Isolation, identification and antioxidant potential of culturable endophytic fungus *Chaetomium* LAV15 associated with leaves of *Tinospora cordifolia*

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Indian medicinal plant *Tinospora cordifolia* is often called Amrita due to several substances in the extract of its parts that potentially act as a strong antioxidant and have been prized in folk and traditional medicinal systems for ages to improve health and treat various diseases. In the present study, we isolated endophytic fungus from the leaves of *Tinospora cordifolia* as *Chaetomium* LAV 15 (Accession number: MK212345) species. Hence, the antioxidant activities of isolated endophytic fungus were assessed using total phenol, total flavonoids, total antioxidant activity and 2,2-diphenyl-1-picrylhydrazyl (DPPH) methods. Further research on Gas chromatography and mass spectrometry (GC-MS) analysis of the isolated endophytic fungus revealed the presence of many impressive compounds such as host metabolites 2,4-di-tert-butylphenol, an important antioxidant metabolite.

Key words: Antioxidant activity, endophytic fungi, GC-MS analysis, *Tinospora cordifolia*, 2,4-Di-tert-butylphenol.

INTRODUCTION

Endophytes are a polyphyletic group of microorganisms including bacteria, ascomycetes, yeast and filamentous fungi present within asymptomatic tissues of plants (Jia *et al*. 2016). They show no detectable effect on plant growth and function. However, among these endophytic fungi have attracted, a great deal of attention because of their larger role in the defensive mechanism, and potential to produce a myriad of medicinally important explored and new secondary metabolites hence, providing molecular models for the development of new drugs for human beings (Fadiji and Babalola, 2020).

Fungal endophytes an assembly of beneficial microbes present inside the tissues of plants, act as -'defensive mutualists' and protect plants from abiotic stresses and provide oxidative stress protection to the host plant by producing various kinds of secondary antioxidants metabolites. Therefore, increased expression of antioxidant compounds by the host plant and the endophytic fungi would counteract stress-induced reactive oxygen. Therefore, abiotic stress protection in the plant is largely a function of oxidative stress protection (Sadeghi *et al.* 2020).

Tinospora cordifolia is an indigenous climber of the tropical region of India belonging to the family Menispermaceae. Much of the research on the medicinal properties of *Tinospora cordifolia* has been conducted in India. It has been widely used in Indian traditional medicines as a source of bioactive natural products for treating various diseases and a source of agrochemicals for many centuries (Bharathi *et al.* 2018). The association of fungi and microbes with plants is old but has gained importance in recent years due to the increased demand for bioactive compounds (Sharma *et al.* 2020).

The present study was conducted to isolated endophytic fungi from leaves of medicinal plant *Tinospora cordifolia* of the semi-arid region. Further, the isolated fungus extracts were studied for antioxidant activities beneficial for human beings. In addition, bioactive compounds present in the extract of potential fungal isolate have also been identified.

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MATERIALS AND METHODS

Collection site and plant material

Healthy and fresh leaves of *Tinospora cordifolia* were collected from a specific location (Latitude 26.917238N and Longitude 75.759450E) in the garden, Khatipura, Jaipur. The plant was taxonomically identified and authenticated by Dr Amit Kotiya (University of Rajasthan, Jaipur), with a voucher specimen (Accession no. RUBL211303) preserved in Department of Botany, University of Rajasthan, Jaipur.

Isolation of endophytic fungi from leaves of Tinospora cordifolia

Isolation of fungal endophytes from leaves of the plant, surface- sterilization was carried out following the protocol described by Bhardwaj *et al*. (2015) with some modifications.

Identification of endophytic fungi

The selected endophytic fungus was identified for morphological characteristics using various standard manuals, microscopic studies by lactophenol cotton blue staining (Sadananda *et al.* 2014), and were identified at the molecular level using ITS 1F and ITS 4 primers.

Molecular identification of endophytic fungus from the leaf of Tinospora cordifolia

The molecular identification of fungus from leaf samples of *Tinospora cordifolia* was performed using DNA sequences of ITS1 and ITS4 (White *et al*., 1990). The gene sequence of the isolated fungi was submitted to the NCBI GenBank database and accession number was obtained. The distance matrix was generated using the RDP database, and the Phylogenetic tree of the isolated fungus was constructed using MEGA 7 (Kumar *et al*. 2016).

Fermentation and extraction

The fermentation and extraction of isolated endophytic fungus *Chaetomium*LAV15 species were done according to the method described by Kumar and Kaushik (2013) with slight modification. Isolated fungus species was then grown *in vitro* in Potato Dextrose broth with pH adjusted to 6.5. The pure fungal strain was subjected to submerged fermentation in Potato Dextrose medium (PDA) for four weeks at 28ºC under a static condition with intermittent shaking by applying OSMAC (One Strain Many Compounds) approach (Bills *et al.* 2008).

Determination of total phenol content

The total phenol content of ethyl acetate extract of the endophytic fungus was estimated using the Folin-Ciocalteau (FC) method with minor modifications, as given by Jayanthi and co-workers Jayanthi and Lalitha (2011).

Determination of total flavonoid content

The total flavonoid contents of the ethyl acetate extract of endophytic fungus were estimated based on the formation of flavonoid-aluminium complex (Saravanan and Parimelazhagan, 2014).

Total antioxidant activity

The total antioxidant assay of ethyl acetate extracts of endophytic fungus was carried out using molybdenum reagent and (Umamaheshwari and Chatterjee, 2008).

DPPH (2,2-diphenyl-1-picrylhydrazyl) assay

The DPPH (1,1-diphenyl-2-picrylhydrazyl) assay was performed by following the method described by Nithya and Xie (Nithya *et al*. 2016; Xie*et al*. 2010) with minor modifications.

%DPPH radical scavenging activityŒ [(Absorbance of Control-Absorbance of the test sample)/ (Absorbance of Control)] ×100

Gas chromatography-Mass spectrometry (GC-MS) analysis

GC-MS did identify bioactive compounds present in the crude extract of the selected endophytic fungus at CEG test house, Jaipur.

The separated compounds' names, molecular weights, and structures were ascertained by comparing the mass spectra with data from the National Institute of Standards and Technology (NIST) libraries.

RESULTS AND DISCUSSION

In this study, fungal endophytes associated with leaves of climber *Tinospora cordifolia,* were studied

to evaluate the production of secondary metabolites.

Isolation and identification of endophytic fungi

In this study, a total of 100 fragments of leaves of the *Tinospora cordifolia* were used to isolate endophytic fungi. Colonization frequency was found to be 7%. Thus, leaves showed a low colonization frequency, which might be because leaves of this climber fall every year resulting in less exposure to microbes, therefore, less colonization.

Based on observation of morphological characteristics such as mycelial appearance after 15 days, mycelia arising from plant part and fungus exhibiting tissue specificity, one dominant fungus from leaf was isolated and characterized for morphological features and identified at the molecular level as *Chaetomium* species.

Selected isolate from leaf was labelled as L1. The endophytic fungus growing out of the leaf has been depicted in Fig. 1 (a).

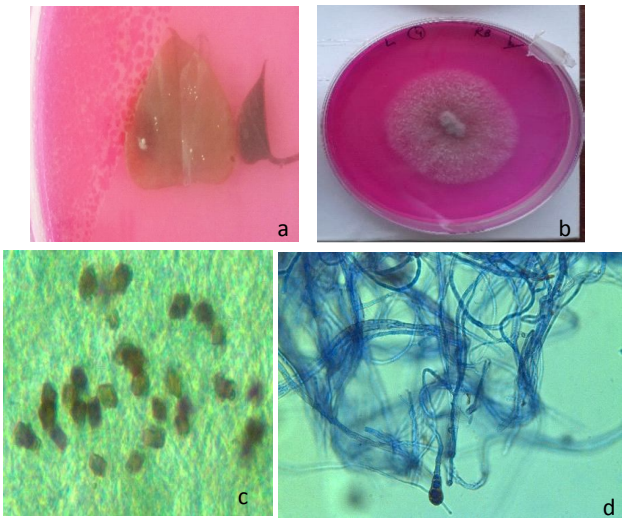

Fig. 1: Endophytic fungus emerging from leaf (a), endophytic fungus on rose bengal agar (b), microscopic view of endophytic fungus from leaf (c &d).

Colony characteristics: As shown in Fig.1 (b) the isolate was rapidly growing. It was cottony white to yellow and spread radially on the plate. Pink to brown diffusing pigment was observed at the edges.

Microscopic characteristics: Fungal slides were prepared and observed under the microscope. Large dark brown, flask-shaped perithecia with hair-like setae on their surface. Ascospores were one-celled, brown in colour and lemon-shaped (Fig.1c). and small conidia on short solitary phialides were observed (Fig. 1d) .

Based on the above macroscopic and microscopic trait, the isolate was initially identified as a *Chaetomium* species.

Isolate obtained from leaves of *Tinospora cordifolia* has been labelled as L1 showed 96 % similarity with *Chaetomium strumarium* voucher SCFUN3011 (Accession number: MG780384.1) based on nucleotide homology and phylogenetic analysis. ITS sequence of isolate L-1 was submitted in GenBank database NCBI gene bank as *Chaetomium* LAV15 (Accession number: MK212345)

The phylogenetic tree of *Chaetomium* LAV 15 was constructed using Mega 7 software using the neighbour adjoining method (Fig. 2).

Hence,isolated fungus L1 isolate from leaves was identified morphologically and at the molecular level as *Chaetomium* species. The identified fungus genus is endophytic to many plants and was also isolated as endophytes from *Ginkgo biloba* (Li *et al*. 2014), *Nyctanthes arbor-tristis* (Gond *et al*. 2012), *Cinnamomum camphor* (Kharwar*et al*. 2014), *Huperzia serrata* (Chen *et al*. 2011) and *Lycopersicon esculentum* (Larran*et al*. 2001).

Chaetomium is a large genus generally found in natural niches such as plant debris. In addition, in this study, we found that this fungus produces pink (initially) to brown (later) diffusing pigments on agar plates. This study reported *Chaetomium* species (*Chaetomium* LAV 15) from leaves of *Tinospora cordifolia*. Similarly, in a separate study, *Chaetomium* species was reported from leaves of host plant viz. *Macleaya cordata*, *Altheae rosea*, *Glycine max*, *Silybum marianum*, *Bauhinia racemosa,* and Mangroves (Rashmi *et al*. 2019). The recovered taxa belong to genera of filamentous ascomycetes group supports the findings of Bhardwaj *et al.*(2015) that most fungal endophytes are filamentous Ascomycota.

Fermentation and extraction

The extract of pure fungal strain was subjected for submerged fermentation in Potato Dextrose

Table. 1: ITS sequence analysis of endophytic fungus (Isolate L1) of *Tinospora cordifolia*

Consensus sequences (bp)	Similarity to species	Percentage similarity	Query coverage	Gen Bank Accession number	Strain designated as
620	*Chaetomium strumarium* voucher SCFUN3011 (Accession Number: MG780384.1)	96%	89%	MK212345	ChaetomiumLAV15

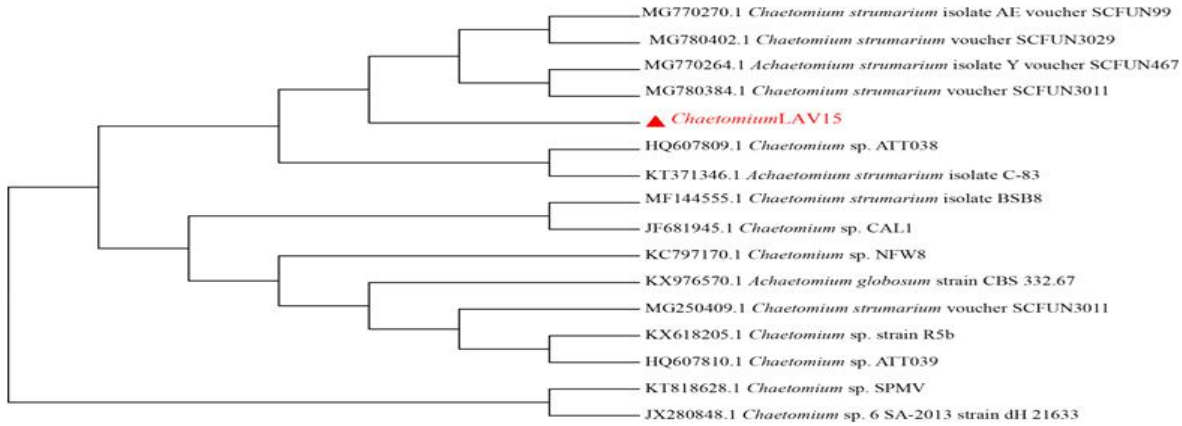

Fig.2: Phylogenetic tree of *Chaetomium* LAV15 constructed using the neighbour adjoining method.

Table 2: DPPH free radical scavenging activity of endophytic fungal isolates (Mean±SE)

Extracts	100 µg/ml,	200 µg/ml	300 µg/ml	400 µg/ml	500 µg/ml
Chaetomium LAV 15	54.7±0.07	55.33±0.065	57.13±0.07	58.65±0.111	68.37±0.149
Ascorbic acid	27.70±0.063	34.63±0.394	57.43±0.149	74.55±0.251	91.29±0.189

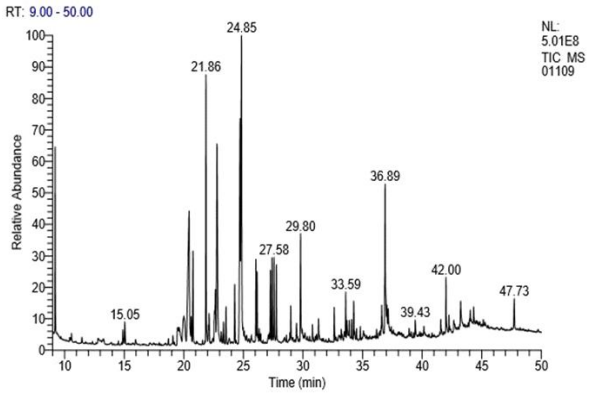

Fig. 3: GC-MS analysis graph of ethyl-acetate extract of *Chaetomium* LAV15

medium (PDA) by applying OSMAC (One Strain Many Compounds) approach (Bills *et al*. 2008). Culture filtrates obtained were extracted with the commonly used solvent ethyl acetate. Since it is a solvent with medium polarity, it could dissolve both polar and non-polar compounds and evaporate easily, extracting typical fungal secondary metabolites. The colour/pigmentation of fermented culture filtrates of *Chaetomium* LAV15 was light yellow.

Total phenolic content (TPC) and total flavonoid content (TFC)

It is known that there is a correlation between phytochemical compounds such as phenolic and

Table 3: Natural products identified in the ethyl acetate extract from the culture filtrate of *Chaetomium* LAV 15 by GC-MS.

Retention Time	Name of the compound	Molecular formula	Molecular weight (gm/mol)	Area%
20.01	Butanoic acid, 3-hydroxy-2,2-dimethyl-, ethyl ester	$C_8H_{16}O_3$	160	3.12
20.45	2-Heptanol, acetate	$C_9H_{18}O_2$	158	10.12
20.78	2,4-Pentadien-1-ol, 3-ethyl-, (2Z)-	$C_7H_{12}O$	112	3.20
21.86	Benzoic acid,3-hydroxy-methyl ester	$C_8H_8O_3$	152	8.77
22.65	Methylparaben	$C_8H_8O_3$	152	1.70
22.79	Cantharidin	$C_{10}H_{12}O_4$	196	10.722
24.28	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206	2.15
24.72	Pyrimidine-6-ol-4-carboxylic acid	$C_5H_4N_2O_3$	140	11.89
24.85	2-Carboxymethyl-3-methyl-cyclopentanecarboxylic acid	$C_9H_{14}O_4$	186	16.18
26.06	7-Hydroxy-6-methyl-oct-3-enoic acid	$C_9H_{16}O_3$	172	2.61
26.15	7-Hydroxy-6-methyl-oct-3-enoic acid	$C_9H_{16}O_3$	172	2.23
27.27	2-Methoxycarbonylmethyl-3-methylcyclopentanecarboxylic acid, methyl ester	$C_{11}H_{18}O_4$	214	2.19
27.41	Tulobuterol	$C_{12}H_{18}CINO$	227	2.48
27.58	7-Oxabicyclo [4.1.0] hept-3-ene-3-carboxylic acid, methyl ester, (. + -.)-	$C_8H_{10}O_3$	154	2.57
27.77	Terrein	$C_8H_{10}O_3$	154	2.27
29.80	Pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-	$C_7H_{10}N_2O_2$	154	4.13
36.61	Diisooctyl maleate	$C_{20}H_{36}O_4$	340	1.44
36.89	Harmine	$C_{13}H_{12}N_2O$	212	5.76
42.00	Hexadecanoic acid	$C_{19}H_{38}O_4$	330	1.78

flavonoid compounds present in the extract and their antioxidant activity. In the present study, the TPC and TFC of ethyl acetate extract from *Chaetomium* LAV 15 were investigated. The TPC and TFC observed in *Chaetomium*LAV15 extract were 9.307±0.0095 and 5.43±0.0360, respectively.

Total antioxidant activity

Extract of endophytic fungus *Chaetomium* LAV 15 showed total antioxidant activity of 60.64±0.112. The above results support the view of Pawle and Singh (2014), that the presence of phenol and flavonoid might be responsible for the total antioxidant activity.

DPPH radical scavenging activity

The ethyl acetate extracts of the endophytic isolate showed excellent scavenging activity compared to the standard till 300 µg/ml concentrations except at higher concentrations.

GC-MS analysis of the crude extract

The identification of compounds in the extract was achieved using data generated from Gas chromatography and mass spectrometry (GC-MS) analysis. The gas chromatography results of fungal crude extracts predicted the presence of several compounds (Tables 4,5 &6) and identified them based on retention time, peak area, molecular weight, and molecular formula. Database of NIST 2.2 (National Institute standard and technology) was used to interpret the mass spectrum of GC-MS.

Isolate *Chaetomium*LAV15 was found to produce Harmine with an area **j** of 5.76. Harmine, an

alkaloid, is reported abundantly produced by several different plants and is well- documented as a major bioactive component with pharmacological properties including anti-cancer and antioxidant (Jain *et al*., 2020; Liu *et al*., 2007). However, as per our knowledge, this is the first report that Harmine is produced by *Chaetomium* sp. 2,4-di-tert-butylphenol (area 2.15j) metabolite has previously been reported in endophytic culture extracts (Chen *et al*. 2015) and has been reported to possess dominant antioxidant activity (Zhao *et al.* 2020). Isolated fungus and their respective host produce 2,4-di-tert-butylphenol, thus establishing the symbiotic host-endophytic fungi relationship. GC-MS analysis of *Chaetomium* LAV15 also showed compounds such as hexadecanoic acid (fatty acid ester), which is reported as a major metabolite of many fungi including ascomycetes, has been reported to be mainly indulged in antioxidant activity (Anisha *et al*. 2017).

Terrein (fungal metabolite) produced by *Chaetomium* LAV 15 has been reported to have antioxidant and other pharmacological activity (Zaehle*et al.* 2014). In addition, Goutam *et al.* (2017), for the first time, reported “Terrein” from endophytic-derived fungus, exhibiting antifungal and anticancer activity.

Interestingly, one of the major metabolites Cantharidin (terpenoids) of *Chaetomium* LAV 15 extract has been secreted by blister beetles as defensive secretion indicating that this fungus might serve as a source of diverse metabolites. Furthermore, it is also found to have effective antioxidant power (Peksel *et al.* 2013).

The results of our study suggest that the nature of identified bioactive compounds are phenol, flavonoids, fatty acid, sterols and terpenoids. These findings greatly highlight the importance of the *Chaetomium* LAV15 as a source of compounds that are potentially active as antioxidants.

CONCLUSION

Our study demonstrates that fungal crude extract of *Chaetomium* LAV15 from *Tinospora cordifolia* has potent antioxidant properties. However, the presence of various bioactive compounds revealed that they produce metabolites, which may not only be involved in the host endophyte relationship but may also have immense bioactive potential for human welfare. These findings, also suggest that *Chaetomium* LAV15 can be used as building block for new research and innovative drugs to help conserve plant.

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REFERENCES

Anisha, C.,Radhakrishnan, E.K. 2017. Metabolite analysis of endophytic fungi from cultivars of *Zingiber officinale*Rosc. identifies myriad of bioactive compounds including tyrosol. *3 Biotech***7**: 146..

Bharathi, C., Reddy, A.H., Nageswari, G., Lakshmi, M., Saumya, D., Vanisri, B., Venkatappa, B. 2018. A review on medicinal properties of *Tinospora cordifolia*. *Int. J. Sci. Res. Rev*. **7**: 585 – 598.

Bhardwaj, A., Sharma, D., Jadon, N., Agrawal, PK. 2015. Antimicrobial and phytochemical screening of endophytic fungi isolated from spikes of *Pinus rouxburghii*. *Arch. Clin. Microbiol.* **6**:1-9

Bills, G.F., Platas, G., Fillola, A., Jiménez, M.R., Collado, J., Vicente, F., Martín, J., González, A., Bur-Zimmermann, J., Tormo, J.R., Peláez, F. 2008. Enhancement of antibiotic and secondary metabolite detection from filamentous fungi by growth on nutritional arrays. *J. Appl.Microbiol.***104**:1644-1658.

Chen, D., Su, A., Fu, Y., Wang, X., Lv, X., Xu, W., Xu, S., Wang, H., Wu, Z. 2015. Harmine blocks herpes simplex virus infection through downregulating cellular NF-êB and MAPK pathways induced by oxidative stress. *Antiviral Res.* 123: 27-38.

Fadiji, A.E.,Babalola, O.O. 2020. Elucidating mechanisms of endophytes used in plant protection and other bioactivities with multifunctional prospects. F*ront. Bioeng. Biotechnol.*8:467.

Gond, SK., Mishra, A., Sharma, VK., Verma, SK., Kumar, J., Ravindra, N., Kharwar, RN., Kumar, A. 2012. Diversity and antimicrobial activity of endophytic fungi isolated from *Nyctanthes arbor-tristis*, a well-known medicinal plant of India. *Mycoscience* **53**:113–121

Goutam, J., Sharma, G., Tiwari, V.K., Mishra, A., Kharwar, R.N., Ramaraj, V., Koch, B. 2017. Isolation and Characterization of “Terrein” an antimicrobial and antitumor compound from endophytic fungus *Aspergillus terreus* (JAS-2) associated from *Achyranthus aspera* Varanasi, India. *Front.Microbiol*. 8:1334.

Jain, S., Panuganti, V., Jha, S.,Roy, I. 2020. Harmine acts as an indirect inhibitor of intracellular protein aggregation. *ACS Omega* **5**: 5620-5628.

Jayanthi, P., Lalitha, P. 2011. Reducing power of the solvent extracts of *Eichhornia crassipes* (Mart.) Solms. *Int. J. Pharmacy and Pharmaceutical Sci*. **3**: 126-128.

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. *Front.Microbiol*. 7:906.

Kumar, S., Kaushik, N. 2013. Endophytic fungi isolated from oil-seed crop *Jatropha curcas* produces oil and exhibit antifungal activity. *PLoS ONE* 8: e56202.

Kumar, S., Stecher, G., Tamura, K. 2016. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol.Evol*. **33**: 1870-1874.

Kharwar, R., Mishra, A., Sharma, V., Gond, S., Verma, SK., Kumar, A., Kumar, J., Singh, D., Goutam, J. 2014. Diversity and Biopotential of Endophytic Fungal Flora Isolated from Eight Medicinal Plants of Uttar Pradesh, India. *Microb. Divers.Biotechnol.Food Secur*. 479-488

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., Higgins, D.G. 2007. Clustal W and Clustal X version 2.0. *Bioinform*. **23**: 2947-2948.

Larran S., Monaco C., Alippi H.E. 2001. Endophytic fungi in leaves of *Lycopersicon esculentum* Mill. *World J.Microbiol. Biotechnol.* **17**: 181-184.

Li, H., Xiao, J., Gao, YQ., Tang, JJ., Zhang, AL., Gao, JM. 2014. Chaetoglobosins from *Chaetomium globosum*, an endophytic fungus in *Ginkgo biloba*, and their phytotoxic and cytotoxic activities. *J. Agric. Food Chem*.**62**:3734–3741.

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 Chem*. 105: 548-554.

Nithya, P.Y., Jelastin, S., Veerabahu, M. 2016. Evaluation of total phenolics, flavonoid contents and in-vitro antioxidant properties of *Catharanthus pusillus* (*Apocynaceae*). *Int. J. Pharm. Sci. Res.***7**: 3021-3027

Pawle, G., Singh, S.K. 2014. Antimicrobial, antioxidant activity and phytochemical analysis of an endophytic species of *Nigrospora* isolated from living fossil *Ginkgo biloba*. *Curr. Res. Environ. Appl. Mycol*. **4**: 1–9.

Peksel, A., Celik, C., Ocal, N., Yanardag, R. 2013. Antioxidant and radical scavenging activities of some norcantharidin and bridged perhydroisoindole derivatives. *J. Serb. Chem. Soc*.**78**:15-25.

Rashmi, M., Kushveer, J.S., Sarma, V.V. 2019. A worldwide list of endophytic fungi with notes on ecology and diversity. *Mycosphere* **10**: 798-1079.

Sadananda, T.S., Govindappa, M., Ramachandra, Y.L. 2014. In vitro antioxidant activity of lectin from different endophytic fungi of *Viscum album* L. *Trop. J. Pharm. Res*. 4: 626-643.

Sadeghi, F., Samsampour, D., Seyahooei, M.A., Bagheri, A., Soltani, J. 2020. Fungal endophytes alleviate drought-induced oxidative stress in mandarin (*Citrus reticulata* L.): toward regulating the ascorbate–glutathione cycle. *Sci. Hortic*. **261**:108991.

Saravanan, S., Parimelazhagan, T. 2014. In vitro antioxidant, antimicrobial and anti-diabetic properties of polyphenols of *Passiflora ligularis*Juss. fruit pulp. *Food Sci. Hum Wellness* **3**:56-64

Sharma, A., Malhotra, B., Kharkwal, H., Kulkarni, G.T., Kaushik, N. 2020. Therapeutic agents from endophytes harboured in Asian medicinal plants. *Phytochem. Rev*. **19**: 691–720.

Umamaheswari, M., Chatterjee, T.K. 2007. *In Vitro* Antioxidant Activities of the Fractions of *Coccinia Grandis* L. Leaf Extract. *Afr. J.Tradit. Complement Altern.Med*. **5**: 61-73.

White, T.J., Bruns, T., Lee, S., Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols: A Guide to Methods and Applications* **18**: 315-322.

Xie J.H., Xie M.Y., Nie S.P., Shen M.Y., Wang Y.X., Li C.2010. Isolation, chemical composition and antioxidant activities of water-soluble polysaccharides from *Cyclocaryapaliurus* (Batal.) Iljinskaja. *Food Chem*. **119**: 1626-1632.

Zaehle, C., Gressler, M., Shelest, E., Geib, E., Hertweck, C., Brock, M. 2014. Terrein biosynthesis in *Aspergillus terreus* and its impact on phytotoxicity. *Chem. Biol*. **21**:719-731.

Zhao, F., Wang, P., Lucardi, R.D., Su, Z., Li, S. 2020. Natural sources and bioactivities of 2,4-Di-tert-butylphenol and its analogs. *Toxins* **12**:35.