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Der Pharma Chemica, 2015, 7(8):112-117
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Phytochemical analysis and *in vitro* anticancer effect of aqueous extract of *Abrus precatorius* Linn

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ABSTRACT

This paper aims to evaluate the in vitro anticancer activity of aqueous extract of Abrus precatorius leaves on the murin mastocytoma cancer cell line (P815), and the phytochemical analysis of the extract as well. The Aqueous extract (ETA), was obtained by traditional method adapted to laboratory conditions and the phytochemical analysis was based on differential staining and precipitation reactions. In vitro anticancer effect was evaluated by the cellular cytotoxicity against the murin mastocytoma cell line (P815). Cellular cytotoxicity was determined by the MTT assay. Phytochemical screening of aqueous extract showed several chemical groups: alkaloids, flavonoids (flavones), tannins, coumarins, sterol, triterpenoids, saponins and reducing compounds. The in vitro anticancer effect, showed a dose dependant cytotoxic effect. It was observed, that the maximum activity of the extract at the highest concentration tested (200µg/mL) was 50% (±1.5). 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 43 and 47 %, the IC50 value of the extract (200µg/mL. Our results suggest that aqueous extract of A. precatorius leaves contains several chemical groups and possess a weak in vitro anticancer effect against P815 tumor cell line.

Keywords: *Abrus precatorius*, phytochemical analysis, *in vitro* anti-cancer activity, P815 cancer cell line.

INTRODUCTION

The World Health Organization (WHO) has classified cancer among non-communicable diseases, which are responsible for 63% of deaths worldwide **[1]**. The World Bank income groups estimated that the incidence of 12.7 million new cancer cases in 2008 **[2]** will rise to 21.4 million by 2030, and low or middle-income countries will be the most affected with nearly two thirds of all cancer diagnoses **[3]**. West Africa is composed of mostly poor countries where cancer is an emergent disease. In 2008, men in the African Region had more than double of the rate of liver cancer while women in this region had the highest incidence of cancer of the cervix uteri worldwide **[4]**. According to WHO estimates, 80% of the rural population of this region has almost exclusively uses traditional medicine for its needs of primary health care **[5]**. This massive use of traditional medicine, composed mainly of

medicinal plants, is related to cultural and economic reasons. This is why WHO encourages countries of this region to promote and integrate traditional medical practices in their health system **[6, 7]**.

Abrus precatorius Linn (*Fabaceae*) is known mainly for its medicinal properties to cure various diseases. The roots, leaves and seeds of this plant are used for different medicinal purpose.

The plant have been reported for neuromuscular effects, neuro-protective, abortifacient, antiepileptic, anti-viral, antimalarial, antifertility, nephroprotective, immunostimulatory properties, antiinflammatory activity and antidiabetic effect.

The plant is considered as a valuable source of unique natural products for development of medicines against various diseases and also for the development of industrial products **[8]**. The ethnomedicinal study carried out in the department of Agboville (Southern Ivory Coast), showed that Abbey and Krobou use the leaves of *Abrus precatorius* to treat gyneco-obstetric disorders and to make easier childbirth **[9, 10]**. In this country *Abrus precatorius* is not know as anticancer potential plant. However several studies revealed that extracts of *Abrus precatorius* possess potential antitumor and anticancer effects **[11, 12]**. This present study, was carried out to study the phytochemical analysis of total aqueous extract of *Abrus precatorius* leaves and to evaluate their potential anticancer effect, against the murin mastocytoma cancer cell line (P815)

MATERIALS AND METHODS

2.1 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 **[10, 13]**.

2.1.1 Preparation of extracts

The decoction is the method recommended in traditional medicine [14]. In our case this decoction realized from already powder dried leaves in the laboratory condition.

100 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. Extraction was repeated several times to obtain a sufficient quantity.

Various extracts of the leaf were prepared for a comparative phytochemical analysis. These various extracts were obtained using different solvents polarities (ethanol, acetate diethyl and hexane) according to the modified Zirihi method **[15]**.

25 g of powder of leaves was subject to maceration under magnetic agitation for 48 hours in 1250 mL of ethanol. Ethanolic mixture was filtered once on cotton wool and then filtered on filter paper (*whatman*). The filtrate was concentrated using a rotary evaporator at 65 °C (HEIDOLPH WB 2000). The concentrate was totally dried in the steam room at 55°C (SELECTA) during 24 hours. Then the ethanolic extract was obtained (EEAP).

To obtain acetic extract (EAAP), 25 g of powder of leaves was subject to maceration under magnetic agitation for 48 hours in 1250 mL of acetate diethyl. Acetatic mixture was filtered twice with cotton wool. The filtrate is left evaporate in the room temperature during 24 hours and dried totally in the steam room at 55 °C during 24 hours. To obtain hexanic extract, 25 g of powder of leaves was macerated under magnetic agitation for 48 hours in 1250 mL of hexanic mixture. After that the extract was filtered twice with cotton wool. The filtrate is left evaporate in the laboratory temperature during 24 hours and dried totally in the stove at 55 °C during 24 hours.

2.1.2 Phytochemical analysis

Phytochemical analysis of aqueous extract of *A. precatorius* leaves was conducted on the basis of differential staining reactions and precipitation using the method by Houghton and Raman **[16]**. The different reactions about active compounds are summarized in Table 1.

Table 1. Phytochemical analysis

Classes of active substances		Specific reagents reactions	Reactions
Alkaloids		Dragendorff. (tetraiodo-bismuthale de potassium)	orange color with precipitate
Polyphenolic compounds	Tanins	reaction of Stiasny ($FeCl_3$)	blue-dark green or black
	Flavonoids	Cyanidine reaction	orange color, red or purple
Quinonic compounds	Coumarins	Bornträger reaction - UV	intense fluorescence
Saponins		Determination of the Foam Index (MI)	Positive test if MI >100 Intense foam
Sterol and triterpenoids		Libermann-Burchard (Anhydride Acetique-H_2SO_4)	violet-blue or green
Reducing compounds		Fehling's reaction	brick-red precipitate

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

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

2.2.1 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 37 ºC.

2.2.2 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 **[17]**. 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 (10^6 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 MicroELISA 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:

% inhibition =100 x (1- A / A_0),

where A0 and A, are the absorbencies of negative control and ETA extract or methotrexate treated cells, respectively.

2.3 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

3.1 Phytochimical analysis

The qualitative phytochemical study revealed the presence of several chemical groups: alkaloids, tannins, flavonoids (flavones), saponines, quinones (coumarins), sterols, triterpènes and reducing compounds. This qualitative phytochemical study shows that all the chemical groups identified at level of the leaves of *Abrus precatorius* find themselves in the traditional preparation (decoction), Table 2.

3.1 *In vitro* anticancer effect

The *In vitro* anticancer activity of the ETA extract was evaluated at 3.12; 6.25; 12.15; 25; 50; 100 and 200 µg/mL against P815 tumor cell. The result is summarized in figure 1. It is shown in this figure that the growth inhibition is dose dependent (Figure1). It was also observed, that the maximum activity of the extract at the highest concentration tested (200µg/mL) was 50% (±1.5) 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 43.35 and 47.50 % (table 3). The IC_{50} value of the extract (200µg/mL) (Table 3), is more higher compared with the Methotrexate (Table 4) used as positive control which

exhibits and IC50 value of 2.5µg/mL (Figure 2). These values of lysis percentage demonstrate that aqueous extract of *Abrus precatorius* leaves have a weak effect on P815 cancer cell line

Table 2. Chemical groups in various extracts of *Abrus precatorius* leaves

Chemical groups		Extracts			
		ETA	EEAP	EAAP	EHAP
Alkaloids		+++	+++	-	-
Polyphenolic compounds	Tannins	+++	-	+++	-
	Flavonoids (flavons)	+++	-	+++	-
Quinones compounds	Coumarins	+++	-	-	-
Saponins		+++	-	-	-
sterols and triterpenoids		+++	+	+	+++
reducing compounds		+++	-	-	-

+: presence.;+++: Intense presence; –: absence

Table 3. Percentage of lysis of cancer cell line according to the concentration of (ETA)

Concentration ETA (µg/mL)	% of Cell Lysis
200	50 ± 1.5
100	47.98 ± 3.5
50	47.05 ± 3.6
25	46.13 ± 3.7
12.5	45.20 ± 3.8
6.25	44.28 ± 3.9
3.12	43.35 ± 4

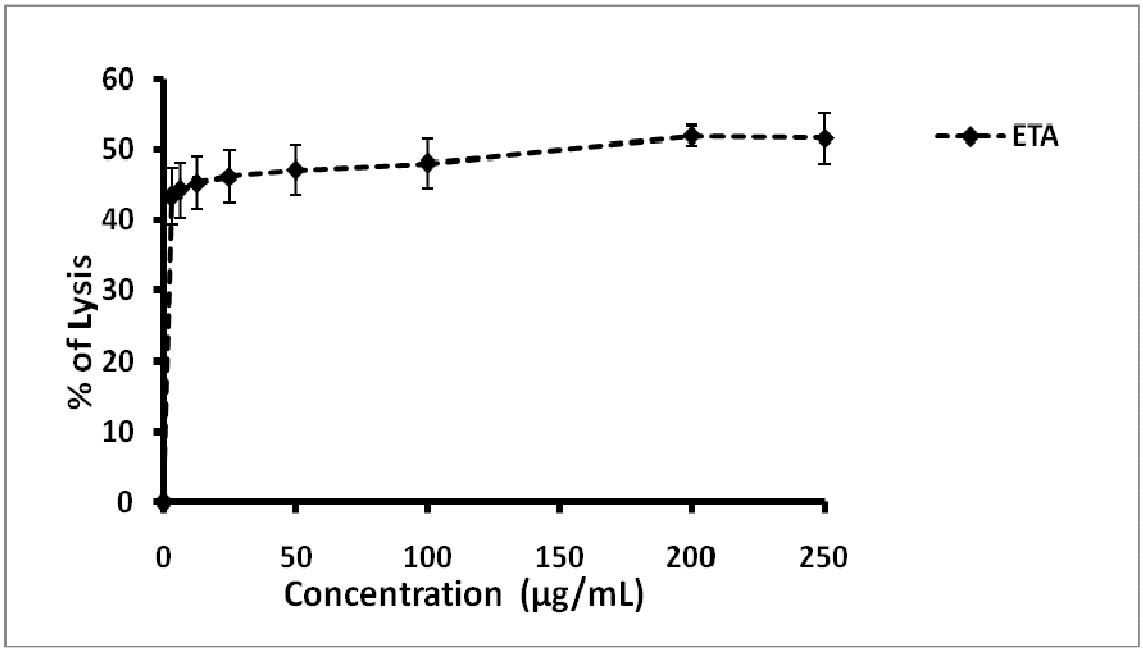

Figure 1*: in vitro* anticancer effect of aqueous extract of *A. precatorius* leaves on P815 cancer cell line
Dose - response curve of aqueous extract of *A. precatorius* leaves on P815 cancer cell line

Table 4. Percentage of lysis of cancer cell line according to the concentration of methotrexate

Concentration Methotrexate (µg/mL)	% of Cell Lysis
65	80.84 ± 3.5
32.5	74.00 ± 3
16.25	67.17 ± 4.33
8.12	60.33 ± 5
4	53.49 ± 4,70
2	46.65 ± 4.50
1	39.81 ± 5.75

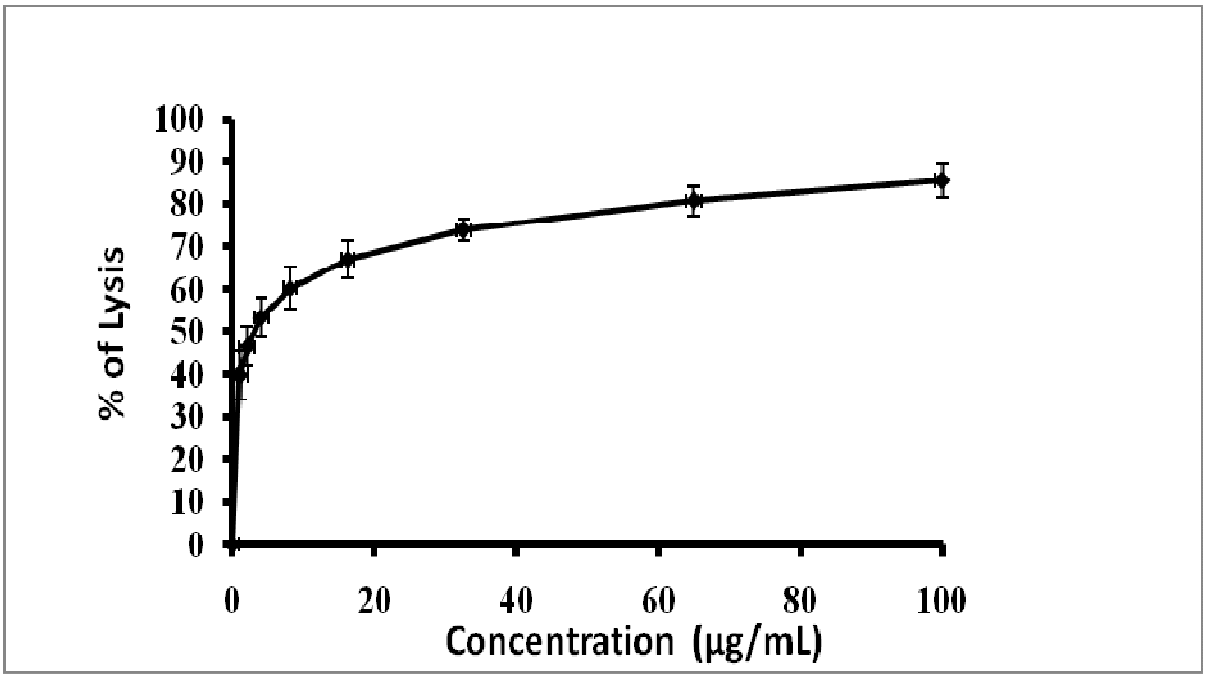

Figure 2: *in vitro* anticancer effect of methotrexate on P815 cancer line
Dose-response curve of methotrexate on P815 cancer cell line

DISCUSSION

The qualitative phytochemical study revealed the presence of several chemical groups: alkaloids, tannins, flavonoids (flavones), saponins, coumarins, sterols, triterpenoids and reducing compounds. This qualitative phytochemical analysis showed that all identified chemical compound in *Abrus precatorius* leaves was found in leaves decoction in the traditional preparation.

From a qualitative point of view, the extraction method used in traditional folk medicine is effective compared to the other studied methods of extraction (Ethanolic, Acetatic and Hexanic). The abundance of active principles confers to the plant the remarkable pharmacological properties, what could justify its multiple therapeutic indications. Our results are in accordance with the results of Konkon et al. **[18]** that showed that all the chemical groups identified in *Mitragyna inermis* leaves finds itself in the traditional preparation (decoction of leaves).

Hence, this study evaluates the potential *in vitro* anticancer activity of aqueous extract of leaves *Abrus precatorius* against murin mastocytoma cells (P815). In this study, aqueous extract was evaluated as promising anticancer solution using MTT assays. The concentration of aqueous extract of *A. precatorius* leaves leading to 50% lysis is 200 µg/mL. Our results are in accordance with the results of Mohammed Shafi Sofi et al **[19]**, that found aqueous extracts of *Abrus precatorius* leaf *exhibits* anticancer activity against human breast cancer cell line MDAMB-231, with IC50 = 98 µg/mL. Sivakumar et al. **[20]** the in vitro cytotoxicity of methanol insoluble fraction of crude red forms of *Abrus precatorius* against A-549 cancer cell lines showed an IC50 value of 175, 100 mg/mL. The lowest cytotoxic activity of water extract was also described by Mir Z Gul et al. **[21]**, which demonstrate no significant cytotoxic activity against four cancer cell lines (Colo-205, Y79, HepG2 and SupT1), whereas ethyl acetate extract and ethanol extract demonstrated significantly effective antiproliferative activities in a concentration dependent manner, with an IC_{50} value of 18.91 and 26.74 µg/mL against Colo-205 and Y79 cells respectively. These results clearly demonstrate that the cytotoxic activity of extracts from *Abrus precatorius* depends not only on the nature of the extract and its chemical composition but also on the target tumor cells. In fact, the molecular mechanisms involved in the cytotoxic activity can be different from cell type to another. These are in accordance with those of Tilaoui et al., **[22]**. and Jaafari et al., **[23].**

Previous studies on different panels of cancer cell lines, described that wide bioactive phytocompounds isolated from *A. precatorius* including tannins, alkaloids, steroids, saponins, terpenoids, and flavonoids demonstrated marked inhibitory effects and have properties to induce apoptosis on various types of cancers **[24-26]**. The water extract of *Abrus precatorius* possessed an abundance of phenolics amounting to 25.48 mg GAE/g DW gallic acid equivalents. The sensitivities of cancer cells to cell death by phenolics compounds are reported by several studies **[27-31]**.

CONCLUSION

This present study confirmed that aqueous extract of *A. precatorius* leaves could be potentially useful for the development of therapeutic agents against cancer. However, further studies should be done to evaluate the in vitro anticancer activity against a panel of cancer cell lines. 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.

Acknowledgements
We express gratitude to the Department of biochemistry, University Felix Houphouet-Boigny of Abidjan (Ivory Coast) and to the Sultan Moulay Slimane University of Beni Mellal, Faculty of Technical and Sciences (Morocco) for providing the facilities for conducting this research

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