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HR-LCMS BASED MYCOCHEMICAL PROFILING OF ENDOPHYTIC *XYLARIA ADSCENDENS* (FR.) FR., IN *WENDLANDIA THYRSOIDEA* (ROTH) STEUD. OF CENTRAL WESTERN GHATS

Vinu K[1*], Krishna V[2], Himani[1] S and Krishnappa M[1*]

[1]Department of Studies and Research In Applied Botany, School of Biological Sciences, Kuvempu University, Shankaraghatta – 577451, Karnataka, India

[2]Department of Studies and Research In Biotechnology and Bioinformatics, School of Biological Sciences, Kuvempu University , Shankaraghatta – 577451, Karnataka, India

*Email: vinukkirishnappa9126@gmail.com

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ABSTRACT

Endophytic fungi are important microbes that exist in the internal tissues of diverse parts of the plant, developing a mutual relationship. The present study was concentrated on the isolation, identification and mycochemical profiling of the fungal endophyte in the healthy and matured leaves of *Wendlandia thyrsoidea* (Roth) Steud. of Chikkamagaluru. The endophytic fungus was identified based on morphological characteristics and molecular characterization. The aim of this study is metabolite profiling by HR-LCMS and molecular characterization of endophytic *Xylaria adscendens* (Fr.) Fr., in *Wendlandia thyrsoidea*, an ethnomedicinally important and widely used plant in Indian subcontinent.

Keywords: HR-LCMS, isolation, identification, ITS, microbes, Xylariales

INTRODUCTION

Fungal endophytes are the microorganisms (Xu *et al.,* 2007, Uzma *et al.,* 2019), that colonize in intercellularly or intracellularly (Saikkonen *et al.,* 1998) without causing any disorder signs or disease symptoms to host plant (Fisher *et al.,* 1984). Fungal endophytes also play an vital function in plant community fitness through imparting resistance to host against specific biotic and abiotic stresses (Gond *et al.,* 2012). The structure and function of endophytic fungal communities is promoted by many factors consisting of geographical locations, host specificities, climatic styles, seasonality, age, host, structure, surrounding flowers variety, body structure (Huang *et al.,* 2015), and colonized tissue specificity (Bhagat *et al.,* 2012). Xylariaceae is one of the biggest and extensively dispensed families of Xylariales (Ascomycota) and more than 300 species of endophytic Xylariaceae were identified and considered as significant source of natural chemical compounds (Yuan *et al.,* 2020) and have also been considered saprophytic and often pathogenic (Chen *et al.,* 2013).

In recent years, the endophytic fungal study was centered on the presence and plant interaction of the endophytes. The prominence on studying of endophytes in medicinal vegetation to discover novel compounds (Jena *et al.,* 2013) and their wide variety of biological activities such as antibiotic, anticancer, antioxidant, anti-inflammatory agents (Chow *et al.,* 2015), antimycotics, immune suppressant's (Nath *et al.,* 2012), antiviral and antimicrobial properties (Devi *et al.,* 2012).

Wendlandia thyrsoidea (Roth) Steud. belongs to Rubiaceae family. The plant is an ethnomedicinal small tree and usually known as mountain Wendlandia. The plant is commonly used for ethnomedicinally like bronchial asthma, skin sicknesses and jaundice treatments. The petroleum ether, ethyl acetate and methanol extracts of leaves showing antimicrobial and analgesic properties (Bodke *et al.,* 2015; Vagdevi *et al.,* 2009).

The present study reveals the isolation, characterization and investigates the metabolite profile of the fungal endophyte in the healthy matured leaves of *Wendlandia thyrsoidea*. This is the first report of endophytic fungi research in *Wendlandia thyrsoidea*, collected from the Central Western Ghats area of Kemmannugundi, Karnataka, India.

MATERIALS AND METHODS

Study area and collection of samples

The matured leaves of *Wendlandia thyrsoidea* (Fig. 3. A) were collected from the central Western Ghats area of Kemmannugundi – Chikkamagaluru during February 2018 (Fig. 1.), situated at 13° 33' 16.92" N and 75° 45' 50.45" E. The samples were screened within 24 h of collection (Nalini *et al.,* 2014).

Isolation of Endophytic fungi

The collected leaves were washed using distilled water and rinsed in using ethanol for 1–2 min and 5% sodium hypochlorite for 5 min. The sterilized leaves cut into 1cm^2 pieces in aseptic condition, then placed on potato dextrose agar (PDA) and incubation at 25° ± 2° C, on 4th day after

incubation the expressed fungal colonies were transferred to PDA for further use (Kour *et al.,* 2008).

Identification of Endophytic Fungi

Morphological and Molecular Identification

The pure culture of endophytic fungi was identified based on morphological characteristics like colony characters (Deepthi *et al.,* 2018). For DNA extraction, the pure endophytic fungal mycelia were transferred into 250 mL Erlenmeyer's flasks containing potato-dextrose broth (PDB) without shaking. After the 5th day mycelial mat was harvested. The genomic DNA turned into isolated the usage of the Qiagen DNeasyR Plant Mini Kit. The extracted DNA was diluted in 10X TE buffer and stored at 4°C.

ITS areas had been amplified using primers (Forward primer and Reverse primer), ITS1 (5′-TCC GTA GGT GAA CCT GCG G- 3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC- 3′). The PCR master mix was prepared to 25µl containing 2.5µl 10X PCR buffer, 2µl of dNTPs, 1µl of each ITS1 and ITS4 primers, 0.25µl of Taq polymerase and 19µl of Sterile dH2O. The PCR changed into achieved with an initial denaturation at 94°C at 5 min followed by using 30 cycles of 30 s denaturation at 94°C, 30 s annealing at 50°C and 1 min extension at 72°C. Final extension for 7 min at 72°C was performed and visualize the PCR merchandise on 1.2% agarose gel containing EtBr (Zakaria and Aziz, 2018) with modifications. The amplified product turned into examined and sequenced the use of the Applied Biosystems. The sequences have been manually edited (Bio Edit Software) and as compared with available facts from GenBank databases (NCBI- BLASTn). After obtaining BLAST outcomes for the phylogenetic evaluation to perceive the endophytic fungi (Lu *et al.,* 2012).

Growth Characteristics on different solid media

The study of mycelial growth, colony characteristics of endophytic *Xylaria adscendens* (Fr.) Fr., on four different media, particularly PDA (Potato Dextrose Agar), CDA (Czapek Dox Agar), SDA (Sabouraud Dextrose Agar) and MEA (Malt Dextrose Agar) was accomplished (Sharma and Pandey 2010).

Secondary metabolite extraction

The pure culture of X. *adscendens* was inoculated in 1000ml Erlenmeyer flask containing 500ml of Potato dextrose broth on a rotary shaker for 15-22 days at 25° by fermentation method (Fig.3.C) The culture filtrate was filtered and extracted with the same volume of Ethyl acetate twice and the extract was rotary evaporator dried for further analysis (Avinash *et al.,* 2015). The ethyl acetate crude extract was analysed by HR-LCMS.

Qualitative analysis of Mycochemicals

Preliminary phytochemical analysis of the ethyl acetate and methanolic extract crude extracts of *Xylaria adscendens* was carried out for the presence of the following metabolites: alkaloids, steroids, flavonoids, tannins, phenols, saponins, glycosides and terpenoids using standard methods (Devi *et al.,* 2012; Ramesha and Srinivas, 2014).

Detection of bioactive compounds by HR-LCMS analysis

For HR-LC-MS analysis, the ethyl acetate extract was sent to IIT-Powai, India for High-resolution Mass Spectrophotometry (HR-LC-MS). Agilent Technologies, USA Model-1290 Infinity UHPLC System,1260 infinity Nano HPLC with Chipcube, 6550 iFunnel Q-TOFs.

Phylogenetic Analysis

The evolutionary record was inferred by using the Maximum Likelihood method and Kimura 2-parameter version (Kimura M., 1980). The tree with the best log likelihood (-1901.67) is shown. The percentage of tree wherein the related taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were acquired automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated the usage of the Maximum Composite Likelihood (MCL) method, and after selecting the topology with advanced log likelihood value. This analysis involved 17 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions with less than 80% site coverage were eliminated, i.e., fewer than 20% alignment gaps, missing data and ambiguous bases were allowed at any position (partial deletion option). There were a total of 513 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar *et al.,* 2018).

RESULTS AND DISCUSSION

Morphological and Molecular Identification

The colony morphology of the endophytic fungi showing white silky mycelia (Fig.3.B), white in colour, after two weeks of mycelial increase later develops into white greenish or brownish shade. The Microscopic studies show hyphae septate and non-sporulating. The pure culture is identified to the species level based on morphological features and deposited in National Fungal Culture Collection, Agharkar Research Institute, Pune, India (Deposition Number: NFCCI-4835) and the ITS sequences were submitted in NCBI-GenBank (Accession Number- MN736511).

Figure.1. Map Showing the study area of plant collection site, Kemmannugundi, Chikkamagaluru, Karnataka, India.

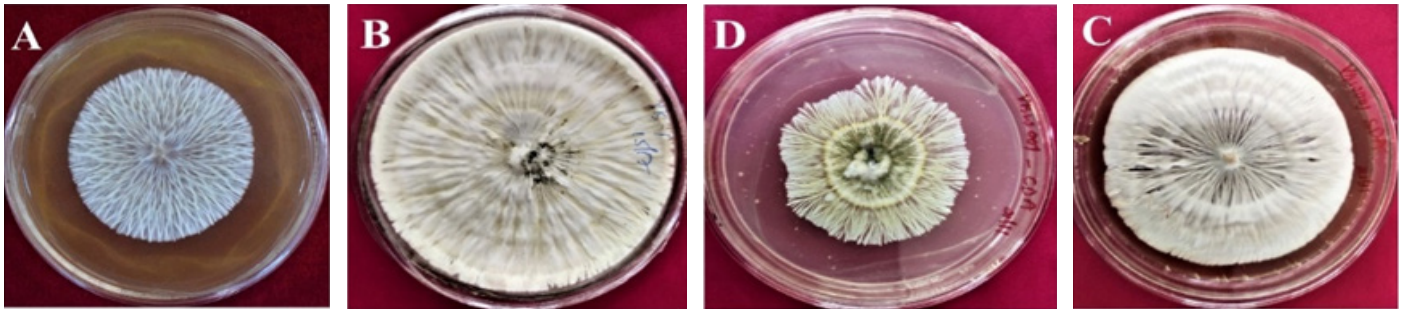

Figure 2. Cultures of *Xylaria adscendens* (Fr.) Fr., on different solid Agar media. A)MEA (Malt Extract Agar), B) PDA (Potato Dextrose Agar), C) SDA (Sabouraud Dextrose Agar), D) CDA (Czapek-Dox Agar).

***Xylaria adscendens*(Fr.) Fr., on different solid Agar media**

The *Xylaria adscendens* was inoculated by using 4 solid media viz, PDA, CDA, SDA and MEA. In PDA (Fig 2.B) confirmed the maximum radial growth, and after weeks colony becomes white greenish colour. After the PDA, SDA (Fig 2.C) indicates the maximum mycelial boom than CDA (Fig.2.D) and MEA (Fig 2.A).

Taxonomy

***Xylaria adscendens* (Fr.) Fr., Nova Acta Regia Soc. Sci. Upsal., Ser. 3, 1: 128 (1851)**

Mycobank 190364

The colony becomes 4- 4.5 cm diameter within 7 days, white striped silky to appressed and radial increase (Fig.3.B). The white cottony mycelium is around point of inoculum and attaining to the lid of Petri dish. After two weeks of inoculation culture at first was white then slowly turned into the greenish colour of the mycelia. The reverse side of the Petri dish is white, and then turns into a light-yellow colour. After four weeks of inoculation in Potato dextrose broth medium stromata (Fig.3. D.) were shaped and initiated. The stromata have commonly white with brownish and cylindrical (Fig.3. E.), to 3.5cm-4cm high x 3-4 mm wide. The teleomorph stage shows, dark shading in the base and white punctuated ostioles on a superficial level. Conidia (Fig.3 G.), oval in shape measuring 10.5-13µm × 4.5-5µm and one celled (100X Magnification) utilizing Binocular magnifying instrument LYNX.

Table1: Comparative and distribution study of *X. adscendens* in earlier reports worldwide.

Strain number	Host	Country	Stage	Stromata			Ascospore		References
				Shape	Size	colour	Size	colour	
-	*Capsicum chinense*	Uganda	Anamorph	-	-	-	-	-	Aigbe.2019
-	-	-	Anamorph and Telomorph	cylindrical	3.5 cm high x 4 mm wide	Cream or orange white	(8.1-)9.9-11.7 x 2.7-3.6	-	Rodrigues *et al.,* (1993)
FLOR 31921	Undetermined decaying hardwood	BRAZIL	Teleomorph	cylindrical with apices sterile and flattened	0.8–53 mm length × 2–7 mm diam,	Cream to very light brown	(9–)11–14.5(–15) × 3–5 µm	dark brown	Pereira *et al.,* 2009
-	-	Africa and South America	Telomorph	cylindric to clavate	3-8(-13) cm x 0.2-2 cm	brownish black, but retaining white areas on apical parts	(10-)11-13(-15) x 3-3.5(-4) ~m,	-	Jack D. Rogers (1984)
-	-	Mexico	Telomorph	cylindrical, terete or flattened	7- 10 cm total length X 3-12	externally reddish brown to dull black	(9.5-)10.5-13(- 14) X 4-5(-5.5) m,	brown	Gonzalez and Rogers (1989)
VKW001	*Wendlandia thyrsoidea* (Roth) Steud.	India	Anamorph and Telomorph	cylindrical	3.5cm-4cm high x 3-4 mm wide	white with brownish	10.5-13µm × 4.5-5µm	brown	**Present paper**

Table2. List of Xylaria species, origin and GenBank accession numbers of the ITS sequences used in phylogenetic analysis. A new generated sequence is in bold.

Species	Accession number	Strain	Origin	References
Daldinia concentrica	MK513839	R22	Portugal	Trovao, J (2019)
X. adscendens	KP133263	R27	USA	Thomas *et al., (*2014)
X. adscendens	**MN736511**	**VKW001**	**INDIA**	**Present paper**
X. adscendens	KP133258	892	USA	Thomas *et al*., (2014)
X. adscendens	KP133287	47.4.2	USA	Thomas *et al*., (2014)
X.arbuscula	AF163029	452.63	Korea	Lee *et al., (*2012)
X. arbuscula	AF163028	454.63	Korea	Lee *et al., (*2012)
X. grammica	JQ341088	D11b3	Germany	Meli and Langer, (2012)
X. grammica	JQ341087	D15b3a	Germany	Meli and Langer, (2012)
X. hypoxylon	MK247386	A205	China	Zhang, H (2018)
Xylaria hypoxylon	MK304075	X189	China	Zhang, H (2018)
X. longipes	MG098261	NW-FVA2228	Germany	Busskamp,J. (2017) (Unpublished)
X. longipes	KY250408	GAB197	USA	Roy and Yombiyeni, (2016) (Unpublished)
X. multiplex	KP133436	1005	USA	Thomas *et al.,* (2014)
X. multiplex	KP133437	1048	USA	Thomas *et al.,* (2014)
X. papulis	JX256827	22809	China	Ma *et al.,* (2012)
X. papulis	GU300100	89021903	Taiwan	Hsieh *et al.,* (2009)

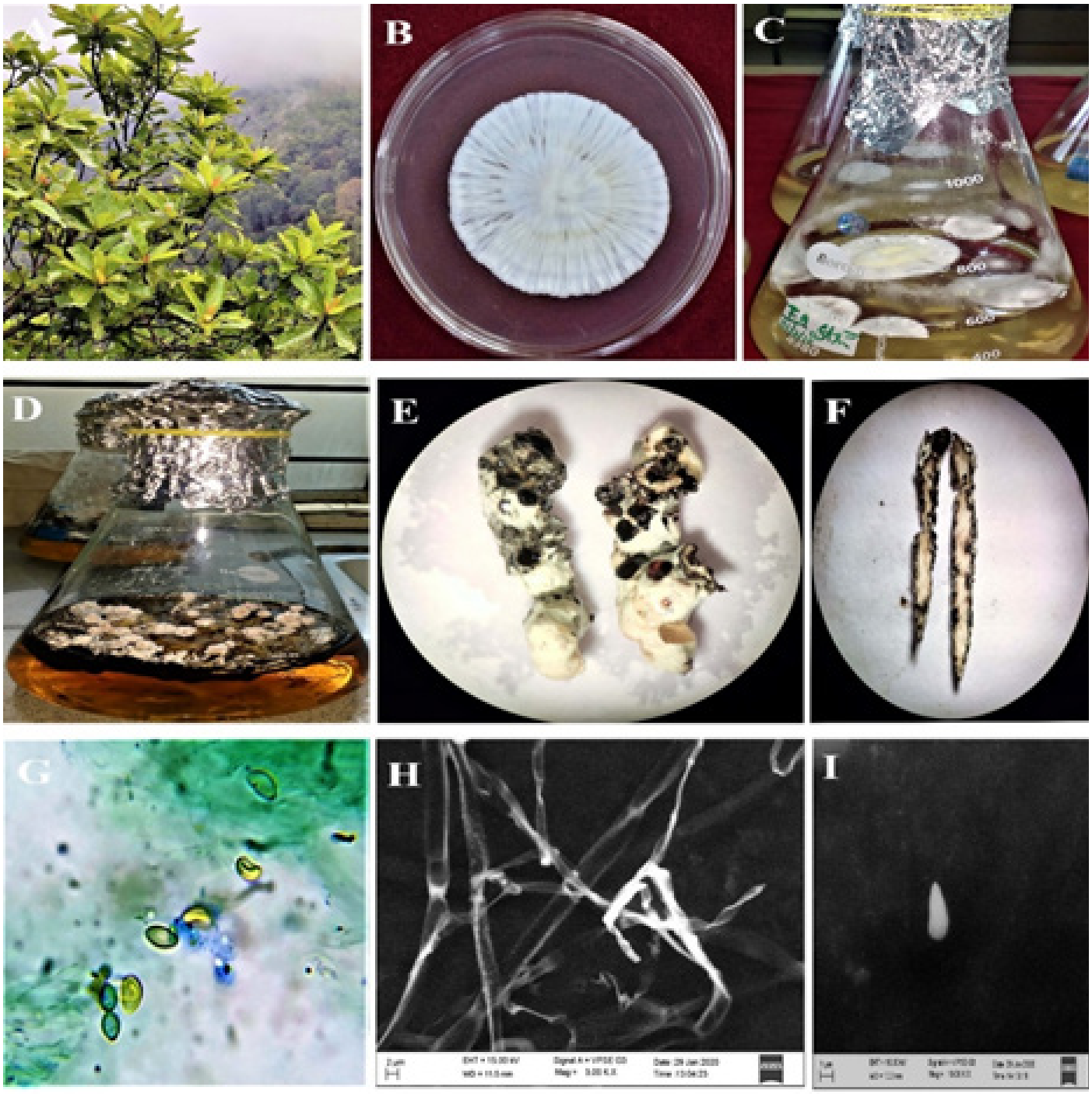

Figure. 3. Isolation, culture and Identification of *Xylaria adscendens* (Fr.) Fr.,A)*Wendlandia thyrsoidea* (Roth) Steud., B)Pure culture , C) Culture in Potato Dextrose Broth (PDB), D) Stromata formation, E) Stromata F)Vertical section , G) Asco spores(10.5-13µm × 4.5-5µm), H) Scanning Electron Microscopic view of Mycelia, I) Scanning Electron Microscopic view of Ascospore.

Distribution

Substrate- Healthy matured leaves of *Wendlandia thyrsoidea* (Roth) Steud.

Material examined- India, Karnataka, Kuvempu University, Department of Applied Botany, *Wendlandia thyrsoidea* (Roth) Steud., 24 November 2018, Vinu K, VKW001.

Phylogenetic Analysis

The phylogenetic study was performed by NCBI-Blastn and MEGA-X software program. Blast search was made to choose the nearest 15 taxa and selected as one outgroup (*Daldinia concentrica* MK513839) of the evaluation (Table.2). The evolutionary examine inferred the use of the ITS sequences with the ClustalW and to determine the most appropriate model to develop a Maximum likelihood phylogenetic tree (Kumar *et al.,* 2018), by the use of MEGA-X software program (Fig.4). The new recorded species is highlighted in blue colour.

Qualitative analysis of Phytochemicals

The mycochemical analysis showed positive results for alkaloids, terpenoids, flavonoids, cardiac glycosides, steroids, phenols, fats and oils. Saponins was found to

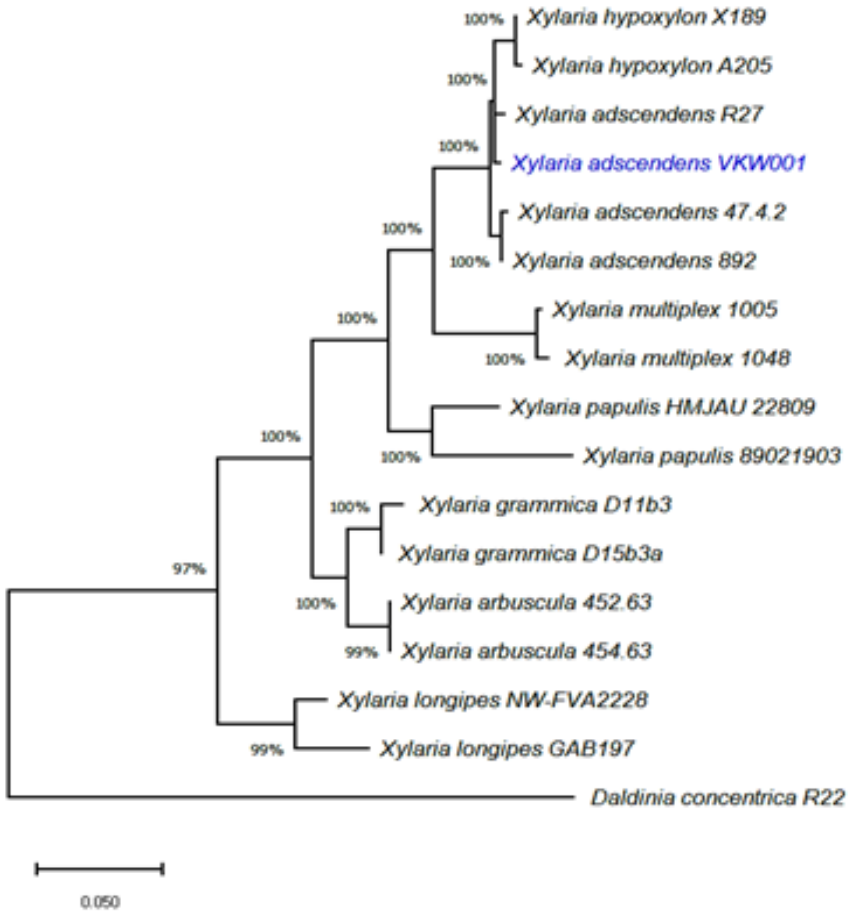

Figure 4. Phylogenetic tree basedon ITS sequence data of *Xylaria adscendens (Fr.) Fr.* It illustrates the relationships between the *Xylaria adscendens* with other Xylaria species.

Discussion

Many researchers have worked on the endophytes in medicinal plants for the study of fungal diversity, plant microbial interactions, symbiotic relationship and the production of novel secondary metabolites for various activities. But there was no report on the endophytic Xylaria *adscendens* (Fr.) Fr., in the *Wendlandiathyrsodia* (Roth) Steud. The plant is commonly called as mountain Wendlandia and used as ethnomedicine for bronchial asthma, skin sicknesses and jaundice treatments. The leaf and flowers having antimicrobial, antioxidant and analgesic properties.

The main study shows, first report of the endophytic fungal research in *Wendlandiathyrsodia* (Roth) Steud. of Kemmannugundi-Chikkamagaluru, Karnataka, India. The isolated non-sporulating endophytic fungi *Xylaria adscendens* (Fr.) Fr., is identified based on morphological appearances of anamorph and teleomorph stages and molecular identification. Rogers (1984) defined the *Xylaria adscendens* teleomorph characteristics were closely linked with our isolated Xylaria sp., like stromata were cylindric to clavate and unbranched or branched from the base and initially white in colour and later turns into brownish black and white in apical parts. The surface was smooth and dark ostioles were present. He reported only from the tropical regions of Africa and South America and the findings conclude that *Xylaria adscendens* differ from *X.mali* in conidia and ascospores were interrelated to *X. hypoxylon*. According to Rodrigues (1993) reveals that *X. adscendens* was endophytic fungi in Euterpe oleracea of Combu. They discussed and reported the taxonomy, descriptions and illustrations of *X. adscendens* and other new Xylaria species (Pereira *et al.,* 2009). Esteban (2012) and his team examined the taxonomic keys of *X. adscendens*. They defined the Stomatal characters like size, width and outer surface. And the shape and size of ascospores. Lin (2016) reported the many numbers of endophytic Xylariales in Basidiomata of Scytinopogon sp.

be absent in all the extracts. Mycochemical analysis was carried out of methanolic and ethyl acetate crude extracts of *Xylaria adscendens*, to determine the presence of chemical components as a potential source for medicinal and industrial uses (Sharma *et al.,* 2016). The active metabolites contain chemical groups such as phenols, flavonoids, terpenoids, alkaloids, tannins, carbohydrates and saponins. Only two phytocomponents were present in ethyl acetate extract, i.e., saponins, flavonoids, phenols and alkaloids whereas methanolic crude extract exhibited all phytocomponents except saponin.In the current study, phytochemical analysis of ethyl acetate extracts of *C. gloeosporioides* extract showed the presence of alkaloids and steroids; whereas *F. oxysporum* extract revealed the presence of flavonoids, phenol and phenolic compounds.

HR-LCMS based mychochemical profiling

Metabolite profiling of ethyl acetate extract of *X. adscendens* using HR-LC-MS was done for the identification of important metabolites (Fig. 5). Based on the chromatogram total of 27 metabolites were identified (Table.4). The most prevailing compounds were identified (Carvedilol (p-Hydroxy), Entandrophragmin, Deoxystreptomycin, Metaxalone Securinine, Indole acetaldehyde and Benazepril (Table.4).

The metabolite profiling of ethyl acetate extract of *X.adscendens* by using HR-LCMS shows several chemical compounds. The major important compounds like Securinine have mainly used for treatment Leukaemia and neurological disorders. Desmethylnortriptyline (desmethylnortriptyline glucuronide) is one of the major mycotoxin was reported first time in *X. adscendens*.

Table.3. Phytochemical analysis of the Methanol and Ethyl acetate extracts of Endophytic *Xylaria adscendens* (Fr.) Fr.,

Phytochemical compounds	Methanolic extract	Ethyl acetate extract
Alkaloids	-	+
Steroids	+	+
Tannins	-	–
Glycosides	+	+
Terpenoids	–	–
Flavonoids	+	+
Saponins	–	–
Phenols	+	+

Note: + Presence, - absence.

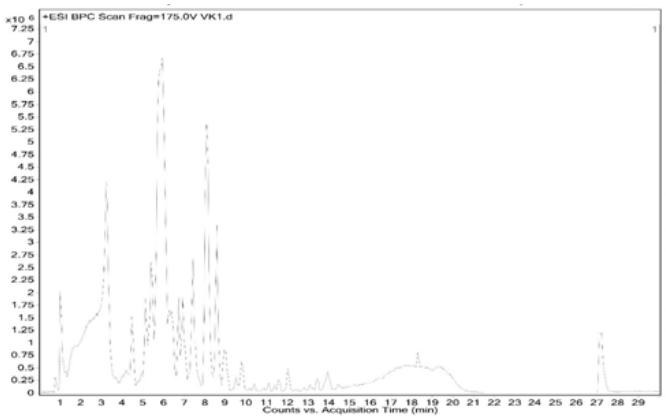

Figure.5. Chromatogram of HR-LCMS based metabolite profiling of Ethyl acetate extract of X. adscendens.

Table4. List of Compounds identified in Ethyl acetate extract of *Xylaria adscendens*(Fr.) Fr., by HR-LC-MS based metabolic profiling.

Metabolites	RT	Mass	m/z	Formula	Biological activities	References
Methyldopate	1.132	239.1156	222.1122	$C_{12}H_{17}NO_4$	Decarboxylase inhibitor with antihypertensive activity.	PubChem
Carbofuran	1.45	221.1052	222.1123	$C_{12}H_{15}NO_3$	Carbamate pesticide	Jaiswal *et al.,* (2017)
Carboxyibuprofen	3.002	236.1045	219.1011	$C_{13}H_{16}O_4$	Nonsteroidal anti inflammatory (NSAID) drug	PubChem
Bisacodyl diphenol glucuronide	4.994	397.2019	420.191	$C_{23}H_{23}N_7$	Stimulant laxative.	Horn *et al., (*2005)
Cilazapril	5.093	417.2279	422.2068	$C_{22}H_{31}N_3O_5$	Treatment of hypertension and congestive heart failure.	Natoff *et al.,* (1990)
3-OMethylisoprotere-nol	5.311	225.1364	208.1331	$C_{12}H_{19}NO_3$	Used as bronchodilator and heart stimulant.	Drug Bank
Carvedilol(p-Hy-droxy)	5.435	422.1837	423.1909	$C_{24}H_{26}N_2O_5$	Cardiovascular drug	Chettupalli *et al., (*2017)
Benazepril	5.436	424.1991	425.2063	$C_{24}H_{28}N_2O_5$	pharmacodynamic and pharmaco-kinetic properties.	Balfour and Goa, (1991).
Sebacic acid	5.637	202.1229	207.1016	$C_{10}H_{18}O_4$	used as intermediate for aromatics and antiseptics.	Jeon *et al., (*2019)
5,8,11-dodecatriynoic acid	5.64	188.0836	189.0909	$C_{12}H_{12}O_2$	Synthesis of Arachidonic Acid	Fryer *et al.,* (1975)
Metaxalone	5.861	221.1048	204.1015	$C_{12}H_{15}NO_3$	Treatment of musculoskeletal conditions	Scaife *et al.,* (2004)
4-[[5-[[(cyclopenty-loxy)carb onyl]amino]-1-meth-yl-1H-indol-3-yl] methyl]-3-methoxy-Benzoic	6.474	422.1843	423.1923	$C_{24}H_{26}N_2O_5$	Treatment of pulmonary disorders	Goverdhan *et al., (*2009)
Entandrophragmin	6.92	844.3689	423.1917	$C_{43}H_{56}O_{17}$	Phytochemical and antisickling activities	Adejumo *et al., (*2011)
epi-4'- hydroxyjas-monic acid	8.008	226.1207	209.1174	$C_{12}H_{18}O_4$	Plant growth regulators	Nakamura *et al.,* (2011)
1-Cyclohexene-acryl-ic acid, 2,6,6-trimethyl-3-oxo-	8.077	208.11	191.1067	$C_{12}H_{16}O_3$	Organoleptic properties	Trenkle *et al.,* (1981)

Continued....

Desmethylnortriptyline (desmethylnortriptyline glucuronide)	8.13	425.1844	408.1806	$C_{24}H_{27}NO_6$	Mycotoxins	Slobodchikova *et al., (*2019)
Dihydrodeoxystreptomycin	8.919	567.2894	568.2967	$C_{21}H_{41}N_7O_{11}$	Aminoglycoside antibiotic, with bactericidal property	PubChem
1-hexadecanoyl-2-heptadecanoyl-3 eicosanoylsn-glycerol	27.178	875.7934	876.8007	$C_{56}H_{108}O_6$	Osmotic diuretic and laxative effects	PubChem
Melanin	1.181	318.0621	317.0552	$C_{18}H_{10}N_2O_4$	Photoprotective and immunological action	ElObeid *et al.,* (2016)
Loxoprofen Metabolite (b-DGlucopyranuronic acid, 1-[a-methyl-4-[(2-oxocyclopentyl) methyl]benzeneace	5.434	422.1593	421.152	$C_{21}H_{26}O_9$	Non-steroidal anti-inflammatory drug (NSAID)	Shrestha *et al.,* (2018)
Cotarnine	5.52	237.1	236.0927	$C_{12}H_{15}NO_4$	Treatment of uterus sub involution	DRUGS NCATS
Securinine	5.751	217.1104	262.1085	$C_{13}H_{15}NO_2$	Leukemia differentiation-inducing agent and neurological related diseases.	Gupta *et al., (*2011)
Carvedilol (m-Hydroxy	6.071	422.1836	421.1763	$C_{24}H_{26}N_2O_5$	Heart failure and high blood pressure.	Dean, (2018)
Indoleacetaldehyde	7.156	159.0684	218.0822	$C_{10}H_9NO$	Involved in the tryptophan metabolism pathway	Pubchem
Deoxystreptomycin	7.488	565.2576	624.2709	$C_{21}H_{39}N_7O_{11}$	Antibacterial agent.	Umezawa *et al., (*1984)
11alpha-(chloromethyl)-1alpha,25-dihydroxyvitamin D3 / 11alpha-(chloromethyl)- 1alpha,25-dihydroxycho	12.433	464.3074	523.3215	$C_{28}H_{45}ClO_3$	Used to heal bone fractures and to treat metabolic bone diseases.	PubChem
5-[2-(hydroxymethyl)-5-methylphenoxy]-2,2-dimethyl-Pentanoic acid (Gemfibrozil M4)	24.089	266.1543	265.1473	$C_{15}H_{22}O_4$	Used to treat hyperlipidemia.	PubChem

Carboxyibuprofen and Loxoprofen were used as Non-steroidal anti-inflammatory drug (NSAID). The Metabolites having many pharmacological activities such as antimicrobial, anti-inflammatory, anticancer, neurological and also agricultural uses.

The overall study reveals the features of anamorph and teleomorph stages of X. *adscendens* and with the help of morphology and molecular identification confirms it is *X. adscendens* and new distribution to the host and the study area. The present work provides the first scientific report on metabolite profiling of the secondary metabolites of endophytic *X. adscendens* isolated in *Wendlandiathyrosidea* of central Western Ghats region, Karnataka, India.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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