Evaluation of four Cameroonian medicinal plants for anticancer, antigonorrheal and antireverse transcriptase activities

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Abstract

Methanol extracts from the leaves, bark and roots of four Cameroonian medicinal plants, *Bersama engleriana*, *Cupressus lusitanica, Vitellaria paradoxa* and *Guibourtia tessmannii* were tested for their *in vitro* cytotoxicity, antigonorrheal and antireverse transcriptase activities. The XTT (2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxyanilide inner salt) assay, the dilution method and reverse transcriptase (RT) assay were used for the investigations. Preliminary phytochemical analysis of the extracts was also conducted using standrad methods. Results showed that all extracts contained compounds belonging to the classes of phenols and terpenoids. They were also able to reduce in dose dependent manner, the proliferation of the cancer THP-1, DU145, HeLa, MCF-7, HepG2 and the normal Vero cells. IC_{50} values below 30 µg/ml were noted with extract from the three parts of *B. engleriana* on at least two of the five studied cancer cell lines, the lowest value of 5.9 µg/ml being obtained with sample from the bark. IC_{50} values below 30 µg/ml were also recorded with extracts from the leaves (on HeLa cells) and bark (on MCF-7) of *G. tessmanii*, and that from the bark of *C. lusitanica* on MCF-7. Extracts from *B. engleriana* and those from

the bark of *V. paradoxa* gave the minimal inhibitory concentrations (MIC) values below 100 µg/ml on most of the ten tested *Nesseria gonorrhoeae* strains. Extracts from *B. engleriana* also inhibited more than 80% the activity of the Human Immuno-deficiency Virus (HIV) enzyme. Finally, the results of the present study provide baseline information for the use of *B. engleriana,C. lusitanica, G. tessmanii, V. paradoxa*.

Keywords: Anticancer; anti-gonorrheal; anti-reverse transcriptase, medicinal plants.

1. Introduction

Cancers diseases are characterized by an abnormal proliferation of cells. They constitute the second cause of mortality behind cardiovascular diseases in developed countries and the third after infectious and cardiovascular diseases in developing countries (Bieche, 2004). The use of plant extracts and derived products in the treatment of cancers is of exceptional value in the control of malignancies, due to the fact that most of the anticancer drugs severely affect the normal cells. It has been recommended that ethnopharmacological usages, such as immune and skin disorders, inflammatory, infectious, parasitic and viral diseases be taken into account when selecting plants used to treat cancer, since these reflect disease states bearing relevance to cancer or a cancer symptom (Cordell et al., 1991; Popoca et al., 1998). On the other hand, *Neisseria gonorrhoeae* infection is a common bacterial sexually transmitted disease that can cause cervicitis, urethritis, proctitis and pelvic inflammatory disease, with long-term sequelae, adverse outcomes of pregnancy, and increased susceptibility to transmission of HIV infection (Rottingen et al., 2001). In Cameroon, there is a rich tradition in the use of herbal medicinal plants for the treatment of various infectious diseases, cancer, inflammation, injuries and other diseases (Adjanohoun et al., 1996). This study was therefore designed to assess the anticancer, antigonorrheal and antireverse transcriptase activities of four Cameroonian plants used locally. *Bersama*

engleriana Engl. (Melianthaceae) is used traditionally to treat cancers, spasms, infectious diseases, HIV infection, male infertility, diabetes and as sexual stimulant (Kupchan et al., 1971; Bowen et al., 1985; Makonnen et Hagos, 1993; Iwu, 1993; Njike et al., 2005; Watcho et al., 2007). *Cupressus lisitanica* Mill. (Cupressaceae) is used in the treatment of rheumatism, cough, skin infections and cancers (Perez et Villavicencio, 1994 ; Duke, 2004); *Guibourtia tessmannii* Harms Leonard (Leguminosae) is used to treat hypertension, worms and infectious diseases (Adjanohoun, 1984); *Vitellaria paradoxa* C. F. Gaertn (Sapotaceae) is used in case of cancer, gastro-intestinal infections, diarrhea, worms, skin disease and leprosy (Soladoye et al., 1989; Ferry et al., 1974).

2. Material and Methods

2.1. Plant material and extraction

Bersama engleriana was collected in January 2007 at Bafou (West Region, Cameroon); *Cupressus lusitanica* and *Vitellaria paradoxa* were collected respectively in February and May 2007 in Yaounde (Centre Region, Cameroon); *Guibourtia tessmannii* was collected in May 2007 at Eseka (Centre Region). They were identified at the Cameroon National Herbarium where the voucher specimens were conserved under the reference numbers 24725/HNC, 66102/HNC, 5670/HNC, 2957/HNC respectively for *B. engleriana, C. lusitanica, G. tessmannii* and *V. paradoxa*.

The air-dried and powdered plant material including leaves, bark and roots for each sample (1 kg) was soaked in methanol (4 L) for 48 h, at room temperature. The methanol extract was concentrated under reduced pressure to give the crude extract (Table 1). This extract was then conserved at 4˚C till further use.

2.2. Preliminary phytochemical investigations

The major secondary metabolite classes such as alkaloids, anthraquinones, coumarins, triterpenes, saponins, phenols and flavonoids were screened for each extract, according to the common phytochemical methods described by Harborne (1973).

2.3. Cell lines and treatments

The studied cell lines included five cancer cells, DU145 prostate cells, HeLa cervix adenocarcinoma cells, HepG2 hepatocarcinoma cells, MCF-7 breast cancer cells, THP-1 leukemia cells and the normal Monkey kidney cells (Vero). They were obtained from the American Type Culture Collection (Rockville, USA). THP-1 cells were maintained in RPMI 1640 containing 100 units/ml penicillin and 100 µg/ml streptomycin and supplemented with heat-inactivated 10% fetal bovine serum (FBS). Others cells were maintained in Dulbecco's minimum essential medium (DMEM) supplemented with 5% fetal calf serum (FCS), gentamicin sulfate (0.004%), glucose (0.57%), and NaHCO3 (0.12%). All Cultured cells were maintained in a humidified environment at 37˚C with 5% CO_2. Doxorubicin (Sigma-Aldrich, Steinheim, Germany) was used as a positive (cytotoxic) control. The concentration of dimethylsulfoxide (DMSO) was kept at or below 0.1% in all experiments.

2.4. Cytotoxicity assays

The cytotoxicity of the plant extracts was evaluated using XTT (2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxyanilide inner salt) assay (Gerlier and Thomasset, 1986; Zee-Cheng,1997; Itharat et al., 2004). Briefly, adherent cells in appropriate culture medium were seeded into 96-well flat-bottomed plates at a concentration of 3.0×10^5 cells/ml. After 24 h, cells were treated with serially diluted sample. The highest concentrations were 400 µg/ml against cancer cells and 3200 µg/ml against Vero cells. After 72 h incubation at 37 ˚C with 5% CO_2 and 95% relative humidity, XTT labeling reagent (50:1) was added and the absorbance (560 nm) read (Gerlier and Thomasset, 1986). For non

adherent cells (THP-1), aliquot of $5x10^4$ cells/ml (obtained from overnight suspension) were seeded in 96-well plates, and extracts were added immediately. After 24 h incubation, plates were treated with XTT solution as above mentioned. Experiments were carried out three times, in triplicate. The concentration of the sample that inhibited 50% cell proliferation (IC50 on cancer cells or EC_{50} on Vero cells) was determined graphically. Doxorubicin, a known cytotoxic agent, was used as positive control. The cell survival percentage was determined using the formula: % cells survival = (ODT/ODC) × 100; ODT and ODC being the absorbance of the test sample-treated group and the control group (0.1% DMSO), respectively. The selectivity index was determined as the ratio EC_{50}/IC_{50}.

2.5. Microbial strains and antigonorrheal assay

N. gonorrhoeae strains used included ATCC 49226, β-lactamase negative WHO (A) and (B), clinical β-lactamase negative (NGCS1-4) and β-lactamase positive (NGCS5-7) strains. They were subcultured on GCB medium (D+ glucose, l-glutamine, carboxylase, Bacto agar, NaOH, pH 7.2), diluted to a concentration of 10^8 CFU/ml in GCB, and incubated at 37 °C in 5%CO_2-enriched atmosphere for 24–48 h. The MICs of all samples were determined by the agar dilution method (Pottumarthy et al., 2006). Gentamicin was used as positive drug control. DMSO was used to dilute all studied samples. Appropriate dilutions of DMSO used to dilute samples (1% for antigonorrheal) served as solvent control. All experiments were carried out in triplicate.

2.6. Anti-HIV investigations: reverse transcriptase (RT) assay

The effects of plant extracts on RT activity *in vitro* were evaluated with recombinant HIV-enzyme, using a non-radioactive HIV-RT colorimetric ELISA kit from Roche, Germany (Ayisi and Nyadedzor, 2003; Harnett et al., 2005). The protocol outlined in the kit was followed using 2 ng of enzyme in a well and incubating the reaction for 2 h at 37 °C. In order to avoid the tannins interference, bovine serum albumin (Fraction V) was added to assay

buffers to a final assay concentration of 0.2%(w/v) to adsorb possible tannins from crude extract (Harnett et al., 2005). Extracts were first tested at 0.2 mg/ml and samples which reduced activity by at least 50% were considered active (Woradulayapinij et al., 2005). Two-fold dilutions (3.13–100 µg/ml) were then made in order to determine IC_{50} values. IC_{50} was the amount of extract required to reduce the reverse transcriptase activity by 50%. The IC_{50} values were determined from the activity/concentration regression curves with at least seven concentration/activity points (Bessong et al., 2005) using Microsoft Excel. Doxorubicin was used as a positive control. The assay was carried out in triplicate.

2.7. Statistics

The one-way ANOVA at 95% confidence level was used for statistical analysis.

3. Results and discussion

The results of the phytochemical studies are summarized in Table 1. It's can be found that all studied extracts contained phenols and terpenoids while alkaloids and coumarins were not detected. Most of the metabolite classes were also detected in the three parts of each plant but flavonoids and tannins were not detected in all parts of *C. lusitanica*. However, compounds from detected metabolites classes were isolated from some of the investigated plants. Sebotin and 2′,4-dihydroxy-4′-methoxy-6′-*O*-β-glucopyranoside, two dihydrochalcone glucoside, and also 3,5-dimethoxy-4′-*O*-(β-rhamnopyranosyl-(1→6)-β- glucopyranoside) stilbene were isolated from the stem bark of *G. tessmanni* (Nkengfack et al., 2001; Fuendjiep et al., 2002). The isolation of triterpenes and saponins from the stem bark of *Bersama engleriana* was previously reported by Tapondjou et al. (2006).

The results of cytotoxicity assay are reported in Table 2. All extracts (except that from the roots of *V. paradoxa*) were able to inhibit (at 400 µg/ml) the proliferation of the cancer THP-1, DU145, HeLa, MCF-7, HepG2 and the normal Vero cells. The American National

Cancer Institute guidelines (NCI) set the limit of activity for crude extracts at 50% inhibition (IC_{50}) of proliferation of less than 30 µg/ml after an exposure time of 72 h (Suffness and Pezzuto, 1990). IC_{50} values below this stringent point were noted with extracts from the three parts of *B. engleriana* on at least two of the five studied cancer cell lines, the lowest (5.9 µg/ml) being obtained with sample from the bark (Table 2). IC_{50} values below 30 µg/ml were also recorded with extracts from the leaves (on HeLa cells) and bark (on MCF-7) of *G. tessmanii*, and that from the bark of *C. lusitanica* on MCF-7. Nevertheless, none of the tested extract was as active as doxorubicin. All extracts showed EC_{50} values above 100 µg/ml and consequently the selectivity indexes of actives samples were >2 (Table 2). These data are of interesting, as it suggests that the extracts are more toxic for cancer cells than on normal cells. When regarding the overall activity of the extracts, it's appears that *B. engleriana* can be considered as a potential anticancer drug. This is in accordance of the preliminary antitumor studies, which previously demonstrated that extracts from different part of *B. engleriana* were able to prevent the induction of crown gall tumors by *Agrobacterium tumefaciens* (Kuete et al., 2008). Though the extracts from *C. lusitanica* were not active on most of the cell lines, the overall activities were moderated. Nevertheless, an interessting IC_{50} value of 13.1 µg/ml was obtained with the bark extract on MCF-7 cells. However, extract from the leaves of this plant induced apoptotic cell death on HeLa, CasKi and HepG2, though the IC_{50} values were noted reported (Lopéz et al., 2002).

The results of the antigonorrheal activity of the extracts are summarized in Table 3. MIC values below 512 µg/ml were detected with the extracts from the bark and roots of *B. engleriana,* and those from the leaves and bark of *V. paradoxa* on all the ten studied *N. gonorrhoeae* strains. All other samples showed selective activities. The stringent point for antimicrobial activities has been set as follow: significant (MIC < 100 µg/ml), moderate (100 < CMI ≤ 625 µg/ml) or weak (CMI > 625 µg/ml) (Kuete, 2010; Kuete and Efferth, 2010). As

consequences, extracts from *B. engleriana* and those from the bark of *V. paradoxa*, can be considered as good sources of antigonorrheal medicine, taking in account the fact that most of the MICs obtained with such samples were below 100 µg/ml. The MIC values obtained with BEL and BER were in some of cases, closer or lower than those of gentamicin on corresponding strains, highlighting the good antigonorrheal potency of *B. engleriana.* Previously, MIC values below 100 µg/ml were reported on fungi of the genus *Candida* and several bacterial species including Gram positive and negative bacteria as well as *Mycobacterium tuberculosis* (Kuete et al., 2008), consolidating therefore the assertion that this plant have a good antimicrobial potential. Few antimicrobial studies were carried out on the plants reported herein. However, essential oil from the leaves of *C. lusitanica* showed antibacterial activity against *Bacillus cereus* and antifungal activity against *Aspergillus niger* (Hassanzadeh et al., 2010). Ethanol, acetone and aqueous extracts of *C. lusitanica* were also reported for their antimicrobial activities against *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Shigella dysenteriae* and *Salmonella typhi* (El-Mahmood et al., 2008). Nevertheless, the work by El-Mahmood et al. (2008) was rather qualitative and growth inhibition was noted at 6.5 mg/ml with the ethanol extract on *K. pneumonia*e and *P. mirabilis*. However, the lowest MIC value (128 µg/ml) recorded herein against the studied *N. Gonorrhoeae* strains indicates that this plant have a moderate antimicrobial activity.

A plant extract is considered active on the reverse transcriptase, if the concentration inhibiting 50% enzyme activity (IC_{50}) is below 200 µg/ml (Woradulayapinij et al. 2005). In this work, extracts from *B. engleriana* exerted such ant-RT activity (Table 4). Samples from this plant inhibited more than 80% the activity of the HIV enzyme, with IC_{50} values of 9.38, 11.95 and 18.75 µg/ml respectively for the extracts of the roots, leaves and bark. However, better IC_{50} value was obtained with the reference compound, doxorubicin. These values clearly show that this plant has a strong anti-RT potential, and this preliminary results should

be further confirmed by the determination of the extract effects on viral replication cell culture tests and their further fractionation. Other samples were found to have poor anti-RT properties. However, they are not used in the traditional medicine for AIDS treatment, therefore consolidating the obtained results.

Finally, the results of the present study provide supportive baseline information for the medicinal use of *B. engleriana,C. lusitanica, G. tessmanii, V. paradoxa*. The overall data indicated that *B. engleriana* could be good source of anticancer, anti-HIV and antigonorrheal medicine.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Tables

Table 1. Plants, extraction yields and phytochemical composition

Samples	Part	index	Extraction yield[a] (%)	Physical aspect of the extract	Chemical group detected[b]
Bersama engleriana	leaves	BEL	10.8	Dark paste	Phenols, flavonoids, tannins, anthraquinones, saponines, terpenoids (Kuete et al., 2008)
	bark	BEB	13.2	Brown powder	Phenols, flavonoids, tannins, anthraquinones, saponines, terpenoids (Kuete et al., 2008)
	roots	BER	9.4	Brown powder	Phenols, flavonoids, tannins, anthraquinones, saponines, terpenoids (Kuete et al., 2008)
Cupressus lusitanica	leaves	CLL	6.2	Dark-green paste	Phenols, flavonoids, tannins, saponines, terpenoids
	bark	CLB	5.8	Brown powder	Phenols, tannins, saponines, terpenoids
	roots	CLR	3.4	brown powder	Phenols, saponines, terpenoids
Guibourtia tessmannii	leaves	GTL	6.7	brown powder	Phenols, flavonoids, tannins, anthraquinones, terpenoids
	bark	GTB	10.5	brown powder	Phenols, flavonoids, tannins, anthraquinones, terpenoids
	roots	GTR	4.3	brown powder	Phenols, flavonoids, tannins, anthraquinones, terpenoids
Vitellaria paradoxa	leaves	VPL	7.9	Green paste	Phenols, flavonoids, anthraquinones, terpenoids
	bark	VPB	13.0	Red powder	Phenols, flavonoids, tannins, anthraquinones, terpenoids
	roots	VPR	4.2	brown powder	Phenols, flavonoids, anthraquinones, terpenoids

[a]The yield was calculated as the ratio of: mass of the extract obtained/mass of the powder extracted
[b]Coumarins and alkaloids were not detected in all extracts

Table 2. Cytotoxicity parameters[a] of the tested extracts and doxorubicin

Plants	Extract (EC_{50})	Cell lines IC_{50} ± SD values[b] and Selectivity index (in parenthesis)				
		THP-1	**DU145**	**HeLa**	**MCF-7**	**HepG2**
Bersama engleriana	BEL (745.0±22.5)	100.0±8.2 (7.45)	**15.7**±1.6 (47.6)	50.8±5.1 (14.7)	**8.6**±6.4 (86.9)	**20.3**±3.1 (36.7)
	BEB (748.2±16.4)	271.6±15.3 (2.8)	83.4±6.8 (9.0)	43.8±3.9 (17.1)	**18.7**±1.2 (40.0)	**21.9**±1.4 (34.2)
	BER (1300.0±24.6)	199.9 ± 11.6 (6.5)	34.4±2.7 (37.81)	**10.9**±0.7 (118.7)	**5.9**±0.6 (221.1)	**19.5**±0.9 (66.5)
Cupressus lusitanica	CLL (1008.6±38.2)	87.6±6.1 (11.5)	74.6±6.1 (13.5)	>400	91.3±0.8 (11.0)	150.6±13.4 (6.7)
	CLB (1580.0±26.2)	81.3±7.2 (19.5)	98.8±6.9 (16.0)	83.1±6.7 (19.0)	**13.1**±0.9 (121.0)	93.8±7.4 (16.9)
	CLR (>3200)	60.8±5.8 (>56.2)	>400	>400	>400	>400
Guibourtia tessmannii	GTL (>3200)	72.4 (>43.1)	44.3±4.1 (>72.2)	**8.8**±0.5 (>363.6)	>400	>400
	GTB (1580±18.8)	99.1±8.4 (16.0)	49.6±2.3 (31.9)	51.6±5.2 (30.6)	**13.3**±1.4 (118.89)	>400
	GTR (>3200)	74.3±7.1 (>43.1)	>400	>400	>400	>400
Vitellaria paradoxa	VPL (2391.2±42.2)	>400	331.6±29.7 (7.2)	133.7±11.8 (7.9)	>400	>400
	VPB (350.0±16.8)	262.1±17.4 (1.34)	162.5±12.8 (2.2)	68.8±5.7 (5.1)	106.0±7.1 (3.3)	>400
	VPR (183.3±11.1)	>400	>400	>400	>400	>400
Doxorubicin (46,4±2.3)		4.8±0.3 (9.6)	<3.1 (>14.8)	<3.1 (>14.8)	<3.1 (>14.8)	5.5±1.1 (8.5)

[a]IC_{50} and EC_{50} values are in µg/ml; Data reported are result of three assays done in triplicate [b](-): Not determined as the inhibition percentage was <50% at 400 µg/ml; Underlined are significant IC_{50} values according to NCI criterion for plant extracts.

Table 3. Anti-gonorrheal activity of the tested extracts and gentamicin

Neisseria gonorrhoeae strains	Studied samples and their MIC values												
	Bersama engleriana			***Cupressus lusitanica***			***Guibourtia tessmannii***			***Vitellaria paradoxa***			**GM**
	BEL	BEB	BER	CLL	CLB	CLR	GTL	GTB	GTR	VPL	VPB	VPR	
ATCC 49226	32	16	32	256	256	512	512	256	-	128	64	256	0.5
WHO A (βL-)	64	16	64	-	512	-	512	512	-	256	32	256	8
WHO B (βL-)	64	64	64	-	-	-	-	-	-	256	64	-	2
NGCS1 (βL-)	128	16	32	512	-	-	256	-	-	512	128	-	1
NGCS2 (βL-)	32	32	32	512	512	-	512	512	512	64	128	256	32
NGCS3 (βL-)	32	16	64	128	-	512	128	512	-	128	64	512	4
NGCS4 (βL-)	32	32	32	-	-	-	-	-	-	64	128	256	16
NGCS5 (βL+)	32	16	32	256	256	-	-	-	-	256	128	128	16
NGCS6 (βL+)	>512	128	256	-	-	-	512	-	512	256	64	256	32
NGCS7 (βL+)	64	16	64	128	-	-	-	-	-	128	64	512	32

GM: gentamicin; clinical strain *NGCS* : *N.gonorrhoeae* clinican strain; (βL+) : β-lactamase positive; (βL-) : β-lactamase negative; (-): >512 µg/ml

Table 4. Reverse transcriptase activity of the tested extracts and doxorubicin

Tested plant	Extracts	Parameters	
		Inhibition percentage (%)±SDat 200 µg/ml	IC_{50} ± SD(µg/ml)
	BEL	89.17±4.55[a]	11.95±2.3[b]
Bersama engleriana	BEB	85.11±4.99[a]	18.75±1.4[c]
	BER	92.51±3.59[a]	9.38±1.0[b]
Cupressus lusitanica	CLL	16.75±2.56[d]	nd
	CLB	6.07±0.36[f]	nd
	CLR	8.16±0.91[e]	nd
Guibourtia tessmannii	GTL	28.96±3.37[b]	nd
	GTB	33.21±2.18[b]	nd
	GTR	19.72±1.3[c]	nd
Vitellaria paradoxa	VPL	33.18±2.48	nd
	VPB	28.06± 2.77[b]	nd
	VPR	12.98±1.08[d]	nd
Doxorubicin at 100 µg/ml		91.22 ±3.99[a]	4.24±0.6[a]

(nd): not determined; values with the same letter in each column are not significantly different (ANOVA, $P<0.05$). Data are results ±SD of three experiments