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In vitro Cytotoxic Effects and Antibacterial Activity of Moroccan Medicinal Plants *Aristolochia longa* and *Lavandula multifida*

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: The aim of this study was the evaluation of *in vitro* cytotoxic and antibacterial activities of organic extracts from *Aristolochia longa* and *Lavandula multifida*.
Study Design: Evaluation of *in vitro* cytotoxic and antibacterial activities of extracts.
Place and Duration of Study: Department of Biology (Faculty of Sciences), between July 2007 and July 2008.
Methodology: The aerial parts were extracted by organic solvents (hexane, dichloromethane and methanol). The antibacterial activity of extracts was tested against *Rhodococcus* strains using the agar well diffusion method and cytotoxic activity was evaluated against three cancerous cell lines using the MTT assay. The chemical composition of extracts was determined using GC-MS.
Results: The results show that the hexanic extract of *A. longa* (AH) and the dichloromethanic extract of *A. longa* (AD) present a good inhibitory effect on the three cancerous cell growth with 15 µg/ml≤IC_{50}≤ 250 µg/ml and a total inhibitory effect on the bacterial growth with inhibition zone 30 mm at 50 mg/ml. Whereas, the extracts of *L. multifida* present less important inhibiting effects on the cell growth, in particular hexanic extract of *L. multifida* (LH) and dichloromethanic extract of

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L. multifida (LD) with 115 µg/ml≤IC_{50}≤ 300 µg/ml. These extracts are also active against the three strains of *Rhodococcus,* with more than 20 mm rings of inhibition at 50 mg/ml. The study of the chemical composition of each these species was undertaken by means of GC-MS. The phytochemical analysis of the extracts studied showed the presence of many chemical compounds which can explain these biological activities. These preliminary results suggest the presence in the extracts of compounds such as linoleic acid chloride; oleic acid and limonene-6-ol, pivalate for *A. longa*, and methyl linolenate; octadecane; oleic acid; 2,3,5,8-tetramethyldecane, phenol, 2-methyl-5-(1-methylethyl) for *L. multifida*.

Conclusion: This study deserves to be pursued to characterize better the asset compounds and to clarify their mechanism of action.

Keywords: Traditional medicine; medicinal plants; Aristolochia longa; Lavandula multifida; GC-MS analysis; antitumor activity; antibacterial activity.

1. INTRODUCTION

Cancer, after cardiovascular diseases, is the second leading cause of death worldwide. It continues to present the largest cause of mortality in the world. Based on worldwide estimate, in 2002, about 10 million people per year are diagnosed with cancer and more than 6 millions die of disease and 24 million persons alive with cancer [1]. Chemotherapy, the conventional cancer treatments used now days is based on synthetic drugs. However, this treatment is expensive, and cause many side effects including such minor ones as vomiting, diarrhea or major ones such as neurological, cardiac, pulmonary, renal toxicity and present limited anti-cancer activity.

Infectious diseases are also the world's leading cause of premature death, killing almost 50 000 people every day. *Rhodococcus equi* infection is commonly encountered in HIV-infected patients; recipients of organ transplants; and in those with lymphoma, chronic renal failure, alcoholism, lung cancer, leukemia, diabetes mellitus, and other states of immunodeficiency.

The most common manifestations of *R. equi* infections are multiple abscesses in the lungs, and extra-pulmonary infections may include wound infections, subcutaneous abscesses, brain abscesses, meningitis, pericarditis, osteomyelitis, cervical adenopathy, endophthalmitis, lymphangitis, or mastoiditis [2]. In recent times, there have been increases in antibiotic resistant strains of clinically important pathogens, imposing the need for a permanent search and development of new drugs [3]. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents [4]. Therefore, actions must be taken to reduce this problem, for example, to control the use of antibiotic, develop research to better understand the genetic mechanisms of resistance, and to continue studies to develop new drugs. Natural products with antibacterial and anti-proliferative activity have received much interest over the past few years. Among the potential sources of new agents, plants have long been investigated. They contain many bioactive compounds that can be of interest in therapeutic. A variety of monoterpenes have been shown to possess cancer chemopreventive and chemotherapeutic properties [5]. On the other hand, Taxol isolated from *Taxus sp* is one of the most important cancer chemotherapeutics used to treat several types of cancer. Morocco is one of the developing countries which have enormous diversity of plants and yet majority stays scientifically neglected and undiscovered. In Morocco, the use of traditional medicine is widespread practice. About 70% of the population uses traditional medicine, mainly herbal plants [6]. The ethnobotanical and ethnopharmacological surveys conducted in different areas allowed the compilation of an inventory of 360 species and more than 500 prescriptions are recorded [7]. Previous studies conducted in our laboratory, demonstrated the antimicrobial and cytotoxic activities of many Moroccan medicinal plants [8,9,10,11,12,13,14]. In our continuous search of the antibacterial activities of Moroccan plants, the extracts of two Moroccan medicinal plants were investigated for their *in vitro* antibacterial and antitumor potential. We sought to contribute to future phytochemical and pharmacological investigations. To the best knowledge, no previous study of the anticancer and antibacterial activity from our extracts has been reported. Antibacterial assays have been performed on *Rhodococcus* species which present a similar morphology and growth characteristics with *Mycobacterium tuberculosis*

(TB). An attempt has been made to discover new anti-TB agents. The extracts were also tested against RD: Embryonal Rhabdomyosarcoma cancerous cell lines, BSR: Kidney adenocarcinoma of hamster and Vero: Monkey kidney cancerous cell lines. Extracts from the following plants were utilized: *Aristolochia longa and Lavandula multifida*. Table 1. introduces taxonomic classification of the plants, vernacular name, different parts of plants collected, traditional use and pharmacological activities.

2. MATERIALS AND METHODS

2.1 Plant Material and Preparation of Extracts

Each plant was dried at room temperature and the powdered materials were then weighed (300 g), and were successively extracted with hexane, dichloromethane and methanol using Soxhlet. The filtrate obtained was concentrated in a rotary evaporator to obtain the crude extract. The crude extracts were kept at 4°C until further uses.

2.2 Analytical Techniques

Gas chromatography-mass spectrometry (GC/MS) analysis of the different extracts was performed on a TRACE GC ULTRA Polaris Q (Thermo Electron Corporation) equipped with non-polar VB-5 (5% phenyl, 95% methylpolysiloxane) capillary column (30 m x 0.25 mm x 0.25 µM film thickness), directly coupled to a mass spectrometer (Polaris Q). The electron ionization energy was set at 70 eV. The oven temperature was programmed from 60°C to 280°C at 4°C/min, then for 280°C to 300°C at 20°C/min. The components of the extracts were identified by comparison of their mass spectra with those in the Willey NIST 7th Edition Library of mass spectra data. The composition of the extract sample was calculated from GC-MS peak areas and given by percentages.

2.3 Cell Viability Assays

The *in vitro* cytotoxic effect of the various extracts was evaluated on RD: Embryonal Rhabdomyosarcoma cancerous cell lines (ATCC N°CCL-136), BSR: Kidney adenocarcinoma of hamster (ATCC N°CCL-10), Vero: Monkey kidney cancerous cell lines (ATCC N°CCL-81). Cells were grown in Dulbecco's Modified Eagle Medium (DMEM) (GIBCO) supplemented with 10% heat-inactivated fetal calf serum and 1% Penicillin-Spreptomycin mixture. Cultures were maintained at 37°C in 5% $CO_2$and 100% relative humidity atmosphere. The effect of the isolated extracts on cell viability was assessed using the 3-(4,5-dimethylthiazol -2-yl)-2, 5 diphenyltetrazolium bromide (MTT) assay, which measures the metabolic activity of mitochondria (Mosmann [15]). MTT assays are presently the preferred methods of cytotoxicity assessment in our laboratory [16,17,9,10]. The tests were conducted on 96-well microplate. Before treatment with extracts, 100 µL medium DMEM (GIBCO) containing 3- $4x10^6$ cells/mL were placed in each well containing DMEM (GIBCO) and cultured at 37°C in 5% CO_2/ humidified air for 24 h. After 24h incubation and attachment, cells were treated with crude extracts. Exactly from the stock solution (80 mg/ml), each extract sample was applied in a series of 6 dilutions (final concentrations ranging from 12.5 µg/mL to 400 µg/mL) in Dimethyl sulfoxid (DMSO 1%). Test solution (100 µL), was added in decreasing concentrations in duplicate. Microplate were then incubated for 48 h at 37°C in air condition of 5% CO_2. After, 20 µl MTT solution (5 mg/ml) (SIGMA) was added to the wells containing cells. The cells were incubated for 4 - 5 h at 37°C in 5% CO_2. Tetrazoluim salts are cleaved to formazen dye by cellular enzyme (only in the viable cells). A solubilization solution (Isopropanol/hydrochloric acid) is added to dissolve the insoluble purple formazen product into coloration solution. The absorbance was measured at 545 nm, using microplate reader (Statfax 2100).

Table 1. Ethnobotanical data and some reported pharmacological activities of plants species used in this study

Plant species	Trivial name	Part plant collected	Traditional use	Pharmacological activities
Aristolochia longa	Bereztem	Tuber	Skin diseases [7]	Cytotoxic and antimicrobial activity [20]
Lavandula multifida	Kohayla	Aerial parts	Gastrointestinal disorders, Rhumatism [27]	Antifungal [28]

2.4 Antibacterial Activity

2.4.1 Microorganisms and inoculum preparation

The bacteria studied were three species of Gram$^+$: *Rhodococcus equi* isolated from poulain (France), *Rhodococcus sp GK1* isolated from soil polluted with petrol (France) and *Rhodococcus sp GK3* obtained from soil. Each isolate was inoculated into sterile medium mixture: $(NH_4)_2SO_4$, Na_2HP_4, KH_2PO_4, Thiamine, ($MgSO_4$, $7H_2O$), ($CaCl_2$, $2H_2O$), ($FeSO_4$, $7H_2O$), ($MnSO_4$, 3H2O), ($ZnSO_4$, 3 H_2O) and agar for solid medium bacteria growth [18].

2.4.2 Agar well diffusion method

The test samples were first dissolved in dimethylsulfoxide (1%) who thus did not affect the microbial growth. The Agar well diffusion method was employed for the determination of antimicrobial activities of the tested extracts. Briefly, the test was performed in sterile petri plates containing medium agar. 30 mL of sterilized medium was poured into sterile petri plates. After solidification, 100 µL of fresh cultures of *Rhodococcus sp* (one microorganism per Petri dish), were swabbed on the respective plates. Then, 100 µL of extracts were placed in wells previously punched over the agar plates using sterile Pasteur pipette, at various concentrations (6.25 mg/ml ; 12.5 mg/ml ; 25 mg/ml ; 50 mg/ml). All Petri plates were then incubated at 30°C for 48 h. The diameters of inhibition zones were measured in millimeters. In addition, The antimicrobial activities of the two selected plant extracts on *Rhodococcus sp* were compared with the commercially available antibiotics. The antibiotic discs such as Chloramphenicol and Ampicillin were placed on the surface of the plates. DMSO 1% was used as negative control. The plates were incubated at 30°C for 48 hours and after incubation the diameter of the inhibition zones were measured in mm and recorded [19].

2.4.3 Time-kill dynamic curves

Rhodococcus sp were grown overnight at 30°C in 25 ml medium broth. Each extracts 50 mg/ml were prepared in DMSO (1%) and placed in viable bacteria and were shaken and incubated at 30°C. The density of the each culture (designed as bacterial growth) was measured by spectrophotometer at a wavelength of 600 nm after each time point indicating the bacterial biomass present in the suspension. The suspension of bacteria strains with no extracts was used as control.

3. RESULTS

3.1 Phytochemical Analysis

Freshly prepared extracts were subjected to a preliminary phytochemical screening for various constituents by GC-MS analysis. Table 2 indicates the major compounds present in different extracts.

3.2 Cytotoxicity Effects

The investigation of the cytotoxic potential of six extracts from *A. Longa* and *L. Multifida* Moroccan plants that are used in traditional medicine for treatment of various diseases, were conducted on three tumor cell lines RD, BSR and Vero. Cancerous cell lines were exposed to increasing concentrations ranging from 12.5 µg/mL to 400 µg/mL. Assay by the MTT assay as described above, indicates that the extracts revealed different cytotoxic activities towards the three cancer cell lines investigated. In general, a dose-dependent decrease in the survival of the three cancerous cell lines.

As shown in Fig. 1 and Table 3, *A. longa* hexanic (AH) extract and *A. longa* dichloromethanic (AD) extract are present a good inhibiting effects with IC_{50} values of 30 µg/ml and 15 µg/ml respectively, with a total inhibitory effect on the RD cell growth at concentration lower than 150 µg/ml. Whereas, the extracts of *L. multifida* presents less important inhibiting effects on the cell growth RD, in particular hexanic extract of *L. multifida* (LH) and dichloromethanic extract of *L. multifida* (LD) with IC_{50} of 115 µg/ml and IC_{50} of 130 µg/ml respectively. On the other hand, Complete inhibitory effect on RD cancerous cell lines was observed at concentration of 400 µg/mL; the concentrations providing 50% inhibition (IC_{50}) values of the hexanic extract of *A. longa* (AH), dichloromethanic extract of *Aristolochia longa* (AD) and methanolic extract of *A. longa* (AM) on the BSR cancerous cell line were 18 µg/mL, 60 µg/mL and 350 µg/mL respectively. For the BSR cancerous cell lines, the result showed that the BSR cancerous cell was more resistant to AM. In the case of *L. multifida* extracts, LH and LD present similar and less inhibiting effects on the BSR with IC_{50} of 250 µg/ml **(**Fig. 2 and Table 2.).

In addition, AM and LM exhibited poor cytotoxicity against Vero cells lines. Concentrations providing 50% inhibition (IC_{50}) values of the hexanic extract of *L. multifida* (LH), dichloromethanic extract of *L. multifida* (LD) on the Vero cancerous cell line were 300µg/ml (Fig. 3 and Table 3). This result showed that Vero cell lines was the most resistant compared to RD and BSR to each plant fractions studied here.

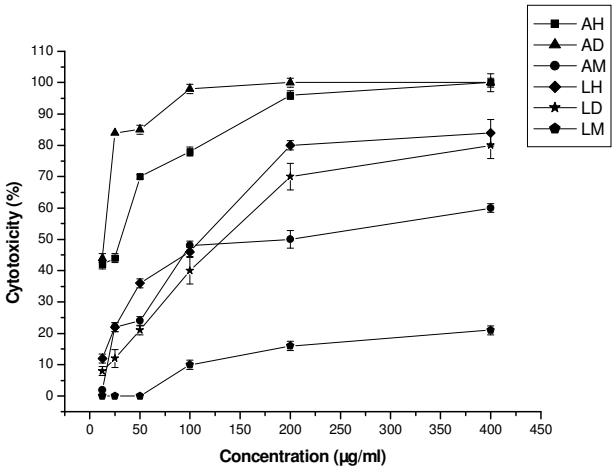

Fig. 1. Cytotoxic activity of extracts from 2 medicinal plants against RD cell lines. AH: Hexanic extract from *Aristolochia longa;* AD: Dichloromethanic extract from *Aristolochia longa;* AM: Methanolic extract from *Aristolochia longa;* LH: Hexanic extract from *Lavandula multifida;* LD: Dichloromethanic extract from *Lavandula multifida;* LM: Methanolic extract from *Lavandula multifida*

Tests were carried out in duplicate. Cells were incubated with different concentrations of extracts at 37°C for 48 h. Data are expressed as means ± SD of three independent experiments

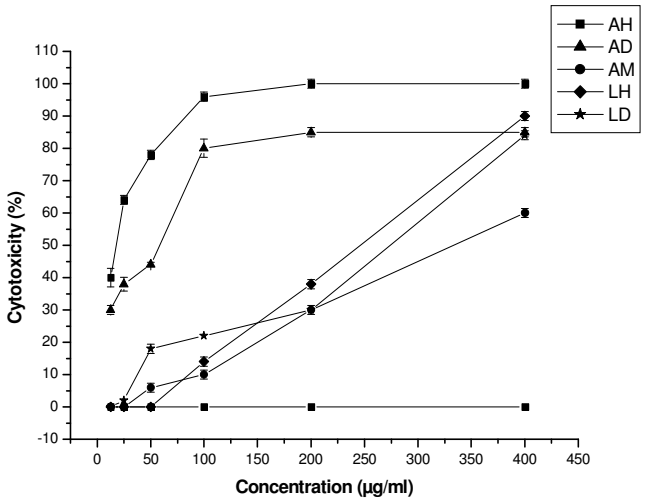

Fig. 2. Cytotoxic activity of extracts from 2 medicinal plants against BSR cell lines. AH: Hexanic extract from *Aristolochia longa;* AD: Dichloromethanic extract from *Aristolochia longa;* AM: Methanolic extract from *Aristolochia longa;* LH: Hexanic extract from *Lavandula multifida;* LD: Dichloromethanic extract from *Lavandula multifida;* LM: Methanolic extract from *Lavandula multifida*

Tests were carried out in duplicate. Cells were incubated with different concentrations of extracts at 37°C for 48 h. Data are expressed as means ± SD of three independent experiments

3.3 Antibacterial Activity

The results of Agar well diffusion and broth dilution methods showed that each extracts showed different degree of growth inhibition. The screening for antibacterial activity indicates that at concentration of 50 mg/ml, the AD was found to possess a relatively high antibacterial activity against R. equi with diameter of inhibition 30 mm (Fig. 4) and 100% of growth inhibition as compared to untreated bacteria (T) (Fig. 5). R. equi showed also high sensitivity to AH with diameter of inhibition 25 mm and a maximum bacterial biomass about 0.23 g/l. LH present a moderate antibacterial activity (diameter of inhibition 18 mm). While, both AM and LM have limited antibacterial activity (diameter of inhibition 12 mm) with bacterial biomass more than 1.2 g/l as compared to control (T^-). In addition, the maximum effect was recorded against *Rhodococcus* sp GK1 with AD at concentration 50 mg/ml (inhibition zone 25 mm) and 100% of growth inhibition (Fig. 6 and Fig. 7). Furthermore, each extract from L. multifida and A. longa extracts were found to be more active at 50 mg/ml concentration against *Rhodococcus* sp GK3 (inhibition zone ranged from 20 to 26 mm). In the case of *A. longa*, AD and AH presented a high antibacterial activity (Fig. 8 and Fig. 9). Chloramphenicol used as reference antibiotics showed important and similar antibacterial activity against all the Rhodococcus population tested with inhibition zone of 30 mm. So, There was no preferential activity against bacteria strains studied. While, the three strains were resistant to ampicillin. In addition, No zone inhibition was observed with DMSO 1%.

4. DISCUSSION

The present study was undertaken to provide comparative data on the *in vitro* cytotoxic and antibacterial activity of different extracts from two Moroccan medicinal plants: *A. longa* and *L. multifida.* For the cytotoxic effects, hexanic extract of *A. longa* (AH) and the dichloromethanic extract of *A. longa* (AD) exhibited a significant cytotoxic effect against the majority of tumor cell lines used. These observations agree with previous research in Family of Aristolochiaceae, in which *A. longa* exhibit strong cytotoxic activity against cancerous cell lines [20,21,22]. In addition, extract of *L. multifida* (LH) and the dichloromethanic extract of *L. multifida* (LD) was found to exhibit less effect on the cells tested. Only minimal cytotoxicity was observed for AM and LM against Vero cell lines. Interestingly, we report here that the differential cytotoxic effect

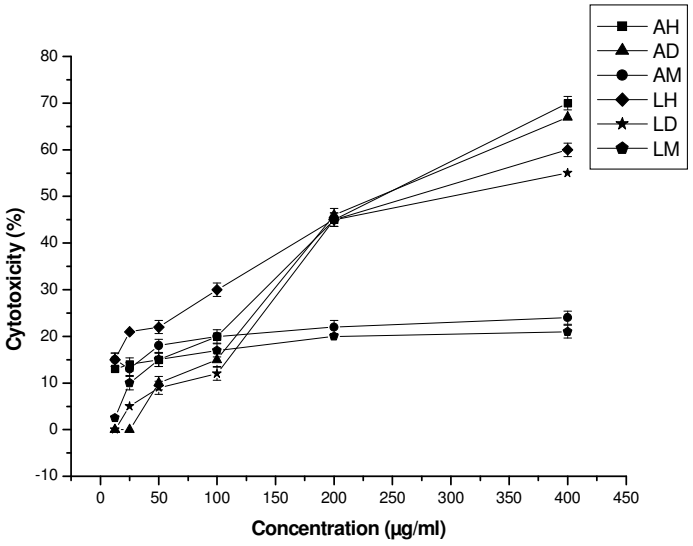

Fig. 3. Cytotoxic activity of extracts from 2 medicinal plants against Vero cell lines. AH: Hexanic extract from *Aristolochia longa;* AD: Dichloromethanic extract from *Aristolochia longa;* AM: Methanolic extract from *Aristolochia longa;* LH: Hexanic extract from *Lavandula multifida;* LD: Dichloromethanic extract from *Lavandula multifida;* LM: Methanolic extract from *Lavandula multifida*

Tests were carried out in duplicate. Cells were incubated with different concentrations of extracts at 37°C for 48 h. Data are expressed as means ± SD of three independent experiments

of these extracts was related not only to their chemical composition but also to the nature of the tumor cell lines and the differential cytotoxicity of these extract against the same cell line is related to the differential composition of such extracts. On the other hand, to evaluate the antibacterial activity of these extracts, we tested their effect on three bacterial strains of Genus *Rhodococcus*. AH and AD were found to exhibit a strong growth inhibition effect on all the bacteria tested. While, no significant activity was observed with AM and LM. Our results agree with previous research in which Aristolochia longa paucinervis was found to have a good in vitro antibacterial activity against Clostridium perfringens and *Enterococcus faecalis* [23]. In addition, previous findings reported an inhibitory effect against *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus faecalis* and *Staphylococcus epidermis* [20]. In the case of *L. multifida*, extract of *L. multifida* (LH) and the dichloromethanic extract of *L. multifida* (LD) exhibited strong antibacterial activity against the three *Rhodococcus* bacteria strains tested. *Rhodococcus equi* is a bacterium identified in a variety of soils, water and animals. *R. equi* infection is rare but the incidence increased with the pandemia of AIDS and organ transplantation. It belongs to Nocardiaceae family which includes *Mycobacterium tuberculosis* (TB) [24]. The problem of tuberculosis has been intensified. No anti-TB drugs have discovered and there is an urgent need to search for an develop effective ant-TB. In this scenario, the two medicinal plants species studied in our laboratory may be looked as an important source of new anti-TB agents.

Table 2. Percentage (%) of the main components of the two plant extracts

Plant extracts	Main components	Percentage (%)
	9,12-Octadecadienoyl chlorid, (Z,Z)-	12.46
	9-Octadecenoic acid (Z)-	43.73
AH	Limonen-6-ol, pivalate	55.84
	(2'-Nitro-2'-propenyl) cyclohexane	19.16
	Heptadecane, 2,6,10,15-tetramethyl-	5.58
	Cycloheptane, 4-methylene -1-methyl-2-	7.07
	(2-methyl-1-propen-1-yl)-1-vinyl	
AD	9.12-Octadecadienoyl chlorid, (Z,Z)-	9.80
	Cholestan-3-ol, 2-methylene-, (3à,5à)-	17.32
	Trans-Z-à-Bisabolene epoxide	10.66
	9-Octadecenoic acid (Z)-	33.14
	d-Glycero-d-ido-heptose	15.42
	Permethylated and reduced product of H3- Glycolipid	73.02
AM	3-Hexadecyloxycarbonyl-5-(2-hydroxyethyl)-4-methylimidazolium ion	23.94
	-Octadecenal	8.36
	1-Gala-1-ido-octose	65.66
	9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)	11.45
	Octadecane	34.33
LH	9-Octadecenoic acid (Z)-	30.48
	Decane, 2,3,5,8- Tetramethyl-	9.76
	Phenol,2-methyl-5-(1-methylethyl)	16.65
	9,12,15-Octadecatrienal	10.60
	9-Octadecenoic acid (Z)-	26.96
LD	Decane, 2,3,5,8-Tetramethyl-	8.52
	1-Octanol, 2-butyl-	11.06
	1-Decanol, 2-ethyl	13.36
	5-Ketofructose	12.68
	Butanoic acid, 4-(2-oxocyclopentyl)-	8.70
LM	8,11,14-eicosatrienoic acid, (Z, Z, Z)-	17.66
	3-Cyclopropyl carbonyl oxytridecane	15.65

AH: Hexanic extract from Aristolochia longa; AD: Dichloromethanic extract from Aristolochia longa; AM : Methanolic extract from Aristolochia longa; LH : Hexanic extract from Lavandula multifida; LD : Dichloromethanic extract from Lavandula multifida; LM : Methanolic extract from Lavandula multifida

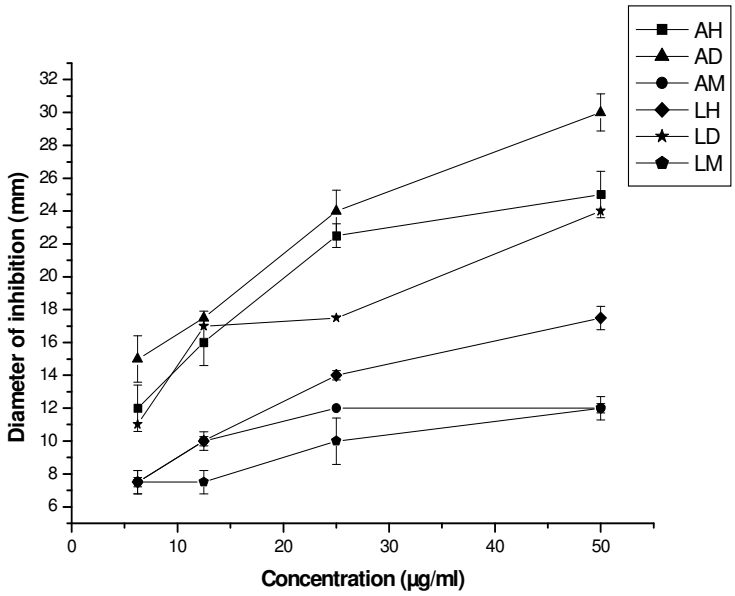

Fig. 4. Antibacterial activity* of extracts from 2 Moroccan medicinal plants against *Rhodococcus equi* as determined by diffusion technique on solid media
***Zone of inhibition (Ø mm)**

Tests were carried out in duplicate. Bacterial cell were incubated with plant extracts at 30°C for 48 h. Data are expressed as means of inhibition zone in mm ± SD of three independent experiments

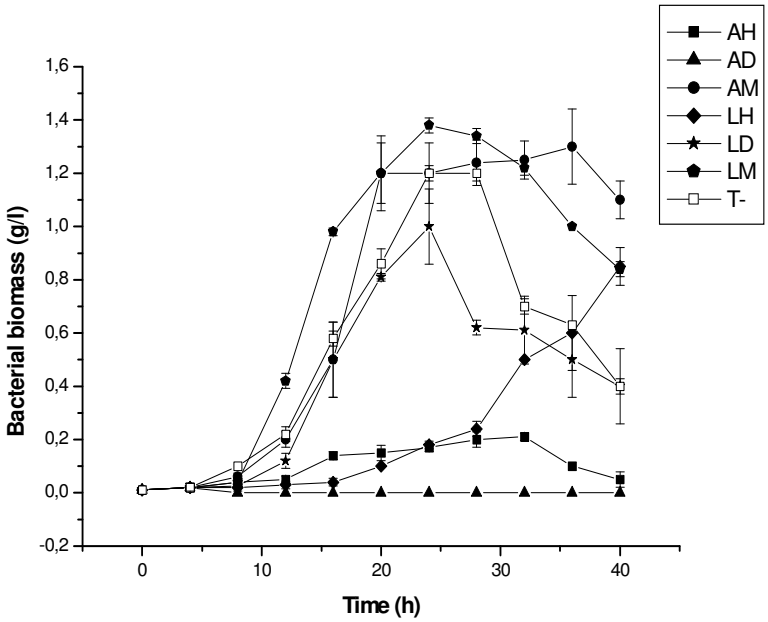

Fig. 5. Bacterial growth registered after 40 h of exposition of each extracts on *Rhodococcus equi*. T-: Untreated bacteria

Tests were carried out in duplicate. Data are expressed as means of bacterial biomass (g/L) ± SD of three independent experiments

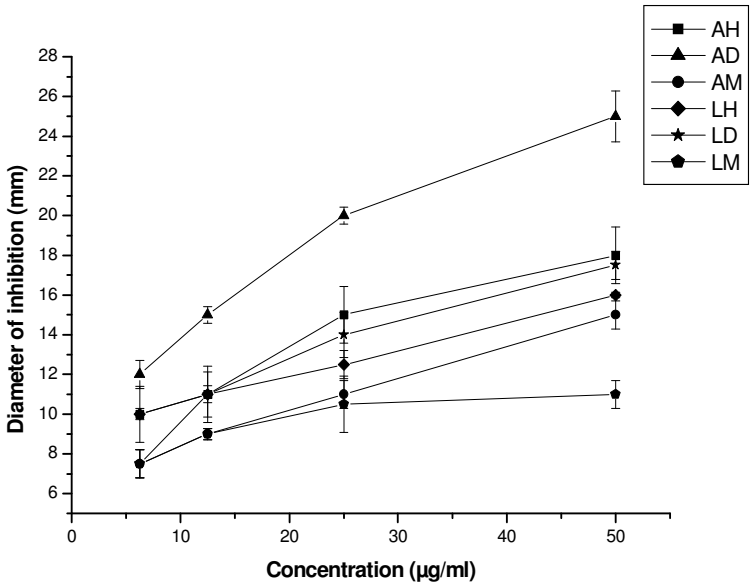

Fig. 6. Antibacterial activity* of extracts from 2 Moroccan medicinal plants against *Rhodococcus sp GK1* as determined by diffusion technique on solid media
***Zone of inhibition (Ø mm)**

Tests were carried out in duplicate. Bacterial Cell were incubated with plant extracts at 30°C for 48 h. Data are expressed as means of inhibition zone in mm ± SD of three independent experiments

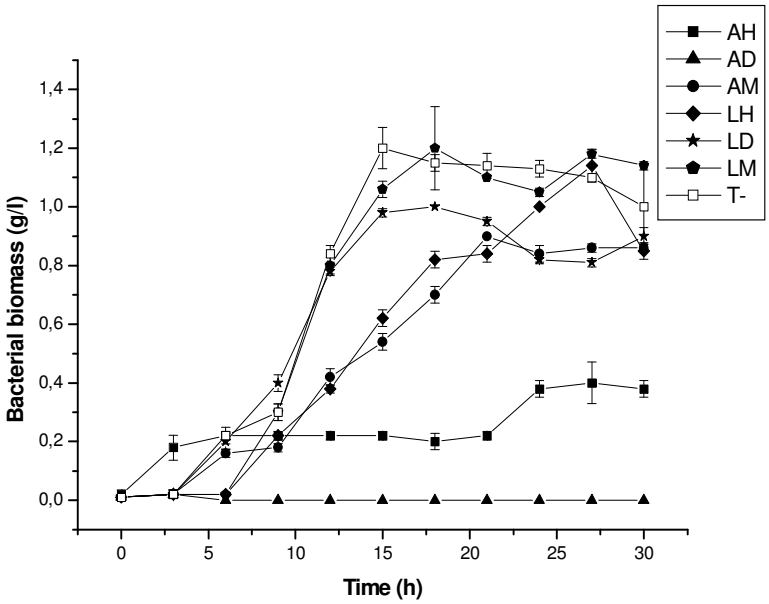

Fig. 7. Bacterial growth registered after 30 h of exposition of each extracts on *Rhodococcus sp GK1*. T⁻: Untreated bacteria

Tests were carried out in duplicate. Data are expressed as means of bacterial biomass (g/L) ± SD of three independent experiments

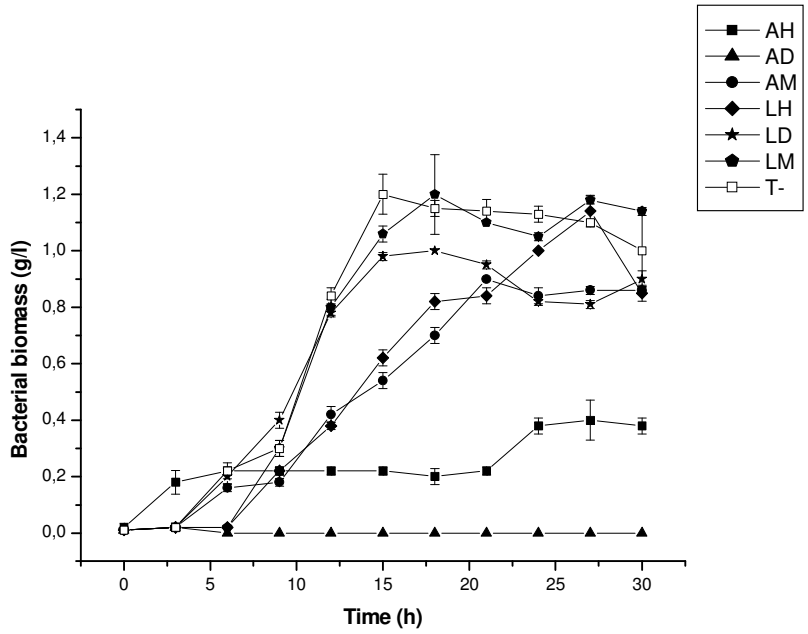

Fig. 8. Antibacterial activity* of extracts from 2 Moroccan medicinal plants against *Rhodococcus sp GK3* as determined by diffusion technique on solid media. * Zone of inhibition (Ø mm)

Tests were carried out in duplicate. Bacterial Cell were incubated with plant extracts at 30°C for 48 h. Data are expressed as means of inhibition zone in mm ± SD of three independent experiments

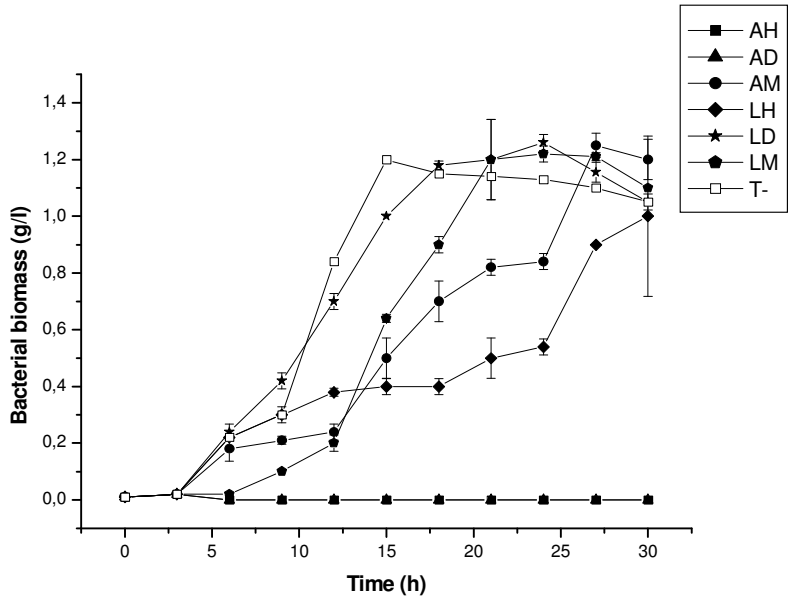

Fig. 9. Bacterial growth (measured as Bacterial biomass (g/l)) registered after 30 h of exposition of each extracts on *Rhodococcus sp GK3*. T: Untreated bacteria

Tests were carried out in duplicate. Data are expressed as means of bacterial biomass (g/L) ± SD of three independent experiments

Table 3. Inhibition concentration (IC_{50} in µg/ml) values from *A. longa* and L. multifida towards RD, BSR and Vero as determined by the MTT assay

		Aristolochia longa			*Lavandula multifida*		
		AH	**AD**	**AM**	**LA**	**LD**	**LM**
IC_{50} (µg/mL)	RD	30±0.70	15±1.06	200±7.78	115±3.53	130±5.09	---
	BSR	18±3.53	60±2.47	350± 8.48	250±3.88	250±4.59	---
	Vero	250±4.94	250±4.80	---	300±4.63	300±7.42	---

AH: Hexanic extract from Aristolochia longa; AD: Dichloromethanic extract from Aristolochia longa; AM: Methanolic extract from Aristolochia longa; LH: Hexanic extract from Lavandula multifida; LD: Dichloromethanic extract from Lavandula multifida; LM: Methanolic extract from Lavandula multifida.

Tests were carried out in duplicate. Cells were incubated with different concentrations of extracts at 37°C for 48 h. Data are expressed IC_{50} values are means ±SD of three independent experiments

The results suggest that the high biological activities of the hexanic and dichloromethanic extract of the two plants may be related to their major compounds : linoleic acid chloride; oleic acid and limonene-6-ol, pivalate for *A. longa*, and methyl linolenate; octadecane; oleic acid; 2,3,5,8-tetramethyldecane, phenol, 2-methyl-5-(1-methylethyl) for *L. multifida*. However, this does not exclude the possibility that the other constituents may account for the biological property of the extracts. The synergistic effects of these active chemicals with other constituents of the extracts should be taken into consideration. The mechanism of action of extracts is not full understood but it is thought to involve walls and membrane disruption by the lipophilic compounds [25,26]. It is then important to develop a better understanding of their mechanisms of biological activity.

5. CONCLUSION

The results obtained in this preliminary study indicate that the hexanic extract and the dichloromethanic extract of the two plants *A. longa* and *L. multifida* were shown to induce significant and dose-dependent inhibitory activities against human and animals cancer cell line (RD, BSR, Vero). There remains interesting to evaluate the cytotoxic activity of selected plants in vitro on other cancer cell lines. In addition, these extracts were found to be more active against the chosen pathogenic bacterial strains (*R. equi*, *Rhodococcus sp GK1*, *Rhodococcus sp GK3*)

This study provides an important basis for further investigation into the isolation, characterization and mechanism of biological compounds. Thus, these plants could be as source for new lead structures in drug design and may be used together with known drugs in the development of pharmacological agents to combat cancer and infectious diseases. Finally, Morocco possesses variety of plant species that might be important sources compounds to treat different diseases.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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