academicJournals

Vol. 7(17), pp. 1182-1189, 3 May, 2013
DOI 10.5897/JMPR12.1167
ISSN 1996-0875 © 2013 Academic Journals
http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

In vitro anticancer activity of some plants used in Moroccan traditional medicine

Abdeljlil DAOUDI[1]*, Amal EL Hamsas EL YOUBI[1], Denyse BAGREL[2] and Lotfi AARAB[1]

[1]Laboratory of Bioactive Molecules, Immunology Unit, Faculty of Sciences and Techniques, University Sidi Mohamed Ben Abdellah, P.O: 2202, Road of Immouzer, Fez, Morocco.
[2]Laboratory Engineering Biochemistry and Molecular Pharmacology, University Paul Verlaine, Metz, France.

Accepted 11 February, 2013

Over time, cancer remains a world public health problem. The use of traditional medicine as alternative treatments of cancer was recognized in 1950s by the US National Cancer Institute (NCI) under the leadership of the late Dr. Jonathan Hartwell. In this context, we are interested to search for bioactive natural substances for cancer therapy used in Moroccan traditional medicine. Based on ethnopharmacological study, we selected four plants with an important therapy against cancer in Moroccan traditional medicine; these are *Aristolochia longa* (L), *Citrullus colocynthis* (L), *Piper cubeba* (L) and *Delphinium Staphisagria* (L). In the pharmacological study, we evaluated the antiproliferative activity of selected Moroccan plants on five different cancer cell lines namely MCF7, HT29, N2A, H5-6 and VCREMS using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay at a dose of 500 µg/ml; lyophilized extracts after 24 h of treatment showed that two plants possess significant cytotoxic activity against cancer cell lines, such as MCF7, HT29, N2A, H5-6 and VCREMS. *P. cubeba* (L) extract showed the inhibition of 98.64 and 91.59% against MCF7 lines and HT29 cell lines, and for *C. colocynthis* (L), 91.84 and 85.58%, respectively. *C. colocynthis* (L) showed a dose-dependent effect on MCF7 and HT29 cell lines with an IC_{50} of 22.0 and 32.5 µg/ml, respectively. This cytotoxicity is induced probably by apoptosis.

Key words: *Aristolochia longa* (L), *Citrullus colocynthis* (L), *Piper cubeba* (L), *Delphinium Staphisagria* (L), cytotoxicity, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), cancer cell lines, IC_{50}.

INTRODUCTION

Cancer is a leading cause of deaths worldwide and accounted for 7.6 million deaths (13% of all deaths) in 2008. According to reports of the World Health Organization (WHO), in 2020, the number of deaths due to cancer will experience a substantial increase in the world to reach 15 million of new incidents (WHO, 2012). The incidence and geographic distribution of cancer are related to multiple factors such as sex, age, race, genetic predisposition and the most important factor which is exposure to environmental carcinogens. Chemical carcinogens such as tobacco smoke as well as azo dyes, aflatoxins and petrol have been clearly implicated in cancer induction in and animals. In addition, exposure to mutagenic agents were also strongly identified as causative agents for cancer (Rastogi et al., 2004).

At present, the limits related to the cost, problems of low efficiency and severe side effects which reduce the quality of life and discourage patients to observe medication protocols which then lead to the progression of cancer and associated complications. In addition, many of these current treatments may limit anticancer activities (Mans, 2000). Therefore, the development and search for novel and effective anticancer agents to overcome the developed resistance and the reduction of severe side effects have become very important issues. During the last decades, natural products have been an

*Corresponding author. E-mail: daoudiabdeljlil@yahoo.fr. Tel: +212676470677. Fax: +212 535608214.

important source of chemotherapeutics. Approximately 60% of drugs currently in use for treatment of cancer have been isolated from or derivatives of natural products (Gordaliza, 2007; Newman, 2007; Ma, 2009).

The most common examples are vinblastine and vincristine obtained from the Madagascar *Vinca rosea* and taxol from *Taxus brevifolia*, and camptothecin from *Camptotheca acuminata* (De Smet, 1997). The traditional Moroccan medicine was written on the uses of medicinal plants for thousands years. Several ethnobotanical studies reported anticancer activities for Moroccan medicinal plants (Bellakhdar, 1997; Hmamouchi, 1996; Merzouki et al., 2000). Some of them have been confirmed recently against cancer *in vitro* (Lamchouri et al., 2000).

In this study, we have contributed to select and to collect four medicinal plants based on previous ethnopharmacological studies. The selected plant extract were evaluated *in vitro* at the anti-proliferative using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (M) assay against five cancer cell lines (MCF7, HT29, N2A, H5-6 and VCREMS); the IC50 was calculated for the extracts from Citrullus colocynthis which have a significant antiproliferative activity and the microscopic observations were carried out to elucidate their probable cytotoxic mechanism.

MATERIALS AND METHODS

Ethnopharmacological study

The ethnopharmacological study was conducted in Fez city (coordinates 34° 03' 00''North 4°58' 59'' west), region of North-Central Morocco. The choice of this city was related to the great number of traditional herbalists in Fez, that meet the needs of researcher cares to collect and exchange information. The ethnopharmacological survey consists of interviewing 100 individual herbalists in different parts of the city. The questioner revealed different plants recommended by the herbalists for clients against various diseases Various conversations and discussion were conducted during the interviews. Verbal information collected was recorded, and the specimens of each plant were deposited as references.

Pharmacological study

Chemicals and reagents

Chemicals and reagents were purchased from Life Technologies and Sigma-Aldrich Co. (St. Louis, MO, USA) and were of analytical grade.

Preparation of plant extracts

The specimens of the purchased plants from herbalists were deposited as voucher specimens (LMB/Number/Year) (Table 1) as reference in the Laboratory of Bioactive Molecules, Faculty of Sciences and Techniques, Fez, Morocco.The plants were washed, dried, crushed and macerated at 10% in phosphate buffered saline (PBS, 15 mM, pH 7.4) overnight at 4°C. The supernatants were filtered on Millipore membrane filter (Whatman filter 0.22 µm) to eliminate non soluble particles. The filtrates were lyophilized separately (FTZ®, Dry System Model 77520 USA), then resuspended at 500 µg/ml concentration in sterile Roswell Park Memorial Institute (RPMI).

Preparation of cancer cell lines

The different cell models used in this study are as follows: the mammary adenocarcinoma MCF-7 was developed from a mammary adenocarcinoma (Soule et al., 1973), this line comes from the ATCC (American Type Culture Collection, USA). The Vcr-R line (originally named VCREMS) corresponding to MCF-7 cells, were selected for resistance to vincristine after treatment with a mutagen (Whelan et al., 1992). The line of colon carcinoma HT29 was characterized by Lesuffleur et al. (1990). The line of rat hepatoma H5-6 (Pitot et al., 1964). The line Neuro-2a, clone N2A established by Klebe and Ruddle (1969) from a brain tumor spontaneous albino mice.

MTT assay

Cancer cell lines MCF7 cells, HT29 cells and VCREMS cells, were cultured in RPMI 1640 medium supplemented with 1.25 mM sodium pyruvate, 2 mM L-glutamine, 10% fetal calf serum, ampicillin 100 U/ml and streptomycin 100 mg/ml, 25 mg/ml amphotericin B.N2A cells and H5-6 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 1 g/L of glucose for N2A and with 5 g/L of glucose for H5-6. These mediums were supplemented with 1.25 mM sodium pyruvate, 2 mM L-glutamine, 10% fetal calf serum, ampicillin 100 U/ml and streptomycin 100 mg/ml, and 25 mg/ml amphotericin B DMEM.Cell lines proliferation were measured by the MTT assay (Mosman, 1983) as described by Daoudi et al. (2008). Briefly, the optimal densities were previously defined to achieve the longest possible treatments within the exponential growth phase and working on the maximum cell growth. The seeding densities of all cell lines used in the study were as follows MCF7: 10^5 Cells/ml; HT29: 15.10^4 Cells/ml; H5-6:10^5 Cells/ml; N2A: 10^5 Cells/ml, and VCREMS: 5.10^4 Cells/ml. Then in 96 well plates, the cell lines were incubated at 37°C in a humidified chamber under an atmosphere of 95% air and 5% CO_2.

The plant extract concentrations (500 µg/mL RPMI) not cytotoxic for host normal cells (Daoudi et al., 2008). Were then added after their incubation for 24h at the suitable concentration for 24h also. Thereafter, 10 µL of MTT solution (5 mg/mL in PBS) was added. After 3 h of incubation, the supernatant was removed and replaced by 100 mL of dimethyl sulfoxide (DMSO). Finally, the optical density was measured at wavelength 560 nm using the spectrophotometer (Dynatech MR5000).

Statistical analysis

Statistical analysis of the results was performed at least three times independently. The results are represented as mean ± standard error of mean (SEM). Their significance was evaluated by the *Student t-test*. The results were considered statistically different when P was less than 0.05 compared to the control.

RESULTS

Ethnopharmacological study

In this part, we have selected plants that were declared significantly by 100 herbalists. Table 1 shows the

Table 1. Ethnopharmacological data collected on plants species used in this study.

Plant species (Family)	Abbreviation	Plants vernacular names	Plant part used	Traditional uses		Voucher specimen
				Ailments treated	Citation as anti cancer (%)	
Ammi visnaga (L) (Apiaceae)	AV	Bachnikha	Seed	A, Db,	0	LMB 01/04
Aristolochia longa (L) (Aristolochiaceae)	AL	Baraztam	Root	Ca,	24	LMB 02/04
Lepidium sativum (L) (Brassicaceae)	LS	Habb archad	Seed	Db, Di, M,	0	LMB 03/04
Sinapis nigra (L) (Brassicaceae)	SN	Khardal	Seed	Co, R,	0	LMB 04/04
Capparis spinosa (L) (Capparidaceae)	CS	Kabbar	Fruit	Db, I, R,	0	LMB 05/04
Citrullus colocynthis (L) (Cucurbitaceae)	CC	Handal	Seed	Ca, Db, H, I, R, Sp,Tu,	21	LMB 06/04
Tetraclinis articulata (L) (Cupressaceae)	TA	Aaraâr	Seed	A, Db, H, I, R, Te,	0	LMB 07/04
Juncus acutus (L) (Juncaceae)	JA	Assmar	Seed	I, K, Te,	0	LMB 08/04
Marrubium vulgare (L) (Lamiaceae)	MV	Marriwa	Flower	B, Ca, Db, H, I, Te,	0	LMB 09/04
Piper cubeba (L) (Piperaceae)	PC	Kebaba	Seed	Ca, R, Sx,	13	LMB 10/04
Delphinium staphysagria (L) (Ranunculaceae)	DeS	Habb'arras	Seed	Ca, Te,	14	LMB 11/04
Datura stramonium (L) (Solanaceae)	DaS	Chdak-jmel	Seed	A, Ca, I, Se,	2	LMB 12/04
Urtica dioïca (L) (Urticaceae)	UD	Hourrayga	Seed	H, I, K,	0	LMB 13/04
Elettaria cardamomum (L) (Zingiberaceae)	EC	Qaâqolla	Seed	Sx,Ca,	6	LMB 14/04

Abb: Abbreviation; A, asthma; B, bilious stimulation; Ca, cancer; Co, constipation ; Db, diabetes; Di, diarrhoea ; H, hypertension; I,infection; K, kidney stone ; M, migraine ; R, rheumatism; Se, sedative; Sp, spasmolytic; Sx, sexual stimulation; Te, teigne; Tu, tuberculosis.

ethnopharmacological data of the investigated plant species, including the species name and vernacular name of the plants, the plant parts used by the patients, the traditional uses and the percentage of importance citation against cancer.

The results presented in Table 1, revealed that the most important plants against cancer were in the following order at: *Aristolochia longa* (L): 24% (AL), *Citrullus colocynthis* (L): 21% (CC), *Delphinium staphisagria* (L): 14% (DeS) and *Piper cubeba* (L): 13% (PC).

Antiproliferative activity of the selected plant extracts

In this part, the antiproliferative activity of the selected plant extracts against cancerous cell lines was evaluated as percentage of viability relative to the control at 24 h after 24 h of treatment at a dose of 500 µg/ml of the plant extract. The IC_{50} values were estimated using GLMTOX software (Maul, 1992). With the exception of the extract of *D. staphisagria* and *A. longa,* the results presented in Figure 1 showed that *P. cubeba* and *C. colocynthis*, have an antiproliferative activities against all cell lines tested compared to control at 24 h, with strong effect against MCF7 and HT29 cell lines.

The results presented in Table 2 and Figure 1 show that the percentages of inhibitory activity compared to control at 24 h is in the following order: [PC 98.64% (±0.60) > CC 91.84 % (±0.60) > AL 83.12% (±0.11) > DeS 33.93% (±0.24)) and (PC 91.59% (±0.10) > CC 85.58% (±0.21) > AL 80.8% (±0.13) > DeS 5.75% (±0.40)] against MCF7 and HT29 cell lines, respectively. The results presented in Figures 2 and 3 show that the inhibitory activities of *P. cubeba* against MCF7 and HT29 cell lines are with an IC_{50} 34.2 µg/ml and with an IC_{50} 70 µg/ml, respectively. On other hand, *C. colocynthis* shows the inhibitory activities with an IC_{50} of 22.0 µg/ml and with an IC_{50} of 32.5 µg/ml against MCF7 and HT29 cell lines, respectively.

Morphological changes of MCF7 line during treatments with *C. colocynthis* (L)

The cytotoxicity of *C. colocynthis* extract against MCF7 proliferation was assessed by microscopical observations of the morphological and cytological changes.

Table 2. Antiproliferative activities of lyophilized extracts against cancer cell lines after 24 h of treatment.

Cell line	After 24 h (% ± SEM)		After 48 h (% ± SEM)			
	Control at 24h	Control at 48h	Treated with extract of			
			CC	AL	DeS	PC
MCF7	100 ± 0.28	+13.69 ± 0.87	-91.84 ± 0.60	-83.12 ± 0.11	-33.93 ± 0.24	-98.64 ± 0.60
HT29	100 ± 0.35	+16.7 ± 0.62	-85.58 ± 0.21	-80.8 ± 0.13	-5.75 ± 0.40	-91.59 ± 0.10
VCREMS	100 ± 0.12	+39.12 ± 0.15	-82.62 ± 0.10	+05.6 ± 0.90	+14.48 ± 0.18	-86.54 ± 0.29
H5-6	100 ± 0.11	+16.83 ± 0.25	-60.96 ± 0.82	-59.82 ± 0.7 7	+41.07 ± 0.65	-74.48 ± 0.28
N2A	100 ± 0.74	+51.65 ± 0.28	-69.88 ± 0.92	-22.47 ± 0.37	+41.87 ± 0.88	-76.08 ± 0.15

After 24 h of culture, cells were treated for 24 h with the plant extracts at concentration 500 µg/ml. The results are calculated as the percentage of cells viability compared to control at 24 h. The results are expressed at (% ± SEM) (N = 6).

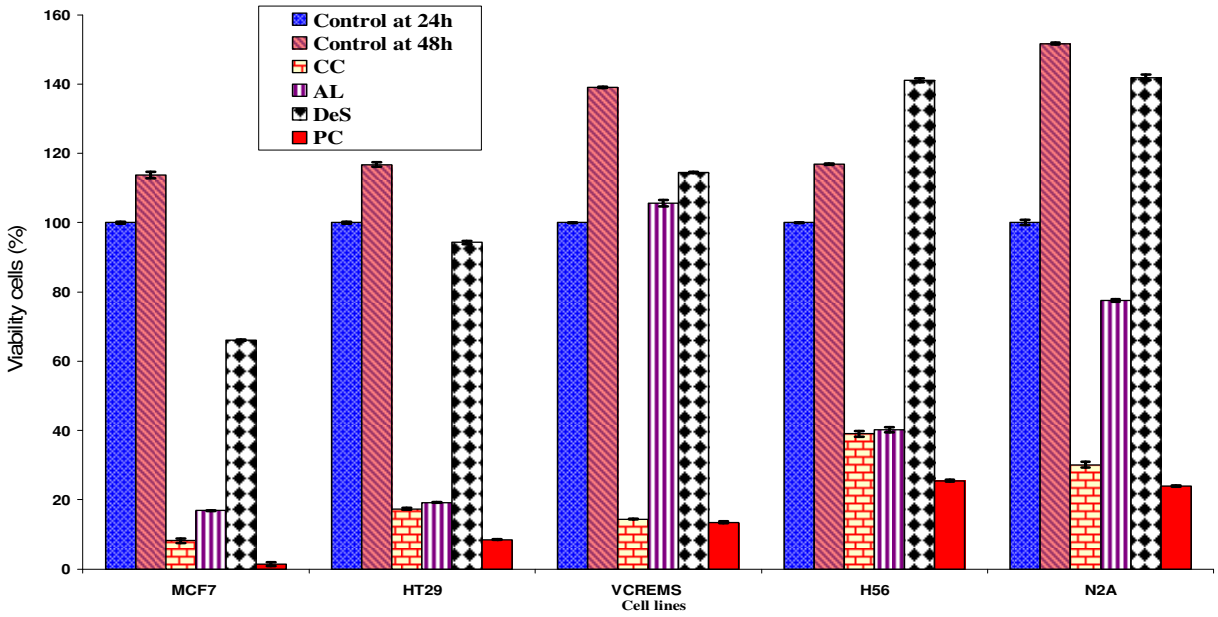

Figure 1. Antiproliferative activities of lyophilized extracts against cell lines after 24 h of treatment. After 24 h of culture, cell lines (MCF7, HT29, VCREMS, H5-6 and N2A) were treated for 24 h with 500 µg/ml of lyophilized extracts. The results are expressed as percentages of cells viability (% ± SEM) to control at 24 and 48 h (N = 6). *Aristolochia longa* (L): (AL), *Citrullus colocynthis* (L): (CC), *Delphinium Staphisagria* (L): (DeS) and *Piper cubeba* (L): (PC).

The microscopic observation of MCF7 cell line after treatment for 24 h at a concentration corresponding to IC_{50} (22.0 µg/ml), reveals a significant morphological changes (Figure 4) with a strictures cytoplasm, nuclear condensations and a detachment of the cells to the mounting bracket for cells treated with a passage in the form of elongated cells to the round, compared to untreated cells, where the previous transformations are absent.

DISCUSSION

The search for anticancer agents from natural resources is a big challenge for researchers worldwide. Several active natural products have been isolated from plants and are used to treat different types of cancer. Ethnopharmacological knowledge is useful to conduct the search for plants with a potential cytotoxic activity.

The present ethnopharmacological study is based on interviews with 100 herbalists practicing treatment with herbal alternative medicine in Fez city, Morocco in order to integrate the information collected between the relationship patient and herbalist in the collecting system of modern health care. Table 1 shows the most important plants sold by 100 herbalists in Fez city (Morocco) against cancer and are arranged in the following order; AL 24%, CC 21%, DeS 14% and PC 13%.

It is worth mentioning that the ethnopharmacological researchers in Morocco who are interested in treatment

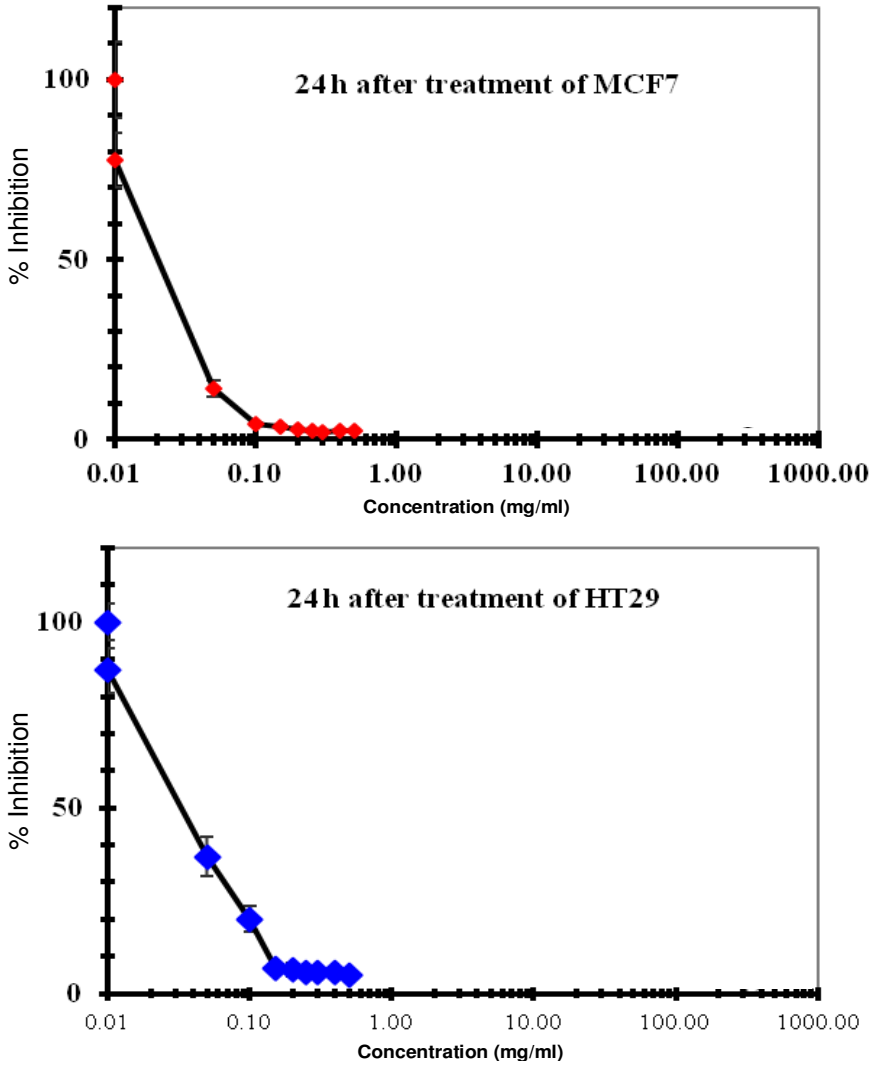

Figure 2. Determination of IC_{50} of the *C. colocynthis* lyophilized extracts on MCF7 and HT29 after 24 h of treatment. IC_{50} is calculated at 5% error using the software GLMTOX. The results are expressed in absolute absorbance ± SEM (N = 6).

fidelity of medicinal plants used against various diseases (Merzouki et al., 2000). Other ethnopharmacological studies aimed primarily at the uses of herbal medicine while neglecting very critical points such as conditions, place and time of collection of used herbs, in addition to right identification and adulteration (Hmamouchi, 1996; Bellakhdar, 1997).

Our field observations revealed that majority of the herbalists interviewed, are interested in the diseases currently incurable by modern medicine (the percentage of citation of cancer is 80%), and also the most frequent pathologies. This study suggested that the herbalists are always looking to accomplish the inability of modern medicine, based rather to give hope to the patient, unlike modern medicine which causes final verdicts.

The pharmacological studies in this work was designed to evaluate the cytotoxicity of selected Moroccan medicinal plants against five cancer cell lines (MCF7, HT29, N2A, VCREMS and H5-6) *in vitro* using MTT assay. The results in Figure 1 showed various degrees of activities for the tested plant against cancer cell lines, some are not cytotoxic on certain cell lines (DeS and AL). The results of the present *in vitro* cytotoxicity of the selected plants did not match the results of the ethno-pharmacological study, which suggests a certain caution should be taken towards the reputations of the uses of herbal medicine in the treatment of various diseases reported by patients or herbalists. In this sense, the previous work reported on the cytotoxicity of a group of plants of family Annonaceae against Fadu, Hep-2, fibro-blasts, HeLa and SVKO3 cell lines (Amaro et al., 1998).

In the previous study, we were interested on *C. colocynthis*, belonging to Moroccan flora. Table 2 shows that the line MCF7 is the most sensitive followed by other

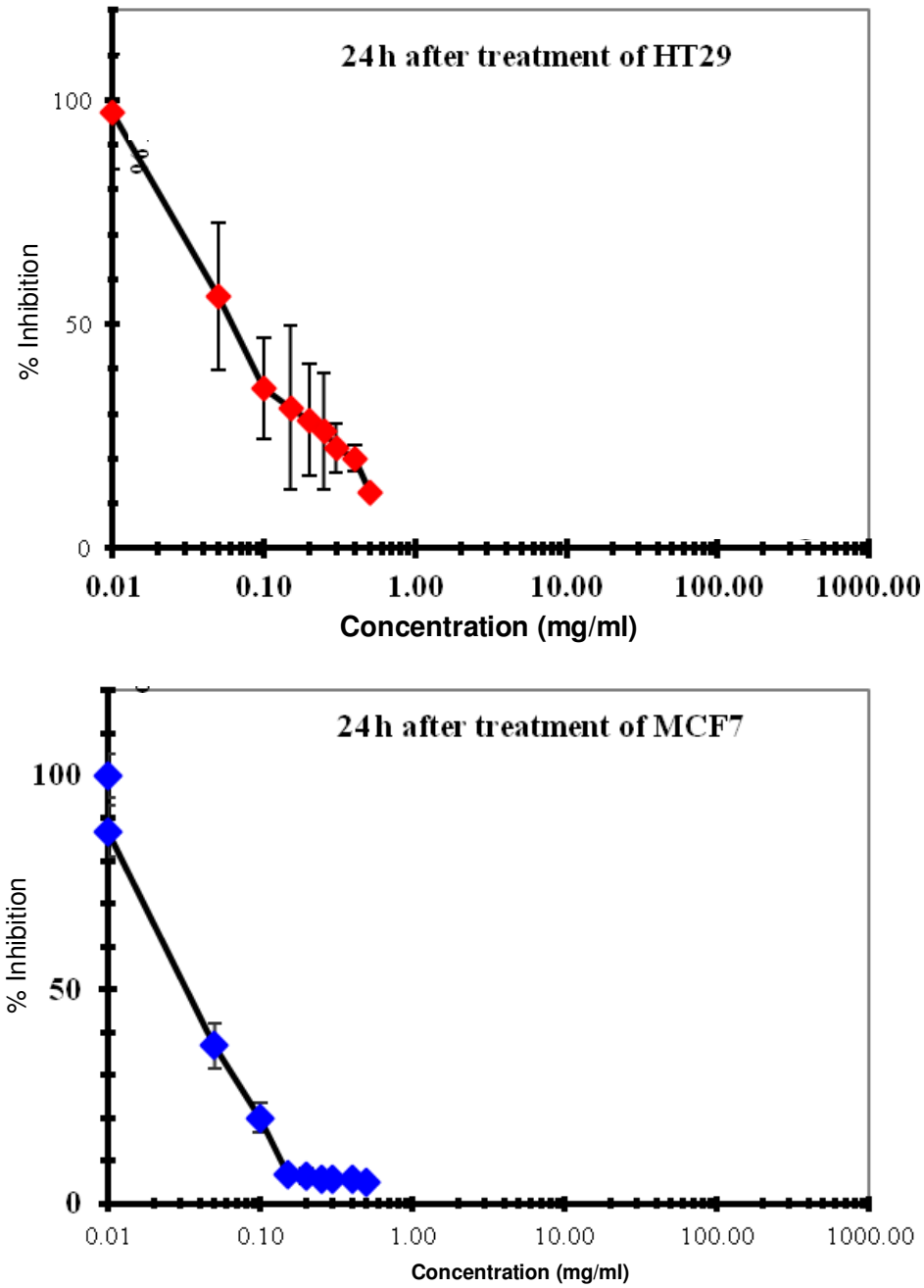

Figure 3. Determination of IC_{50} of the *P. cubeba* lyophilized extracts on MCF7 and HT29 after 24 h of treatment. IC_{50} is calculated at 5% error using the software GLMTOX. The results are expressed in absolute absorbance ± SEM (N = 6).

cell lines. It could be deduced that the cytotoxicity of cancer cells depends not only on the type of plant extract but also on the sensitivity of cancer cell lines. The dose response shows that the IC_{50} of *C. colocynthis* calculated on the cancer cell lines MCF7 and HT29 are important, respectively (22.0 and 32.5 µg/ml) after 24 h after treatment, this represents a significant sensitivity of the line MCF7 to extract *C. colocynthis*. The American National National Cancer Institute (NCI) assigned a significant cytotoxic effect of promising anticancer product for future bio-guided studies if it exerts an IC_{50} value < 30 µg/ml (Suffnes, 1990). The obtained results indicate that *C. colocynthis* were shown to induce significant and dose-dependent inhibitory activities against MCF7 and HT29 cancer cell lines.

The search for the cytotoxic mechanism action of *C.*

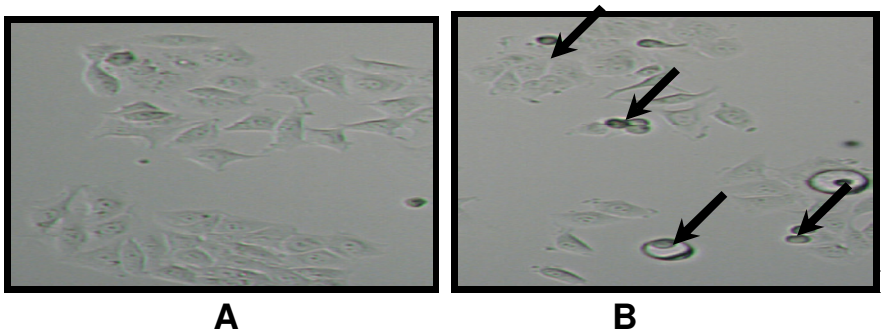

Figure 4. Morphological changes of MCF7 cells treated by *C. colocynthis* lyophilized extracts. The results are revealed by microscope after 24 h of treatment. A: untreated cells, B: treated cells. The arrows indicate apoptosis of MCF7 cells.

colocynthis on line MCF7 (most sensitive) appears to act by inducing apoptotic morphological changes (Figure 4). Indeed treatment of the MCF7 protein extract has led to changes in overall cell morphology of a stretched elongated shape to a round cell, indicating that the treatment caused impairment of protein filaments of cellular organization.

This morphological change could also affect intracellular signaling by molecules such as PKB, resulting in inhibition of survival signaling. The same hypothesis was obtained by other studies that showed an antiproliferative activity of cucurbitacin glucoside isolated from *C. colocynthis* by an interruption in the form of elongated cells to an appearance of the round shape during processing lines MCF7 and MDA-MB -231 (Tannin-Spitz et al., 2007).

Conclusion

In this study we are demonstrated *in vitro* that the *Citrullus colocynthis* L, family Cucurbitaceae, widely distributed in north-central Morocco regions, as a potential source of anticancer drug against five cancers cell lines (MCF7, HT29, N2A, VCREMS and H5-6) with a promising activity against MCF7cell line. Further investigations are necessary for the isolation and characterization of bioactive constituents and elucidation of the mechanism of inhibition of proliferation by induction of apoptosis caspase-dependent and involving a mitochondria-mediated signaling pathway. Moreover, it will be interesting to run some *in vivo* models to evaluate the anti-tumor activity of these plant extracts in future.

ACKNOWLEDGEMENTS

The authors are grateful to Professor D. Bagrel for the use of her Laboratory (Laboratory of Molecular Engineering and Pharmacological Biochemistry, University Paul Verlaine, Metz, France) and they also thanks Professor A. Ennabili for the identification of the plant materials (National Institute of Medicinal and Aromatic Plants Taounate, Morocco).

REFERENCES

Amaro MI, Orfila L, Colman T (1998). Cytotoxic effects in vitro of the extracts of plants from annonaceous in tumoral and normal cell. Toxicol. Lett. 95:153-153.

Bellakhdar J (1997). La pharmacopée Marocaine Traditionnelle, Médecine Arabe Ancienne et Savoirs Populaires. Ibis Press, Paris, France.

Daoudi A, Benbouker H, Bousta D, Aarab L (2008). Screening of fourteen Moroccan medicinal plants for immunomodulating activities. Moroccan J. Biol. 5:24-30.

De Smet PA (1997). The role of plant derived drugs and herbal medicines in healthcare. Drugs. 54:801-840.

Gordaliza M (2007). Natural products as leads to anticancer drugs. Clin. Tran. Oncol. 9:767-776.

Hmamouchi M (1999). Les Plantes Médicinales Marocaines: Utilisations, Biologie, Ecologie, Chimie, Pharmacologie, Toxicologie, Lexique. Rabat Institute, Morocco.

Klebe RJ, Ruddle FH (1969). Neuroblastoma: Cell culture analysis of a differentiating stem cell system. J. Cell Biol. 43:69a.

Lamchouri F, Settaf A, Cherrah Y, Hassar M, Zemzami M, Atif N, Nadori EB, Zaid A, Lyoussi B (2000). In vitro cell toxicity of *Peganum harmala* alkaloids seeds on cancerous cell-lines. Fitoterapia 71:50-54.

Lesuffleur T, Barbat A, Dussaulx E, Zweibaum A (1990). Growth adaptation to methotrexate of HT-29 Colon Carcinoma Cells is Associated with their Ability to Differentiate into Columnar Absorptive and Mucus-secreting Cells. Cancer Res. 50:6334-6343.

Mans DR, da Rocha AB, Schwartsmann G (2000). anticancer drug discovery and development in Brazil: targeted plant collection as a rational strategy to acquire candidate anticancer compounds. Oncol., 5:185-198.

Maul A (1992). Application of generalized linear models to the analysis of toxicity test data. Environmental. Monitoring and Assessment. 23:153-163.

Ma X, Wang Z (2009). Anticancer drug discovery in the future: an evolutionary perspective. Drug Disc. Today 14:1137-1142.

Merzouki A, Ed-derfoufi F, Molero Messa J (2000). Contribution to the knowledge of Rifian traditional medicine. II: Folk medicine in Ksar Lakbir district (NW Morocco). Fitoterapia. 71:278-307.

Mosmann T (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J.

Immunol. Methods 65:55-63.
Newman DJ, Cragg GM (2007). Natural products as sources of new drugs over the last 25 years. J. Nat. Prod. 70:461-477.
Pitot HG, Peraino C, Morse PAJr, Potter VR (1964). Hepatomas in Tissue Culture Compared with Adapting Liver *in vivo*. Natl. Cancer Inst. Monit. 13:229-245.
Rastogi T, Hildesheim A, Sinha R (2004). Opportunities for cancer epidemiology in developing countries. Nat. Rev. Cancer 4:909-917.
Soule HD, Vazquez J, Long A, Albert S, Brennan M (1973). A cell line from a pleural effusion derived from a breast carcinoma. J. Nat. Cancer Inst. 51:1409-1416.
Suffnes M, Pezzuto JM (1990). Assays related to cancer drug discovery. In: Hostettmann,K. (Ed). Methods in Plant Biochemistry: Assays for Bioactivity. Academic Press, London 6:71-133.
Tannin-Spitz T, Grossman S, Dovrat S, Gottlieb HE, Bergman M (2007). Growth inhibitory activity of cucurbitacin glucosides isolated from *Citrullus colocynthis* on breast cancer cells. Bioch. pharmacol., 73:56-67.
Whelan RD, Waring CJ, Wolf CR, Hayes JD, Hosking LK, Hill BT (1992). Over-expression of P-glycoprotein and glutathione S-transferase pi in MCF-7 cells selected for vincristine resistance *in vitro*. Int. J. Cancer 52:241-246.
WHO (2012). World Health Organization warns of growing "crisis of suffering". Report of the world health 1997/ Press release/ (www.who.int). Geneva, Switzerland.