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UPLC-MS Quantification and Anticancer Potential of Ximenia Americana Hydro-Acetonic Crude Extract Leaves

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ABSTRACT

Ximenia americana is a plant of the Ivorian pharmacopeia used in the treatment of various diseases. The present study aims firstly, to quantify by UPLC-MS some phenolic compounds contained in Ximenia americana and secondly, to evaluate its cytotoxic potential against 6 cancerous cells lines. UPLC-MS quantitative analysis revealed the presence of flavonoids (13%), gallotannins (5%), phenolic acids (0.7%), ellagic acid (0.3%) and an abundance of condensed tannins (81%). The cytotoxicity study indicated an antiproliferative behavior notable of this plant.

Keywords: Ximenia americana, Phenolic compound, UPLC-MS, Anticancer potential

INTRODUCTION

Ximenia americana Linn. is a thorny shrub of Olacaceae family. Its sheets are narrowly elliptic, measuring 3 cm to 7 cm long on 3 cm broad. Its ellipsoidal fruits, of yellow color sulphur with maturity, measuring up to 3 cm long. A species of the regions of savanna, Ximenia americana is widespread of Senegal in Cameroun [1]. Among others pathologies, it is used to treat conjunctivitis, malaria, jaundice, diarrhoea, fever [2], sexual impotence and leprosy [3]. Several work undertaken on this vegetable species show that it possesses antioxidant [4], antiseptic, astringent [3], analgesic [5], antimicrobial, antifungal [6], antiviral [7], antipyretic [8] and anticancer [9] properties. Its chemical composition highlights the presence of several secondary metabolites (anthracenes, sterols and polyterpenes, steroids, saponins, reducing sugars, coumarins) with prevalence in flavonoids and tannins [10]. The objective of this study was to quantify by UPLC-MS the phenolic compounds and to evaluate the biological effect of the leaves of Ximenia americana against various cancerous cells lines.

MATERIALS AND METHODS

Hydro-acetonic crude extract preparation (HACE)

Ximenia americana leaves were collected in Toumodi (Côte d'Ivoire) in June 2010, then identified in the herbarium of the National Center of Floristic (CNF) of Felix Houphouët-Boigny university (Abidjan-Cocody) by the emeritus professor AKÉ-ASSI Laurent. They were cleaned, dried in a room air conditioned during 7 days, then pulverized with an electric grinder (RETSCH, standard SM 100). The hydroacetonic extract has been obtained after maceration under magnetic agitation (24 h) of vegetable powder (5g) previously treated by petroleum ether in CH_3COCH_3 (100 mL, 70%). The macerated was filtered then lyophilized to provide a lyophilisate for the phenolic compounds quantification and the cytotoxicity test.

Phenolic quantification

The contents of the phenolic compounds were evaluated according to the method described by N'Gaman et al. [11]. The lyophilisate (1 mg) was dissolved in H_2O-CH_3OH mixture (1 mL, 50:50 v/v) by a vortex. The mixture was filtered with a disposable filter (porosity 0.2 µm), then diluted to 1/10 for injection. LC-MS analysis was performed on a Waters Acquity UPLC chain coupled to a mass spectrometer (Brucker Amazon X) by UPLC-ESI-IT-MS^n. A C18 column Waters Acquity BEH 150 × 1 mm reverse phase particles (1.7 µm diameter) was used at 35°C. The mobile phase: solvent A (H_2O+1% of HCO_2H) and B (CH_3OH + 1% of HCO_2H). Gradient used: 2% to 30% of B (0-10 min), 30% of B (10-12 min), 30% to 75% of B (12-25 min), 75% of B (25-30 min), 90% of B (30-35 min), 90-2% of B (35-38 min) and 2% of B (38-43 min). Rate of flow: 0.08 ml/min; volume of injection: 0.5 µL. UV-visible spectra were recorded from 250 to 600 nm with a pitch of 2 nm. The contents of phenolic phytochemicals were calculated from the surfaces integration of the molecular peaks at wavelength of maximum absorbance of each compound in the UV; and an external calibration with the corresponding standard or a neighbouring molecule. The standards and the wavelengths used for the quantification was Gallic acid at 280 nm for the dosage of the gallotannins, ellagic acid at 360 nm for the dosage of the ellagitannins, caffeic acid at 320 nm for the dosage of hydroxycinnamic acids, quercetin 3-O-glucoside at 360 nm for the dosage of flavones and flavonols, protocatechic acid at 280 nm for the dosage of phenolic acids (except gallic acid) and epicatechin at 280 nm for the dosage of flavanones and condensed tannins. The concentrations have been expressed in equivalent of the reference molecule for the molecular family.

Cell cultures

The cancerous cells coming from American Type Collection Culture (ATCC) were cultivated in a medium "Eagle" modified by Dulbecco (DMEM) (Sigma-Aldrich) supplemented with fetal calf serum (FCS) (10%), 2 mM of L-glutamine and 1% (v/v) of Penicillin Streptomycin at 37°C in a humidified atmosphere with CO_2 (5%). Breast cancer stem (MDA-MB 231), melanoma cancer stem (MDA-MB 435 and B16F10), colon cancer stem (Caco-2) and brain cancer stem (glioma cells SNB75 and C6) were used for HACE cytotoxicity activity evaluation.

HACE cytotoxicity test

Cell survival was assessed by colorimetric MTT (3 bromide (4,5-dimethylthiazol-2-yl)-2,5-diphenyl) test (Sigma Aldrich) [12]. Cells were cultured in 100 µL environment and incubated for 24 h. After obtaining an adherent cell layer, the environment was decanted and replaced with the extract at concentrations ranging from 0.125 to 2 mg/mL. After 72 h, the wells were emptied. The cells were washed with PBS and incubated with 100 µl of culture containing MTT (2 mg/mL) for 4 h at 37°C and CO2 (5%). Color intensity produced indicates the relative number of living cells, determined at 570 nm using a micro plate reader (Multiscan Labosystems). The viability percentage (VP) was calculated with the equation 1.

$$VP=(Abs\ sample/Abs\ control) \times 100 \quad (1)$$

The statistical analysis of all the data was made with the software Microsoft Office Excel 2013.

RESULTS AND DISCUSSION

Phenolic phytochemicals quantification

The chromatograms obtained at various wavelengths show thirty phenolic phytochemicals among which phenolic acids, gallotannins, condensed tannins and flavonols (**Figure 1**). The chromatographic characteristics of the main quantified compounds have been presented in the **Table 1**.

HACE phenolic phytochemicals quantification gave by ascending order: ellagic acid (0.3%), phenolic acids (gallic, protocatechic and other) (0.7%), gallotannins (5%), flavonols (13%) and condensed tannins (81%). Thus, a significant content of flavonoids (24.845 mg/g of powder) and condensed tannins (152.185 mg/g of powder) was observed. This presence provides a good antioxidant power to X. americana. These results are comparable as a whole with those resulting from work already published [10,13,14]. However, the absence of anthocyanes was observed (**Figure 1**, UV 520 nm), which contradicts some results that we reported before [4]. Indeed, in hot acidic medium, the condensed tannins are depolymerized to engender anthocyanidols [15].

HACE cytotoxic character

The **Figure 2** shows the viability percentage (VP) of different cancerous cells lines treated by HACE during 72 h, with different concentrations ranging from 0.125 to 2 mg/mL.

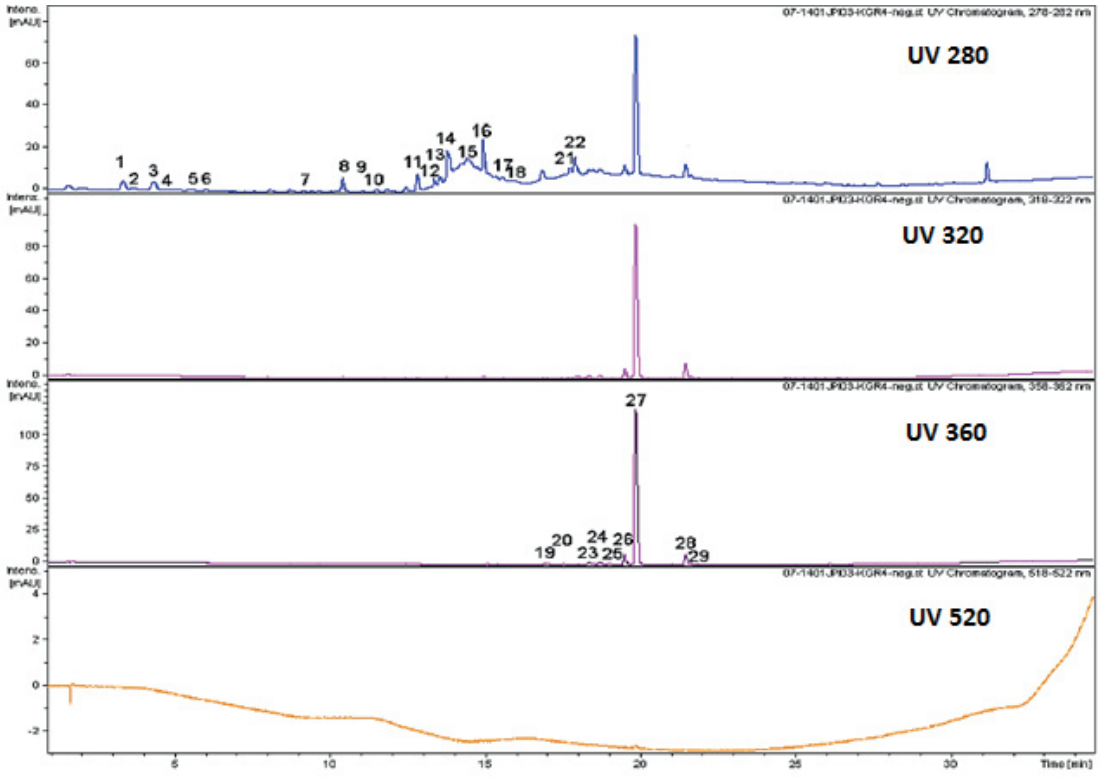

Figure 1: LC-MS chromatograms at various wavelengths of HACE

Table 1: UPLC-MS/MS quantification of HACE phytophenols

Peaks	RT (min)	Areas	Identified compound	Molar mass	Content (mg/g of powder)
1	3.3	38.55	Gallic acid	170	1.04
2	3.6	10.45	Gallotannin	322	0.54
3	4.3	45.54	Gallotannin	344	2.49
4	4.8	5.43	Gallotannin	332	0.29
5	6	3.97	Protocatechic acid	155	0.21
6	6.3	0.86	Gallotannin	322	0.04
7	9.1	3.06	Phenol acid	314	0.15
8	10.4	37.36	Gallotannin	484	2.87
9	11.1	3.05	Condensed tannin	578	76.09
10	11.5	7.29	Gallotannin	484	0.56
11	12.8	37.72	Condensed tannin	730	7.57
12	13.3	21.23	Gallotannin	454	1.53
13	13.5	15.83	Condensed tannin	1169	5.09
14	13.7	106.87	Condensed tannin	881	25.89
15	14.4	40.26	Condensed tannin	1017	11.26
16	14.9	71.24	Condensed tannin	441	8.64
17	15.5	7.21	Condensed tannin	1168	2.32
18	15.9	8.05	Condensed tannin	1322	2.93
19	16.9	8.39	Flavonol	616	0.37
20	17.5	3.87	Flavonol	592	0.17
21	17.7	17.65	Gallotannin	510	1.43
22	17.9	51.11	Condensed tannin	882	12.40
23	18.3	19.41	Ellagic acid	302	0.65
24	18.7	13.37	Flavonol	434	0.42
25	18.9	5.44	Flavonol	434	0.17
26	19.5	48.55	Flavonol	434	1.52
27	19.8	638.84	Flavonol	448	20.61
28	21.4	41.10	Flavonol	432	1.28
29	21.6	7.34	Flavonol	600	0.32

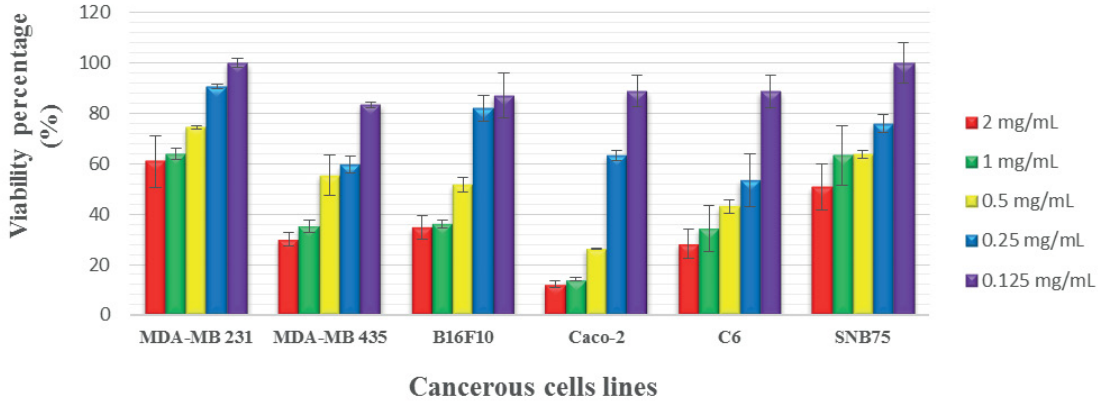

Figure 2: Viability percentage of cancerous cells lines

A considerable toxicity of HACE was noticed against the majority of the cancerous cells lines to high concentrations. In addition, a good activity was remarked against Caco-2, with VP going of 12% (2 mg/mL) to 88% (0.125 mg/mL). It seems correlative to the existence of phenolic phytochemicals in HACE. Indeed, some works showed that certain polyphenols would inhibit the cellular proliferation [16] and could consequently contribute to the prevention of various cancers [17]. It is the case of the flavonoids which act in all the stages of the carcinogenesis (initiation, promotion and progression) [18].

CONCLUSION

UPLC-MS quantitative analysis realized on the hydro-acetonic extract resulting from Ximenia americana leaves highlighted several families of phenolic secondary metabolites. Among these, we note an abundance of condensed tannins (81%, corresponding to 152.185 mg/g of powder). The cytotoxicity test also revealed a considerable anticancer potential against the majority of the cancerous cells lines, in particular against Caco-2. The presence with profusion of the phytophenols in HACE, would explain the anticancer activity of Ximenia americana. Thus an answer is brought which probably seems a rational explanation of the use of the plants in the cellular proliferation traditional therapy.

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