RESEARCH ARTICLE

Evaluation of antidiabetic and antioxidant potential of endophytic fungi isolated from medicinal plants

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Abstract

Introduction: Endophytic microorganisms residing in the tissues of the higher plants are gaining increasing importance as sources of novel therapeutic molecules. In the present study, isolation and screening of endophytes for alpha-glucosidase inhibitory and antioxidant potential was carried out which could have application in the management of diabetes mellitus (DM) and related complications. DM has become a major health concern worldwide, and development and progression of diabetic related complications are accelerated by reactive oxygen species which are generated and accumulated due to hyperglycemia. **Material and Methods:** Endophytic fungi were isolated from different healthy plants after surface sterilization and screened for the production of alpha-glycosidase inhibitors and antioxidant potential. Antioxidant potential was characterized using 2,2-diphenyl-1-picryl hydrazyl and ferric reducing antioxidant power assays. Total phenolic content (TPC) was estimated by Folin–Ciocalteu assay. Thin-layer chromatography-based phytochemical analysis was conducted using different visualization reagents. In a selected culture, BRY-1 identification of phenolic compounds was carried out using reversed-phase-high-performance liquid chromatography. **Results:** Sixty-six cultures were isolated from indigenous medicinal plants and screened for α-glucosidase inhibitors (AGI) and antioxidant potential. Majority of isolates belonged to *Alternaria* and *Aspergillus* sp. Maximum AGI activity (91.57%) was exhibited by VRJ-3, while good antioxidant potential of 92% was observed in BRY-1, BRY-2, and AZAI-12 cultures. A positive correlation was found between TPC and antioxidant potential; however, no such correlation existed between TPC and AGI. **Conclusion:** These studies reveal the potential of endophytic fungi as sources of pharmaceutically important molecules. AGI activity in species of *Fusarium* and *Alternaria* is being reported for the first time. These isolates can be selected and subjected to further studies to determine the nature of the components.

Key words: Alpha glucosidase inhibitor, antioxidant activity, endophytes

INTRODUCTION

Endophyte refers to indigenous microflora of plants, colonizing the living plant tissue without causing symptoms of any disease.[1] Nearly all vascular plant species explored to date are known to host a variety of endophytes. Endophytes are present in almost all organs of a given plant host and some are even seed borne.[2] The endophytic fungi are a source of unprecedentedly novel bioactive compounds which have found wide-ranging pharmaceutical applications such as anticancer, immunosuppressant, and antiviral.[3] With huge biodiversity of endophytic fungi in higher plants, there is an enormous scientific interest for isolating them and investigating the bioactive properties of their extracts.[4] Diabetes is considered as a major health concern worldwide as the global prevalence of diabetes among adults over 18 years of age which has risen from 4.7% in 1980 to 8.5% in 2014.[5] Diabetes is categorized into two types - diabetes mellitus (DM) Type 1 and DM Type 2. DM Type 2 (formerly known as insulin independent) accounts for 95% of the cases of DM. The α-glucosidase inhibitors (AGI) are very promising in the control of DM (Type 2) as they inhibit glycoside hydrolases enzymes in striated border of the small intestine, thereby slowing down the breakdown of carbohydrates into glucose. Due to this, less glucose is

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Received: 18-08-2017
Revised: 14-11-2017
Accepted: 26-11-2017

available for absorption, as a result post-prandial blood glucose level is lowered.[6] The commercially available AGIs such as acarbose, voglibose, and miglitol are being currently used in the treatment of post-prandial hyperglycemia.[7,8] Their regular use has induced side effects such as liver toxicity, diarrhea, and other gastrointestinal complications.[9,10] It has also been established that biochemical pathways initiated by hyperglycemia lead to generation and accumulation of reactive oxygen species (ROS). The ROS activates stress signaling pathways, thereby escalating oxidative stress, which is explained as a reason for development and progression of diabetic-related complications.[11] Thus, antioxidants with the ability to quench free radicals can help in subsidizing the symptoms of diabetes.[12,13] Besides this, antioxidants are also gaining importance in the treatment of cardiovascular diseases, arthritis, pulmonary diseases, etc.[14] Therefore, search for novel, better, safe therapeutic agents with novel properties is always a priority. Endophytes with their immense biosynthetic potential can serve as sources of these novel compounds. Keeping this in view, the present study was conducted to explore the endophytes isolated from medically important plants for AGI and antioxidant potential.

MATERIALS AND METHODS

Isolation of Endophytic Fungi

Stem and leaves from healthy plants of *Azadirachta indica*, *Bryophyllum, Vinca rosea,* and *Calotropis gigantea* were collected and thoroughly washed with tap water followed by sterilized distilled water. They were surface sterilized in 70% ethanol for 2–3 min, rinsed with distilled water, and thereafter treated with sodium hypochlorite 4% for 3 min. Before plating, plant pieces were again rinsed in distilled water. Surface sterilization was confirmed by plating water obtained after the last wash. The aspectially cut plant segments were placed on Petri dish containing potato dextrose agar (PDA) supplemented with antibacterial antibiotic chloramphenicol (200 µg/ml). The plates were incubated at 30°C. Fungal mycelia originating from plant parts were picked and purified and maintained on PDA slants. These slants were stored at 4°C for further experimental use and were subcultured periodically.[15]

Culture Identification

Isolated fungal cultures exhibiting AGI activity and antioxidant potential were identified on the basis of colony and microscopic morphology using slide culturing method. A thin layer of PDA was cut into small squares and was aseptically placed on a glass slide. The agar block was point inoculated with fungal cultures, and a sterile coverslip was placed over it gently. The complete assembly was incubated at 30°C for 24–30 h and thereafter examined under light microscope for identification using standard taxonomic keys.[16]

Production of Fungal Extract

A plug was cut with a cork borer (8 mm diameter) from the periphery of activated fungal culture and inoculated in Erlenmeyer flasks (250 ml) containing 50 ml malt extract broth. These flasks were then incubated at 30°C on the rotary shaker at 250 rpm for 10 days. After incubation, equal volume (50 ml) of ethyl acetate was added. Extraction was carried out on a rotary shaker at 120 rpm and 40°C for 1.5 h. This was repeated twice with fresh solvent each time. The organic phase was then concentrated on rota vaporizer (Buchi) at 40°C, and the concentrated samples were suspended in 1 ml of deionized water.[17]

In Vitro AGI Assay

The AGI assay was performed using ρ-nitro phenyl-α-D-glucopyranoside as a substrate. Reaction mixture was prepared by adding 50 µl of phosphate buffer (50 mM, pH-6.8), 10 µl of α-glucosidase enzyme from *Saccharomyces* sp. (1U/ml), and 20 µl of test sample followed by incubation for 5 min at 37°C. After incubation, 20 µl substrate was added and incubated for 30 min at 37°C. Reaction was terminated by adding 50 µl of sodium carbonate. All the reactions were performed in triplicates in 96-well microtiter plate. Absorbance was taken at 405 nm and activity was calculated using the following formula:

$$\text{Inhibition of } \alpha-\text{glucosidase } (\%) = \frac{(\Delta A \text{ control} - \Delta A \text{ Sample})}{\Delta A \text{ control}} \times 100$$

ΔA control is the absorbance of the control reaction (containing all reagents except the test compound), and ΔA sample is the absorbance of the test compound.[15]

α-amylase Inhibitory (AAI) Assay

Reaction mixture was prepared by adding 40 µl of fungal extract, 200 µl of phosphate buffer (pH 6.9), and 40 µl (24 U/ml) porcine pancreatic amylase. It was incubated at 37°C for 10 min followed by addition of 50 µl of starch (1%) and incubation at 37°C for 20 min. Reaction was terminated by adding 0.5 ml DNS followed by incubation in boiling water for 5 min. The reaction mixture was diluted with 5 ml of distilled water and absorbance was read at 540 nm. For each concentration, blank tubes were prepared by replacing the enzyme solution with 40 µl of buffer. Control representing enzyme activity was prepared in a similar manner, without extract. The experiments were repeated thrice using the same protocol.[15]

The reaction was represented by following equations:

$$\text{Soluble starch} \xrightarrow{\alpha\text{- amylase}} \text{glucose + maltose}$$

$$\text{Reducing sugars + 3,5-dinitrosalicylic acid} \longrightarrow \text{orange red colored products}$$

The AAI activity was calculated using the formula:

$$\% \text{ inhibitory activity} = \frac{\text{Absorbance}_{540}(\text{Control}) - \text{Absorbance}_{540}(\text{Extract})}{\text{Absorbance}_{540}(\text{Control})} \times 100$$

Phytochemical Tests

The extract was spotted on silica plate with the help of capillary. The spots were air dried. After drying, spots were sprayed with 2,2-diphenyl-1-picryl hydrazyl (DPPH) for evaluation of antioxidant property (dot blot method). Selected cultures were then subjected to different reagents, namely, fast blue B, $FeCl_3$, ninhydrin, and Dragendorff to study the nature of compounds.[18]

DPPH Radical Scavenging Assay

DPPH assay was performed to measure the scavenging activity of selected methanolic fungal extracts. The scavenging activity was measured by the bleaching of purple-colored methanol solution of DPPH treated with fungal extracts. Diluted fungal extract (1.5 ml) was added to 1.5 ml of methanolic solution (0.2 mM) of DPPH. After 30 min incubation at room temperature, the absorbance was read against a blank at 517 nm.[19] Ascorbic acid was used as a positive control. Scavenging activity was calculated according to the following equation.

$$\%\text{DPPH radical scavenging activity} = \frac{\text{absorbance of blank} - \text{absorbance of sample}}{\text{absorbance of blank}} \times 100$$

Where blank is the absorbance of control reaction, and sample is the absorbance of test compounds.

Ferric Reducing Antioxidant Power (FRAP) Assay

In this assay, 10 µl of sample was mixed with 15 µl of 0.1 M sodium phosphate buffer (pH 6.6) and 15 µl of potassium ferricyanide (1% w/v) followed by incubation at 50°C for 20 min. After 20 min incubation, 15 µl of trichloroacetic acid (10%) was added in reaction mixture and mixed well. Distilled water (55 µl) and ferric chloride (110 µl, 10% w/v) were added, and the absorbance was measured at 700 nm. An increased absorbance of the reaction mixture indicates increased reducing capability. L-ascorbic acid was taken as a reference standard. All experiments were carried out in triplicates.[20]

Determination of Total Phenolic Content (TPC)

TPC of the extract was determined using Folin–Ciocalteu assay. In this assay, reaction mixture containing 10 µl of extract and 100 µl of Folin–Ciocalteu reagent were mixed well and allowed to stand for 5 min. 80 µl of 7.5% of sodium carbonate solution was added and kept in the dark at room temperature for 30 min. The absorbance was measured at 750 nm with a spectrophotometric microtiter plate reader. TPC of the extracts was expressed as gallic acid equivalents (GAE).[21]

Detection of Phenolics

Detection of phenolics in fungal extracts was performed by high-performance liquid chromatography (HPLC) using PDA detector (Dionex UVD 340U UV/VIS) on Microsorb-MV 100-5 C 18 (250 mm × 4.6 mm ID, 5 µM) column (Agilent, USA). Phenolics were detected using solvent system comprising of 70:30 (0.1% acetic acid in water and methanol), respectively, at the flow rate of 3 ml/min.[22]

Statistical Analysis

Each experiment was performed in triplicate. To calculate standard error and mean, Microsoft Office Excel 2007 (Microsoft Corp, USA) was used, and Pearson correlation analysis was performed using the BioEstat 5.3 software.

RESULTS

Isolation and Identification

A total of 66 cultures were isolated from indigenous healthy plants, namely, *V. rosea, C. gigantea, A. indica,* and *Bryophyllum* and screened for glycosidase inhibitory potential and antioxidant properties [Table 1].

Twenty-four endophytic fungal cultures were isolated from *V. rosea*. Majority of cultures were identified on morphological basis to belong to two genera *Alternaria* and *Aspergillus* [Figure 1a and b]. A few cultures were found to be non-sporulating and could not be identified on morphological basis. AGI activity in the range of 55–92% was exhibited by five cultures. Two cultures, namely, VRJ-3 and VRJ-5 evinced high AGI activity being 91.54% and 81.61%, respectively. None of the culture exhibited inhibitory activity against alpha-amylase. Dot blot assay revealed antioxidant activity in four cultures with the maximum being in VRJ-3.

Ten cultures were isolated and screened for AGI from *C. gigantea*. Majority of cultures were found to be *Alternaria* sp. and two cultures, namely, AKL-1 and AKL-16 were identified to be *Fusarium* and *Penicillium,* respectively [Figure 1c and d]. Six cultures exhibited AGI activity in the range of 55–87.4% with the maximum being found in AKL-4 (87.4%) followed by AKL-9 (84.4%). Activity against alpha-amylase was found to be insignificant. Four cultures

Table 1: AGI, AAI, and antioxidant activities of endophytic fungi isolated from various medicinal plants

Plants	Total number of isolates	Isolates exhibiting activities	% AGI activity	% AAI activity	Antioxidant activity (dot blot assay)
V. rosea	24	VRJ 3	91.54	–	+++
		VRJ 5	81.61	–	–
		VRJ-A14	65.93	–	+
		VRJ-A9	57.77	–	+
		VRJ-B5	59.67	–	+
C. gigantea	10	AKL-1	68.5	–	–
		AKL-16	72.28	–	–
		AKL-12	76.55	–	–
		AKL-9	84.4	–	++
		AKL-4	87.4	–	+++
		AKL-13	55.65	–	–
		AKL-5	≤50	–	+
		AKL-2	–	–	+
A. indica	17	AZAI-1	–	–	+++
		AZAI-2	–	–	+++
		AZAI-3	≤50	–	+
		AZAI-4	≤50	–	+
		AZAI-7	64.62	–	++
		AZAI-12	61.21	–	+++
		AZAI-13	–	–	+
Bryophyllum	15	BRY-1	66.04	–	+++
		BRY-2	–	–	+++
		BRY-3	51.34	–	+++
		BRY-5	–	–	+++
		BRY-9	55.04	–	+

Low positive (+), moderate positive (++), high positive (+++), negative (–). *V. rosea: Vinca rosea, A. indica: Azadirachta indica, C. gigantea: Calotropis gigantean*, AGI: α-glucosidase inhibitors, AAI: α-amylase inhibitory

(AKL-2, AKL-4, AKL-9, and AKL-5) were found to possess antioxidant capacity.

Seventeen cultures were isolated from *A. indica* and screened for the presence of AGI. Inhibitory activity was found to be relatively low in these isolates as compared to *Calotropis* and *V. rosea* isolates. Activity in the range of 20–30% was observed in ten cultures. Only two cultures showed activity above 50% in the range of 61–65%. Maximum inhibitory activity was possessed by AZAI-7 followed by AZAI-12. Antioxidant activity was found in seven cultures as detected by dot blot method. Isolates identified morphologically belonged to genera *Colletotrichum*, *Fusarium*, *Aspergillus*, and *Penicillium* [Figure 1e-g]. No significant activity was noticed against alpha-amylase.

Fifteen cultures were isolated and screened from *Bryophyllum*. Inhibitory activity was demonstrated by three cultures in the range of 50–66%. Two cultures BRY-1 (66.04 ± 2.87%) followed by BRY-9 (55.04 ± 1.98) evinced a good inhibitory activity against alpha-glucosidase enzyme. Antioxidant capacity of five cultures, namely, BRY-1, BRY-2, BRY-3, BRY-5, and BRY9 identified on the basis of dot blot method. None of the cultures demonstrated any considerable inhibitory activity against alpha-amylase.

Phytochemical Analysis

Cultures exhibiting activity were subjected to phytochemical tests. Most of cultures produced positive results on staining with both fast blue b and $FeCl_3$ indicating the presence of phenolics. A few cultures, namely, AZAI-3, BRY-3, BRY-9, and AKL-2 were positive for fast blue b and ninhydrin but negative for $FeCl_3$ indicating the presence of amine group [Table 2]. Using Dragendorff reagents, the presence of alkaloids was detected in extracts AZAI-2, AZAI-1, BRY-1, and AZAI-7. It can be inferred that these fungal extracts consisted of a mixture of compounds.

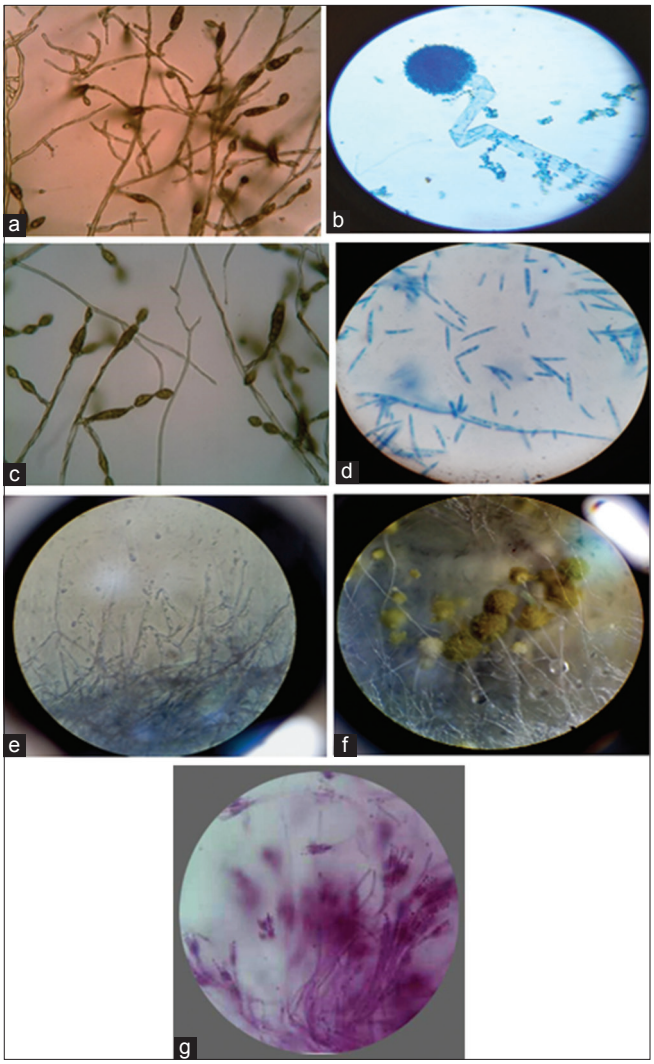

Figure 1: Microscopic view of (a) *Alternaria* sp. (VRJ-3) (b) *Aspergillus* sp. (VRJA-14) (c) *Alternaria* sp. (AKL-9) (d) *Fusarium* sp. (AKL-1) (e) *Colletotrichum* sp. (AZAI-13) (f) *Aspergillus* sp. (AZAI-4) (g) *Penicillium* sp. (AZAI-2)

Antioxidant Potential

DPPH scavenging radical assay

Antioxidant potential of compounds was determined by their hydrogen-donating capacity. DPPH is organic nitrogen radical which accepts hydrogen atom and attains a stable state. The antioxidant activity was proportional to the disappearance of purple color. This reaction is stoichiometric in terms of a number of hydrogen atoms absorbed. The decrease in absorption at 517 nm signifies antioxidant ability. All the cultures stained with DPPH in dot blot test were observed to inhibit the formation of free radicals with high scavenging activity of >90% being observed in cultures obtained from *Bryophyllum*, namely, BRY-2 (92.55%) and BRY-1 (92.05%). Cultures, namely, AZAI-2, VRJ-3, and AKL-9 from *A. indica, V. rosea,* and *C. gigantea,* respectively, also showed high DPPH free radical scavenging activity which illustrates their high hydrogen-donating capacity.

FRAP

High electron-donating capacity of substance corresponds to their high antioxidant potential. To assay the reducing capacity of fungal cultures, FRAP assay was conducted. In this assay, colorless ferric tripyridyltriazine complex is reduced to blue-colored ferrous-(2,4,6-tripyridyl-s-triazine)$_2$ and measured at 593 nm. Cultures BRY-2, AZAI-12, BRY-1, and AKL-5 exhibited high activity ranging from 75% to 85% indicating their electron-donating potential, while cultures AZAI-3, AZAI-13, and BRY-9 showed activity in the range of 35–45%. Value of FRAP is directly proportional to its antioxidant capacity, as in this assay, antioxidant acts as reducing agent by donating electrons to ferric ion.

The results obtained in the DPPH and FRAP assay indicated high activities in cultures, namely, BRY-1, AZAI-12, and BRY-2 [Figure 2].

TPC

The cultures were also quantified for TPC. TPC is expressed in GAE. Maximum TPC was observed in culture BRY-1 (1.76 ± 0.24 mg/ml GAE) followed by BRY-2 (1.65 ± 0.21 mg/ml GAE) and AZAI-12 (1.45 ± 0.15 mg/ml GAE). A significant positive correlation was observed between FRAP and TPC with r = 0.7393 ($P < 0.05$). Correlation between TPC and DPPH free radical scavenging activity was also studied, and it was observed to be significantly positively correlated with r = 0.649 ($P < 0.05$). These results indicated that extracts having more phenolic content tend to serve as strong antioxidants.

The fungal extract (BRY-1) exhibiting maximum phenolic content was subjected to HPLC for analysis of phenolic compounds. The extract possessed variable concentration of different phenolic compounds. Eleven phenolics were detected, namely, gallic acid, catechin, chlorogenic acid, epicatechin, caffeic acid, umbelliferone, coumaric acid, rutin, ellagic acid, tert-butylhydroquinone, and kaempferol [Figure 3].

DISCUSSION

In the present study, endophytes have been isolated from indigenous medicinal plants, namely, *A. indica, Bryophyllum, C. gigantea,* and *V. rosea*. A number of researchers have worked on these plants and reported their antidiabetic and antioxidant potential.[23-26] It is possible that endophytes residing in these plants could be producing the same bioactive compounds as a outcome of genetic transfer.[3] It is exemplified in case of yews (known for taxol production) where endophytes isolated from it were also found to produce taxol.[27] Sixty-six cultures were isolated and purified which mainly consisted of *Penicillium, Aspergillus, Colletotrichum*, *Fusarium,* and *Alternaria*. Maximum cultures isolated belonged to *Aspergillus* and *Alternaria* sp. Yadav *et al*. (2016) and Momsia and Momsia (2013) have demonstrated

Table 2: Phytochemical analysis and antioxidant activities of endophytic isolates

Isolate	Staining reagent				TPC (GAE mg/ml)	FRAP (ascorbic acid equivalent mg/ml)	DPPH (% free radical scavenging activity)
	Fast blue B	$FeCl_3$	Ninhydrin	Dragendorff			
AZAI-2	+	+	+	+	0.43 ± 0.03	71.5 ± 2.24	89.85 ± 2.74
AZAI-1	++	+	+	+	0.84 ± 0.13	74.3 ± 1.35	83.55 ± 2.64
AZA1-4	++	+	–	–	0.19 ± 0.07	63.9 ± 1.22	64.35 ± 1.95
AZAI-3	+	–	+	–	0.19 ± 0.07	37.5 ± 1.64	39.6 ± 1.68
AZAI-12	++	++	++	–	1.45 ± 0.15	80.5 ± 1.32	92.27 ± 2.88
AZAI-13	+	+	+	–	0.26 ± 0.11	46.1 ± 1.33	53.33 ± 1.56
AZAI-7	++	+	–	+	0.28 ± 0.04	55.1 ± 2.94	76.73 ± 1.68
BRY-1	+++	+++	–	+	1.76 ± 0.24	80.4 ± 1.59	92.05 ± 2.87
BRY-2	+	+++	–	–	1.65 ± 0.21	80.4 ± 1.52	92.55 ± 1.48
BRY-5	+++	+++	+	–	0.92 ± 0.17	72.5 ± 1.54	81.75 ± 1.27
BRY-3	+	–	+	–	0.3 ± 0.01	73.9 ± 2.11	81.75 ± 2.41
BRY-9	++	–	+	–	0.19 ± 0.04	42.4 ± 1.54	53.81 ± 2.74
AKL-9	++	++	–	–	0.93 ± 0.21	65.8 ± 1.74	88.9 ± 2.74
AKL-2	++	–	+	–	0.21 ± 0.08	63.5 ± 1.95	67.8 ± 2.98
AKL-4	+	+	–	–	0.86 ± 0.13	70.5 ± 1.42	82.94 ± 1.28
AKL-5	+	+	–	–	0.98 ± 0.16	80.7 ± 1.87	86.75 ± 1.59
VRJ-3	+++	+++	–	–	0.95 ± 0.23	77.6 ± 1.36	83.54 ± 2.55
VRJA14	+	+	–	–	0.39 ± 0.21	69.5 ± 2.55	71.32 ± 2.87
VRJA9	+	–	–	–	0.48 ± 0.11	73.5 ± 1.95	77.25 ± 2.44
VRJB5	+	+	–	–	0.41 ± 0.17	71.2 ± 2.32	75.64 ± 1.95

Low positive +, moderate positive ++, high positive +++, negative –. TPC: Total phenolic content, GAE: Gallic acid equivalents, FRAP: Ferric reducing antioxidant power, DPPH: 2,2-diphenyl-1-picryl hydrazyl

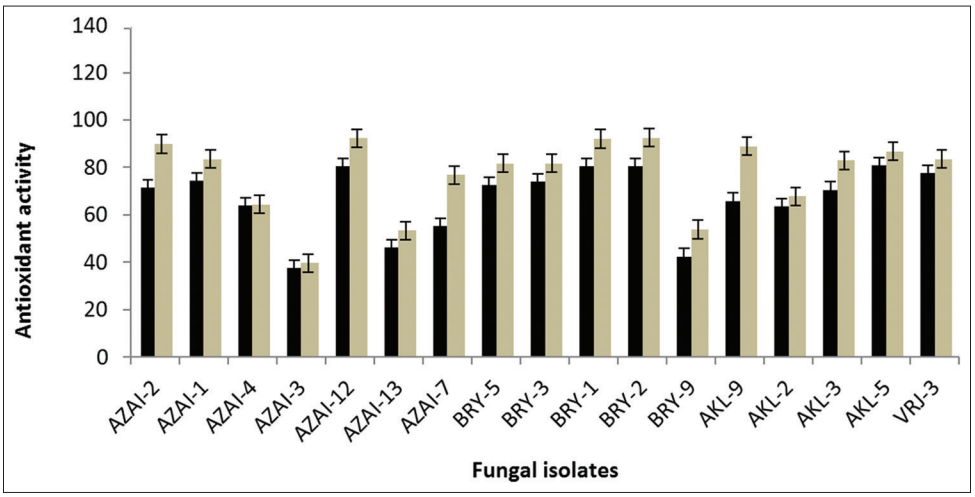

Figure 2: Comparative 2,2-diphenyl-1-picryl hydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) activities of fungal isolates. The error bar in the figure represents the standard error (SE) (±1 SE). ■ FRAP activity, ▭ DPPH activity

high frequency of *Aspergillus* sp. and *Alternaria* sp. in endophytes isolated from *Eugenia jambolana* Lam. and *Catharanthus roseus*.[28,29] The fungal extracts were evaluated for their antidiabetic and antioxidant potential. Many endophytic cultures were found to possess good AGI activity, namely, AKL-4, AKL-9, VRJ-3, and VRJ-5 and most of these belonged to the genus *Aspergillus*. AGI activity of *Aspergillus* species, namely, *A. awamori*, *A. aculeatus,* and *A. terreus* has been documented by many workers.[15,30,31] Artanti *et al.* (2014) have reported AGI activity in *Colletotrichum* sp.

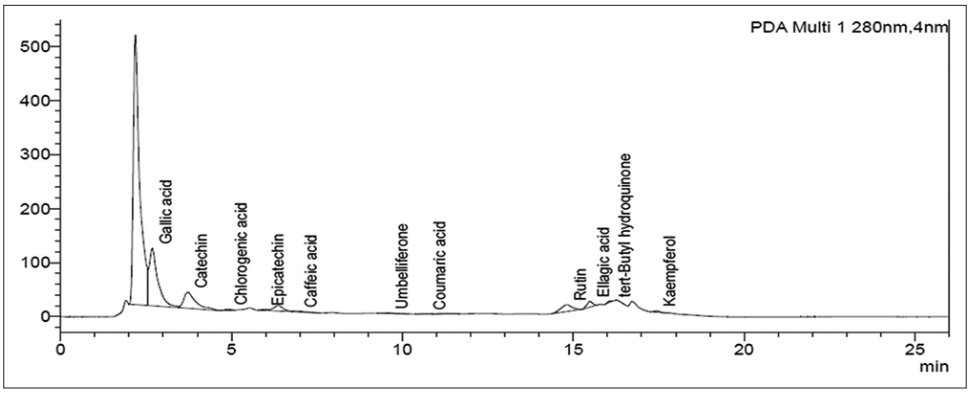

Figure 3: Reversed-phase-high-performance liquid chromatography chromatogram showing the presence of phenolics inthe extract of BRY-1

from *Taxus sumatrana*.[32] Although many other endophytic fungi have also been reported as producers of AGIs,[15,17,32] a survey of literature revealed no previous reports on AGI production by *Fusarium* and *Alternaria*. The cultures AKL-9 and VRJ-3 showing the presence of phenolics in phytochemical tests were found to possess high inhibitory activity. Singh *et al*. (2015) have documented the presence of a phenolic AGI from *Cladosporium*. Inhibitory activity against α-glucosidase enzyme by phenolics was reported by Apostolidis *et al*. (2006) who found that inhibition was proportional to phenolic content.[33] Kwon *et al*. (2008) also studied the positive correlation between phenolic content and AGI activity of wine and tea extracts.[34] On the other hand, cultures such as BRY-2 and BRY-1 having high phenolic content did not show good AGI activity. . Extract of cultures such as VRJ-5, AKL-1, and AKL-16 having good AGI activity was phytochemically identified to be non-phenolics in nature. Their inhibitory activity could be due to non-phenolic inhibitors. Biochemically, the AGI are known to belong to different groups, namely, protein, sugars, curcuminoid, or phenols.[15,35]

Researchers have documented less or no alpha-amylase inhibitory activity in extracts possessing good AGI and antioxidant potential.[34] Similarly, in the present study, none of the fungal cultures were found to exhibit alpha-amylase inhibitory activity.

The antioxidant potential was estimated using DPPH and FRAP. DPPH test determined the fungal extract's ability to scavenge free radicals which are highly reactive, while FRAP assay demonstrated the reducing power of antioxidants. In the present study, the average values of antioxidant activities achieved by DPPH and FRAP assays were found to have a strong positive Pearson correlation ($r = 0.91$, $P < 0.05$).

This correlation signifies that the compounds have high electron-donating capacity (reducing iron complex) in FRAP and same compounds served as hydrogen sources for DPPH radical. The results obtained in the DPPH and FRAP assay indicated high activities in cultures, namely, BRY-1, AZAI-12, and BRY-2. It can be postulated that these cultures have both electron- and hydrogen-donating potential which enhanced their antioxidant property. Similar results were documented by Ascêncio *et al*. (2014) where DPPH and FRAP activities of endophytic fungal extracts have been found to be significantly correlated.[36] There was also a strong correlation between TPC and antioxidant potential as determined by DPPH and FRAP ($r = 0.649$, $r = 0.7393$ respectively) at $P < 0.05$. Similar strong positive correlation between TPC and antioxidant capacity was observed by Benzie and Szeto (1999) in extracts of 25 different teas.[37] In another study, a combination of water extracts of cranberry (75%) and oregano (25%) having high TPC was found to possess high antioxidant capacity.[33] HPLC analysis of fungal extract of BRY-1 revealed the presence of a number of phenolic compounds. Researchers have documented the presence of various phenolic compounds in endophytic fungi and their role in antioxidant potential.[38] Phenolics such as quercetin, rutin, isoquercitrin, and chlorogenic acid have also been reported to inhibit alpha glycosidases.[22,39,40]

CONCLUSION

These studies reveal the potential of endophytic fungi as sources of pharmaceutically important molecules. Cultures possessing high AGI activity as well as good antioxidant potential were isolated in the present study. These cultures on account of their dual activity would be a better therapeutic agents. These isolates can be selected and subjected to further studies to determine the nature of the components responsible for the activity. Besides, AGI activity in *Fusarium* and *Alternaria* is being reported for the first time.

ACKNOWLEDGMENT

The authors, Jasleen Kaur and Rajvir Kaur, acknowledge the grant of fellowship under UPE (University with Potential for Excellence) scheme of University Grants Commission, New Delhi, India.

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Source of Support: Nil. **Conflict of Interest:** None declared.