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ORIGINAL RESEARCH

Antifungal activity of *Cardiospermum halicacabum* L. (Sapindaceae) against *Trichophyton rubrum* occurs through molecular interaction with fungal Hsp90

Roberta Gaziano[1,*]
Elena Campione[2,*]
Federico Iacovelli[3]
Daniele Marino[1]
Francesca Pica[1]
Paolo Di Francesco[1]
Stefano Aquaro[4]
Francesco Menichini[4]
Mattia Falconi[3]
Luca Bianchi[2]

[1]Microbiology Section, Department of Experimental Medicine and Surgery, University of Rome 'Tor Vergata', Rome, Italy; [2]Department of Systems Medicine, University of Rome 'Tor Vergata', Rome, Italy; [3]Department of Biology, University of Rome 'Tor Vergata', Rome, Italy; [4]Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, Rende, Italy

*These authors contributed equally to this work

Correspondence: Mattia Falconi
Department of Biology, University of Rome 'Tor Vergata', Via della Ricerca Scientifica 1, 00133 Rome, Italy
Tel +39 6 7259 4025
Email falconi@uniroma2.it

Luca Bianchi
Department of Systems Medicine, University of Rome 'Tor Vergata', Viale Oxford 81, 00133 Rome, Italy
Email luca.bianchi@uniroma2.it

Introduction: Dermatophytosis is a superficial fungal infection limited to the stratum corneum of the epidermis, or to the hair and nails, and constitutes an important public health problem because of its high prevalence and associated morbidity. Dermatophyte fungi, especially 2 species, *Trichophyton rubrum* and *Trichophyton mentagrophytes,* are the predominant pathogens. Topical antifungal drugs, mainly azoles or allyamines, are currently used for the treatment of dermatophytoses, although in some cases, such as in nail and hair involvement, systemic treatment is required. However, therapeutic efficacy of current antifungal agents can be limited by their side effects, costs, and the emergence of drug resistance among fungi. Plant extracts represent a potential source of active antimicrobial agents, due to the presence of a variety of chemical bioactive compounds. In the present work, we evaluated in silico and in vitro the antifungal activity of an extract of the medicinal plant *Cardiospermum halicacabum* against *T. rubrum* suggesting a potential interaction with Hsp90 as playing an important role in both pathogenicity and drug susceptibility of *T. rubrum*.

Methods: We investigated in vitro the effect of different concentrations of *C. halicacabum* (from 500 to 31.25 µg) against a clinical isolate of *T. rubrum*. Furthermore, using a computational assessment, the interaction between different *C. halicacabum* active compounds and the fungal Hsp90 was also investigated.

Results: Our results indicate a clear-cut antifungal activity of the total plant extract at the highest concentrations (500 and 250 µg). Among all tested *C. halicacabum* compounds, the luteolin and rutin molecules have been identified in silico as the most important potential inhibitors of Hsp90. Based on these data, luteolin and rutin were also individually assessed for their antifungal activity. Results demonstrate that both substances display an antifungal effect, even if lower than that of the total plant extract.

Conclusion: Our data indicate a strong fungistatic effect of *C. halicacabum* against *T. rubrum*, suggesting its potential therapeutic efficacy in the treatment of dermatophytoses. Additionally, *C. halicacabum* compounds, and particularly luteolin and rutin, are all possible Hsp90 interactors, explaining their fungistatic activity.

Keywords: *Trichophyton rubrum*, dermatophytoses, *Cardiospermum halicacabum*, antifungal activity, Hsp90, molecular modeling, molecular docking, rutin, luteolin

Introduction

Dermatophytosis are superficial mycotic infections limited to the stratum corneum of the epidermis, or to the hair and nails, and represent some of the most common dermatologic diseases seen worldwide. Among dermatophytosis, onychomycosis is a chronic fungal infection of the fingernails or toenails that causes discoloration,

thickening, and separation from the nail bed. Onychomycosis occurs in 10% of the general population but is more common in older adults; the prevalence is 20% in those older than 60 years and 50% in those older than 70 years. The increased prevalence in older adults is related to peripheral vascular disease, immunologic disorders, and diabetes mellitus. Onychomycosis is widely believed to be only a cosmetic problem, but it can be uncomfortable and can lead to cellulitis in older adults and foot ulcers in patients with diabetes.[1,2] Fungal nail infections can be caused by many different types of fungi, most often dermatophytes, yeasts (*Candida*), and nondermatophytes. Among dermatophytes, *Trichophyton rubrum* and *Trichophyton mentagrophytes* are the most common agents of onychomycosis.[3] Onychomycosis is more difficult to treat than most dermatophytosis because of the inherent slow growth of the nail. The treatment depends on the clinical type of the onychomycosis, the number of affected nails, and the severity of nail involvement.[4] Topical treatments with amorolfine 5%, ciclopirox 8% nail lacquer, and tioconazole 28% solution, which need to be applied for prolonged periods of time and require strong adherence by the patients, are limited to superficial onychomycosis, and generally are unable to cure onychomycosis because of insufficient nail plate penetration. A systemic treatment with azole and allylamine is always required in proximal subungual onychomycosis and in distal lateral subungual onychomycosis involving the lunula region.[3,5] The use of topical agents in combination with systemic therapy is also shown to be more effective in severe toenail onychomycosis and increases the cure rate.[6] However, treatment for this illness is long term, and recurrences are frequently detected. Furthermore, treatment is limited by the high cost of conventional antifungal drugs; their adverse effects included headache, gastrointestinal problems, rash, and the emergence of drug resistance among fungal pathogens.

Therefore, there is a continuous and urgent need to discover novel antifungal agents and to identify new potential molecular drug targets for the treatment of fungal infections. Recently, scientific interest in medicinal plants has arisen as a new area due to the increased efficiency of plant-derived drugs for the treatment of various diseases. In this scenario, *Cardiospermum halicacabum*, belonging to the Sapindaceae family, is an herbaceous climber widely distributed in tropical and subtropical regions. It is seen all through the plains of Africa, America, Bangladesh, India, Malacca, and Pakistan. Several chemical constituents have been isolated from its, ie, β-arachidic acid, apigenin, apigenin-7-*O*-glucuronide, chrysoeriol-7-*O*-glucuronide,

rutin, and luteolin-7-*O*-glucuronide.[7–9] Various fatty acids were also isolated from seed oil.[10] The plant-based herbal products like gel, cream, shampoo, spray, medical drops, and pills are commercially available and are helpful in dry itchy skin and scalp and in the treatment of chronic skin diseases, including psoriasis.[8,11] The plant has been used in Ayurveda and folk medicine for a long time in the treatment of rheumatism, lumbago, cough, hyperthermia, nervous diseases (epilepsy and anxiety disorders), as a demulcent in orchitis, and in dropsy.[12–15] Various pharmacological actions of *C. halicacabum* have been investigated in animal models. Particularly, *Cardiospermum* reduces cyclophosphamide (CTX)-induced toxicity and oxidative stress in mice and also regulates *iNOS* and *COX-2* gene expression in LPS-stimulated macrophages.[16] Furthermore, remarkable antipyretic and antiulcer activities of *Cardiospermum* have been shown in rats.[17] The plant also shows antihyperglycemic activity against streptozotocin-induced diabetes in rats and antidiarrheal potential in mice.[18] The ethanol extract of the plant suppresses the production of TNF-α and nitric oxide in human peripheral blood mononuclear cells.[19] Additionally, the anti-inflammatory, analgesic, and vasodepressant activities of this plant have also been described.[20] The leaf extract of *C. halicacabum* also possess appreciable antibacterial activity, antifungal activity against *Candida albicans*, antimalarial activity, and antifilarial activity; so this plant could potentially be used for the treatment of infectious diseases.[21–23]

Evaluation of the interconnection among drug resistance, stress response, and the signaling pathways activated in this processes has revealed Hsp90 as a novel potential cellular target for antifungal drugs.[24–26] Hsp90 is an adenosine triphosphate (ATP)-dependent eukaryotic molecular chaperone. Its involvement in the pathogenicity and resistance of *C. albicans*, *Aspergillus fumigatus*, and *T. rubrum* to azole and echinocandin antifungals are well established.[27–29] Recently, some natural products such as geldanamycin (a benzoquinone ansamycin antibiotic binding to the ATP-binding pocket of Hsp90) and resorcyclic acid lactone produced by certain fungal species have been studied as inhibitors of Hsp90.[30] The recent application of some Hsp90 inhibitors as anticancer therapy has raised considerable interest in this protein as a target for new antifungal therapies.[31] Within this context, the aim of the present study was to investigate the effect of *C. halicacabum* on the in vitro growth of *T. rubrum*. Furthermore, as suggested by molecular docking analysis, 2 of the most promising *C. halicacabum* active compounds have been tested, confirming their remarkable role in the inhibition of fungal growth.

Materials and methods

Plant material

The seeds of *C. halicacabum* were collected in Modica (Sicily, Italy) and authenticated by Dr NG Passalacqua, Natural History Museum of Calabria and Botanic Garden, University of Calabria, Italy. A voucher specimen (CLU 22013) was deposited at the Herbarium of University of Calabria.

Extraction procedure

The seeds (431 g) of *C. halicacabum* were exhaustively extracted by maceration with 1,500 mL of methanol (4×72 hours). The resulting solutions were evaporated under reduced pressure to give the extract (yield of 1.42%). The extract of *C. halicacabum* was analyzed for its total phenol and flavonoid content as previously described.[9]

Materials

A stock solution of *C. halicacabum* was prepared by dissolving 1 g of the plant extract in 1 mL of methanol. Further dilutions with sterile PBS were made to a final concentration of 10 mg/mL. Luteolin-7-*O*-glucuronide, rutin, and amphotericin B (Fungizone) were purchased from Sigma Aldrich (St Louis, MO, USA). Luteolin and rutin were dissolved in methanol to a concentration of 10 mg/mL and then diluted with sterile PBS to the desired concentrations ranging from 500 to 62.5 µg. Amphotericin B was dissolved in sterile water and diluted with physiological solution to appropriate concentration of 250 µg/mL.

Determination of antifungal activity

The *T. rubrum* strain used in this study was obtained from a clinical sample according to routine hospital laboratory procedures. The fungal strain was grown on Sabouraud dextrose agar (Difco, Detroit, MI, USA) supplemented with chloramphenicol at 30°C for 5–7 days. The antifungal activity in vitro of *Cardiospermum* extract and of its compounds luteolin and rutin was investigated by using a disc diffusion assay (Kirby–Bauer method). The antifungal drug amphotericin B was used as positive control. Briefly, a sterile swab dipped into the fungal suspension, adjusted to 2.0 McFarland standard turbidity, was spread uniformly over the entire surface of Sabouraud dextrose agar plate. Sterile filter paper discs were placed onto the surface of the agar plate and individually impregnated with 50 µL of twofold serial dilutions of *Cardiospermum* extract, ranging from 500 to 31.25 µg/disc, or the flavonoid compounds luteolin and rutin, ranging from 500 to 62.5 µg/disc, or amphotericin B ranging from 12 to 1.5 µg/disc. Plates were then incubated at 30°C

for 48 hours and checked for clear zones of inhibition. PBS with 1% of methanol was used as negative control.

Molecular docking simulation methods

Protein–ligand molecular docking has been used to evaluate binding mode and energy between *T. rubrum* Hsp90 and 13 *C. halicacabum* active compounds. The ATP natural cofactor has been included in the analysis. The docking simulations have been executed using the AutoDock Vina 1.1.2 program through the AutoDock/Vina PyMOL plugin (http://wwwuser.gwdg.de/~dseelig/adplugin.html) (The PyMOL Molecular Graphics System Version 1.5.0.4, Schrödinger, LLC, New York, NY, USA).[32,33] The 13 compounds and ATP structural files (SDF file) have been obtained from the PubChem compound database (https://pubchem.ncbi.nlm.nih.gov), converted into mol2 files, and filled with hydrogens using the Open Babel program.[34] In the absence of *T. rubrum* Hsp90 X-ray structure, the crystal structure of Hsp90 from *Saccharomyces cerevisiae*, deposited in the Protein Data Bank (PDB, http://www.rcsb.org/pdb) with PDB ID code 2CG9 whose sequence shares around 76% of identity and 95% of query coverage with *T. rubrum* Hsp90-like protein (Uniprot sequence ID: F2SVB4) (http://www.uniprot.org/uniprot/F2SVB4), has been used as a template to build the *T. rubrum* Hsp90 model structure as reported in Figure 1.[35] The model has been
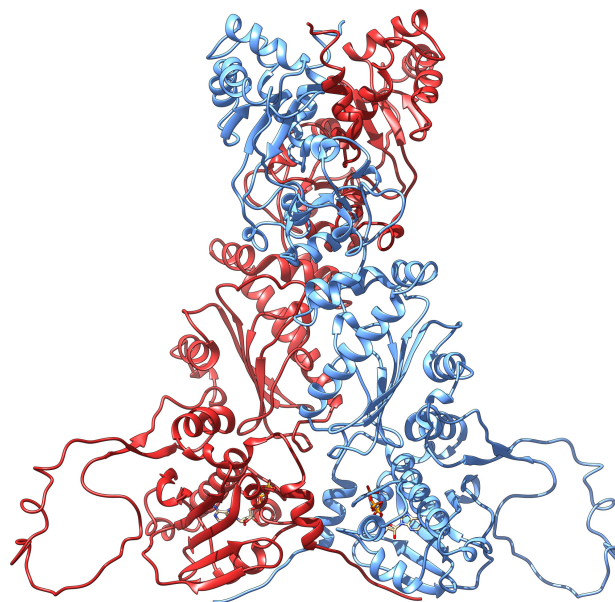
Figure I Structural model of *T. rubrum* Hsp90 dimeric structure generated using as a template the crystal structure of Hsp90 from *Saccharomyces cerevisiae* (PDB ID: 2CG9). **Notes:** Blue and red colors indicate the 2 monomers. This image has been generated using the Chimera program.[42]
Abbreviation: PDB, Protein Data Bank.

generated using the SWISS-MODEL protein modeling tool (http://www.swissmodel.expasy.org) and, after a structural check, has been used as a receptor for the molecular docking simulations of ATP, the natural Hsp90 cofactor, and *C. halicacabum* compounds in the ATP-binding site.[36] The side chains belonging to the Hsp90 ATP-binding site (Glu32, Asn36, Asp78, Phe83, Asn91, Ser98, Gln118, Val121, Phe123, Arg375) have been considered rotatable to simulate the receptor flexibility during the docking procedure. The dimensions of the chosen docking box in Å were as follows: $X=22.50$; $Y=26.25$; $Z=22.50$. The AutoDock/Vina program selects, for each docking simulation, 10 ligand poses representing the cluster centroids of all the evaluated solutions. Each docking simulation run takes about 40′ (elapsed real time) on a dedicated Intel i7-4700 CPU workstation.

Results
Antifungal activity of *C. halicacabum* against *T. rubrum*

T. rubrum is the most important causative agent of dermatophytoses. The antifungal activity of *C. halicacabum* against *T. rubrum* has never been studied. For the first time, this study aims to primarily evaluate the effect of the medicinal plant *C. halicacabum* on *T. rubrum* growth in vitro. Our experimental results (Figure 2) show a considerable inhibitory effect on fungal growth in the areas treated with 500 or 250 µg of *C. halicacabum* extract, whereas concentrations <250 µg (125, 62.5, and 31.25 µg) apparently did not affect the growth of the fungus. It is interesting to note that the inhibition zone diameter of the total extract at 500 µg/disc overlaps approximately with that of the standard antifungal amphotericin B at the highest concentration used (12 µg/disc), suggesting that this natural extract has a significant potential antifungal

efficacy, as a function of the dose employed in the in vitro experiments.

Interactions of *C. halicacabum* active compounds in *T. rubrum* Hsp90 ATP-binding site

Previous works have been demonstrated that *Cardiospermum* inhibited the growth of bacterial as well as fungal strains, but the mechanisms underlying the antifungal activity still as yet incompletely understood. Recently, Hsps have been implicated in several fungal processes, including pathogenicity.[29] Herein, we investigated by a molecular docking approach the interactions between a total of 13 compounds of *Cardiospermum* and *T. rubrum* Hsp90 ATP-binding site. As reported in Table 1, a group of 6 molecules, showing energies between −10 and −11 kcal/mol (kaempferol, apigenin, cholecalciferol, all-trans-retinoic acid, quercetin, and chrysoeriol), may be considered as potential competitive inhibitors of the ATP natural ligand characterized by an interaction energy of −10.2 kcal/mol. Another set of compounds (calycosin-7-*O*-β-D-glucopyranoside, 1-hentriacontanol, pentadecanoic acid, 3,4-dihydroxybenzoic acid, and 3,4-dihydroxybenzaldehyde) show energies between −9.3 and −6.2 kcal/mol indicating a lower affinity, though still sufficiently high for the calycosin molecule, in comparison with the preceding group. Among all tested compounds, the rutin and luteolin molecules have been identified as the most important potential inhibitors of Hsp90. In fact, their structures, as observed with the ATP molecule, completely fill the Hsp90 ATP-binding site in the N terminal domain of the *T. rubrum* model structure, establishing a large number of interactions (Figures 3 and 4). In particular, these molecules are fully stabilized by several
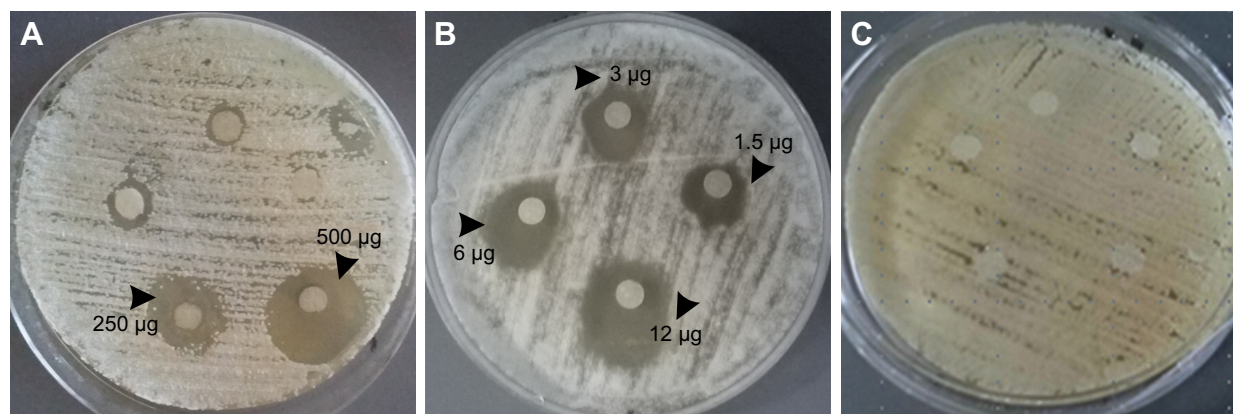
Figure 2 Disc diffusion assay of antifungal activity of *Cardiospermum halicacabum* against *Trichophyton rubrum*.
Notes: Fungal culture was incubated at 30°C with different concentrations of *C. halicacabum* total extract, as described in the "Materials and methods" section (**A**). Amphotericin B at 12, 6, 3, and 1.5 µg/disc was used as positive control (**B**). As a negative control, PBS with 1% of methanol was used (**C**). After 48 hours of incubation, a clear inhibition zone was observed in the areas treated with 500 or 250 µg of *Cardiospermum* extract solution. One of 3 representative experiments is shown.

Table 1 Docked compounds' structures and energies

Compound name	PubChem CID	Docking energy (kcal/mol)
Rutin	5280805	−12.1
Luteolin	5280637	−11.9
Kaempferol	5280863	−10.6
Apigenin	5280443	−10.4
Cholecalciferol	5280795	−10.4
All-trans-retinoic acid	444795	−10.2
Quercetin	5280343	−10.1
Chrysoeriol	5280666	−10.1
Calycosin-7-*O*-β-D-glucopyranoside	5318267	−9.3
1-Hentriacontanol	68345	−7.3
Pentadecanoic acid	13849	−7.0
3,4-Dihydroxybenzoic acid	72	−6.6
3,4-Dihydroxybenzaldehyde	8768	−6.2
ATP	5957	−10.2

Notes: List of docked *Cardiospermum halicacabum* compounds. Docking energies have been obtained through molecular docking simulation using a structural model of *Trichophyton rubrum* Hsp90 as a receptor. The energy value obtained with the Hsp90-ATP redocking is indicated for comparison. The PubChem (https://pubchem.ncbi.nlm.nih.gov) CIDs are also shown.
Abbreviations: ATP, adenosine triphosphate; CIDs, compound identification numbers.

hydrophobic contacts and hydrogen bonds established with the ATP-binding site residues. Similar interaction energies have been obtained for the rutin and luteolin compounds (−12.1 and −11.9 kcal/mol, respectively), showing values about 2.0 kcal/mol lower than that obtained with the redocking of the ATP molecule (Table 1). These results strongly indicate that *C. halicacabum* compounds are all possible Hsp90 interactors, explaining its high fungistatic effect, although rutin and luteolin may be considered as

the most efficient competitive inhibitors of the primary chaperon Hsp90.

Luteolin and rutin exert a direct antifungal activity against *T. rubrum*

Based on the data obtained by molecular docking study, the in vitro antifungal activity of the 2 *Cardiospermum* bioactive flavonoids, luteolin and rutin, has been also experimentally investigated. The results presented in Figure 5A show that luteolin-7-*O*-glucoside at the highest concentration of 500 µg exhibited a significant antifungal effect which, however, was considerably lower than that of the total extract of *C. halicacabum* (Figure 2A), but higher than that of rutin (Figure 5B). The inhibition zone diameters induced by luteolin and rutin, at the highest tested concentrations, overlap with those of amphotericin B at 3 and 1.5 µg, respectively (Figure 2B).

Discussion

Dermatophytes are fungal pathogens that commonly cause superficial infections of keratinized structures such as skin, nails, and hair in immunocompetent individuals. The prevalence rate of superficial mycotic infection worldwide has been found to be 20%–25%. However, in rare cases, dermatophytosis may be invasive, such as in Majocchi's granuloma, a dermatophyte infection of dermal and subcutaneous tissue. Patients with deep dermatophytosis may have a history of uncontrolled superficial fungal infections such as tinea unguium and tinea cruris. Depending on the amplitude and the site of infection, dermatophytoses can be difficult to cure and systemic treatment with azole, allylamine, and
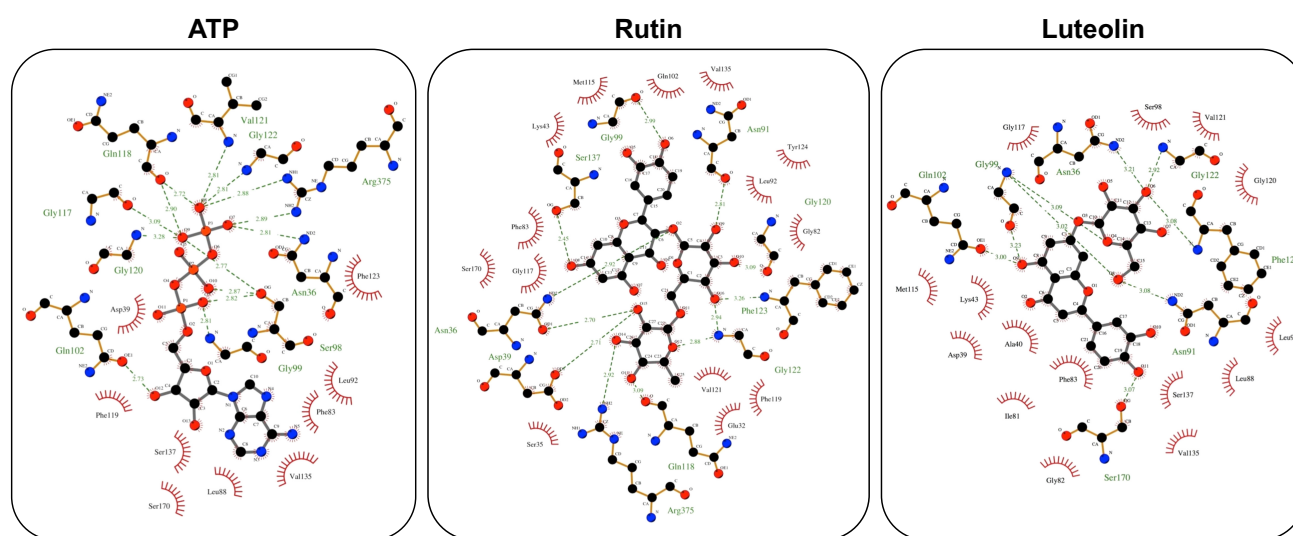
Figure 3 Schematic view of the best molecular docking complex between *Trichophyton rubrum* Hsp90 and ATP (left panel), rutin (central panel), and luteolin (right panel).
Notes: The residues interacting through hydrogen bonds (green dashed lines) are shown as ball-and-stick images, while the residues in contact with the ligands are indicated by arches with rays. This image has been produced using the LigPlot+ software (European Bioinformatics Institute, Cambridgeshire, UK).[43]
Abbreviation: ATP, adenosine triphosphate.

ATP   **Rutin**   **Luteolin**
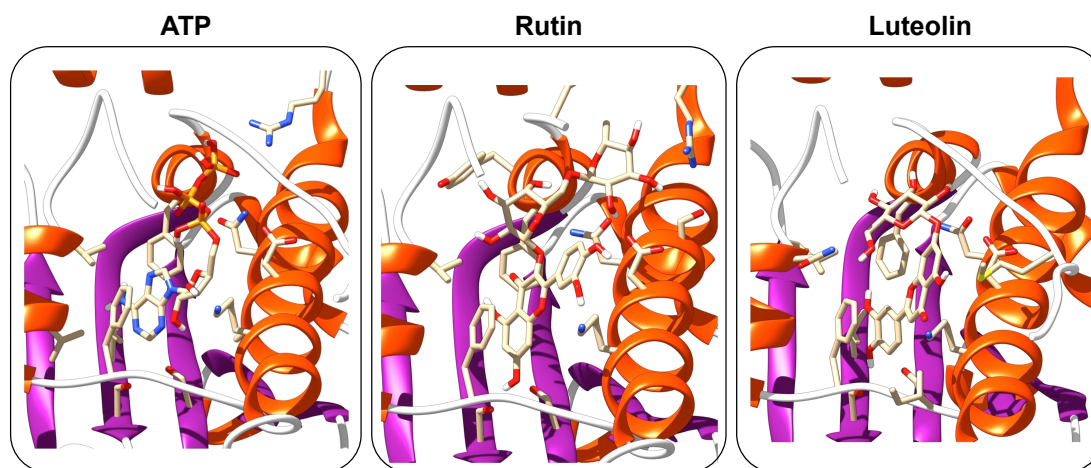
Figure 4 Best molecular docking complex between *Trichophyton rubrum* Hsp90 and ATP (left panel), rutin (central panel), and luteolin (right panel).
Notes: The β-strands are represented by violet arrows, while the α-helices and the loops are shown as orange spirals and light gray wires, respectively. The ligand molecules, hosted in the Hsp90 ATP-binding site, are indicated by stick models colored by atom type. This picture has been generated using the Chimera program.[42]
**Abbreviation:** ATP, adenosine triphosphate.

griseofulvin is always required. Particularly, terbinafine, the orally available allylamine antifungal agent, is considered to be a first-line drug for the treatment of dermatophytosis owing to its good mycological and pharmacokinetic profile.[37] Sertaconazole is a newer topical imidazole antifungal drug used in Europe for the treatment of superficial skin mycoses such as dermatophytosis.[38]

However, to date, despite the advent of new antifungal agents for topical and systemic treatment of superficial mycotic infections, an increase in the number of fungi that are resistant to the antifungal drugs currently in clinical use has been reported. Moreover, the toxic side effects of conventional antifungal therapies make dermatophytosis an infection increasingly difficult to treat. Furthermore, fungi are eukaryotic organisms like mammals, and so the number of

antifungal drugs targeting structures that are unique to fungi is extremely limited. Thus, the development of innovative antifungal strategies to overcome drug resistance and toxicity of the actually available antifungal drugs is undoubtedly a current medical need for the effective treatment of mycotic infections.

Recently, there is also an increasing interest in plant-derived products for their potential application in the treatment of mycotic infections. Particularly, *C. halicacabum* is a climbing plant which has been used in Chinese medicine for a long time owing to its natural anti-inflammatory and antioxidant properties. In the past years, a significant antibacterial and antifungal activity in vitro of *C. halicacabum* has also been described. Shareef et al[39] reported that ethanol extract proved to have a strong inhibition activity against *S. cerevisiae* and
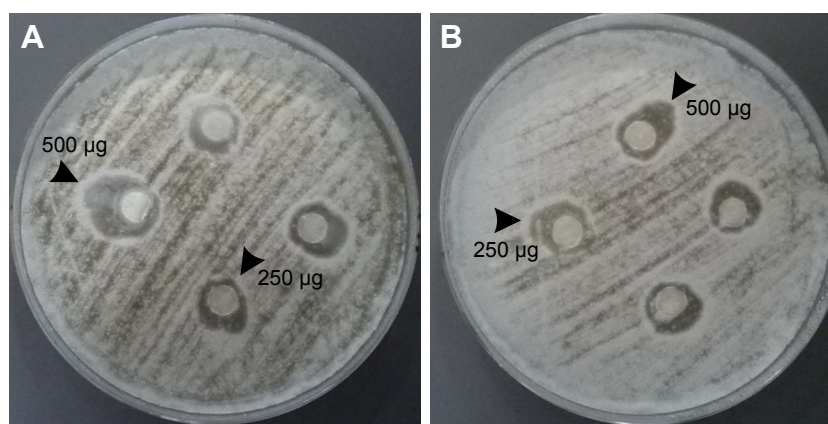
Figure 5 Disc diffusion assay of antifungal activity of luteolin and rutin against *Trichophyton rubrum*.
Notes: Fungal cultures were incubated at 30°C with luteolin (**A**) or rutin (**B**), at 500, 250, 125, and 62.5 μg/disc. After 48 hours of incubation, a significant inhibition zone was observed in the area around the disc impregnated with 500 μg of each compound. One of 3 representative experiments is shown.

Aspergillus niger, whereas in case of *C. albicans* ethanol, aqueous, and oil extracts recorded only a moderate inhibition activity. Seed oil was also active against animal pathogens such as *Micosporillum gypsiccus* and *T. mentagrophyte*. By contrast, ethanol, aqueous and oil extracts did not influence the in vitro growth of *Trichophyton longifusis*, *Trichophyton tonsurans*, and *Microsporum canis*.[39] Moreover, an important antimicrobial effect of ethanolic extract of *C. halicacabum* against *C. albicans* and multidrug resistant human pathogenes, such as *Staphylococcus aureus* and *Escherichia coli*, was also described by Jeyadevi et al.[21]

Among dermatophytes, *T. rubrum* is the main fungus that commonly causes deep and invasive infections, especially in immunocompromised patients, including those undergoing solid organ or bone marrow transplantation, those with diabetes mellitus, hematological malignancy, human immunodeficiency disorder, and those using long-term steroid therapy. On the basis of these evidences, in the present study we investigated the effect of *C. halicacabum* against *T. rubrum* growth. The results show that *C. halicacabum* total extract is able to exert an efficient dose-dependent inhibitory activity in vitro on *T. rubrum.*

The molecular mechanism by which *C. halicacabum* exerts its antimicrobial activity is still unclear. The antibacterial activity of phenol- and flavonoid-rich plant extracts could be attributed to their ability to form complexes with the bacterial cell wall.[40]

On the other hand, it is known that Hsp90 is conserved from bacteria to eukaryotic cell, and recent works indicate that in fungi Hsps are implicated in several processes, including pathogenicity, phase transition in dimorphic fungi, and antifungal drug resistance.[41] Cowen et al[25] demonstrated that in vitro treatment with geldanamycin, an inhibitor of Hsp90 which interferes with the ATP-binding domain, reduced the emergence of resistance to azoles and echinocandins in human pathogens such as *C. albicans* and *A. fumigatus*.[28] Furthermore, chemical inhibition of Hsp90 resulted in increased susceptibility of *T. rubrum* to itraconazole and micafungin, and decreased its growth in human nails in vitro.[29] These evidences prompted us to also investigate, using molecular docking approaches, whether *C. halicacabum* compounds may directly interfere with the ATP-binding site of *T. rubrum* Hsp90. Interestingly, among a total of 13 plant compounds assessed, the flavonoids luteolin and rutin have been identified as the most important potential inhibitors of Hsp90, although all tested plant compounds have been shown to have affinity for Hsp90. These data are supported by our in vitro experiments which show a direct antifungal activity exerted individually by luteolin and rutin against *T. rubrum*, even though their antifungal efficacy was lower than that of the total plant extract. Overall, these results suggest that each single bioactive compound presumably interacts directly with the ATP-binding pocket of Hsp90, and all together can work in agonist and synergistic way, potentiating the antifungal action of the total plant extract. Furthermore, the Hsp90 interaction may be an important molecular mechanism underlying the antifungal property of *C. halicacabum*, but it would not be the only 1 orchestrating fungal morphogenesis and virulence. The antifungal efficacy of *C. halicacabum* may be due to multiple molecular interactions among its bioactive compounds and different target sites in a single or multiple intracellular pathways that are critical for the fungal growth. On the basis of these evidences, we propose that the antibacterial activity exerted by flavonoids is due not only to their interaction with the cell wall of bacteria, but also to their ability to directly interfere with the Hsp90 function.

Conclusion

Our results demonstrate that the whole-plant extract of *C. halicacabum* could possibly be a great source of bioactive agents capable of inhibiting the fungal growth by interfering with Hsp90, which can be considered a potential target for novel antifungal strategies. Overall, these evidences suggest that *C. halicacabum* extract, alone or in combination therapy with lower doses of conventional antimycotic drugs to reduce their side effects, can represent a new potential therapeutic strategy for the treatment of infections caused by fungi which show resistance to the current antifungal therapies. However, all studies investigating the antifungal activity of *C. halicacabum* have been carried out in vitro. Further investigations in experimental animal models of mycotic infections are necessary to assess in vivo the efficacy and safety of this natural compound for future potential translation of research into clinical practice.

Author contributions

All authors contributed toward data analysis, drafting and critically revising the paper, gave final approval of the version

to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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