CZECH MYCOLOGY 71(2): 187–203, DECEMBER 19, 2019 (ONLINE VERSION, ISSN 1805-1421)

# Antioxidative and L-asparaginase potentials of fungal endophytes from *Rauvolfia densiflora* (*Apocynaceae*), an ethnomedicinal species of the Western Ghats

NAGUVANAHALLY SOMASHEKAR BHAVANA[1], HARISCHANDRA SRIPATHY PRAKASH[2], MONNANDA SOMAIAH NALINI[1]*

[1] Department of Studies in Botany, University of Mysore, Manasagangotri, Mysore, IN-570 006, Karnataka, India
[2] Department of Studies in Biotechnology, University of Mysore, Manasagangotri, Mysore, IN-570 006, Karnataka, India
*corresponding author: nmsomaiah@gmail.com

Bhavana N.S., Prakash H.S., Nalini M.S. (2019): Antioxidative and L-asparaginase potentials of fungal endophytes from *Rauvolfia densiflora* (*Apocynaceae*), an ethnomedicinal species of Western Ghats. – Czech Mycol. 71(2): 187–203.

This study focuses on the isolation and molecular characterisation of fungal endophytes from an ethnomedicinal plant, *Rauvolfia densiflora* (*Apocynaceae*), and an evaluation of antioxidative and L-asparaginase potentials. Fungal endophytes were isolated from leaf and stem parts of *R. densiflora* collected from the site of Talacauvery, Western Ghats, India. The highest colonisation frequency was found in leaves (95.5%), followed by stem parts (63.5%).

A total of 19 fungal morphotypes belonging to 11 genera were identified by analysing the ITS sequences of the endophytes. The strains were screened for asparaginase production by qualitative plate assay and quantification by Nesslerisation. Maximum activity was recorded in the *Penicillium chrysogenum* culture (3.77 IU/ml). Submerged fermentation and ethyl acetate extraction were carried out to obtain secondary metabolites for the evaluation of total phenolic content (TPC) and antioxidative potentials. The TPC of fungal extracts ranged from 12.37 to 89.29 mg GAE/g dry extract and the $IC_{50}$ value of scavenging activity from 26.64 to 547.23 µg/ml. The *P. chrysogenum* strain (MH392736) was found to have the potentially highest total phenolic content and a high antioxidant capacity. This is the first report on the characterisation of fungal endophytes from *R. densiflora* and their antioxidative and L-asparaginase potentials.

**Key words:** India, endophytic fungi, *Penicillium chrysogenum*, antioxidant, L-asparaginase, phenolic content.

**Article history:** received 2 August 2019, revised 16 November 2019, accepted 29 November 2019, published online 19 December 2019.

**DOI:** https://doi.org/10.33585/cmy.71205

Bhavana N.S., Prakash H.S., Nalini M.S. (2019): Antioxidační a L-asparaginázový potenciál endofytických hub z *Rauvolfia densiflora* (*Apocynaceae*), léčivé rostliny ze Západního Ghátu. – Czech Mycol. 71(2): 187–203.

Studie je zaměřena na izolaci a molekulární charakteristiku houbových endofytů z léčivé rostliny *Rauvolfia densiflora* (*Apocynaceae*) a hodnocení jejich potenciálu v tvorbě L-asparaginázy a antioxidačních účinků. Endofyty byly izolovány z částí listů a stonků *R. densiflora* z oblasti Talacauvery

v Západním Ghátu (Indie). Listy byly kolonizovány houbami podstatně více než stonky (95,5 % oproti 63,5 %).

Celkem bylo zaznamenáno 19 houbových morfotypů, identifikovaných na základě sekvencí ITS a zařazených do 11 rodů. U získaných kmenů byla hodnocena produkce asparaginázy – nejvyšší aktivita enzymu byla zjištěna v kultuře *Penicillium chrysogenum* (3.77 IU/ml). Dále byl zjištěn celkový obsah fenolických látek (TPC) a antioxidační potenciál sekundárních metabolitů – TPC se pohybuje v rozsahu 12.37 až 89.29 mg GAE/g vysušeného extraktu a hodnota $IC_{50}$ (vyjadřující antiradikálovou aktivitu) od 26.64 do 547.23 µg/ml. Potenciálně nejvyšší obsah fenolických látek i antioxidační kapacita byla zazanamenána taktéž u kmene *P. chrysogenum* (MH392736). Práce tak uvádí první přehled houbových endofytů z *R. densiflora* a jejich antioxidační a L-asparaginázový potenciál.

## INTRODUCTION

Endophytes are now considered a reliable source of natural bioactive products. Tropical and temperate forests are regarded to be the most diverse terrestrial ecosystems, possessing the highest number and diversity of endophytic fungi (Strobel 2002). Due to the relationships which endophytes have developed with their host plants over time, they make a number of biologically active compounds, which are classified as antibiotics, antioxidants, anticancer agents, volatile antimicrobial agents, immunosuppressive compounds, plant growth promoting agents and insecticides (Strobel 2018). Fungal endophytes have been recognised as a repository of unique secondary metabolites with potential beneficial activities. They have strong impacts on their host plants by increasing their growth and fitness, strengthening their tolerance to abiotic and biotic stresses, thereby promoting the accumulation of secondary metabolites (Jia et al. 2016).

Enzymes are biocatalysts produced by living cells for specific biochemical reactions. Endophytic fungi synthesise a wide range of enzymes and natural products exhibiting a wide variety of biological catalysts. They are also valued for their ability to synthesise extracellular enzymes like amylase, asparaginase, cellulase, chitinase, laccase, lipase, protease and tyrosinase (Rana et al. 2019). Most of the enzymes are applied as anti-proliferative agents. L-asparaginase (L-asparagine aminohydrolase, enzyme code EC 3.5.1.1) is a potential anti-tumour medicine. Tumour cells require asparagine for their growth, but asparaginase converts it to aspartate and ammonia, resulting in antiproliferative activity which tumour cells cannot survive (Paul et al. 2019). L-asparaginase for chemical formulation is derived from bacterial sources such as *Escherichia coli* and *Erwinia chrysanthemi*, which has led to several immunological and hypersensitive reactions. However, L-asparaginase from eukaryotic organisms like fungi has been suggested as an alternative source with fewer side effects (Ashok et al. 2019).

As part of the continuing efforts to find novel antioxidants from natural resources, fungal endophytes were investigated on their potential to form reservoirs of antioxidants. The antioxidative potentials of endophytic fungi may be due to the secretion of phenolics and flavonoid compounds into the medium. Recently, antioxidants have become a topic of interest since they act as radical scavengers and inhibit lipid peroxidation and other free radical mediated processes. Therefore they are capable of protecting the human body from several diseases attributed to reactions of radicals. The use of synthetic antioxidants in preventing free-radical damage has been reported to involve toxic side effects, thus necessitating the search for natural antioxidants and free-radical scavengers (Radulovic et al. 2007).

The Western Ghats of Southern India is a recognised hot spot area of plant endemicity. It represents biologically rich, biogeographically unique treasure house for species richness. Several research reports on the isolation of endophytes from medicinally important plants of the Western Ghats have been published (Nalini et al. 2014, Akshatha et al. 2014, Manasa & Nalini 2014, Das et al. 2017, Sheik & Chandrashekar 2018). Medicinal plants found growing in forests harbour both endophytic fungi and actinomycetes which have yielded bioactive metabolites (Akshatha et al. 2014, Das et al. 2017, 2018). Plants of the *Apocynaceae* family comprise of 392 genera and 5,140 species worldwide, distributed in tropical, subtropical and temperate regions. The family is represented by 30 genera and 60 species in the form of trees and shrubs in India (Endress & Bruyns 2000). They have a wide array of traditional uses here and are a rich source of drugs used in medicine.

The *Apocynaceae* include a large number of ethno-medicinal plants, which harbour fungal endophytes. Manasa & Nalini (2014) reported the evaluation of different members of this family for the occurrence of fungal endophytes. Other *Apocynaceae* members like *Aspidosperma tomentosum* (Savi et al. 2019), *Chonemorpha fragrans* (Clarance et al. 2019), *Catharanthus roseus* (Jariwala & Dessai 2018, Shukla et al. 2018, Dhayanithy et al. 2019, Sudharshana et al. 2019), *Hemidesmus indicus* (Shobha et al. 2019), *Picralima nitida* (Nwachukwu et al. 2018), *Rauvolfia tetraphylla* (Alurappa & Chowdappa 2018), *Vinca major*, *V. herbacea* and *V. minor* (Leylaie & Zafari 2018) were evaluated for their endophytic fungal associations.

*Rauvolfia densiflora* Benth. ex Hook. f. [currently classified as *Rauvolfia verticillata* (Lour.) Baill.], known as 'Dense-flowered snake root', is a repository of many biologically active compounds and is known as a source of many pharmacological and medicinally important phytochemicals (Iqbal et al. 2013). The plant parts are used in ethnomedicinal cures for various ailments by distinctive tribes of southern and eastern India and Nepal (Das et al. 2009, Lalitharani et al.

2011). The isolation of fungal endophytes from this species has been attempted (Huang et al. 2007), but the identification and molecular characterisation of the isolates have not been carried out. Contrary to the fungi, the endophytic actinomycetes from this species and their biological activities are documented (Akshatha et al. 2016).

Microbial asparaginase has also been comprehensively studied for its therapeutic applications. There is an increasing effort to identify and characterise endophytic fungi associated with enzymatic and antioxidative potentials, signifying that medicinal properties of plants are related to their imperative biological properties. Therefore, the present study was undertaken to investigate the isolation and molecular identification of endophytic fungi from *R. densiflora* and to assess the ability of these fungi to produce the L-asparaginase enzyme and to determine their antioxidative potentials. To the best of our knowledge, this study provides the first attempt to characterise the endophytic fungi from this plant.

## MATERIAL AND METHODS

Collection of plant samples. Plant samples of *R. densiflora* were collected from healthy shrubs growing in the forests of Kodagu District, in the Talacauvery site of the Western Ghats (12°17' to 12°27' N and 75°26' to 75°33' E; Fig. 1), Karnataka, India, during the month of June 2014. A total of five stem samples and five leaves were collected from five plants. Parts of the main stem measuring 1.0 cm diameter and 5.0 cm long, plus the leaves were excised using sterile pliers. The fresh cut ends of plant samples were placed separately in zip-lock polythene bags, stored at 4 °C and brought to the laboratory. The samples were processed within 48 hours of collection. A herbarium specimen of the plant has been deposited in the Botanical Survey of India (BSI), Western Circle, Pune, India with registered accession number 136245.

Isolation of the endophytic fungi. The collected plant samples were subjected to surface sterilisation procedures. Before processing, the samples were rinsed in running tap water to remove debris and dust. The leaf and stem pieces were surface-sterilised by sequential rinsing in 70% (v/v) ethanol for one minute, 3.5% (v/v) of sodium hypochlorite for two minutes, and finally with sterile distilled water two to three times to remove traces of the sterilants. Aliquots of the sterile distilled water used in the final rinse were poured onto Water Agar medium (WA 2%, w/v) to check the effectiveness of the surface-decontamination process (Schulz et al. 1998). A total of 200 segments of both leaves and stems of *R. densiflora* were plated for the isolation and identification of endophytic fungi. The plates were wrapped using Clean Wrap™ Cling film® (Xiamen, China) and

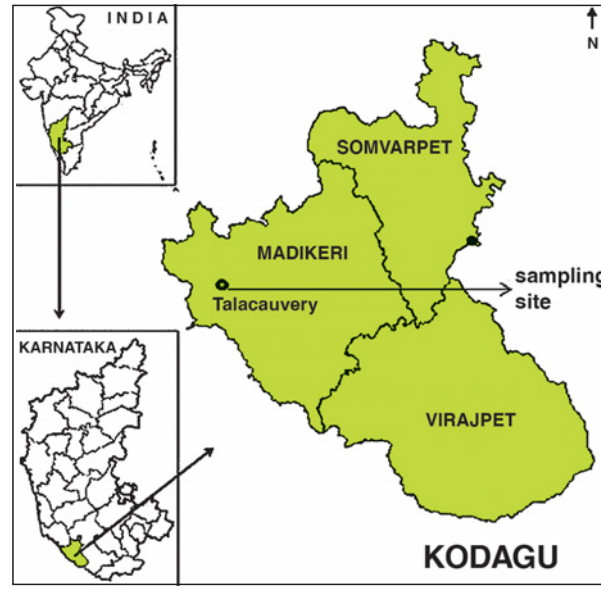


**Fig. 1.** Map showing the location of plant sample collection from its natural habitat in the Western Ghats.

incubated at 27 ± 2 °C with 12 hours of light and dark cycles for 4–6 weeks, and monitored regularly to check the growth of endophytic fungal emergence from the edges of the fragments. Individual hyphal tips were then transferred separately onto fresh Potato Dextrose Agar (PDA) slants and incubated at 27 ± 2 °C for 10–15 days, and pure cultures were maintained at 4 °C for further analysis.

Morphology and microscopic identification of endophytic fungi. The morphological study was performed by inoculating the endophytic fungal isolates on PDA plates and incubating them for 7 days. The isolates were identified based on colony morphology and microscopic observation. Parameters like colony characteristics, and filamentous or mat type growth were studied for the morphological identification. The slides of the fungal cultures were prepared by tease mount method using lactophenol cotton blue stain (NICE, Kerala, India) and observed under a light microscope (Quasmo, Haryana, India) at a magnification of 400×. The identification was based on the observation of mycelia, fruiting bodies, conidial characters according to standard identification manuals (Domsch et al. 1980, Singh et al. 1991, Barnett & Hunter 1998, Mathur & Kongsdal 2003) to the morphotype level.

Molecular characterisation of fungal endophytes using ITS-PCR and electrophoresis. Mycelial plugs from 19 morphologically different endophytic fungi were inoculated into Potato dextrose broth (PDB, Hi Media, Mumbai, India) and grown in still culture at 27 ± 2 °C for 7–10 days. Fungal

mycelia were separated from cultures, filtered and harvested. They were placed in polythene zip lock covers and stored at –20 °C. Genomic DNA was extracted from the freeze-dried mycelial mat using the cetyltrimethylammonium bromide (CTAB) method with trivial modifications (Ausubel et al. 1994). The concentration of DNA was measured using a nanospectrophotometer (Thermo Fischer 2000c, Bangalore, India) at 260 and 280 nm.

The DNA was amplified with the PCR technique using a PCR 18 kit (Chromous Biotech Pvt. Ltd., Bangalore, India). The target regions of the rDNA, ITS1, ITS2 regions and 5.8S gene were amplified using ITS1 (5'-TCCGTAGGTGAACCTGCG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') primers. The PCR was performed in a thermal cycler (Mastercycler Gradient, Eppendorf, Germany) by using the following programme: 94 °C for 2 min (initial denaturation), 35 cycles of 94 °C for 1 min (denaturation), 47 °C for 15 s (primer annealing), 72 °C for 30 s (primer extension), followed by 10 min of final extension at 72 °C. Subsequently, the amplified products were analysed with horizontal agarose gel electrophoresis through 1% agarose gel supplemented with ethidium bromide along with the 100bp DNA marker. DNA bands on the gel were visualised under a UV light trans-illuminator (Geldoc XRT, BioRad, Hercules, USA) and documented.

The amplified PCR products were sent to Chromous Biotech Pvt. Ltd. (Bangalore, India) for sequencing. Sequencing similarity searches were performed for the obtained fungal sequences and compared with ITS sequence data from strains available from the GenBank database (National Centre for Biotechnology Information website; http://www.ncbi.nlm.nih.gov/) by using the BLAST sequence match routines.

Data analysis. The colonisation frequency (%CF) of the endophytes was calculated and determined using the following formula:

$$\%CF = \frac{\text{Number of segments colonised by an endophyte}}{\text{Total number of tissue segments plated}} \times 100$$

Screening of fungal endophytes for L-asparaginase production. Qualitative analysis of L-asparaginase by plate assay. The isolated fungal endophytes were subjected to rapid screening of L-asparaginase production by the plate assay method (Gulati et al. 1997) on modified Czapek Dox's (MCD) agar medium [Saxena & Sinha 1981; glucose 2.0 g/l, L-asparagine 10 g/l, potassium dihydrogen phosphate ($KH_2PO_4$) 1.52 g/l, potassium chloride (KCl) 0.52 g/l, magnesium sulphate ($MgSO_4 \cdot 7H_2O$) 0.52 g/l, copper nitrate ($Cu(NO_3)_2 \cdot 3H_2O$) 0.001 g/l, zinc sulphate ($ZnSO_4 \cdot 7H_2O$) 0.001 g/l, ferrous sulphate ($FeSO_4 \cdot 7H_2O$) 0.001 g/l and agar 20 g/l] with an initial pH of 6.2, supplemented with 0.009% (v/v) phenol red indicator prepared from a stock solution of 2.5% of the dye in ethanol. At a low pH (acidic), phenol red is yellow while it turns pink at a high pH (alkaline),

therefore a pink zone is formed around endophytic fungal isolates producing L-asparaginase. The control plates were prepared with modified Czapek Dox's medium without asparagine (substituted with 0.001 g/l of $KNO_3$ as the only nitrogen source) and with phenol red indicator. The prepared medium was autoclaved and poured into sterilised plates. The MCD plates were divided into four quadrants and inoculated with mycelial plugs from four different isolated fungi. The plates were then incubated at 27 ± 2 °C for five days. The released ammonia reacts with water in the medium, which results in an increase of the pH of the medium (alkaline). Isolates showing pink zones were inoculated on individual MCD agar medium plates to confirm the enzyme activity.

Quantitative assay for enzyme production. For the production of L-asparaginase in liquid state, the mycelial discs from positive agar plates were inoculated into modified Czapek Dox's broth and incubated at 30 °C in an orbital shaker (GeNei™, Bangalore, India) for 5 days at 120 rpm. The crude enzyme sample was extracted by centrifugation and the supernatant containing the enzyme was used for further analysis by means of Nesslerisation. The reaction mixture consisted of 0.5 ml of 0.04 M L-asparagine, 0.5 ml of 0.5 M Tris-HCl buffer (pH 8.2), 0.5 ml of enzyme obtained from culture filtrate and 0.5 ml of distilled water. The samples were incubated for 30 min at 27 °C to stop the enzymatic reaction; 0.5 ml of 1.5 M trichloroacetic acid (TCA) was added. This was followed by pipetting 0.1 ml of the mixture into fresh tubes containing 3.7 ml of distilled water and 0.2 ml of Nessler's reagent and incubated at 27 °C for 20 minutes, after which the absorbance of the samples was measured at 450 nm using a UV-Visible spectrophotometer (TPL Technology Pvt. Ltd., Bangalore, India). A blank was prepared by adding TCA followed by an enzyme sample. One international unit (IU) of L-asparaginase is expressed as the amount of enzyme that catalyses the formation of 1 µmol of ammonia per minute at 27 °C (Imada et al. 1973).

Submerged fermentation and extraction of secondary metabolites. Mycelia from actively growing 7-day-old endophytic pure cultures were inoculated aseptically into 500 ml of PDB contained in Erlenmeyer flasks in duplicates. The inoculated flasks were incubated at 27 ± 2 °C for 21 days. The flasks were examined for any contamination. Culture broths were filtered through muslin cloth. The supernatant was transferred to a separating funnel, to which an equal volume of ethyl acetate (1:1 v/v) was added, and extracted thrice by strong agitation. The extract obtained in this way was concentrated in a Rotary flash evaporator and stored in vials for further use.

Determination of total phenolic content. The total phenolic content (TPC) in the endophytic fungal extracts was determined using Folin-Ciocalteu (FC) reagent following a minor modification (Liu et al. 2007). The ex-

tracts were mixed with 1 ml of FC reagent and 2 ml of sodium carbonate (20% w/v). The mixture was left to stand in the dark for 45 minutes with intermittent shaking for colour development. The absorbance of the resulting blue colour was measured at 765 nm using UV-VIS spectrophotometer (DU 730 "Lifesciences", Beckman Coulter, Indianapolis, USA). Gallic acid was used as a reference standard for plotting the calibration curve. The total phenolic content was calculated from the calibration curve and the results were expressed as milligramme of Gallic acid equivalent (GAE)/g of the dry extract.

Determination of antioxidant activity with DPPH radical scavenging assay. The method described by Pannangpetch et al. (2007) was used for determination of the scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH, Sigma-Aldrich, Bangalore, India) free radical with slight modifications. An amount of 0.001 mM of DPPH prepared in methanol was mixed with the fungal extracts. The mixture was vortexed thoroughly and left to stand in the dark for 20 minutes at room temperature (25 ± 1 °C). A blank was set with methanol (SD Fine-Chem Ltd., Mumbai, India). The absorbance of each solution was measured spectrophotometrically at 517 nm. Ascorbic acid was used as standard reference. The scavenging activity of the DPPH radical was calculated as follows:

$$\% \text{ scavenging} = \frac{Ac - As}{Ac} \times 100$$

Ac = absorbance of control; As = absorbance of test sample.

The $IC_{50}$ value (µg/ml) is the effective concentration at which DPPH radicals were scavenged by 50% and it was obtained by linear regression analysis.

## RESULTS

### Molecular characterisation and occurrence of endophytic fungi

A total of 318 isolates were obtained from 400 tissue segments distributed over 19 endophytic taxa (five of them belonging to Eurotiomycetes and 14 of them belonging to Sordariomycetes). The relative colonisation frequency of endophytic fungi in leaf and stem parts along with their molecular characterisation is shown in Tabs. 1 and 2. Besides single species of other genera, four species of *Colletotrichum*, three species of *Fusarium*, three species of *Neocosmospora* and two species of *Aspergillus* were recovered.

A high colonisation frequency was reported for *Penicillium chrysogenum*, which was found to be the dominant species in leaves (48.0% in leaf segments), followed by *Chaetomium globosum* (average 9.75%), *Neocosmospora solani* (8.0%), *Aspergillus carneus* (7.25%) obtained from both leaves and stems. Besides

**Tab. 1.** Molecular characterisation of fungal endophytes isolated from *Rauvolfia densiflora* with GenBank accession numbers, the closest match of ITS sequence in the NCBI database, and their sequence similarity.

| Fungal endophytes | Isolate code | GenBank Accession Number | Length (bp) | Closest GenBank match #ITS | Sequencing similarity (%) |
|---|---|---|---|---|---|
| *Aspergillus carneus* (Tiegh.) Blochwitz | RD-WG-33 | MK424326 | 526 | HQ889708 | 523/525 (99%) |
| *Aspergillus versicolor* (Vuill.) Tirab. | RD-WG-03 | MK415240 | 520 | KP131640 | 516/520 (99%) |
| *Bionectria ochroleuca* (Schwein.) Schroers & Samuels | RD-WG-32 | MK682679 | 503 | HQ637283 | 501/503 (99%) |
| *Chaetomium globosum* Kunze | RD-WG-16 | MK415057 | 528 | KU184610 | 528/528 (100%) |
| *Clonostachys rosea* (Link) Schroers, Samuels, Seifert & W. Gams | RD-WG-15 | MH393295 | 389 | KX421414 | 389/389 (100%) |
| *Colletotrichum boninense* Moriwaki, Toy, Sato & Tsukib. | RD-WG-26 | MK446916 | 535 | MF062469 | 527/535 (99%) |
| *Colletotrichum cliviicola* Damm & Crous | RD-WG-34 | MK426287 | 534 | MH142471 | 530/534 (99%) |
| *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. | RD-WG-22 | MK424265 | 426 | JQ936123 | 425/427 (99%) |
| *Colletotrichum* sp. | RD-WG-31 | MK685173 | 540 | JQ341134 | 526/528 (99%) |
| *Fusarium fujikuroi* Nirenberg | RD-WG-09 | MK446915 | 497 | KJ000439 | 496/497 (99%) |
| *Fusarium oxysporum* Schltdl. | RD-WG-11 | MH393293 | 495 | MG136706 | 495/495 (100%) |
| *Fusarium* sp. | RD-WG-13 | MK446912 | 525 | KY775948 | 521/526 (99%) |
| *Neocosmospora haematococca* (Berk. & Broome) Nalim, Samuels & Geiser | RD-WG-28 | MK446936 | 526 | GU134901 | 519/525 (99%) |
| *Neocosmospora solani* (Mart.) L. Lombard & Crous | RD-WG-10 | MK418238 | 521 | KF897901 | 506/520 (97%) |
| *Neocosmospora* sp. | RD-WG-29 | MK446913 | 519 | LT746276 | 518/522 (99%) |
| *Paecilomyces penicillatus* (Höhn.) Samson | RD-WG-06 | MH393291 | 543 | JX012226 | 543/543 (100%) |
| *Penicillium chrysogenum* Thom | RD-WG-02 | MH392736 | 531 | MG818936 | 531/531 (100%) |
| *Sarocladium kiliense* (Grütz) Summerbell | RD-WG-08 | MK426286 | 559 | MH393397 | 555/560 (99%) |
| Unidentified species of *Eurotiales* | RD-WG-17 | MK450305 | 529 | KP963597 | 525/525 (100%) |

*P. chrysogenum*, also *Aspergillus versicolor*, *Paecilomyces penicillatus*, *Sarocladium kiliense*, *Fusarium oxysporum*, *Fusarium* sp., *Clonostachys rosea*, an unidentified species of *Eurotiales*, *Neocosmospora* sp., *Colletotrichum* sp., *Aspergillus carneus* and *Colletotrichum cliviicola* expressed tissue specificity by occurring only in leaf fragments, while *Fusarium fujikuroi*, *Colletotrichum boninense*, *Neocosmospora haematococca* and *Bionectria ochroleuca* were recovered only from stem segments.

## Qualitative and quantitative analysis of L-asparaginase by means of plate assay and enzyme production

A total of 14 fungal strains demonstrated the ability to produce the L-asparaginase enzyme. In the positive strains, formation of a pink zone around the colonies was distinct. The positive fungal strains produced L-asparaginase activities in the

**Tab. 2.** Colonisation frequencies of fungal endophytes isolated from plant parts of *Rauvolfia densiflora* (200 segments were plated from leaf and stem for frequency analysis).
Abbreviations: I – number of isolates; CF – colonisation frequency.

| Fungal endophytes | Leaf | | Stem | | Total %CF |
|---|---|---|---|---|---|
| | I | %CF | I | %CF | |
| *Aspergillus carneus* | 08 | 4.0 | 21 | 10.5 | 7.25 |
| *Aspergillus versicolor* | 02 | 1.0 | – | – | 0.5 |
| *Bionectria ochroleuca* | – | – | 04 | 2.0 | 1.0 |
| *Chaetomium globosum* | 04 | 2.0 | 35 | 17.5 | 9.75 |
| *Clonostachys rosea* | 06 | 3.0 | – | – | 1.5 |
| *Colletotrichum boninense* | – | – | 19 | 9.5 | 4.75 |
| *Colletotrichum cliviicola* | 07 | 3.5 | – | – | 1.75 |
| *Colletotrichum gloeosporioides* | 11 | 5.5 | 03 | 1.5 | 3.5 |
| *Colletotrichum* sp. | 06 | 3.0 | – | – | 1.5 |
| *Fusarium fujikuroi* | – | – | 06 | 3.0 | 1.5 |
| *Fusarium oxysporum* | 09 | 4.5 | – | – | 2.25 |
| *Fusarium* sp. | 14 | 7.0 | – | – | 3.5 |
| *Neocosmospora haematococca* | – | – | 22 | 11.0 | 5.5 |
| *Neocosmospora solani* | 15 | 7.5 | 17 | 8.5 | 8.0 |
| *Neocosmospora* sp. | 02 | 1.0 | – | – | 0.5 |
| *Paecilomyces penicillatus* | 03 | 1.5 | – | – | 0.75 |
| *Penicillium chrysogenum* | 96 | 48.0 | – | – | 24.0 |
| *Sarocladium kiliense* | 06 | 3.0 | – | – | 1.5 |
| Unidentified species of *Eurotiales* | 02 | 1.0 | – | – | 0.5 |

– = endophyte not detected

range of 0.400 ± 0.06 – 3.778 ± 0.08 IU/ml (Tab. 3). Eleven of these strains were considered to have a relatively high asparaginase activity (1.648 ± 0.19 – 3.778 ± 0.08 IU/ml), eight of them from leaf and three isolates from stem parts of the plant. A significantly high L-asparaginase activity was found for endophyte *Penicillium chrysogenum* isolated from leaves with 3.778 ± 0.08 IU/ml of enzyme. *Colletotrichum boninense* (2.992 ± 0.07 IU/ml) from stems, *Fusarium oxysporum* (2.900 ± 0.08 IU/ml) from leaves, *C. gloeosporioides* (2.724 ± 0.06 IU/ml) from leaves, *Paecilomyces penicillatus* (2.308 ± 0.09 IU/ml) from leaves, and *Neocosmospora haematococca* (2.306 ± 0.07 IU/ml) from stems exhibited considerably significant L-asparaginase activity.

## Total phenolic content

The total phenolic content (TPC) of the 19 endophytic fungal extracts is graphically represented in Fig. 2. The TPC of the extracts ranged from 12.37 ±

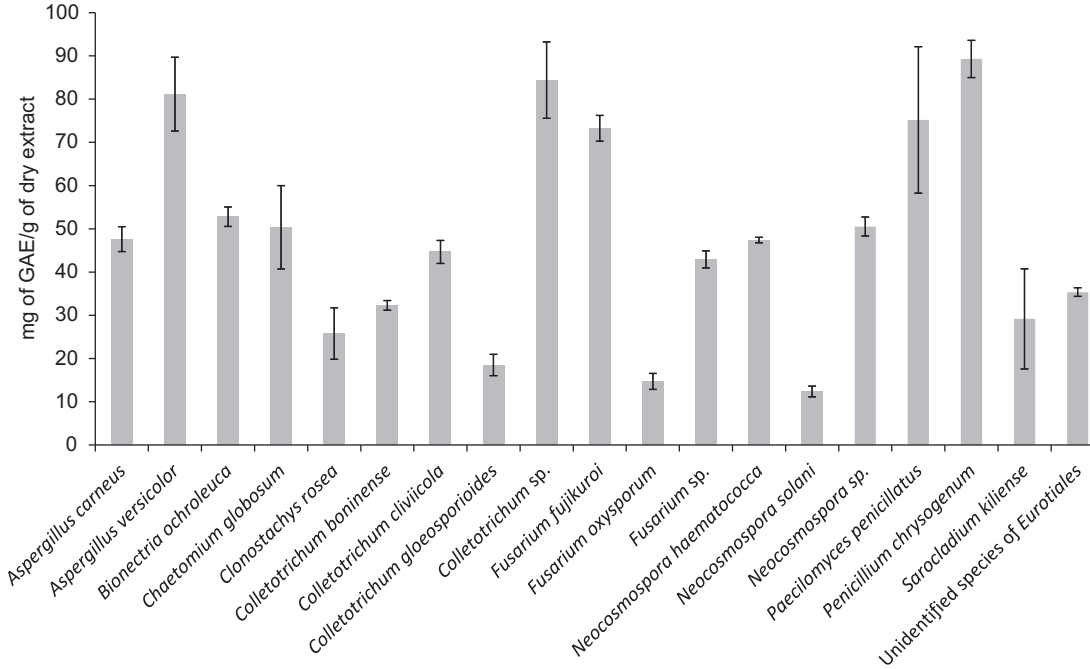


**Fig. 2.** Total phenolic content of fungal strains associated with *Rauvolfia densiflora*. Data are reported as mean ± SD of three independent analyses.

1.24 to 89.29 ± 4.30 mg GAE/g dry extract. The extract of *Penicillium chrysogenum* exhibited a high total phenolic content (89.29 ± 4.30 mg GAE/g dry extract), followed by *Colletotrichum* sp. (84.39 ± 8.84 mg GAE/g) and *Aspergillus versicolor* (81.15 ± 8.54 mg GAE/g). *Paecilomyces penicillatus* and *Fusarium fujikuroi* showed a phenolic content ranging from 75.19 ± 16.94 mg GAE/g to 73.24 ± 2.99 mg GAE/g dry extract, respectively.

**Determination of antioxidant activity**

The 19 fungal extracts were tested for DPPH radical scavenging potential (Tab. 3). The results showed that the $IC_{50}$ value of the fungal extracts varied in scavenging activity from 26.64 ± 2.57 to 547.23 ± 15.83 µg/ml. *Penicillum chrysogenum* showed a high radical scavenging activity of 26.64 ± 2.57 µg/ml followed by *Aspergillus versicolor* with 96.74 ± 2.17 µg/ml. *Paecilomyces penicillatus* (126.37 ± 3.49 µg/ml), *Bionectria ochroleuca* (131.22 ± 11.05 µg/ml) and *Neocosmospora* sp. (143.59 ± 11.96).

**Tab. 3.** Estimation of L-asparaginase enzyme production and antioxidant activity of fungal endophytes associated with *Rauvolfia densiflora*.
Abbreviations: IU – international unit; DPPH – 1,1-diphenyl-2-picrylhydrazyl; $IC_{50}$ – Inhibitory concentration 50%. Data are reported as mean ± SD of three independent analyses.
Letters in superscript denote: $^{L}$ – leaf isolate; $^{S}$ – stem isolate.

| Fungal endophytes | Enzyme activity (IU/ml) | DPPH radical scavenging capacity ($IC_{50}$ µg/ml) |
|---|---|---|
| *Aspergillus carneus* $^{L}$ | 0.910 ± 0.07 | 195.53 ± 8.33 |
| *Aspergillus versicolor* $^{L}$ | 1.891 ± 0.07 | 96.74 ± 2.17 |
| *Bionectria ochroleuca* $^{S}$ | – | 131.22 ± 11.05 |
| *Chaetomium globosum* $^{L}$ | – | 305.46 ± 11.72 |
| *Clonostachys rosea* $^{L}$ | – | 338.14 ± 14.41 |
| *Colletotrichum boninense* $^{S}$ | 2.992 ± 0.07 | 436.95 ± 14.03 |
| *Colletotrichum cliviicola* $^{L}$ | 0.400 ± 0.06 | 350.83 ± 14.70 |
| *Colletotrichum gloeosporioides* $^{L}$ | 2.724 ± 0.06 | 308.09 ± 9.85 |
| *Colletotrichum* sp. $^{L}$ | 1.959 ± 0.59 | 164.69 ± 10.55 |
| *Fusarium fujikuroi* $^{S}$ | 1.917 ± 0.04 | 302.82 ± 9.34 |
| *Fusarium oxysporum* $^{L}$ | 2.900 ± 0.08 | 216.00 ± 15.00 |
| *Fusarium* sp. $^{L}$ | – | 200.94 ± 9.16 |
| *Neocosmospora haematococca* $^{S}$ | 2.306 ± 0.07 | 215.32 ± 4.17 |
| *Neocosmospora solani* $^{S}$ | 0.688 ± 0.04 | 232.13 ± 18.88 |
| *Neocosmospora* sp. $^{L}$ | 1.648 ± 0.19 | 143.59 ± 11.96 |
| *Paecilomyces penicillatus* $^{L}$ | 2.308 ± 0.09 | 126.37 ± 3.49 |
| *Penicillium chrysogenum* $^{L}$ | 3.778 ± 0.08 | 26.64 ± 2.57 |
| *Sarocladium kiliense* $^{L}$ | – | 285.56 ± 9.59 |
| Unidentified species of *Eurotiales* $^{L}$ | 1.698 ± 0.08 | 547.23 ± 15.83 |

– = enzyme activity not detected by plate assay

## DISCUSSION

In the present study, a total of 19 endophytic fungal taxa belonging to 11 different genera were obtained. A higher relative colonisation frequency of endophytic fungi was found in leaves than in stems. This may have been because the plant leaves are nutrient-rich and have a larger surface area than the stem and are thin-walled, which enhances endophytic fungal colonisation.

In the current study, the fungal strain *Penicillium chrysogenum*, isolated from leaf segments, showed a high relative colonisation frequency (48.0%). This species was formerly reported as a fungal endophyte from *Rauvolfia serpentina* (D'Souza & Hiremath 2015). Reports on the isolation of *Penicillium* sp. and *P. citrinum* from *R. serpentina* as endophytes have been documented before (D'Souza & Hiremath 2015, Sahu et al. 2016).

Endophytic fungi, with their capability of their L-asparaginase production, have gained considerable attention owing to their cost-effective and eco-friendly nature. Fungal strains examined for enzyme activity exhibited a reaction of L-asparaginase hydrolysing L-asparagine to ammonia. Uzma et al. (2016) reported significantly higher L-asparaginase activity from Western Ghats endophytic fungi such as *Fusarium* sp. (79.66 ± 0.29 IU/ml), *Rhizopus* sp. (72.33 ± 1.30 IU/ml) and *Aspergillus* sp. (72.33 ± 0.29 IU/ml). Our results are on par with the original work by Theantana et al. (2009), in which the quantitative activity of 53 positive out of 82 strains of endophytes ranged from 0.014 to 1.5 IU/ml. Uzma et al. (2016), although having followed the paper by Theantana et al. (2009), failed to discuss the abnormally high enzyme activity they found, the calculation of which is not documented in the published paper. In our study, of the isolates screened, endophytic fungal strain *P. chrysogenum* showed a considerable high enzyme activity of 3.778 ± 0.08 IU/ml, higher than the activity of *Fusarium verticillioides* isolated from *Tabernaemontana heyneana* from the Western Ghats (Manasa & Nalini 2014). The L-asparaginase activity of *Penicillium* sp. 4 URM 7287 (1.28 IU/g) from Brazilian tropical dry forest (Silva et al. 2018) and *Eurotium* sp. (1.304 IU/ml) from Jalgaon Garden (Jalgaonwala & Mahajan 2014) was found to be lower in comparison with our investigation. Earlier studies revealed that *Penicillium* spp. (Pádua et al. 2019) and *P. chrysogenum* (Oliveira et al. 2017) are producers of L-asparaginase.

Medicinal plants and their endophytes are important resources for natural products. The antioxidant capacities of endophytic fungal cultures significantly correlate with their total phenolic contents, suggesting that phenols are also the major antioxidant constituent of the endophytes. In the current study, fungal extracts of endophytes isolated from *Rauvolfia densiflora* showed phenol contents ranging from 12.37 ± 1.24 mg GAE/g to 89.29 ± 4.30 mg GAE/g. Phenolic contents of microbial extracts are usually much lower than those of plant extracts, but comparable studies exhibit different results. Phytochemical analysis of leaf extracts of *R. densiflora* showed that the leaves have a lower total phenolic content (4.5 ± 1.08 mg GAE/g to 39 ± 2.6 mg GAE/g) in different solvent systems (Akshatha et al. 2015). In contrast, the phytochemical analysis of entire plant extracts of *R. densiflora* was reported to exhibit the highest total phenolic contents in different solvent systems (31.31 ± 0.5 mg GAE/g to 107.67 ± 1.5 mg GAE/g) (Pandurangamurthy et al. 2015). Conversely, also a report on the TPC of *R. serpentina* with a value of 245.21 mg GAE/100 g fresh weight (Islam 2018) has been presented.

Among the 19 endophytic fungal extracts tested for the ability to scavenge DPPH in the present study, *Penicillium chrysogenum* ($IC_{50}$ of 26.64 ± 2.57 µg/ml) exhibited the potentially highest antioxidant capacity. This value is higher than that of the methanol leaf extracts of *R. densiflora* ($IC_{50}$ 76 ± 2.89 µg/ml) tested by Akshatha et al. (2015). Also, the free-radical scavenging capacity of acetone ex-

tract of *R. serpentina* ($IC_{50}$ of 69.1 ± 0.43 µg/ml) (Chauhan et al. 2016) was found to be lower, which is distinctly different from root extracts of *R. vomitoria* ($IC_{50}$ of 3.56 ± 1.67 µg/ml) (Sonibare & Akpan 2016). However, some investigators have revealed that there is no correlation between total phenolic content and antioxidant activity (Yadav et al. 2014).

## CONCLUSIONS

This study provides insight into the diversity of the endophytic fungal community isolated from *Rauvolfia densiflora*. This is the first report on the characterisation of fungal endophytes from *R. densiflora* growing in the Western Ghats. Of the 19 endophytic fungi screened for L-asparaginase and antioxidative potential, *Penicillium chrysogenum* exhibited significant results, being the relatively most frequent coloniser of leaf parts of the plant. Currently, we have initiated the secondary metabolite profiling of *P. chrysogenum* extracts to test the antiproliferative potentials in cancer cell lines. Understanding mechanisms of the antiproliferative potentials of *P. chrysogenum* may provide new insights into tumour biology, drug discovery, development of alternative drug combinations and pharmaceutical applications.

## ACKNOWLEDGEMENTS

The authors thank the Institution of Excellence (IOE) for Biodiversity & Sustainable Development, Vijnan Bhawan, and the University of Mysore for providing the necessary facilities to carry out the study.

## REFERENCES

AKSHATHA J.V., NALINI M.S., D'SOUZA C., PRAKASH H.S. (2014): Streptomycete endophytes from antidiabetic medicinal plants of the Western Ghats inhibit alpha-amylase and promote glucose uptake. – Letters in Applied Microbiology 58: 433–439. DOI: https://doi.org/10.1111/lam.12209

AKSHATHA J.V., PRAKASH H.S., NALINI M.S. (2015): Antioxidative and α-amylase inhibitory potentials of medicinal plants from the Western Ghats of southern India. – Der Pharmacia Lettre 7(5): 10–17.

AKSHATHA J.V., PRAKASH H.S., NALINI M.S. (2016): Actinomycete endophytes from the ethno medicinal plants of Southern India: antioxidant activity and characterization studies. – Journal of Biologically Active Products from Nature 6: 166–172. DOI: https://doi.org/10.1080/22311866.2016.1191971

ALURAPPA R., CHOWDAPPA S. (2018): Antimicrobial activity and phytochemical analysis of endophytic fungal extracts isolated from ethno-pharmaceutical plant *Rauwolfia tetraphylla* L. – Journal of Pure and Applied Microbiology 12(1): 317–332. DOI: https://doi.org/10.22207/JPAM.12.1.38

ASHOK A., DORIYA K., RAO J.V., QURESHI A., TIWARI A.K., KUMAR D.S. (2019): Microbes producing L-asparaginase free of glutaminase and urease isolated from extreme locations of Antarctic soil and moss. – Scientific Reports 9: 1423. DOI: https://doi.org/10.1038/s41598-018-38094-1

AUSUBEL F.M., BRENT R., KINGSTON R.E., MOORE D.D., SEIDMAN J.G., SMITH J.A., STRUHL K. (1994): Current Protocols in Molecular Biology. – Wiley, New York.

BARNETT H.L., HUNTER B.B. (1998): Illustrated genera of imperfect fungi, 4th edition. – APS Press, St. Paul.

CHAUHAN P.K., AVINASH THORY R., NAGRAIK R. (2016): Comparative evaluation of phytochemical and in-vivo antioxidant activities of two endangered plant species of Western Himalayas. – Biological Forum 8(2): 234–239.

CLARANCE P., LALITHA J., SALES J., KHUSRO A., AGASTIAN P. (2019): Anticancer activity of camptothecin producing endophytes isolated from *Chonemorpha fragrans* (Moon) Alston (*Apocynaceae*). – Research Journal of Biotechnology 14(5): 74–82.

DAS S., KHAN M.L., RABHA A., BHATTACHARJYA D.K. (2009): Ethnomedicinal plants of Manas National Park, Assam, Northeast India. – Indian Journal of Traditional Knowledge 8(4): 514–517. http://nopr.niscair.res.in/handle/123456789/6273

DAS M., PRAKASH H.S., NALINI M.S. (2017): Antioxidative properties of phenolic compounds isolated from the fungal endophytes of *Zingiber nimmonii* (J. Graham) Dalzell. – Frontiers in Biology 12(2): 151–162. DOI: https://doi.org/10.1007/s11515-016-1441-z

DAS M., PRAKASH H.S., NALINI M.S. (2018). Bioactive sesquiterpene, plasticizer and phenols from the fungal endophytes of *Polygonum chinense* L. – Annals of Microbiology 68: 595–609. DOI: https://doi.org/10.1007/s13213-018-1367-6

DHAYANITHY G., SUBBAN K., CHELLIAH J. (2019): Diversity and biological activities of endophytic fungi associated with *Catharanthus roseus*. – BMC Microbiology 19(22): 1–14. DOI: https://doi.org/10.1186/s12866-019-1386-x

DOMSCH K.H., GAMS W., ANDERSON T. (1980): Compendium of soil fungi. – Academic Press, New York.

D'SOUZA M.A., HIREMATH K.G. (2015): Isolation and bioassay screening of medicinal plant endophytes from Western Ghats forests, Goa, India. – International Journal of Advanced Research in Biological Sciences 2(8): 176-190.

ENDRESS M.E., BRUYNS P.V. (2000). A revised classification of the *Apocynaceae* s.l. – The Botanical Review 66(1): 1–56. DOI: https://doi.org/10.1007/BF02857781

GULATI R., SAXENA R.K., GUPTA R. (1997): A rapid plate assay for screening L-asparaginase producing micro-organisms. – Letters in Applied Microbiology 24: 23–26. DOI: https://doi.org/10.1046/j.1472-765x.1997.00331.x

HUANG W.Y., CAI Y.Z., XING J., CORKE H., SUN M. (2007): A potential antioxidant resource: endophytic fungi from medicinal plants.- Economic Botany 61 (1): 14-30. DOI: https://doi.org/10.1663/0013-0001(2007)61%5B14:APAREF%5D2.0.CO;2

IMADA A., IGARASI S., NAKAHAMA K., ISONO M. (1973): Asparaginase and glutaminase activities of micro-organisms. – Journal of General Microbiology 76(1): 85–99. DOI: https://doi.org/10.1099/00221287-76-1-85

IQBAL A.A.M., KHAN F.A.K., ANSARI I., QURAISHI A., KHAN M. (2013): Ethno-phyto-pharmacological overview on *Rauvolfia densiflora* (Wall) Benth. ex Hook. f. – International Journal of Pharmacutical and Phytopharmacological Research 2(5): 372–376.

ISLAM R. (2018): Leaf phenolics and pigments along with their antioxidative potential in some medicinal plants. – Asian-Australasian Journal of Food Safety and Security 2(2): 86–92.

JALGAONWALA R.E., MAHAJAN R.T. (2014): Production of anticancer enzyme asparaginase from endophytic *Eurotium* sp. isolated from rhizomes of *Curcuma longa*. – European Journal of Experimental Biology 4(3): 36–43.

JARIWALA B., DESSAI B. (2018): Isolation and identification of endophytic fungi from various medicinal plants. – BMR Microbiology 4(1): 1–7.

JIA M., CHEN L., XIN H.L., ZHENG C.J., RAHMAN K., HAN T., QIN L.P. (2016): A friendly relationship between endophytic fungi and medicinal plants: A systematic review. – Frontiers in Microbiology 7: 906. DOI: https://doi.org/10.3389/fmicb.2016.00906

LALITHARANI S., KALPANADEVI V., TRESINA SORIS P., MARUTHAANDIAN A., MOHAN V.R. (2011): Ethnomedicinal plants used by Kanikkars of Agasthiarmalai Biosphere Reserve, Western Ghats. – Journal of Ecobiotechnology 3(7): 16–25.

LEYLAIE S., ZAFARI D. (2018): Antiproliferative and antimicrobial activities of secondary metabolites and phylogenetic study of endophytic *Trichoderma* species from *Vinca* plants. – Frontiers in Microbiology 9: 1–16. DOI: https://doi.org/10.3389/fmicb.2018.01484

LIU X., DONG M., CHEN X., JIANG M., LV X., YAN G. (2007): Antioxidant activity and phenolics of an endophytic *Xylaria* sp. from *Ginkgo biloba*. – Food Chemistry 105(2): 548–554. DOI: https://doi.org/10.1016/j.foodchem.2007.04.008

MANASA C., NALINI M.S. (2014): L-asparaginase activity of fungal endophytes from *Tabernaemontana heyneana* Wall. (*Apocynaceae*), endemic to the Western Ghats (India). – International Scholarly Research Notices 2014: 1–7. DOI: https://doi.org/10.1155/2014/925131

MATHUR S.B., KONGSDAL O. (2003): Common laboratory seed health testing methods for detecting fungi. – International Seed Testing Association, Geneva.

NALINI M.S., SUNAYANA N., PRAKASH H.S. (2014). Endophytic fungal diversity in medicinal plants of Western Ghats, India. – International Journal of Biodiversity 2014: 1–9. DOI: https://doi.org/10.1155/2014/494213

NWACHUKWU C.U., NGWOKE K.G., EZE P.M., EBOKA C.J., OKOYE F.B.C. (2018): Secondary metabolites from *Curvularia* sp., an endophytic fungus isolated from the leaves of *Picralima nitida* Durand and Hook. (*Apocynaceae*). – Tropical Journal of Natural Product Research 2(5): 209–213. DOI: https://doi.org/10.26538/tjnpr/v2i5.2

OLIVEIRA M.M., ARAUJO N.K., MEDEIROS D., PEDROSA M.F.F., ASSIS C.F. (2017): Evaluation of L-asparaginase production by microorganisms. – In: Proceedings of National Bioprocesses Symposium and Enzymatic Hydrolysis of Biomass Symposium, 3–6 September 2017, Aracaju, Brazil.

PÁDUA A.P.S.L., FREIRE K.T.L.S., OLIVEIRA T.G.L., SILVA L.F., MAGALHÃES G.R.A., AGAMEZ-MONTALVO G.S., SILVA I.R., BEZERRA J.D.P., SOUZA-MOTTA C.M. (2019): Fungal endophyte diversity in the leaves of the medicinal plant *Myracrodruon urundeuva* in a Brazilian dry tropical forest and their capacity to produce L-asparaginase. – Acta Botanica Brasilica 33(1): 39–49. DOI: https://doi.org/10.1590/0102-33062018abb0108

PANDURANGAURTHY G., CHANDRASHEKAR K.B., LOKESH S. (2015): Phyto-chemical and pharmacological evaluation of ethno medicinal plant drugs (EMP) and tribal medicine formulation (TMF) used by tribal practitioners for wound therapeutics in the region of Biligirirangana hills, Karnataka**.** – International Journal of Research in Applied Natural and Social Sciences 3(7): 41–66.

PANNANGPETCH P., LAUPATARAKASEM P., KUKONVIRIYAPAN V., KUKONGRIYAPA C., KONGYINAYOES B., AROMDEE C. (2007): Antioxidant activity and protective effect against oxidative hemolysis of *Clinacanthus nutans* (Burm. f.) Lindau. – Songklanakarin Journal of Science and Technology 29(1): 1–9.

PAUL T., MONDAL A., BANDYOPADHYAY T.K. (2019): Isolation, purification, characteri**z**ation and application of L-ASNase: A review. – Recent Patents on Biotechnology 13(1): 33–44. DOI: https://doi.org/10.2174/1872208312666181012150407

RADULOVIC N., STANKOV-JOVANOVIC V., STOJANOVIC G., SMELCEROVIC A., SPITELLER M., ASAKAWA Y. (2007): Screening of in-vitro antimicrobial and antioxidant activity of nine *Hypericum* species from the Balkans. – Food Chemistry 103: 15–21. DOI: https://doi.org/10.1016/j.foodchem.2006.05.062

RANA K.L., KOUR D., SHEIKH I., YADAV N., YADAV A.N., KUMAR V., SINGH B.P., DHALIWAL H.S., SAXENA A.K. (2019): Biodiversity of endophytic fungi from diverse niches and their biotechnological applications. – In: Singh B.P., ed., Advances in endophytic fungal research, pp. 105–144. Springer Nature Switzerland, Basel. DOI: https://doi.org/10.1007/978-3-030-03589-1_6

Sahu R., Kumar S., Aharwal R.P., Sandhu S.S. (2016): Antibacterial activity of isolated endophytic fungi from *Rauvolfia serpentina* (L.) Benth. ex Kurz. – International Journal of Pharmacy and Pharmaceutical Sciences 8(11): 38–42. DOI: https://doi.org/10.22159/ijpps.2016v8i11.9733

Savi D.C., Aluizio R., Glienke C. (2019): Brazilian plants: An unexplored source of endophytes as producers of active metabolites. – Planta Medica 85(8): 619–636. DOI: https://doi.org/10.1055/a-0847-1532

Saxena R.K., Sinha U. (1981): L-asparaginase and glutaminase activities in the culture filtrates of *Aspergillus nidulans*. – Current Science 50: 218–219.

Schulz B., Guske S., Dammann U., Boyle C. (1998): Endophyte-host interaction II. Defining symbiosis of the endophyte-host interaction. – Symbiosis 25: 213–227.

Sheik S., Chandrashekar K.R. (2018): Fungal endophytes of an endemic plant *Humboldtia brunonis* Wall. of Western Ghats (India) and their antimicrobial and DPPH radical scavenging potentiality. – Oriental Pharmacy and Experimental Medicine 18(2): 115–125. DOI: https://doi.org/10.1007/s13596-018-0297-y

Shobha M., Bharathi T.R., Sampath Kumara K.K., Prakash H.S. (2019): Diversity and biological activities of fungal root endophytes of *Hemidesmus indicus* (L.) R. Br. - Journal of Pharmacognosy and Phytochemistry 8(1): 273–280.

Shukla S.T., Rudani M.G., Gohil K.J., Patel N.A., Habbu P.V., Mishra R.K., Gupta A. (2018): Antioxidant and anticancer activities of endophytic crude fraction of Sadabahar [*Catharanthus roseus* (L.) G. Don] in rats. – International Journal of Unani and Integrative Medicine 2(4): 26–34. https://www.researchgate.net/publication/331131299

Silva L.F., Freire K.T.L.S., Araújo-Magalhães G.R., Agamez-Montalvo G.S., Sousa M.A., Costa-Silva T.A., Paiva L.M., Pessoa-Junior A., Bezerra J.D.P., Souza-Motta C.M. (2018): *Penicillium* and *Talaromyces* endophytes from *Tillandsia catimbauensis*, a bromeliad endemic in the Brazilian tropical dry forest, and their potential for L-asparaginase production. – World Journal of Microbiology and Biotechnology 34(11): 162. DOI: https://doi.org/10.1007/s11274-018-2547-z

Singh K., Frisvad J.C., Thrane U., Mathur S.B. (1991): An illustrated manual on identification of some seed-borne Aspergilli, Fusaria, Penicillia and their mycotoxins. – Danish Government, Institute of Seed Pathology for Developing Countries, Hellerup.

Sonibare M.A., Akpan G.U. (2016): In-vitro callus induction and antioxidant activity of *Rauwolfia vomitoria* Afzel. (*Apocynaceae*). – Nigerian Journal of Pharmaceutical Research 12(2): 105–115.

Strobel G. (2002): Rainforest endophytes and bioactive products. – Critical Reviews in Biotechnology 22(4): 315–333. DOI: https://doi.org/10.1080/07388550290789531

Strobel G. (2018): The emergence of endophytic microbes and their biological promise. – Journal of Fungi 4(57): 1–19. DOI: https://doi.org/10.3390/jof4020057

Sudharshana T.N., Venkatesh H.N., Nayana B., Manjunath K., Mohana D.C. (2019): Anti-microbial and anti-mycotoxigenic activities of endophytic *Alternaria alternata* isolated from *Catharanthus roseus* (L.) G. Don.: molecular characterization and bioactive compound isolation. – Mycology 10(1): 40–48. DOI: http://doi.org/10.1080/21501203.2018.1541933

Theantana T., Hyde K.D., Lumyong S. (2009): Asparaginase production by endophytic fungi from Thai medicinal plants: cytotoxicity properties. – International Journal of Integrative Biology 7(1): 1–8.

Uzma F., Konappa N.M., Chowdappa S. (2016): Diversity in the extracellular activities of fungal endophytes isolated from medicinal plants of the Western Ghats, Karnataka. – Egyptian Journal of Basic and Applied Sciences 3: 335–342. DOI: https://doi.org/10.1016/j.ejbas.2016.08.007

Yadav M., Yadav A., Yadav J.P. (2014): In vitro antioxidant activity and total phenolic content of endophytic fungi isolated from *Eugenia jambolana*. – Asian Pacific Journal of Tropical Medicine 7(1): 256–261. DOI: https://doi.org/10.1016/S1995-7645(14)60242-X