



Assessment of Phytochemicals, Antioxidant Activity and Enzyme Production of Endophytic Fungi Isolated from Medicinal Plant Sources

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Article History	Abstract
<p>Received: 28 Sept 2023 Revised: 21 Oct 2023 Accepted: 02 Nov 2023</p> <p>CC License CC-BY-NC-SA 4.0</p>	<p><i>Endophytic fungi are mitosporic and meiosporic ascomycetes that asymptotically reside in the internal tissues of plants beneath the epidermal cell layer, where fungi colonise healthy and living tissue via quiescent infections. Endophytes are important components of microbial diversity. Endophytic fungi isolated from medicinal plants more likely exhibit pharmaceutical potentials. These plentiful natural products isolated from endophytes represent a huge reservoir which offers an enormous potential for exploitation for medicinal, agricultural and industrial uses. There has been a great interest in endophytic fungi as potential producers of novel, biologically active products. Endophytes are believed to carry out a resistance mechanism to overcome pathogenic invasion by producing secondary metabolites. Globally, there are at least one million species of endophytic fungi in all plants which can potentially provide a variety of structurally unique natural products such as alkaloid, benzopyranones, chinones, flavanoids, phenols, steroids, xanthenes and others. Therefore, there is an ample opportunity to unearth novel and interesting endophytic microorganisms with significant therapeutic efficacy. The objectives of our current study are to isolate endophytic fungi from specific medicinal plants found locally and carry out their characterization and isolation, followed by qualitative and quantitative assessment of secondary metabolites produced by them and study their antioxidant and enzyme activities. The present study, therefore would highlight the growing concept that the bioactive compounds produced by the endophytes not only establishes host endophyte relationship but also have an immense chance of application in the field of medicine, agriculture and industry.</i></p> <p>Keywords: <i>Endophytic fungi, phytochemicals, enzyme activity, medicinal plants</i></p>

1. Introduction

The term 'endophyte' includes all organisms that grow inside plant tissues without causing disease symptoms. This is contrasted to epiphytes, which refer to the organisms on the outside of the plant (Petrini *et al.*, 1991). Endophytic fungi are an unexplored group of organisms that have enormous potentials for new pharmaceutical substances. Endophytic fungi have attracted great attention in the past few decades due to their ability to produce novel secondary metabolites for medical, agricultural and industrial use. Endophytic fungi are also considered as an outstanding source of bioactive compounds due to their ability to occupy any plants at any environment Endophytic fungi live in their host plants and due to this, they must develop certain chemical strategies that favor their existence. By producing metabolites, the endophytic fungi either protect the host from animal or herbivore attack or from other pathogenic microbes' infection that will decrease the fungi's colonization (Jeffrey *et al.*, 2008). These metabolites, when isolated and characterized, have potential for use in industries, agriculture and in medicines. They represent an important and quantifiable estimate of at least 1 million species of fungal diversity mostly belonging to the class Ascomycetes, Basidiomycetes and Deuteromycetes (Kumar *et al.*, 2014). The bioactive metabolites produced by endophytic fungi originate

from different biosynthetic pathways and belong to diverse structural groups such as terpenoids, steroids, quinones, phenols, coumarins etc. Endophytes therefore, represent a chemical reservoir for new compounds such as, anticancer, immunomodulatory, antioxidant, antiparasitic, antiviral, antitubercular, insecticidal etc. for use in the pharmaceutical and agrochemical industries (Kaul *et al.*, 2012).

Endophytes perform antioxidant activity against reactive oxygen species (ROS) like superoxide, hydroxyl radical and hydrogen peroxide produced in the metabolic process within the living cells of all organisms. Fungal endophytes may act as an alternative easily available low-cost source of antioxidant. Many endophytes synthesize bioactive compounds that can be used by plants for defense against pathogens and some of these compounds may be valuable as pharmaceutical drugs (Strobel *et al.*, 2003). Antimicrobial resistance has been a major health issue and still presents threat to healthcare systems globally. Thus, this global problem has led to the increase in researches featuring endophytic fungi, particularly those isolated from medicinal plants for their potential as sources of new antibiotics (Jia *et al.*, 2016). Like other organisms invading plant tissues, endophytic fungi produce extracellular hydrolases as a resistance mechanism against pathogenic invasion and to obtain nutrition from host. Fungal enzymes are used in food, beverages, confectionaries, textiles and leather industries to simplify the processing of raw materials. They are often more stable than enzymes derived from other sources. The array of enzymes produced differs between fungi and often depends on the host and their ecological factors. Such enzymes include pectinases, cellulases, lipases, xylanase, phosphatase, proteinase, lignocellulase, ligninase (Sunitha *et al.*, 2013).

2. Materials And Methods

Collection of plant materials:

Healthy leaves of *Centella asiatica* and *Aloe vera* were collected from Purba Bardhaman district of West Bengal. The plant materials were brought to the laboratory in sterile polythene bags and processed within eight hours on the same day after collection to reduce the chance of contamination in the isolation process of the fungal endophytes.

Isolation of endophytic fungi:

Leaves collected from both the plants (*Centella asiatica* and *Aloe vera*) were at first washed with running tap water for 30 minutes and then washed three times with sterilized distilled water. After the process of washing with water, the plant materials were subjected to surface sterilization by treating sequentially with ethanol (70%) for 1 minute, NaOCl (3%) for 3 minutes and again ethanol (70%) for 30 seconds, followed by washing with sterilized distilled water for 2 times. After surface sterilization the leaves were soaked with sterilized Whatman No. 1 filter paper. The leaves were then cut with the help of sterilized cork borer to obtain round leaf discs (diameter 3mm). The leaf discs were transferred separately into the Petri plates (three segments in each plate) containing sterilized Potato Dextrose Agar (PDA) medium supplemented with streptomycin (100 units/ml) for isolation of endophytic fungi. The plates were then sealed with parafilm, incubated for 7 days at 30°C and inspected at two days interval for observing the fungal endophytes coming out of the samples. The organisms which initiate to grow during the period of incubation were transferred to PDA slants to develop pure cultures.

Identification of endophytic fungi:

The isolated fungal endophytes were initially identified based on morphological features involving cultural characteristics and light microscopic observations following the protocol mentioned in the relevant literatures wherever applicable (Hunter *et al.*, 1998). The identification of dominant isolates was done.

Calculation of colonization frequency:

Percentage of colonization frequency (CF %) of each fungal endophyte was calculated using the following formula (Hata *et al.*, 1995):

$$CF (\%) = \frac{\text{Number of leaf or stem segments colonized by the fungus}}{\text{Number of segments screened}} \times 100$$

Preparation of fungal extract:

The four dominant fungal endophytes isolated from *Centella asiatica* and *Aloe vera* were grown separately in 500 ml Erlenmeyer flask containing 100 ml of PDB media. each of the dominant fungi were transferred to Erlenmeyer flasks (250ml) containing PDB media (100 ml) separately and were subjected to incubation at 30 ± 1°C for 15 days. After 15 days the culture filtrates were obtained by

filtering and the culture filtrates were considered as samples for assessment of different qualitative and quantitative tests for different bioactive compounds.

Qualitative detection of bioactive compounds:

The fungal crude extracts were considered to detect the presence of compounds namely alkaloids, phenols, flavonoids, saponins, terpenoids and tannins by the use of standard protocol (Chowdhury *et al.*, 2018).

Alkaloids:

The fungal extracts were evaporated to dryness separately in a boiling water bath. Each residue was then subjected to dissolve in 5 ml of 2 (N) HCl and the mixture was centrifuged at 10,000 rpm for ten minutes; the supernatant obtained was divided into three equal portions. One portion was mixed with a few drops of Meyer's reagent; one with equal amount of Dragendroff's reagent and the other with equal amount of Wagner's reagent. The creamish precipitate (Meyer's reagent), the brown precipitate (Dragendroff's reagent) and the orange precipitate (Wagner's reagent) indicates the presence of alkaloids in the fungal extract.

Flavonoids:

In a test tube 0.5 ml of crude extract and dilute HCl (10 drops) were added, with a small piece of zinc and the mixture was subjected to boiling for a few minutes. Presence of flavonoids in the samples can be detected by the development of reddish pink or dirty brown colour.

Phenols:

The extract (2ml) was mixed with 2 ml of ddH₂O. Few drops of FeCl₃ (5%, neutral pH) solution were added and appearance of dark green color indicates the presence of phenolic compounds in the extract.

Tannins:

The fungal crude extract was mixed with alcoholic FeCl₃ reagent. Formation of a bluish black colour and disappearance of the color on addition of few drops of dilute H₂SO₄, followed by the formation of yellowish-brown precipitate indicates the presence of tannins.

Terpenoids:

The fungal extract (2ml) was mixed with CHCl₃ (2ml) and subjected to evaporation to dryness. Then concentrated H₂SO₄ (2ml) was added. An appearance of reddish brown colour in the inner face indicates the presence of terpenoids.

Saponins:

The Frothing test generally performed to detect the presence of saponins in the sample. The crude fungal extract was subjected to shake vigorously with ddH₂O and allowed to stand for 10 minutes. No frothing indicated the absence of saponins and stable frothing more than 1.5 cm in height indicates the presence of saponins.

Quantitative determination of total phenol content:

The quantitative estimation of phenolics in fungal extracts was performed using spectrophotometric methods (Murthy *et al.*, 2011). Methanolic solution of the extract (1 mg/ml) was used for the analysis. Methanolic extract (0.5 ml) was mixed with 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% NaHCO₃ to make a reaction mixture. The reaction mixture was then incubated at 37°C for 45 min. Blank set was simultaneously prepared by adding 0.5 ml methanol, 2.5 ml 10% Folin-Ciocalteu reagent (prepared with water), and 2.5 ml of 7.5% of NaHCO₃. The absorbance was checked using UV- VIS spectrophotometer at 765 nm wavelength. Three replicates for each sample were prepared and the mean value of absorbance was recorded. A standard curve using gallic acid was prepared. The content of phenolic compounds in the fungal extracts was estimated and expressed as mg of gallic acid eq. per g dry weight of extract. The values were expressed as mean ± SD.

Quantitative determination of total flavonoid content:

The total flavonoid content was estimated by the aluminum chloride assay method. Each test sample (1 ml) was mixed with 4 ml of ddH₂O and 0.3 ml of sodium nitrite solution (5% w/v), the mixture was allowed to stand for 5 min. Aluminum chloride solution (10%, 0.3 ml) was added to the sample mix and after 1 minute 0.2 ml of NaOH (1 M) was added. The volume was made up to 10 ml with ddH₂O and mixed well, allowed to stand until the appearance of yellow colour. The optical density was recorded at a wavelength of 510 nm in UV-VIS spectrophotometer. Total flavonoid content was estimated after

calibrating with the standard curve prepared using different concentrations (100-1000 µg/ml) of quercetin (Sigma, Extra pure) and expressed as mg of quercetin equivalent (QE) per g dry weight of fungal extract. The values were expressed as mean ± SD.

Antioxidant Assay:

Determination of antioxidant activity by phosphomolybdenum assay:

The total antioxidant activity of the cell free extract was determined following mainly the relevant methods with certain modifications (Hata *et al.*, 1995). The cell free extracts of the selected dominant endophytic fungi isolated from *C.asiatica* and *A.vera*, grown in potato dextrose broth at 29 ± 2°C for 18 days, were obtained by filtering the grown cultures through Whatman filter paper No. 1. The cell free extracts were then evaporated to dryness at 60°C temperature. The solid residue obtained after evaporation was dissolved in methanol (concentration 1 mg/ml) and was used for determining total antioxidant activity of the sample. Each sample (1 ml) was mixed with 2 ml reagent solution prepared by mixing ammonium molybdate (4mM), sodium phosphate (28mM) and sulfuric acid (0.6M) in the ratio of 1:1:1]. The test tubes containing sample mixture were incubated at 30°C for 60 minutes to develop green colour. Three replicates were maintained for each set. The absorbance was measured at 665 nm wavelength against a blank (methanol). The antioxidant activity of the fungal extracts was calculated from the standard curve prepared by using ascorbic acid and results were expressed as mg of ascorbic acid equivalent per gm of the dry weight of extract.

Determination of antioxidant activity by DPPH free radical scavenging assay:

1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was carried out following the protocol mentioned in the relevant literatures (Yadav *et al.* 2012). The cell free extracts of the selected five dominant endophytic fungi isolated from *C.asiatica* and *A.vera* grown in PDB media, were evaporated to dryness at 60°C temperature. The solid residue obtained after evaporation was dissolved in ethanol (concentration 1 mg/ml) and was used to assay DPPH free radical scavenging activity. The sample extract of 0.5 ml (equivalent to 500µg) was mixed with 2.5 ml of 0.5 mM methanolic solution of DPPH and incubated for 30 min, kept at 37°C temperature in dark condition to develop yellow colour. Three replicates were maintained for each set. After 30 min, the absorbance was measured at 517 nm wavelength against a blank (ethanol). Free radical scavenging activity was expressed as a percentage. The results were expressed as a mean % value ± SD. The percentage of the DPPH radical scavenging was calculated as

$$\text{Inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 10$$

Enzyme Assay:

Assay of amylase:

The production of enzyme by fungal endophytes was qualitatively determined by using Hankin and Anagnostakis method (Jianwen *et al.*, 2004). Amylase enzyme activity was assessed by growing the fungi on glucose yeast extract peptone (GYP) agar medium (glucose-1g, yeast extract 0.1g, peptone 0.5g, agar 16 g, distilled water 1000 mL and pH 6) containing 1% soluble starch. After 5 days incubation, the plates with fungal colony were flooded with 1% iodine in 2% potassium iodide. The appearance of clear zone surrounding the colony was considered positive for amylase enzyme.

Assay of protease:

Protease assay was performed by growing the fungi on GYP agar medium amended with 0.4 % gelatin and adjusted the pH to 6. After 5 days of incubation, plates were flooded with saturated aqueous ammonium sulphate. The undigested gelatin will precipitate with ammonium sulphate and digested area around the fungal colony would appear as clear zone.

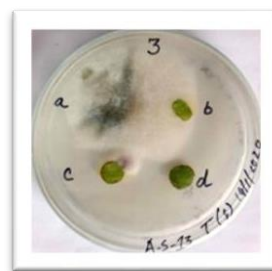
3. Results and Discussion

Colonization of leaves of Aloe Vera and Centella asiatica by fungal endophytes:

The surface sterilized leaves of *Aloe Vera* and *Centella asiatica* were placed in PDA medium and were incubated for seven days. After seven days, growth was observed. The endophytic fungal colony grew out from the leaves of *Aloe Vera* and *Centella Asiatica*



1(a)



1(b)

Fig. 1(a) Emergence of endophytic fungi from leaf segments of *Aloe vera*

1(b) Emergence of endophytic fungi from leaf segments of *Centella asiatica*

Morphological characteristics of the isolated fungal endophytes:

The fungal that endophytes emerged from the leaves of the two plants were selected on the basis of higher colonisation frequency. The isolated fungi were identified partially based on their morphological characteristics and microscopic observation.

Table 1 Morphological characteristics of the isolated endophytes

PLANT	ENDOPHYTIC FUNGI ISOLATED	MORPHOLOGICAL CHARACTERISTICS
<i>Aloe vera</i>	<i>Fusarium sp.</i>	<p><u>Front:</u> Growth -slow, Color- greenish black(central), white(peripheral) Nature-puffed and cottony</p> <p><u>Reverse:</u> Color- greenish black(central), off- white(peripheral)</p>
<i>Aloe vera</i>	<i>Aspergillus sp.1</i>	<p><u>Front:</u> Growth -moderate, Color- dark green(central), white(middle), green(peripheral) Nature-powdery and cottony</p> <p><u>Reverse:</u> Color- yellowish white(centre), greenish white(peripheral)</p>
<i>Aloe vera</i>	<i>Aspergillus sp.2</i>	<p><u>Front:</u> Growth -slow, Color- milky white Nature- cottony Exudates present</p> <p><u>Reverse:</u> Color- yellowish green(central), off- white(peripheral)</p>
<i>Centella asiatica</i>	<i>Penicillium sp.</i>	<p><u>Front:</u> Growth -very slow, Color- pinkish(central), white(peripheral) Nature-compact and cottony Grooves present</p> <p><u>Reverse:</u> Color- blackish(central),off- white(peripheral)</p>

The morphological views and the microscopic views of the dominant fungal endophytes are given in the following pictures.

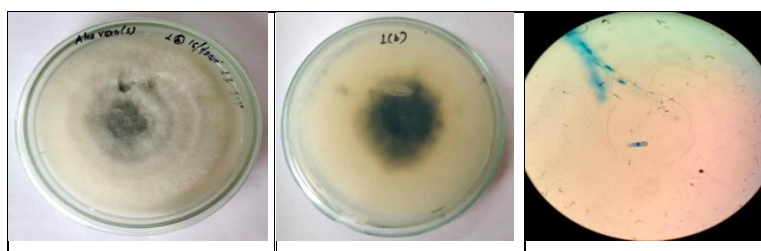


Figure 2(a)(i)

Figure 2 (a)(ii)

Figure 2(a)(iii)

Fig. 2 (a) Colony morphology of *Fusarium sp.* (i): front view (ii) back view (iii) microscopic view

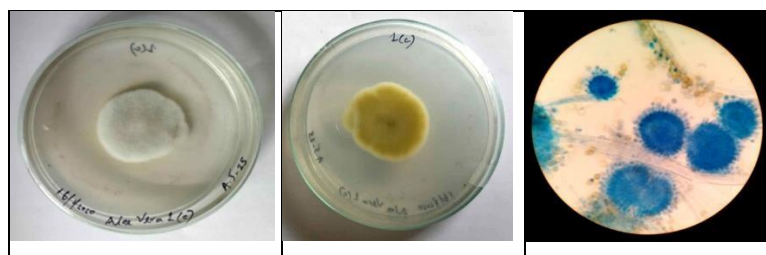


Figure 2(b)(i) Figure 2 (b)(ii) Figure 2(b)(iii)

Fig. 2 (a) Colony morphology of *Aspergillus sp.* (i): front view (ii) back view (iii) microscopic view



2(c) (i) 2(c) (ii) 2(c) (iii)

Fig. 2 (c) Colony morphology of *Penicillium sp.* (i): front view (ii) back view (iii) microscopic view

Determination of colonization frequency:

Table 2 Determination of colonization frequency

Plant name	Plant's part	No. of leaf discs	No. of fungi isolated	Isolated endophytic fungi	CF%
<i>Aloe vera</i>	Leaves	20	4	<i>Fusarium sp.</i>	20
<i>Aloe vera</i>	Leaves	20	3	<i>Aspergillus sp.1</i>	15
<i>Aloe vera</i>	Leaves	20	2	<i>Aspergillus sp.2</i>	15
<i>Centella asiatica</i>	Leaves	20	3	<i>Penicillium sp.</i>	10

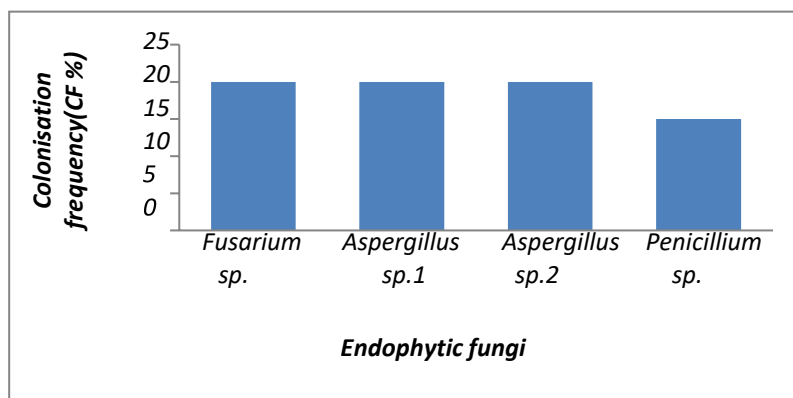


Fig 3: Graphical representation of the colonization frequencies of endophytic fungi isolated from *Aloe vera* and *Centella asiatica*

Qualitative detection of Bioactive compounds from endophytic fungal extract:

Table 3 Qualitative detection of Bioactive compounds from endophytic fungal extract

Endophytic fungi	Phenol	Flavonoids	Saponins	Terpenoids	Alkaloids	Tannins
<i>Fusarium sp.</i>	+	++	-	+	+	-
<i>Aspergillus sp.1</i>	+	+	-	+	+	++
<i>Aspergillus sp.2</i>	+	++	-	+	+	-
<i>Aspergillus sp.2</i>	+	+	-	++	+	-

Quantitative assay of total phenolic content from endophytic fungal extract:

Table 4 Quantitative assay of total phenolic and flavonoid content from endophytic fungal extract

Endophytic fungi isolated from leaves	Phenol content (from culture filtrate) mg/g	Flavonoid content (from culture filtrate) mg/g
<i>Fusarium sp.</i>	1.77±0.25	0.88±0.03
<i>Aspergillus sp.1</i>	2.05±0.13	1.02±0.02
<i>Aspergillus sp.2</i>	1.42±0.10	0.62±0.05
<i>Penicillium sp.</i>	0.87±0.02	0.56±0.03

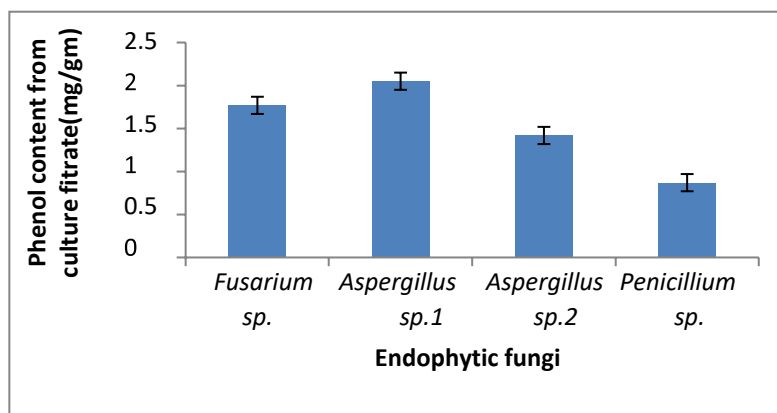


Fig. 4: Graphical representation of the phenol content of the culture filtrate (mg/gm) .“I” on top of the bar denotes the value of standard deviation (±SD)

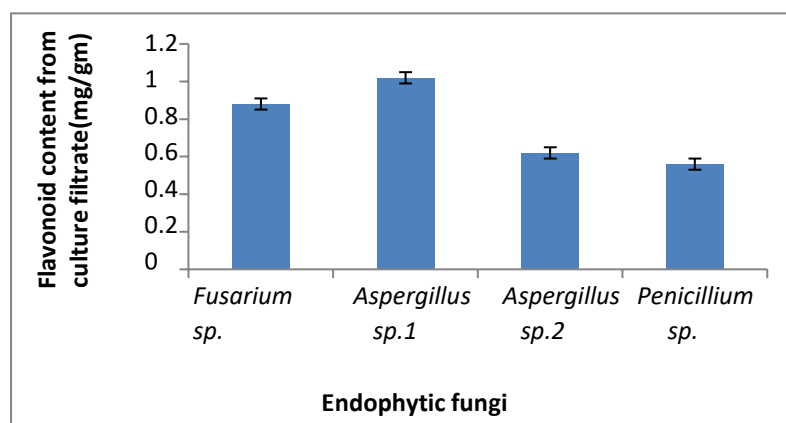


Fig 5: Graphical representation of the flavonoid content of the culture filtrate (mg/gm) .“I” on top of the bar denotes the value of standard deviation (±SD)

Antioxidant activity of endophytic fungi:

Table 5 Antioxidant activity of endophytic fungi

Endophytic fungi isolated from leaves	Antioxidant activity equivalent as mg. of ascorbic acid/gm of dry weight of extract	Percentage of Inhibition (DPPH Assay) (%)
<i>Fusarium sp.</i>	0.80±0.06	66.01±0.03
<i>Aspergillus sp.1</i>	2.45±0.06	91.67±0.73
<i>Aspergillus sp.2</i>	0.79±0.02	74.21±0.16
<i>Penicillium sp.</i>	0.62±0.03	30.02±1.05

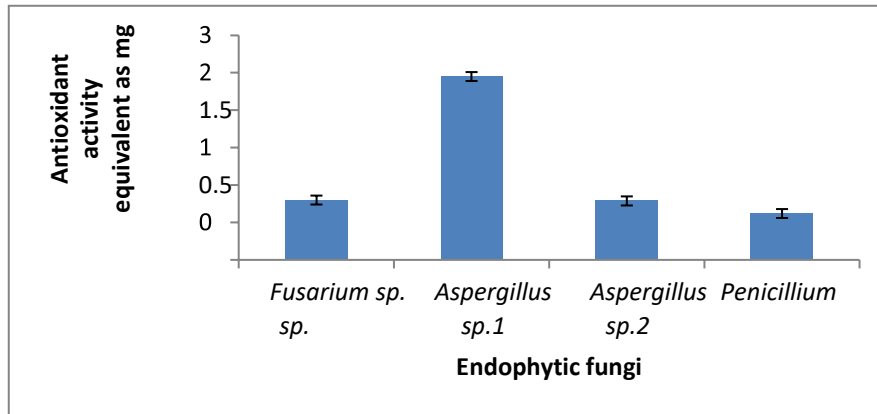


Fig. 6: Graphical representation of the antioxidant activity of the culture filtrate (equivalent as mg of ascorbic acid per gram of dry weight of extract). “I” on top of the bar denotes the value of standard deviation (\pm SD)

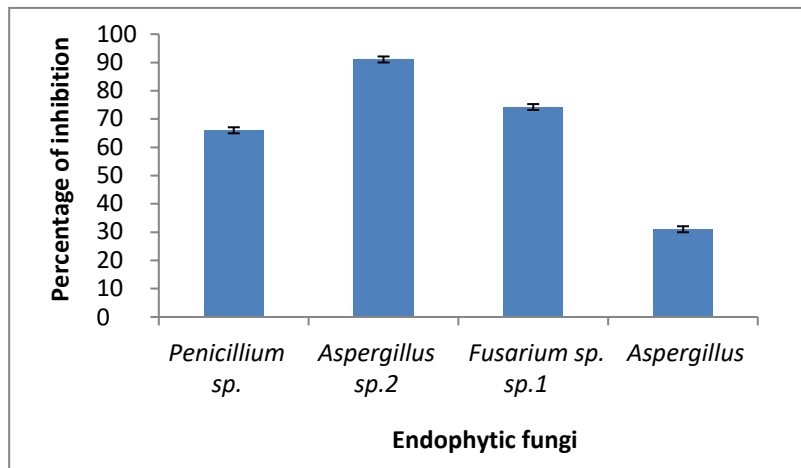


Fig 7: Graphical representation of % inhibition of DPPH radical activity of fungal culture filtrate by free radical scavenging assay, “I” on top of the bar denote the value of standard deviation (\pm SD)

Enzyme assay of endophytic fungi:

Table 6 Enzyme Assay of endophytic fungi

Endophytic fungi isolated from leaves	Presence of amylase	Presence of protease
<i>Fusarium sp.</i>	+	+
<i>Aspergillus sp.2</i>	+	+
<i>Penicillium sp.</i>	+	+

The production of amylase and protease by the isolated endophytic fungi are shown in the following pictures:

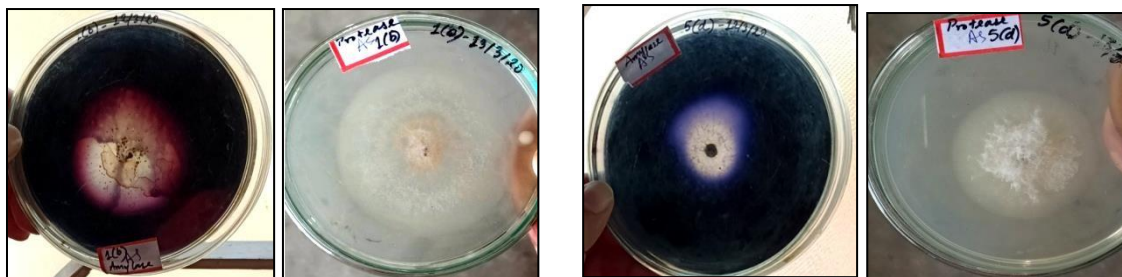


Figure 8(a)(i):

Amylase activity of *Fusarium sp.*

Figure 8(a)(ii):

Protease activity of *Fusarium sp.*

Figure 8(b)(i):

Amylase activity of *Aspergillus sp.2*

Figure 8(b)(ii):

Protease activity of *Aspergillus sp.2*

Discussions

Aloe vera and *Centella asiatica* are well known medicinal plants. In the present study, the segments of the leaves of *Aloe vera* and *Centella asiatica* were harvested with maintenance of appropriate conditions. The leaf segments of both the plants were given in potato dextrose agar (PDA), which ultimately led to the emergence of fungal endophytic growth from the leaf segments after seven days. Among the fungal endophytes, *Fusarium sp.*, *Aspergillus sp.1*, *Aspergillus sp. 2* and *Penicillium sp.* were selected for further studies as they have high colonization frequency. The most dominant fungal endophytes were *Fusarium sp.* with colonization frequencies of 20%, followed by *Aspergillus sp. 1* and *Aspergillus sp.2* with colonization frequencies of 15 % each. The four dominant fungal endophytes isolated from *Centella asiatica* and *Aloe vera* were grown separately in PDB media. After 15 days the culture filtrates were obtained by filtering and the culture filtrates were considered as samples for assessment of different qualitative and quantitative tests for different bioactive compounds. All the fungal strains showed positive activity for phenol, flavonoid, alkaloid and terpenoid production. Qualitatively, *Aspergillus sp1.* showed high flavonoids production; *Aspergillus sp2.* showed high production of tannins and *Fusarium sp.* showed high flavonoid production. Quantitatively, total phenol content was assessed by spectrophotometric method using gallic acid as standard. Quantitative estimation of total flavonoids was done by aluminum chloride assay. Highest phenol and flavonoid content were observed in *Aspergillus sp .1.*, having phenol content of 2.05 ± 0.13 mg/gm and flavonoid content of

1.02 ± 0.02 mg/gm, followed by *Fusarium sp.* having phenol content of 1.77 ± 0.25 mg/gm and flavonoid content of 0.88 ± 0.03 mg/gm. The ethanolic extracts of the four dominant fungal endophytes were subjected to Antioxidant activity assay by both phosphomolybdenum and DPPH free radical scavenging assay. Highest antioxidant activity in the culture filtrate was noted in *Aspergillus sp.1* (2.45 ± 0.06) mg/gm followed by *Fusarium sp.* (0.80 ± 0.06) mg/gm isolated from *Aloe vera*. Among the dominant isolates of *Aloe vera* and *Centella asiatica*, highest free radical scavenging activity was shown by *Aspergillus sp.1* ($91.67\pm 0.73\%$), followed by *Aspergillus sp.2* ($74.21\pm 0.16\%$), *Fusarium sp.* ($66.01\pm 0.03\%$) and *Penicillium sp* ($30.02\pm 1.05\%$). It was noted that the fungi which produced highest antioxidant activity by phosphomolybdenum assay also showed highest free radical scavenging activity by DPPH assay. It was observed that the fungal endophytes that had maximum phenol content were also found to possess highest free radical scavenging activity. It can be analyzed that the presence of phenolic compound plays a role in increasing the antioxidant activity. The dominant fungal endophytes were then subjected to screening for production of enzyme amylase by Hankin and Anagnostakis method and screening was also done to check production of enzyme protease by ammonium sulphate precipitation method. *Penicillium sp.* isolated from *C. asiatica* and *Fusarium sp.*, *Aspergillus sp.1* and *Aspergillus sp.2* isolated from leaves of *A. vera* showed positive amylase and protease activity. The present study revealed the existence of such important endophytic fungi like *Aspergillus sp.1*, *Aspergillus sp.2*, *Fusarium sp.* and *Penicillium sp.* on leaves of common medicinal plants *Aloe vera* and *Centella asiatica*, which can be isolated and used for obtaining different phytochemicals, antioxidants and enzymes which have potential application in many fields.

4. Conclusion

From the present study, we can conclude that the leaves of both the medicinal plants *Aloe vera* and *Centella asiatica* have many endophytic fungal strains; the dominant ones found in this study being *Penicillium sp.* isolated from *C. asiatica* and *Fusarium sp.*, *Aspergillus sp.1* and *Aspergillus sp.2* isolated from leaves of *A. vera*. These fungal strains have the capability to produce large amount of secondary metabolites, especially phenols, flavonoids, tannins and terpenoids. Also, fungal species like *Aspergillus sp* and *Fusarium sp* have very high free radical scavenging activity. *Penicillium sp* and *Fusarium sp.* have high amylase and protease enzyme production activity. The present study, therefore highlights the growing concept that the bioactive compounds produced by the endophytes not only establishes host endophyte relationship but also have an immense chance of an application in the field of medicine, agriculture and industry.

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