TANG HUMANITAS MEDICINE

Review

Recent advances in pharmacologic study of anticancer natural products from medicinal plants in Morocco

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ABSTRACT

The aim of this study is to collate all available data on experiments reporting the antiproliferative, cytotoxic effects of plants and natural products in Morocco in the last two decades. A bibliographic investigation was carried out by analyzing recognized books and peer-reviewed papers, consulting worldwide accepted scientific databases (Scirus, Embase, HighWire, MEDLINE/PubMed, LILACS, Ovid, ScienceDirect, SciELO, Google Scholar). We used medical subject heading terms and the words 'anticancer', 'antiproliferative', 'antineoplastic', 'antitumoral', 'cytotoxic', 'Morocco', to identify relevant articles. Moroccan plants with attributed anti-cancer properties studied as plant extracts that have been evaluated for cytotoxic effects, antitumoral effects, plants with active compounds tested on cancer cell lines, and plants with active compounds that have been assayed on animal models were chosen for this research. In the present study, interest is focused on experimental research conducted on medicinal plants, particularly those which show antiproliferative or cytotoxic activities alongside bioactive components. A total of 20 plant species belonging to 12 families have been identified as active or promising sources of phytochemicals with antiproliferative properties. The plant families, which cover all the species studied in this field, are Lamiaceae (7 species) and Asteraceae (4 species); the most studied species being *Argania spinosa* (Sapotaceae) and *Arisarum vulgare* (Araceae), *Thymus* Genus (Labiateae) and *Peganum harmala* (Zygophyllaceae). Based on the search results, it is recommended to increase the number of experimental studies and to begin conducting clinical trials with Moroccan plants and their active compounds selected by *in vitro* and *in vivo* activities.

Keywords antiproliferative, anticancer, cytotoxic, medicinal plants, Morocco

INTRODUCTION

The number of cancer deaths has increased dramatically; the overall trend is due to variations of the causes of cancer leading to different types of cancer. These variations make the task of finding effective treatments very difficult. Although cancer is the world's second biggest killer after cardiovascular disease, it is one of the most preventable non-communicable chronic diseases. The WHO reported that cancer killed 7.6 million people in 2005, three quarters of whom were in low- and middle- income countries. By 2015, that number is expected to rise to 9 millions and increase further to 11.5 million in 2030. Up to 40% of all cancer deaths can be avoided (WHO).

Cancer chemotherapy is an important alternative to surgery and radiation to treat successfully some types of solid tumors, lymphomas, and leukemias, and many clinically approved cytotoxic and antiproliferative anticancer drugs are available, both of synthetic and natural product (microbial and plant) origin (Chabner et al., 2005; DeVita et al., 2008). Due to the high mortality rates of cancer and the absence of effective chemotherapy, there is a continued need for new alternatives for treatment and prevention.

Plants used in folklore medicine continue to be an important source of discovery and development of novel therapeutic agents. Particularly, there has been a long standing interest in the identification of natural products derived from medicinal plants for developing cancer therapeutics and considerable attention has been paid to identifying chemopreventive naturally occurring substances that inhibit, delay or reverse the process of carcinogenesis. A number of phenolic substances, particularly those present in plants (*Ganoderma lucidum*, turmeric, pomegranate, garlic, etc), have shown very encouraging results.

In different regions of Morocco many traditional healers use various medicinal plants for treating of various cancers. This review revealed that many of medicinal plants used by the Moroccan population are reported to have scientific evidence. The objective of this review is to collate all available data on experiments reporting the antiproliferative and cytotoxic effects of plants and natural products in Morocco in the last decade.

METHODS

A bibliographic investigation was carried out by analyzing recognized books and peer-reviewed papers, consulting worldwide accepted scientific databases from the last decade (Scirus, Embase, HighWire, MEDLINE/PubMed, LILACS, Ovid, ScienceDirect and SciELO databases) using medical subject heading terms and the words 'anticancer',

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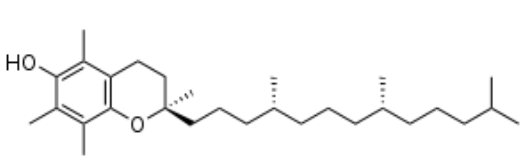

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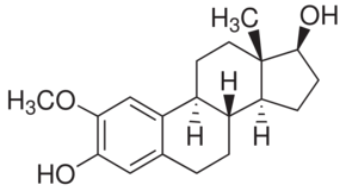

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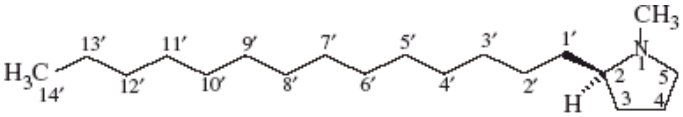

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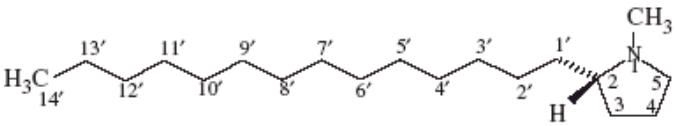
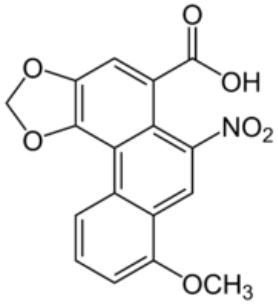


2*S*-bgugaine : (**2**)

R-BGUGAINE AND S-BGUGAINE



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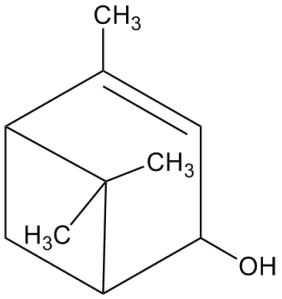

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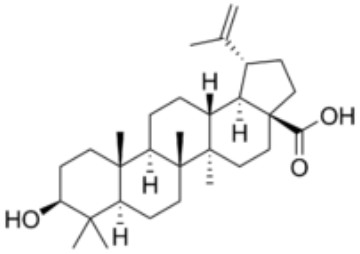

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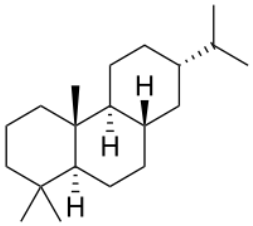

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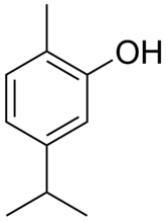

CARVACROL

Fig.1. Structure of some active chemical constituents.

'antiproliferative', 'antineoplastic', 'antitumoral', 'cytotoxic', 'Morocco', to identify relevant articles. We read the titles and abstracts of all articles in an initial screen, obtaining full text unless there was clear evidence that the article would not be eligible.

In the present study, interest is focused on experimental research conducted on anticancer plants particularly those which show antiproliferative or cytotoxic activity alongside bioactive components.

RESULTS

***Argania spinosa* (Sapotaceae)**

Medicinal uses:

The Argan tree (*Argania spinosa*) is an endemic tree in western-south of Morocco and the forest of Argan has recently been classified as a "Biosphere Reserve" by UNESCO. There is much evidence of the beneficial effects of Argan oil on human health. The Berbers tribes have long relied on Argan oil as a key element of their diet, as a skin and hair moisturizer, and as a treatment for minor wounds and ailments from rashes to diabetes (Lybbert et al., 2003) http://www.informaworld.com/smpp/title%7Edb=all%7Econtent=t713667234%7Etab=issueslist%7Ebranches=17 - v17.

Argan oil is an ideal ally for protecting the skin from fungal growths and sunburn, as well as an antiseptic for minor wounds. It is also used to treat skin diseases such as neurodermatitis and psoriasis, due to its analgesic and antiinflammatory properties. Argan oil possesses a maximum inhibition of the *in vitro* and *ex vivo* platelet aggregations induced by different agonists (Mekhfi et al., 2008). One of the most interesting nutritional properties of Argan oil is its ability to reduce harmful cholesterol levels (LDL), and treat high blood pressure (Drissi et al., 2004). Moreover, it has been demonstrated that Argan oil can improve some of the metabolic and insulin signaling abnormalities associated with high-fat/high-sucrose (HFHS) diets in which 6% of the fat is replaced by Argan oil feeding (Samane et al., 2006). It has been shown that Argan oil improves hyperglycemia in streptozotocin-induced diabetic rats and has a beneficial effect in the Oral Glucose Tolerance Test model and subchoronic treatment (Bellahcen et al., 2012; Bnouham et al., 2008).

Anticancer investigation:

Tocopherols and saponins extracted from the argan tree and 2-methoxyestradiol exhibit a dose-response cytotoxic effect and antiproliferative action on DU145 and LNCaP cell lines. The best antiproliferative effect of tocopherols is obtained with DU145 and LNCaP cell lines (28 µg/ml and 32 µg/ml, respectively, as GI50). The saponins fraction displayed the best antiproliferative effect on the PC3 cell line with 18 µg/ml as GI50 (Drissi et al., 2006).

Polyphenols and sterols extracted from virgin argan oil and 2ME(2) exhibited a dose-response cytotoxic effect and antiproliferative action on the three tested cell lines. The antiproliferative effects of the polyphenols were similar for the DU145 and LNCaP cell lines; the GI(50) (Defined as the concentration inhibiting growth by 50% in comparison with the control) was respectively 73 and 70 µg/ml. The

antiproliferative effects of sterols were 46 and 60µg/ml as GI(50) for the DU145 and LNCaP cell lines. For the PC3 cell line, the best antiproliferative effect was obtained by Argan sterols with GI(50) = 43 µg/ml. On the other hand, nuclear morphology analysis has shown an increased proportion of pro-apoptotic of nuclei in LNCaP cells treated with IC(50) of polyphenols or sterols compared to control cells (Bennani et al., 2007; Bennani et al., 2009).

Samane et al. (2006) have prepared various extracts of the Argan fruits, namely keel, cake and Argan oil extracts, which they tested on the HTC hepatoma cell line for their potential to affect cellular insulin responses.

Cell viability was measured by Trypan Blue exclusion and the response to insulin evaluated by the activation of the extracellular regulated kinase (ERK1/2), ERK kinase (MEK1/2) and protein kinase B (PKB/Akt) signaling components. None of the extracts demonstrated significant cytotoxic activity. Certain extracts demonstrated a bi-phasic effect on ERK1/2 activation; low doses of the extract slightly increased ERK1/2 activation in response to insulin, whereas higher doses completely abolished the response. In contrast, none of the extracts had any significant effect on MEK, whereas only a cake saponin subfraction enhanced insulin-induced PKB/Akt activation. The specific action of the Argan oil extracts on ERK1/2 activation can be considered as an antiproliferative action. The authors have thus tested other transformed cell lines (HT-1080 and MSV-MDCK-INV cells) and found similar results. Inhibition of ERK1/2 activation was also associated with decreased DNA synthesis as evidenced by [(3)H]thymidine incorporation experiments. These results suggest that the products of *Argania spinosa* may provide a new therapeutic avenue against proliferative diseases.

Recently, ElBabili et al. (2010) have evaluated the potential antioxidant and antimalarial activities as well as the activity against human breast cancer cells (MCF7) of Argan fruit extracts using *in vitro* models to validate the traditional use of this plant. They found good antimalarial activity (IC50 35 to >100 µg/ml) and human breast cancer cell activity (IC50 42 to >100 µg/ml) (ElBabili et al., 2010).

***Arisarum vulgare* (Araceae)**

Medicinal uses:

Arisarum vulgare Targ. Tozz . (Araceae) is native to the Mediterranean region (Bellakhdar, 1997). The used parts are the roots. It is used against skin cancer. It is vulnerary. However, it was shown that *Arisarum vulgare* could be involved in the toxicologic symptoms observed after consumption of this plant's tubers by humans and animals. Numerous original alkaloids are present in the tubers of *Arisarum vulgare* (Melhaoui and Belouali, 1998).

The signs of the toxicity are irritation of the mucous membrane, oral pain, mouth and pharynx tumefaction, vomiting, intestinal pain and mydriasis. It can produce death by asphyxia (Bellakhdar, 1997). The tubers of this plant contain alkaloids (Melhaoui and Belouali, 1998) and irniine is one of the toxic alkaloids of this plant. It induces significant hepatotoxicity, DNA damage and oxidative stress which lead to cell death by necrosis and/or by apoptosis (Rakba et al., 1999). Bgugaine is another strong toxic alkaloid of this plant. It can induce important hepatotoxicity and significant DNA damage (Rakba et al., 2000).

Anticancer investigation:

The 2-alkylpyrrolidine R-bgugaine, a natural alkaloid isolated from tubers of *Arisarum vulgare* Targ. Tozz. (Araceae), and its isomer S-bgugaine obtained by an asymmetric synthesis have been examined for their cytotoxic activities on two cancerous cellular lines: the murine mastocytoma cell line P815 and the human laryngeal carcinoma cell line Hep. These two alkaloids exhibited important cytotoxic activities on these two cancerous cellular lines. The concentrations required to induce 50% of lysis (IC(50)) for R-bgugaine and S-bgugaine alkaloids were 10 and 5 µg/mL, and 5 and 100 µg/mL, respectively, for the mastocytoma P815 and carcinoma Hep, compared with those of Adriamycine (5 µg/mL) for P815 cell line and 5 µg/mL for Hep cell line), taken as positive controls (Benamar et al., 2009).

***Aristolochia baetica* (Aristolochiaceae)**

Medicinal uses:

Aristolochia baetica L. is a native plant of Morocco used in traditional medicine. The roots of *A. baetica* are used for constipation and the whole plant is applied for external use against ringworm (Bellakhdar, 1997).

Anticancer investigation:

In order to systematically evaluate the potential activity of *Aristolochia baetica* L. on human breast cancer, four different polarity extracts were assessed *in vitro* for their antiproliferative effects on MCF-7 cells. As a result, several extracts showed potent cell proliferation inhibition on MCF-7 cells. The chloroform extract of *A. baetica* (IC50: 216.06 +/- 15 µg/mL) was the most active. Thin layer chromatography examination of the bioactive extracts of *A. baetica* showed the presence of aristolochic acid (Chaouki et al., 2010).

***Artemisia herba-alba* Asso., (Asteraceae)**

Medicinal uses:

A. herba-alba is widely used in Moroccan folk medicine for the treatment of different health disorders. It is used in oriental Morocco to treat diabetes and hypertension (Bnouham et al., 2002; Skiker et al., 2010; Ziyyat et al., 1997). This plant is used as an anthelmintic, poison antidote, emmenagogue, diuretic, tonic, depurative and cholagogue (Bellakhdar, 1997).

Anticancer investigations:

In this study, eighteen volatile compounds were identified by GC-MS analysis of the essential oil obtained from the plant's aerial parts. The main volatile constituent in *A. herba-alba* was found to be a monoterpene, Verbenol, contributing to about 22% of the total volatile components. The essential oil showed significant antiproliferative activity against the acute lymphoblastic leukaemia (CEM) cell line with 3 µg/mL as the IC50 value (Tilaoui et al., 2011).

***Daphne gnidium* (Thymeleaceae)**

Medicinal uses:

Daphne gnidium is native in Spain, Northern Africa, Western Asia, Southeastern Europe and Southwestern Europe. This plant was also naturalized elsewhere (FDA). The leaves are used as decoction. It is used for hair care and against tinea. This plant can be abortive. It produces also headaches, shivering, paleness, pupil dilatation, mouth and lip swelling, difficulties of the deglutition, diarrhea and digestive spasms, convulsions and pulmonary disorders. It can produce death (Bellakhdar, 1997; Bruneton, 1996). This plant contains tannins, a vesicant resin, which is a mixture of daphnetoxin and mezerein. It also contains coumarinic products (daphnoside, ombelliferone, and daphnoretin) (Bruneton, 1996). The acute toxicity of daphnetoxin was revealed at LD50 = 0.25 or. Unk (Duke, 1992).

Anticancer investigation:

Daphne gnidium was evaluated for its potential activity in breast cancer. Four extracts from this plant of different polarity

were tested for their antiproliferative effects on MCF-7 cells. Results from viability assays showed the potent antiproliferative capacities of the hexane (IC(50)-48 h: 630 +/- 16 µg/ml), dichloromethane (IC(50)-48 h: 112 +/- 7 µg/ml) and ethyl acetate extracts (IC(50)-48 h: 263 +/- 9 µg/ml). On the other hand, the methanol extract was inactive. LDH tests revealed the cytotoxicity of the hexane extract as opposed to two others. The characterization of the ethyl acetate extract showed its dose-dependent pro-apoptotic effect. Surprisingly, it was observed that activation of the inducible cyclooxygenase-2 followed the kinetics of apoptosis development. On the other hand, the dichloromethane extract showed the distinct effect on COX-2 activity as a function of the dosage used. A low dose seemed to inhibit COX-2 activity; whereas a high dose seemed to increase it. These findings suggest that *Daphne gnidium* L. might be of potential chemopreventive interest (Chaouki et al., 2009).

***Inula viscosa* (Asteraceae)**

Medicinal uses:

It is a Mediterranean species. It is used by women in order to increase body weight. The powder of the roots is used against tuberculosis and lung diseases (Bellakhdar, 1997).

Anticancer investigation:

I. viscosa was tested for its potential cytotoxic effects on the human cervical cancer cell lines SiHa and HeLa, harbouring HPV16 and HPV 18 respectively. MTT (Tetrazolium blue) colorimetric assay was used to evaluate the viability of cell cultures in the presence of the extracts. The extract from *Inula viscosa* exhibited marked cytotoxic effects on the two cell lines. The IC50 was 54 µg/ml in SiHa cells and 60 µg/ml in HeLa cells (Merghoub et al., 2009).

***Juniperus thurifera* var. Africana; *Juniperus phoenicea* (Cupressaceae)**

Six new diterpenic acids isolated as their methyl ester derivatives, together with two new isovalerate derivatives of p-methoxycinnamyl alcohol and linalool, were isolated from the leaves of *Juniperus thurifera* var. africana and *Juniperus phoenicea*, which is grown in Morocco. The structures of these compounds were established by using spectroscopic techniques, including 2D NMR spectra. The cytotoxicity of the abietane diterpenoids was tested against five cell lines: A-549 (human lung carcinoma), H-116 (human colon carcinoma), PSN1 (human pancreatic adenocarcinoma), T98G (human caucasian gioblastoma), and SKBR3 (human breast carcinoma). Compounds 1–3 showed an inhibitory activity against H-116 at 2.5 µg/ml (Barrero et al. 2004).

***Lavandula dentata* (Lamiaceae)**

Medicinal uses:

The genus *Lavandula* contains a high number of species. Lavender is one of the most useful medicinal plants and several therapeutic effects of lavender, such as sedative, spasmolytic, antiviral and antibacterial activities, have been reported (Imelouane, 2009). In Morocco *L. dentata* is used to treat gastro-intestinal disorders, liver diseases and renal pathologies (Bellakhdar, 1997).

Anticancer investigation:

The *in vitro* cytotoxicity of the essential oils of the aerial part and flowers of *Lavandula dentata* on five (P388D1, PC3, V79, U-373 MG, MCF7) human cancer cell lines was examined. The citotoxicity of two cancer lines (P388D1, U-373) was significantly stronger than that of the aerial part of *L. dentate* (Imelouane et al., 2010).

***Nigella sativa* (Ranunculaceae)**

Medicinal uses:

It is a Mediterranean species. It grows also in Europe and it is cultivated in India (Bellakhdar, 1997). The powder of the seeds of *N. sativa* is used against the cold, influenza, sinusitis, asthma, paralysis and haemorrhoids and dental pain. It is galactoguogue (Bellakhdar, 1997).

Anticancer investigation:

Ait Mbarek et al. (2007) had evaluated the *in vitro* and *in vivo* anticancer effects of *Nigella sativa* L. seed extracts. The essential oil (IC50 = 0.6%, v/v) and ethyl acetate (IC50 = 0.75%) extracts were more cytotoxic against the P815 cell line than the butanol extract (IC50 = 2%). Similar results were obtained with the Vero cell line. Although all extracts had a comparable cytotoxic effect against the ICO1 cell line, with IC50 values ranging from 0.2 to 0.26% (v/v), tests on the BSR cell line revealed a high cytotoxic effect of the ethyl acetate extract (IC50 = 0.2%) compared to the essential oil (IC50 = 1.2%).

In vivo, using the DBA2/P815 (H2d) mouse model, the results showed that the injection of the essential oil into the tumour site significantly inhibited solid tumour development. Interestingly, the administration of the essential oil into the tumour site inhibited the incidence of liver metastasis development and improved mouse survival (Ait Mbarek et al., 2007).

***Origanum compactum* (Lamiaceae)**

Medicinal uses:

This medicinal plant is native to Morocco. It is used against intestinal pain, lung diseases, the cold and influenza (Bellakhdar, 1997).

Anticancer investigation:

In order to systematically evaluate the potential activity of *Origanum compactum* Benth. (Lamiaceae) on human breast cancer, four different polarity extracts were assessed *in vitro* for their antiproliferative effects on MCF-7 cells. As a result, several extracts showed potent cell proliferation inhibition on MCF-7 cells. Ethyl acetate of *O. compactum* (IC50: 279.51 +/- 16 µg/mL) was the most active. A thin layer chromatography examination of the bioactive extract of *O. compactum* showed the presence of betulinic acid, respectively (Chaouki et al., 2010).

Recently, in another study, the samples (essential oil and extracts) of *O. compactum* were subjected to a screening for antioxidant (DPPH and ABTS assays) and antimalarial activities and against human breast cancer cells. The ethyl acetate extract (30 mg/L) and ethanol extract (56 mg/L) showed activity against human breast cancer cells (MCF7) (El babili et al., 2011).

***Ormenis eriolepis* (Asteraceae)**

Medicinal uses:

It is used to treat gastro-intestinal diseases. It is also vermifuge and used for its effects on the female reproductive system

Anticancer investigation:

O. eriolepis was tested for its potential cytotoxic effects on the human cervical cancer cell lines SiHa and HeLa, harbouring HPV16 and HPV 18 respectively. MTT (Tetrazolium blue) colorimetric assay was used to evaluate the viability of cell cultures in the presence of the extracts. The extract from *O. eriolepis* exhibited marked cytotoxic effects on the two cell


Argania spinosa


Argania spinosa almonds


Nigella sativa


Nigella sativa (seeds)


Aristolochia baetica


Daphne gnidium


Lavandula dentata


Juniperus thurifera


Origanum compactum


Peganum harmala


Peganum harmala seeds


Retama monosperma


Thymus broussonetii

Warionia saharae Benth &Coss


Withania adpressa

Fig. 2. Pictures of some described plants.

lines. The IC50 was 94 µg/ml in SiHa cells and 96 µg/ml in HeLa cells (Merghoub et al., 2009).

***Peganum harmala* (Zygophyllaceae)**

Medicinal uses:

Peganum harmala is native to Northern Africa, West Tropical Africa; Arabian Peninsula, Western Asia, Soviet Middle Asia, China, Indian Subcontinent and Europe (FDA).

The used parts are seeds (maceration and poultice). It is used for hair care and development. It is also used for feminine sterility, colds, intestinal pain, cardiac and hepatic diseases, and hemorrhoids (Bellakhdar, 1997). This plant can be toxic at high doses, particularly in children. The signs of toxicity are vomiting, vertigo, hyperthermia, headaches, deep sleep, cardiac disorder, convulsions, paralysis, anuria, hyperuremia, mydriasis, paralysis of the nervous system center and death by respiratory arrest (Bellakhdar, 1997). This plant contains alkaloids, particularly, in the roots and the seeds. These alkaloids are peganine, harmaline, harmine, and harmalol. The fumigation destroys some alkaloids and consequently it decreases the toxicity of the plant (Bellakhdar, 1997). Harmaline, the active principle of the plant seeds, and its derivatives, cause visual troubles, loss of coordination, agitation, delirium, and, at high doses, it can produce paralysis. In orally-loaded Wistar rats the LD50 was 2.7 g/kg. In chronic studies, an aqueous extract of *P. harmala* administered orally for six times a week at doses of 1, 1.35 and 2g/kg during 3 month period increased transaminases. Histologic study showed liver degeneration and spongiform changes in the central nervous system (CNS) in rats treated with 2g/kg dose but not at the therapeutic dose of 1g/kg (Lamchouri et al., 2002).

Anticancer investigation:
In vitro, proliferation of tumoural cell lines (Med-mek and UCP-Med carcinoma, UCP-Med sarcoma and Sp2/O-Ag14) was significantly reduced by varying concentrations (10 to 120 µg/ml) of total alkaloid extracts of *Peganum harmala* seeds (collected in Morocco) during the first 24 h of contact. A cell lysis effect occurred after 24 h and progressed to complete cell death within 48 to 72 h depending on the alkaloid concentration.

The active principle at a dose of 50 mg/kg given orally to syngenic BALB/c mice 40 days was found to have significant antitumoural activity. *In vivo* experiments were performed with the Sp2/O cell-line grafted subcutaneously (Lamchouri et al., 1999). The alkaloidic fraction of the methanol extract of *Peganum harmala* seeds showed the same effect as total alkaloids in the cells lines cited above (Lamchouri et al., 2002).

***Retama monosperma* (Fabaceae)**
Medicinal uses:
R monosperma grows in Spain and North Africa, particularly in the Saharan region. It is used to induce vomiting. It is also purgative and vermifuge. The roots of this plant cause abortion. It is a toxic plant (Bellakhdar, 1997).

Anticancer investigation:
R. monosperma was tested for its potential cytotoxic effects on the human cervical cancer cell lines SiHa and HeLa, harbouring HPV16 and HPV 18 respectively. An MTT (Tetrazolium blue) colorimetric assay was used to evaluate the viability of cell cultures in the presence of the extracts. The extract from *R. monosperma* exhibited marked cytotoxic effects on the two tested cell lines. The methanolic extracts from *Retama monosperma* showed IC50's of 99 µg/ml in SiHa cells and 112 µg/ml in HeLa cells (Merghoub et al., 2009). Recently, an experiment showed that the *Retama monosperma* L. dichloromethane fraction (Rm-DF) was the most active extract, exhibiting significant cytotoxic activities on both cell lines in a dose-dependent manner after 72 h of treatment. IC50 values obtained were 14.57 ± 4.15 µg/ml and 21.33 ± 7.88 µg/ml, for SiHa and HeLa cell lines respectively. Moreover, the analysis of Rm-DF by CG/MS revealed the presence of five known quinolizidine alkaloids as well as sparteine (10.97%), L-methyl cytisine (9.11%), 17-oxosparteine (3.49%), lupanine (0.93%) and anagyrine (39.63%) (Merghoub et al., 2011).

***Thymus* Genus (Lamiaceae)**
Medicinal uses:
The same uses as *Origanum compactum*. It is used to treat gastro-intestinal diseases and a cold. It used also for mouth diseases and throat pain (Bellakhdar, 1997).

Anticancer investigation:
Jaafari et al., 2007 have evaluated the chemical compositions and antitumoral activities of extracts for eleven species and chemotypes of Moroccan thyme. Chemical analysis of the essential oils and different extracts confirmed that Moroccan thyme is characterised by its richness and its diversity. The major compounds the most encountered in these extracts are carvacrol, thymol, borneol and paracymene. The chemical composition of thyme extract can also vary according to the geographical localization. Thus, *Thymus satureoides* is represented by 3 chemotypes and *Thymus broussonettii* by 2 chemotypes. The antitumoral activities of the extracts as well as those of two pure compounds (carvacrol and thymol) were evaluated against the P815 mastocytoma tumor cell line. The results show that Moroccan thyme extracts have an important cytotoxic effect and that carvacrol is the most important cytotoxic product. Indeed, essential oils with high amount of carvacrol (*Thymus algeriensis*, *Thymus broussonettii*, *Thymus maroccanus*, *Thymus leptobotris*) are more cytotoxic. Interestingly, no cytotoxic effect of these essential oils was observed on the human normal cells but only on the proliferative cells (Jaafari et al., 2007).

Other experimental work has been done to evaluate the antitumor effect of the Moroccan endemic thyme (*Thymus broussonettii*) essential oil (EOT). It was investigated *in vitro* using the human ovarian adenocarcinoma IGR-OV1 parental cell line OV1/P and its chemoresistant counterparts OV1/adriamycin (OV1/ADR), OV1/vincristine (OV1/VCR), and OV1/cisplatin (OV1/CDDP). All of these cell lines elicited various degrees of sensitivity to the cytotoxic effect of EOT. The IC50 values (mean +/- SEM, v/v) were 0.40 +/- 0.02, 0.39 +/- 0.02, 0.94 +/- 0.05, and 0.65 +/- 0.03% for OV1/P, OV1/ADR, OV1/VCR, and OV1/CDDP, respectively. Using the DBA-2/P815 (H2d) mouse model, tumors were developed by the subcutaneous grafting of tumor fragments of similar size obtained from P815 (murin mastocytoma cell line) injected into the donor mouse. Interestingly, the intra-tumoral injection of EOT significantly reduced solid tumor development. Indeed, by the 30th day of repeated EOT treatment, the tumor volumes of the animals were 2.00 +/- 0.27, 1.35 +/- 0.20, and 0.85 +/- 0.18 cm(3) after injections with 10, 30, or 50 µL per 72 h (six times), respectively, as opposed to 3.88 +/- 0.50 cm(3) for the control animals. This tumoricidal effect was associated with a marked decrease of mouse mortality.

These data indicate that the EOT which contains carvacrol as the major component has an important in vitro cytotoxic activity against tumour cells resistant to chemotherapy as well as a significant antitumor effect in mice (Ait Mbarek et al., 2007).

***Warionia saharae* Benth &Coss (Asteraceae)**
Medicinal uses:
It is native species in Morocco. It is used against rheumatism pain, to treat jaundice, the crisis of epilepsia and gastro-intestinal diseases (Bellakhdar, 1997).

Anticancer investigation:
Using cytotoxicity against the KB cancer cell line (ATCC CCL17) as a lead, the bioactivity-guided fractionation of the MeOH-soluble part of the DCM extract of *Warionia saharae* leaves led to the isolation of six new cytotoxic guaianolide type sesquiterpene lactones (1 - 6). Besides the two guaianolides showing the common 6,7-trans fused lactone ring (3 and 4), four compounds exhibiting the more rare 6,7-cis configuration (1,2 and 5,6) were also isolated. Compounds 1, 2, 5, and 6 showed an unprecedented ether bridge between C-2 and C-4. The structures were deduced from extensive 1D and 2D NMR spectroscopy ((1)H, (13)C, DQF-COSY, HSQC, HMBC, ROESY), as well as mass spectrometry (EI and HR-MALDI). Cytotoxicity testing against the KB cancer cell line revealed IC(50) values of 1.0 (1), 4.5 (2), 1.7 (3), 2.0 (4), 3.3 (5), and 5.5 (6) μg/mL (Hilmi et al., 2002).

***Withania adpressa* (Solanaceae)**
Medicinal uses:
Withania adpressa Coss. is a medicinal plant endemic to Moroccan Sahara. The infusion and the powdered leaves are used against intoxications. (Bellakhdar, 1997).

Anticancer investigation:
Extracts of *Withania adpressa* Coss., were tested for their cytotoxicity towards a panel of cancer cell lines (Hep2, HT29, RD, Vero and MDCK), using the (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide). The bioassay-guided fractionation of this plant extracts results in a novel withanolide 14α,15α,17β,20β-tetrahydroxy-1-oxo-(22*R*)-witha-2,5,24-trien olide, and the already identified withanolides F and J extract, semi-purified fractions and pure compounds exhibit potent cytotoxicity against human cancer cell lines tested, in a dose-dependent manner. Morphological features of treated Hep2 cells with the novel withanolide and characteristic DNA fragmentation revealed that the cytotoxicity was due to the induction of apoptosis. These results suggest that withanolides from *W. adpressa* Coss. hold potential as antiproliferative agents (Abdeljebbar et al., 2009).

DISCUSSION

Plants have played an important role as a source of effective anticancer agents, and over 60% of currently used anticancer agents are derived from natural sources, including plants, marine organisms and micro-organisms (Cragg et al., 2005; Newman et al., 2003). Moreover, behind the antineoplastic agents marketed in western countries and Japan, it was revealed that of the 155 compounds in total introduced since the 1940s, 47.1% were either unmodified natural products (25 compounds, 16.1%) or semi-synthetic derivatives of natural products (48 compounds, 31.0%) (Kinghorn et al., 2009).

Plants contain many phytochemicals with various bioactivities, including anticancer activity. Many authors have stated that natural products play an important role in the development of drugs, especially for the treatment of cancer. Podophyllotoxin, camptothecin, vinblastine and paclitaxel are only four examples of lead natural anticancer drugs within the broad arsenal of natural compounds whose structural modification has led to more potent and less toxic compounds than the prototype (Gordaliza, 2007).

Although research of the natural products for anticancer purposes is at the beginning stages in Morocco and are preliminary, the results have been promising. In fact, the search for improved cytotoxic agents continues to be an important line in the discovery of modern anticancer drugs (Gordaliza, 2007).

The Moroccan flora is made up of almost 4200 species belonging to 130 families (940 genera). It reflects the richness of the Moroccan flora. In addition, this flora contains northern species, Saharan, Mediterranean, African, and cosmopolitan species. The endemic species constitute 5% of the genera and 19% of the Moroccan species (Bellakhdar, 1997; Bnouham et al., 2010). This richness is also explained by the great variety of bioclimatic areas in Morocco (Saharan bioclimatic, arid bioclimatic, semiarid, sub wet, wet, bioclimatic of the high mountains) (Bellakhdar, 1997).

In the present study we have described 20 species that belong to 12 families. The plant families, which cover the species studied in this field, are Lamiaceae (7 species) and Asteraceae (4 species), with the most studied species being *Argania spinosa* (Sapotaceae) and *Arisarum vulgare* (Araceae), *Thymus* Genus (Lamiaceae) and *Peganum harmala* (Zygophyllaceae).

The most used method to study the anticancer activities of these natural products was the evaluation of their activities against cancer cell lines such as MCF-7 which is a breast cancer cell line, HT1080, a human fibrosarcoma cell line, the MSV-MDCK-INV invasive variant of Moloney sarcoma virus (mos) transformed MDCK cells UCP-Med and Med-mek Carcinoma, and UCP-Med Sarcoma.

Sp2/O-Ag14: This cell line is a non-Ig-secreting or synthesising line derived from a cell line created by the fusion of BABL/c mouse spleen and the mouse myeloma P3X63Ag8.

IGR-OV1 parental cell line human: ovarian adenocarcinoma OV1/P and its chemoresistant counterparts OV1/adriamycin (OV1/ADR), OV1/vincristine (OV1/VCR), and OV1/cisplatin (OV1/CDDP). P815 is a murin mastocytoma cell line). tumors were developed by the subcutaneous grafting of tumor fragments of similar size obtained from P815 (murin mastocytoma cell line) injected in a donor mouse. Human laryngeal carcinoma cell line Hep and KB cancer cell line (ATCC CCL17) were also used in anticancer experiments. KB cells have been reported to contain human papillomavirus 18 (HPV-18) sequences. HPV16, HPV18: The human papillomavirus (HPV) are members of the papillomavirus family of viruses that are capable of infecting humans. Other cancer cell lines have been used, such as the human colon adenocarcinoma grade II cell line, the RD cell line, derived from a human rhabdomyosarcoma, PC3 which is a human prostate cancer cell lines are the "classical" cell lines of prostatic cancer and U-373 MG which is a human glioblastoma-astrocytoma cell line was also used in anticancer experiment. Finally some authors have used P388D1 which is a murine macrophage cell line which spontaneously secretes a plasminogen activator or V79 developed after the spontaneous transformation of cells isolated from the lungs of a normal Chinese hamster (male).

Generally, these results of experiments suggest that the products of *Argania spinosa* (polyphenols, sterols, tocopherol and saponins) may provide a new therapeutic avenue against proliferative diseases.

However, it seems that research on the anticancer natural products did not arrive at the clinical stage. Furthermore, it has been concluded in a review about the therapeutic potential of Argan oil that a lack of clinical data constitutes a serious weakness in our knowledge of Argan oil; therefore, it is difficult to correlate the reported pharmacological activities to any potential clinical relevance (Manfalouti et al., 2010).

Several extracts from *Origanum compactum* and *Aristolochia beatica* showed potent cell proliferation or inhibition on tumoral cell lines. Other plants like *O eriolepis*, *Retama monosperma* and *Inula viscosa* showed cytotoxic effects on the human cervical cancer cell lines. The alkaloidic

fraction of *Peganum harmala* seeds showed an antiproliferative effect on tumour cells lines.

R-bgugaine and S-bgugaine alkaloids isolated from *Arisarum vulgare* were highly efficient against mastocytoma P815 and carcinoma Hep.

The citotoxicity effect of the essential oil of *Lavendula dentata* was efficient on two types of human cancer lines. Another essential oil of the *Nigella sativa* L. seed is the basis for a rare extract tested *in vivo* (injection of the essential oil into the tumor) for anticancer effect. On the other hand, this tumoricidal effect was associated with a marked decrease of mouse mortality. Another essential oil isolated from *Thymus* has an important in vitro cytotoxic as well as a significant antitumor effect in mice.

Researchers believe that the antioxidant and antiinflammatory natural products can play very important roles against cancer. These natural products can be consumed as food additives in the prevention and treatment of cancer. Indeed, *in vivo* and *in vitro* studies indicate that the products already inhibit the proliferation of cancer cells by acting at various points in their development and promote the production of enzymes that help the body to fight against the proliferation of cancer cells. It is hoped that many of these natural products can be used as the basis for future generations of anticancer drugs usable in humans.

The overall findings from this study suggest that relative to the dozen plants tested, most of them are cytotoxic to cancer cell lines at low concentrations. These plants should be further explored for anticancer constituents, application to other types of tumour cells, and could be considered for future CAM strategies that apply to suppressing the growth of malignant tumours.

In different regions of Morocco many traditional healers use various medicinal plants for treating various cancers. This review revealed that many of medicinal plants used by the Moroccan population are reported to have scientific evidence.

However, this review indicates that no clinical trials have been performed with Moroccan plant extracts or their active compounds. Moreover, it seems that it is time to increase the number of experimental studies and to begin to conduct toxicological studies and clinical trials with the potential bioactive compounds selected by *in vitro* and *in vivo* activities. Also, the mechanisms of action by which these plants extracts and their active compounds exert anticancer should be undertaken.

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CONFLICT OF INTEREST

The author has no conflicting financial interests.

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