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Diversity Guided Antibacterial Bioactive Metabolites from Endophytic Fungi of *Withania somnifera* (L.) Dunal

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ABSTRACT: *Withania somnifera* (L.) Dunal, is commonly acknowledged as ashwagandha or as Indian ginseng. It is used in Indian system of traditional medicine and it exhibits broad spectrum therapeutic properties. The sum of 165 endophytic fungi belonging to twenty-two different species was isolated from 250 tissue fragments analyzed. The Shannon and Simpson diversity indices were greater in stem trailed by root, leaf, flower and seed. The phytochemical analysis showed that 91% of endophytic fungal isolates produced terpenoids, 60% flavonoids, 42% steroids, 40% quinones and 54% tannins. Further, fungal isolates were analyzed by agar plug method for antibacterial activity; *Talaromyces radicus* (WSR2) and *Aspergillus niger* (WSR1) isolated from root of *W. somnifera* exhibited broad range activity against all the Gram-positive and Gram-negative pathogenic bacteria tested. These two endophytic fungi were subjected to molecular identification and sequences submitted to Gen Bank and accession numbers obtained *Talaromyces radicus* - MN099424.1, *Aspergillus niger* - MN099425.1. The GC-MS analysis of *Talaromyces radicus* (WSR2) ethyl acetate crude extract demonstrated the existence of eighteen compounds.

Keywords: Endophytic fungi, Diversity, Bioactive metabolites, *Withania somnifera.*

INTRODUCTION

Endophytic fungi colonize the internal parts of healthy plant tissues growing on the ground or under the ground. They colonize all the parts of living plants including stems, leaves, flowers and roots without causing any adverse negative effects, symptoms or disease (Hirsch and Braun 1992). The current research has discovered and evaluated that, at least 1 million species of endophytic fungi reside within healthy plant parts (Dreyfuss and Chapela 1994). These endophytic fungi symbolizes a significant and assessable constituent of biodiversity in fungi and are acknowledged to have impact on plant diversity (Sanders, 2004; Krings *et al.,* 2007). The endophytic fungi are reported in all major lineages of terrestrial plants and also in natural and anthropogenic communities extending from arctic to tropics (Baron and Rigobelo 2022). They encompass polyphyletic cluster of fungi exhibiting tremendous diversity and dwell an inimitable biological niche of inhabiting plant tissues without causing any illness or any destructive symptoms. The endophytic fungi play substantial physiological (Malinowski and Belesky 2006) and ecological (Tintjer and Rudgers 2006) roles in the life span of host plants. The ubiquity of these symbiotic endophytic fungi is clear but, diversity, host-range and geographical dispersals are unknown (Arnold, 2007). Endophytic fungi are presently well-thought-out as significant constituent of biodiversity. The dispersal of endophytic mycoflora diverges within different parts of host plants (Mc Arthur *et al*., 2021; Yadav *et al.,* 2022). An aggregate of 1.5 million fungi are reported to be present on different niche of earth and endophytic fungi itself constitutes 7%. The 4,20,000 plant species occur in nature and merely few have been fully studied for their endophytic fungal association; out of 1.5 million fungi, only around 80,000 to 1,00,000 fungi have been described till date (Hawksworth and Rossman 1997). The endophytic fungi are alleged to be reservoir of biologically and structurally inimitable natural products. A large number of antifungal compounds structurally similar to flavonoids, alkaloids, steroids, terpenoids, quinones and peptides has been described from endophytic fungi (Ahmadi, 2022). The endophytic fungi are reported to harvest a surfeit of substances that are of significance in contemporary industry, agriculture and medicine; the endophytic fungal metabolites include anticancer compounds, immune suppressants, innovative antibiotics and anti-mycotics (Strobel and Daisy 2003; Mitchell, 2008; Tiwari and Bae 2022).

The bioactive metabolites secreted by endophytic fungi intensify the malleability of host plants with its associated endophytic fungi and enhance tolerance to biotic and abiotic stresses. In addition, the endophytic fungi associated with its host plants persuades the manufacturing of abundant quantity of recognized and naturally dynamic secondary metabolic products which

are utilized and adopted by human beings as therapeutic assets (Long *et al.,* 2023**;** Firáková *et al.,* 2007; Rodriguez *et al.,* 2009). Numerous research data have validated the aptitude of endophytic fungi to harvest innumerable metabolites including antimicrobial compounds (Souza *et al.,* 2004; Siqueira *et al*., 2008; Pinheiro *et al.,* 2013), extracellular enzymes (Teske, 1994), antitumor compounds (Bezerra *et al*., 2012; Chandra, 2012) and hormones inducing plant growth promoters.

The application of endophytic fungi and its metabolites has provoked auxiliary study and steered towards discovery of novel compounds exhibiting potential uses in pharmaceutical industries (Meng *et al.,* 2011; Wang and Dai 2011). There is always a pronounced probability of discovering new drugs from endophytic microorganisms which can be effectively used in treating diseases in animals and humans (Kumar and Kaushik 2013). The study of endophytic fungi and relationship with its associated host plant will throw light on both evolution and ecology: the fruition of endophyte - plant cooperation, environmental factors that impacts the path of endophyte - host plant relations (Saikkonen *et al*., 1998).

Withania somnifera (L.) Dunal, usually known as Indian ginseng or ashwagandha is an economically significant plant widely used as medicine. It is accepted since the primitive times as traditional herb in Indian system of medicine to enhances stamina, energy, strength, management of numerous disorders like asthma, bronchitis, diabetes, ulcer, leukoderma, rheumatoid arthritis (Subbaraju, *et al.,* 2006). This plant has exhibited wide spectrum healing ability, used to enhance memory and also as anti-stress, immune-modulatory, cardioprotective, neuroprotective, antidiabetic, nerve tonic and antioxidant (Visavadiya *et al.,* 2007; Dar *et al.,* 2015).

Withania somnifera is an imperative tropical therapeutic plant belonging to Solanaceae family (Yang et al., 2007). The various preparatory forms of Ashwagandha like oil, smoke, powder, decoction and poultice have been successfully used to treat ailments related to intestine, rheumatoid arthritis, nervous system, veneral diseases also, as an energy booster to treat weakness in geriatric.

Several anolides obtained from *W. somnifera* have proved to exhibit anti-inflammatory, antibacterial, antitumor, and immune modulatory activities (Budhiraja and Sudhir 1987; Lee *et al.,* 2022). The pastes of leaves and roots of ashwagandha are smeared on inflated cervical glands or other glands to reduce edema and pain; for treating vata disease and weakness ashwagandha oil massage therapy is done. The juice of Ashwagandha leaves are used as eardrops to treat ear discharge; black ashes of the roots are applied for healing of blisters. The paste made from powdered dry leaves of ashwagandha is effectively used in treatment of wrinkle skin, wound, burns, premature graying, ageing of hair and also, used as sunscreen (Saini *et al*., 2023). *Withania somnifera* is the most commonly used medicinal plant for treatment of many ailments as it is a reservoir of several bioactive metabolites. Hence, in this study an effort was done to catalogue and determine the diversity analysis of endophytic fungi associated with different parts of *Withania somnifera*. The phytochemical, antibacterial activities of isolated endophytic fungi were evaluated and several antimicrobial metabolites present in fungal extracts were detected by GC-MS/MS analysis.

MATERIALS AND METHODS

A. Isolation of endophytic fungi

Withania somnifera healthy plants (showing no visual disease symptoms) were collected from Central Institute for Medicinal and Aromatic Plants (CIMAP), Allalasandra, Yelahanka, Bengaluru (Identification No. Bot/Ws/014/22). The endophytic fungi were isolated from flower, seeds, leaf, root and stem of *W. somnifera* following the modified procedure (Zhu *et al.,* 2008). The samples were removed, cleaned with running tap water, sliced into 0.5 cm^2 pieces and sterilized using 95% ethanol (60 sec), 4% NaOCl (300 sec), 95% ethanol (60 sec) and washed3 times using sterile distilled water. The disinfected pieces of the plant parts were inoculated on Potato Dextrose Agar (PDA) plate amended with 50µg/mL tetracycline under aseptic conditions. The inoculated plates were maintained in dark conditions at 30ºC and daily observed for fungal growth. The pure fungal cultures were grown on PDA slants and maintained at 4 ºC.

B. Identification of endophytic fungi

The endophytic fungi obtained from various parts of *W. somnifera* were identified based on morphological characteristics using standard manuals like (Barnett and Hunter 1998).

C. Data analysis

The frequency or occurrence of endophytic fungi inhabiting various parts of *W. somnifera* such as flower, seeds, stem, leaves and roots were determined (Larran *et al.,* 2002). The absolute frequency (f), relative frequency (fr), isolation rate (IR), colonization rate (CR) of the endophytic fungi isolated from each part of the plant was determined. The Shannon-Wiener index (H') and Simpson's (D') diversity indices, evenness (J) and species richness (S) were determined as described by Magurran (Magurran and Magurran 1988).

D. Identification of selected endophytic fungi by molecular methods

The prospective endophytic fungus was identified based on their ribosomal DNA (18S rDNA gene) sequences. The full length ITS sequences obtained was further analyzed using BLAST in NCBI database. The total score obtained and the highest homology was determined for further investigation. The rDNA sequence of prospective endophytic fungi was submitted to NCBI and accession numbers obtained.

E. Cultivation of endophytic fungi

The endophytic fungi were inoculated in 100 mL PDB and incubated at 28 ± 2 ºC, 120rpm for one week under dark conditions. The fungal culture was filtered through cheese cloth, mycelia removed, and filtrate was extracted using double the quantity of ethyl acetate and further concentrated in vacuum to remove organic

solvent (Wang *et al.*, 2007). The ethyl acetate extracted culture filtrate was utilized for phytochemical analysis.

F. Phytochemical analysis using ethyl acetate crude extract of endophytic fungi

The crude ethyl acetate extract of endophytic fungi were used to detect the presence of different Phytochemical following standard methods (Sofowora, 1993; Farnsworth, 1996; Rangari, 2002).

Tests for detection of flavonoids (Alkaline reagent): 2 mL of fungal extract was taken in a test tube and few drops of NaOH (20%) was added and mixed, the appearance of intense yellow color becoming colorless on addition of dilute hydrochloric acid, indicated the presence of flavonoids.

Test for detection of steroids: The ethyl acetate crude fungal extract was dissolved in chloroform and equal volume of concentrated H_2SO_4 was added. The formation of bluish red to cherry colour in chloroform layer and green fluorescence colour in acid layer indicated the presence of steroids.

Test for Quinone: Small amount of ethyl acetate crude fungal extract was added to concentrated Hydrochloric acid (HCl) and the formation of yellow colored precipitate indicated the presence of Quinones.

Test for Coumarins: Alcoholic or aqueous extract + 10% NaCl. Formation of yellow colour indicated Coumarins.

Test to detect Saponins (Foam test): 2 mL crude ethyl acetate fungal extract was mixed with 6ml water. The mixture was vigorously shaken and formation of persistent foam indicated presence of saponins.

Test for detection of Phenols (Ferric chloride test): 2 mL of crude ethyl acetate fungal extract was added to 5% ferric chloride (aqueous). The formation of deep blue or black color indicated the presence of phenols.

Test to detect Terpenoids: 2 mL of crude ethyl acetate fungal extract was added to 1mL chloroform followed by few drops of concentrated sulphuric acid. The formation of reddish brown precipitate indicated the presence of terpenoids.

Test for detection of tannins: 2 mL crude ethyl acetate fungal extract was added to 10 % ferric chloride (alcoholic) solution. The appearance of blue or greenish color indicated the presence of tannins**.**

G. Gas chromatography–mass spectrometry (GC-MS) analysis

The crude ethyl acetate extract of selected endophytic fungi was used for GC-MS analysis to determine the chemical characterization of metabolites. GC-MS analysis was accomplished in an Agilent 240 MS series chromatograph furnished with an ion trap mass-spectrometer 28. The 50 µL of fungal extracts were separated on Agilent 19091J- 433 column (30m × 0.25mm × 0.25µm), carrier gas used was helium. The solvent used was methanol - water of linear gradient ranging between 30% to 100% methanol with in 30min time interval and flow rate was 1 mL/ min at 325 °C. The compounds eluted were monitored using ion mass spectrometer and PDA detector. The compounds present in the fungal extracts were identified by comparing the spectrum of GC-MS/MS with NISTVer.2.1 MS data library

The GC-MS/MS spectrum obtained using fungal extract was interpreted by comparing with the NIST (National Institute Standard and Technology) library hosting more than 62,000 patterns. The chemical formula, molecular mass and chemical names of the compounds present in the endophytic fungal extracts were determined

H. Assessment of antibacterial activity

The endophytic fungi isolated from different parts of *W. somnifera* were evaluated to exhibit antibacterial property by used agar plug method. The test bacteria used were Gram-positive (*Staphylococcus aureus* MTCC - 3160 and *Bacillus subtilis* MTCC - 10619) and Gram-negative (*Escherichia coli* MTCC- 443, *Proteus vulgaris* MTCC - 426, *Klebsiella pneumoniae* MTCC - 109) bacteria procured from MTCC (Microbial Type Culture Collection), Institute of Microbial Technology, Chandigarh, India were used for this experiment. The turbidity of the bacterial culture broth was compared with 0.5 McFarland standard which accounted for roughly 1.5×10^8 cfu/ mL.

The endophytic fungi associated with *W. somnifera* were screened to detect their antibacterial activity by agar plug diffusion method (Devaraju and Satish 2011). The test bacteria matching 0.5 McFarland's standard turbidity were inoculated on Mueller-Hinton Agar (MHA) plates to obtain a lawn culture. The mycelia discs (4mm) from edges of each actively growing endophytic fungi (one-week old culture) were aseptically placed on the wells bored on MHA plates pre-seeded with test bacteria. The PDA discs without fungi were used as negative control. The plates were wrapped using para film and refrigerated at 4 °C for 8 h to allow the diffusion of metabolites. The plates were incubated for 18-24h at 37 °C, visible inhibition zone was determined using a ruler in milli-meter (mm). The experiments were done in triplicates and mean value of inhibition zone was documented.

I. Statistical analysis

The test trials were accomplished in triplicates and means of inhibition zones were determined statistically and Duncan multiple range tests were done with SPSS software program version 20.

RESULTS AND DISCUSSION

A. Diversity analysis of the endophytic fungi isolated from Withania somnifera

The 50 segments of different parts of *Withania somnifera* – seed, leaf, stem, root and flower was taken for isolation of endophytic fungi (Fig. 1). The total of 165 endophytic fungi were isolated from 250 tissue segments, the fungi belonged to twenty-two different species. The sixty-four endophytic fungi were isolated using root segments, 49 using stem, 37 with leaf, 4 using seed and 11 with flower segments (Fig. 2); fungi isolated belonged to Deuteromycota and Basidiomycota under *Dothideomycetes*, *Eurotiomycetes*, *Sordariomycetes*, and *mucoromycotina* groups. The *Talaromyces radicus* and *Aspergillus niger* were the recurrent isolates from root; *Penicillium* sp., and *Fusarium oxysporum* were frequent isolates from stem; *Aspergillus flavus* and *Alternaria* sp. were dominant

isolates from leaf; *Aspergillus niger* and *Nigrospora* sp., were frequent isolates from seed; *Cladosporium* sp., was significantly isolated from flower (Table 1). The *W. somnifera* associated endophytic fungal colonization was diverse in different plant parts. The colonization rate and isolation rate were highest in root (CR = 38.78%, IR = 1.28) followed with stem (CR = 29.69%, IR = 0.98), leaf (CR = 22.42%, IR = 0.74), flower (CR = 6.66%, IR = 0.22), seed (CR = 2.42%, IR = 0.08). The stem reported highest Shannon diversity index (H') (H'= 0.94) followed with root (H'= 0.84), leaf (H'= 0.61), flower (H'= 0.5) and seed (H'= 0.3). The stem reported high Simpson diversity index (D') (D'= 0.88) with a maximum of 11 species, followed with root (D'=0.8) with 12 species, leaf (D'= 0.74) with 6 species, flower (D'= 0.7) with 4 species and seed (D'= 0.66) with 2 species. The highest Shannon evenness index (J') was in seed (J'=1) followed with leaf (J'= 0.88), stem (J'= 0.79), root (J'= 0.78) and flower (J'= 0.53) (Table 2). All the parts of *W. somnifera* hosted different species of endophytic fungi.

The present findings are in accordance with Riya and Sohrab (2022) who have reported the presence of endophytic fungi in various parts of *W. somnifera*. These results are in harmony with the findings of Khan *et al*. (2010) who also stated different parts of *W. somnifera* hosted endophytic fungi and colonization frequency was highest in stem. The present findings also agree with the findings of Tenguria and Khan (2015) who reported that *Aspergillus niger* and *Alternaria alternata* were frequently obtained in different parts of *W. somnifera*. Palem *et al*. (2015) have previously reported *Talaromyces radicus* associated with *Catharanthus roseus* produced vinblastine and also vincristine, capable of inducing apoptotic cell death.

B. Molecular Identification of selected fungal endophyte

The endophytic fungi exhibiting broad spectrum antibacterial property were further confirmed by molecular identification (chromous biotech Pvt. Ltd). The DNA was isolated using cetyltrimethylammonium bromide (cTAB) protocol and confirmed on 1% agarose gel (Fig. 3a). The isolated DNA was subjected to polymerase chain reaction (PCR) using 18S rDNA primers NS1: GTAGTCATATGCTTGTCTC and C-18L: GAAACCTTGTTACGACTT for amplification of ITS regions (Shweta *et al.*, 2010). The PCR amplified regions were visualized on 1% agarose gel using UV transilluminator (Fig. 3b). The amplified DNA fragment was sequenced and compared with fungi showing homology using Blast search. The sequences were submitted to Gen Bank to obtain NCBI accession numbers; *Talaromyces radicus* - MN099424.1, *Aspergillus niger* - MN099425.1; both these fungi were isolated from roots of *W. somnifera*.

C. Phytochemical investigation of endophytic fungi

The phytochemical investigation was done for all endophytic fungi obtained from different parts of *W. somnifera*. The 91% endophytic fungi produced terpenoids, 60% produced flavonoids, 42% showed positive for steroids, 40% showed the presence of quinones and 54% demonstrated the presence of tannins. The phenol, saponins, coumarins were absent in all the endophytic fungi tested (Table 3). This result agrees with Li *et al*. (2015). Devi *et al*. (2012) also have described the production of terpenoids, flavonoids and steroids in endophytic fungi of *Salvia miltiorrhiza* and *Centella asiatica* plants. Chaudhuri *et al.* (2012) have previously reported the phytochemical constituents of *W. somnifera* exhibited considerable antioxidant and free radical scavenging activity.

D. Screening for antibacterial activity of endophytic fungi associated with W. somnifera

The endophytic fungi obtained from different parts of *W. somnifera* were screened by agar plug method against *Staphylococcus aureus* and *Bacillus subtilis* (Gram-positive bacteria); *Escherichia coli*, *Proteus vulgaris* and *Klebsiella pneumonia* (Gram-negative bacteria) to determine their antibacterial activity (Fig. 4a-e). The 51% of endophytic fungi associated with different parts of *W. somnifera* exhibited antibacterial activity for the pathogenic bacteria tested (Table 4).

The highest inhibition zone against *B. subtilis* was by *Talaromyces radicus* - WSR2 (34mm) followed by *Chrysosporium tropicum* - WSR3 (32.33mm); *Rhizopus* sp. - WSR9 (28.33mm), *Alternaria* sp. - WSR10 (23mm), *Fusarium oxysporum* - WSSt4 (20.66mm), *Aspergillus ochraceus* - WSSt11 (20.33mm), *Aspergillus niger* - WSR1 (20.33mm), *Alternaria alternata* - WSR12 (17.33mm), *Fusarium oxysporum* - WSR11 (16.33mm), *Penicillium* sp. - WSSt7 (14.66mm), *Nigrospora* sp. - WSR8 (13.66mm), *Aspergillus terreus* - WSSt1 (12.33mm). The highest inhibition zone for *E. coli* was recorded in *Alternaria alternata*- WSR12 (32mm) followed by *F. oxysporum* - WSR11 (25.33mm), *F. oxysporum* – WSSt4 (21.66mm). The highest zone of inhibition against *K. pneumoniae* was recorded in *T. radicus* - WSR2 (21.66mm) followed by *A. niger* - WSR1 (19mm). The highest zone of inhibition against *P. vulgaris* was recorded in *Talaromyces radicus* - WSR2 (35.33mm) followed by *Chrysosporium tropicum* - WSR3 (32mm), *Rhizopus* sp.- WSR9 (30.66mm). The highest zone of inhibition against *S. aureus* was recorded in *Aspergillus niger* - WSR1 (18mm) followed by *T. radicus* -WSR2 (17.66mm).

Among the endophytic fungi screened, *Talaromyces radicus* (WSR2) and *Aspergillus niger* (WSR1) isolated from root of *W. somnifera* exhibited broad antibacterial activity against all Gram-positive and Gram-negative pathogenic bacteria tested. The 11% endophytic fungi associated with different parts of *W. somnifera* inhibited the growth of *S. aureus* and 37% inhibited *B. subtilis*, 42% inhibited *E. coli*, 42% inhibited *P. vulgaris* and 8% inhibited *K. pneumoniae*.

The present findings are correlated with Atri et al. (2020) who have reported that the endophytic fungi isolated from *W. somnifera* exhibited significant antibacterial activity. The current results agree with Owais *et al.* (2005); Salini *et al.* (2014) who have recorded the effectiveness of endophytic fungi in inhibiting the pathogenic microorganisms. The endophytic fungi isolated from medicinal plant *Dillenia*

indica is reported to exhibit considerable antibacterial activities (Bora *et al*., 2023). The antimicrobial activity was significantly reported in endophytic fungi isolated from *Zygophyllum madavillei* (Yehia *et al*., 2020). The silver nano particles synthesized from the endophytic fungus of *Withania somnifera* exhibited significant antibacterial activity (Singh *et al.*, 2015). Srinivas *et al.* (2015) recorded the endophytic fungi isolated from medicinal plants exhibited considerable antimicrobial activity.

3.5. GC-MS investigation of the selected endophytic fungal extracts

The endophytic fungi associated with *Withania somnifera* exhibiting greater absolute frequency, relative frequency, phytochemicals and antibacterial activities were chosen for GC-MS analysis. The crude ethyl acetate extracts of two endophytic fungi – *Talaromyces radicus* and *Aspergillus niger* obtained from root of *Withania somnifera* were chosen for GC-MS analysis. The chromatograms of the two endophytic fungal extracts depicted the existence of thirty-seven compounds and recognized based on molecular weight, retention time, peak area and molecular formula. The catalog of National Institute standard and Technology (NIST) was used for elucidation of GC-MS spectrum which has above 62,000 samples. The mass spectrum of unidentified compounds was matched up with the spectrum of identified compounds deposited in the NIST library. The structure, molecular weight and names of bioactive compounds present in the test samples were thus ascertained and tabulated.

The GC-MS spectrum of *Talaromyces radicus* (WSR2) ethyl acetate crude extract showed the presence of eighteen metabolites (Table 5; Fig. 5a-b) which included Maltol with Retention time (RT) - 44.40, Molecular Formula (MF) - $C_6H_6O_3$, Molecular Weight (MW) - 126, Similarity Index (SI) - 635, Reversed Search Index (RSI) - 719; 1,3,5-Trioxane with RT - 3,29, MF- $C_3H_6O_3$, MW - 90, SI - 839, RSI - 945; Diethanolamine with RT – 5.45, MF – $C_4H_{11}NO_2$, MW - 105, SI: 360, RSI - 822; Formic acid hydrazide with RT - 5.16, MF - CH_4N_2O, MW - 60, SI - 698, RSI - 961; 2-Propenoic acid, 2-hydroxyethyl ester with RT - 6.57, MF - $C_5H_8O_3$, MW - 116, SI - 574, RSI - 741; Cyclotrisiloxane, hexamethyl with RT - 7.93, MF - $C_6H_{18}O_3Si_3$, MW - 222, SI - 847, RSI - 873; 2-Thiazolidinecarboxamide, 2-methyl with RT - 10.73, MF - $C_5H_{10}N_2OS$, MW - 146, SI - 700, RSI - 824; Methanamine, 3- Furanmethanol with RT – 9.50, MF – $C_5H_6O_2$, MW - 98, SI - 817, RSI - 839; Oxime-, methoxy-phenyl with RT – 12.30, MF – $C_8H_9NO_2$, MW - 151, SI - 794, RSI - 807; 6-Oxa-bicyclo[3.1.0]hexan-3-one with RT – 13.62, MF- $C_5H_6O_2$, MW - 98, SI - 816, RSI - 854; 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-2-one with RT – 18.98, MF - $C_6H_8O_4$, MW - 144, SI - 782, RSI - 813; 2H-Pyran-2,6(3H)-dione with RT – 20.20, MF - $C_5H_4O_3$, MW - 112, SI -770, RSI - 931; 1,3-Cyclopentanedione,4-methyl with RT - 24.97, MF - $C_6H_8O_2$, MW - 112, SI - 596, RSI - 756; Trans-4-Nonene with RT - 28.42, MF - C_9H_{18}, MW - 126, SI - 536, RSI - 687; 2,5-Dimethyl-4-hydroxy-3(2H)-furanone with RT – 35.22, MF - $C_6H_8O_3$, MW - 128, SI - 660, RSI - 736; 6-methyl-2-pyrazinylmethanol with RT - 35.81, MF - $C_6H_8N_2O$, MW -124, SI - 550, RSI - 628; 2,4,5-Trihydroxypyrimidine with RT - 37.36, MF - $C_4H_4N_2O_3$, MW - 128, SI - 769, RSI - 824; D-Alanine, N-propargyloxycarbonyl-tridecyl ester with RT - 39.71, MF - $C_{20}H_{35}NO_4$, MW -353, SI - 683, RSI - 731.

The compounds obtained in our study are also reported previously by many researchers. The maltol has been reported by Kadhim *et al*. (2016) in *Candida albicans* and exhibited significant antibacterial activity. Cho *et al.* (2008) have reported the presence of maltol in medicinal herbs and is used as potent medicine for preventing various diseases and also used in skin care. Pandey *et al.* (2014) also has reported the presence of maltol in *Limonia acidissima* fruit and it is used as antibacterial, antioxidant and flavor enhancer. Thompson *et al.* (2004) have reported the metal maltol complexes exhibits significant hyperglycaemic activity. The 1, 3, 5 Trioxane obtained in our study is reported by Strobel (2014) in endophytic fungus *Hypoxylon* sp. producing fuel related hydrocarbons. The 1, 3, 5 Trioxane was also reported from *Aspergillus brasilensis* isolated from root of *Baliospermum montanum* (Jagannath *et al.*, 2021). The cyclotrisil oxane hexamethyl is also obtained from West Anatolian olive (*Oleaeuropaea* L.) leaves and has exhibited significant antimicrobial activity (Keskin *et al.*, 2012). Mousa and Raizada (2013) reported that oxime moieties when introduced to sordarin amplified antifungal activity against *Candida glarrata* and *C. albicans*. The GC-MS analysis of *Cinnamomum zeylanicum* also has discovered the presence of 6-oxa-bicyclo [3.1.0] hexan-3-one and reported to have both antibacterial and antifungal activity (Hameed *et al*., 2016). Rodin *et al.* (1965) has reported that 2, 5-Dimethyl-4-hydroxy-3(2H)-furanone as aroma enhancer.

The GC-MS spectrum of the ethyl acetate extract of *Aspergillus niger* (WSR1) discovered the existence of nineteen compounds (Table 6 and Fig. 6a-b) which included 1, 2-Benzisothiazol-3-amine tbdms with RT - 6.04, MF - $C_{13}H_{20}N_2SSi$, MW - 264, SI - 465, RSI - 626; 2,5-Dimethyl-1-ethylcyclotetrazenoborane with RT - 41.41, MF - $C_4H_{11}BN_4$, MW - 126, SI - 556, RSI - 728; Methoxyacetic acid with RT - 4.90, MF - $C_3H_6O_3$, MW - 90, SI - 878, RSI - 907; 2-Furancarboxaldehyde with RT - 6.63, MF - $C_5H_4O_2$, MW - 96, SI - 554, RSI - 859; 2-Furanmethanol with RT - 9.15, MF - $C_5H_6O_2$, MW - 98, SI - 551, RSI - 712; Ethoxy(methoxy)methylsilane with RT - 10.83, MF - $C_4H_{12}O_2Si$, MW - 120, SI - 589, RSI - 741; Oxine-,methoxy-phenyl with RT - 11.70, MF - $C_8H_9NO_2$, MW - 151, SI - 773, RSI - 782; 2-Cyclopenten-1-one,2-hydroxy- with RT - 12.94, MF - $C_5H_6O_2$, MW - 98, SI - 818, RSI - 975; Cyclotrisiloxane, hexamethyl with RT - 7.73, MF - $C_6H_{18}O_3Si_3$, MW - 222, SI - 869, RSI - 885; 2,4-Dihydroxyl-2,5-dimethyl-3(2H)furan-3-one with RT - 18.16, MF - $C_6H_8O_4$, MW - 144, SI - 832, RSI - 862; Phenyl-pentamethyl-disiloxane with RT - 14.29, MF - $C_{11}H_{20}OSi_2$, MW - 224, SI - 616, RSI - 695; 2H-Pyran-2, 6(3H)-dione with RT - 19.18, MF - $C_5H_4O_3$, MW - 112, SI - 668, RSI - 936; Oxazolidine,2,2-diethyl-3-methyl with RT - 20.20, MF - $C_8H_{17}NO$, MW - 143, SI - 585, RSI – 885; 2,3,5-Trioxabicyclo[2.1.0]pentane,1,4-bis(phenylmethyl)

with RT - 24.41, MF - $C_{16}H_{14}O_3$, MW - 254, SI - 537, RSI - 789; 2,5-Dimethyl-4-hydroxy-3(2H)-furanone with RT - 31.04, MF - $C_6H_8O_3$, MW - 128, SI - 609, RSI - 770; Bis(succinimido) methanone with RT - 27.02, MF – $C_9H_8N_2O_5$, MW - 224, SI - 501, RSI - 703; 2,5-Dimethyl-4-hydroxy-3(2H)-furanone with RT - 35.19, MF - $C_6H_8O_3$, MW - 128, SI - 665, RSI - 672; 1-Propanone,1-(2-furanyl) with RT - 33.79, MF - $C_7H_8O_2$, MW - 124, SI - 654, RSI - 807; D-Alanine, N-propargyloxycarbonyl-,isohexylester with RT - 35.70, MF -$C_{13}H_{21}NO_4$, MW - 255, SI - 735, RSI - 788.

The compounds obtained in our study are also reported previously by many researchers. The 1.2-benzisothiazol-3-amine has been reported in ethanol root extract of *Acacia karroo* and exhibited antibacterial activity (Maroyi, 2017). The methoxy acetic acid obtained in our endophytic fungi is also reported as an active metabolite of ester phthalates extensively used as viscosity, gelling and stabilizing agent in industry. It is also described to curb prostate cancer cell augmentation by initiating apoptosis (Parajuli *et al*., 2015). The 2-Furanmethanol derivatives are reported previously to exhibit antioxidant, anti-inflammatory and wound healing properties (Devasvaran and Yong 2016). The cyclotrisiloxane, hexamethyl produced by endophytic fungi in this study is also reported in the aqueous extract of West Anatolian olive (*Olea europaea* L.) leaves and has considerable antimicrobial activity (Keskin *et al.,* 2012). The 2, 4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one is also reported in *Aspergillus niger* isolated from dried fruits and has significantly inhibited *Staphylococcus aureus, Pseudomonas aeruginosa*, *Proteus mirabilis, E. coli* and *Klebsiella pneumoniae* exhibiting broad spectrum antibacterial property (Hameed *et al.,* 2015). The 2,5-dimethyl-4-hydroxy-3(2H)-furanone (DMHF) is a compound with fragrance and is found in various fruits and has significant therapeutic properties including antimicrobial activity inhibiting nosocomial pathogens (Sung *et al.,* 2007). The D-alanine, N-propargyloxycarbonyl-, isohexyl ester is obtained from the biodrugs extracted from kiwi fruit (Dong *et al.,* 2019). The D-alanine, N-propargyloxycaronyl-, isohexyl ester is also reported in Inky cap mushroom (*Coprinus* sp.) and possess antifungal activity against various pathogenic wilt causing *Fusarium* sp. (Jeeva and Krishnamoorthy 2018). The bioactive metabolites possessing antibacterial properties were also reported from endophytic *Talaromyces trachyspermus* (Farhat *et al*., 2022).

The compounds like cyclotrisiloxane, hexamethyl; oxime, methoxy-phenyl; 2, 4-dihydroxy-2, 5-dimethyl-3(2H)-furan-3-one; 2, 5-dimethyl-4-hydroxy-3(2H)-furanone are reported in both the endophytic fungi *Talaromyces radicus* and *Aspergillus niger* obtained from root of *W. sominifera*. The total of thirty-seven bioactive compounds are obtained in the ethyl acetate extracts of two endophytic fungi *Talaromyces radicus* and *Aspergillus niger* obtained from root of *W. sominifera* indicates that the endophytic fungi are reservoirs of countless such biologically active metabolites which needs to be explored and applied for the benefit of mankind and nature. The selection of host plant is of significant importance when working with endophytic fungi. The exploration of endophytic fungi is a promising field and all plants surely harbour fungi with several bioactive contents and activities (Nisa *et al.,* 2018).

Table 1: Frequency of endophytic fungi isolated from different parts of *Withania somnifera.*

Sr. No.	Endophytic Fungi	Root		Stem		Leaf		Seed		Flower		Total	
		F	fr%	f	fr%	f	fr%	f	fr%	F	fr%	F	fr%
1	*Talaromyces radicus*	23	35.93	0	0	0	0	0	0	0	0	15	9.09
2	*Alternaria alternata*	5	7.81	0	0	0	0	0	0	0	0	5	3.03
3	*Aspergillus* sp.	4	6.25	0	0	0	0	0	0	0	0	4	2.42
4	*Helminthosporium* sp.	4	6.25	0	0	0	0	0	0	0	0	4	2.42
5	*Aspergillus niger*	15	23.43	2	4.08	0	0	2	50	0	0	6	3.63
6	*Nigrospora* sp.	3	4.68	6	12.24	1	2.7	2	50	0	0	17	10.3
7	*Chrysosporium tropicum*	1	1.56	0	0	0	0	0	0	0	0	1	0.6
8	*Colletotrichum* sp.	1	1.56	0	0	0	0	0	0	0	0	1	0.6
9	*Fusarium oxysporum*	2	3.12	9	18.36	0	0	0	0	0	0	11	6.66
10	*Alternaria* sp.	3	4.68	2	4.08	10	27.02	0	0	1	9.09	16	9.69
11	*Rhizopus* sp.	2	3.12	4	8.16	0	0	0	0	1	9.09	28	16.96
12	*Cladosporium* sp.	1	1.56	0	0	0	0	0	0	5	45.45	6	3.63
13	*Penicillium* sp.	0	0	11	22.44	2	5.4	0	0	0	0	8	4.84
14	*Aspergillus flavus*	0	0	0	0	15	40.54	0	0	0	0	15	9.09
15	*Cladosporium cladosporioides*	0	0	0	0	4	10.81	0	0	0	0	4	2.42
16	*Fusarium* sp.	0	0	0	0	5	13.51	0	0	0	0	5	3.03
17	*Penicillium notatum*	0	0	0	0	0	0	0	0	4	36.36	4	2.42
18	*Aspergillus terreus*	0	0	1	2.04	0	0	0	0	0	0	1	0.6
19	*Pestalotiopsis* sp.	0	0	2	4.08	0	0	0	0	0	0	2	1.21
20	*Curvularia* sp.	0	0	2	4.08	0	0	0	0	0	0	2	1.21
21	*Nodulisporium sp.*	0	0	5	10.2	0	0	0	0	0	0	5	3.03
22	*Aspergillus ochraceus*	0	0	5	10.2	0	0	0	0	0	0	5	3.03
Total		64	100	49	100	37	100	4	100	11	100	165	100
Colonization rate (CR%)		38.78%		29.69%		22.42%		2.42%		6.66%		100%	
Isolation Rate (IR)		1.28		0.98		0.74		0.08		0.22		0.66	

Note: f-absolute frequency, fr-relative frequency

Table 2. Diversity, evenness and species richness of endophytic fungi isolated from different segments of *Withania somnifera.*

Withania somnifera parts	H'	D'	J'	S
Root	0.84	0.80	0.78	12
Stem	0.94	0.88	0.79	11
Leaf	0.61	0.74	0.88	6
Seed	0.3	0.66	1	2
Flower	0.5	0.7	0.53	4

Note: H'=Shannon frequency, D'=Simpson frequency, J'=Evenness, S=Species richness

Table 3: Phytochemical analysis of ethyl acetate extract of endophytic fungi from *Withania somnifera.*

Fungal isolate	Plant part	Endophytic fungi	Phytochemical test							
			Flavonoids	Steroids	Phenols	Quinones	Tannins	Saponins	Coumarins	Terpenoids
WSR1	Root	*Aspergillus niger*	+	+	-	+	+	-	-	+
WSR2		*Talaromyces radicus*	+	+	-	+	+	-	-	+
WSR3		*Chrysosporium tropicum*	+	+	-	+	-	-	-	-
WSR4		*Aspergillus* sp.	+	+	-	+	-	-	-	+
WSR5		*Helminthosporium* sp.	+	-	-	-	-	-	-	+
WSR6		*Colletotrichum* sp.	+	-	-	+	+	-	-	+
WSR7		*Cladosporium* sp	+	-	-	-	+	-	-	+
WSR8		*Nigrospora* sp.	+	+	-	-	+	-	-	+
WSR9		*Rhizopus* sp.	+	-	-	-	-	-	-	+
WSR10		*Alternaria* sp.	+	-	-	-	-	-	-	+
WSR11		*Fusarium oxysporum*	-	-	-	-	-	-	-	+
WSR12		*Alternaria alternata*	+	+	-	-	-	-	-	+
WSL1	Leaf	*Aspergillus flavus*	-	+	-	+	+	-	-	+
WSL2		*Alternaria* sp.	+	-	-	-	+	-	-	+
WSL3		*Penicillium* sp.	+	+	-	+	+	-	-	+
WSL4		*Nigrospora* sp.	-	+	-	-	+	-	-	-
WSL5		*Fusarium*sp.	-	+	-	-	+	-	-	+
WSL6		*Cladosporium cladosporioides*	-	-	-	+	+	-	-	+
WSF1	Flower	*Penicillium notatum*	-	-	-	+	-	-	-	+
WSF2		*Aspergillus* sp.	+	-	-	-	+	-	-	+
WSF3		*Rhizopus*sp.	+	+	-	-	+	-	-	+
WSF4		*Cladosporium* sp.	-	+	-	+	-	-	-	+
WSSt1	Stem	*Aspergillus terreus*	-	-	-	-	-	-	-	+
WSSt2		*Aspergillus niger*	-	-	-	-	-	-	-	+
WSSt3		*Nigrospora* sp.	+	+	-	-	+	-	-	-
WSSt4		*Fusariumo xysporum*	+	-	-	+	+	-	-	+
WSSt5		*Alternaria* sp.	+	-	-	+	+	-	-	+
WSSt6		*Rhizopus*sp.	-	-	-	-	+	-	-	+
WSSt7		*Penicillium* sp	-	+	-	+	-	-	-	+
WSSt8		*Curvularia* sp	-	-	-	-	+	-	-	+
WSSt9		*Pestalotiopsis* sp.	-	-	-	-	-	-	-	+
WSSt10		*Nodulisporium* sp.	+	-	-	-	-	-	-	+
WSSt11		*Aspergillus ochraceus*	+	-	-	-	-	-	-	+
WSS1	Seed	*Aspergillus niger*	-	-	-	-	-	-	-	+
WSS2		*Nigrospora* sp.	+	+	-	+	+	-	-	+

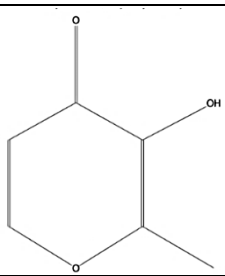
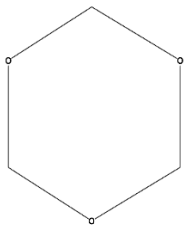
Note: + Present, - Absent

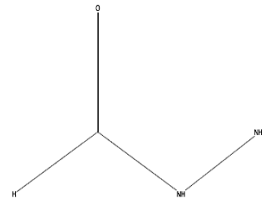
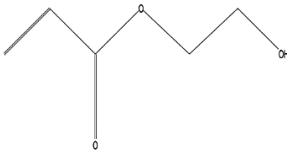
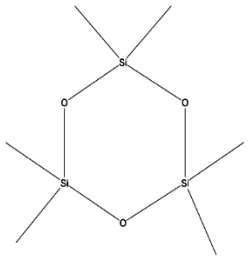
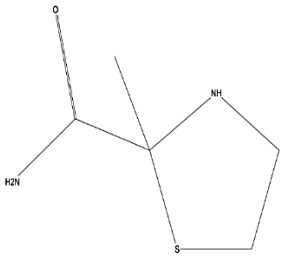
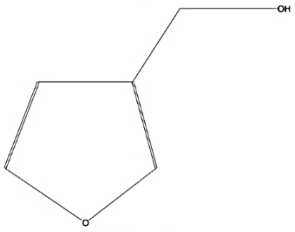
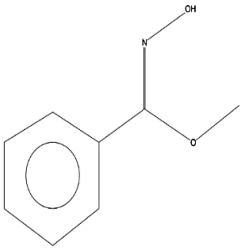
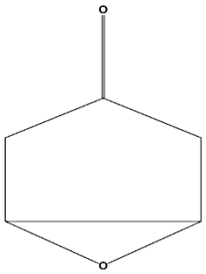
Table 4: Antibacterial activity of endophytic fungi isolated from *Withania somnifera* by agar plug method.

Sr. No.	Fungal isolate	Plant parts	Endophytic fungi	Zone of Inhibition in mm ±SD; n=3				
				P. vulgaris	***E. coli***	***K. pneumoniae***	***S. aureus***	***B. subtillis***
1	WSR1	Root	*Aspergillus niger*	18.66±1.52 ^{de}	11.33 ±1.52^{b}	19 ± 1^{c}	18.33 ±1.52^{c}	20.33 ±2.51^{g}
2	WSR2		*Talaromyces radicus*	35.33 ± 0.57^{i}	18.66 ± 1.15^{f}	21.66 ± 1.52^{d}	17.66 ±2.51^{c}	34 ± 1.73^{k}
3	WSR3		*Chrysosporium tropicum*	32 ± 2^{h}	12.66 ± 0.57^{bc}	0^{a}	0^{a}	32.33± 1.52^{j}
4	WSR4		*Aspergillus* sp.	13.66 ± 1.52^{b}	13 ± 1.73^{c}	13.66 ± 1.52^{b}	0^{a}	11.66 ± 1.52^{b}
5	WSR5		*Helminthosporium* sp.	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
6	WSR6		*Colletotrichum* sp.	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
7	WSR7		*Cladosporium* sp.	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
8	WSR8		*Nigrospora* sp.	13 ± 1.73^{b}	18.33 ± 0.57^{f}	0^{a}	0^{a}	13.66 ±1.52^{bcd}
9	WSR9		*Rhizopus* sp.	30.66 ± 1.15^{h}	0^{a}	0^{a}	0^{a}	28.33± 1.52^{i}
10	WSR10		*Alternaria* sp.	20.66 ± 1.52^{ef}	14.66 ± 1.52^{d}	0^{a}	0^{a}	23 ± 2^{h}
11	WSR11		*Fusarium oxysporum*	23 ± 1^{g}	25.33 ± 0.57^{h}	0^{a}	0^{a}	16.33±0.57^{ef}
12	WSR12		*Alternaria alternata*	19.33 ± 1.52^{e}	32 ± 2^{hi}	0^{a}	11.66±1.52^{b}	17.33±1.5^{f}
13	WSL1	Leaf	*Aspergillus flavus*	16.66 ± 1.15^{cd}	13 ± 1^{c}	0^{a}	0^{a}	0^{a}
14	WSL2		*Alternaria* sp.	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
15	WSL3		*Penicillium* sp.	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
16	WSL4		*Nigrospora* sp.	16.66 ± 0.57^{cd}	15.66 ± 0.57^{e}	0^{a}	0^{a}	0^{a}
17	WSL5		*Fusarium* sp.	22.33 ± 0.57^{fg}	0^{a}	0^{a}	0^{a}	0^{a}
18	WSL6		*C.cladosporioides*	0^{a}	21.33 ± 1.52^{g}	0^{a}	0^{a}	0^{a}
19	WSF1	Flower	*Penicillium notatum*	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
20	WSF2		*Aspergillus* sp.	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
21	WSF3		*Rhizopus* sp.	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
22	WSF4		*Cladosporium* sp.	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
23	WSSt1	Stem	*Aspergillus terreus*	16.66 ± 1.15^{cd}	13 ± 1.73^{c}	0^{a}	0^{a}	12.33±1.52^{bc}
24	WSSt2		*Aspergillus niger*	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
25	WSSt3		*Nigrospora* sp.	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
26	WSSt4		*Fusarium oxysporum*	16.66 ± 0.57^{cd}	21.66 ± 1.52^{gh}	0^{a}	0^{a}	20.66±2.08^{gh}
27	WSSt5		*Alternaria* sp.	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
28	WSSt6		*Rhizopus* sp.	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
29	WSSt7		*Penicillium* sp	0^{a}	14.66 ± 0.57^{d}	0^{a}	0^{a}	14.66±1.15^{cde}
30	WSSt8		*Curvularia* sp	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
31	WSSt9		*Pestalotiopsis* sp.	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
32	WSSt10		*Nodulisporium* sp.	22.33 ±0.57^{fg}	0^{a}	0^{a}	11 ± 1^{b}	0^{a}
33	WSSt11		*Aspergillus ochraceus*	0^{a}	11.5 ± 1.15^{b}	0^{a}	0^{a}	20.33± 1.52^{g}
34	WSS1	Seed	*Aspergillus niger*	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
35	WSS2		*Nigrospora* sp.	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
Positive control				22±0.89^{fg}	21±1^{gh}	20±0.93^{cd}	17±0.9^{c}	20±0.89^{g}

Note: Positive control: Ampicillin 10µg/mL; in each column, mean values followed by thesameletter are not significantly different according to DMRT at $p < 0.05$.

Table 5: GC-MS analysis of metabolites in the ethyl acetate crude extract of *Talaromyces radicus* isolated from the root of *Withania somnifera.*

Sr. No.	Constituents	RT	MF	MW	SI	RSI	Structure
1.	Maltol	44.43	$C_6H_6O_3$	126	635	719	
2.	1,3,5-Trioxane	3.29	$C_3H_6O_3$	90	839	945	
3	Diethanolamine	5.45	$C_4H_{11}NO_2$	105	360	822	

4	Formic acid hydrazide	5.16	CH_4N_2O	60	698	961	
5	2-Propenoic acid, 2-hydroxyethyl ester	6.57	$C_5H_8O_3$	116	574	741	
6	Cyclotrisiloxane, hexamethyl-	7.93	$C_6H_{18}O_3Si_3$	222	847	873	
7	2-Thiazolidinecarboxamide, 2-methyl-	10.73	$C_5H_{10}N_2OS$	146	700	824	
8	3-Furanmethanol	9.50	$C_5H_6O_2$	98	817	839	
9	Oxime-, methoxy-phenyl-	12.30	$C_8H_9NO_2$	151	794	807	
10	6-Oxa-bicyclo[3.1.0]hexan-3-one	13.62	$C_5H_6O_2$	98	816	854	

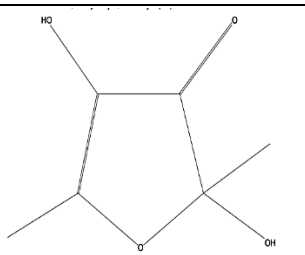
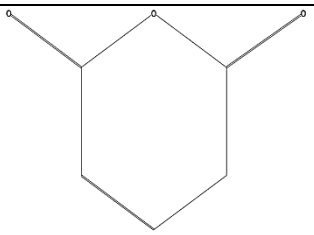
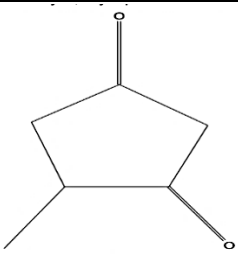
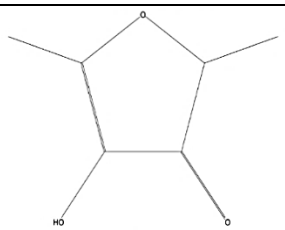
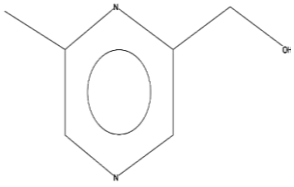
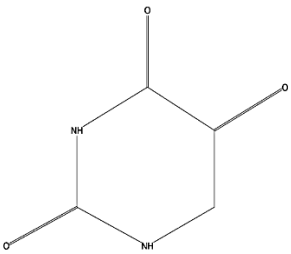
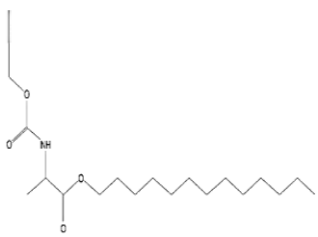
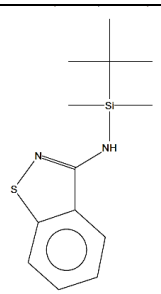
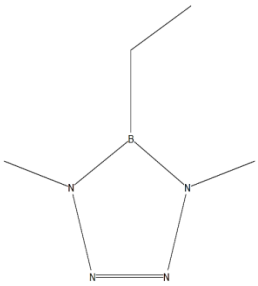
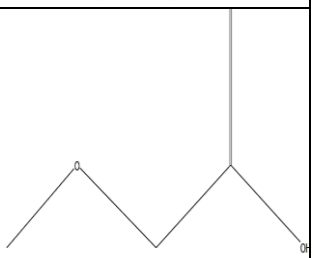
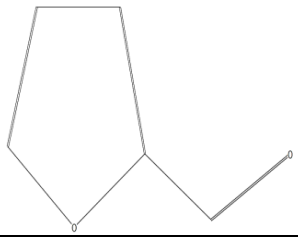
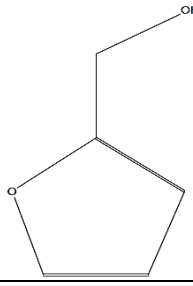
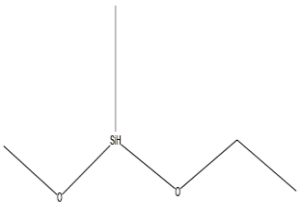
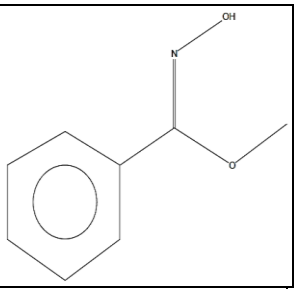
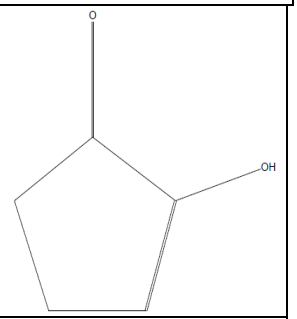
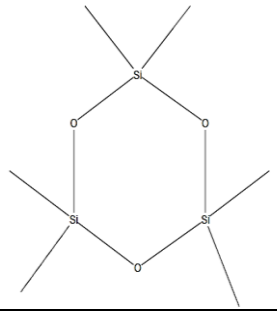
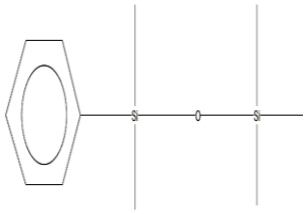
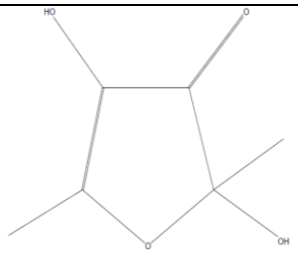
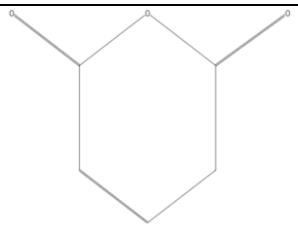
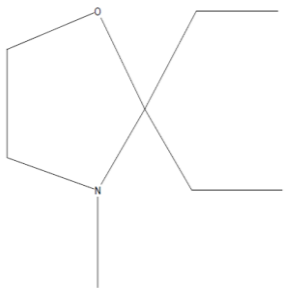
11	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	18.98	$C_6H_8O_4$	144	782	813	
12	2H-Pyran-2,6(3H)-dione	20.20	$C_5H_4O_3$	112	770	931	
13	1,3-Cyclopentanedione, 4-methyl-	24.97	$C_6H_8O_2$	112	596	756	
14	Trans-4-Nonene	28.42	C_9H_{18}	126	536	687	
15	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	35.22	$C_6H_8O_3$	128	660	736	
16	6-Methyl-2-pyrazinylmethanol	35.81	$C_6H_8N_2O$	124	550	628	
17	2,4,5-Trihydroxypyrimidine	37.36	$C_4H_4N_2O_3$	128	769	824	
18	D-Alanine, N-propargyloxycarbonyl-, tridecyl ester	39.71	$C_{20}H_{35}NO_4$	353	683	731	

Table 6: GC-MS analysis of metabolites in the ethyl acetate crude extract of *Aspergillus niger* isolated from root of *Withania somnifera.*

Sr. No.	Constituents	RT	MF	MW	SI	RSI	Structure
1.	1,2-Benzisothiazol-3-amine tbdms	6.04	$C_{13}H_{20}N_2SSi$	264	465	626	
2.	2,5-Dimethyl-1-ethylcyclotetrazenoborane	41.41	$C_4H_{11}BN_4$	126	556	728	
3.	Methoxyacetic acid	4.90	$C_3H_6O_3$	90	878	907	
4.	2-Furancarboxaldehyde	6.63	$C_5H_4O_2$	96	554	859	
5.	2-Furanmethanol	9.15	$C_5H_6O_2$	98	551	712	
6.	Ethoxy(methoxy)methylsilane	10.83	$C_4H_{12}O_2Si$	120	589	741	

7.	Oxime-, methoxy-phenyl	11.70	$C_8H_9NO_2$	151	773	782	
8.	2-Cyclopenten-1-one, 2-hydroxy	12.94	$C_5H_6O_2$	98	818	975	
9.	Cyclotrisiloxane, hexamethyl	7.73	$C_6H_{18}O_3Si_3$	222	869	885	
10.	Phenyl-pentamethyl-disiloxane	14.29	$C_{11}H_{20}OSi_2$	224	616	695	
11.	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	18.16	$C_6H_8O_4$	144	832	862	
12.	2H-Pyran-2,6(3H)-dione	19.18	$C_5H_4O_3$	112	668	936	
13.	Oxazolidine, 2,2-diethyl-3-methyl	20.20	$C_8H_{17}NO$	143	585	885	

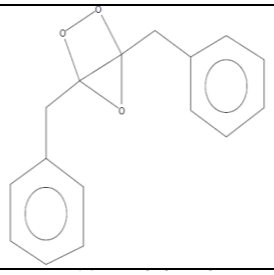
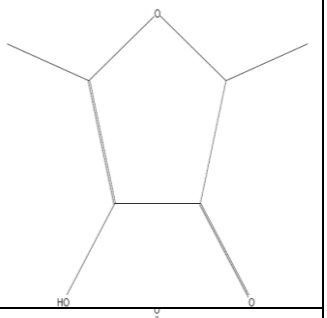
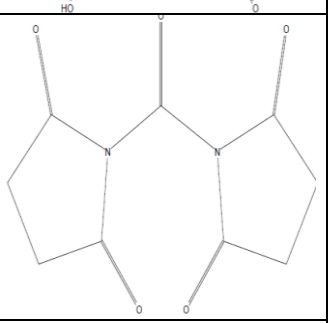
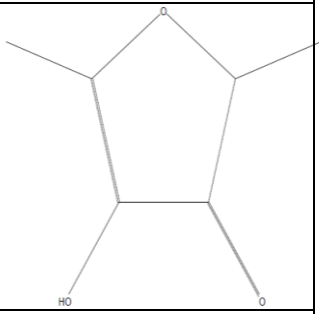
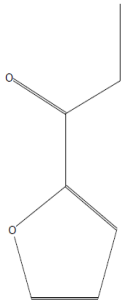
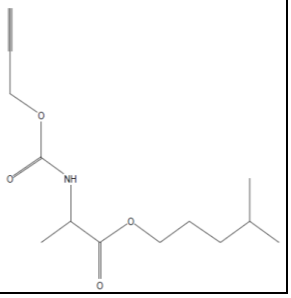
14.	2,3,5-Trioxabicyclo[2.1.0]pentane, 1,4-bis(phenylmethyl)	25.41	$C_{16}H_{14}O_3$	254	537	789	
15.	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	31.04	$C_6H_8O_3$	128	609	770	
16.	Bis(succinimido) methanone	27.02	$C_9H_8N_2O_5$	224	501	703	
17.	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	35.19	$C_6H_8O_3$	128	665	672	
18.	1-Propanone, 1-(2-furanyl)	33.79	$C_7H_8O_2$	124	654	807	
19.	D-Alanine, N-propargyloxycarbonyl-, isohexyl ester	35.70	$C_{13}H_{21}NO_4$	255	735	788	

Fig. 1. *Withania somnifera* plant and its parts. a) *W. somnifera* growing in field. b) Stem, c) Seed, d) Root, e) Leaves, f) Flower.

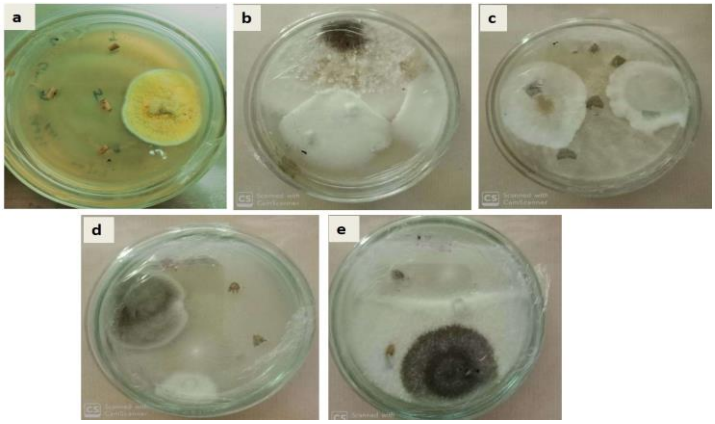


Fig. 2. The endophytic fungi of *Withania somnifera* emerged from different tissue segments on PDA. a) Endophytic fungi from root segments b) Stem segments c) Leaf d) Flower e) Seeds.

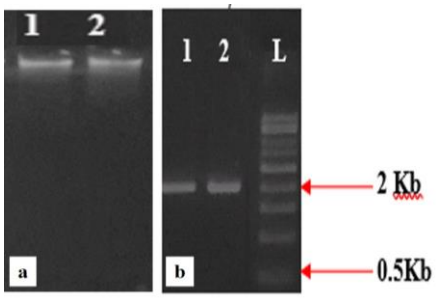


Fig. 3a. Genomic DNA loaded on 1% agarose gel, **3b.**PCR amplicon (~1.8kb) loaded on 1% agarose gel.

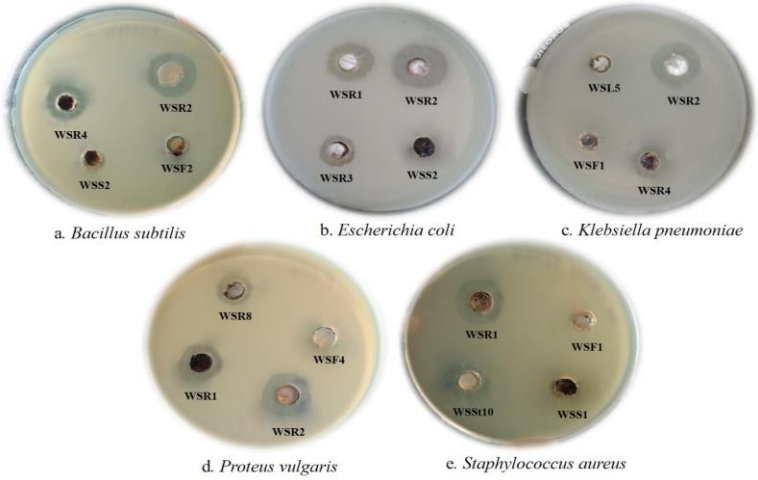


Fig. 4 (a–e) Antibacterial activity of endophytic fungi isolated from different parts of *Withania somnifera* by agar plug method.

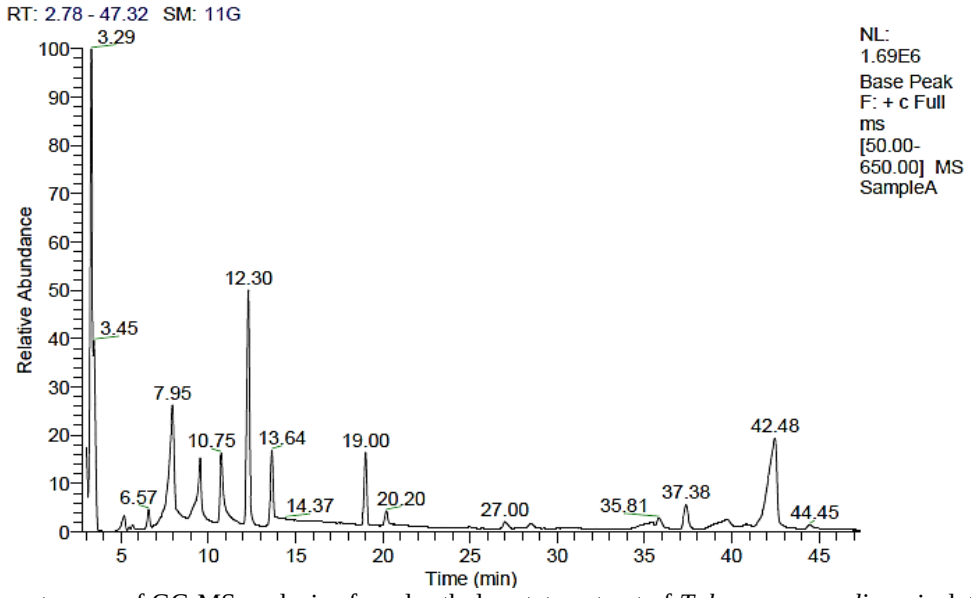


Fig. 5a. Chromatogram of GC-MS analysis of crude ethyl acetate extract of *Taloromyces radicus* isolated from root of *Withania somnifera*.

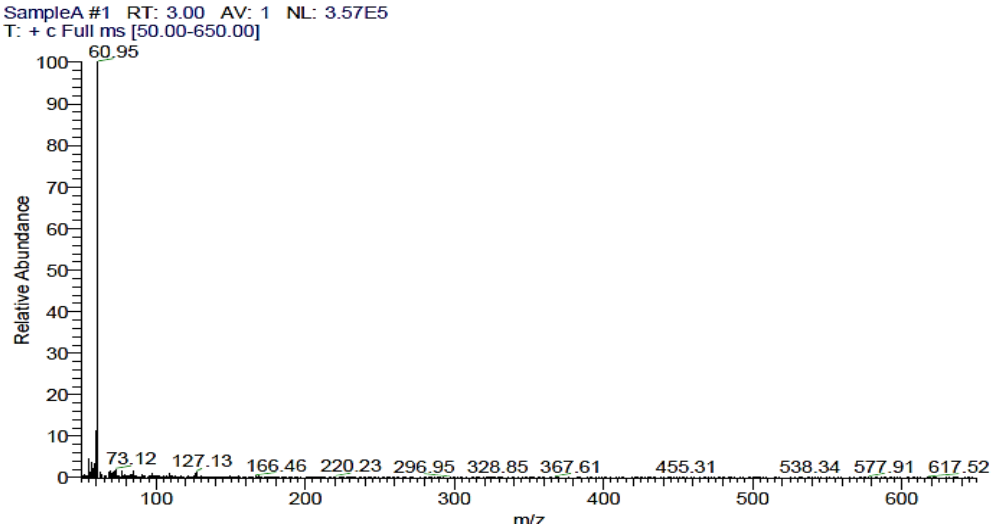


Fig. 5b. GC-MS spectrum of ethyl acetate extracts of *Taloromyces radicus* isolated from root of *Withania somnifera*.

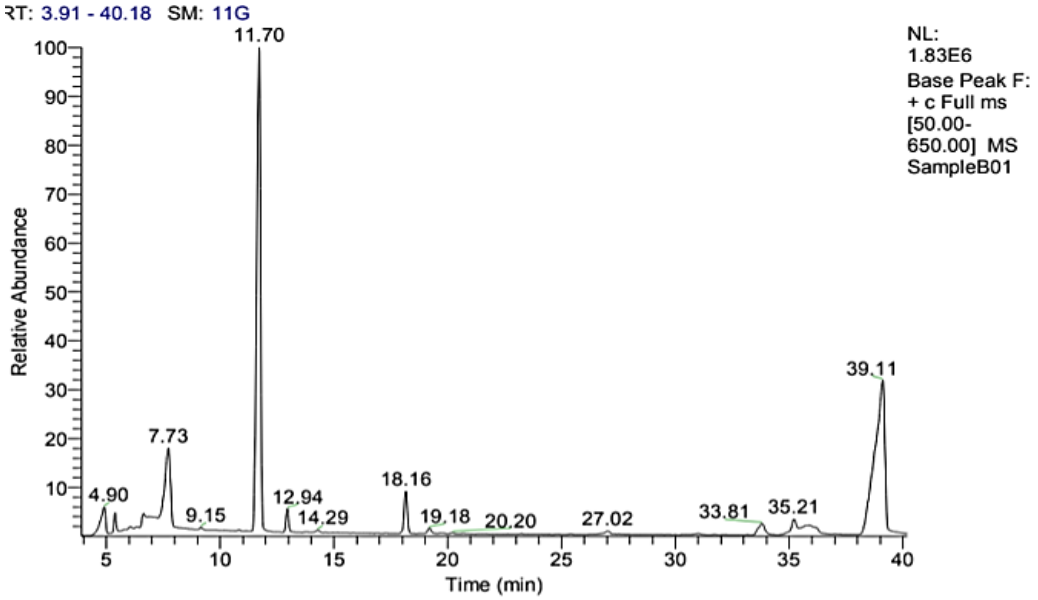


Fig. 6a. Chromatogram of GC-MS analysis of crude ethyl acetate extract of *Aspergillus niger* isolated from root of *Withania somnifera*.

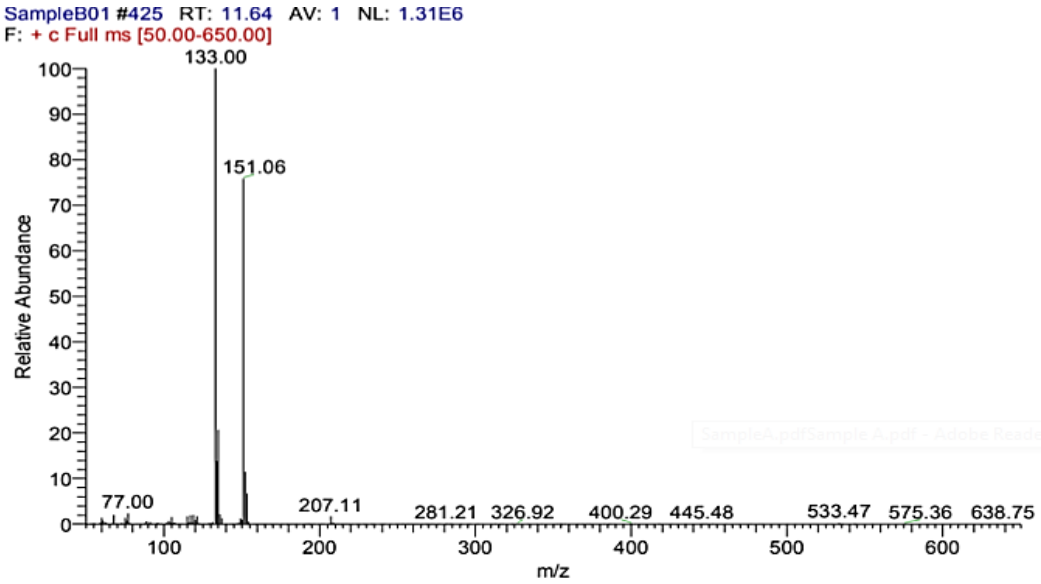


Fig. 6b. GC/MS/MS spectrum of ethyl acetate extracts of *Aspergillus niger* isolated from root of *Withania somnifera*.

CONCLUSIONS

The present results revealed that the endophytic fungi associated with the medicinal plants are tremendous reservoirs of many bioactive metabolites which can be used for the betterment of mankind and nature.

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