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(RESEARCH ARTICLE)

In vitro antifungal activity of selected plant extracts against *Candida albicans*, *Microsporum canis*, and *Trichophyton rubrum*

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Abstract

Superficial fungal infections caused by *Candida* species and dermatophytes remain frequent clinical problems, increasing the interest in medicinal plants as potential sources of antifungal agents. This study evaluated the *in vitro* antifungal activity of extracts from *Allium sativum* L., *Lawsonia inermis L., Pinus halepensis*, and *Olea europaea* L. against *Candida albicans*, *Microsporum canis*, and *Trichophyton rubrum*. Crude, oily, aqueous, and hydroalcoholic extracts were prepared according to the plant material and extraction suitability. Antifungal activity against *C. albicans* was assessed using the disk diffusion method, while dermatophyte inhibition was evaluated by an agar surface exposure method. The most important result was obtained with crude garlic extract, which produced the strongest inhibition against *C. albicans*, with a 70 mm inhibition zone. Oily garlic extract also showed significant activity, with a 32 mm inhibition zone. In contrast, hydroalcoholic henna extract showed only moderate activity against *C. albicans*, and hydroalcoholic *P. halepensis* bark extract showed weak activity. Aqueous olive leaf extract, aqueous *P. halepensis* extract, and oily *P. halepensis* extract showed no detectable inhibition. Against *M. canis* and *T. rubrum*, both crude and oily garlic extracts completely inhibited visible fungal growth, whereas hydroalcoholic henna extract was inactive. These findings highlight *A. sativum* as the most promising plant tested, with marked antifungal activity against both yeast and dermatophyte models. However, further studies using minimum inhibitory concentration assays, chemical profiling, extract standardization, and cytotoxicity testing are required before any therapeutic or pharmaceutical application can be proposed.

Keywords: *Allium sativum;* Antifungal Activity*; Candida albicans; Lawsonia inermis; Microsporum canis; Trichophyton rubrum*

1. Introduction

Superficial mycoses are among the most common fungal infections in humans and primarily involve the skin, hair, nails, and mucosal surfaces. Their etiological agents include yeasts, particularly *Candida albicans*, and keratinophilic dermatophytes such as *Microsporum canis* and *Trichophyton rubrum*. Dermatophytes colonize keratinized tissues and are responsible for tinea capitis, tinea corporis, tinea pedis, onychomycosis, and related clinical presentations. Their epidemiological significance is reinforced by chronicity, recurrence, zoonotic transmission in some species, and the long duration of therapy often required [1,2].

Current antifungal therapy relies on azoles, allylamines, polyenes, and other synthetic or semisynthetic agents. Although these drugs are effective, their use can be limited by toxicity, drug interactions, recurrence, cost, reduced adherence

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during prolonged treatment, and the emergence of reduced susceptibility in some fungi. For research purposes, standardized antifungal susceptibility testing, particularly broth microdilution and disk diffusion methods, provides a framework for comparing antifungal activity. However, plant extracts present additional methodological complexity because their biological effect is influenced by extraction solvent, phytochemical profile, concentration, viscosity, and diffusion capacity in agar [3-6].

Medicinal plants remain an important reservoir of bioactive molecules. *Allium sativum* L. (garlic) contains organosulfur compounds, especially allicin and related thiosulfinates, which have documented antimicrobial and antifungal properties. *Lawsonia inermis* L. (henna) is rich in lawsone, phenolics, flavonoids, and tannins, compounds that may contribute to antimicrobial activity. *Olea europaea* L. leaves contain oleuropein, hydroxytyrosol, and other phenolics whose antimicrobial activity has been reported, although antifungal effects can be inconsistent depending on the extraction procedure and concentration. *Pinus halepensis* contains phenolic compounds, terpenoids, and tannins that may justify evaluation against fungi, particularly when traditional use suggests antiseptic or cicatrizing properties [7-15].

The objective of the present study was to evaluate and compare the *in vitro* antifungal activity of selected plant extracts from *A. sativum, L. inermis, P. halepensis*, and *O. europaea* against *C. albicans* and, for the most relevant extracts, against *M. canis* and *T. rubrum*. The working hypothesis was that extract type and solvent polarity would strongly influence antifungal response.

2. Materials and Methods

2.1. Study setting

The experimental work was conducted in the pharmacognosy laboratory of the Department of Pharmacy, University Ferhat Abbas Setif 1, and in the mycology laboratory of CHU Setif over a two-month period. The study focused on the preparation of selected plant extracts and the *in vitro* evaluation of their activity against fungal species associated with superficial mycoses.

2.2. Plant material

Four plants were selected: *A. sativum* L. bulbs, *L. inermis* L. leaves, *P. halepensis* bark, and *O. europaea* L. leaves. Garlic bulbs were collected from the Beni Mouhli region in Setif. Dried henna leaves originating from Biskra were obtained from a herbalist and pulverized before extraction. Olive leaves of the Azemour variety were collected during flowering, dried in the shade, and powdered. *Pinus halepensis* bark pieces were collected, cut, shade-dried, and finely powdered (figure 1).

Figure 1 The various plants selected to assess antifungal activity *in vitro*. (A) *Allium sativum* powder; (B) *Lawsonia inermis* powder;(C) *Pinus halepensis* powder; (D) *Olea europaea* powder

2.3. Fungal strains and identification

Three fungal species were used: *C. albicans, M. canis*, and *T. rubrum*. The strains were obtained from clinical samples and maintained on Sabouraud chloramphenicol agar. *C. albicans* was identified by germ tube formation, chlamydospore production, and carbohydrate assimilation testing. Dermatophytes were identified using macroscopic colony morphology and microscopic features, including macroconidia and microconidia morphology (figure 2).

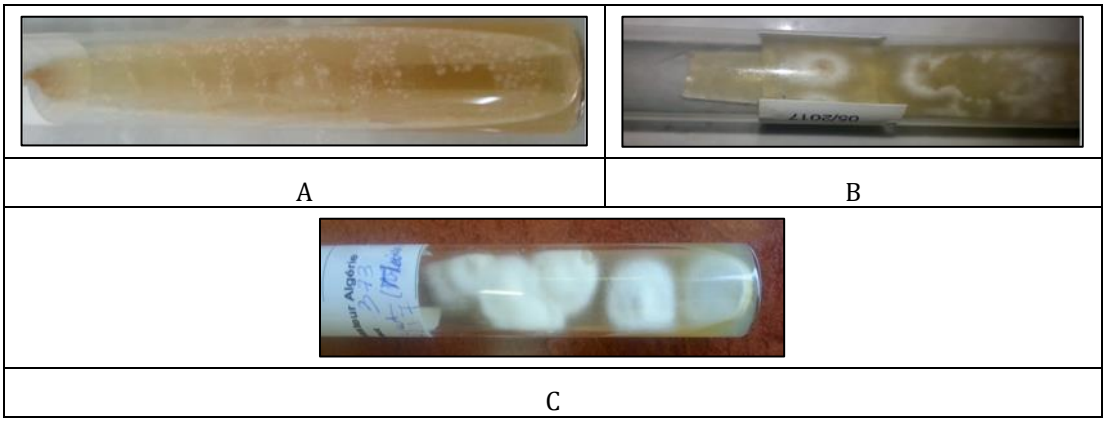

Figure 2 The different fungal species to assess antifungal activity *in vitro*. (A) *Candida albicans*; (B) *Microsporum canis*;(C) *Trichophyton rubrum*

2.4. Preparation of plant extracts

Crude garlic extract was obtained by grinding fresh bulbs, filtering the homogenate through gauze, centrifuging at 3800 rpm for 15 min, and collecting the supernatant [13]. Oily garlic extract was prepared by macerating chopped garlic in olive oil for 24 h followed by filtration. Hydroalcoholic henna extract was prepared by macerating leaf powder in 70% ethanol for 24 h, filtering, and concentrating the filtrate [17]. Aqueous olive leaf extract was obtained by macerating leaf powder in warm water at 45 °C, followed by agitation and successive filtrations [18]. *Pinus halepensis* bark extracts were prepared as aqueous, hydroalcoholic, and oily extracts by maceration, homogenization, and filtration.

2.5. Inoculum preparation

For each fungal species, a standardized inoculum was prepared in sterile physiological saline. The turbidity was adjusted using a densitometer to obtain an inoculum of approximately 10^6 CFU/mL. The suspension was corrected by adding fungal culture or sterile saline when turbidity was respectively too low or too high.

2.6. Antifungal assay against *Candida albicans*

The antifungal activity against *C. albicans* was evaluated using the disk diffusion method. The standardized inoculum was spread over Sabouraud chloramphenicol agar using sterile swabs. Sterile disks of 6 mm diameter were impregnated with the tested extracts and placed on the inoculated agar surface. Blank disks, ethanol-impregnated disks, and olive-oil-impregnated disks served as negative controls. Plates were incubated at 37 °C for 48 h. Inhibition zones were measured in millimeters, including the 6 mm disk diameter [6].

2.7. Antifungal assay against dermatophytes

For *M. canis* and *T. rubrum*, the selected extracts were evaluated using a modified agar-based screening method adapted from previously described antifungal assays against dermatophytes [15]. After fungal inoculation, the agar surface was exposed to the tested extract. Plates were incubated at 25 °C for 7 days. Growth was interpreted in comparison with the untreated control. Any visible fungal growth, even weak growth, was considered negative for complete inhibition.

2.8. Data presentation

Results are presented as inhibition zone diameters for *C. albicans*, and as presence or absence of visible growth for *M. canis* and *T. rubrum*. Because this preliminary study did not include concentration gradients or biological replicates sufficient for inferential statistics, no statistical comparison was performed. The results should therefore be interpreted as screening data rather than definitive susceptibility endpoints.

3. Results

3.1. Activity against *Candida albicans*

The antifungal response against *C. albicans* differed markedly between extracts. Negative controls, including the blank disk, ethanol, and olive oil, did not produce inhibition zones. The strongest activity was observed with crude garlic extract, followed by oily garlic extract. Hydroalcoholic henna extract and hydroalcoholic *P. halepensis* extract produced smaller inhibition zones, whereas aqueous olive leaf extract and the aqueous and oily *P. halepensis* extracts were inactive (table 1).

Table 1 Antifungal activity of plant extracts against *Candida albicans*

Extract tested	Code	Inhibition zone (mm)
Blank disk	V	6
Ethanol	A	6
Olive oil	HO	6
Crude garlic extract	BA	70
Oily garlic extract	HA	32
Hydroalcoholic henna extract	H	12
Aqueous *Pinus halepensis* bark extract	AP	6
Oily *Pinus halepensis* bark extract	HP	6
Aqueous *Olea europaea* leaf extract	O	6
Hydroalcoholic *Pinus halepensis* bark extract	HAP	8

6 mm = disc only = no inhibition.

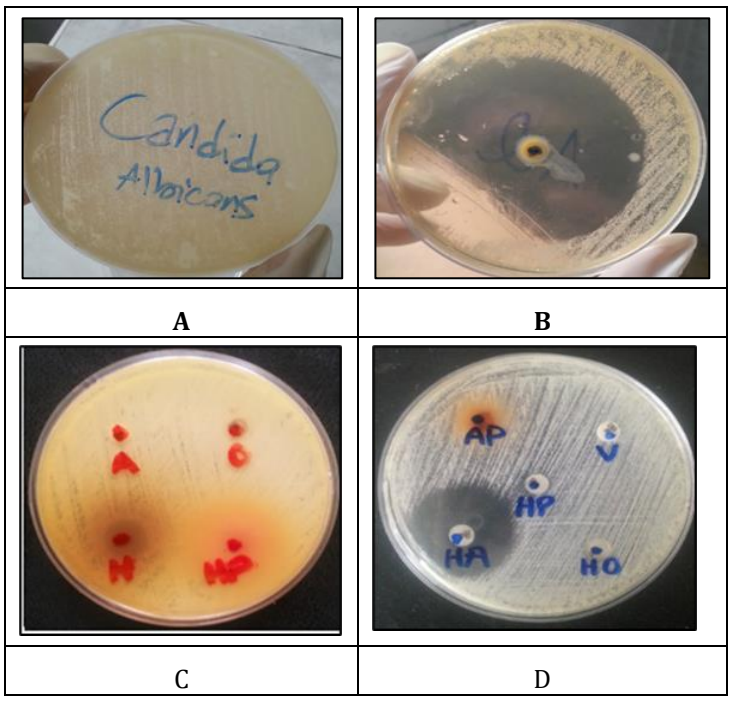

Figure 3 Antifungal activity of plant extracts against *Candida albicans*. (A) Control without extract; (B) crude garlic extract; (C) aqueous and hydroalcoholic extracts of plants; (D) plant oil extracts

3.2. Activity against *Microsporum canis*

Both crude and oily garlic extracts completely inhibited visible growth of *M. canis* after 7 days of incubation. In contrast, hydroalcoholic henna extract did not prevent fungal growth under the conditions tested (table 2).

Table 2 Antifungal activity of selected extracts against *Microsporum canis*

Extract tested	Visible growth after incubation
Control without extract	+
Crude garlic extract	-
Oily garlic extract	-
Hydroalcoholic henna extract	+

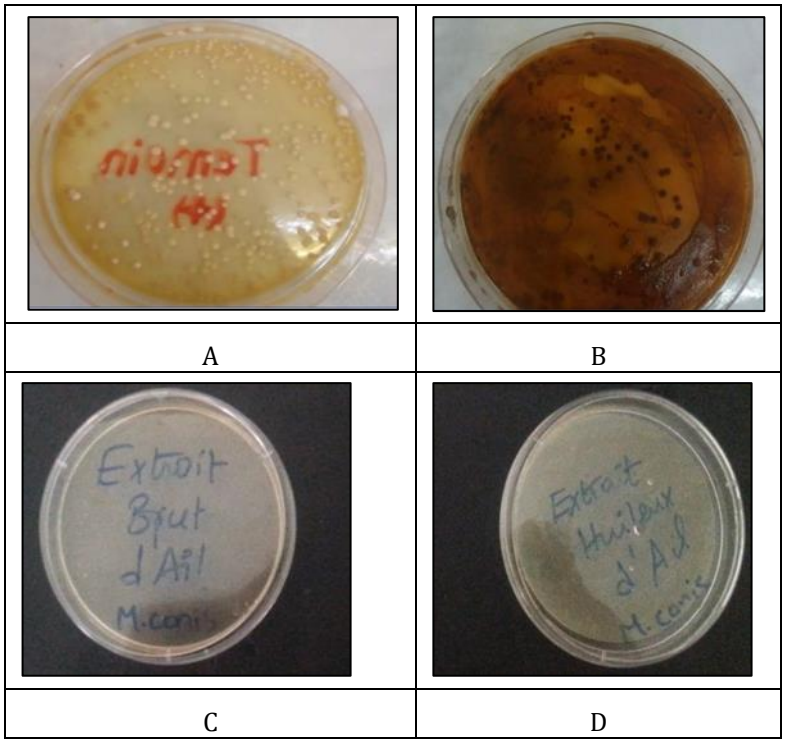

Figure 4 Antifungal activity of plant extracts against *Microsporum canis*. (A) Control without extract; (B) hydroalcoholic henna extract; (C) Crude garlic extract; (D) Oily garlic extract

3.3. Activity against *Trichophyton rubrum*

The pattern observed for *T. rubrum* was similar to that obtained for *M. canis*. Crude and oily garlic extracts produced complete inhibition of visible growth, whereas hydroalcoholic henna extract was inactive (table 3).

Table 3 Antifungal activity of selected extracts against *Trichophyton rubrum*

Extract tested	Visible growth after incubation
Control without extract	+
Crude garlic extract	-
Oily garlic extract	-
Hydroalcoholic henna extract	+

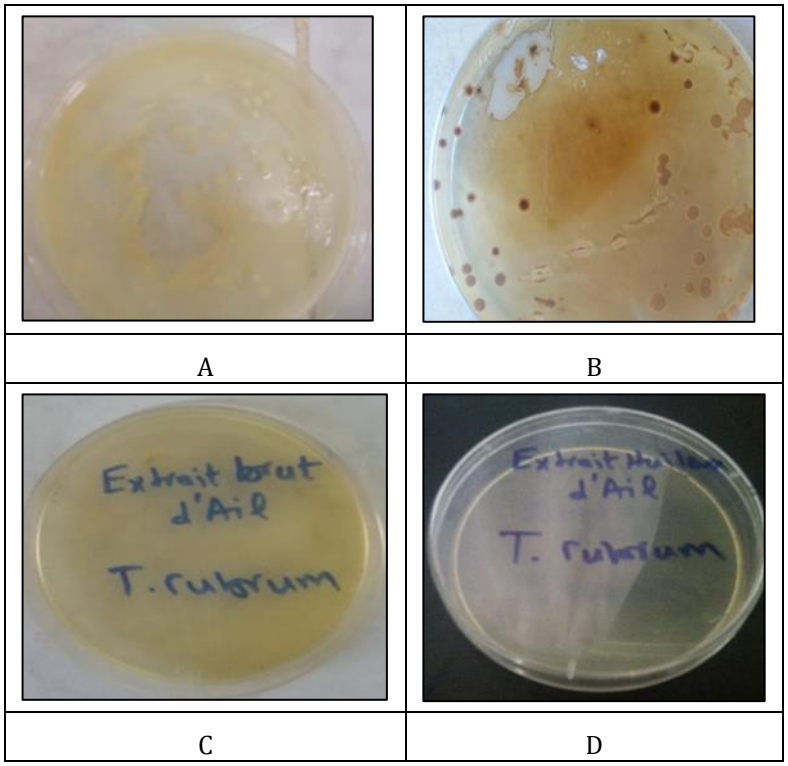

Figure 5 Antifungal activity of plant extracts against *Trichophyton rubrum*. (A) Control without extract; (B) hydroalcoholic henna extract; (C) Crude garlic extract; (D) Oily garlic extract

4. Discussion

This study demonstrates a clear hierarchy of antifungal activity among the tested plant extracts. The most important finding is the strong in vitro activity of *A. sativum* extracts, particularly the crude extract, against *C. albicans* and the two dermatophytes tested. The inhibition zone of 70 mm against *C. albicans* is substantial for a crude natural preparation and indicates a high level of diffusible antifungal activity under the experimental conditions. The negative results obtained with blank disks, ethanol, and olive oil strengthen the interpretation that the observed inhibition was attributable to plant-derived constituents rather than to the solvent system.

The superior activity of crude garlic extract compared with oily garlic extract is chemically plausible. Fresh garlic contains alliin and alliinase in separate cellular compartments. Tissue disruption allows enzymatic conversion of alliin into allicin, a reactive thiosulfinate widely associated with antimicrobial activity. Allicin can react with thiol-containing enzymes and proteins, which may disrupt essential fungal metabolism, impair membrane-associated functions, and reduce fungal growth [8,9]. Since allicin and related compounds are chemically labile, the extraction procedure, time between preparation and testing, temperature, pH, and solvent environment can markedly influence activity [10]. The crude extract probably preserved a broader fraction of polar and semi-polar reactive sulfur compounds, whereas the oily extract may have favored less polar constituents and reduced diffusion through agar [7].

The complete inhibition of *M. canis* and *T. rubrum* by both garlic preparations is particularly relevant because dermatophytes are clinically important keratinophilic fungi involved in persistent cutaneous infections. However, the binary growth/no-growth endpoint used for dermatophytes should be interpreted conservatively. It demonstrates visible inhibition under a screening condition, but it does not quantify potency. Without concentration-response testing, minimum inhibitory concentration (MIC), minimum fungicidal concentration (MFC), and subculture-based viability assays, it is not possible to determine whether the extracts were fungistatic or fungicidal. This distinction is essential for any future pharmaceutical development [12].

Hydroalcoholic henna extract showed only moderate activity against *C. albicans* and no complete inhibition of the two dermatophytes. This result does not necessarily contradict previous studies reporting antifungal effects of *Lawsonia inermis* extracts against *Candida albicans*, *Trichophyton rubrum* and *Microsporum canis* [14]. Rather, it highlights the dependence of plant-extract bioactivity on extraction and processing conditions. Lawsone and other phenolic constituents may be affected by extraction temperature, extraction time, solvent composition and solvent-to-plant ratio

[15,16]. If the extract was concentrated by heating, thermolabile or oxidation-sensitive compounds could have been partially degraded. Moreover, hydroalcoholic extracts may contain active compounds, but at concentrations insufficient to completely inhibit dermatophytes under the method used.

The weak activity of hydroalcoholic *P. halepensis* bark extract against *C. albicans* suggests that some antifungal constituents may be extracted by ethanol-water mixtures, but at a level insufficient for strong inhibition. The inactivity of aqueous and oily *P. halepensis* extracts supports the view that solvent polarity was a determinant of extract performance. *P. halepensis* is known to contain phenolics, terpenoids, resinous compounds, and tannin-like constituents, whose availability depends strongly on plant part, geographical origin, extraction solvent, and concentration [18]. In the present screening, the hydroalcoholic extract was the only *P. halepensis* preparation that produced a detectable inhibition zone.

The absence of antifungal activity for the aqueous *O. europaea* leaf extract against *C. albicans* is scientifically defensible and should not be interpreted as proof that olive leaves lack antifungal potential. Olive leaves contain oleuropein, hydroxytyrosol, and related phenolics, but reported antifungal effects vary considerably between studies. Recent findings indicate that olive leaf extracts may show weak, inconsistent, or concentration-dependent activity against *C. albicans* [19,20]. The negative result in this study may reflect insufficient extraction efficiency, degradation of active secoiridoids, low concentration of extractable active compounds in the tested cultivar, or limited diffusion of active constituents in agar.

A key methodological limitation is the use of agar diffusion as the main readout. This method is simple and useful for preliminary screening, but it is not equivalent to standardized MIC determination. Zone size depends not only on antifungal potency but also on molecular diffusion, extract viscosity, compound solubility, inoculum density, and interaction with the culture medium [7]. This limitation is particularly important when comparing aqueous, hydroalcoholic, and oily extracts. A poorly diffusing oily extract may appear less active than a hydrophilic extract even if it contains biologically active compounds. Therefore, the present results are best interpreted as preliminary evidence of extract-level activity rather than standardized susceptibility values.

These findings are coherent and generate a strong research direction. Garlic extracts, particularly crude extract, emerged as the most promising candidate but it would be premature to jump directly from *in vitro* inhibition results to therapeutic claims; however, these data justify the continued development of antifungal preparations based on *A. sativum.*

5. Conclusion

The present *in vitro* screening showed that plant extracts differ substantially in antifungal activity depending on botanical source and extraction procedure. Crude *A. sativum* extract was the most active preparation against *C. albicans*, followed by oily garlic extract. Both garlic extract completely inhibited visible growth of *M. canis* and *T. rubrum*. Hydroalcoholic henna extract showed moderate activity against *C. albicans* but was inactive against dermatophytes under the tested conditions. Hydroalcoholic *P. halepensis* bark extract showed weak activity against *C. albicans,* whereas aqueous olive leaf extract and the remaining *P. halepensis* extracts were inactive. These findings support the antifungal potential of *A. sativum* as a candidate for further investigation, but they require confirmation using standardized quantitative assays, chemical characterization, toxicity testing, and formulation studies.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

I declare and all co-authors that we participated in the design, execution and analysis of the document and that I approve the final version. In addition, there is no conflict of interest in connection with this document, and the material described is not in the process of being published nor is it intended for publication elsewhere.

Statement of ethical approval

The fungal strains were obtained from clinical laboratory material used for routine diagnostic purposes. No identifiable patient information is included in this manuscript. The study was conducted in accordance with standard laboratory practices and the principles of confidentiality and anonymity.

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