

## Full Length Research Paper

# A study of the biological activity of methanol extracts of some medicinal plants from South Africa

Pascal O. Bessong<sup>1\*</sup>, Luis B. Rojas<sup>2</sup>, Larry C. Obi<sup>1</sup>, Peter M. Tshisikawe<sup>3</sup>, Eunice O. Igunbor<sup>4</sup><sup>1</sup>Department of Microbiology, University of Venda, Thohoyandou, South Africa<sup>2</sup>Laboratoire de Chimie des Substances Végétales, Centre de Recherche en Chimie Moléculaire, Université de Bordeaux 1, and Instituto de Investigaciones, Facultad de Farmacia, Universidad de Los Andes, Venezuela.<sup>3</sup>Department of Biological Sciences, University of Venda, Thohoyandou, South Africa.

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The use of medicinal plants for AIDS-related conditions is common in South Africa. In order to establish an antiviral rationale for the use of these plants we screened fractions of the methanol extracts of medicinal plants for activity against HIV-1 reverse transcriptase (RT) and integrase (IN). The n-butanol fraction obtained from the crude methanol extracts of the roots of *Bridelia micrantha* (Hochst) Baill. (Euphorbiaceae) was observed to be as the most active inhibiting the RNA-dependent-DNA polymerization (RDDP) activity of HIV-1 RT with an IC<sub>50</sub> of 7.3 g/ml. However, it had no activity on the 3'-end processing activity of HIV integrase. Bioassay-guided fractionation of the n-butanol fraction yielded friedelin and -sistosterol, which did not inhibit the RDDP of RT or 3'-end processing functions of IN even at a concentration of 500 M. An uncharacterized fraction obtained in the bioassay-guided fractionating process inhibited the RDDP with an IC<sub>50</sub> of 9.6 g/ml, but had no inhibition on IN.

Phytochemical screening indicated the presence of flavonoids and tannins in the uncharacterized fraction.

**Key words:** HIV-1; reverse transcriptase; integrase; medicinal plants; inhibition; Venda; South Africa.

## INTRODUCTION

Infection with human immunodeficiency virus continues to pose a serious public health challenge worldwide owing to the lack of preventive or curative therapeutics. Several attempts are being made in the search for better anti-HIV agents either through molecular modeling of antagonists to viral enzymes and structural proteins or through the evaluation of plant-derived molecules to identify possible lead anti-HIV compounds. South Africa is one of the countries with the heaviest HIV burden, with an estimated prevalence of 25% as of 2004 when women presenting for their first antenatal clinic visits were considered for baseline analysis. Running parallel to the high HIV and AIDS prevalence is the frequent use of decoctions from medicinal plants as treatment for AIDS and AIDS-related conditions (Morris, 2002; Motsei et al., 2003). Apart from

anecdotal evidence of the benefits of plants extracts on the well-being of HIV/AIDS patients, more data is required on the antiviral properties of medicinal plant preparations. We report here the biological activity of fractions of the crude methanol extracts of six medicinal plants from Venda, Limpopo Province of South Africa for anti-HIV properties.

## MATERIALS AND METHODS

We have previously described the inhibitory properties of the crude aqueous and methanol extracts of a selection of medicinal plants identified in the HIV/AIDS pharmacopoeia of traditional healers in Venda, Limpopo Province, South Africa on HIV-1 reverse transcriptase and integrase functions (Bessong et al., 2005). In the present report, we screen for inhibitory effects of the acetyl acetate, butanol and water fractions obtained from the crude methanol extracts of six medicinal plants on the RNA-dependent-DNA polymerization of HIV-1 RT, and the 3'-processing function of HIV-1 integrase (IN). HIV-1 RT is responsible for the conversion of viral RNA to viral DNA, while integrase catalyses the integration of

\*Corresponding author. E-mail: [bessong@univen.ac.za](mailto:bessong@univen.ac.za). Tel: +27 15 962 8301. Fax: +27 15 962 4749.

proviral integration, two nucleotides are removed from each of the 3'-end by integrase to produce new 'hydroxyl ends' (CA-3'OH) in a reaction called 3'-end processing activity. After entering the nucleus, the 5' ends of processed viral DNA are covalently joined to the host DNA leading to integration. Reverse transcription and integration are essential for infection to be established.

The plants and plant parts investigated in the present report included the roots of *Bridelia micrantha* (Euphorbiaceae), *Combretum molle* (Combretaceae), *Elaeodendron transvaalensis* (Celastraceae), *Mucana coriacea* (Fabaceae), *Vernonia stipulacea* (Asteraceae), and leaves of *Sutherlandia frutescens* (Fabaceae) and *Ricinus communis* (Euphorbiaceae). The procurement and preparation of the crude methanol extracts of these plants have been reported (Bessong et al., 2005). All the reagents used were of analytical grade, unless stated otherwise. The acetyl acetate, butanol and water fractions were obtained as follows: About 20 g of the methanol extract was dissolved in a methanol: water solution (80:20, v/v) followed by the addition of hexane (analytical grade) in a Buchner funnel. The hexane fraction which usually contains fatty materials was separated from the methanol/water soluble fraction. The latter was evaporated at 40°C to dryness under reduced pressure. This residue was resuspended in 50 ml of distilled water. To obtain the acetyl acetate fraction, 30 ml of acetyl acetate (analytical grade) was added to the suspension, vigorously shaken for about 5 min and centrifuged at 5000 rpm for 10 min. This was done two more times, and the acetyl acetate soluble fraction was aspirated each time, pooled, evaporated to a small volume and then lyophilized. To obtain the butanol and water soluble fractions, the water soluble fraction plus undissolved matter derived from the acetyl acetate acquisition process was resuspended in 30 ml of n-butanol, vigorously shaken and centrifuged as previously mentioned. The water soluble fraction was evaporated at 60°C to dryness, while the butanol soluble fraction was evaporated at 40°C to a small volume and then lyophilized.

The inhibition of reverse transcription activity was evaluated by measuring the incorporation of methyl-<sup>3</sup>H thymidine triphosphate (<sup>3</sup>H TTP) by RT using polyadenylic acid-oligodeoxythymidilic acid (polyA-dT) as a template-primer in the presence and absence of the respective fractions. Details of the methodology have been described (Bessong et al., 2004). Assessment of the anti-IN activity was measured by evaluating the ability of the test substances to block the production of a 19 base pair product from a 21 base pair model substrate. The methodology used was as previously reported (Parissi et al., 2000; Bessong et al., 2005). The fractions were tested at a concentration of 1, 10 and 100 g/ml on RT function and 0.1, 1, 10 and 100 g/ml for activity against HIV-1 IN.

#### Bioassay-guided fractionation

Due to the appreciable activity of the n-butanol extract of *B. micrantha* on RT we carried out a bioassay-guided fraction in an attempt to isolate an active component. Five grams of the n-butanol soluble fraction was subjected to vacuum column chromatography making use of silica gel, 60-200 microns (Interchim, France). Fractions 1a-7a were obtained using a solvent comprising petroleum ether : acetyl acetate in the following volume to volume ratios 95:5, 90:10, 80:20, 70:30, 60:40, 50:50, 30:70. Fractions 8a-11a were obtained with 100% acetyl acetate, 100% distilled methanol, methanol : water (60:40), and water, respectively. Three hundred ml were used in each elution. All fractions were evaporated to a small volume and allowed to cool to obtain potential crystallization. Through this way, a white precipitate was obtained from fractions 2a and 3b which crystallized out of a yellow aqueous phase. The precipitate in fraction 2a (0.21 g) was subjected to flash chromatography using petroleum ether : acetyl acetate (21:1) as solvent to obtain 26 fractions (1b-26b). Tubes were left overnight for solvents to escape from which

fractions 6b-14b yielded fine needle-like crystals. The crystals were combined in trichloromethane and evaporated to dryness and labeled as substance X.

Fraction 4a and 5a gave similar profiles on thin layer chromatography (TLC) and were combined (0.33 g) and also subjected to flash column chromatography with 5 g of silica gel to obtain 26 fractions (1c-26c). According to TLC profiles, making use of petroleum ether : acetyl acetate (21:1) as the mobile phase, fractions 9c-15c were pooled and evaporated to dryness yielding a white precipitate of 0.075 g. The latter was further subjected to flash column chromatography using dichloromethane as the solvent with 4 g of silica gel to yield 23 tubes (1d-23d). Tubes 10-23 were combined and evaporated to dryness (0.040 g). This product was labeled as substance Y.

#### Phytochemical screening

A precipitate (0.01 g) was obtained from fraction 8a upon the addition of chloroform. Due to lack of enough quantities for extensive chemical and spectroscopic analysis, qualitative assays for alkaloids, flavonoids, tannins and saponins were performed using standard methods to provide an indication of the possible constituents of this substance. The presence of alkaloids was determined by adding a few drops of freshly prepared Dragendorff's reagent to a suspension of fraction 8a and observed for the presence of a precipitate. To test for flavonoids, a yellow coloration was sought in a revelation test with AlCl<sub>3</sub> after TLC, which employed acetyl acetate : formic acid (20:0.1) as the mobile phase. Quercetin (Sigma) was used as a positive control. To test for tannins two methods were used. Firstly, 10 l of a 0.1% FeCl<sub>3</sub> solution was added to approximately 1mg of the test substance. The development of a blue black coloration was indicative of the presence of tannins. In the second method, a solution of the test substance was added to a suspension of commercial gelatin. The formation of a white precipitate was indicative of the presence of tannins. Commercial tannin (Sigma) was used as a positive control. Checking for saponins involved the vigorous shaking of an approximately 4 ml suspension of the substance for about 15 s and observed for a relatively stable froth formation.

## RESULTS AND DISCUSSION

The n-butanol fraction of *B. micrantha* was the most active with an IC<sub>50</sub> of 7.3 g/ml against the RDDP function of HIV-1 RT. In general, the n-butanol fractions were more inhibitory. The inhibitory profiles of all the fractions tested are presented in Table 1. No inhibition was observed with the water soluble fractions (Data not shown). The electrophoretic band intensity of the 3'-end processing product formed in the absence of test substance was comparable to the bands formed in the presence of each of the extract when the 12% polyacrylamide gel electrophoretic profiles were analyzed by an NIH image apparatus. This means none of the fractions inhibited IN activity at the concentrations tested (Data not shown). <sup>1</sup>H and <sup>14</sup>C nuclear magnetic resonance data, MS, UV and infrared spectroscopic data revealed that substance X and Y were the known phytosterols friedelin and -sistosterol, respectively. Upon evaluation, friedelin and -sistosterol did not inhibit

**Table 1.** Degree of inhibition of the RNA-dependent-DNA polymerization activity of HIV-1 reverse transcriptase by the acetyl acetate and n-butanol fractions of South African medicinal plants.

Plant	RDDP activity inhibition			
	Acetyl acetate fraction		n-butanol fraction	
	% inhibition at 100 g/ml (±sd)	IC50 (g/ml) <sup>a</sup>	% inhibition at 100 g/ml (±sd)	IC50 (g/ml) <sup>b</sup>
<i>B. micrantha</i> (roots)	91±3.2	10.4	98.6±2.1	7.3
<i>E. transvaalensis</i> (roots)	0.0	183.8	48.6±5.3	53.1
<i>M. coriacea</i> (roots)	97.3±5.1	11.5	97.7±3.7	12.6
<i>R. communis</i> (roots)	0.0	196.3	22.2±4.8	160.7
<i>S. frutescens</i> (leaves) <sup>a</sup>	-68±3.6	410.4	-15±1.1	296.3
<i>V. stipulacea</i> (roots)	12.1±2.8	330.2	00.0	310.1

The acetyl acetate and n-butanol fractions were obtained from the respective crude methanol extracts.

<sup>a</sup>Fractions of the leaves of *S. frutescens* were noted to stimulate RT activity. This property was also observed with the crude methanol extract.

<sup>b</sup>IC50 values were obtained from activity/concentration curves considering the mean of three independent determinations.

HIV-1 reverse transcription or the 3'-end processing activity even at a concentration of 500 M.

The uncharacterized fraction (8a) inhibited RT activity with an IC50 of 9.6 g/ml but had no activity on IN. Phytochemical screening indicated that the presence of flavonoids and tannins and negative for alkaloids and saponins. Friedelin and phenolic derivatives such as gallic and ellagic acids have been isolated from the stem-bark of *B. micrantha*, and caffeic acid from the leaves of the same plant (Pegel and Rogers, 1968). In addition, friedelin and  $\beta$ -sistosterol (Boonyaratavej et al., 1991) have been isolated from the roots of the related species *B. ferruginea*, while Onunkwo et al. (1996) indicated the presence of flavonoids. Generally, flavonoids are thought to have anti-HIV RT properties (Amzazi et al., 2003), and are seen to be of potential therapeutic value (Asres et al., 2005).

Many plant species are rich in sterols and sterolines which have been credited to have immuno-modulatory effects and boost the vitality of AIDS patients (Bouic et al., 2001; Bouic, 2002). *B. micrantha*, investigated herein, is generally used among the VhaVendas of South Africa to cure diarrhea and stomach discomfort, common illnesses in AIDS. Whether -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. It is suggested that the acetyl acetate fraction obtained from the n-butanol soluble fraction of the methanol extract of the roots of *Bridelia micrantha* be further investigated for a possible principle inhibitory to HIV-1 reverse transcriptase.

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