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Chromatographic Analysis and Cytotoxic Effect of Extracts of *Abrus precatorius* a Medicinal Plant with Strong Anticancer Potential

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ABSTRACT

This paper aims to evaluate the cytotoxic effect of ethanolic extract of Abrus precatorius leaves on the murinmastocytoma cancer cell line (P815), and chromatographic study of aqueous extract as well. The Aqueous extract was obtained by decoction and ethanolic was obtained by maceration. The chromatographic study was carried out on a thin layer and on a chromatographic column. The cytotoxicity was sought by determining the percentage of lysis of cancer cells and the inhibitory concentration 50% (IC50) by the MTT assay. Chromatographic analysis in thin layer with increasing of solvent polarity (Hexane / acetate of ethyl) showed the different spots depending on solvent polarity. The column chromatography allowed separating two fractions. A dose dependent cytotoxic effect of the extract was observed on the cancer cell line (P815). It was observed that the ethanolic extract has the best cytotoxic effect with an IC50 of 43.94 µg/mL different from aqueous extract which has a weak cytotoxic effect with an IC50 of 200 µg/mL against 2.5 µg/mL for methotrexate. Ours results suggest that A. precatorius leaves contains several chemical groups and possess a potential anticancer effect in vitro against P815 tumor cell line.

Keywords: *Abrus precatorius*, Chromatographic study, In vitro anticancer activity, P815 cancer cell line

INTRODUCTION

Cancer is one of major cause of death in developed and developing countries [1]. Its treatment protocols depend on chemotherapy, radiotherapy and surgical intervention [2]. West Africa composed of mostly poor countries where cancer is an emergent disease. In 2008, men in this region of Africa had a doubly high rate of liver cancer while women had the highest cervical cancer cases worldwide [3]. The evolution of cancer in this part of Africa is very worrying, which is why WHO encourages countries in these regions to promote and integrate traditional medical practices into their health systems [4]. In the search for means of combating these diseases, man has used the medicinal properties of many cultivated and wild plants to combat these disturbing ailments. Among the many plant species exploited, *Abrus precatorius* is probably the best known of the 17 species of the genus Abrus. This climbing plant from the Fabaceae family is known mainly for its medicinal properties to cure various diseases [5]. The roots, leaves and seeds of this plant are used for different medicinal purpose [6]. En Inde, cesfeuillessontutilisées pour guérir la fièvre, les troubles d'estomac, l'asthmeet la bronchite [7]. In Nigeria leaf decocted is used in the treatment of diabetes [8]. In Côte d'Ivoire, the leaves of *Abrus precatorius* are used in the south of the country, among the Abbey and Krobou peoples of Agboville, to facilitate childbirth for women [9].The plant have been reported for activité antimicrobienne [10], Abortifacient effect, Antidiarrheal activity, Antifertility effect [11] and antidiabetic effect [12]. Several studies revealed that extracts of *Abrus precatorius* exhibit anticancer activity against human breast cancer cell line MDAMB-23 [13], against A-549 cancer cell lines [14], against four cancer cell lines (Colo-205, Y79, HepG2 and SupT1) [15]. The aqueous extract of *A. precatorius* leaves contains several chemical groups and possess *in vitro* anticancer effect against mastocytoma cancer cell line (P815) [16]. This present study, was carried out to study chromatographic aqueous extract and cytotoxic effect of ethanolic extract of *Abrus precatorius leaves* against the murinmastocytoma cancer cell line (P815).

MATERIALS AND METHODS

Plant collection

The leaves of *Abrus precatorius* were collected in an urban area of Abidjan (Southern Ivory Coast) in month of October 2014. The plant had already been identified at the National Centre Floral of Abidjan (Ivory Coast) on the issue: *Abrus precatorius* (Fabaceae): Aboudé-Mandéké (Ivory Coast), 23 May 1990 N'Guessan Koffi 165 [9].

Preparation of extract

The aqueous extract of the leaves of *Abrus precatorius* was prepared by decoction according to the method of Lébri*et al.*, [17]. 10 g of powder of leaves were introduced into a triple-neck round-bottom of 250 mL, 100 ml of distilled water were added. A round-bottom was topped with a cooler connected to a faucet opened by pipe. The round-bottom is put down into a warm balloon (ELECTROMANTLE) maintained in a constant temperature of heating during one hour. After cooling, the mixture is filtered with cotton wool three times and the obtained filtrate was moved in the stove (SELECTA) at 55°C during 24 h. The extract was dried and the aqueous extract (ETA) was obtained.

Chromatographic analysis

Thin Layer Chromatography

Thin Layer Chromatographic Analysis was performed with three (3) solvent mixing systems (System 1: Ethyl Acetate / Hexane (1/9); System 2: Ethyl Acetate / Hexane (2/8) System 3: Ethyl acetate / Hexane (3/7) One (1) mg of the sample (aqueous extract, ethanol, acetatic, hexane) was diluted in a small quantity of Dichloromethane (spot solution). A sample deposit in small spots (3 to 4 times) in the form of dots using a capillary was carried out.

The chromatographic plate was placed in the saturated tank of the mixing system. The development of the chromatogram was followed until the arrival of the solvent at the upper front. The revelation of the constituents on the plate was carried out under a UV lamp: Ultra violet (λ = 254 nm and 365 nm) and by iodine vapor. For each constituent, the frontal ratio or the retention factor (Rf) was calculated according to the following formula:

$$Rf = \frac{\text{Distance traveled by the component}}{\text{Distance traveled by the eluent front}}$$

Column chromatography

Column chromatography was carried out in an attempt to obtain different fractions of the total aqueous extract of the leaves of *Abrus precatorius* in order to make their identifications according to the method of Aljerf [18].The silica deposit was prepared from (one) 1 g of extract mixed with silica, the whole taken up in a quantity of hexane and the contents were concentrated by evaporation in a rotary evaporator (HEIDOLPH WB 2000). The mixture is carried out at the top of a glass column (55 x 2) filled with 60 Å silica gel in hexane. The fractionation was carried out by successive applications of an ethyl acetate / hexane mixture (1: 9; 2: 8; 3: 7). 10 ml fractions were collected and the solvent was removed by rotary evaporation (HEIDOLPH WB 2000) at a temperature of 65°C

In vitro anticancer effect of aqueous extract of *A. precatorius*

In vitro anticancer effect of ethanolic extract of *A. precatorius* leaves was conducted in the Laboratory of biological engineering, Faculty of Sciences and Techniques, Sultan MoulaySlimane University of Beni-Mellal (Maroc).

Tumor cell line and culture

The mastocytoma tumor cell line, were grown in RPMI1640 (Sigama-Aldrich) supplemented with 10% heat-inactivated Fetal Bovine Serum (FBS) (Sigama-Aldrich), 1% penicillin-streptomycin, and 0.2% sodium bicarbonate (Sigama-Aldrich), under a fully humidified atmosphere of 95% air and 5% CO_2 at 37ºC.

Cytotoxicity assay

Cellular cytotoxicity was determined by the MTT reduction assay. This Colorimetric assay is based on the capacity of mitochondria succinate dehydrogenase enzymes in living cells to reduce the yellow water soluble substrate 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) into an insoluble, colored formazan product which is measured spectrophotometrically [19]. Growing concentrations of the tested extract (solubilised in Dimethyl sulfoxide (DMSO): 3, 12; 6, 25; 12,5; 25; 50; 100 and 200 µg/mL) were applied to the wells of a 96-well plate containing the confluent cell monolayer (106 cells per well) in duplicate. Methotrexate as positive control drug was added in the same concentrations and conditions. After 48 h of incubation, 20 µL of the MTT solution [5 mg/mL in Phosphate buffered saline (PBS)] was added. After incubation in the same conditions for 4 h, the plates were treated with a mixture of HCl /Isopropanol (24:1) to dissolve the blue intracellular formazan product. One hour later, the plates were read on a Micro ELISA reader using two wavelengths (540 and 630 nm). DMSO was used as negative control. The median inhibitory concentration (IC_{50}) was calculated as the concentration of the sample that leads to 50% of cell lysis comparatively to the negative (positive) control.

The relative inhibition of cell proliferation was calculated by the formula:

$$\% \text{ inhibition} = 100 \times (1 - A / A_0),$$

where A_0 and A, are the absorbencies of negative control and ethanolic extract or methotrexate treated cells, respectively.

Statistical Analysis

Data are reported as means±SEM of 3 experiments Statistical differences were assessed by analysis of standard deviation using the Student's t test , with the level of significance set at $p<0.05$

RESULTS

Chromatographic study of extracts from the leaves of *A. precatorius*

Thin Layer Chromatography (TLC)

The phytochemical analysis of the aqueous extract of the leaves of *Abrus precatorius* revealed the presence of several chemical groups (alkaloids, tannins, flavonoids (flavones), saponins, quinone compounds (coumarins), sterols and triterpenes and reducing compounds) [12]. Analysis of the dry extract (aqueous) by thin-layer chromatography after UV detection and iodine vapor reveals the presence of 4; 5 and 6 different Rf spots with the systems (Ethyl acetate / Hexane: 1/9; 2/8 and 3/7) (Table 1).The number of different Rf spots was also revealed on the TLC plates with the dry extracts obtained from different organic solvents. The ethanolic extract exhibits 5 spots with the systems (Ethyl Acetate / Hexane: 1/9; 2/8 and 3/7). The acetate extract respectively 5 and 4 spots with the systems (Ethyl acetate / Hexane: 1/9; 2/8 and 3/7) and the hexane extract respectively 4; 6 and 5 with (Ethyl acetate / Hexane: 1/9; 2/8 and 3/7) (Table 1).

Table 1: Number of spots observed on the TLC plate of different extracts from the leaves of *Abrus precatorius.*

Extracts	**ETAAP**			**EEAP**			**EAAP**			**EHAP**		
Eluent (proportion) Ethyl acetate / Hexane	1/9	2/8	3/7	1/9	2/8	3/7	1/9	2/8	3/7	1/9	2/8	3/7
Number of spots	4	5	6	5	5	4	5	5	4	4	6	5
Rf	0.07 0.12 0.37 0.7	0.15 0.20 0.45 0.57 0.7	0.12 0.3 0.4 0.52 0.62 0.75	0.02 0.08 0.13 0.32 0.72	0.10 0.22 0.38 0.51 0.78	0.07 0.12 0.37 0.7	0.05 0.1 0.22 0.54 0.73	0.13 0.15 0.24 0.54 0.81	0.13 0.27 0.54 0.78	0.13 0.43 0.51 0.84	0.16 0.30 0.40 0.51 0.59 0.78	0.13, 0.24 0.40, 0.59 0.81

Column chromatography

The chromatographic study on a column using the elution solvent of increasing polarity (Ethyl acetate / Hexane: 1/9; 2/8 and 3/7) made it possible to obtain two fractions called LA1 and LA2 of appearance oily, of respective mass 100 mg and 300 mg per 1 g of dry extract (ETAAP).On TLC plate, the LA1 fraction presents a single spot and the LA2 fraction presents two spots (Table 2).

Table 2: Numbers of spots revealed in fractions LA1 and LA2 on TLC plate.

Fractions	**Aspect**	**Mass (mg)**	**Yied (%)**	**Analysis**		
				Eluent (Proportion)	**Numbers of spots**	**Rf**
Fraction LA1	Oily	100	10	Ethyl acetate / Hexane (3/7)	1	0.75
Fraction LA2	Oily	300	30	Ethyl acetate / Hexane (3/7)	2	0.62-0.75

***Invitro* anticancer effect**

The *Invitro* anticancer activity of ethanolic extract of *Abrus precatorius* was evaluated at 3.12; 6.25; 12.5; 25; 50 ; 100; 200 and 400 µg/mL.) against P815 tumor cell. The result is summarized in Figure 1. The results show that ethanolic extract has a best inhibitory effect on the growth of tumor cells compared to the aqueous extract which are less 20% at a concentration 200 µg/mL (Table 3). The ethanolic extract has aninhibitory effect which increases with concentration, this effect is similar to that of Methotrexate used as a reference (Figure 3). It was also observed, that the maximum effect of the ethanolic extract at the highest concentrations tested (400µg/mL) was 72,20 % (± 4,60) and (200 µg/mL) was 63.54% (±4,28) of lysis. Further, on lower doses of 3.12; 6.25; 12.15; 25 and 50 µg/mL percent growth inhibition observed by the extract was between

6,25% (±1,70) and 43.94 % (±3,30) (Table 3). The IC50 of the ethanol extract is 43.94 µg/mL (Figure 1), this IC50 is lower than that of the aqueous extract which are 200 µg/mL (Figure 2). The IC50 of ethanolic extract is more higher compared with the Methotrexate (2.5µg/mL) used as positive control (Figure 3).

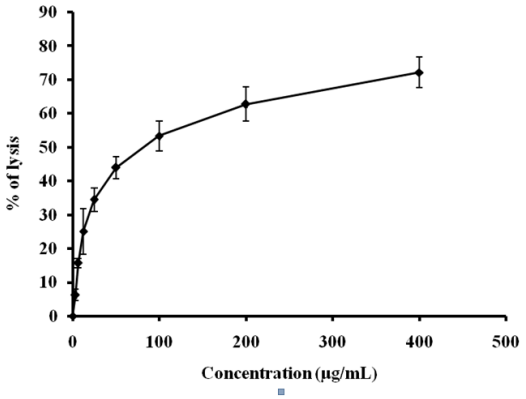

Figure 1*: in vitro* anticancer effect of ethanolic extract of *A. precatorius* leaves on P815 cancer cell line
Dose - response curve of ethanolic extract of *A. precatorius* leaves on P815 cancer cell line.

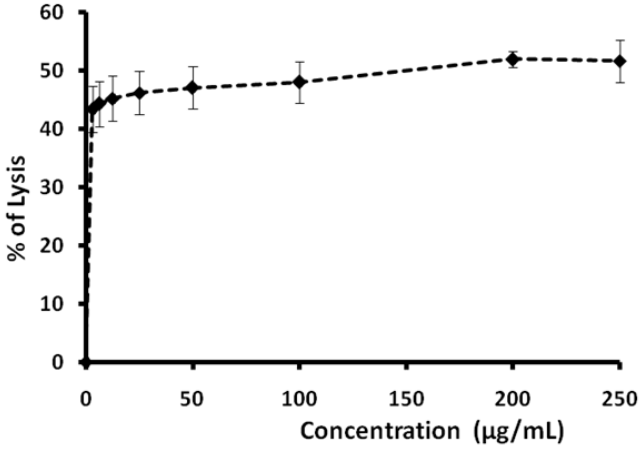

Figure 2: *In vitro* anticancer effect of aqueous extract (ETA) of *A. precatorius* leaves on P815 cancer line [16]
Dose-response curve of aqueous extract of *A. precatorius* on P815 cancer cell line.

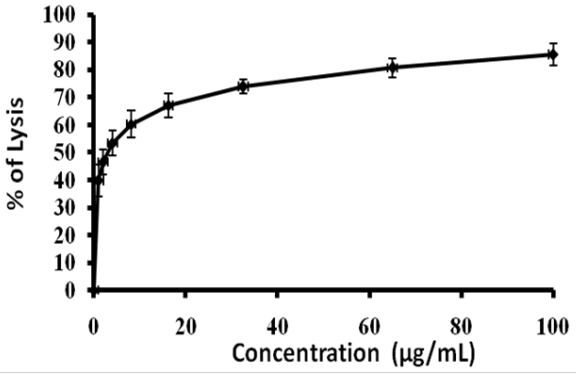

Figure 3: *in vitro* anticancer effect of methotrexate on P815 cancer line
Dose-response curve of methotrexate on P815 cancer cell line.

Table 2: Percentage of lysis of cancer cell line according to the concentration of etanol extract.

Concentration (µg/mL)	400	200	100	50	25	12.5	6.25	3.12
% of cell lysis (ethanolic extract)	72.20 ± 4.60	62 ;78 ± 5.07	53.36 ± 4.47	43.94 ± 3.30	34.52 ± 3,46	25.09 ± 6.71	15.67 ± 1.41	6.25 ± 1.70

DISCUSSION

The qualitative phytochemical study of the total aqueous extract of the leaves of *Abrus precatorius* showed that the plant contained alkaloids, tannins, flavonoids (flavones), saponins, quinone compounds (coumarins), sterols, triterpenes and reducing compounds [17]. The thin-layer chromatographic study of the different extracts (aqueous, ethanolic, acetate and hexane) made it possible to demonstrate the presence of several spots of different frontal ratio. Moreover, the chromatographic study on a column of the aqueous dry extract allowed to obtain two fractions, one containing a stain and the other two stains of different RF. The results of chromatographic studies confirm the richness of the extracts in active chemical compounds which could explain the traditional use of *Abrus precatorius* to treat many diseases. Work on the plant has isolated several groups of secondary compounds including alkaloids, steroids and triterpenoids, isoflavanoquinones, anthocyanins, tannins, flavonoids and phenolic compounds [20]. The root, leaf and stem containing (L +) abrin, glucosides (abralin, hemagglutinin), (N-methyltryptophan and urease) are traditionally used in the treatment of cough [21]. Studies have showed that compounds isolated from the *Abrus precatorius* plant (tannins, alkaloids, sterols, triterpenes and flavonoids) have anti-tuberculosis and antiplasmodial properties [22]. Hence, this study evaluates the potential *in vitro* anticancer activity of ethanolic extract of leaves *Abrus precatorius* against murinmastocytoma cells (P815). In this study, the cytotoxicity of extract was sought by determining the percentage of lysis of cancer cells and the inhibitory concentration 50% (IC50) by the MTT assay.

The results showed that the ethanolic extract of the leaves of *Abrus precatorius* exerts a dose-dependent inhibitory effect on the growth of cancer cells. The concentration of extract leading to 50% lysis is 43.94 µg/mL. The effect of the extract on the cancer cell line has been compared with that of methotrexate, which is a molecule used in the treatment of cancer. The control product showed a dose-dependent inhibitory effect with an IC50 (IC50=2.5 µg/mL) much lower than the extract. However, the inhibitory concentration 50 of the ethanolic extract (IC50 = 43.94 µg / mL) is lower than that of the aqueous extract of the leaves of *A. precatorius* ((IC50=200 µg /mL). The dose-dependent inhibitory effect of the ethanolic extract on the growth of cancer cells may be due to the action of the active ingredients contained in the extract. The results obtained are close to Tilaoui *et al.*,[23]. These authors have shown that the essential oil rich in monoterpenes extracted from the plant *Artemisia herba-alba* has a dose-dependent cytotoxic effect on the acute lymphoblastic leukemia (EMF) blood cancer cell line in humans (IC50=3 µg / mL).Several studies carried out using extracts of the *A precatorius* plant on different lines of cancer cells are in agreement with the results obtained with the ethanolic extract of the leaves of *Abrusprecatorius*. Sivakumar *et al.*,[14]. have shown that the crude insoluble methanolic fraction extracted from the seeds of *Abrus precatorius* exerts an effect on the cancer cell line A-549 (IC50 = 175 mg/ml). Mohammed Shafi Sofi *et al.*,[13]. Showed that the aqueous extract of the leaves of *Abrus precatorius* possesses anti-cancer activity on the MDAMB-231 female breast cancer cell line (IC50 = 98 µg/mL). Low cytoxic activity of the aqueous extract of the leaves of *Abrus precatorius* has also been described by Mir Z Gul *et al.*,[15]. Their work showed non-significant cytotoxic activity of the aqueous extract on four human tumor cell lines (Colo-205, Y79, HepG2 and SupT1) and unlike acetate and ethanolic extracts. Lebri *et al.*, [16]. Also showed a weak cytotoxic activity in the aqueous extract of the leaves of *Abrus precatorius* on a murine mastocytoma P815 tumor cell line. These results show that the cytotoxic activity obtained with the ethanolic extract of the leaves of *Abrus precatorius* is better than that obtained with the aqueous extract. The results clearly demonstrate that the cytotoxic activity of the extracts of *Abrus precatorius* depends not only on the nature of the extract and its chemical composition, but also on the target tumor cells.

CONCLUSION

This present study confirmed that *A. precatorius* leaves could be potentially useful for the development of therapeutic agents against cancer. The ethanolic extract of *Abrus precatorius* showed the best cytotoxic effect. Furthermore, the study, the *in vivo* anticancer activity and the molecular mechanisms involved in such activity are needed to facilitate integration of *Abrus precatorius* as an anticancer herbal medicine.

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