Molecular profiling and antioxidant as well as anti-bacterial potential of polyphenol producing endophytic fungus-Aspergillus austroafricanus CGJ-B3

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ABSTRACT

Fungal endophytes are less studied group of microorganisms with vast therapeutic potential. A polyphenol producing endophytic fungus was isolated from Zingiber officinale rhizome. The ethyl acetate extract of Aspergillus austroafricanus (EAE) was tested against five human pathogenic bacteria by disc diffusion method. Antioxidant activity of EAE was determined by DPPH, H2O2 and nitric oxide radical-scavenging methods. DNA protection from the OH radicals was tested by agarose gel electrophoresis. High-performance liquid chromatography was used to determine the total yield of polyphenols. The identity of the endophytic fungus was established as A. austroafricanus CGJ-B3 (GenBank accession No. KT780617) based on rDNA and phylogenetic analysis. EAE showed significant antioxidant, antimicrobial activity and DNA damage protection capacity. The HPLC analysis showed the presence of polyphenols such as p-coumaric acid, ferulic acid and cinnamic acid and the content was about 0.392 ± 0.08 µg/mg, 4.35 ± 0.16 µg/mg and 1.976 ± 0.11 µg/mg, respectively. A. austroafricanus CGJ-B3 isolated from Z. officinale is a promising potential pharmaceutical agent and can be used as an alternative source of polyphenols like p-coumaric acid, ferulic acid and cinnamic acid.

1. Introduction

Plants are the reservoir of therapeutic compounds like phenolics, flavonoids, terpenoids and alkaloids. Amongst these, polyphenols and flavonoids play a very vital role in the antioxidant, free radical-scavenging and other pharmacological activities (Gangwar et al. 2014). So, the polyphenol producing medicinal plants are serving the needs of pharmaceutical industries since many years (Hosseinzadeh et al. 2015).

Zingiber officinale is one of the important tropical medicinal plants which belongs to the family Zingiberaceae. This plant was used as an ingredient in Ayurveda and Siddha medicine since ages for treating various ailments (Kumar et al. 2011). The rhizome of this plant is one of the ingredients in food, beverages, medicines and cosmetics. Different parts of Z. officinale have been reported to show an array of medicinal properties such as analgesic, anti-inflammatory, antiulcer, antioxidant (Ma et al. 2004; Chrubasik et al. 2005; Ghasemzadeh et al. 2010; Nanjundaiah et al. 2011a), anticancer (Miyoshi et al. 2003; Shukla & Singh 2007), antimicrobial (Aghazadeh et al. 2016), anticoagulant (Srivas 1984), fibrinolytic, prebiotic (Helal et al. 2014), immunomodulatory (Gupta & Chaphalkar 2015), hepatoprotective, larvicidal (Kumar et al. 2011) and anti-Limnatisnilotic activities (Bahmani et al. 2013). It is a potent inhibitor of proton–potassium ATPase activity as well as the ulcer-causing organism, Helicobacter pylori (Siddaraju & Dharmesh 2007).

Endophytes are the microorganisms that inhabit the plant organs and can colonise internal plant tissue without causing any apparent harm to the host (Petrini et al. 1993). These organisms harbour numerous therapeutic molecules like phenolics, flavonoids, terpenoids and alkaloids (Kaul et al. 2012). According to Zhao et al. (2010), endophytic fungi have the capacity to produce similar compounds as that of the host plants. Isolation of potent bioactive compounds from the endophytic fungi is in great demand as it has several beneficial applications in pharmaceutical, agricultural and pharmaceutical industries. Endophytic fungal extracts have been reported to possess antimicrobial, anticancer,
antimalarial (Wiyakrutta et al. 2004; Santos et al. 2012), antitumor (Li et al. 2005), antidiabetic (Ushasri & Anusha 2015), antimicrobial (Supaphon et al. 2013; Hussain et al. 2014; Shen et al. 2014; Rao et al. 2015) and antioxidant activity (Liu et al. 2007; Yadav et al. 2014). Many endophytes have been isolated from Z. officinale and these endophytes have been reported to possess antimicrobial (Ginting et al. 2013), antioxidant activity (Bussaban et al. 2003). Anisha and Radhakrishnan (2015) have isolate ß-gliotoxin-producing endophytic fungus, Acremonium sp. from Z. officinale. Even though some of the endophytes have been isolated from Z. officinale, still many are underexploited for the therapeutic potential. So, the aim of our current investigation was to isolate the novel endophytes from Z. officinale and to evaluate the antioxidant, antimicrobial, DNA protection activity. We had successfully isolated the endophytes from the rhizomes of Z. officinale. The fungus was identified as Aspergillus sp. which was capable to produce polyphenols and flavonoids which is a rarely reported in the literature.

2. Materials and method

2.1. Chemicals used

Agarose and Folin–Ciocalteu reagent were purchased from Sisco Research Laboratories. λ-DNA was purchased from Bangalore Genei, India. Ferrous sulfate heptahydrate (FeSO₄·7H₂O), hydrogen peroxide, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, TRIS base, butylated hydroxyanisole, ethidium bromide, gallic acid, ethylene diaminetetraacetic acid (EDTA) and thiobarbituric acid were purchased from Sigma Chemicals (USA). All other chemicals used were of analytical grade.

2.2. Collection of plant material

Z. officinale rhizome was collected in the month of February 2014, Somawarpet (T), Kodagu (D) Karnataka, India. The Latitude and longitude is 12.46700N 75.96700E and a plant was authenticated by Dr. Jagat Timmaiah, Assistant professor, Dept. of Botany, Mangalore University, Cauvery campus, Madikeri, Karnataka, India.

2.3. Isolation of endophytic fungus from Z. officinale rhizome

The rhizome of Z. officinale was washed with running tap water to remove soil debris followed by washing with distilled water. The samples were cut into small pieces (0.6–0.8 cm) and rinsed with 70% alcohol. The alcohol washed pieces were again rinsed with 0.5% sodium hypochloride followed by washing thrice with sterile distilled water. The sterilised samples were transferred aseptically to PDA media containing 150 mg/L chloramphenicol (Higginbotham et al. 2013).

2.4. Scanning electron microscopic observation of Aspergillus austroafricanus CGJ-B3

The pure cultures of isolated endophytic fungal colony characters were studied and observed under the Scanning Electron Microscope (Model No. LEO 425 VP, Electron Microscopy Ltd. (Cambridge, UK). The methods mainly referred to (Qiu et al. 2010) with slight modification. The specimens were first fixed with 2.5% glutaraldehyde in phosphate buffer (PBS, 0.2 M, pH 7.0) for 6 h and then washed three times with PBS. They were postfix with 1% (w/v) osmium tetroxide (OsO₄) in PBS for 1 h and again washed three times with PBS. The specimens were dehydrated by a graded series of ethanol (10–100%) for about 10–15 min at each step; finally, they were dehydrated in a critical point dryer and coated with gold palladium and observed the microscopic images under SEM (Qiu et al. 2010).

2.5. Molecular identification

Molecular identification was achieved by sequencing the partial sequence of 28S rDNA. The fungus was grown and cultured in potato dextrose broth at 28 ± 2°C temperature for 4 days. The genomic DNA was isolated and the 28S rDNA was amplified using forward 5’ACC CGC TGA ACT TAA GC 3’ and reverse 5’GGT CCG TGT TTC AAG ACG G 3’ primers.

2.6. Phylogenetic analysis

The evolutionary history was inferred using the neighbour-joining method (Saitou & Nei 1987). The bootstrap consensus tree inferred from 1000 replicates was
2.7. Preparation of fungal mat culture
The endophytic fungal isolate was cultured in 1000 mL conical flask containing 350 mL potato dextrose broth and 10% of starter culture inoculum was inoculated and incubated in an incubate shaker at 100 rpm at 28 ± 1°C for 3 days followed by incubation 28 ± 1°C for 18 days in a static condition (Cui et al. 2011).

2.8. Extraction of secondary metabolites
Extraction of secondary metabolites was carried according to the procedure explained by Higginbotham et al. (2013), with slight modification. Liquid culture with evident growth was mixed with an equal volume of ethyl acetate (Merck, India). The mixture was blended with the help of pastel and mortar. The resulting homogenate was filtered with Whatman* qualitative filter paper, Grade one and extracted thrice with an equal volume of ethyl acetate. The aqueous and organic layer were dried and stored at −20°C for further use (Higginbotham et al. 2013). The extract was labelled as endophytic ethyl acetate extract of A. austroafricanus (EAE).

2.9. Preliminary phytochemical analysis
Freshly prepared EAE was subjected to standard methods of phytochemical analyses to detect the presence of phyto-constituents, viz. flavonoids, carbohydrates, glycosides, saponins, tannins and alkaloids (Poojary et al. 2015).

2.10. Determination of total phenolic content
Total phenolic content was measured according to the method of (Yadav et al. 2014), with minor modifications. Standard tannic acid (0.1 mg/mL) and EAE (1 mg/mL) were taken in a tube and 0.5 mL of Folin–Ciocalteu reagent (1:1) and 2.5 mL of sodium carbonate (20%) were added. The final volume was made up to 10 mL by adding the distilled water. The absorbance of the reaction mixture was measured at 760 nm in UV spectrophotometer (Optima Tokyo, Japan). Gallic acid was used as standard and total phenolic content was expressed as milligram equivalents of gallic acid.

2.11. Total flavonoids content
The total flavonoids contents in EAE were determined spectrophotometrically according to the method of Qiu et al. (2010). Standard quercetin was employed to make the standard curve. EAE (1 mg/1 mL) was mixed with 1 mL of 2% aluminium chloride (AlCl₃·6H₂O) methanolic solution. The reaction mixtures were incubated at 25 ± 1°C for 15 min, and then the absorbance was measured at 430 nm in UV spectrophotometer. The total flavonoids content was expressed as milligram quercetin equivalent.

2.12. Antioxidant activity
2.12.1. Reducing power assay
Reducing power was determined according to the method of Poojary et al. (2015) with modifications. Standard (0.1 mg/mL) and diluted EAE (1 mg/mL) were taken in different tubes. Phosphate buffer (2.0 mL, 0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide were added to reaction mixture and incubated at 50°C for 20 min. Trichloroacetic acid (2.5 mL, 10%), 2.5 mL distilled water and 0.5 mL of 0.1% ferric chloride were added and mixed well. The absorbance of the reaction mixture was measured at 700 nm UV spectrophotometer.

2.12.2. Total antioxidant activity by phosphomolybdate reagent
Total antioxidant capacity of the EAE was determined according to the method of Kanner et al. (1994), with slight modifications. The EAE (1 mg/mL) was taken in a test tube and mixed with a mixture of 3 mL of phosphomolybdate reagent (0.6 M H₂SO₄, 4 mM ammonium molybdate and 28 mM sodium phosphate) and incubated at 95°C for 90 min. The absorbance of the solution was measured at 695 nm in a
UV spectrophotometer (Optima, Tokyo). The total antioxidant content of the EAE was calculated as the ascorbic acid equivalent.

2.12.3. Determination of free radical-scavenging activity by DPPH method
The free radical-scavenging activity by DPPH method is carried out by using the procedure as explained by Brand-Williams et al. (1995), with slight modifications. Standard ascorbic acid (0.1 mg/mL) and diluted EAE (0.1 mg/mL) were taken different tubes. The volume of each test tube was made up to 1 mL using methanol and 3 mL of 0.1 M DPPH (Sigma, USA) was added and mixed thoroughly with the help of vertex machine (REMI, India). The reaction mixture was incubated at dark for 30 min and absorbance was measured at 517 nm in UV spectrophotometer (Optima, Tokyo). DPPH activity was calculated by using the following equation,

\[
\text{Inhibition (\%)} = \left( \frac{A_{\text{control}} - A_{\text{test or standard}}}{A_{\text{control}}} \right) \times 100
\]

2.12.4. Hydroxyl radical-scavenging activity
Hydroxyl radical-scavenging activity was assayed by deoxyribose method (Halliwell et al. 1987), with slight modifications. Hydroxyl radicals were generated by using Fenton reagent (Ascorbate-EDTA-H$_2$O$_2$-Fe$^{3+}$ method). In this method, the total reaction mixture containing 2-deoxy-2-ribose (2.6 mM), ferric chloride (20 µM), H$_2$O$_2$ (500 µM), as well as phosphate buffer (100 µM, pH 7.4) was mixed with ascorbic acid (100 µM) and EAE (100–500 µg/mL). The reaction mixture was incubated at 37°C for 1 h to initiate the reaction. After incubation, 0.8 mL of the reaction mixture was added to the 2.8% TCA (0.8 mL), followed by 1% TBA (1 mL) and 0.1% SDS (0.2 mL); the reaction mixture was then heated at 90°C for 20 min to obtain colour, later cooled and 1 mL of double distilled water was added. The absorbance was read at 532 nm and the percentage inhibition was calculated by following equation:

\[
\text{Hydroxyl radicals inhibition \%} = \left( 1 - \frac{A}{A_0} \right) \times 100
\]

where $A_0$ is the absorbance of the negative control (without sample) at 532 nm, and $A$ is the absorbance at 532 nm of the reaction mixture containing the sample.

2.12.5. Nitric oxide radical-scavenging assay
Nitric oxide radical inhibition was estimated using Griess Ilosvory reaction Patel et al. (2010). The reaction mixture (3 mL) containing 2 mL of 10 mM sodium nitroprusside, 0.5 mL saline phosphate buffer and 0.5 mL of standard solution or EAE extract (100–500 µg/mL) was incubated at 25°C for 150 min. After incubation, 0.5 mL of the reaction mixture was mixed with 1 mL sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for the completion of the reaction of diazotisation. After this, a further 1 mL of the naphthylethenediamine dihydrochloride was added, mixed and was allowed to stand for 30 min at 25°C. The concentration of nitrite was assayed at 546 nm and was calculated with the control absorbance of the standard nitrite solution. Buffer was used as a blank and ascorbic acid was taken as the standard solution. The percentage inhibition was calculated using the formula:

\[
\text{\% scavenging activity} = \left( \frac{A_{\text{control}} - A_{\text{test or std}}}{A_{\text{control}}} \right) \times 100
\]

where $A_{\text{control}}$ is the absorbance of control and $A_{\text{test or std}}$ is the absorbance of test or standard, respectively.

2.13. Antimicrobial activity
Antimicrobial activity of EAE was tested by disc diffusion method against five pathogenic strains namely, Escherichia coli (ATCC-118), Bacillus subtilis (ATCC-441), Staphylococcus aureus (ATCC-7443), Staphylococcus epidermidis (ATCC-435) and Pseudomonas aeruginosa (ATCC-424) (Arora & Kaur 1999). The plates containing brain heart infusion medium (Himedia, India) were spread with 0.3 µL of 24 h starter culture inoculum. Discs made of Whatman® qualitative filter paper, grade 1 (6 mm diameter) containing 100, 200 and 400 µg/disc of EAE were placed on the bacterial lawn. The plates were incubated at 37°C for 24 h. The zone of inhibition was measured.

2.14. λ-DNA nicking assay
Oxidative λ-DNA damage prevention potential of EAE was assayed according to the protocol of Ghanta et al. (2007). λ-DNA (0.5 µg) with and without EAE (200 µg) was incubated with Fenton
reagent (1 mM FeSO₄, 25 mM H₂O₂ in tris buffer 10 mM, pH 7.4) in a final reaction volume of 30 µL for 1 h at 37°C. The relative difference between oxidised and native DNA was analysed on 1% agarose gel prepared in tris-acetate-EDTA buffer (pH 8.5). The gel was run at 50 V for 3 h at room temperature and documented (Uvitec Company, software platinum 1D, UK) and the band intensity was determined.

2.15. HPLC analysis

EAE samples were filtered with 0.45 µm PS membrane filter prior to high-pressure liquid chromatography (HPLC) analysis. HPLC was done according to the Le et al. (2007), with slight modifications. The system was equipped with a photodiode array detector (Agilent-Model 1200 series). The analysis was performed on a grace smart reverse phase C-18 column (250 mm × 4.6 mm; 5 µm) at a flow rate of 1 mL/min, using an injection volume of 20 µL and detected at 280 nm. The mobile phases employed were water/methanol/acetic acid (83:15:2). The standard phenolic compounds such as gallic acid, p-coumaric acid, cinnamic acid, ferulic acid, gentisic acid, syringic acid and vanillic acid were used for the identification and quantification of phenolic acids present in the EAE.

2.16. Statistical analysis

Each experiment was conducted in triplicate and the data were reported as mean ± SD with the help of graph pad prism 5.0 software. The difference between the control and treated groups is determined by one-way ANOVA test (Bonferroni multiple comparison test).

3. Results

3.1. Isolation and characterisation of endophytic fungus

A polyphenol and flavonoid producing fungus was isolated from the rhizome of Z. officinale. Greenish to greyish white colonies of the fungal strain grown up to 3–4 cm diameter on a PDA plate after 7 days of incubation at room temperature. The rear side of the plate was yellowish due to the secretion of pigment by the fungus (Figure 1).

3.1.1. Scanning electron microscopic study of endophytic fungus

Based on the SEM images of mycelium and conidia, fungus was identified as the species of the genus Aspergillus. The conidial spores were 0.3–0.4 µm size in showing similarity to conidial spores of Aspergillus sp. (Figure 2).

3.1.2. Molecular identification

The identity of the endophytes was established by comparing the 28S rDNA sequence. The phylogenetic tree was constructed with the help of Bootstrap values (1000 replications) based on multiple sequence alignment using the MEGA-6 software. The endophytic fungus was identified as A. astroafricans as it shared 99% similarity with the sequence data deposited in GenBank (GenBank Accession No.: KT780617) (Figure 3).

3.2. Phytochemical analysis

The phytochemical screening of EAE revealed the presence of phenolics and flavonoids. However, the extract did not show any positive results for alkaloid, saponins, glycosides, carbohydrates and steroids.

Figure 1. Isolation and pure culture of endophytic fungus from Z. officinale. (a) Control plate. (b) Growth of the endophytic fungi on Z. officinale.
3.3. Antioxidant activity

The EAE was analysed for the total polyphenol, flavonoid content. Polyphenol was estimated using Folin–Ciocalteu’s reagent method and the flavonoid was estimated by making it react with aluminium chloride. Tannic acid and quercetin were used as the standard for total phenol and flavonoid content determination, respectively. A volume of 80 ± 11.50 µg/mg of phenol and 16.0 ± 0.46 µg/mg of flavonoid were present in EAE. The EAE also showed significant reducing power (15 ± 0.81 µg/mg) as well as total antioxidant activity (82 ± 0.78 µg/mg) (Figure 4).

EAE was also studied for its free radical-scavenging activity by three methods namely, DPPH, H$_2$O$_2$ and nitric oxide radical-scavenging activity. Ascorbic acid was used as the standard in DPPH and nitric oxide-scavenging activity while mannitol was used as a standard in H$_2$O$_2$ radical-scavenging study. EAE showed varying degree of antioxidant activity in the three methods used for the study. DPPH radical-scavenging activity of the EAE was comparable to the standard ascorbic acid. The radical-scavenging activity of the EAE was concentration dependent (Figure 5).

3.4. Antimicrobial activity

The EAE was also tested for antimicrobial activities against a panel of four bacterial and a fungal
pathogen by disc diffusion method. Results of the antimicrobial activity were shown in Figure 6. The antimicrobial potential of EAE was found to be in the range of moderate to a significant level. The antimicrobial activity of the fungal extract was found to be dose dependent. The zone inhibition was ranging from 7 to 24 mm.

3.5. Protection of $\lambda$-DNA from the free radicals

Incubation of Fenton radicals with $\lambda$-DNA for 1 h resulted in the total disappearance of DNA bands on 1% agarose gel compared to DNA control and DMSO vehicle (Lane 2). However, the addition of 200 µg of EAE to the mixture of $\lambda$-DNA and Fenton reagent prevented the radical induced DNA damages (Lane 3) as shown in Figure 7.

3.6. HPLC analysis

The polyphenols in EAE were identified by using reverse phase C-18 column in HPLC. Different peaks with respect to retention time confirmed the presence of polyphenols in EAE (Figure 8). The EAE showed the presence of $p$-coumaric acid (0.392 ± 0.08 µg/mg), ferulic acid (4.35 ± 0.16 µg/mg) and cinnamic acid (1.976 ± 0.11 µg/mg). The fungal extract was quantitated for the presence of phytochemicals and the quantity of individual chemical.

4. Discussion

Endophytes present in the medicinal plants share the therapeutic properties of the host plants (Zhao et al. 2010). These characters of the endophytes make them the best source of medicinal compounds instead of plants as they have several advantages over plants (such as the purification and mass cultivation). Z. officinale is a medicinal plant and its medicinal properties are exploited since ages. This plant
The phytochemical analysis of EAE showed the presence of polyphenol and flavonoids as the major constituents. Phenols and flavonoids are well-documented phytochemicals responsible for the antioxidant potential of the natural products (Ghasemzadeh et al. 2010; Nanjundaiah et al. 2011a). Different groups of researchers have reported contradictory results about the correlation between the total phenolic content and antioxidant potential of the extract. Yadav et al. (2014) have described about the positive correlation between the antioxidant capacities of the extract to the phenolic constituents. Even our study showed a good relation between the phenolic content and antioxidant capacity. The hydroxyl group present in the phenols may be involved in radical-scavenging. This is in agreement with the findings of Yadav et al. (2014) and Huang et al. (2005).

Even though the antioxidant activity was measured with various protocols, DPPH method is extensively used to assess the radical generation inhibition potential of the molecules. The accuracy and repeatability of DPPH method makes it a method of choice for most of the researchers (Brand-Williams et al. 1995). This method primarily works on the power of the molecule to scavenge proton radical. The characteristic absorbance of DPPH decreases with the increase in proton radical scavengers (Singh & Rajini 2004). In our study, EAE showed a dose-dependent DPPH radical-scavenging activity. This trend may be due to the hydrogen donating capability of the compounds present in EAE. It is well established that the compounds scavenge DPPH radical by donating hydrogen (Chen & Ho 1995).

Many researchers have reported the relation between the antioxidant activity and reducing power. The reducing power of the extract is due to the presence of reductons which have the capacity to stop the free radicals by donating hydrogen as well as by preventing peroxide formation (Singh & Rajini 2004). Our data on reducing power may be attributed to the hydrogen donation capacity as the extract is rich in phenols and flavonoids.

Even though $H_2O_2$ presents in small quantities in the human body, it will rapidly decompose to produce hydrogen radicals. These radicals have deleterious effects on biomolecules such as lipids and DNA (Gülçin et al. 2005). The EAE showed hydrogen peroxide-scavenging activity. Our results are in agreement with the studies of Saeed et al. (2012). The
phenolic groups present in the extract could neutralise the \( \text{H}_2\text{O}_2 \) by donating the electron. EAE also showed the nitric oxide radical-scavenging potential. Nitric oxide radicals have the capability to alter the structure as well as the function of biomolecules. The reaction of nitric oxide (NO) with superoxide will result in a more toxic peroxynitrite amines which undergo decomposition to \( \text{OH}^- \) and \( \text{NO}_2^- \) (Pacher et al. 2007; Awah 2010).

Secondary metabolites derived from fungi were used as a model structure to develop several antimicrobial agents. In the present study, the EAE showed moderate antimicrobial activity against the pathogens. According to Hussain et al. (2014), lower antimicrobial activity against the microorganisms may be due to the presence of active compounds in lesser amounts (Hussain et al. 2014). Our study is in agreement with the observation of Hussain et al. (2014).

Photolysis of \( \text{H}_2\text{O}_2 \) release hydrogen radicals which intern damage the DNA. The radicals generated may break single or double strand (Patel et al. 2010). Chemicals that prevent the generation of free radical can protect the DNA damage. Antioxidant secondary metabolites of endophytic fungus are the key mediators of protection of DNA damage by free radicals. Similar results have been reported by Ma et al. (2004) and Ruma et al. (2013).

The HPLC analysis revealed the presence of various phytochemicals such as \( p \)-coumaric acid, ferulic acid, cinnamic acid etc. These phytochemicals are known to be antioxidant molecules and are used in many therapeutic studies. Interestingly Siddaraju and Dharmesh (2007) have reported the chemical contents of \( Z. \text{ officinale} \) and it biological properties. Our study also reports a similar result highlighting the association of microorganisms with medicinal plants and medicinal properties.

5. Conclusion

We have successfully isolated an endophytic fungus \( A. \text{ austroafricanus} \) CGJ-B3 from the inner tissues of \( Z. \text{ officinale} \) rhizome. The preliminary study on this fungus showed its capacity to produce polyphenol and flavonoids which are rare in the fungus \( A. \text{aspergillus} \) sp. This is the first report on the isolation of polyphenol and flavonoid producing \( A. \text{aspergillus} \) sp. from the rhizomes of \( Z. \text{ officinale} \). The fungal extract showed antioxidant, antimicrobial and DNA damage protection potential. The medicinal properties of this endophytic fungus may be attributed to the different phenolic compounds such as \( p \)-coumaric acid, ferulic acid, cinnamic acid etc. Our study showed the possibility of \( A. \text{ austroafricanus} \) CGJ-B3 in the development of a potent drug against free radical-related disorders such as aging, arthritis, diabetes, cancer etc. Further studies on this organism at the molecular level will aid in optimising the production of bioactive compounds in a larger scale.

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Disclosure statement

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