due to diminished viability of the cells induced by the extracts. The extracts selectively inhibited viral infectivity of the cells. In earlier studies, correlations between anti-HIV-1 vector activity and anti-wild type HIV-1 activity have been demonstrated. In one of such studies, Steinstraesser et al. (2005) demonstrated in an assay that the porcine defensin (protegrin-I) showed more than three-fold higher activity against the wild type HIV-1 than against the corresponding lentiviral vector. It was also shown that some antiviral medicinal plant extracts showed up to ten-fold higher activities against wild type lentiviruses as against the corresponding lentiviral vector particles (Esimone et al., 2005). This is very instructive and points to the reliability of the high through-put viral vector particles-based assay in the screening of putative substances for antiviral activities. It also suggests that the extracts screened in this study using the lentiviral HIV-I vector could show higher antiviral activities when used against wild type HIV-I virus. The pattern of inhibition of the HIV lentiviral vector suggests that the extracts either directly interacted with the vector particles inhibiting the envelope protein or that it interacted with some host cells-derived components of the viral particle which could be the lipid membrane derived from the cell or the cellular membrane proteins that are often incorporated in lentiviral particles during budding (Gould et al., 2003).

Interestingly, some studies and reports suggest that extracts from *M. oleifera* could be deployed for its nutritive and immune-boosting properties to support PLWHA. The proceedings of the 14th International AIDS Conference held in Barcelona, Spain in 2002 included a recommendation that *M. oleifera* powder be considered as an alternative treatment to boost the immune systems of HIV-positive patients in Africa who would otherwise not receive antiretroviral drugs or, in fact, any treatments at all (Burger et al., 2002). Similarly, the widespread concomitant use of *M. oleifera* with antiretroviral drugs by PLWHA in East Africa, the benefits, as well as the potential for herb/drug interactions has also been documented (Monera and Maponga, 2012). The outcome of this study, which showed that early steps in the infectivity of HIV-1 lentiviral vector particles are inhibited by leaf extracts of *M. oleifera,* could lend credence to its widespread use among PLWHA. The study suggests that, apart from its nutritive values and strengthening of the immune system which are widely reported, retroviral inhibitory activities of *M. oleifera* leaf extracts could partly explain the benefits and improvement in quality of life claimed by PLWHA in the use of this medicinal plant as supplement.

Conclusion

In this study, *M. oleifera* Lam. leaf extracts showed potent

and selective inhibition of HIV-1 infectivity and could serve as source of antiretroviral lead molecule. The outcome of this investigation could partly explain the benefits claimed in the use of extracts of *M. oleifera* in the management/treatment of People Living with HIV/AIDS in African Traditional Medicine.

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