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Biofilm Inhibition of *Inula viscosa* (L.) *Aiton* and *Globularia alypum* L. Extracts Against *Candida* Infectious Pathogens and In Vivo Action on *Galleria mellonella* Model

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The increasing importance of fungal infections has fueled the search for new beneficial alternatives substance from plant extracts. The current study investigates the antifungal and antibiofilm activity of *Inula viscosa* (L.) *Aiton* and *Globularia alypum* (L.) leaves extracts against *Candida* both in vitro and in vivo. The inhibition of planktonic and sessile *Candida albicans* and *Candida glabrata* growth using both leaf extracts are evaluated. Moreover; an in vivo infection model using *Galleria mellonella* larvae; infected and treated with the extracts are performed. All extracts show fungicidal activity; with a minimum fungicidal concentration (MFC) ranging from 128 to 512 µg mL^{-1} against the two selected strains of *Candida*. In particular, the best results are obtained with methanolic extract of *I. viscosa* and *G. alypum* with an MFC value of 128 µg mL^{-1}. The extracts are capable to prevent 90% of biofilm development at minor concentrations ranging from 100.71 ± 2.49 µg mL^{-1} to 380.4 ± 0.92 µg mL^{-1}. In vivo, tests on *Galleria mellonella* larvae show that the extracts increase the survival of the larvae infected with *Candida*. The attained results reveal that *I. viscosa* and *G. alypum* extracts may be considered as new antifungal agents and biofilm inhibiting agents for the pharmaceutical and agro-food field.

1. Introduction

Fungi infect billions of people each year and despite the high number of contaminations and high mortality rates, fungal diseases have received so far little attention. Usual antifungals used to treat fungal infections are ineffective, and there are few new antifungals in development. There are few drugs available to treat fungal diseases, and the increased.

Resistance to the present drugs has raised concerns about treatment efficacy in the future.[1] To effectively combat fungal diseases, new antifungals must be developed. In this context, there has been an increasing interest in alternative therapeutic interventions, and the use of plant extracts as adjuvants in antifungal therapy has been highlighted in recent years.[2] In comparison with bacterial infectious diseases there are few studies on antifungal activity of plant extracts,[3] and the majority of these research are focused on plant fungal pathogens and food fungal contaminants[4–6]; as of yet, only a scattering number of plant-derived extracts have been revealed to possess antifungal potential against human and animal pathogens.[7–10]

Candida albicans and *Candida glabrata* represent 60% of *Candida* species existing in the human body and are also the most widespread infective *Candida* species, being responsible for over than 400 000 life-threatening infections worldwide annually.[11,12] This fungus can change from commensalism to active infection and is attached to its numerous virulence traits. Biofilm growth is a critical process that allows the fungus to adhere and to proliferate on medically implanted devices as well

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as host tissue, resulting in potentially fatal infections. Biofilms are complex communities of filamentous and yeast cells encased in an extracellular matrix that confers increased antifungal drug resistance.[12] Furthermore, microorganisms in a biofilm have different growth characteristics and gene expression patterns than planktonic counterparts.[13] As well, the mainly mutual spots for fungal contaminations related with biofilms are the oral cavity, lungs, burn wounds, the lower reproductive tract, the gastrointestinal tract, skin, intravascular, and the insertion site of urinary catheters.[14] For this aim, researchers have given an interest in novel options, obtained from bioresources and various biologically active plant-derived medicinal compounds, for detachment or inhibition of biofilm formation, their bioactive compounds they have a potent role not only in the treat-ment of biofilm-based diseases, but also generally they are healthfuller and fewer side effects compared with synthetic compounds.[15,16]

On the other hand, the invertebrate *Galleria mellonella* larvae (wax moth) has been established as an alternate model that has attracted attention because of the working simplicity and reliability in the assessment of infections induced by different human pathogens, in the discovery of new virulence genes, as well as in the evaluation of toxicity and efficacy of antimicrobial agents.[17]

In this context, the current research was designed to examine the activity of *Globularia alypum* (L). and *Inula viscosa* (L.) *Aiton* leaves extracts against *Candida albicans* ATCC 10231, and *Candida glabrata* PMC 0849 biofilms using the biofilm inhibition ratio (%). Furthermore, *Galleria mellonella* was utilized as in vivo infection model through the analysis of the mortality rate in survival assays. Previously these plant extracts have been investigated for their polyphenolic profile by HPLC-DAD/ESI-MS analysis and the volatile content assessed by GC-MS, along with the evaluation of their antioxidant and antidiabetic activities.[18,19] Based on the results obtained, such species might be proposed as promising herbal medicines, due to their activity against fungal pathogens such as *Candida glabrata* and *Candida albicans*. The attained findings from this study reinforce the understanding about the overall medicinal properties of *I. viscosa* and *G. alypum*.

2. Results

2.1. Anti-Candida Activity of Crude Extracts

The MIC and MFC for Methanol (MetOh), Ethyl acetate (EtOAc), and chloroform extracts of *G. alypum* and *I. viscosa* against two fungal strains are presented in Tables S1 and S2 (Supporting Information).

All extracts tested showed activity against fungal growth of *Candida* strains, antifungal potential is estimated by assessing strain growth in the presence of increasing doses of extracts, so all extracts are able to inhibit fungal growth at low concentrations, specifically EtOAc and MetOh extracts for both plants with (MIC = 64 μg mL^{-1} and CMF = 128 μg mL^{-1}); versus *C. albicans* and (MIC = 128 μg mL^{-1} and CMF = 256 μg mL^{-1}); against *C. glabrata*, Chloroformic extracts were also able to inhibit fungal growth, However, MIC and CMF were higher compared to the other extracts (MIC = 512 μg mL^{-1}, CMF = 256 μg mL^{-1}) and (MIC = 256 μg mL^{-1}, CMF = 512 μg mL^{-1}) for *C. albicans* and *C. glabrata* respectively.

2.2. In Vitro Biofilm Inhibition Assay of the Investigated Extracts

The biofilm forming ability is an important characteristic associated with the pathogenicity of *Candida* and in the present study the effect of *G. alypum* and *I. viscosa* leaves extracts versus the mature biofilms of two *Candida* strains, namely *C. albicans* ATCC 10231 and *C. glabrata* PMC 0849 was tested with results illustrated in **Figure 1**. The inhibiting biofilm capacity of the extracts is reported as Minimal Inhibiting Concentration Biofilm ($MICB_{50}$) in Tables S3 and S4 (Supporting Information). The obtained results using different concentrations of *G. alypum* and *I. viscosa* extracts against *Candida* biofilm formation were extremely interesting. These revealed that *G. alypum* and *I. viscosa* extracts have a dose-dependent effect on the biofilm formation in two strains, It was found that the addition of *G. alypum* and *I. viscosa* Ethyl acetate, Methanol, and chloroform extracts at different concentrations (512–32 μg mL^{-1}) present the highest inhibition of 90% of biofilm formation with *G. alypum* and *I. viscosa* extracts at 512 μg mL^{-1} with favor for Methanol and Ethyl acetate extracts which inhibited the biofilm formation with approximately 90% at 512 μg mL^{-1} followed by the chloroform extracts for the two studied plants.

2.3. In Vivo Antifungal Activity Evaluation

Both extracts were further tested on *C. albicans* ATCC 10231, *C. glabrata* PMC 0849 using *Galleria mellonella* model with results presented in **Figure 2** and **3**.

2.4. Statistical Analysis

Statistical analysis is reported in Tables S5 and S6 (Supporting Information).

3. Discussion

The antifungal capacity of G. *alypum* and *I. viscosa* leaves extracts could be associated to its high content of polyphenol the synergistic interactions between different minor compounds.

In terms of biofilm forming ability the obtained results, illustrated in Figure 1, using different concentrations of *G. alypum* and *I. viscosa* extracts against *Candida* biofilm formation, revealed that both extracts have a dose-dependent effect on *Candida* biofilm formation. It was found that EtOAc and MetOh extracts inhibited 90% of biofilm formation at 512 μg mL^{-1} (Figure 1). The results of the minimum biofilm-inhibiting concentrations of each extract are shown in Tables S3 and S4 (Supporting Information), with values ranging from 100.71 ± 2.49 to 380.84 ± 0.92 μg mL^{-1}.

The results obtained indicate that ethyl acetate extracts from both plants were the most effective in inhibiting *C. albicans* and *C. glabrata* biofilms with $CMIB_{50}$ = 100.71 ± 2.49 and 100.88 ± 0.38, while chloroform extracts from both plants were the least effective compared to the others with inhibitory concentrations ranging from 280.21 ± 0.56 to 380.84 ± 0.92 μg mL^{-1}.

This finding demonstrates that the plants extracts present an important antifungal activity preventing the development of 50%

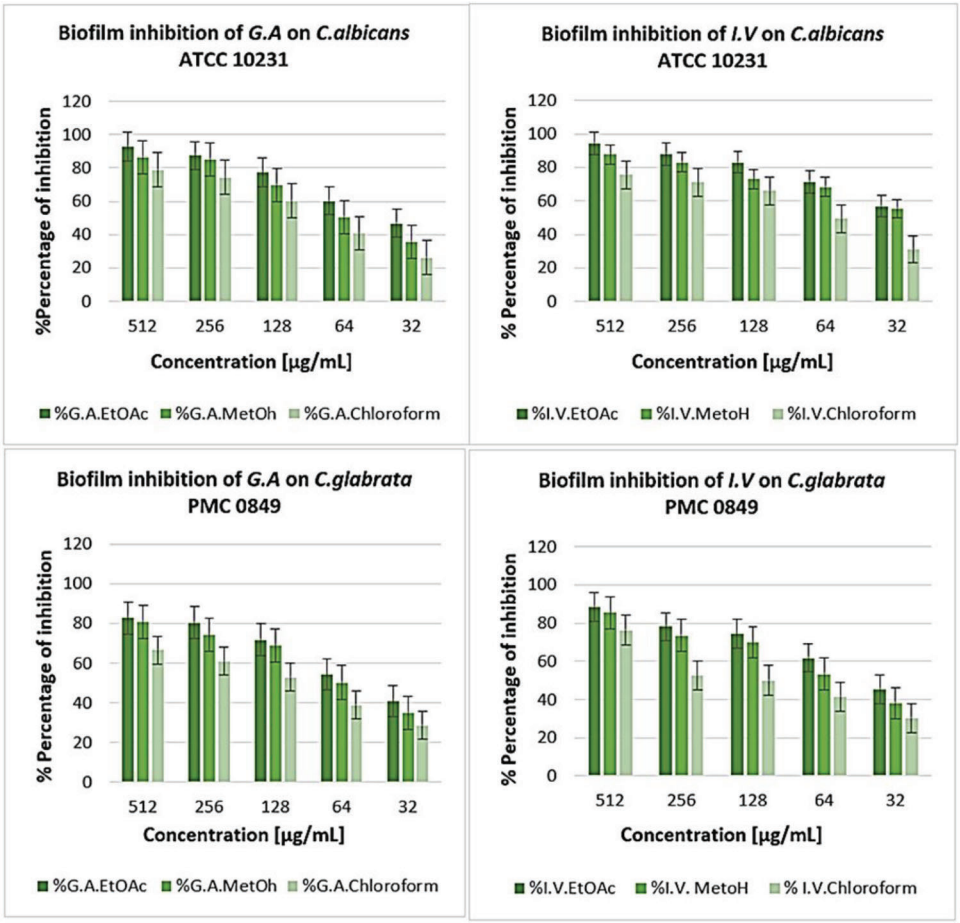

Figure 1. The activity of *I. viscosa* and *G. alypum* extracts against the mature biofilms of *C. albicans* ATCC 10231 and *C. glabrata* PMC 0849. The value is expressed as the median of at least three independent replicates.

of *C. albicans* and *C. glabrata* biofilms. The examined antifungal activity depends on the solvent used for the extraction process, seemingly owing to the differences in solubility of the bioactive compounds with solvent polarity.[20,21] Likewise, EtOAc, MetOh, extracts of *G. alypum* and *I. viscosa* leaves reveal potent antifungal and biofilm inhibiting activity, followed by chloroform extract. In fact, solvents with different polarities can extract individual polyphenols to different degrees, and this could account for the different antimicrobial activities of the extracts. The variations in the activity profiles are possibly due to the capability of the extracting solvent to dissolve the bioactive compounds depending on their polarities. On the other hand, the antifungal activity of both leaves' extracts could be related to its polyphenolic content.[18,19,20–29]

The chemical analysis of the phenolics profile of the extracts of *I. viscosa* and *G. alypum* was determined in our previous works[18,19] using HPLC-DAD-ESI/MS analysis, revealed the presence of 22 chemical compounds, for *I. viscosa* extract and 20 chemical compounds for *G. alypum* extract. Concerning *I. viscosa* chemical compounds, five of the identified compounds are phenolic acids, namely caffeic acid, galloylquinic acid, two isomers of di-O-Caffeoylquinic acids and rosmarinic acid, while the rest of compounds is represented by flavonoids *viz*. derivatives of quercetin, luteolin, naringin and apigenin. On the other hand, *G. alypum* extract characterization showed the identification of Three different phenolic compounds classes (quinic acid, gallic acid, and gallic acid ethyl ether), flavonoids (derivatives of quercetin and kaempferol), and several iridoids. Such data are in agreement with previously published data reporting the chemical characterization of such extracts.[30–41] Many of these compounds were reported to contribute to several biological properties such as antioxidant, antifungal, anti-inflammatory, antimicrobial, cytotoxicity, antiproliferative, and anticancer.[18,30,40–48]

Besides, *G. alypum* is one of the most used plants regions of North Africa for managing several disorders such as, cardiovascular diseases, diabetes, and various cancerous lesions of the stomach and liver.[49] Its extracts have been demonstrated in several studies revealing its wealthy of bioactive compounds, especially phenolics compounds, e.g. globularin, 6-hydroxyluteolin-7-O-glucoside, syringin, Quinic acid, Gallic acid, Kaempferol, Quercetin, Coumaroyl hexosyl glucitol, 6-O caffeoyl-3,4-dihydrocatalpol, 6′-O-caffeoylcatalpol,6-hydroxyluteolin 7-O-glucoside, caffeoylhexoside derivative, dihydroxyphenylethyl-caffeoyl-glucoside, 6-methoxyluteolin, hydroxy phenylethyl-feruloyl-glucoside, and 6′-O-caffeoylverbascoside.[50–53]

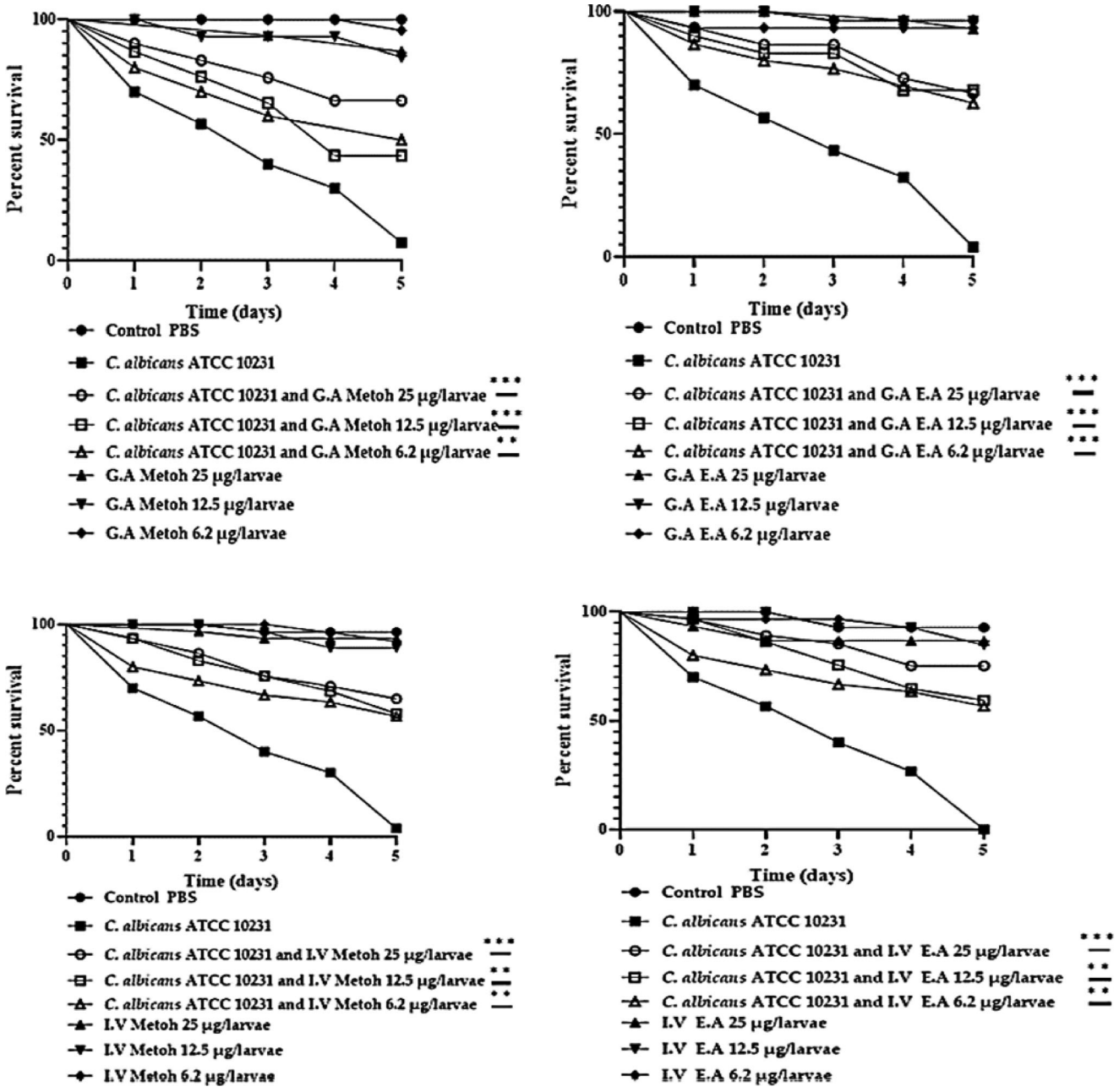

Figure 2. % of Survival rate of *G. mellonella* infected with *C. albicans* ATCC 10231 and treated with *I.viscosa* and *G.alypum* extracts after 24, 48,72, 96, and 120 h of incubation.

Several investigations have demonstrated the mechanism of action of phenolic compounds against fungi and could be caused by the perturbation of the lipidic membrane. In 2010 Sung and Lee proved that phenolic acids possibly engender interruption of ions transport,[54] although five years later Teodoro et al.[46] suggested that the carboxylic and hydroxyl acid groups of phenolic compounds have a significant role in destabilizing the fungal cytoplasmic membrane.

Mohti et al, have shown that the ethanolic fraction of *I. viscosa* leaves and flower buds can be used effectively as antimicrobial agents against *C. albicans*-associated infections, with MICs ranging from 125 to 250 µg mL^{-1}, this study identified 3,5-di-caffeoylquinic acid, padmatin, and isorhamnetin-3-O-(6-O-feruloyl)-glucoside as the main components of these extracts (Mohti et al., 2020). Another study by Mssillou et al. (2021), to evaluate the antimicrobial power of Moroccan *I. viscosa* leaf extracts against two fungal strains, *C. albicans* and *A. niger*, both ethanol and ethyl acetate extracts showed remarkable activity on both strains, with MICs ranging from 0.87 to 10 mg mL^{-1}.[29]

The antimicrobial activity of the methanolic extract of *G. alypum* from northeastern Tunisia was tested by the serial dilution method against two *Candida* species (*C. albicans* ATCC 90028,

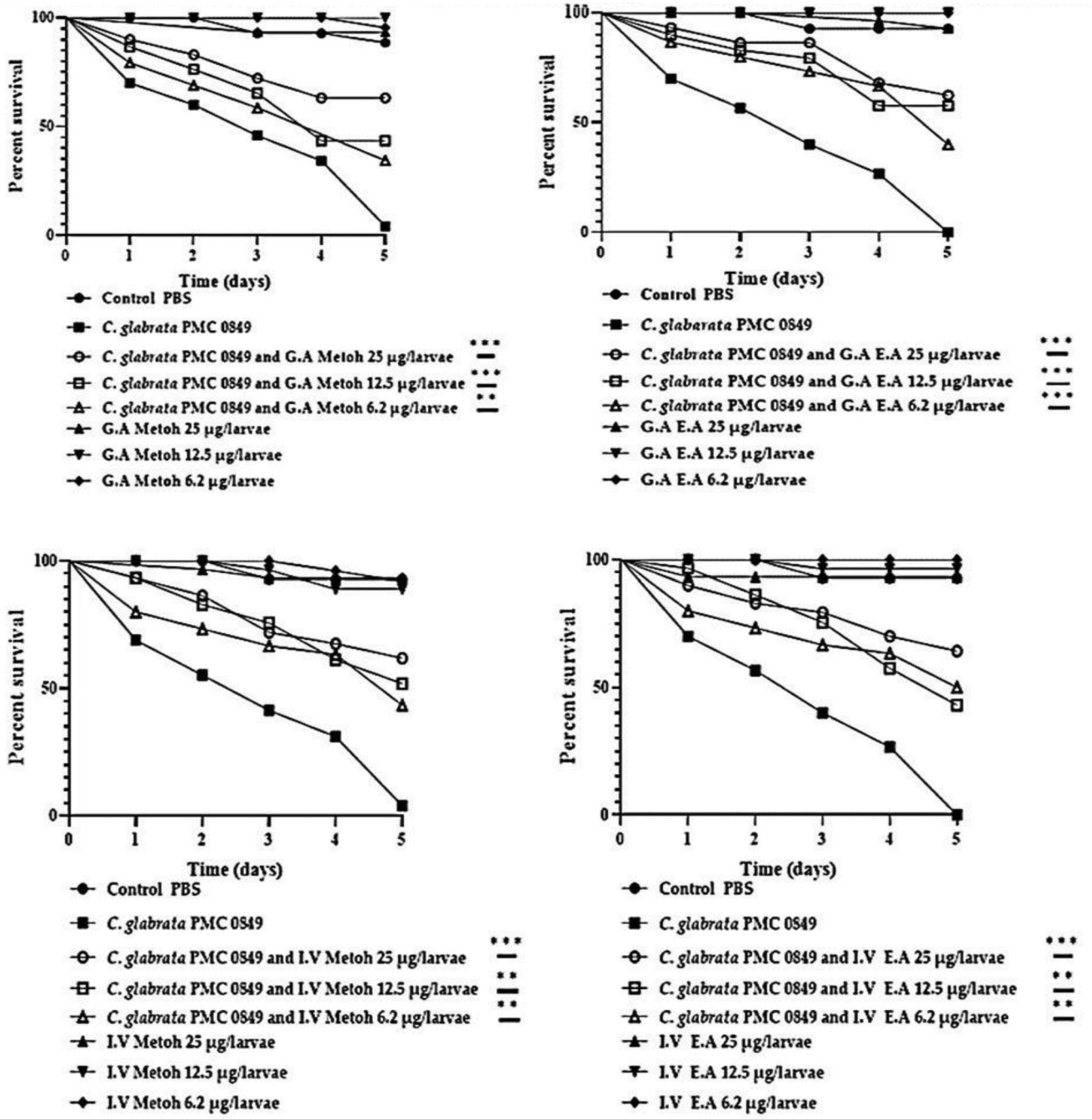

Figure 3. % of Survival rate *of G. mellonella* infected with *C. glabrata* PMC 0849 and treated with *I. viscosa* and *G.alypum* extracts after 24, 48,72, 96, and 120 h of incubation.

C. creusi ATCC 6258),[55] the extract has a significant antifungal action, particularly against *C. albicans* ATCC 90028 (MIC = 2560 µg mL^{-1}), explaining that this activity may be due to the presence of trans-cinnamic (45.14%), cirsiliol (3.21%), and caffeic acid (0.55%) detected by LC-MS.

Consequently, the present research has enriched information on the antimicrobial potential of organic extracts of *I. viscosa*, and *G. alypum*, it has also confirmed the popular use of these species in traditional medicinal practices, also indicating that soxhlet extraction from the leaves could be a safe and valuable source of broad-spectrum antimicrobial agents, this activity of the crude extracts is probably related to their chemical composition rich in phenolic compounds.

One of the virulence factors of *C. albicans* and *C. glabrata* is their ability to form biofilms,[56] *C. albicans* and *C. glabrata* biofilms were sensitive to *G. alypum* and *I. viscosa* extracts. Biofilm attachment was reduced by ethyl acetate and methanolic extracts. The extract was found to be

more active against biofilm formation by *C. albicans* and *C. glabrata*.

By comparing the minimum biofilm inhibitory concentrations with the minimum growth inhibitory concentrations achieved against the two fungal strains, we can deduce that the concentration required to reduce 50% of the mature biofilm is approximately twice the minimum growth inhibitory concentration. These results show that fungal cells in a biofilm matrix are more resistant to antimicrobial agents than planktonic cells, which has been reported in several studies.[57,58]

Biofilm inhibition could be explained by the presence of phenolic compounds, particularly flavonoids, in the extracts studied. Indeed, flavonoids such as quercetin, apigenin, luteolin, and rutin have been shown to be effective in biofilm inhibition.[59,56,60] Adhesion to living or inanimate surfaces is considered the first step in biofilm formation by microorganisms. Consequently, targeting this crucial step reduces microbial virulence by blocking its cell adhesion potential.[61]

Studies have suggested the use of several enzymatic approaches and plant extracts for the disruption of preformed microbial biofilms.[62,63,64,65] In this study, we observed significant inhibition of cell attachment to the 96-well plastic surface after treating cells with various concentrations of extracts from both plants. Thus, our study contributes to alternatives that can be used to inhibit biofilm formation.

The antifungal effect of the extracts could be due to the phenolic acids and flavonoids present in the chemical composition of the extracts, which has already been elucidated by chromatographic techniques. In addition, the solvents used for extraction may also affect the antifungal effect and also the phenolic compound content in each extract, which explains the variations in the results, other compounds present in natural extracts may act synergistically with phenolic acids to enhance the overall antifungal effect.

A study was carried out by Rocha et al, in which the MICs of kaempferol and quercetin were evaluated on strains of *C. orthopsilosis*, *C. metapsilosis*, and *C. parapsilosis*. It was analyzed that kaempferol and quercetin decreased the metabolic activity and biomass of all fungal strains, showing that these compounds can therefore be used as antifungals.[66]

Another study was conducted by Fu Y et al, showing the antibiofilm effect of luteolin on interrupting the production of *Candida* biofilm matrix components thus blocking biofilm formation and increasing antifungal treatment.[67]

In several research, *G. mellonella* model has been served as an adequate, simple, and reasonable option for the assessment of antimicrobial substances efficiency in vivo; the inoculation of the tested extracts at 25, 12.5, and 6.25 µg mL^{-1} in uninfected of *G. mellonella* larvae did not modify the rate of survival. The infection of *G. mellonella* with *C. albicans* and C. *glabrata* (10^6 CFU/larvae) resulted in the mortality of all larvae within 5 days. In fact, this effect was prevented when the larvae were injected with a single dose of 25, 12.5, and 6.25 µg mL^{-1} which resulted in a survival rate ranging from 80% to 60% in 5 days after infection for both tested plants extracts. The immunological response of *G. mellonella* against microbial infection characterized by melanogenesis that is the complex process by which the pigment melanin is generated. Nevertheless, the excess of production of this color (black) has been associated to microorganisms caused death.[68,69] The obtained results are presented in Figure 2 and 3, highlighting a positive correlation confirming the previously achieved results on the beneficial property of these extracts in contaminated larvae. Overall, we suggest that extracts of *I. viscosa* and *G. alypum* may represent an alternative therapy for controlling fungal infections induced by *C. albicans* and *C. glabrata*, as they have the ability to decrease fungal virulence, which could improve the host immune response to infection.

According to the statistical analysis, reported in Tables S5 and S6 (Supporting Information), showing the effect of *I. viscosa* and *G. alypum* extracts on the inhibition of mature biofilms of *C. glabrata and C. albicans*, a significant difference ($p > 0.05$) between the means of the % of inhibition of *C. glabrata* PMC 0849 and *C. albicans* ATCC 10231 biofilms was noted.

4. Conclusion

The development of various drug-resistant strains in the last time owing to the widespread and frequent use of several antibiotics has encouraged the exploration of alternatives especially new molecules derived from plants extracts. The current study revealed that *I. viscosa* and *G. alypum* leaves extracts were up to interrupt biofilm formation by a robust biofilm producer isolate, and this antibiofilm action was possibly related to the reduce of viability of cells inside the biofilm. To the best of our knowledge, this is the first time that the antibiofilm activity of *G. alypum* and *I. viscosa* leaves extracts has been described against biofilm producers *C. albicans* and *C. glabrata*. Consequently, the use of such extracts might be regarded as viable options to antibiotics/antimicrobial substances in the agro-food and pharmaceutical field. Additionally, the in vivo antifungal activity of both extracts against *C. albicans* ATCC 10231, and *C. glabrata* PMC 0849 improved the survival rate of *Galleria mellonella* larvae thus revealing the effectiveness of these plants extracts as a potential candidate to drug development for treatment of *Candida albicans* and *Candida glabrata* infections. As a screening tool for antifungal assessment, *G. mellonella* thus offering a valuable alternative to mammalian models in terms of rapidity and inexpensiveness.

5. Experimental Section

Plant Material and Extraction: The leaves of *G. alypum* and *I. viscosa* were carefully cleaned with water to eliminate soil and dust particles, prior to be air-dried under shade at ambient temperature for 2 weeks and powdered. For extracts preparation, three different solvents were used, namely methanol, ethyl acetate, and chloroform; the extraction process was conducted using the Soxhlet apparatus: powdered sample (50 g) was extracted with 250 mL of each solvent, afterward, the extracts obtained were eliminated of solvent using a rotary evaporator, the obtained crude extracts were saved in a refrigerator (−4 °C) in airtight bottles.

Growth Conditions and Fungal Strains: *Candida albicans* ATCC 10231 and *Candida glabrata* PMC 0849 were selected for the experiment for biofilm and *Galleria mellonella* assays; the strains were routinely cultured in Sabouraud dextrose agar (SDA) plates media and incubated for 24 h in an incubator at 36 °C, to be used freshly for the experiments. Thereafter, for experimental use, the density of fungal cultures was rectified turbidimetrically at a wavelength of 530 nm to 10^4–10^6 colony forming units (CFU) per mL in a sterile solution of RPMI.

Antifungal Susceptibility Testing of C. albicans and C. glabrata: The antifungal potential of the extracts was evaluated by the broth microdilution

method on the report of a standardized method for yeasts by the Clinical and Laboratory Standard Institute.[22,23] Briefly, the crude plants extracts were dissolved successively in RPMI 1640 broth to achieve the final dilution to be established, the concentration of extracts ranging from 2 to 1024 μg mL^{-1}. *C. albicans* and *C. glabrata* strains were developed on Sabouraud dextrose agar for 24 h at 37 °C. Then, cell suspensions of the strains were prepared in RPMI 1640 medium buffered to pH 7.0. The fungal inoculum was diluted in RPMI to a final concentration of 10^3–10^4 CFU mL^{-1}. Subsequently, 100 μL of each concentration was dispersed in a 96-well plate, and 100 μL of the final fungal dilution was added to each well. The incubation was performed at 36 °C for 48 h. the Miconazole was applied as positive control at concentration ranging from 0.25 to 32 μg mL^{-1}. The antifungal capacity was examined in three independent tests presented in Triplicate. In fact, the minimum inhibitory concentration (MIC) was considered as the concentration that produced no visible fungous development, and the minimum fungicidal concentration (MFC) was determined by inoculating 10 μL taken from the wells with no turbidity in MIC determination into an SDA medium Petri dish. The MFC was considered as the smallest concentration that entirely blocked the growth in the incubation conditions earlier designated.

In Vitro Antibiofilm Activity of Extracts Against C. albicans and C. glabrata Biofilms: The formation of biofilm was evaluated in sterile polystyrene 96-well microplates by the crystal violet method,[24] and the capability of *G. alypum* and *I. viscosa* extracts in inhibiting or decreasing the biofilm formation was revealed. Briefly, a volume of 160 μL of RPMI was added in the first line and 200 μL in the sterility line, then 100 μL in the other lines, and an equal volume of 40 μL of plant extracts was added to the first line; afterward, a dilution of 1:2 was performed to reach the final concentration. At last, 100 μL of *Candida* suspension (104 CFU mL^{-1}) was pipetted except for the sterility line without fungal culture. (Final volume was 200 mL in each well). The concentrations of extracted range from 32 to 512 μg mL^{-1}. The plates were incubated at 37 °C for 48 h, without shaking to permit the cells to affix to the surface. Following incubation, the supernatant was aspirated carefully so as not to touch and disrupt the biofilm, then, the wells were washed softly with 200 μL of phosphate-buffered saline (PBS) to eliminate non-adherent cells. The remaining yeast was subsequently stained with 200 μL of 0.1% crystal violet solution and incubated at ambient temperature for 15 min, The plates were then washed two times with sterile distilled water to eliminate the unabsorbed stain. The wells were destined by adding 100 μL of ethanol (95%). Finally, 80 μL of ethanol was transferred to a new 96-well plate and the absorbance was measured at 590 nm using a Microplate reader. Each assay was performed at least three times on two separate experiments for each compound tested four repetitions.

Galleria Mellonella In Vivo Survival and Toxicity Assays: *Galleria mellonella* larvae killing assays were evaluated according to the protocol described by Cairone et al..[25] Larvae were kept at 4 °C and were used within 1 week. *G. mellonella* larvae (300–400 mg) were randomly dispersed in groups (n = 10/group); three groups were inoculated with a volume of 10 μL of each extract (25, 12.5, and 6.25 μg mL^{-1}), with or without 1.106 cells of *C. albicans* ATCC 10231 and *C. glabrata* PMC 0849. For the treatment model, *Candida* suspension was delivered behind the last proleg on the opposite side to the extract injection site, and three negative control groups one group was inoculated with PBS only, in order to examine the impact of any negative effect from the injection process: one group was injected with *Candida* suspension only, while the last group with no inoculation as a control to evaluate the viability. The same process was applied for the assessment of the toxicity of the extracts and three groups for each extract (one for each concentration) were inoculated with (25, 12.5, and 6.25 μg mL^{-1}), respectively with *I. viscosa* and *G. alypum* extracts. Larvae were placed into Petri dishes and were incubated at 37 °C and controlled daily for survival for 120 h and were noted as dead when they did not move in response to touch. Each test was performed at least in triplicate.

Statistical Analysis: Assays were conducted in triplicates and results were expressed as mean values ± standard deviation (SD). Data were examined by one-way analysis of variance (ANOVA) by the Statistical Package for Social Science Software (SPSS 26.0). The *G. mellonella* survival rate was presented via Kaplan–Meier curves. (GraphPad Prism Software Inc 9.0, San Diego, CA, USA). A p-value lower than 0.05 was considered statistically significant.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

F.A. and G.S. performed conceptualization. F.A. and F.E.M. performed methodology. F.E.M. acquired the software. F.A., A.L., and G.S. performed validation. F.E.M. and F.C. performed a formal analysis. F.A. and F.E.M. performed an investigation. G.S. acquired resources. F.A. and J.B. wrote the original draft preparation. F.C. and F.E.M. wrote reviews and editing. A.L. performed visualization. G.S., F.C., and A.L. performed supervision. G.S. and A.L. performed project administration. All authors read and agreed to the published version of the manuscript.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

antifungal agents, biofilm inhibition, *Galleria mellonella* model, *Globularia alypum* (L.), *Inula viscosa* (L.) aiton

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