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Original Article

Endophytic fungal diversity and bioactive potentials: investigating antimicrobial and antioxidant activities

Djouza Salmi[1], Rawaf Alenazy[2*], El-hafid Nabti[3],Mohammed Alqasmi[2], Fawaz M Almufarriji[4], Maryam S. Alhumaidi[5], Mohammed A. Thabet[6], Ajar Nath Yadav[7,8,9], Karim Houali[1]

[1] LABAB Laboratory, Faculty of Biological and Agronomic Sciences, Mouloud MAMMERI University of Tizi-Ouzou, Algeria
[2] Department of Medical Laboratory, College of Applied Medical Sciences-Shaqra, Shaqra University, Shaqra 11961, Saudi Arabia
[3] Renewable Energy Management Laboratory, Faculty of Nature and Life Sciences, University of Bejaia, Bejaia 06000, Algeria
[4] Medical Laboratories Department, College of Applied Medical Sciences in Al-Quwayiyah, Shaqra University, Saudi Arabia
[5] Department of Biology, College of Science, University of Hafr Al Batin, P. O. Box 1803, Hafr Al Batin 31991, Kingdom of Saudi Arabia
[6] Department of Biology, Faculty of Science, Al-Baha University, Al-Baha City 65431, Saudi Arabia, KSA
[7] Department of Genetics, Plant Breeding & Biotechnology, Dr. Khem Singh Gill Akal College of Agriculture, Eternal University, Baru Sahib, Sirmour-173101, Himachal Pradesh, India
[8] Centre of Research Impact and Outcome, Chitkara University, Rajpura-140401, Punjab, India
[9] Chitkara Center for Research and Development, Chitkara University, Baddi-174103, Himachal Pradesh, India

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Abstract

This study investigates the colonization of endophytic fungi in nettle leaf tissues and evaluates their antibacterial and antioxidant activities. Using an inverted optical microscope, extensive fungal colonization was observed in all leaf parts, with hyphae prevalent in epidermal cells, parenchyma cells, and vascular tissues. 144 endophytic fungal isolates were isolated from 800 leaf fragments, indicating an 18% retention rate. ANOVA analysis revealed significant differences ($p < 0.001$) in colonization frequencies among 20 subjects, with subject 3 showing the highest frequency (40%) and subject 11 the lowest (2. 5%). Ethyl acetate extracts of the three most abundant endophytic fungi demonstrated notable antibacterial activity against both Gram-positive and Gram-negative bacteria, including methicillin-resistant Staphylococcus aureus (MRSA). Inhibition zones ranged from 9. 5 to 15. 16 mm, with minimum inhibitory concentrations (MICs) between 0. 19 to 25 mg/mL. Alternaria sp. exhibited the highest antimicrobial activity against MRSA. Antioxidant activity was assessed using the DPPH radical scavenging test and FRAP method. All extracts showed substantial free radical scavenging properties, with IC50 values close to those of standards like BHT. Alternaria sp. had the highest antioxidant activity, followed by Epicocum sp. and Ulocladium sp. The FRAP method confirmed high reducing potential, with Alternaria sp. again exhibiting the highest activity. These findings highlight the potential of endophytic fungi in nettle leaves as sources of antimicrobial and antioxidant agents, with significant implications for pharmaceutical and biotechnological applications.

Keywords: Alternaria, Antibacterial activity, Antioxidant, Endophytic fungi, MRSA, Urtica dioica.

1. Introduction

The introduction Endophytic fungi are well defined as organisms colonizing the endophytic space of plant tissues without destroying the host plant. They interact with the host plant and influence their metabolic process to produce diverse molecules with multiple activities [1,2]. Currently, endophytic fungi attracted the great attention of researchers due to their wealth of natural compounds with remarkable pharmacological potential [3,4]. Endophytic fungi can provide a wide variety of new metabolites that are a promising alternative due to the possibility of their production on an industrial scale. This makes it possible to both reduce production costs and preserve the plant kingdom [5].

Urtica dioica L., has been used in the form of infusions to treat several ailments such as rheumatic and stomach pains, gout and hair loss [6]. Studies have shown that aqueous extracts of the plant can decrease prostate adenoma in cancer patients [7], and that leaf extract can inhibit platelet aggregation [8], considerably reduce total cholesterol concentration [9] and improve overall liver function [10]. The same extract has also been shown to suppress the activation of histamine production via the NF-kB pathway [11], as well as the secretion of cytokines and chemokines asso-

* Corresponding author.
E-mail address: ralenazy@su. edu. sa (R. Alenazy).
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ciated with allergic reactions [12]. At the same time, other studies have demonstrated antiulcer, analgesic effects, and significant antibacterial and antioxidant activities [13].

Subsequent studies have shown that endophytic fungi can either exhibit the same activities as their host plants or possess novel activities by secreting unique phytochemical components not found in the plant itself [14,15]. Additionally, some endophytics can biotransform plant-produced molecules into metabolites with important biological activities [16,17]. Steroids, isocoumarins, alkaloids, phenols, flavonoids and terpenoids belong to a class of secondary metabolites produced by endophytic fungi with various antioxidant biological activities [18]. A major challenge in the search for biologically active natural products is the low concentration of these compounds in nature. Endophytic fungi offer the potential to produce these compounds industrially and in large quantities[19]. This approach would not only increase productivity but also preserve the environment and biodiversity while reducing the cost of drugs. The present work focuses on evaluating the fungal diversity of *Urtica dioica* leaves and determining the antibacterial and antioxidant activities of ethyl acetate extracts from the most abundant fungal isolates.

2. Materials and methods

2. 1. Staining *Urtica dioica* leaves tissues for detection of foliar endophytic fungi

The staining method developed by [20] for analysis of root fungi was modified to allow optimal visualization of fungi in nettle leaves. The method described here has proven effective for analyzing roots of a variety of plants. The plant was collected from Larba Nath Irathen (Tizi-Ouzou, Algeria). Wash healthy leaves of uniform maturity and appearance under running water to remove dirt. Leaves were randomly selected and cleaned by placing them in a pasteurization oven containing 10% KOH. Temperature was set at 90°C for 30 min and samples were removed from the pasteurization oven. Leaves were rinsed with tap water, bleached with 10% H_2O_2 for 10 min to take off pigmentation, and then placed in 10% lactic acid for 4 min. Depigmented leaves were rinsed with distilled water (dH_2O) for three minutes, then stained with trypan blue and placed in pasteur oven at 90°C for two hours. Stained leaves were stored in acid glycerol until growth. Place each leaf on a microscope slide containing a few drops of glycerinated gelatin. A cover slip is placed on the sheet and pressed firmly to facilitate analysis at high magnification. Analyzes were performed using one hundred WETZLAR microscopes at different magnifications.

2. 2. Isolation and identification of endophytic fungi

To isolate endophytic fungi, healthy nettle leaves with no obvious disease symptoms are carefully selected and wash the leaf samples thoroughly under running tap water to eliminate attached dust and dirt. Under sterile environment, the surface of leaf was soaked with 96% ethanol for 1 min, then treated with 10% sodium hypochlorite for 4 min, and then rinsed with 96% ethanol for 30s. and last wash with sterile distilled water and blotted dry on sterile filter paper [21]. Then the leaves were cut into 1 cm^2 pieces and 4 pieces were aseptically placed on plates containing solid potato dextrose agar (PDA) with chloramphenicol (100 µg/ml) added toinhibit the growth of bacterial population. Then for obtaining pure culture colonies were streaked on a new PDA plate and colonization frequency (CF) was calculated by as standard method given by [22]. Colonization frequencies of individual taxa were calculated similarly. All fungal species present on segmented leaves were identified using microscopic observation of mycelium, asexual/sexual spores, and colony morphology [23].

2. 3. Fermentation and extraction of endophytic fungi

Only three endophytic fungi were selected *Alternaria* sp., *Epicocum* sp. *Ulocladium* sp. and were obtained in PDA plates and incubated at 28°C for 6 days. Then the agar blocks of growing pure cultures were placed into 250 mL Erlenmeyer flasks containing 100 mL of culture medium and each fungus was cultured on potato dextrose broth (PDB) [24]. Each bottle was incubated for 4 weeks at 28°C with regular shaking and checked for contamination. After completion of incubating period, the fermentation broth was filtered through muslin cloth to eliminate mycelium and then centrifuged at 5000×g/10 min /4°C. After that, supernatant was extracted with an equal volume of ethyl acetate. Then, organic phase was collected and solvent was evaporated under reduced pressure at 40°C and samples were stored at -20°C.

2. 4. Determination of antibacterial activity

The antimicrobial activity of endophytic fungal extracts was evaluated in comparison with a reference strain obtained from a food safety laboratory (ANSES. F-94700, Maisons-Alfort, France). 5 bacteria of Gram-positive such as *Bacillus cereus* ATCC 10876, *Staphylococcus aureus* MU 50, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* LGA 251, *Staphylococcus aureus* ATCC 43300 methicillin-resistant *Staphylococcus aureus* (MRSA) and three bacteria of Gram-negative including *Citrobacter freundii* ATCC 8090, *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 4352.

First, bacterial strains were introduced in sterile Muller-Hinton (MH) agar plates and incubated at 37°C for 18 h. The concentrated crude extract was diluted with dimethyl sulfoxide (DMSO). For each extract, saturate sterilized 5 mm diameter disks with 20 µL of crude extract and place them on MH agar plates containing bacteria and adjust the turbidity to 0. 5 McFarland in sterile water and each experiment was performed in triplicate. A negative control was used by saturating a filter paper disk with DMSO. Moreover, chloramphenicol disks were utilized as reference control. The incubation of plates was monitored for about 18h at 37°C and the antimicrobial activity was assessed by the diameter (mm) measurement of growth inhibition zone of pathogenic bacteria using a standard [25].

The minimum inhibitory concentration (MIC) of the extracts was determined in Mueller-Hinton broth [26]. The concentrated crude extract was diluted using serial dilution technique and sequentially diluted to 0. 19, 0. 39, 0. 78, 1. 56, 3. 12, 6. 25, 12. 50, and 25 mg/mL. Inoculums were made in the same culture medium with a density adjusted to McFarland turbidity standard 0. 5 (10^8 colony forming units (CFU/mL)). After that, add 1 mL of inoculums in each 1 mL dilution. The tubes were placed in an incubator at 37°C for 24 h. The microbial growth was recorded by taking optical density (OD) at 620 nm. The MIC value is expressed in mg/mL and sample-free medium and microorganism-free medium was used as control.

2. 5. Determination of antioxidant activity

2. 5. 1. DPPH° radical scavenging activity

Crude extracts antioxidant activity was evaluated by free radical scavenging capacity of DPPH (1,1-diphenyl-2-trinitrophenylhydrazine) following to [27] measured with adjustments. Serial dilutions of leaf and endophytic fungal extracts were carried out ranging from 50 to 450 µg/mL. Positive controls were prepared using: ascorbic acid, quercetin and BHT. For negative controls and blank values, methanolic and methanolic solutions of DPPH were used, respectively. After that mix DPPH methanol solution (0. 1 mM, 1. 95 mL) with 50 µL of extract. The reaction mixture was incubated in the dark room temperature for 30 min. The absorbance was taken at 517 nm in spectrophotometer. The decreased absorbance of mixture indicates the free radical activity is higher and the DPPH scavenging capacity was calculated by standard method given by [27].

2. 5. 2. Reducing power

The reducing power was determined by using the method given by [28]. One mL of different concentrations of leaves and endophytic fungi extracts were combined with phosphate buffer containing 2. 5 mL, 0. 2 M, pH 6. 6 and potassium ferricyanide containing 1% in 2. 5 mL and incubated for 20 min at 50°C. Then add 2. 5 mL of 10% trichloroacetic acid in mixture and centrifuge at 3000×g for 10min. Then separate upper layer of 2. 5 mL solution and mix it with 2. 5 mL distilled water and 0. 1% $FeCl_3$ in 0. 5 mL. Then absorbance was measured at 700 nm against black and ascorbic acid, quercetin and BHT were used as the reference standards.

2. 6. Statistical analysis

All experiments were examined at least three times independently. The data were analyzed by ANOVA using the PAST free software and expressed as mean ± SD.

3. Results

3. 1. Location of endophytic fungi in the leaf tissue of *Urtica dioica*

An inverted optical microscope was used to observe the occurrence of endophytic fungi inside nettle leaf tissues. We found all-round and spread colonization of all parts of the leaves. Hyphae are the main fungal structures observed and are commonly found in epidermal cells, parenchyma cells, and vascular tissue. Hyphae are found moving between parenchyma cells and sometimes aggregate into mycelium. Endophytic fungi are also observed in the stomata and urticarial hairs, spores are also visualized (Figure 1).

3. 2. Diversity and frequency of colonization of endophytic fungi

A total of 800 leaf fragments were analyzed, and 144 endophytic fungal isolates were obtained. This equates to a retention rate of 18%. The analysis by ANOVA displayed highly significant differences ($p < 0. 001$) between the colonization frequencies of 20 analyzed subjects. The highest frequency was in subject 3 (40%) followed by subject 2 (37. 5%), while the lowest frequency was in subject 11 with 2. 5% (Figure 2).

A total of 144 isolates were obtained, and more than 7% remained in culture as sterile mycelium. The remaining isolates sporulated in culture and were identified into 11 genera. The most common genus was *Alternaria*, accounting for approximately 27% of the total. (Figures 2 and 3).

3. 3. Antibacterial activity of fungal extracts

In the present study, the antibacterial activity of ethyl acetate extracts of the three most abundant endophytic fungi in nettle leaves was investigated. The study was carried out against Gram-positive bacteria with strains of methicillin-resistant *Staphylococcus aureus* (MRSA) and Gram-negative bacteria: *Escherichia coli* ATCC 25922, *Klebsiella pneumonia* ATCC 4352 and *Citobacter freundii* ATCC 8090.

The study of antibacterial activity revealed that virtually all the extracts studied showed fairly significant zones of inhibition against the majority of the bacteria studied, with inhibition diameters of between 9. 5 and 15. 16 mm (Figure 4 and Table 1), albeit less than that obtained with chloramphenicol. The impurity and complexity of the crude extracts used could no doubt explain this difference. The minimum inhibitory extract concentrations (MICs) were determined and revealed as potential Gram +ve and Gram –ve bacterial inhibitors, with MIC values oscillating between 0. 19 to 25 mg/mL.

The analysis also reported antimicrobial activity against MRSA strains. The results revealed that *Alternaria* sp. showed the highest antimicrobial activity against these

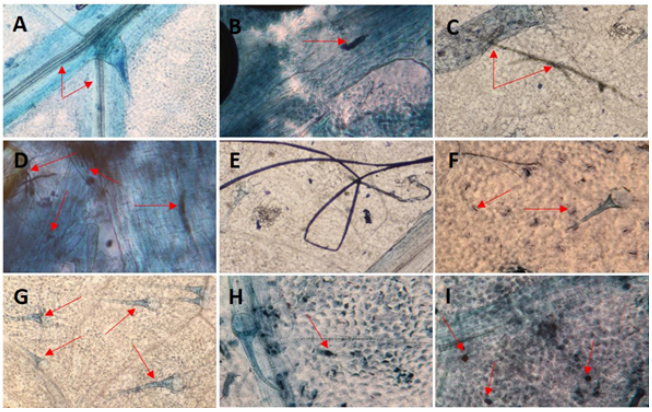

Fig. 1. Microscopic observations (X 400) of inter- and intracellular colonization of the leaf tissue of *Urtica dioica* by endophytic fungi. (A): at the level of vascular tissues. (B): mycelial masses. (C): at parenchyma level. (D, E): hyphal structures. (F): at stomata, (G): at urticating hairs, (H, I): spores of endophytic fungi

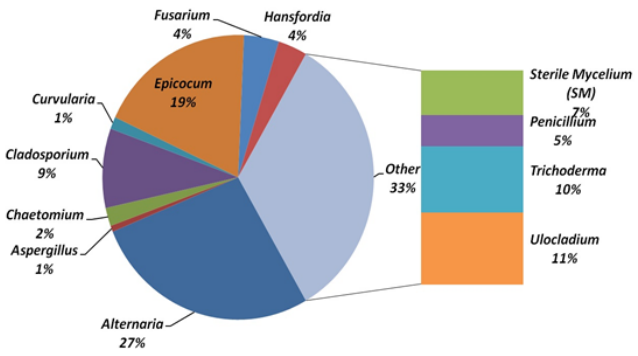

Fig. 2. Percentage abundance of mycoendophytes in the leaf tissue of Urtica dioica.

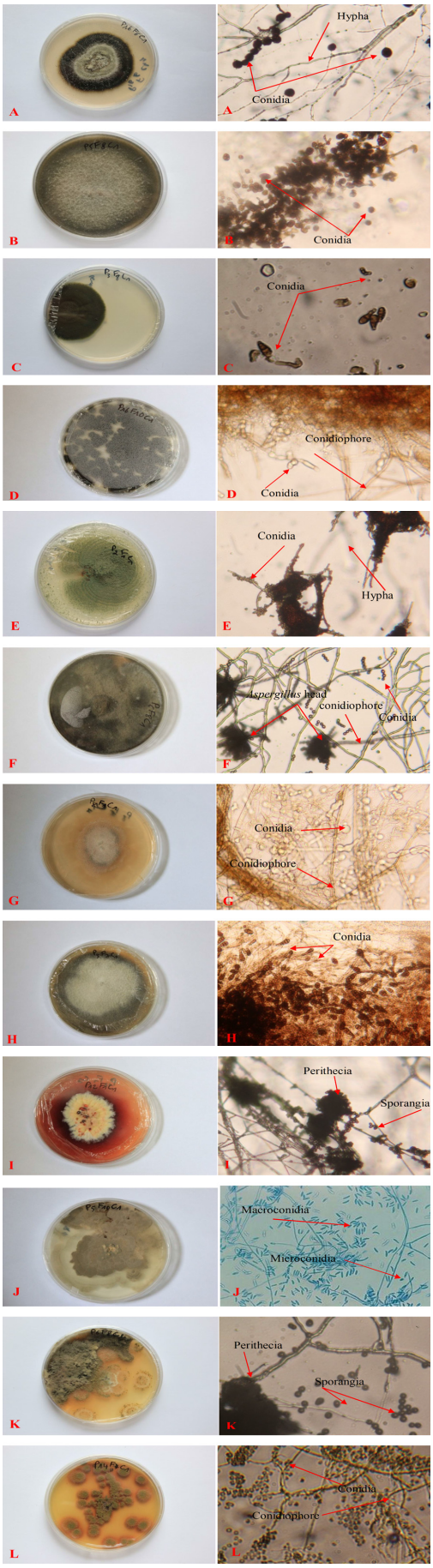

Fig. 3. Endophytic fungi colonizing *Urtica dioica* leaf tissue: under a light microscope (X400) and in culture on PDA medium: A: *Epicocum* sp. , B: *Ulocladium* sp. , C: *Curvularia* sp. , D: *Cladosporium* sp. , E: *Trichoderma sp.* , F: *Aspergillus sp.* , G: *cladosporium sp.* , H: *Alternaria sp,* I: *Hansfordia* sp. , J: *Fusarium sp.* , K: *Chaetomium sp*, L: *Penicillum sp*.

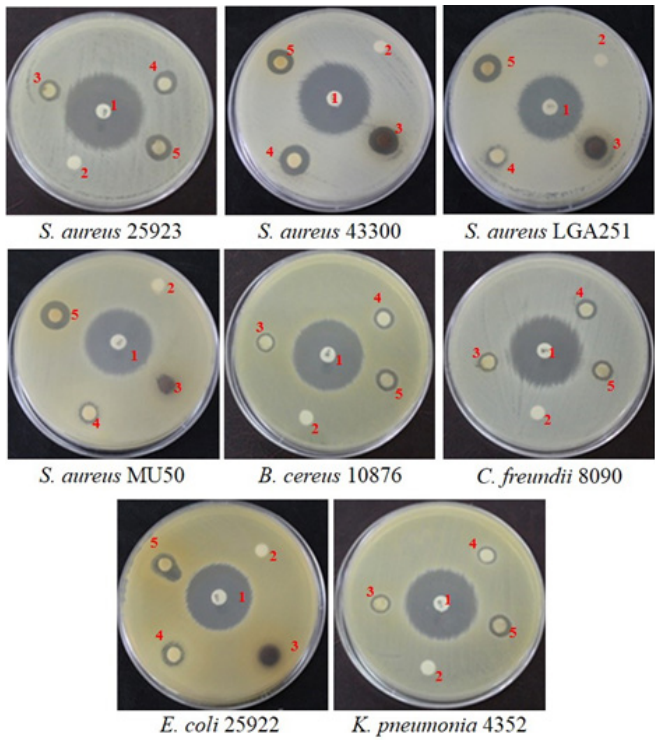

Fig. 4. Antibacterial activity of ethyl acetate extracts of Urtica dioica leaf endophytic fungi, obtained by agar diffusion method (1: Chlorphenicol, 2: DMSO, 3: Ulocladiumsp. extract, 4: Epicocum sp. extract, 5: Alternaria sp. extract).

strains, with inhibition ranging from 14. 16 to 15. 5 mm. Other extracts also showed significant antimicrobial activity against these strains, with MIC values ranging from 1. 56 to 0. 39 mg/mL.

3. 4 Antioxidant activity of extracts

The antioxidant activity of ethyl acetate extracts of the three endophytes was investigated firstly on the basis of the DPPH radical activity test, which consists in determining the free radical and odd electron scavenging capacity of DPPH, and secondly on the basis of their ferrous ion chelating capacity. The DPPH radical test is based on the reduction of DPPH- radicals in the presence of an antioxidant. Evaluation of the antioxidant activity of the various extracts against the DPPH- radical is expressed by 50% inhibitory concentration (IC50), which corresponds to concentration of extract required to inhibit the initial DPPH-concentration by 50%. IC50 is inversely proportional to anti-free radical activity. All IC50s are calculated from linear regression equations of inhibition percentages as a function of the extracts tested concentration (Figure 5).

The results show that all the extracts have interesting free radical scavenging properties, manifesting themselves with low IC50 values due to their ability to give up electrons to reduce the DPPH- radical. On the other hand, all IC50 values for various extracts are higher than those marked by the standards ascorbic acid (55. 82 ± 2. 24 µg/mL), quercetin (86. 63 ± 3. 86 µg/mL) and BHT (114. 63 ± 2. 48 µg/mL). This shows that all extracts have a lower reducing capacity than the referents. *Alternaria* sp. , showed the highest free radical scavenging activity, with an IC50 (139. 27 ± 1. 56 µg/mL) close to that obtained by the BHT standard, followed respectively by *Epicocum* sp. extract (157. 85 ± 6. 74 µg/mL) and *Ulocladium* sp. extract (268. 13 ± 3. 62 µg/mL).

Table 1. Antibacterial activity of ethyl acetate extracts of *Urtica dioica* foliar endophytic fungi.

Bacterial strain	*Alternaria* sp.		*Epicocum* sp.		*Ulocladium* sp		Chloramphenicol	DMSO
	IZ (mm)	MIC (mg/ml)	IZ (mm)	MIC (mg/ml)	IZ (mm)	MIC (mg/ml)	IZ (mm)	IZ (mm)
***S. aureus* 25923**	14. 83±0. 28	0. 78	12. 83±0. 28	0. 78	10. 83±0. 28	1. 56	30. 83±0. 28	-
***S. aureus* 43300**	15. 5±0. 28	0. 39	12. 16±0. 76	1. 56	11. 83±0. 28	3. 12	29±0. 5	-
***S. aureus* MU50**	14. 16±0. 76	0. 78	12. 83±0. 28	0. 78	11. 5±1. 32	6. 25	27. 33±0. 57	-
***S. aureus* LGA251**	14. 5±0. 5	0. 78	12. 5±0. 57	1. 56	11. 66±1. 04	3. 12	27. 83±0. 28	-
***E. coli* 25922**	11. 83±0. 28	1. 56	10. 83±0. 28	3. 12	10,83±0,76	3. 12	27. 16±0. 28	-
***K. pneumonia* 4352**	10. 83±0. 28	3. 12	11,83±0,28	3. 12	9. 16±1. 04	25	23. 66±0. 57	-
***B. cereus* 10876**	11±0. 5	1. 56	10. 16±0. 5	6. 25	9. 5±0. 5	25	27. 83±0. 76	-
***C. freundii* 8090**	11. 83±0. 28	1. 56	9. 50±0. 86	6. 25	9. 5±0. 86	25	27. 83±0. 76	-

In order to assess the reducing power of the extracts, the FRAP (Ferric Reducing Antioxidant Power) method was applied to all extracts. This method is based on the reduction of Fe3+ contained in the potassium ferrocyanide complex (K3 $Fe(CN)_6$) to $Fe2^+$ by an electron from these extracts. To compare the activities of different extracts, the procedure is the graphic determination of PR0. 5, which is defined as the concentration of a compound in µg/ml that gives an optical density of 0. 5 at 700 nm and is inversely proportional to the reducing power (Figure 6).

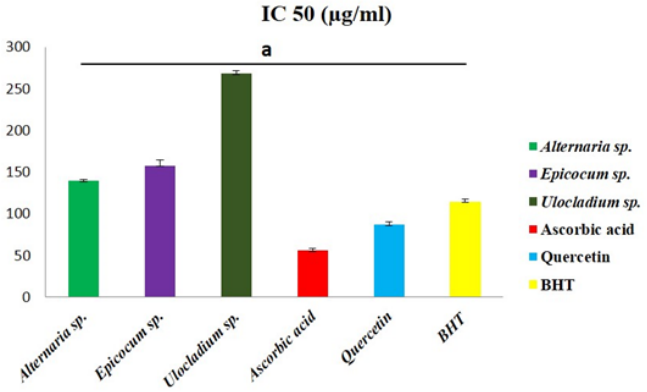

Fig. 5. DPPH radical scavenging activity of extracts and standards expressed with IC 50 values (µg/ml) (bars are the mean of three independent experiments ± SD, a: P <0. 1).

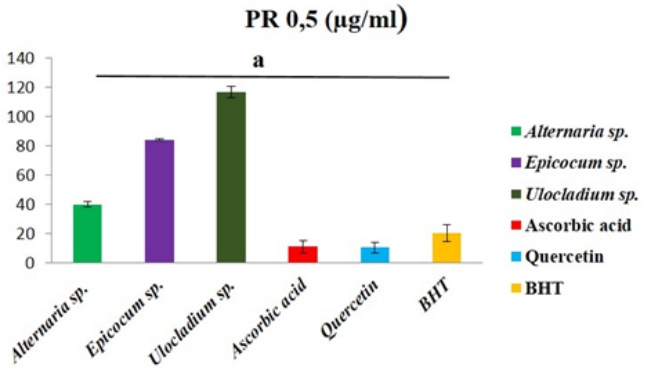

Fig. 6. Reducing power of extracts and standards expressed with PR0. 5 values (µg/ml) (results are the mean of three independent experiments ± SD, a : P <0,05)

4. Discussion

The role of endophytic fungi within a plant and their interactions with the host plant, other endophytes, and associated organisms are still not well understood. Despite this, the microbial diversity present in different plant species, coupled with the diverse metabolites produced by endophytic fungi, presents an opportunity for discovering novel bioactive molecules with various biotechnological applications. Additionally, several studies have highlighted the beneficial role of endophytic microorganisms in aiding host survival by directly influencing plant metabolism, enabling resistance to extreme temperatures, drought conditions, and the presence of phytopathogens. Therefore, the traditional use of the plant and the region it inhabits are crucial factors to consider when isolating endophytes [1,2].

Endophytic fungi are those that live inside plant tissues without causing harm to the host species [2]. [29] used light microscopy to observe endophytic fungi colonizing the intercellular and extracellular spaces of *Luehea divaricata* leaves. Colonization was observed in palisade parenchyma, sclerenchyma, adaxial epidermis, and conductive vessels, suggesting a close interaction between endophytes in multiple structures and nutritional subniches of the host. These results corroborate microscopic study data on colonization of nettle leaves by endophytic fungi. Likewise, [30] studied colonization of *Phoenix dactylifera* L. palm leaves by endophytic fungi and observed a high density of colonizing hyphae in the intercellular and extracellular spaces of the parenchyma. [31] also noted fungal hyphae attached to palisade parenchyma cells in *Citrus lemon* leaves.

According to the study, [32] state that intracellular space contains large amounts of inorganic and organic nutrients that can support the concentrations of endophytic fungi observed in grasses. Therefore, these fungi colonize intracellular spaces that represent protected ecological niches with little competition from other organisms due to nutrient richness.

Variations in endophyte abundance on host plants are influenced by host species, developmental stage of plant sampling [33], environmental conditions [34] plant location, altitude, exposure, vegetation and such as the age of the organization [35]. Some studies have also shown that endophytic fungal colonization is related to climatic factors such as rainfall. Infection in leaves is via rain-disper-

sed conidia and increases with time and rainfall [36, 37]. These factors may determine the dispersal and germination of endophytic fungal spores [38]. Nettle leaf endophytes were abundant and diverse. A total of 144 isolates were obtained, and more than 7% remained in the culture as sterile mycelium. The remaining isolates sporulated in culture and were identified into 11 genera. The most common genus is *Alternaria*, accounting for approximately 27% of the total.

The genera *Aspergillus* and *Curvularia* are the rarest with only 1% (Figure 2). Our study showed that nearly 7% of endophytic fungi remained in the form of mycelium after cultivation on agar.

These sterile mycelia have been sorted out as endophytes from a variety of plants [39]. In a study, medicinal plant *Tripterygium wilfordii*, sterile mycelium (23. 6%) was isolated as the second most common taxon [40]. In our study, we noted the presence of dark-colored septate fungal endophytes, particularly *Curvularia* and *Alternaria*. Dark septate endophytes primarily inhabit root tissue [41]. However, they are frequently isolated from leaves and are not restricted to mycorrhizal tissue. Dark septate fungi help plants resist abiotic stress caused by free radicals [42]. They act as natural plant antioxidants. *Alternaria* dominance was connected to its greater potential to colonize the leaves of *U. dioica*, which favours help in development and growth [43], or its harmful effect on other fungi [44]. Numerous studies have reported the predominance of *Alternaria*in different plants [45,46]. It is well documented that *Alternaria* sp. have been model plant pathogenic fungi, but they are also parasites or saprophytes. Although, *Alternaria* sp. has been reported as an endophytic fungus sorted out from numerous medicinal plants [47-49].

Several different substances produced by endophytic fungi present numerous biological activities such as hormonal, antitumor, cytotoxic, antiviral, immunosuppressive, antiparasitic, antimicrobial and antioxidant activities, among others [18]. In line with the present study, we found that several endophytic fungi showed antibacterial effect. In a report, *Acremonium sclerotigenum* extract isolated from *Terminalia bellerica* leaves exhibited strong antimicrobial activity toward *B. cereus*, *B. subtilis, S. aureus,E. coli* and *P. aeruginosa* with an inhibition diameter ranging from 3. 5 to 10 mm [50].

Two extracts of *Aspergillus* sp. isolated from *Terminalia brownii* leaves exhibited antimicrobial activity against *S. aureus* [51]. *Pestalotiopsis* species ethyl acetate- extracts showed antibacterial activity with inhibition rates above 75% against five bacterial species, including *Bacillus subtilis*, *E. coli*, *P. fluorescens* and *S. aureus* [52]. Various extracts of endophytic fungi namely *Papulaspora immersa* and *Arthrinium state* were sorted out from *Smallanthus sonchifolius* and each showed antibacterial activity. *P. immersa*, the ethyl acetate extract inhibits the growth of *P. aeruginosa*, *S. aureus* and *Kocuria rhizophila*, while the n-butanol and aqueous extracts show antimicrobial activity against *S. aureus* and *E. coli* growth [53]. The methanolic extract of *Colletotrichum gloeosporioides*, a new endophytic fungus obtained from healthy leaves of *Vitex negundo* L. showed antibacterial activity against *S. aureus*, *Bacillus subtilis*, *E. coli* and *P. aeruginosa* [54].

[54] reported several methicillin-resistant *Staphylococcus aureus* (MRSA) also susceptible to methanolic extracts of *Colletotrichum gloeosporioides*, with MIC values reported to be between 31. 25 and 250 µg/mL. Furthermore, when combining the methanol extract with the antibiotics namely penicillin and vancomycin, showed synergistic effect, resulting in significantly lower MIC values for each antibiotic.

Our results are all the more interesting because most clinical *S. aureus* isolates are resistant to multiple antibiotics [55] and methicillin-resistant *S. aureus* remains a major problem worldwide. Land limits the selection of effective antibiotics. Prevent and treat very common infections in hospitals and the community [56]. Instead, we found that *Alternaria* sp. exhibited stronger antibacterial activity than other mushrooms. *Alternaria* sp. is known to be the source of many bioactive substances with different structures and biological activities [57,58]. Pyrone derivatives of endophytic fungus *Alternaria tenuissima* from the traditional Chinese medicinal plant Salvia also showed antibacterial activity against test strains [59]. [60] reported that tenuazonic acid is the active ingredient against *Mycobacterium tuberculosis* H37Rv in *Alternaria alternata* (MIC 250 µg/mL), making it promising anti-tuberculosis drug. The antibacterial activity of crude endophytic fungal extracts obtained in our work may indicate that further studies on these potent extracts may lead to the identification of metabolites with efficient in treating drug-resistant bacterial infections.

Free radicals are also known the induction oxidative damage in body and lead to many illnesses [61,62]. Numerous studies have reported that medicinal plants and their endophytes could provide a range of potential antioxidant molecules [63]. Other authors also suggested that the medicinal properties of the host could be due to the fact that its endophytic microorganisms are capable of producing secondary metabolites [64]. *U. dioica* is known as a plant that produces antioxidant substances. Thus, [65] demonstrated that aqueous nettle extracts possess the same ability to scavenge DPPH free radicals as standard BHA and also demonstrated the same extract's ability to chelate iron ions. [66] succeeded in demonstrating nettle anti-aging activities, involved in inhibition of enzymatic activities, which they believe are due to the plant antioxidant capacity. Our study showed that endophytic fungi are also a promising source of antioxidant compounds.

Several studies have been conducted on antioxidant potential of endophytic fungi. [67] reported that antioxidant activity of fungal endophytes of *Syzygium samarangense* L., with fungus *Lasiodiplodia venezuelensis*, were sorted out from leaves and found to be the most efficient (IC50 = 49. 96 µg/mL) for fungal extract. [68] evaluated the activity of endophytic fungus *Aspergillus nidulans*, isolated from *Jatropha curcas* L. , and reported very promising results for DPPH sequestration, with an IC50 of 5. 40 µg/mL. [69] examine metabolite extracts produced by *Botryosphaeria dothidea* and obtaine an IC50 of 206 µg/mL. The present study stated that leaf endophytic fungi of *Urtica dioica* are promising sources of antioxidant substances since the metabolic extracts of all isolates tested showed an IC50 < 1000 µg/mL.

Extracts of fungus *Alternaria* sp. showed the highest antioxidant activity as compared with other fungi. Other studies have also reported the antioxidant activity of *Alternaria* fungi obtained from several plant species such as crude extracts of *A. Alternata* from *Coffea arabica* [70]

and *Alternaria* sp. from *Mussaenda luteola*, [71] exhibited antioxidant activity. In parallel, studies have revealed the presence of different types of antioxidants in endophytic *Alternaria* species present in *Aegle marmelos* [72]. In order to insight into the potential role of endophytic fungi in the regulation of abiotic and biotic stresses in the plant, [73,74] investigated ROS detoxification enzyme activities such as catalases and superoxide dismutases. *A. sorghi* showed the best activities compared to other isolated fungi.

Microorganisms serve as abundant sources of phenolic compounds. These microbial metabolites can be produced under controlled conditions at a faster rate compared to obtaining them from plants. This accelerated production from microbial sources offers an economic advantage by reducing costs. By optimizing the cultivation conditions for the endophytic fungi of *U. dioica* leaves, it may be possible to enhance the biosynthesis of bioactive phenolic compounds. Achieving higher concentrations of these beneficial molecules from the endophytic fungi should be an objective for future research endeavors.

5. Conclusion

Rapidly increasing information on endophyte biodiversity, natural products, potential uses and biotechnological applications is found in a rich literature, and should be reviewed regularly for interested readers. As reviewed here, the endophytic fungi have abundant biodiversity and are useful in pharmaceuticals, agriculture, and industry. *Urtica dioica* has been adopted and used both as a source of nutrients and as a traditional medicine for decades. It is abundant in phytonutrients and contains a variety of bioactive phytoconstituents, including polyphenols and flavonoids. This work has shown that foliar endophytic fungi of *Urtica dioica* possess metabolites with significant antioxidant and antibacterial activities. *Alternaria* sp. Exhibits efficient activities as compared to other fungi. Thus, production of certain secondary metabolites by the endophyte can facilitate its dominance within the plant's biological niche or even protect the plant against invasive and harmful pathogens. Further research at molecular level is necessary to identify the active components and gene sections encoding these components, that can contribute to the large-scale production of bioactive metabolites intended for use in the pharmaceutical industry. In conclusion, the identification of targets related to the signaling pathway influenced by *U. dioica* could pave the way for future drug development for treatment of many disorders.

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Conflict of Interests

The author has no conflicts with any step of the article preparation.

Consent for publications

The author read and approved the final manuscript for publication.

Ethics approval and consent to participate

No human or animals were used in the present research.

Informed consent

The authors declare that no patients were used in this study.

Availability of data and material

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

Authors' contributions

Conceptualization, D. S. and K. H. ; methodology, D. S. AND H. N; software, R. A. ; validation, K. H. , R. A. and H. N. ; formal analysis, D. S. ; investigation, D. S. and M. A; resources, F. M. A. ; data curation, D. S. ; writing—original draft preparation, D. S. and H. N; writing—review and editing, D. S. AND K. H. ; visualization, M. S. A and M. A. T. ; supervision, K. H. , R. A. and A. N. Y. ; project administration, K. H. ; funding acquisition, K. H. All authors have read and agreed to the published version of the manuscript. ”

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