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Research Article

Phytochemical Screening and Antifungal Activity of Phases Obtained From the Extracts of *Juglans Regia* L., *Lawsonia Inermis* L. and *Pistacia Lentiscus* L.

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ABSTRACT
The present work aimed to evaluate the anti-dermatophyte activity and to investigate the chemical composition of *Pistacia lentiscus* leaves (PLE), *Lawsonia inermis* (LIN) and the bark of *Juglans regia* (JRE). A bioguided phytochemical screening has revealed the presence of four major chemical groups (tannins, flavonoids, sterols and terpenoids and saponins) in total extracts of our three selected plants. These chemical groups are known for their antimicrobial activities could be responsible for the observed antifungal activities.
The TLC on silica gel and polyamide and the HPLC have confirmed the results of characterization of reactions. The resulting chromatograms revealed under UV before and after the detection Neu reagent gave an idea about the nature of phenolic compounds, mainly flavonoids. Revelation to the Liebermann-Burchard reagent and FeCl3 showed the presence of sterols, terpenes and tannins. Flavonoids extracts of phases of ethyl acetate, ED, water and 1B identified by UV-Visible spectrophotometry confirm the presence of molecules of Flavonol, Flavone, Chalcone, Aurone, Isoflavone present in the three plants. The activity of the extracts obtained by successive phases of confrontation was notable for Candida albicans, single strain inhibited by most of the different fractions (ED, AC and 1B) of three plants. This partition has enabled us to confront the possible consequences of phytochemical fractionation and loss induced activity.
Keywords: Juglans regia, Lawsonia inermis, Pistacia lentiscus, fractions, antifungal activity.

INTRODUCTION

Dermatophytes are a group of fungi capable of digesting keratin and cause ringworm [1]. Statistics show an increase in fungal infections and an increase in the resistance of many pathogens to current treatments [2]. Faced with these data, despite significant progress, the number of antifungal remains insufficient. Indeed, fungi are endowed with formidable adaptability and we must constantly find new drugs or new drug combinations to fight against the emergence of resistant species. An effective alternative to these chemical treatments is the development of herbal medicine, large reservoir of active ingredients.
Lawsonia inermis (Lythraceae) is characterized by its multiple uses against eczema, fungal infections, astringent, antiseptic, antifungal, leshmanicide, healing of wounds and injuries [3-5]. *Pistacia lentiscus* (Anacardiaceae) known for its antitiviral, antibacterial, healing activity, antiinflammatory, antifungal, burns and used in the treatment of eczema [6-14]. *Juglans regia* (Juglandaceae) is used in the treatment of the skeleton, and used as astringent, antiseptic, antifungal and antibacterial and keratinizing fungicide [11].
The aim of the present study is to determine the chemical characterization of the extracts of the previous cited three plants and the *in vitro* evaluation of anti-dermatophyte activity of the phases obtained from these extracts.

Materials and methods

Plant material and chemical study

The plant material was obtained from plants growing in their natural habitat or from herbalists. Their identification was performed by an experienced taxonomist and voucher specimens were deposited in the herbarium of the Laboratory of Medical Botany and Pharmacognosy, Department of Pharmacy, Faculty of Medicine, University Constantine 3. The dried plants were sprayed just before extraction using a cutting mill. The extraction of the powders obtained (30g) was made by cold soaking in a hydroalcoholic solution of 70% ethanol (1:5, v / v), stirred on a magnetic plate (700 r / min for 30 min). The solutions are then clarified by filtration on Whatman paper, concentrated by vacuum evaporation of alcohol, and lyophilized to provide a crude extract dry roughly dry pulverulent consistency. These are stored until analysis at low temperature (-10 ° C). For phytochemical analysis and evaluation of the *in vitro* biological activity of the studied species, extraction of total principles with major chemical groups was necessary. The extraction was done in the Laboratory of Medical Botany and Pharmacognosy, Department of Pharmacy, Faculty of Medicine, University

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Table 1. Chemical screening of the methanolic extracts of the different plants species tested.

	Tanins	Sterols & terpenoids	Coumarins	Flavonoids	Alkaloids	Saponins
Lawsonia inermis (leaves)	+++	+++	---	+++	---	+++
Pistacia lentiscus (leaves)	++++	++++	----	+++	---	++++
Juglans regia (bark)	++++	+++	---	+++	---	++++

---: negative reaction; +++: Positive reaction

Table 2. *In vitro* antifungal activity of the different plant fractions on selected strains.

	Pistacia lentiscus L.			*Juglans regia* L.			*Lawsonia inermis* L.		
	DE	AC	1B	DE	AC	1B	DE	AC	1B
T. mentagrophyte	-	-	-	-	-	-	-	-	-
T. rubrum	-	-	-	-	+	-	+	+	-
M. canis	-	-	-	-	-	-	-	-	-
C. albucans	+	+	+	+	+	+	+	+	+

+: little activity ; - : No activity.

of Constantine 3. Plant material used consists of leaves of *Pistacia lentiscus* L. and *Lawsonia inermis L.* and bark of *Juglans regia* L. The plant material harvested and dried at ambient laboratory temperature was sprayed using an electric cutter mill. The extract was prepared by the method described by Zirihi and Kra [15].

Table 3. Fraction concentrations of different tested extracts (at 420nm).

	Fractions		
	AC	DE	1B
Lawsonia inermis L.	0.203	0.629	0.416
Juglans regia L.	0.415	0.365	1.536
Pistacia lentiscus L.	0.408	0.506	0.982

The phytochemical analysis is performed on the basis of solubility tests, precipitation reactions and staining characteristics to highlight the major chemical groups. For this purpose, several types of reagents were used. Most tests are performed according to the method of Harborne [16] and Bruneton [17]. Reactions characterization focused on the research, powders plants, and major chemical groups. These tests have information on the chemical composition of plants.

In vitro anti-dermatophyte activity assay

The obtained phases ED, AC and 1B are evaporated to dryness then taken up in 4-5 ml of methanol to determine the greatest inhibitory activity on dermatophytes. The antifungal activity of methanol extracts was tested *in vitro* by the agar diffusion method [18,19] using four strains: *Candida albicans* yeast and three dermatophytes: *Trichophyton rubrum*, *Trichophyton mentagrophytes* and *Microsporum canis*. The antifungal activity is performed by comparing the diameter growth of the strains in the presence and absence of plant preparations tested, monitoring and control of the antifungal (Griseofulvin). The diameter of the colony growth was measured using a caliper.

RESULTS

Chemical characterization

The phytochemical screening is recorded in Table 1 and figures (1, 2, and 3); it reveals the presence or absence of a group of secondary metabolites.

The test for the metabolites in different extracts LIN, PLE and JRE gave positive reactions. The analysis of the experimental results led to the following conclusions:

- Flavonoïds, saponins, tannins, sterols and terpenoïds are present in all plants studied in varying amounts.
- The presence of sterols, steroids and tannins in significant quantity was confirmed respectively by solid reaction with the Liebermann Burchardt giving a violet color and with the dilute solution of ferric chloride (FeCl3 to 2%) to give a blue color. Indeed, four groups of bioactive compounds are identified: sterols and triterpenes, flavonoids, tannins and saponins are present in our frankly methanolic crude extracts of leaves of *Lawsonia inermis* L. and *Pistatacia lentiscus* L. and in the bark of *Juglans regia* L. However coumarin compounds and alkaloïds are found in no part of the three plants studied. This is not in agreement with the work of some authors. This result is due to the choice of solvent and extraction method.

Antifungal activity of the extracts of phases

In order to estimate the antifungal potential of our extracts, we considered it important to complete our study by

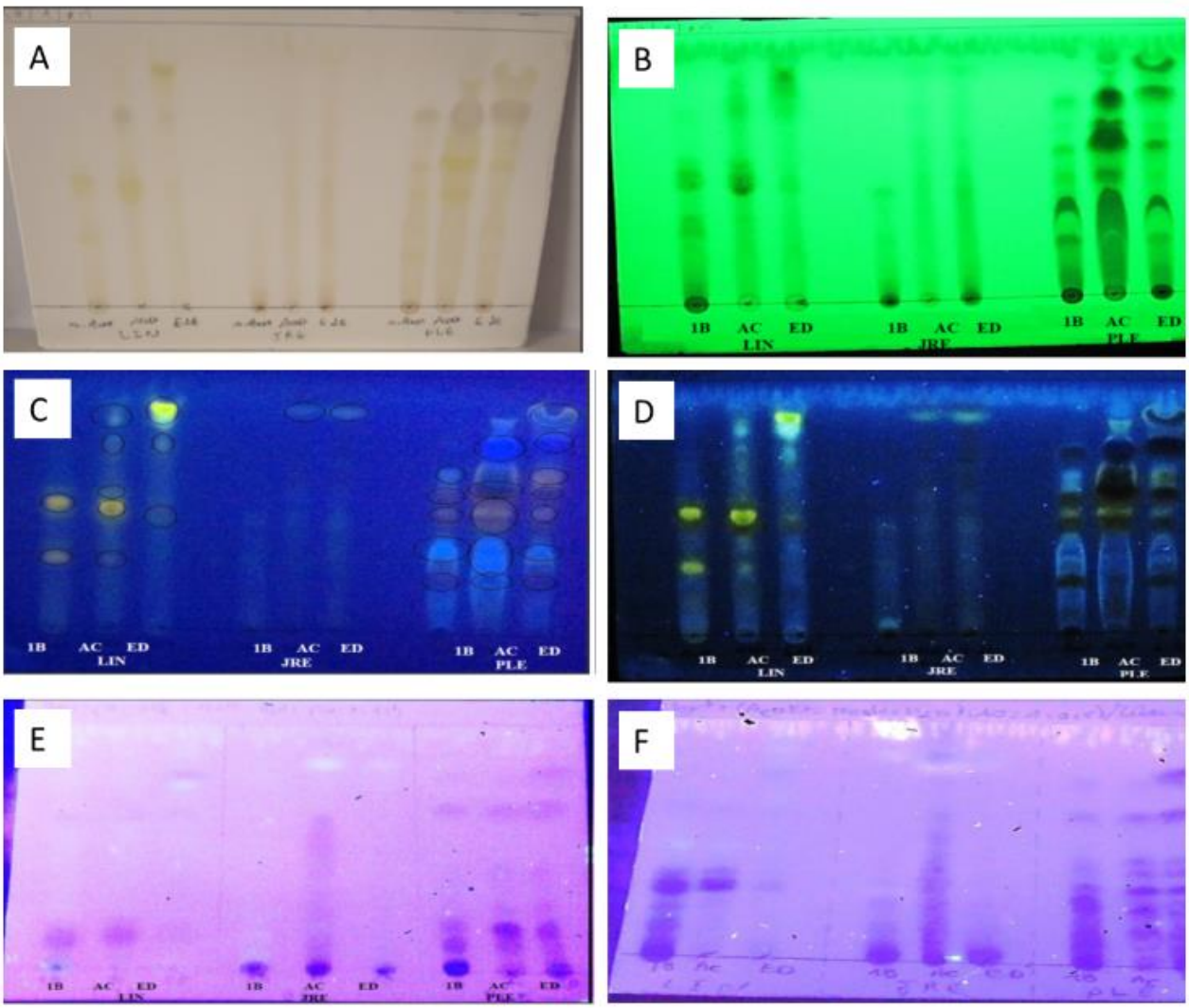

Figure 1. Analytical TLC of the different plant extracts. A: on silica gel developed in ethyl acetate/methanol/water solvent (eye vision); B: on silica gel developed in ethyl acetate/methanol/water solvent (UV 254nm); C, D: Flavonoïds on silica gel developed in Neu reagent system (365 nm); E: On silica gel before revelation; F: On silica gel after revelation in Libermann-Burchard reagent

assessing the activity of the phases obtained by FIFO with solvents of increasing polarity by the diffusion method on agar medium. The strains tested are those used in our previous study in the evaluation of the activity of total extracts. The results obtained are shown in tables 2 and 3.

No activity or little activity was found in our samples for most strains mainly *Trichophyton mentagrophyte* and *Microsporum canis* which were not sensitive to tested extracts. About *Trichophyton rubrum*, it developed a slight sensitivity for both phase's ethyl acetate and diethyl ether extracts of leaves of *Lawsonia inermis* and only diethyl ether Phase bark extracts of *Juglans regia*. However, *Candida albicans* was the only strain inhibited by most of the fractions (DE, AC and 1B) of the three plants.

DISCUSSION

The three plants used in our screening are recognized biologically active in traditional medicine with convincing results [21-24]. These are revealing too little or no activity in our tests, let us move more contingencies regarding the lack of response in our *in vitro* tests. Traditional medicine uses the juice of the leaves, the chemical composition is impossible to reproduce experimentally as well as any solvent associations and synergies of compounds that can take place naturally.

The fresh plant is used in traditional medicine. In this study, for conservation reasons, we took advantage of dry plants. This preliminary drying step may cause chemical changes of the compounds and cause loss of activity.

Tested concentrations may be insufficient to visualize a significant positive activity. Indeed, the lack of *in vitro* activity is not synonymous with lack of real activity and on the contrary, the *in vitro* activity does not guarantee a future activity. It is totally impossible to reproduce in full the factors involved in the interaction skin /worms/ antifungal [25]. We can only get close to developing models (solvents and pharmacological tests) and we should always keep a critical eye about our results. A positive result will therefore always be validated by the result of clinical tests. The characteristics of the analysis technique are essential

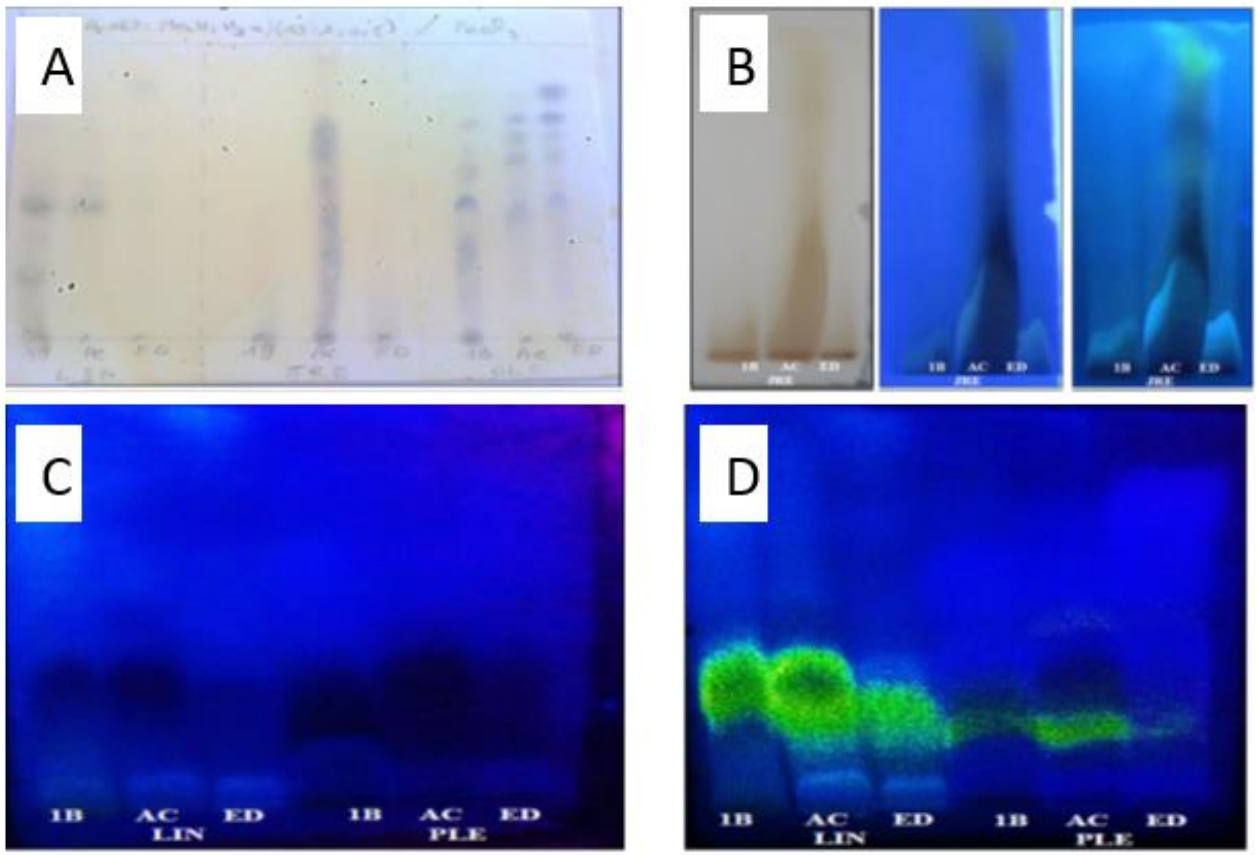

Figure 2. Chromatograms of *Pistacia lentiscus* L., *Lawsonia inermis* L. and *Juglans regia* L. A: Analytical TLC on silica gel revealen on $FeCl_3$ reagent; B: Chromatogram of *Juglans regia* on DC6 polyamide gel developed in MEC/MeOH/EtOH/Petroleum ether (visible, 256nm, 356nm); C, D: Chromatogram of *Pistacia lentiscus* L. and *Lawsonia inermis* L. on DC6 polyamide gel developed in MEC/MeOH/EtOH/Petroleum ether (256nm, 356nm)

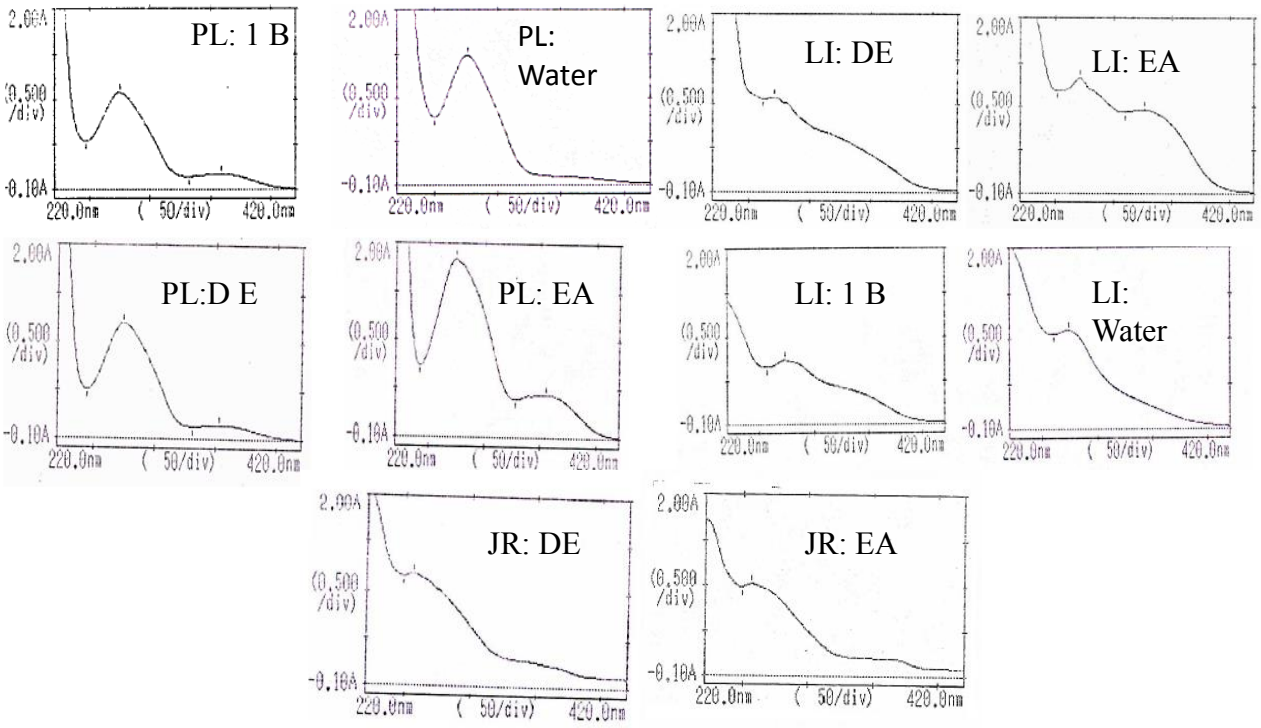

Figure 3. UV absorption specters of the different plant extracts in methanol. 1B (1 butanol), DE (diethylic ether), EA (ethyl acetate)

to ensure the operator of its sensitivity, specificity and reproducibility.

CONCLUSION

The activity of the extracts obtained by successive phases of confrontation was notable for *Candida albicans*, single strain inhibited by most of the different fractions (DE, AC and 1B) of three tested plants *J. regia, P. lentiscus* and *L. inermis*. This partition has enabled us to confront the possible consequences of phytochemical fractionation and loss induced activity. The various classes of compounds have complementary and synergistic properties, for totum effect.

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