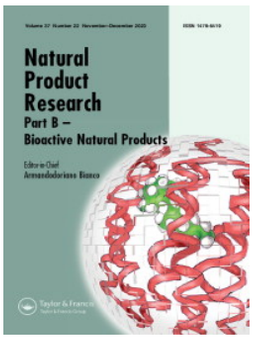

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SHORT COMMUNICATION

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Bioactive saponin profiling of endophytic fungi from *Asparagus racemosus*

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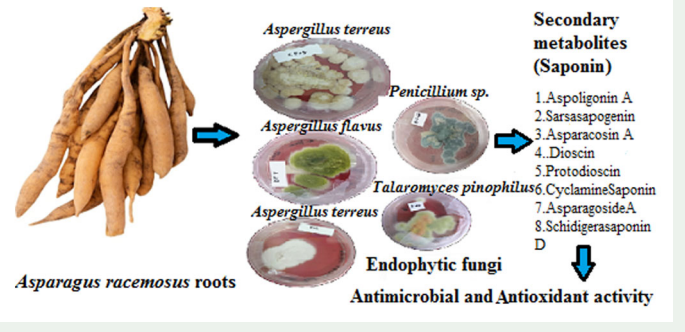
ABSTRACT

Thirty-five distinct endophytic fungi were isolated from the roots of *Asparagus racemosus*. Five out of 35 isolates were found to be efficient saponins producers and they were identified as *Aspergillus terreus* (E.F-1), *Aspergillus flavus* (E.F-7), *Penicillium sp.* (E.F-12), *Talaromyces pinophilus*(S-26), and *Aspergillus terreus* (Y-2) based on 18 sr RNA sequencing. The crude extracts of endophytic fungi were screened using High-performance liquid chromatography (HPLC) for quantitative analysis of saponin. The crude extracts of endophytic fungi were also characterised using FT-IR spectroscopy and mass spectrometry. The IR spectra of all five endophytic fungi crude extracts revealed the presence of –OH,-CH Alkyl,-CH_3,-C-O-C,-C=C,-C=O stretching, which indicated the presence of saponin. Eight types of saponins recognised by mass spectrometry were Cyclamine saponin, Aspoligonin A, Sarsapogenin, Asparacosin A, Schidigera saponinD5, Aspargoside A, Dioscin, and Protodioscin. Endophytic fungi extracts also exhibited antimicrobial activity and antioxidant activity.



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KEYWORDS
Asparagus racemosus; endophytic fungi; saponins observation; phylogenetic analysis; antimicrobial activity

1. Introduction

Asparagus racemosus Wild is a diploid spinous under-shrub distributed throughout India's tropical and subtropical parts and is commonly called Shatavari. This plant is

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recommended for cure in threatened abortion, lactation in women, and normalising the uterus and hormone changes that occur during pregnancy (Garg and Gupta 2010). The roots of this herb are reported to have several biological activities like antiulcer, anticancer, antioxidant (Parihar and Hemnani 2004), anti-diabetic, antitussive, antihyperlipidemic, antistress, antidepressant, antianxiety, immunomodulatory, anti-inflammatory, anti urolithiatic, antibacterial, and antidiarrhea (Mandal et al. 2000). Major constituents of *Asparagus racemosus* include lignin, acemannan, oligosaccharides, quercetin, rutin, racemoside, diosgenin, hyperoside, sarsasapogenin, steroidal saponins, and triterpene saponins (Iqbal et al. 2017). *Asparagus racemosus* is now considered endangered in its natural habitat and recognised as vulnerable (Bopana and and Saxena 2008). Therefore, there is a need to discover a strategy to decrease the dependency on plants. Research on endophytic fungi is nowadays focused to overcome this problem. It is believed that screening for secondary metabolites from endophytic fungi from medicinal plants is a promising way to overcome the dependency on the plants. Secondary metabolites producing endophytic fungi are an excellent approach to fulfill the excessive demand for medicinal plants. Many researchers have proved that endophyte fungi are a new and potential source of novel natural products (Guo et al. 2008; Suryanarayanan et al. 2009). Secondary metabolites isolated from endophytes belong to diverse structural classes, including alkaloids, peptides, steroids, terpenoids, phenols, quinones, saponins, and flavonoids (Gunasekaran et al. 2017). Some endophytic fungi have developed the ability to produce similar bioactive secondary metabolites as those from the host plants (Ludwig-Mülle 2015). Keeping this in view, the present investigation aimed to isolate, screen, characterisation, and evaluation of the microbial activity of saponins-producing endophytic fungi from the roots of *Asparagus racemosus*.

2. Results and discussions

2.1. Isolation and screening of endophytic fungi

A total number of 35 endophytic fungi were isolated from the roots of *Asparagus racemosus*. After screening crude extracts of the isolates by HPLC (High-Performance liquid chromatography), only five were found to be efficient saponin producers. Table 1 depicts saponin production in 1 mg of crude extract of each isolate. A Maximum amount of saponin (5441.54 ng/mg) was observed in isolate E.F-1 (*Aspergillus terreus*). The retention time was found to be 1.7 ± 0.1 min. Figures S1–S7 showed the calibration curve and chromatogram of all five isolates (extracts) along with the standard. Wu et al. (2012) reported saponin-producing endophytic fungi G22 (*Penicillium sp.*)

Table 1. Concentration of saponin in all endophytic fungi extracts.

Strains	Retention time (RT)	Mean area	Standard deviation (SD)	Relative standard deviation (RSD)%	Saponin concentration (ng/mg)
E.F-1	1.8	246455414.333	5026679.87682	2.04	5441.54
E.F-7	1.7	23498483.3333	454854.798041	1.94	518.83
E.F-12	1.7	51216601.3333	546209.563388	1.07	1130.82
S-26	1.6	4215837.6667	72946.1853789	1.73	93.08
Y-2	1.6	7796482	69252.4592993	0.89	172.14

from *Aralia elata*. They observed that G22 produced the highest concentration of saponins (2.049 mg/mL). Complete details of the five isolates given in Tables S1 and S2.

2.2. Molecular phylogenetic analysis of endophytic fungi

The interpretation of the evolutionary tree of E.F-1, E.F-7, E.F-12, and Y-2 closest to *Aspergillus terreus* (GU227345) with 100%, *Aspergillus flavus* (MT635198.1) with 100%, *Penicillium citrinium* (KF758801) with 99.94%, *Aspergillus terreus* (KX550911.1) 99.81% of similarity respectively, while S-26 was found closest to *Talaromyces funiculosus* (KT148636) and *Penicillum purpurogenum* (DQ365947) with a similarity of 100% and was assigned as *Talaromyces pinophilus* (Figures S8–S12). The obtained sequence data have been submitted to GenBank under the accession numbers MN744345 (E.F-1), MN744415 (E.F-7), MN744382 (E.F-12), MN744414 (S-26), and YMN744381(Y-2). The isolates were identified as E.F-1 (*Aspergillus terreus*), E.F-7 (*Aspergillus flavus*), E.F-12 (*Penicillium sp.*), S-26 (*Talaromyces pinophilus),* and Y-2 (*Aspergillus terreus*). All five strains have been deposited at the Institute of Microbial Technology (IMTECH) Chandigarh with the accession numbers MTCC 13031(E.F-1), MTCC 13032(E.F-7), MTCC 13033 (E.F-12), MTCC 13034(S-26), and MTCC 13040(Y-2). In previous studies, researchers isolated PN8 and PN17 saponin-producing endophytic fungi isolated from *Panax notoginseng* belonged to *Fusarium* sp. and *Aspergillus* sp. respectively with similarity of 99.9% (Jin et al. 2017).

2.3. Antimicrobial assay

Antimicrobial activity of the selected endophytes was performed against nine pathogenic microorganisms *B. subtilis* (MTCC-441), *E.coli* (MTCC-16521), *S. aureus* (MTCC-3160), *S. entrica* (MTCC-1253), *S. gordonii* (MTCC-2695), *P. florescence* (MTCC-664), *A. oryzae* (MTCC-5341), *F. oxyspora* (MTCC-10270), and *A. niger* (MTCC-281). In our study, five isolated endophytic fungi extracts showed maximum antifungal activity as compared to antibacterial activity this may be due to the antagonistic behavior of endophytic fungi. All the endophytic fungal extracts showed antimicrobial activity against *B.subtilis* at MIC 64 μg mL^{-1}, whereas E.F-7, E.F-12, S-26, and Y-2 showed antimicrobial activity at MIC 64 μg mL^{-1} against *A. niger* and *A. oryzae*. In the case of *F. oxyspora* three endophytic fungi extracts (E.F-7, E.F-12, and S-26) showed antimicrobial activity at MIC 64 μg mL^{-1} instead E.F-1 and Y-2 showed antimicrobial activity at MIC 128 μg mL^{-1}. Three endophytic fungi extracts (E.F-12, S-26, and Y-2) showed activity against *S.aureus* at MIC 64 μg mL^{-1}. In addition to this E.F-7 showed activity at MIC 128 μg mL^{-1} while E.F-1 did not show any antimicrobial activity. S-26 solely showed antimicrobial activity against *S. entrica* and *E. coli* at MIC 64 μg mL^{-1} whereas E.F-7, E.F-12, and Y-2 showed activity at MIC 128 μg mL^{-1} against *S. entrica*. E.F-1 did not show any antimicrobial activity against *S. entrica* (Table S3). No activity was observed against *S. gordonii* and *P. florescence*. In the past, researchers (Huang et al. 2001; Prabavathy and Nachiyar 2012; Wu et al. 2012; Jin et al. 2017) also reported that endophytic fungi have antimicrobial capacity against pathogenic microbes like *Fusarium sp., Trichoderma sp., Neurospora sp., Stachybotrys sp., Curvularia sp., Verticillium sp., Yersinia sp.,*

V. anguillarum, *Shigella sp.*, *V. parahaemolyticus*, *C. albicans*, *P. expansum*, *A. niger*, *S. agalactiae*, *F.oxyspora*, *R.solani*, *S. sclerotiorum*, *G. fujikuroi*, *P. grisea*, *G. zeae*, *F. oxysporum*, *f. vasinfectum*, *S.aureus*, *B.subtilis*, *E.coli*, *Pseudomonas*, *Klebsiella*, *S. typhi*, *S. typhimurium*, *S. enteritidis*, *A. hydrophila* etc.

2.4. Antioxidant assay

The crude extracts of saponins-producing endophytic fungi were evaluated for their capacity for antioxidant activity. The result of the antioxidant assay indicates that all five saponin-producing endophytic fungi possessed antioxidant capacity. *Aspergillus flavus* (E.F-7) showed maximum antioxidant activity of 86.66% followed by *Penicillium sp*. (E.F-12) which showed 75.4% antioxidant activity and *Talaromyces pinophilus* (S-26) showed moderate antioxidant activity of 56.13%. On the other hand, *Aspergillus terreus* (Y-2) and *Aspergillus terreus* (E.F-1) showed comparatively low antioxidant activity of 35.26% and 31.7%, respectively. The antioxidant activity of standard (Caffeic acid) was 87.24%. The antioxidant activity of *Aspergillus flavus* (E.F-7) was almost equivalent to caffeic acid (Figure S13). Similar result was also carried out by Li et al. (2015), Prabukumar et al. (2015), Pan et al. (2017), Gunasekaran et al. (2017), da Silva et al. (2020), Sharaf et al. (2022)in endophytic fungi. They confirmed that the presence of saponins, phenols, flavonoids, steroids, tannins, alkaloids, anthraquinone, and terpenoid compounds in endophytic fungi showed strong antioxidant activity.

2.5. FT-IR analysis

Figure S14 depicts the IR spectra of all five endophytic fungi extracts. Table S4 shows the bands with their ranges. The IR spectra of each endophytic fungus extract compared with the IR spectra of saponin standard revealed similar functional groups OH, C-H, CH_3, C=C, C=O, C-O, and C-O-C indicating the presence of saponins. The extra peaks seen in E.F-1 and E.F-7 indicate the purification requirement of crude extract. C-O-C reveals glycoside linkages. Our results are in agreement with the previous reports on saponins. They observed –OH, -C=O, C-H, C=C, and C-O-C absorption, which verified the presence of saponin in medicinal plants (Kareru et al. 2008). OH, C=O, C-H, C=C, and C-O were also observed in *Sapindus mukorossi* indicating the presence of saponins (Almutairi and Ali 2015). Bajad and Pardeshi (2016) also reported –OH, -CH_3, C=O, C=C, and C-O stretching in the crude extract of *Sapindus emarginatus*. The existence of O–H stretching, C=C stretching, CH_2, CH_3, C–O stretching, =CH bending represented the occurrence of steroidal saponin (PSI, PSII, PSVI, and PSVII) in *Paris* plant (Yang et al. 2018).

2.6. Mass spectrometry analysis

By comparing the obtained molecular mass from mass spectrometry of crude extracts of endophytic fungi with available data, we confirmed the presence of saponins compounds. We have found eight types of saponin compounds in all five endophytic fungi extracts (Table S5). The mass spectrum of eight saponins compounds was shown

(Figures S15–S22). In a previous study, thirty-one steroidal saponins were observed from *Dioscorea zingiberensis* C.H Wright (Zhu et al. 2010). Timosaponin BII, Diosgenin, Sarsapogenin, Timosaponin AIII, Mangiferin, Neomangiferin, Icariside I, Icariside II, and Emodin were detected in *Anemarrhena asphodeloides* (Sun et al. 2012). A quantitative comparative study of components present in *Dioscorea nipponica* and *D.panthaica* reveals Protodioscin, Protogracillin, Pseudoprotodioscin, Dioscin, Gracillin, Polyphyllin V, and Diosgenin (Tang et al. 2013). Rajasekhar et al. (2019) detected eight saponins in AGAF (Active fraction of *A. gonoclados*). Jayashree et al. (2015) and Kashyap et al. (2020) reported saponins (Protodioscin, Sarsapogenin, Asparacosin A, Asparoside B, Asparenydiol, Aspoligonin A, Schidegera saponin D5, Nysol, Shatavarin I, V, VII, VIII, IX, X, Nysol, and Ferulic acid) in *Asparagus racemosus* .Our finding suggests that endophytic fungi produced almost similar bioactive products from *Asparagus racemosus* roots (Host plant).

3. Conclusions

The present study supports the hypothesis that endophytes of ethno-healthful plants may be a good source of medicinal valuable useful secondary metabolites. *Asparagus racemosus*'s saponins were also observed in endophytic fungi. This may be due to long co-evolution (Strobel and Daisy 2003) and gene transfer mechanisms in endophytic fungi from their host plant (Kusari et al. 2009). This is the first study to isolate similar saponins producing endophytic fungi from the roots of *Asparagus racemosus*. Recently researchers extracted several secondary metabolites of plants from endophytic fungus and these metabolites are widely used for the treatment of many severe diseases (Aly et al. 2011).

Geolocation information

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Disclosure statement

No potential conflict of interest was reported by the authors.

References

Almutairi MS, Ali M. 2015. Direct detection of saponins in crude extracts of soapnuts by FTIR. Nat Prod Res. 29(13):1271–1275.

Aly AH, Debbab A, Proksch P. 2011. Fungal endophytes: unique plant inhabitants with great promises. Appl Microbiol Biotechnol. 90(6):1829–1845.

Bajad PN, Pardeshi AB. 2016. Qualitative and quantitave analysis of saponin as bioactive agent of sapindusemarginatus.
Bopana N, Saxena S. 2008. In vitro propagation of a high value medicinal plant: asparagus racemosusWilld. In Vitro CellDevBiol-Plant. 44(6):525–532.
da Silva MHR, Cueva-Yesquen LG, Júnior SB, Garcia VL, Sartoratto A, de Angelis DDF, de Angelis DA. 2020. Endophytic fungi from Passiflora incarnata: an antioxidant compound source. Arch Microbiol. 202(10):2779–2789.
Garg R, Gupta VB. 2010. Adaptogenic activity of milk and aqueous decoction of asparagus racemosuswilld. In acute and chronic stress paradigms in mice. J Cell Tissue Res. 10(2):2281–2286
Gunasekaran S, Sathiavelu M, Arunachalam S. 2017. In vitro antioxidant and antibacterial activity of endophytic fungi isolated from Mussaendaluteola. J Appl Pharm Sci. 7(8):234–238.
Guo B, Wang Y, Sun X, Tang K. 2008. Bioactive natural products from endophytes: a review. Appl Biochem Microbiol. 44(2):136–142.
Huang Y, Wang J, Li G, Zheng Z, Su W. 2001. Antitumor and antifungal activities in endophytic fungi isolated from pharmaceutical plants Taxus mairei, Cephalataxusfortunei and Torreya grandis. FEMS Immunol Med Microbiol. 31(2):163–167.
Iqbal M, Bibi Y, Raja NI, Ejaz M, Hussain M. 2017. Review on therapeutic and pharmaceutically important medicinal plant Asparagus officinalis L. J Plant Biochem Physiol. 5:180.
Jayashree GV, Kumar KH, Krupashree K, Rachitha P, Khanum F. 2015. LC–ESI–MS/MS analysis of Asparagus racemosusWilld. roots and its protective effects against t-BHP induced oxidative stress in rats. Ind Crops Prod. 78:102–109.
Jin Z, Gao L, Zhang L, Liu T, Yu F, Zhang Z, Guo Q, Wang B. 2017. Antimicrobial activity of saponins produced by two novel endophytic fungi from Panax notoginseng. Nat Prod Res. 31(22): 2700–2703.
Kareru PG, Keriko JM, Gachanja AN, Kenji GM. 2008. Direct detection of triterpenoid saponins in medicinal plants. Afr J Trad Compl Alt Med. 5(1):56–60.
Kashyap P, Muthusamy K, Niranjan M, Trikha S, Kumar S. 2020. Sarsasapogenin: a steroidal saponin from Asparagus racemosus as multi target directed ligand in Alzheimer's disease. Steroids. 153:108529.
Kusari S, Lamshöft M, Spiteller M. 2009. Aspergillus fumigatus Fresenius, an endophytic fungus from Juniperus communis L. Horstmann as a novel source of the anticancer pro-drug deoxypodophyllotoxin. J Appl Microbiol. 107(3):1019–1030.
Li YL, Xin XM, Chang ZY, Shi RJ, Miao ZM, Ding J, Hao GP. 2015. The endophytic fungi of Salvia miltiorrhizaBge. f. alba are a potential source of natural antioxidants. Bot Stud. 56(1):5–7.
Ludwig-Müller J. 2015. Plants and endophytes: equal partners in secondary metabolite production? Biotechnol Lett. 37(7):1325–1334.
Mandal SC, Nandy A, Pal M, Saha BP. 2000. Evaluation of antibacterial activity of Asparagus racemosusWilld root. Phytother Res. 14(2):118–119.
Pan F, Su TJ, Cai SM, Wu W. 2017. Fungal endophyte-derived Fritillaria unibracteata var. wabuensis: diversity, antioxidant capacities in vitro and relations to phenolic, flavonoid or saponin compounds. Sci Rep. 7(1):1–14.
Parihar MS, Hemnani T. 2004. Experimental excitotoxicity provokes oxidative damage in mice brain and attenuation by extract of Asparagus racemosus. J Neural Transm (Vienna). 111(1): 1–12.
Prabavathy D, Nachiyar CV. 2012. Study on the antimicrobial activity of Aspergillus sp isolated from Justicia adathoda. IJST. 5(9):1–4.
Prabukumar S, Rajkuberan C, Ravindran K, Sivaramakrishnan S. 2015. Isolation and characterization of endophytic fungi from medicinal plant Crescentia cujete L. and their antibacterial, antioxidant and anticancer properties. Int J Pharm Pharm Sci. 7(11):316–321.
Rajasekhar A, Peddanna K, Vedasree N, Munirajeswari P, Nagaraju N, Badri KR, Chippada AR. 2019. Antidiabetic activity of root tubers of Asparagus gonoclados Baker in streptozotocin induced diabetic rats. J Ethnopharmacol. 242:112027.

Sharaf MH, Abdelaziz AM, Kalaba MH, Radwan AA, Hashem AH. 2022. Antimicrobial, antioxidant, cytotoxic activities and phytochemical analysis of fungal endophytes isolated from OcimumBasilicum. Appl Biochem Biotechnol. 194(3):1271–1286

Strobel G, Daisy B. 2003. Bioprospecting for microbial endophytes and their natural products. Microbiol Mol Biol Rev. 67(4):491–502

Sun Y, Du Y, Liu Y, Chang L, Ren Y, Cao L, Sun Q, Shi X, Wang Q, Zhang L. 2012. Simultaneous determination of nine components in Anemarrhena asphodeloides by liquid chromatography-tandem mass spectrometry combined with chemometric techniques. J Sep Sci. 35(14): 1796–1807. doi:10.1002/jssc.201200026.22807362

Suryanarayanan TS, Thirunavukkarasu N, Govindarajulu MB, Sasse F, Jansen R, Murali TS. 2009. Fungal endophytes and bioprospecting. Fungal Biol Rev. 23(1-2):9–19.

Tang Y, Yi T, Chen H, Zhao Z, Liang Z, Chen H. 2013. Quantitative comparison of multiple components in Dioscoreanipponica and D. panthaica by ultra-high performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry. Phytochem Anal. 24(4): 413–422.

Wu H, Yang H, You X, Li Y. 2012. Isolation and characterization of saponin-producing fungal endophytes from Aralia elata in Northeast China. Int J Mol Sci. 13(12):16255–16266.

Yang Y, Jin H, Zhang J, Wang Y. 2018. Determination of total steroid saponins in different species of Paris using FTIR combined with chemometrics. J AOAC Int. 101(3):732–738.

Zhu J, Guo X, Fu S, Zhang X, Liang X. 2010. Characterization of steroidal saponins in crude extracts from Dioscoreazingiberensis CH Wright by ultra-performance liquid chromatography/electrospray ionization quadrupole time-of-flight tandem mass spectrometry. J Pharm Biomed Anal. 53(3):462–474.

Nomenclature

ng	Nanogram
mg	Milligram
mL	milliliter
mM	millimolar
μg mL^{-1}	Microgram per milliliter
m/z	Mass to charge
°C	Celsius
ppm	Parts per million
H	Hour
μL	Microliter
μm	Micrometer
mm	millimeter
cm^{-1}	Per centimeter
%	Percentage
T	Transmission