



**Ethnobotanic survey,
cytotoxicity, in vitro study
and identification of anti-
HIV active compounds from
plants used in congolese
pharmacopoeia**

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Objectives



Background

General Objective :

- To improve the effectiveness of traditional Plant-based treatments and to develop new drugs against HIV.

Specific objectives

- Identify plants used against Influenza RNA virus disease in Brazzaville_Congo;
- Assess cytotoxicity of selected plants extracts;
- Assess the anti-HIV1 non-toxic plants triterpenoid rich fractions in vitro
- Identify active compounds.



Methods (1)



- **Ethnobotanic survey**

Interview of traditional healers with questionnaires;

- **Selected plants criteria:**

- used against nausea, headache, asthenia, fever, dizziness, chills, cough, cold, most cited, no or less anti-HIV studies

- **Plant materials**

- Botanical identification at the botanical laboratory of centre d'Etudes sur les Ressources Végétales, Brazzaville-Congo.

- **Preparation of extracts or fractions**



Methods (2)



- **Cytotoxicity study**

LD50 of extract in KB and Vero cells lines

- **Cell Lines and Virus**

T-lymphoblastoid tumor cell line CEM-4, virus strain from congolese patients(CCID50= 10^5 /ml)

- **Glycohydrolase enzymes inhibition**

(**Harnett et al., 2005**) Positive control:

Acarbose P-nitrophenyl-alpha-D-glucopyranose(alpha-D-glucosidase) And p-nitrophenilbeta-D-glucuronidase(beta-glucuronidase)

- **HIV reverse transcriptase (RT), integrase**

None radioactive colorimetric ELISA Kit(Roche, Germany), positive control: Adriamycin



Methods (3)



- **Cellular assays for HIV1 inhibition**

Anti-NF-KB activity using NF-KB-dependent luciferase assay (Marquez et al., 2005),
positive control: Mesuol

- **Virus binding assay**

Interaction between recombinant soluble CD and HIV1 envelop glyp. gp120 using ELISA Kit, flow cytometric binding evaluation, Positive control: monoclonal antibody

- **Chemical analysis**

Phytochemical classic test, Coupling HPLC/UV/Mass and TLC

- **Statistical analysis**

ANOVA, Ducann s multiple-range tests. $P < 0,05$



Results (1)



Selected plants for 20 cited by 22 persons

Plant	Citation%
Quassia africana(Qa)	38
Uapaca paludosa(Ua)	25
Cassia siamea(Cs)	20
Milletia Versicolor(Mv)	17

Extraction yield and cytotoxicity of plant LD50($\mu\text{g/ml}$)

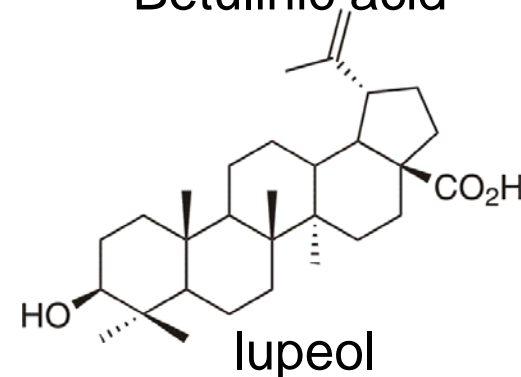
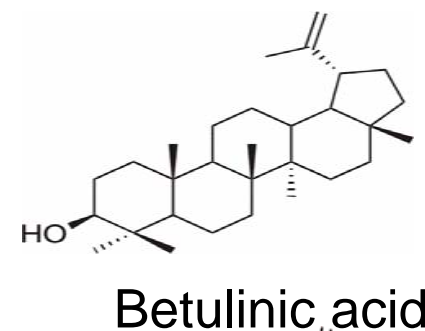
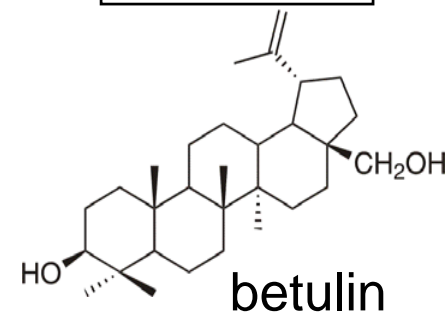
Pant extract	% Yield	Vero	KB
Qa	1,1	1,2	2,5
Up	1,5	70	68
Cs	1	76	80
Mv	1,5	69	75



Results (2)

Anti- HIV1 activity of various compound IC50 (µg/ml)

fractions	RT	Integrase	Alpha-glu	Binding	NF-KB
FQa	1	0,5	10	0,08	0,5
FCs	22	5	1,2	0,4	18
FMv	18	13	12	1,8	15
Con.	0,8		1	0,05	1
	Adriamicin		Acarbose	OKT-4	Mesul





Discussion & Conclusions



- Plant compounds are potentially anti-HIV1 drugs
- Fup, FCs and FMv showed significant anti-HIV binding activity;
($IC_{50}=0,08\mu\text{g/ml}$; $0,4\mu\text{g/ml}$; $1,8\mu\text{g/ml}$)
- Fup exhibited anti-HIV1 (NF-KB and RT $IC_{50}=0,5\mu\text{g/ml}$; $1\mu\text{g/ml}$);
- FCs showed good anti- α -glucosidase activity $IC_{50}=1,2\mu\text{g/ml}$;
- Effect is related to lupeol and betulinic acid and its derivatives.



Future perspectives



- Assess acute and chronic toxicity of these fractions ;
- Doing clinical trials ;
- Domesticated these species and produce anti-HIV1 herbal medicines;
- isolate the active principle and study the pharmacokinetics
- Assess anti-HIV2 activity.