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A pilot study of antioxidant potential of endophytic fungi from some Sudanese medicinal plants

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ABSTRACT

Objective: To evaluate the total phenolic content (TPC) and total antioxidant capacity (TAC) of ethyl acetate extracts of 21 endophytic fungi isolated from five Sudanese medicinal plants: *Calotropis procera* (*C. procera*), *Catharanthus roseus* (*C. roseus*), *Euphorbia prostrate* (*E. prostrata*), *Vernonia amygdalina* (*V. amygdalina*) and *Trigonella foenum-graecum* (*T. foenum-graecum*). **Methods:** Crude extracts of endophytic fungi and their host plants were tested by classical Folin-Ciocalteu colorimetric method to determine the TPC, also TAC was estimated using DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging *in vitro* method. **Results:** Among the endophytes, endophytic fungus *Aspergillus* sp. from *T. foenum-graecum* seeds demonstrated the highest both total phenolic content in term of Gallic Acid Equivalent (89.9±7.1 mg GAE/g) and antioxidant activity for DPPH radical scavenging assay (IC_{50}:18.0±0.1 μg/mL). A high positive linear correlation (R^2=0.999 1) was found between TAC and TPC of endophytic fungi isolated from *V. amygdalina*. **Conclusion:** The present study revealed that some endophytic fungi from the five Sudanese medicinal plants could be a potential source of novel natural antioxidant compounds.

1. Introduction

Endophytes are organisms that colonize internal plant tissues without causing apparent harm to their host[1]. Endophytic fungi from medicinal plants are a potential antioxidant resource[2]. *Vernonia amygdalina* (*V. amygdalina*) Del. (Asteraceae), *Calotropis procera* (*C. procera*) Ait. (Asclepiadaceae), *Catharanthus roseus* (*C. roseus*) L. (Apocynaceae), Euphorbia prostrata (*E. prostrata*) Ait. (Euphorbiaceae), and *Trigonella foenum-graecum* (*T. foenum-graecum*) L. (Fabaceae) are medicinal plants that have several uses in Sudanese folk medicine. Their extracts have shown some biological activities including antiproliferative activity and antioxidant potential[3]. However, the endophytes mycoflora of these five plants have not been investigated. As part of our ongoing efforts towards finding novel antioxidant agents from natural resources we investigated, for the first time, total phenolic content and total antioxidant capacities of some endophytic fungi from these medicinal plants.

2. Materials and methods

Fresh leaves and stems of *C. procera*, *C. roseus*, *E. prostrata*, *V. amygdalina* were collected from Khartoum (15 ° 38 ′ N 32 ° 32 ′ E) and *T. foenum-graecum* seeds were obtained from Khartoum local market. The plants were identified by Dr. Haider Abdalgadir, taxonomist in the Medicinal and Aromatic Plants Research Institute (MAPRI) in Khartoum (Sudan).

Endophytic fungi were isolated from different parts of the collected

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medicinal plants after surface sterilization as described by Zhang P *et al*[4]. The sterilized pieces were cultivated on Potato Dextrose Agar (PDA) medium which was amended with Chloramphenicol (500 mg/L) to suppress bacterial growth. The efficiency of the surface sterilization procedure was confirmed by plating the final rinse water. Furthermore, the endophytic fungi were subcultured in order to obtain pure cultures, numbered and reserved at 4 ℃. Identification of the fungal strains was based on the morphology of cultures or hyphae, the characteristics of the spores, and reproductive structures if the feature were discernible[5]. The cultures which failed to sporulate were grouped as mycelia sterilia[6].

Each fungal strain was cultivated on 20 petri dishes PDA, and was incubated at 30 ℃ for 7-15 d. The solid fungal culture was crushed and extracted with ethyl acetate overnight, filtered, evaporated and preserved at 4 ℃.

Dry leaves and stems of *V. amygdalina*, *C. procera*, *C. roseus*, *E. prostrata* and seeds of *T. foenum-graecum* were ground into fine powder. Each sample (20 g) was extracted with ethyl acetate overnight, filtered, evaporated and stored at 4 ℃.

Total phenolic contents were determined using the Folin–Ciocalteu method as described by Wolfe K *et al*[7]. The absorbance of the resulting was measured with spectrophotometer at 760 nm using a microtiter plate reader (Synergy HT Biotek, logiciel GEN5). Analysis was done in triplicate for each extract. Quantification was based on the standard curve of gallic acid. The results were expressed as Gallic Acid Equivalent (GAE), *i.e.*, mg gallic acid/g.

Total antioxidant capacity (TAC) of the extracts was estimated using DPPH *in vitro* method as described by Yagi S *et al*[8]. The absorbance was measured spectrophotometrically at 517 nm using a microtiter plate reader (Synergy HT Biotek, logiciel GEN5). Ascorbic acid was used as reference antioxidant compound. Each analysis was done in triplicate. The IC_{50} value was calculated from the linear regression of plots of concentration of the test sample against the mean percentage of the antioxidant activity. Results were expressed as mean±SEM and the IC_{50} values obtained from the regression plots (Sigma PlotsR 2001, SPSS Science) had a good coefficient of correlation, (R^2=0.998).

3. Results

A total of 21 endophytic fungal strains were isolated from 5 Sudanese medicinal plants: three endophytic fungi from *V. amygdalina*, five from *T. foenum-graecum*, four from *C. procera*, five from *C. roseus*, and four from *E. prostrata*. The isolated fungal strains were classified into 12 different taxa (Table 1). Ten strains belong to Ascomycetes, whereas seven strains belong to fungal class Deuteromycetes, four strains were failed to sporulate and were grouped as mycelia sterilia. This group of fungi is a common problem concerning the identification of endophytic fungi[9].

TPC of ethyl acetate crude extracts of 21 endophytes and different parts of their host plants were estimated using the classical Folin-Ciocalteu colorimetric method as shown in (Figure 1). It was found that the five medicinal plants contained TPC values ranged from (0.5±0.1) (*T. foenum-graecum* seeds extract) to (32.7±2.9) mg GAE/g) (*V. amygdalina* stem extract). TPC values of 21 endophytes revealed variations ranged from (13.6±1.0) to (89.9±7.1) mg GAE/g. Two *Aspergillus* spp. of both *C. procera* and *T. foenum-graecum* showed the highest TPC values (77.2±7.5) and (89.9±7.1) mg GAE/g respectively).

The antioxidant potential using DPPH radical scavenging assay was investigated for the 21 ethyl acetate extracts of endophytic fungi and their medicinal host plants. Table 2 showed that TAC IC50 values of the medicinal host plants ranged from (50.0±1.7) μg/mL (*V. amygdalina* stem) to no activity (*T. foenum-graecum* seeds). The endophyte extracts revealed extremely wide range of IC_{50} values, from (18.0±0.1) μg/mL for *Aspergillus* sp. isolated from *T. foenum-*

Table 1
Taxonomic identification of the endophytic fungi isolated from various organs of *V. amygdalina*, *C. procera*, *C. roseus*, *E. prostrata* (stems, leaves) and *T. foenum-graecum* (seeds).

	Fungal taxon	Host plant name	Host plant organs
Ascomycetes	*Alternaria* sp.	*C. procera*	Leaves
	Bipolaris sp. 1	*C. roseus*	Leaves
	Bipolaris sp. 2	*E. prostrata*	Leaves+Stem
	Curvularia sp. 1	*C. roseus*	Leaves
	Curvularia sp. 2	*V. amygdalina*	Leaves+Stem
	Chaetomium sp. 1	*C. roseus*	Stem
	Chaetomium sp. 2	*T. foenum-graecum*	Seeds
	Chaetomium sp. 3	*V. amygdalina*	Leaves
	Drechslera sp.	*E. prostrata*	Leaves+Stem
	Emericella sp.	*C. roseus*	Leaves
Deuteromycetes	*Aspergillus* sp. 1	*C. procera*	Stem
	Aspergillus sp. 2	*T. foenum-graecum*	Seeds
	Cladosporium sp. 1	*C. procera*	Leaves
	Cladosporium sp. 2	*V. amygdalina*	Leaves
	Paecilomyces sp.	*E. prostrata*	Leaves+Stem
	Phoma sp.	*C. roseus*	Stem
	Mycelia sterilia sp.1	*C. procera*	Stem
	Mycelia sterilia sp. 2	*T. foenum-graecum*	Seeds
	Mycelia sterilia sp. 3	*T. foenum-graecum*	Seeds
	Mycelia sterilia sp. 4	*T. foenum-graecum*	Seeds

graecum to (2 686.0±51.7) μg/mL for *Phoma* sp. in *C. roseus*.

Table 2

IC_{50} values of 21 endophytic fungi and their host plants by DPPH radical scavenging assay. Values are means ± SD of three analyses.

Crude extract	DPPH (μg/mL)
Ascorbic acid	5.0±0.1
C. procera (S)	668.0±8.1
C. procera (L)	388.0±7.2
Alternaria sp. (L)	236.0±8.3
Aspergillus sp. 1 (S)	58.0±0.4
Cladosporium sp. 1 (L)	1 142.0±1.3
Mycelia sterilia sp.1 (S)	1 030.0±3.0
T. foenum-graecum (seeds)	*
Aspergillus sp. 2 (Ss)	18.0±0.1
Chaetomium sp. 1 (Ss)	70.0±0.3
Mycelia sterilia sp.2 (Ss)	1 013.0±4.2
Mycelia sterilia sp.3 (Ss)	933.0±5.3
Mycelia sterilia sp.4 (Ss)	1 070.0±3.2
V. amygdalina (S)	50.0±1.7
V. amygdalina (L)	63.0±1.8
Chaetomium sp. 3 (L)	252.0±5.1
Cladosporium sp. 2 (L)	480.0±3.9
Curvularia sp. 1 (L+S)	461.0±5.5
C. roseus (S)	1 119.0±2.6
C. roseus (L)	113.0±0.4
Bipolaris sp. 1 (L)	1 556.0±1.5
Chaetomium sp. 3 (S)	405.0±5.2
Curvularia sp. 2 (L)	105.0±2.7
Emericella sp. (L)	137.0±1.3
Phoma sp. (S)	2 686.0±51.7
E. prostrate (L+S)	203.0±7.6
Bipolaris sp. 2 (L+S)	2 305.0±23.4
Drechslera sp. (L+S)	1 074.0±7.7
Ulocladium sp. (L+S)	1 348.0±5.6
Paecilomyces sp. (L+S)	122.0±0.4

* indicated not active, (L) leaves, (S) stem, (Ss) seeds and (L+S) leaves and stem.

A high positive linear correlation (R^2=0.999 1) was found between TAC and TPC of endophytic fungi isolated from *V. amygdalina*. Endophytic fungi isolated from *T. foenum-graecum* and *C. procera* showed moderate correlation (R^2=0.680 8 and 0.515 6, respectively).

4. Discussion

The majority of the fungal genera isolated from the Sudanese plants were common endophytes (*Alternaria*, *Cladosporium*, *Phoma*, *Chaetomium*, *Drechslera*, *Curvularia*, *Bipolaris*, *Paecilomyces*, *Emericella* and *Aspergillus*). However Ulocladium were reported only few times as endophytes[10]. The low diversity of the endophytes of the Sudanese medicinal plants may be due to the climate where it is extremely arid for most of the year with about nine months with average rainfall lower than five mm. Some authors[11] reported that a significant variation was detected in the colonization frequency of endophytic species in relation with the environmental factors such as rainfall and atmospheric humidity.

Ethyl acetate is selective solvent which extract low and high molecular weight polyphenols. Despite the high TAC of stems and leaves of *V. amygdalina* IC_{50}: (50.0±1.7) μg/mL and (63.0±1.8 respectively), their endophyte extracts showed low TAC IC_{50}: (252.0±5.1) to (480.0±3.9) μg/mL. In contrario the seed extract of *T. foenum-graecum* had no antioxidant activity while Aspergillus sp. 2, isolated from the seeds, showed significant TAC (18.0±0.1) μg/mL. These results indicated that no correlation between the TACs of the endophytes and the host plants can be established. The main factor is the fungal genus, indeed *Aspergillus* spp. were recorded the highest TAC. The highest TAC and TPC were obtained with *Aspergillus* spp. extracts isolated from both *C. procera* IC_{50}: (58.0±4.0) μg/mL, TPC: (77.2±7.5) mg GAE/g and *T. foenum-graecum* (IC_{50}: (18.0±0.1) μg/mL, TPC: (89.9±7.1) mg GAE/g. These results are in accordance with Yadav M *et al*[12] who reported that various species of *Aspergillus* strains showed the highest TPC with 58 to 60 mg

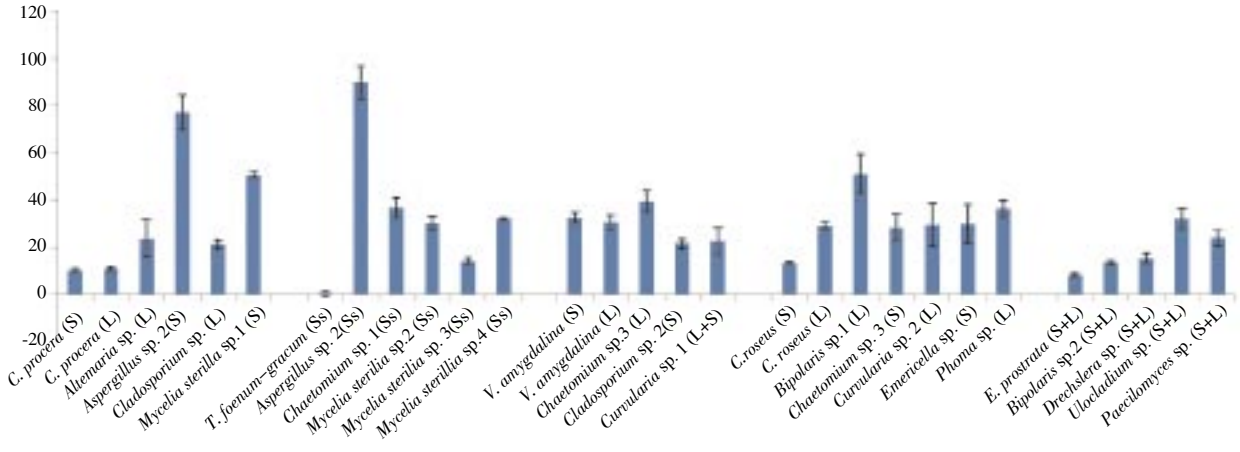

Figure 1. Total phenolic content in ethyl acetate extracts of endophytes and their host plants.

(S) Stem, (L) leaves (Ss) Seeds and (S+L) stem and leaves. Values are means ± SD of three determinations.

GAE/g. It is noted that crude extract of *T. foenum-graecum* seeds from Sudan revealed no antioxidant activity that could be explained by the low concentration of TPC (0.5±0.1) mg GAE/g. In contrario previous works reported that seed ethyl acetate crude extract of *T. foenum-graecum* demonstrated strong antioxidant activity in relation with high phenolic content (106.316 mg GAE/g)[13].

In conclusion, in this study we investigated the diversity of endophytic fungi of 5 Sudanese medicinal plants. The 21 endophytes were identified and classified. *Mycelia sterilia*, and *Chaetomium*, were the dominant fungal taxa isolated. The endophyte diversity was poor in comparison with the results obtained with plants growing in other countries. Our findings revealed the first report on endophytic fungi of 5 Sudanese medicinal plants. Some of them were worthy with phenolic compounds and may serve as potential source of natural antioxidants. The *Aspergillus* sp. endophyte of *T. foenum-graecum* was revealed significant antioxidant activity alongside this strain was rich with phenolic compounds, this fungus strain is recommended for further investigations.

Conflict of interest statement

We declare that we have no conflict of interest.

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