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Ethnopharmacological studies of *Mesembryanthemum nodiflorum*

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Abstract

Ethnopharmacological surveys conducted in Morocco allowed us to identify many plants species, the most prescribed and used in traditional medicine to treat cancer. From these species, we chose to study *Mesembryanthemum nodiflorum.* We prepared various extracts (alkaloid, cyclohexane, dichloromethane and methanolic) of the *Mesembryanthemum nodiflorum* aerial parts, the phytochemical studies revealed that the plant contains sterols, sapogenines, triterpenes, tannins and alkaloids. The chemical analysis of cyclohexane extract results in the identification of known constituents and the alkaloid extract has been studied by different chromatographic methods to isolate many fractions and to study their chemical composition. These investigations revealed the presence of hordenin. Cytotoxic activities were screened by an *in vitro* assay system of growth inhibition against two human cancer cell line, namely breast cancer cell line (MCF7) and cervix adenocarcinoma (HeLa), and the results demonstrated that all various extract of *Mesembryanthmum nodiflrum* did not show significant cytotoxic activity on MCF and Hela cells, at concentrations ranging from 31.25 to 1000 µg/ml. The antioxidant activity of various extracts of *M. nodiflo-rum* was evaluated by DPPH test and showed that all extract exhibited higher radical scavenging activity, as to standard used, Trolox. The dichloromethane extract of *Mesembryanthemum nodiflorum* present an anti-radical activity estimated to 94.39 ±0.51% ($p<0.001$). These results suggest that the products of *Mesembryantemum nodiflorum* may provide a new therapeutic avene.

Keywords: *Mesembryanthemum nodiflorum*, Cytotoxicity, Antioxidant activity, hordenin

Introduction

The area of medicinal and aromatic plants is experiencing a high research activity and development across the world in order to better understand the natural resources used and to discover new sources of raw materials. According to the World Health Organization, 80% of people in developing countries resort to herbal medicine through traditional medicine (Sheeal Verma et al., 2008).Nowadays, the exploitation of medicinal and aromatic plants has blossomed, given the close relationship imposed by the added value of this sector to the economical and the social impact. Currently, and for better exploitation of new active ingredents, healers propose to make a scientific assessment, their remedies and recipes of traditional medicine, often used in treating various diseases, including cancer (Gonzalez-Tejero et al., 2008). Like other developing countries, by its rich and diverse flora, and by increased use of medicinal plants, Morocco has to force the validation of the traditional use of plants that is based primarily on literature and experimental searches. Indeed, Morocco has great potential in plants, with about 42,000 plant species in 150 families' devisees and 940 genera, about 1500 introduced species have been catalogued (J. Bellakhdar, 1997; Tahraoui A. et al., 2007; J. El-Hilaly et al., 2003). Morocco is one of the Mediterranean countries with a long medical tradition and expertise in traditional herbal (Scherrer et al., 2005). Our work is a contribution to the promotion of industry and medicinal and aromatic plants whose objective is to evaluate the cytotoxic and antioxidant activity of organic extracts of the *Mesembryanthemum nodiflrum* plant, antioxidant properties (Hanen F, et al., 2009) and antiviral (Sassi AB et al., 2008) were associated with gender and Mesembryanthemum this species and to our knowledge, has never been the subject of chemical and pharmacological investigations.

Materials and methods

Study area

The ethnopharmacological survey was performed in the different cities of Morocco: Rabat, Sale, Agadir and Taza. The sites were selected preferentially based on the information obtained from the healers and botanical experts with a different climatic conditions and biotopes, presented diverse flora.

Ethnopharmacological survey

The ethnopharmacological survey was carried out for six month (01 September - March 2009) and was performed with the permission of Public Health and local authorities. The information on the medicinal use of plants has been recorded, a total of 72healers were interviewed and have been informed about the objective of this study. The information collected included: name of herbalist and the drug used: botanical and vernacular name, date and place of gathering information, indication, the geographical and ecological distribution of the species, part of the plant being used (leaves, fruit, aerial part, root, seeds), method(s) of preparation and details of administration, including the approximate amounts and number of doses per day.The ethnobotanical data has been analyzed using quantitative methods of data analyses. Descriptive statistics like percentage and frequency distribution have been used to analyze the data collected through interview.

Plant Material

Mesembryanthemum nodiflorum plant (Aizoaceae) was collected fromTiznit region (S of Morocco) with respecting the United Nations Convention of Biodiversity and with assistance of traditional medical practitioner. The plant was identified with botanist of scientific institute (Pr. M. Ibn Tatou). A voucher specimen (RAB 77767) was deposited in the Herbarium of Scientific Institute, University Mohammed V–Rabat–Morocco

Classical extraction

500 g of plant material areal parts were extracted in approximately 500 mL cyclohexane for 6 h using Soxhlet apparatus, cyclohexane containing the extract was then filtered through Whatman paper the extraction followed by removal of the solvent on rotary evaporator (Rotavap: Buchi). The residue of the plant was successively extracted with dichloromethane and methanol by maceration at room temperature (25°C) over period of 24 hours. The extracts were then concentrated at reduced pressure, to give a total of cyclohexane, dichloromethane and methanolic crude extracts equal to 11.25; 2.75 and 74.25 g respectively, the remaining extracts were finally dried in the oven at 30°C for two hours to ensure the removal of any residual solvent, the remaining extracts were finally dried in the oven at 30°C for two hours to ensure the removal of any residual solvent. The cyclohexane extract was analyzed by GS–MS and led us to identify height compounds.

Alkaloidic extraction

One kilogram of powdered areal parts of *M. nodiflorum* was extracted at room temperature with CH_2Cl_2/NH_4OH. The extract was subjected to an alkaloidal extraction procedure and produced a total of 1.794 g. The extract was chromatographied over silica gel and eluted with a mixture of CH_2Cl_2/NH_4OH) (99/1) followed by a gradient of $CH_2Cl_2/MeOH/NH_4OH$) as mobile phase. This separation led to 58 fractions. Similar fractions were pooled. Fraction (40–46) yielded 26 mg was then analyzed by GS–MS and led to identify four compounds.

Qualitative phytochemical analysis

Various types of phytoconstituents: Alkaloids, flavonoids, tannins, anthocyanin and triterpenes etc. may be present in the plant. Qualitative phytochemical analyses of all the extracts were being carried out by using standard methods (Rasineni GK et al 2008, Evans and Trease 1983).

Toxicological study

Experimental animals

Swiss mice (20-25g) (IOPSOffa) were acquired from the animal experimental center of Mohammed V-Souissi University, medicine and pharmacy faculty – Rabat. They were housed three per plastic cage, at constant room temperature (23 ± 1 °C) with free access to water and at ad-libitum feeding and maintained on a 12 h: 12 h day/night. Care of the mice were in compliance with the guidelines of the guide for the care and use of laboratory animals (Commission on life science, national research council 1996)

Evaluation of acute toxicity

Acute toxicity test was performed according to the World Health Organization (WHO) guideline (WHO 2000) and the Organization of Economic Co-operation and Development (OECD) guideline for testing of chemicals 423 (OECD 2001). The extracts of plant were administered by orally way in a single dose (2000 mg / kg) of body weight. The control group received only the water. Mice were continuously observed for 6h after treatment and intermittently for 4h and over period of 24h (Twaij, 1983), all signs of toxicity and deaths and their latencies were recorded. At the end of the study animals were sacrificed for macroscopic tissue examination and the LD_{50} was determined.

Antioxidant activity

DPPH radical scavenging capacity estimation

The free radical scavenging activity of the extracts of *M. nodiflorum* aerial parts were measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH)according to the method of Sanchez-Moreno C (2002).0.2 mmol l^{-1} solution of DPPH in methanol was prepared and 0.5 ml of this solution was added to 2.5 ml of plant extracts and were allowed to stand at room temperature for 30 min, and then absorbance was read using a UV-VIS spectrophotometer at 517 nm against blank samples. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The radical-scavenging activity (RSA) was calculated as a percentage of DPPH discoloration, using the equation:

$$\% \text{ RSA} = [(Ac-Ap)/Ac] \times 100$$

Ac: the absorbance of the control reaction; Ap: the absorbance of test samples

Where (Ac) is the absorbance value of the DPPH blank sample, and (Ap) is the absorbance value of the test solution. (Ap) was evaluated as the difference between the absorbance value of the test solution and the absorbance value of its blank.

In vitro cytotoxic activity assay

Cell lines and culture medium

Cervical cancer cell lines obtained from the American Type Culture Collection (ATCC) (HeLa) and MCF-7 (breast adenocarcinoma) were used in this study. Cells were grown at 37°C in humidified 5% CO_2 and 100% relative humidity atmosphere in Dulbecco's Modified Eagle Media (DMEM) (1% glutamine, 100 U/ml Penicillin-Spreptomycin mixtures and 10% fetal bovine serum).

Cytotoxicity assay

Cytotoxicity of sample on tumor cells was measured by microculture tetrazolium (MTT) assay (Mosmann 1983). For the assays, 96-well microplates were seeded with 100 µl medium containing 10, 000 cells in suspension. After 24 h incubation and attachment, the

cells were treated with 6 fourfold dilution of crude extracts. Exactly from the stock solution (40 mg/ml), each extract sample was applied in a series of 6 dilutions (final concentrations ranging from 31.25 to 1000 µg/ml) with a final DMSO concentration of 0.1% and was tested in quadruplicate. After 48 h incubation, cell viability was determined by adding (Sigma) tetrazolim salt as cytotoxicity indicator and by reading absorbance at 590 nm with a scanning multiwell spectrophotometer (Spectra Count, Packard, Ont., Canada). Tetrazolium salts are cleaved to formazan dye by cellular enzymes (only in the viable cells). The level of absorbance directly correlates to the metabolically active cells. Vinblastine was used as a positive control.

Statistical Analysis

The statistical analysis was performed by one-way ANOVA analysis of variance test, and results were considered to be statistically significant with a 95 % confidence level ($P<0.05$).The data are expressed as mean ± SD (standard deviation).

Results

Ethnopharmacological survey

The data recorded in table 1 are arranged in alphabetical order according and which contains the scientific, vernacular and common name of the plant, its ecological distribution, the part of the plant and the preparation used the therapeutic indication and the frequency (number of informants).During the field study, 35 local plants distributed in 33 genera belonging to 19 families were found to be used to treat cancer,The most dominant families in the study were Lamiaceae (8 species) and Asteraceae (4 species).Other families with low number are listed below: Aizoaceae (1), Leguminosae (1), Apiaceae (2), Liliaceae (2), Apocynaceae (1), Pinaceae (1) Arsitolochiaceae (1), Punicaceae(1), Poaceae (1) Berberidaceae(1), Ranunculaceae (1), Capparaceae(1), Tamaricaceae (1), Caryophyllaceae(2), Violaceae (1), Cruciferae(1), Zingiberaceae (1), Euphorbiaceae (1), Zygophylaceae (1).The analysis of collected questionnaires showed that 10 medicinal plants are the most used, The species most commonly cited are *Aristolochia longa*(90%), *Berberis vulgaris* (88%), *Euphorbia resinifera* (72%), *Cuminum cyminum*(60%), *Pimpinella anisum* (70%), *Anacyclus Perythrum* (56%), *Corrigiola telephiifolia* (50%) and*Mesembryanthemum nodiflorum* (48%) were reported from all the informants, followed *Origanum vulgare* (53%), *Nigella sativa* (50%). Different parts of medicinal plants were used. Among the different plant parts, leaves (12 species) and seeds (11 species) were most frequently used for the treatment of diseases followed by root (7 species), flowers (4 species), aerial parts (4 species), Bulb (2 species), fruit (1 species), stem (1 species) and bark (1 species).

Mode of preparation, route of administration and indication

Local people employed variety of methods in order to prepare remedies, plant parts were generally consumed in the form of a decoction (8 species), macerated material or as infusion (7) using water but occasionally remedies were prepared with honey (17) or powder of plants with water (2). The solubility of active components in water made it commonly used in the traditional medicine preparation. Honey may be used for their properties to disso-

Table 1. Medicinal plants used in traditional medicine in Morocco for treatment of cancer.

Botanical name (Voucher No)	Type of Cancer	Part used		Reported activities	Frequency (%)
Aizoaceae					
Mesembryanthemum nodiflorum (2716)	Digestive	AP	Pd with hy	P. Sathiyamoorthy et al, 1999. Falleh Hanen et al, 2009	48
Apiaceae					
Angelica Archangelica (2881)	Digestive, kidney	Lf, Sd, R	decoct.	Nikolay A. Spiridonov et al, 2005; A. Bogucka-Kocka et al, 2008	30
Cuminum cyminum (2902)	Lung, digestive	Sd	pd with wt	K. Aruna et al, 1992	60
Apocynaceae					44
Nerium oleander (4631)	Cervical	Lf	decoct.	Luay J. Rashan et al, 2011	
Arsitolochiaceae					
Aristolochia longa (6135)	General	R	pd with hy	Amar Djeridane et al, 2010	90
Asteraceae					
Artemisia herba-alba (3989)	Breast,	AP	infus.	F. Bakkali et al., 2005 " cytotoxicity in yeast	5
Anacyclus Perythrum (3949)	Liver, lung	R	pd with hy	N.D	56
Cnicus benedictus (4100)	Digestive, Liver	Lf, Fl	decoct.	V.Steenkamp; M.C. Gouws. 2006	
Inula viscosa (3690)	Digestive, kidney	Lf, Fl	pd with hy	Rozenblat et al., 2008	7
Berberidaceae					
Berberis vulgaris (24037)	Liver,	R	pd with hy	Silvia Letasˇiova´ et al., 2005	88
Capparaceae					
Capparis spinosa (60734)	Leukemia	Ft , Fl	pd with hy	Ze-Kwan La,Tzi-Bun Ng;2009	3
Caryophyllaceae					
Corrigiola telephiifolia (5905)	Cervical, Liver	R	pd with hy	N.D	50
Herniaria glabra (5902)	Digestive, Kidney	Sd	decoct.	N.D	2
Cruciferae					
Lepidium sativum (NI)	Digestive	Sd	pd with hy	N.D	1
Euphorbiaceae					
Euphorbia resinifera (6370)	General	AP	pd with hy	Noureddine Mazoir et al. 2008	72
Lamiaceae					
Ajuga iva (5865)	Breast	Lf , St	pd with hy	N.D	6

Lavandula officinalis (5793)	uterus	Lf	Infus.	N.D	2
Marrubium vulgare (5821)	Digestive	Lf, R	Decoct.	N.D	21
Ocimum basilicum (NI)	Liver, digestive	Lf	Decoct.	Jiradej Manosroi et al., 2005	20
Mentha pulegium L. (5759)	Gingival, lung	Lf, R	Infus.	N.D	24
Rosmarinus officinalis (5797)	lung, liver, digestive	Lf	Decoct.	Abdelfatteh El Omri et al., 2010	9
Thymus vulgaris (5768)	Digestive, gensive	WP	Infus.		11
Thymus ssp. (5768)	Digestive	AP	Infus.	M.J. Gonc¸ alves et al., 2010	8
Leguminosae					
Trigonella foenum-graecum (00337)	Digestive	Sd	pd with hy	Amr Amin et al., 2005	40
Liliaceae					
Allium sativum (7476)	General	B	infus.	F.H. Abdalla et al, 2010 ; Thakur Uttam Singh et al, 2009	14
Allium cepa L. (7476)	General	B	infus.	Andrade-Vieira et al 2011	2
Linaceae					
Linum usitatissimum (3945)	leukemia	Sd	pd with hy	N.D	8
Pinacées					
Pinus halepinsis (NI)	Esophagi	Sd	pd with wt	ND	3
Punicaceae					
Punica granatum 2516	Skin	Rd	decoct.	Ephraim P. Lansky, Robert A. Newman, 2007	9
Poaceae					
Hordeum vulgare 8277	Liver, intestin	Sd	pd with hy	N.D	3
Renonculaceae					
Nigella sativa L 10359	Liver	Sd	pd with hy	S.M.K. Swamy, B.K.H. Tan, 2000	27
Tamaricaceae					
Tamarix articulata (13501)	General	Lf	pd with hy	ND	2
Violaceae					
Viola Odorata 5045	Liver, lung	Lf, Fl	Decoct.	Wenjun He et al., 2011	4
Zingiberaceae					
Zingiber officinale et al., 2011 3 (NI)	General	R	pd with hy	Xiao-Lan Cheng	
Zygophylaceae					
Peganum harmala (76716)	Liver, digestive	Sd	pd with hy	F. Lamchouri et al., 1999	12

AP: aerial parts, Lf: leaf, Sd: seed, R: Root, Fl: Flowers, pd with hy: Powder with honey, pd with wt: powder with water, decoct: decoction, infus: infusion, B: Bulb, NI : not identified in Scientific Institute.

Table 2. Qualitative Phytochemical analysis of various extracts of*Mesembryanthemum nodiflorum*

Identification tests	Compounds	Quantity
Cyanidrine	Flavonoids	-
Hclreaction	Anthocyane	-
Stiasnyreagent	Tanins	++
Leibermann-Burchard	Triterpenes	+++
Dragendorff	Alkaloids	+
Anisaldehyde	Steroidsand sapogenines	++

(+): presence; (++): considerable presence; (+++): abundance; (-): absence

lve active phytochemicals that are not water soluble. Oral (34 species) was the most commonly used route of administration, and was followed by nasal (1). The ethnobotanical survey has revealed that the plants preparations are used against many types of cancer: digestive (10 species), liver (8), lung (4), breast (2), kidney (2), cervical (1), Esophagi (1), uterus (1), skin (1), gingival (1) and others (6).

Chemical Composition of Moroccan M. nodiflorum plant

Preliminary phytochemical screening of various extracts of *Mesembryanthemum nodiflorum* showed the presence of sterols, sapogenines, triterpenes, tannins and alkaloids (Table 2). Main attraction of phytochemical screening was presence of tannins and alkaloids in maxim-um of extracts, these phytoconstituents were known to show medicinal activity (Rasineni GK et al., 2008). The results obtained by GC-MS analyses of the alkaloidic fraction of *M. nodiflorum* show that four compounds were identified in this extract: hordenin;(M)3-Hydro-xycarbofuran; (M) Phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy; (R)124-Cyclopenta-netrione-3-(2-pentenyl).In the cyclohexanic extract were identified 8 componenents:2-Heptanal, Trans 2,4-Decadiena; Pentadecanone, 6, 10, 14- trimethyl; n-Tricosane; n-Pentacosane; n-Heptacosane; n-Nonacosane; Hentriacontane. The components found were identified by com-paring their mass spectra with literature data and commercial mass spectra library

Acute toxicity

The oral administration of a single dose (2000 mg/kg bodyweight) of *M. Nodiflorum* extracts (cyclohexane, dichloromethane and methanol) to mice did not cause death within the fourteen days of the study. The evolution of the weight varied in the mice weighed daily. Based on the symptoms observations animals under positive control group treated orally with the *M. Nodiflorum*, Mice were perturbed with abdominal contraction during the first 30 minutes in 80% of mice. These effects are disparate in 1h after the treatment. Generally At a dose of 2000 mg/kg, the extracts of the plants don't lead to mortality by orally way. Under the system of global harmonization of Chemicals (GHS), this product is classified Category 5, which the higher LD50 is 2000mg/kg.

Free Radical Scavenging Activity

Antioxidant activity measured in extracts obtained using DPPH assay was measured three times to test the reproducibility of the assays. The DPPH radical scavenging is a comm only used method to evaluate the ability of plant extracts to scavenge free radicals generated

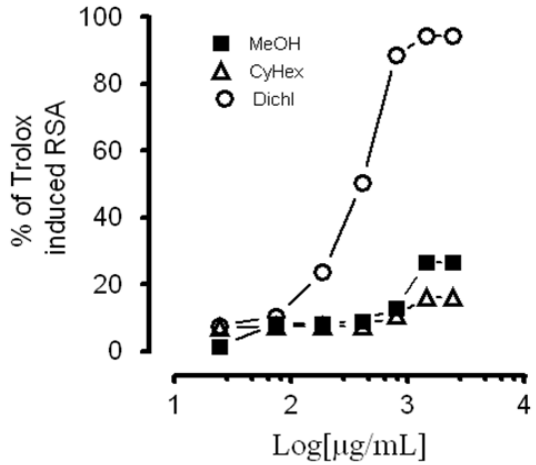

Figure 1. Scavenging effects of leaves of *Mesembryanthemum nodiflorum* cyclohexane, dichloromethane and methanol extracts on DPPH radicals. Trolox was used as a positive control. The free radical scavenging activity Percent of scavenging was plotted via the log of the concentration. All samples were run in triplicate (n=3).

from DPPH reagent (Chung et al., 2006; Angel Gabriel Rajamma et al 2012). Figure 1 shows that the DPPH scavenging activity in extracts was concentration-dependent (increasing from 15.625 µg/ml to 500 µg/ml) and the dichloromethane extract was able to inhibit the formation of DPPH radicals with a percentage inhibition of 94.39 ± 0.51% at the highest concentration. At this concentration, the DPPH radical scavenging capacity of the dichloromethane extract of *M. nodiflorum* was almost similar to the trolox ($P<0.001$). The methanolic extract shows a relatively low activity (26.55 ± 2.60%) followed by the cyclohexanic extract with a percentage inhibition of 16.12 ± 1.41%, which is practically ineffective, no significant difference is observed for those extracts ($p> 0.05$).

Cytotoxic activity

The methods most widely used for measuring cell proliferation and viability, as parts of an antitumor screening of new drugs are those using tetrazolium salts. For this study, we used the MTT assay, which is the first colorimetric method applied for the quantification of cell proliferation (F. Denizot et al., 1986; J. Carmichael et al., 1987), for its reliability, sensitivity and its many benefits. The cytotoxic activity was evaluated on two human cell lines, cervical (HeLa) and breast adenocarcinoma cell lines (MCF7), by measuring cell viability using the MTT assay. Cells were treated for 48h by different extracts (cyclohexane, dichloromethane and methanol) at different concentrations ranging from 1000 to 31.25 ug / ml. The data are presented in figure 1. No significant cytotoxic effect is observed on two cancer cells *in vitro*, the IC_{50} of the various extracts is more or equal to 1 mg / ml.

Discussion and conclusion

The DPPH free radical assay has been extensively used for screening antioxidant activity because it can accommodate many samples in a short period and it is sensitive enough to detect active ingredients at low concentrations. For each extract, the capacity to scavenge the DPPH radical depends on its concentration. The value of the effective concentration, which reduces 50 % of the initial concentration of DPPH (EC_{50}) that is inversely proportional to the

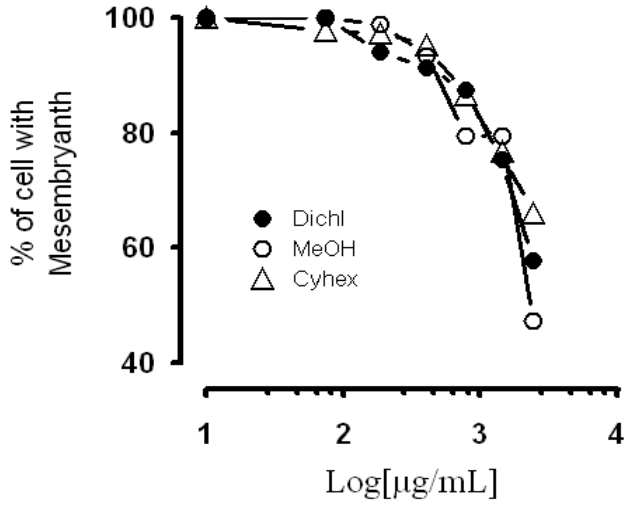

Figure 2. Cytotoxic activity of cyclohexane, dichloromethane and methanol crude extracts against Hela cells; Cells were incubated with different concentrations of the plant extracts (ranged from 31.25 to 1000 µg/ml) for 48 h.Cell viability was determined by the MTT assay (n=4).Viability curves: Percentage viability = absorbance of test wells/absorbance of control wells) × 100) plotted against the concentration of extract.

antiradical efficiency of the tested product. For each extract, the ability to scavenge the radical DPPH was concentration dependent; the value of the effective concentration that reduces 50% of the initial concentration of DPPH (EC_{50}) is inversely proportional to the antiradical efficiency of the tested product. The dichloromethane extract showed great efficacy in reducing the DPPH radical with an EC_{50} of 112.3 µg ml^{-1} ($p < 0.001$). This activity can be explained by the presence of a large proportion of tannins in this extract. Studies have shown the ability of tannins to react with the DPPH radical. The cyclohexane extract is less active due to a small amount of phytoactive compounds, which explains its relatively small effect, that further suggests this activity results from the involvement of several compounds even those, which are present in small amounts; for example, the moderate activity observed with the methanol extract (EC_{50} = 438,4 µg ml^{-1}).

The study of the cytotoxic effect of different extracts prepared from the plant was conducted in order to identify other therapeutic properties other than antioxidant activity; for instance, antitumor activity. Since ancient times and through their therapeutic properties, plants have always been an inexhaustible source of potentially bioactive substances. In the field of cancer, plants have an interesting alternative for screening of numerous cytotoxic molecules. Meanwhile, drugs that are derived from plants and used in cancer chemotherapy are numerous. Morocco has a rich flora in marked endemism, and a great knowledge of medic-ation derived from Medicinal Plants, which constitute an asset to the search for new thera-peutic molecules, particularly in the field of cancer. Antineoplastic drugs are generally selected for their antiproliferative effect on cancer cell lines in culture. The techniques used to evaluate the antiproliferative or cytotoxic effect in vitro are characterized by their simplicity of implementation, their specificity, sensitivity which make them preferred over all the techniques using in vivo models (G. Eisenbrand et al., 2002) .

The IC_{50} value of 50% of cell viability accounts for the benefit of the substance tested as an anticancer agent. The NCI (National Cancer Institute) believes that to be a good candi-

date, the IC_{50} value has to be less than or equal to 30 ug / ml. Substances that showed a significant cytotoxic effect may be used for further biodirected studies (Suffness and Pezzuto, 1999). The choice of the plant is justified by its anticancer and antipyretic properties in traditional medicine and also as an emetic in acute intoxications, such as those associated with arsenic (Bellakhdar, 1997). Its richness in alkaloids thrilled us to study its possible cytotoxic effect (Sweilam, NZ, BS El-Menshawi et al, 1990). The cytotoxic effect was evaluated by studying the antiproliferative activity on two human cancer cell lines HeLa and MCF7. These lines remained refractory to the addition of three extracts of the plant and IC_{50} values obtained are greater than 1 mg / ml. It is possible that this result is related to the tumor origin of human cancer cell line used. Indeed, the NCI (National Cancer Institute) recommends testing new anticancer agent of 60 strains belonging to seven categories tissue: leukemia, melanoma, lung, colon, kidney, brain and ovary (MR Boyd, 1989)

Conflict of interest

There is no conflict associated with authors of the paper.

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