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Inhibition of HIV-1 enzymes, antioxidant and anti-inflammatory activities of *Plectranthus barbatus*

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ABSTRACT

Ethnopharmacological relevance: Plectranthus barbatus is widely used in African countries as an herbal remedy to manage HIV/AIDS and related conditions.
Aim of the study: To investigate the HIV-1 inhibitory, anti-inflammatory and antioxidant properties of *P. barbatus* and thereby provide empirical evidence for the apparent anecdotal success of the extracts.
Materials and methods: Ethanolic extract of *P. barbatus*'s leaves was screened against two HIV-1 enzymes: protease (PR) and reverse transcriptase (RT). Cytotoxicity of the extract was determined through measuring tetrazolium dye uptake of peripheral blood mononuclear cells (PBMCs) and the TZM-bl cell line. Confirmatory assays for cytotoxicity were performed using flow cytometry and real-time cell electronic sensing (RT-CES). The free radical scavenging activity of the extract was investigated with 2,2-diphenyl-1-picrylhydrazyl while the anti-inflammatory properties of the plant extract were investigated using a Th1/Th2/Th17 cytometric bead array technique.
Results: P. barbatus extract inhibited HIV-1 PR and the 50% inhibitory concentration (IC_{50}) was 62.0 μg/ml. The extract demonstrated poor inhibition of HIV-1 RT. Cytotoxicity testing presented CC_{50} values of 83.7 and 50.4 μg/ml in PBMCs and TZM-bl respectively. In addition, the extract stimulated proliferation in HIV negative and positive PBMCs treated. RT-CES also registered substantial TZM-bl proliferation after extract treatment. The extract exhibited strong antioxidant activity with an IC_{50} of 16 μg/ml and reduced the production of pro-inflammatory cytokines indicating anti-inflammatory potential.
Conclusion: This is the first demonstration of the *in vitro* anti HIV-1 potential of *P. barbatus* including direct activity as well as through the stimulation of protective immune and inflammation responses. The low cytotoxicity of the extract is also in agreement with the vast anecdotal use of this plant in treating various ailments with no reported side-effects.

1. Introduction

Plectranthus is a genus that belongs to the Lamiaceae family. This genus is distributed worldwide and contains about 300 species with 45 of these being used ethnobotanically on the African continent (Lukhoba et al., 2006). *Plectranthus* species has numerous ethnomedicinal uses such as in antibacterial, antiviral, anti-malaria and antifungal phytotherapy (Lukhoba et al., 2006). *Plectranthus barabatus* Andr. is regarded as one of the most important medicinal species of the genus *Plectranthus. P. barbatus* originated from north-eastern Africa and is found in sub-Saharan Africa as an invasive species (Rice et al., 2011). For centuries it has been used in folk medicine of Brazil, tropical Africa and China as well as in Hindu and Ayurvedic traditional medicine (Lukhoba et al., 2006; Maioli et al., 2010).

P. barbatus is used in antispasmodic therapy, where it is specifically used for the treatment of gastric disturbances associated with intestinal spasms (Almeida, 2003). In Kenya, the plant is reportedly used traditionally as antimalarial phytotherapy (Nguta et al., 2010a, 2010b). *P. barbatus* has also been reported to be a herbal remedy against candida which was proven in an *in vitro* pharmacological study (Runyoro et al., 2006).

A number of diterpenoids have been isolated from *P. barbatus.* These are the most common group of compounds found in *Plectranthus* plants (Abdel-Mogib et al., 2002). The medicinal properties of *P. barbatus* can probably be attributed to terpenoids.

Surveys carried out in Uganda, Tanzania and Namibia on the use of medicinal plants for HIV/AIDS treatment revealed *P. barbatus* as one of the commonly used herbal remedies in managing HIV/AIDS and the associated opportunistic infections. The aerial parts of this plant are administered orally by traditional medicine

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practitioners as a decoction or an infusion to treat HIV/AIDS, oral candidiasis, herpes simplex, hepes zoster and skin rashes (Chinsembu and Hedimbi, 2010; Kisangau et al., 2011, 2007; Lamorde et al., 2010). Exactly what the mechanism of action of the extract is, or why patients experience alleviation of symptoms (during or after its use) is unknown. Because HIV replicates in a highly oxidized environment (Gil del Valle et al., 2013), demonstrating substantial natural antioxidant activity in *in vitro* extracts of *P. barbatus* will contribute some mechanistic information. Also, direct anti-viral (as in the inhibition of viral enzymes) or anti-inflammatory activities, if exhibited by the extract, presents explanations for how the plant may slow disease progression. To be entirely useful, if the preceding effects exist, these abilities must go hand in hand with a lack of, or very low cytotoxicity.

HIV infected individuals even when successfully treated with highly active anti-retroviral therapy (HAART), continue to deal with incidences of malignancy, cardiovascular disease (CVD), metabolic, bone, renal and liver disease, very much like aging populations (Nixon and Landay, 2010). There is also ample evidence supporting the notion that inflammation plays a role in these conditions in the general population, while also having become leading causes of morbidity and mortality in HIV-infected individuals in settings where HAART is routinely used. Compared with the general population, high-sensitivity C-reactive protein (hs-CRP), interleukin 6 (IL-6), d-dimer, and certain other inflammatory biomarkers are significantly elevated during HIV infection (Nixon and Landay, 2010). Any product/compound that decreases these types of inflammatory biomarkers therefore has a potential use in improving the quality of life of infected individuals. The balance between the pro-inflammatory cytokines IL-1, IL-6 and tumor necrosis factor-alpha, which up-regulate HIV expression, and IL-10, which can act both as an anti-inflammatory cytokine and a B-cell stimulatory factor plays an important role in the progression to AIDS (Breen, 2002). Because HIV/AIDS is also known to affect IFN and IL-17 (Williams et al., 2013) where their levels serve as indicators of disease progression, the anti-inflammatory behavior of the extract was investigated by assessing its effect on all the mentioned relevant pro-inflammatory cytokines simultaneously.

The aims of this study were therefore the investigation of the HIV-1 inhibitory properties, cytotoxicity, anti-inflammatory and antioxidant abilities of *P. barbatus* in order to determine whether the traditional claims are supported by actual pharmacological effects. The idea was to link the HIV/AIDS ethnomedicinal use of the plant to *in vitro* studies in order to validate the anecdotal claims in favor of *P. barbatus*. A lot of work has been done on *P. barbatus*; there is however nothing published on how or why extracts of this plant may be specifically assisting HIV/AIDS patients.

2. Material and methods

2.1. Plant material

Plectranthus barbatus Andr. leaves (74.8 g) were collected at the Botanical garden of the University of Pretoria during April 2011. Plant identification was done in the H.G.W.J Schweikerdt herbarium of the University and a voucher specimen (117198) is kept in the herbarium.

2.2. Extraction

Fresh leaves were blended in ethanol and filtered. The filtrate was concentrated under reduced pressure at 50 °C. The residue was re-dissolved in ethyl acetate to exclude highly polar tannin compounds, non-specific enzyme inhibitors. The ethyl acetate fraction (lipophilic fraction) was dried and stored in the dark at 4 °C until use. The ethyl acetate extract was reconstituted in dimethyl sulfoxide (DMSO), which provides a sterile environment, before each biological assay. Further dilutions to obtain desired extract concentrations were done in either cell culture media for cytotoxicity or buffer for HIV-1 enzyme assays.

2.3. Cytotoxicity assay using MTT and real time cell sensing

Tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was used to assess the cytotoxicity of *P. barbatus* in TZM-bl cells (Mosmann, 1983). Briefly, cells were seeded in a 96-well tissue culture treated plate at 1×10^4 cells per well in the absence or presence of various gradient *P. barbatus* extract concentrations (100–3.125 μg/ml). Incubation was carried out for 72 h at 37 °C in humidified air with 5% CO_2. Auranofin was used as a positive control for cytotoxicity because it is a known toxic compound with antitumor activity (Mirabelli et al., 1985). Other control wells included a negative control (cells & medium only), background control for extract and a DMSO control. Plates were read on a Multiskan Ascent reader at 550/690 nm. The 50% cytotoxic concentration (CC_{50}) was calculated using Graphpad Prism (Graphpad Software Inc. California, USA).

In addition to using MTT for the detection of cytotoxicity a real time-cell electronic sensing (RT-CES) device, *x*Celligence (Roche Diagnostics, Mannheim, Germany) was used to monitor proliferation of the TZM-bl cells in the presence of *P. barbatus* extract. The system monitors cellular events in real time without the incorporation of labels by measuring electrical impedance across interdigitated gold micro-electrodes integrated on the bottom of special tissue culture plates. Increasing attachment of cells to the electrodes increases electrode impedance which is displayed as Cell Index (CI) (Abassi et al., 2009; Atienzar et al., 2011). TZM-bl cells were seeded at 1×10^4 cells per well. Three concentrations of *P. barbatus* extract (100, 50.4 and 25 μg/ml) were tested alongside 10 μM auranofin, positive control for toxicity. An untreated TZM-bl cells control was also included. Cell treatment monitoring was carried out over a period of 72 h.

2.4. Effect of the extract on PBMC proliferation

Ethical approval for obtaining blood samples from consenting donors was granted by the Faculties of Natural and Agricultural Sciences and Health Sciences Ethics Committees (EC080506-019; 163/2008, University of Pretoria, South Africa). Freshly isolated uninfected and infected PBMCs were plated in 96 well plates (Costar, Corning Incorporated, USA) at 1×10^5 cells per well and treated with the extract at final concentrations of 100, 50, 25, 12.5, 6.25 and 3.125 μg/ml. The number of viable cells was detected after 72 h using 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS) solution (Promega, Promega Corporation, USA). Control wells included a toxicity control auranofin (Mirabelli et al., 1985) and the plates were read at 492/690 nm. The percentage viability was calculated relative to an untreated control of cells only and the CC_{50} values were determined using Graphpad Prism (Graphpad Software Inc. California, USA).

The flow cytometric analysis of lymphocyte proliferation was measured using carboxylfluorecein succinimidyl ester (CFSE). The technique monitors the effect of a treatment, in this case extract, on mitotic activity of the *T* cell population based on the visualization of eight to 10 discrete cycles of cell division (Fulcher and Wong, 1999; Lyons, 2000). HIV negative and positive PBMCs were labeled with CFSE and extract treatment of the labeled cells was done at two concentrations (100 and 50 μg/ml) for 72 h. Prior to flow cytometric analysis, propidium iodide was incorporated to

exclude background staining from dead cells (Fonteh et al., 2011). Data was acquired on a FACSAria (BD BioSciences, California, USA) and analyzed using FlowJo Version 7.6.1 (TreeStar Inc., Oregon, USA).

2.5. HIV-1 protease fluorogenic assay

This assay was a modified version of the assay performed by Lam et al. (2000). The fluorogenic HIV protease (PR) substrate 1 (Arg-Glu(EDANS)-Ser-Gln-Asn-Tyr-Pro-Ile-Val-Gln-Lys(DABCYL)-Arg) was dissolved in DMSO to make a 1 mM stock. The stock fluorogenic substrate was diluted to 10 μM using assay buffer (0.1 M sodium acetate, 1 M NaCl, 1 mM EDTA and 1 mM DTT, pH 4.7). An aliquot of the substrate (10 μM, 49 μl) and 1 μl of HIV-1 PR solution (1 μg/ml; Bachem, Switzerland) were added to the reaction mixture in an assay buffer in the presence or absence (untreated control) of the extract to make a final reaction volume of 100 μl. The mixture was incubated at 37 °C for 1 h in black 96 well assay plates. The fluorescence intensity was measured at an excitation wavelength of 355 nm and an emission wavelength of 460 nm using a synergy microplate spectrofluorometer (BioTek, Analytical & Diagnostic products, South Africa). Acetyl pepstatin (AP) was used as a positive control for HIV-1 PR inhibition. The blank treatment consisted of assay buffer with only the substrate and an untreated control of enzyme and substrate was also included. The percentage inhibition was calculated based on the formula: 100−[(Test reagent RFU−background RFU)/(untreated control RFU−blank) × 100] where RFU = relative fluorescence units.

2.6. HIV-1 reverse transcriptase colorimetric assay

The effect of the crude extract on reverse transcription was tested using a reverse transcriptase (RT) colorimetric assay kit from Roche Diagnostics (Mannheim, Germany) and purified recombinant HIV-1 RT (Merck, Darmstadt, Germany). The assay was performed according to the method previously described (Fonteh et al., 2009). Extract was tested at two concentrations 100 and 50 μg/ml. The enzyme (0.2U) was incubated for 1 h with the extract at 37 °C. Subsequent 1 h incubations involved addition of an antibody conjugated to peroxidase that binds to the digoxigenin-labeled DNA. In the final step, the peroxidase substrate solution [2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid)] is cleaved by the peroxidase enzyme, producing a colored reaction product. Two positive controls were used; doxorubicin, a known HIV-1 RT inhibitor (Kuete et al., 2010b; Mbaveng et al., 2011) and an in-house natural product inhibitor active against HIV-1 RT (Fonteh et al., 2009). The absorbance of the samples was read at 405 nm with a reference wavelength of 492 nm using a microtiter plate reader (Multiskan Ascent; Thermo Labsystems; USA) and was directly correlated to the level of RT activity in the sample.

2.7. Antioxidant activity: DPPH free radical scavenging assay

The free radical scavenging activity of the extract was evaluated using a modified method previously described by Kuete et al. (2010a). The extract dissolved in ethanol was mixed with a 90 μM 2,2-diphenyl-1-picrylhydrazyl (DPPH) ethanol solution to give final extract concentrations of 6–100 μg/ml. Incubation of extract with DPPH was done in the dark at room temperature for 30 min. The absorbance values were measured at 550 nm (Multiskan Ascent; Thermo Labsystems; USA) and converted into percentage of antioxidant activity (Marxen et al., 2007). A known antioxidant, ascorbic acid, was used as a standard control. IC_{50} values were calculated using Graphpad Prism (Graphpad Software Inc. California, USA).

2.8. Anti-inflammatory activity: cytometric bead array

The effect of the extract on cytokine production was evaluated using the Human Th1/Th2/Th17 cytometric bead array (CBA) kit (BD Biosciences, San Jose, California) (Williams et al., 2013).

PBMCs were isolated from blood samples of healthy individuals ($n=4$). Cells were seeded at a concentration of 1×10^6 cells per well and treated with two non-cytotoxic concentrations of the extract, 25 and 50 μg/ml. The supernatant was collected after 24 h incubation and stored at −20 °C until cytokine analysis. Cytokine levels in the supernatant were analyzed following the protocol outlined in the kit. The supernatant (50 μl) was mixed with the cytokine capture beads and the detector reagent, phycoerythrin (PE)-conjugated detection antibodies, to form sandwich complexes. These complexes were measured using a FACSArray Bioanalyzer (BD Biosciences, San Jose, CA, USA) in order to identify the concentrations of specific cytokines. The limits of detection for each cytokine were as follow: 2.6 pg/ml for IL-2, 4.9 pg/ml for IL-4, 2.4 pg/ml for IL-6, 4.5 pg/ml for IL-10, 3.8 pg/ml for TNF, 3.7 pg/ml for IFN-ɣ and 18.9 pg/ml for IL-17 A.

3. Results and discussion

3.1. Effect of P. barbatus extract on the viability of TZM-bl and PBMCs

The CC_{50} of *P. barbatus* extract in TZM-bl cells using MTT was 50.4 ± 2.7 μg/ml (Table 1) while RT-CES revealed a different cytotoxicity patterns. With RT-CES (Fig. 1), 100 μg/ml (iii) of the extract resulted in < 50% of TZM-bl cells being viable after 72 h

Table 1
Cytotoxicity and HIV-1 inhibitory activities of *P. barbatus* ethanolic extract.

Sample	CC_{50} (μg/ml) ± SD[a]		IC_{50}(μg/ml) ± SD	
	TZM-bl	PBMCs	HIV-1 RT	HIV-1 PR
P. barbatus	50.4 ± 2.7	84.7 ± 2.2	> 100	62.0 ± 0.2
Auranofin[b]	< 10	< 10	–	–
Acetyl pepstatin[c]	–	–	–	< 0.3
Doxorubicin[d]	–	–	< 25	–
BBR[d]	–	–	0.3 ± 0.1	–

[a] Thevalues represents CC_{50} and IC_{50} ± SD indicating the effect of *P. barbatus* extract on the viability of TZM-bl cells and PBMCs (detected with MTT and MTS) as well as the inhibitory effect on HIV-1 enzymes.
[b] Positive control for toxicity (concentration in μM).
[c] Known inhibitor (positive control) of HIV-1 protease.
[d] Positive control for HIV-1 reverse transcriptase (BBR concentration in mg/ml).

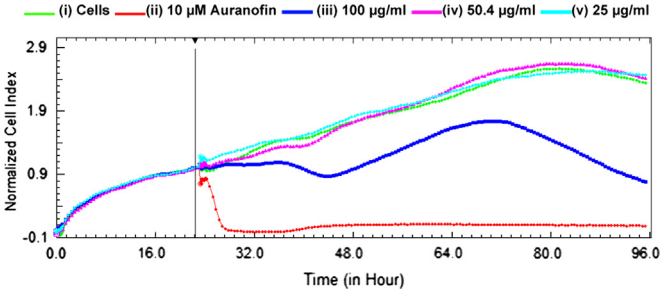

Fig. 1. Effect of *P. barbatus* on TZM-bl cells as generated by real-time cell analysis. The cells were exposed to 100, 50.4 and 25 μg/ml of the extract and these are represented by the different colored curves. Auranofin (ii) was used as a positive control for toxicity. Each data point was normalized against the time point just before extract addition (24 h).

compared to the control cells (i) and this was expected because of what was observed with MTT. The cell index for 100 μg/ml (iii) was low compared to that of control cells (i) indicating the reduction of cell viability at that concentration. Other concentrations tested; 50.4 (iv) and 25 μg/ml (v) were not toxic towards TZM-bl cells. *P. barbatus* has been reported to generally have low toxicity (Figueiredo et al., 2010) and the data obtained in the current study suggests that *P. barbatus* extract concentrations ≥100 μg/ml may result in high toxicity. The CC_{50} for Auranofin, positive control for toxicity, was < 10 μM in TZM-bl and PBMCs.

P. barbatus was less toxic to PBMCs with a CC_{50} of 84.7 ± 2.2 μg/ml (Table 1). These results were confirmed with flow cytometry whereby there was no significant difference ($p < 0.05$) in lymphocytes proliferation of untreated HIV negative (−) PBMCs (42%) and lymphocytes treated with 100 μg/ml of the extract (40%). However, a marginally higher cell division of 47% was observed for lymphocytes treated with 50 μg/ml of the *P. barbatus* extract (Fig. 2). Flow cytometry can be used on adherent or suspension cells while RT-CES is applicable to adherent cells, TZM-bl cells, explaining why the different techniques were applied for confirmation assays as they were.

A different trend was observed in HIV positive (+) lymphocytes (Fig. 3); in the absence of the extract the control cells resulted in 19% cell division (compared to the 42% in uninfected, untreated cells) and a 2.8% cell division was observed with the parent cells of HIV+ lymphocytes treated with 100 μg/ml of the extract. A lymphocyte division of 54% was observed with cells treated with 50 μg/ml of the *P. barbatus* extract. The CFSE data suggests that the high extract concentration tested, 100 μg/ml (Fig. 3B), was toxic to PBMCs while 50 μg/ml was less toxic (Fig. 3C). CFSE analysis allowed for the tracking of daughter cells from the parent population of cells (Lyons, 2000) and the cell division into daughter cells was clearly observed. Lower concentrations of extract had a comparable proliferative effect on infected/uninfected cells.

3.2. Anti-HIV-1 potential of *P. barbatus*

P. barbatus extract inhibited HIV-1 PR, 70% inhibition, at 100 μg/ml ($IC_{50} = 62.0 \pm 0.2$ μg/ml) (Table 1). The lowest concentration tested, 50 μg/ml, gave less than 50% HIV-1 PR inhibition. The positive control, acetyl pepstatin, inhibited HIV-1 PR by 97% at 10 μg/ml and its IC_{50} was < 0.3 μg/ml. The extract had weak inhibitory activity against HIV-1 RT ($< 50\%$). Positive control for HIV-1 RT, doxorubicin, exhibited an $IC_{50} < 25$ μg/ml. The IC_{50} of an in-house natural product inhibitor was 0.3 ± 0.1 mg/ml. These results suggests that the effectiveness of the traditional use of *P. barbatus* in managing HIV/AIDS (Chinsembu and Hedimbi, 2010; Kisangau et al., 2011; Lamorde et al., 2010) could be through the inhibition of HIV-1 PR. The use of herbal remedies for HIV/AIDS treatment is widely practised traditionally and it is of utmost importance to test the efficacy of plants used, in this case *P. barbatus*. There is no literature reporting the *in vitro* anti-HIV-1 activity of *P. barbatus*, making this study the first to link the *in vitro* inhibition of HIV-1 PR to the traditional use of this plant in HIV/AIDS treatment. Inhibition of HIV-1 PR could be attributed to diterpenoid compounds which are often described as the active ingredients identified in *P. barbatus* (Alasbahi and Melzig, 2010a, 2010b).

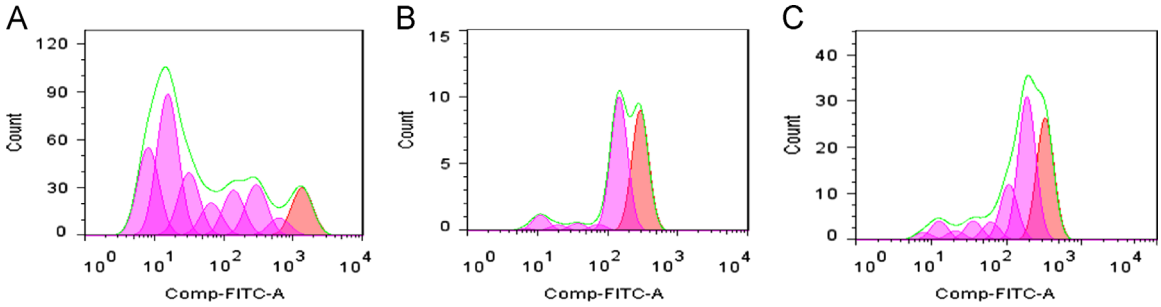

Fig. 2. The 3 day response of CFSE-labeled HIV-PBMCs treated with the *P. barbatus* extract. The orange peak represents the parent cells and pink peaks are daughter populations as a result of cell division. (A) Cell division of 42% was observed in untreated PBMCs. (B) Parent cells of PBMCs treated with 100 μg/ml resulted in 40% division of parent to daughter cells. and (C) PBMCs treated with 50 μg/ml of the extract had the highest cell division of 47%. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

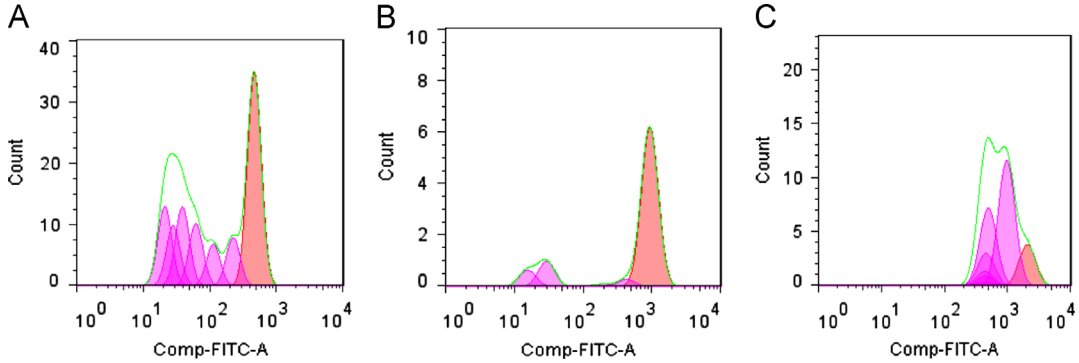

Fig. 3. Effect of *P. barbatus* extract on the proliferation of CFSE-labeled HIV+ PBMCS. The orange peak represent the parent cells and pink peaks are daughter populations as a result of cell division. (A) %cell division in control PBMCs was 19% (B) Only 2.8% cell division was obtained with PBMCs treated with 100 μg/ml and (C) A high cell division of 54% was observed in PBMCs treated with 50 μg/ml of the extractwhich was comparable to uninfected cells treated with a similar extract concentration. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.3. Antioxidant properties of P. barbatus

The results of the antioxidant activity of *P. barbatus* extract summarized in Fig. 4 showed a dose-dependent response. The IC_{50} of the extract was 15.8 ± 0.3 μg/ml and vitamin C, the positive control, had an IC_{50} of 1.1 ± 0.02 μg/ml. The IC_{50} value of *P. barbatus* obtained in this study was twice lower than that previously reported by Maioli et al. (2010), where an IC_{50} of 35.8 ± 0.3 μg/ml was obtained in aqueous leaves extract of *P. barbatus* using a similar DPPH assay.

The good antioxidant properties of *P. barbatus* possibly contribute to the reduction of the viral load in HIV-infected patients that traditionally uses this plant. Oxidative stress, which is caused by the accumulation of reactive oxygen species, has been linked to the activation of HIV-1 leading to the development of AIDS (Gil del Valle et al., 2013; Gil et al., 2003; Vaira et al., 1990). Antioxidants such as vitamin E and C have been shown to reduce oxidative stress in HIV patients and in the process contribute towards a reduction in viral load (Allard et al., 1998).

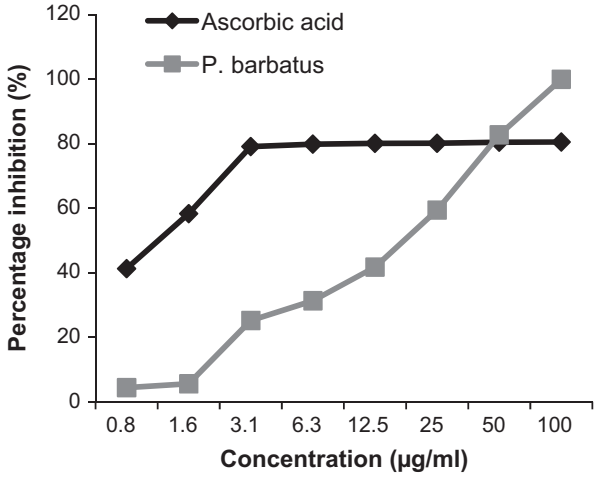

Fig. 4. DPPH antioxidant potential of *P. barbatus* extract. Ascorbic acid was used as a positive control. IC_{50}s were 15.8 ± 0.3 and 1.1 ± 0.02 μg/ml for extract and ascorbic acid respectively.

3.4. P. barbatus inhibits pro-inflammatory cytokines

The two non-cytotoxic concentrations of *P. barbatus* tested, 50 and 25 μg/ml, resulted in the reduction of cytokine production for all cytokines tested including pro-inflammatory cytokines IL-2, IL-6, IL-10, TNF and IL-17A (Fig. 5).

The ability of *P. barbatus* extract to inhibit inflammatory cytokines can be linked to its traditional use in treating various ailments (Lukhoba et al., 2006). Anti-inflammatory properties were previously detected by Matu and Staden (2003) in *P. barbatus* from Kenya when the methanolic extract of the leaves inhibited cyclooxygenase (COX-1), an enzyme involved in the cascade of events resulting in pain and inflammation. The current study identified another anti-inflammatory mechanism of *P. barbatus* which is through the inhibition of pro-inflammatory cytokines. These results can also be linked to anti-HIV properties of this plant; low levels of IL-17A in HIV-1 infection is associated with non-progression (Yue et al., 2008). IL-17A along with other cytokines such as IL-2 and IL-6 are elevated during the different stages of HIV infection (Keating et al., 2011; Nixon and Landay, 2010; Worsley et al., 2010; Yue et al., 2008). Therefore lowering the production of these cytokines especially IL-17A could contribute to slow or non-progression of HIV infection. The IFN-ɣ paradox in HIV infection needs better understanding. There is a need to control antiretroviral effects of IFN-ɣ during HIV infection. The ability of the extract in this study to lower IFN-ɣ production can possibly be of clinical importance during late stages of HIV infection. High levels of IFN-ɣ have been detected during progression of HIV to AIDS with no reported anti-HIV effect (Abel et al., 2002; Biswas et al., 1992; Francis et al., 1992). From data presented here it seems possible that *P. barbatus* ingestion contributes to a slower disease progression during HIV infection by inhibiting cytokines involved in this pathogenesis.

The *in vitro* anti-HIV-1 data obtained in this study is the first for *P. barbatus*. The ability of *P. barbatus* to inhibit HIV-1 protease confirms the ethnobotanical claims of this plant and supports its continuous traditional use in managing HIV/AIDS and associated opportunistic infections. Synergistic interactions between compounds in crude extracts have been reported to occur (Ma et al., 2009). Hence, the need to first test crude extracts before purification of bioactive compounds. The study shows that *P. barbatus* extract contain inhibitory compounds active against HIV-1 PR.

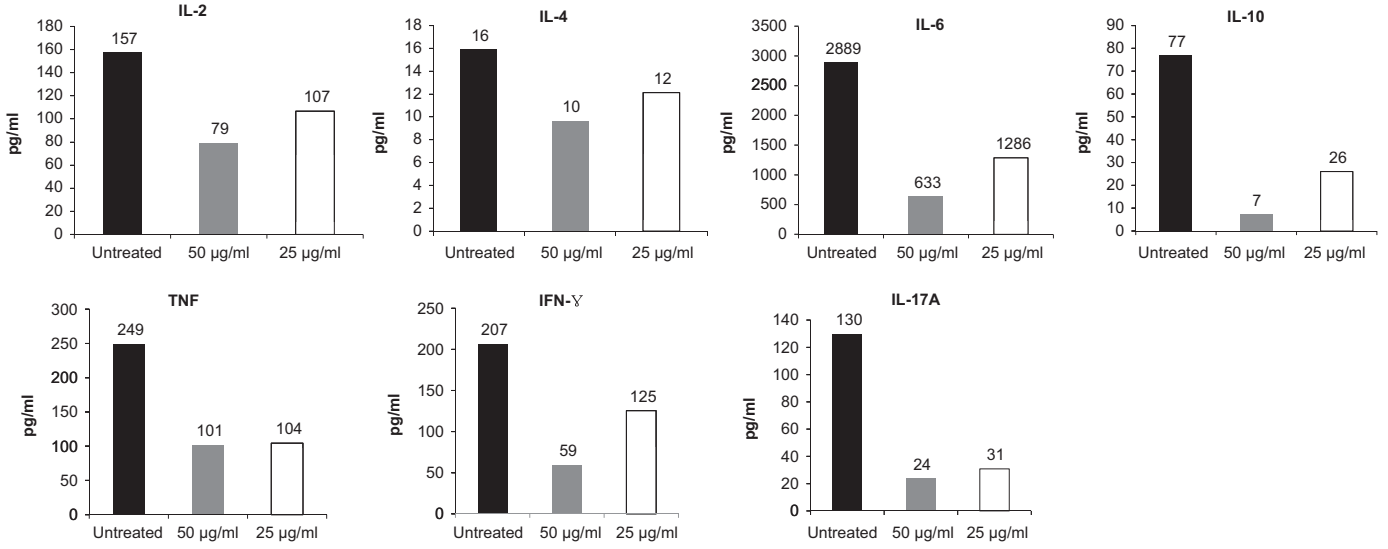

Fig. 5. Effect of *P. barbatus* extract on cytokine production in PBMCs. The values are mean cytokine concentrations of PBMCs from 4 HIV negative individuals. Cells were exposed to medium only (untreated control) and two non-cytotoxic concentrations of the extract, 50 and 25 μg/ml. The extract significantly ($p < 0.05$) reduced the production of IL-2, IL-6, IL-10, TNF, IFN-ɣ and IL-17A.

Since the chemistry of *P. barbatus* has been vastly reported in the past 30 years, it will be of interest for further investigation to be carried out through *in silico* analysis of the compounds isolated from *P. barbatus*. Further work will also involve purification of the active ingredient(s) in order to determine the exact mechanism of action that occurred with HIV-1 PR inhibition which seems to aid in the health of HIV/AIDS patients treated with that plant.

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Appendix A. Supplementary information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jep.2013.06.019.

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