Molecular Biology Reports (2020) 47:423–432
https://doi.org/10.1007/s11033-019-05145-3

ORIGINAL ARTICLE

Assessment of alpha glucosidase inhibitors produced from endophytic fungus *Alternaria destruens* as antimicrobial and antibiofilm agents

Jasleen Kaur[1] · Preeti Sharma[1] · Rajvir Kaur[1] · Sukhraj Kaur[1] · Amarjeet Kaur[1]

Received: 30 April 2019 / Accepted: 16 October 2019 / Published online: 23 November 2019

Abstract

Diabetes is considered as a major health concern worldwide and patients with diabetes are at high risk for infectious diseases. Therefore, α-glucosidase inhibitors possessing antibacterial activity along with the ability to inhibit biofilms would be better therapeutic agents for diabetic patients. In the present study, two fractions (AF1 and AF2) possessing α-glucosidase inhibitory activity were purified from an endophytic fungus *Alternaria destruens* (AKL-3) isolated from *Calotropis gigantea*. These were evaluated for their antimicrobial and antibiofilm potential against human pathogens. AF1 exhibited broad spectrum antimicrobial activity against all the tested pathogens. It also significantly inhibited biofilm formation and dispersed the preformed biofilm at sub-optimal concentrations. AF2 possessed lesser activity as compared to AF1. The active compounds were purified using semi preparative HPLC. Some of the active compounds were identified to be phenolic in nature. The active fractions were also determined to be non-mutagenic and non-cytotoxic in safety analysis. The study highlights the role of endophytic fungi as sources of α-glucosidase inhibitors with antimicrobial potential which can have application in management of diabetes.

Keywords Endophytes · *Alternaria destruens* · α-Glucosidase inhibitors · Antibacterial · Biofilm inhibition · Biofilm dispersion

Abbreviations

AGI	α-Glucosidase inhibitors
BHI	Brain heart infusion
CHO	Chinese hamster ovary
DM	Diabetes mellitus
DMSO	Dimethyl sulfoxide
DOX	Doxorubicin
FTIR	Fourier transform infrared spectroscopy
HPLC	High performance liquid chromatography
MTT	(3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyl-2H-tetrazolium bromide
OD	Optical density
*p*NPG	*ρ*-Nitro phenyl-α-D-glucopyranoside
TLC	Thin layer chromatography

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11033-019-05145-3) contains supplementary material, which is available to authorized users.

✉ Amarjeet Kaur
amarjeetmicro2@gmail.com

Jasleen Kaur
jasleenbal2@gmail.com

Preeti Sharma
preetisharma2925@gmail.com

Rajvir Kaur
Rajvir2204@gmail.com

Sukhraj Kaur
drsukhraj@gmail.com

[1] Department of Microbiology, Guru Nanak Dev University, Amritsar, Punjab 143005, India

Introduction

α-Glucosidases (E.C.3.2.1.20) are glycoside hydrolase enzymes which play a vital role in digestion of carbohydrates. These enzymes also help in the processing of cell surface oligosaccharides, which have crucial roles in cell to cell recognition during various infections, metastasis and immune responses [1]. Thus, agents possessing the ability to inhibit α-glucosidase enzyme can be used as therapeutics against many carbohydrate linked diseases such as Diabetes mellitus (DM) type 2. α-Glucosidase inhibitors (AGIs) have also been reported for their antiviral and antifungal activities [2–5]. But they have not been much explored for their

antibacterial potential. People with diabetes are more susceptible to infections, due to dysfunction of immune system caused by high blood sugar [6, 7]. Diabetes-related health issues, such as nerve damage and reduced blood flow to the extremities can also enhance the vulnerability to infection. Another challenge encountered by diabetic patients is the delayed healing of wounds due to hindrance caused by biofilm formation by the infecting microorganisms [8]. Therefore, AGIs possessing antibacterial activity along with the ability to inhibit biofilms would be better therapeutic agents for diabetic patients. Thus, exploration of such novel enzyme inhibitors is necessitated. Microorganisms such as endophytes, residing in unique ecological niches can serve as sources of novel biomolecules [9]. Endophytes are microorganisms (mostly fungi and bacteria) that inhabit plant hosts for all or part of their life cycle without causing any apparent symptoms on them [10]. They are rich sources of bioactive compounds with novel chemical skeletons and diverse biological activities [9–13].

Though AGIs from many endophytic fungi have been reported [14–16], their antimicrobial and antibiofilm potential has not been explored. In previous studies conducted in our lab, an endophytic fungus *Alternaria destruens* (AKL-3) isolated from *Calotropis gigantea* was found to yield partially purified fractions possessing high AGI [17]. The culture was used in the present study to determine its antimicrobial potential. The antimicrobial activity and the ability of partially purified fractions to inhibit as well as disperse preformed biofilms of pathogenic bacteria was determined.

Materials and methods

Microorganisms

For production of AGI, an endophytic fungus *A. destruens* AKL-3 isolated in previous study from healthy plants of *C. gigantea* and exhibiting an AGI potential of 93.4% was used. For determination of antimicrobial activity various pathogenic microorganisms used were *Staphylococcus aureus* MTCC 96, *Salmonella enterica* MTCC 733, *Klebsiella pneumoniae* MTCC 7407, *Listeria monocytogenes* MTCC 657, *Shigella flexneri* MTCC 1457, *Vibrio cholerae* MTCC 3906, *Escherichia coli* MTCC 119 and *Pseudomonas aeruginosa* MTCC 7925. All the indicator strains used were procured from Microbial Type Culture Collection, Institute of Microbial Technology (IMTECH), Chandigarh, India. *Candida albicans* (clinical isolate) was procured from Department of Microbiology, Government College, Amritsar, Punjab, India. For mutagenicity testing microorganism used was his$^-$ *Salmonella typhimurium* strain MTCC 1251. All the strains were preserved in 20% glycerol stocks at − 20°C.

Production of secondary metabolites

The production and purification of inhibitory compounds was carried out as described in the previous study [17]. Briefly, the production was carried out on malt extract broth for 10 days and the inhibitory compounds were extracted with ethyl acetate. The ethyl acetate extract was concentrated using rotary evaporator, redissolved in HPLC grade water and subjected to silica gel chromatography using chloroform: ethyl acetate: formic acid in 5:4:1 ratio as solvent system. As previously reported, AGI activity was observed in fractions no. 11 (AF1) and 14 (AF2) exhibiting different thin layer chromatography (TLC) profiles [17]. The antimicrobial and antibiofilm effects of these fractions were determined.

In vitro α-glucosidase inhibitory assay

The α-glucosidase inhibition assay was performed using ρ-nitro phenyl-α-D-glucopyranoside (pNPG) as a substrate. The reaction mixture was prepared by adding 50 µl of phosphate buffer (50 mM, pH 6.8), 10 µl of *Saccharomyces* sp. α-glucosidase (1U ml^{-1}), 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. The reaction was terminated by adding 50 µl of sodium carbonate. All the reactions were performed in triplicates in 96-well microtitre plate. Appropriate blank containing only the substrate and buffer was used. Absorbance was taken at 405 nm using microtitre plate reader (BIORAD 680XR) and activity was calculated using the following formula:

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

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 [17].

Determination of antimicrobial activity

Antimicrobial activity was determined by agar gel diffusion assay [18] against various pathogenic bacteria and yeast strains. The bacterial and *Candida* strains were maintained in brain heart infusion (BHI) and Sabouraud media (HiMedia laboratories, Mumbai, India), respectively. The pathogenic indicator strains were inoculated in the respective media and incubated overnight for 24 h at 37 °C. The indicator cultures at 0.1 OD_{580} were spread onto BHI agar plates. Thereafter, wells were cut in the plates and 100 µl of filter sterilized (0.2 µm) AF1 and AF2 at a concentration of 130 µg ml^{-1} were added to the wells. The plates were incubated at 4 °C for 4 h to allow the diffusion of AF1 and

AF2 into the agar media and thereafter incubated at 37˚C for 24 h. The plates were observed and zones of inhibition were measured in mm. Ampicillin (10 mg l^{-1}) was used as reference standard.

Biofilm inhibitory activities of active fractions

Sub-optimal concentrations for the inhibitory fractions were determined and used for evaluation of antibiofilm potential by using modified crystal violet assay [19]. Active fractions AF1 and AF2 of *A. destruens* AKL-3 at concentrations ≥ 22.5 µg ml^{-1} showed lethal effects on the tested pathogens. Therefore, the effect of sub-optimal concentrations viz. 22.5 and 11.25 µg ml^{-1} on the biofilm formation by *P. aeruginosa, C. albicans, E. coli* and *S. enterica* was studied in 96-well microtiter plate (Tarsons Product Pvt. Ltd., Kolkata). Autoclaved BHI/Sabouraud broth (100 µl) was added to each well along with 100 µl of AF1/AF2 and 20 µl of overnight grown indicator organisms having OD_{580} of 0.1. The microtiter plate was incubated at 37 °C for 48 h to allow biofilm formation in the wells. After 48 h the plate was gently washed 3 times with autoclaved distilled water to remove non-adherent cells. The adherent cells were fixed by using 200 µl methanol for 15 min and the plate was emptied and air dried. The fixed biofilm was stained by adding 200 µl of 2% crystal violet for 5 min and the excess stain was removed by washing under running tap water till the color faded. The stain was extracted from the adherent cells by using 160 µl of 33% glacial acetic acid in distilled water and OD_{595} was measured using microtitre plate reader. The control wells contained sterile distilled water instead of inhibitory fractions of *A. destruens* AKL-3. The experiment was conducted in triplicates. The percentage inhibition was calculated as,

$$\text{Percentage inhibition} = 100 - \left[\left(OD_{595}\text{of sample} \times 100\right) / OD_{595}\text{of control}\right]$$

Effect of active fractions on preformed biofilm

The effect of sub-optimal concentrations (22.5 and 11.5 µg ml^{-1}) of AF1 and AF2 was studied on the dispersion of preformed biofilms of *P. aeruginosa, C. albicans, E. coli* and *S. enterica* was studied. Biofilm was developed in 96-well microtiter plate by adding 100 µl of autoclaved BHI broth along with 20 µl of overnight grown indicator organisms having OD of 0.1 at 595 nm. After 24 h incubation at 37 °C, non-adherent cells were removed by gentle pipetting without disrupting biofilm [19]. AF1 and AF2 (100 µl) were added to each well along with 100 µl autoclaved BHI broth. In the control wells instead of inhibitor 100 µl of autoclaved BHI/Sabouraud broth was added. The plates were incubated at 37 °C for 48 h. The experiment was conducted in triplicates. After incubation, quantification of biofilm was done as described previously.

Thermo stability of inhibitors

To check the stability of the inhibitory compounds under different thermal conditions, 100 µl AF1 and AF2 was pre incubated at different temperatures (40, 50, 60, 70, 80, 90 and 100 °C) for different time intervals (15, 30, 45 and 60 min) and the AGI activity was assayed.

Purification and characterization of inhibitors

The active fractions AF1 and AF2 obtained after silica gel chromatography were subjected to semi preparative High Performance Liquid Chromatography (HPLC) (Shimadzu, Kyoto, Japan).The active fractions were chromatographed on Microsorb MV C18 (100 × 10 mm ID, 10 µm) column (Enable, Spinco Biotech, Chennai, India), with a mobile phase of acetonitrile/acidified water in the ratio of 45:55 for AF1 and 50:50 for AF2 in an isocratic manner with a flow rate of 3 ml min^{-1} and at a detection wavelength of 254 nm. Water used in mobile phase was acidified with 0.1% trifluoroacetic acid. The peaks exhibiting AGI activity were collected and characterized.

Biochemical analysis

To determine the nature of the inhibitors various biochemical tests were performed. Different visualization reagents viz. Dragendroff's reagent for alkaloids, $FeCl_3$ and Fast Blue B for phenols, ninhydrin for amine group, p-anisaldehyde for the detection of steroids and terpenoids were used in TLC based assays [20].

HPLC based detection of phenolics

HPLC based identification of phenolics was also carried out in inhibitory fractions. HPLC was performed using PDA detector (Dionex UVD 340 U UV/VIS) on Microsorb-MV 100-5 C18 (250 × 4.6 mm ID, 5 µM) column (Agilent, USA). The solvent system used was 0.1% acetic acid in water and methanol (70:30) at a flow rate of 3 ml min^{-1}. On comparison with standards, the presence of ellagic acid (P3) and kaempferol (P5) in case of active fraction AF1, and rutin (P2) in active fraction AF2 was indicated. Thereafter, authentication was done by using standard addition protocol. Ten µl of standard phenolic was added to the purified collected peak and then subjected to HPLC. The hiking of peak at particular retention time of standard without significant deformations was observed. This procedure was repeated three times. The active peak P2 of active fraction

AF1, which was observed to be non-phenolic was subjected to mass spectrometry and Fourier transform infrared spectroscopy (FTIR).

Mass spectrometry

Mass spectrum of P2 of AF1 was obtained from Micromass Q-TOF micro mass spectrometer in +ve ESI mode at source temperature, 110°C. Gases used were N_2 at pressure 6–7 bars and Argon at pressure of 5–6 bar, with flow of 0.4 ml min^{-1}. The mass spectrum was acquired with scan range of m/z from 100 to 1000. The species formed were identified from their respective m/z.

FTIR

Peak 2 was also subjected to FTIR (Vertex70, Bruker, Germany) for identification of functional groups. All the measurements were carried out in the frequency range 400–4000/cm with 4/cm resolution. The sample was loaded directly in liquid form and spectrum was taken.

Safety evaluation

The mutagenic effect of inhibitory compounds was studied by Ames test as proposed by Maron and Ames (1983) [21] using his^- *S. typhimurium* strain (MTCC 1251, IMTECH). For toxicity testing, 0.1 ml of bacterial culture and 0.1 ml of inhibitory fractions were added to 5 ml of top agar and poured onto the minimal agar plates followed by incubation at 37 °C for 48 h. To determine the spontaneous reversion, sodium azide (5 µl of 17.2 mg ml^{-1}) was used as a positive control while water was used as negative control. After incubation for 48 h, the number of revertant his + bacterial colonies were scored. The cytotoxicity of AF1 and AF2 was determined at a single concentration (120 µg ml^{-1}) by MTT assay [22]. Chinese Hamster Ovary (CHO) cell line obtained from National Centre for Cell Science, Pune, Maharastra, India was grown on Dulbecco's modified Eagle's medium supplemented with streptomycin (100 U ml^{-1}), gentamycin (100 g l^{-1}), amphotericin B (0.25 µg ml^{-1}) and 10% fetal bovine serum (Himedia). Cells were incubated in a CO_2 incubator (5%CO_2; 90% relative humidity) at 37 °C. In 96 well plate, 5×10^3 cells were added in each well and incubated at 37 °C, 5% CO_2 for 24 h. The cells were treated with inhibitory fractions for 48 h, washed and 100 µl of fresh medium with 20 µl MTT solution (5 mg ml^{-1}) was added in each well. The cells were incubated at 37 °C, 5% CO_2 for 4 h. After incubation, the medium was removed and formazan product was dissolved in 100 µl of dimethyl sulfoxide (DMSO) and shaken for 10 min. Absorbance at 550 nm was measured by using microplate reader. Doxorubicin (DOX) was used as positive control. Cell growth inhibition in percentage was calculated by using formula:

$$\% \text{ Cell growth} = \left(OD_{550}\text{of treated cells} / OD_{550}\text{of control}\right) \times 100$$

Statistical analysis

Each experiment was performed in triplicate. To calculate standard error and mean, microsoft office excel 2007 (Microsoft Corp, USA) was used and student *t* test analysis was performed using the SPSS v17.0 software.

Results

The endophytic fungus *A. destruens* used in the present study exhibited 93.4% inhibition against α-glucosidase enzyme. Partial purification of the ethyl acetate extract yielded two active fractions designated as AF1 and AF2 that exhibited 87.75 and 72.11% inhibition, respectively [17]. These active fractions were assessed for their antimicrobial and antibiofilm potential.

Antimicrobial activity

The active fractions, AF1 and AF2 were studied for their antimicrobial activities at a concentration of 130 µg ml^{-1}. AF1 showed antibacterial effects against all the tested pathogens, whereas, AF2 had antibacterial activity only against 4 pathogens (Table 1). AF1 showed maximum zone of inhibition (53 mm) against *S. enterica* and minimum against pathogenic yeast, *C. albicans* (30 mm). The zones of inhibition obtained with AF1 were larger as compared to AF2 except in case of *V. cholerae*. AF2 showed good inhibitory activity against *V. cholerae* (48 mm), whereas, for other tested pathogens less activity was observed (Fig. 1a–g).

Table 1 Antimicrobial activity of AF1 and AF2 from *A. destruens* AKL-3 against different pathogens

Test organism	Zone of inhibition (mm) ± SD	
	AF1	AF2
S. enterica	53.00 ± 0.16	16.00 ± 0.14
Sh. flexneri	47.00 ± 0.25	–
V. cholerae	47.00 ± 0.31	48.00 ± 0.24
E. coli	44.00 ± 0.24	–
P. aeruginosa	44.00 ± 1.14	10.00 ± 0.11
S. aureus	43.00 ± 0.85	11.00 ± 0.45
C. albicans	22.50 ± 0.20	17.50 ± 0.11

Effect of inhibitor on biofilm formation

Effect of sub-optimal concentrations (22.5 and 11.25 µg ml^{-1}) of AF1 and AF2 was studied on the biofilm formation by various pathogens viz. *P. aeruginosa*, *E. coli*, *S. enterica* and *C. albicans*. Both the fractions inhibited the biofilm formation in a dose-dependent manner, however, AF1 at both the tested concentrations showed significantly ($p < 0.01$) higher inhibition of the biofilm formation in all the four test organisms as compared to AF2 (Fig. 2). AF1 at 22.5 µg ml^{-1} exhibited maximum inhibition of biofilm formation by *P. aeruginosa* (58.15%) followed by *C. albicans* (52.16%). Whereas, AF2 at the same concentration showed maximum inhibition of biofilm formation by *C. albicans* (28.54%) followed by *S. enterica* (23.2%). Similar trends were observed at the lower concentration also.

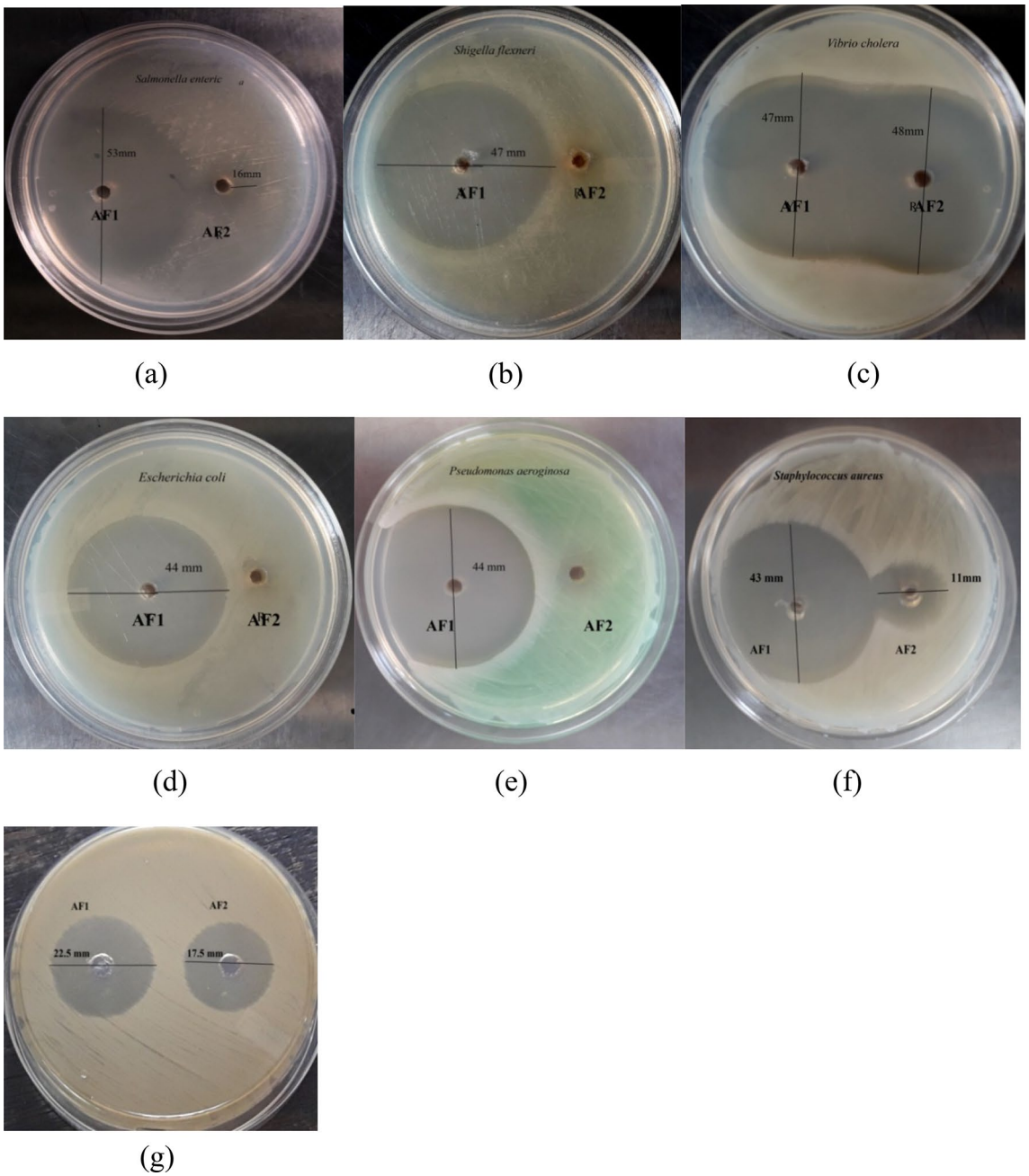

Fig. 1 Antimicrobial activities of AF1 and AF2 obtained from *A. destruens* AKL-3 against various pathogens as demonstrated by agar gel diffusion assay **a** *S. enterica*, **b** *Sh. flexneri*, **c** *V. cholerae*, **d** *E. coli*, **e** *P. aeruginosa*, **f** *S. aureus*, **g** *C. albicans*

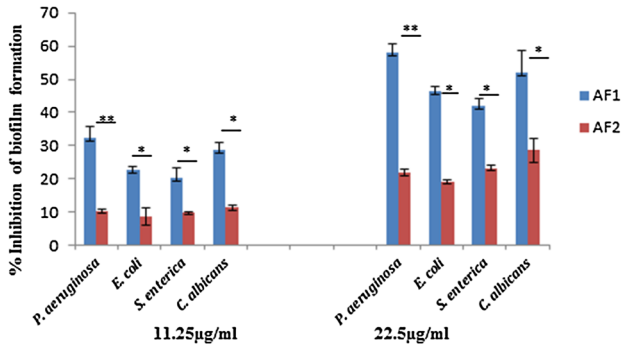

Fig. 2 Percentage inhibition of biofilm formation of test pathogens by AF1 and AF2 at different concentrations. Error bars are representative of standard deviation of the three independent experiments performed in triplicates.**Significant at $p < 0.01$, *Significant at $p < 0.05$

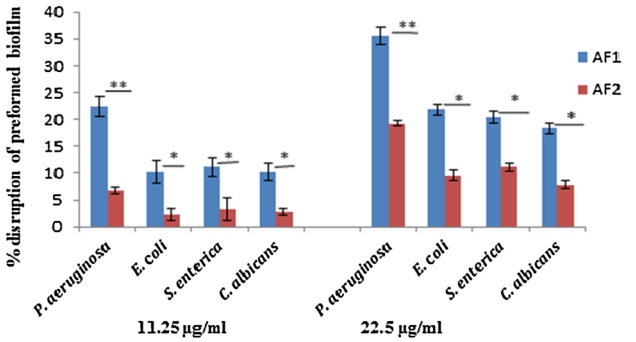

Fig. 3 Disruption of preformed biofilm of test pathogens by AF1 and AF2 at different concentrations. Error bars are representative of standard deviation of the three independent experiments performed in triplicates. **Significant at $p < 0.01$, *Significant at $p < 0.05$

Effect of inhibitor on dispersion of biofilms

The effect of AF1 and AF2 on the dispersion of pre-formed biofilms was also evaluated. AFI was observed to be more effective in dispersing the preformed biofilms of all the pathogens than AF2. At the higher tested concentration of 22.5 µg ml^{-1}, AF1 caused maximum dispersion of biofilm of *P. aeruginosa* (35.58%) followed by *E. coli* (21.84%). At the same concentration AF2 showed 19.2% dispersion of biofilm of *P. aeruginos*a (Fig. 3).

Thermostability of AF1 and AF2

The thermostability of AF1and AF2 was checked at different temperatures for different time intervals. Both the active fractions were found to be thermostable. AF1 retained 60% inhibitory activity after treatment at 100 °C for 60 min. AF2 was found to be more thermostable retaining 90% activity even after 60 min treatment at 100 °C (Online Resource 1).

Purification of inhibitory compounds

AF1 and AF2 were subjected to semi preparatory HPLC. In AF1, six peaks were collected but activity was observed in three peaks at retention times of 6.97 (P2), 7.36 (P3), and 11.52 (P5) min. The isolated peaks exhibited 76.23, 65.12 and 64.82% AG inhibitory potential, respectively (Fig. 4a). In case of AF2, five peaks at retention times of 5.29 (P1), 6.10 (P2), 6.97 (P3), 8.63 (P4) and 11.27 (P5) were collected and examined for AG inhibitory activity (Fig. 4b). It was observed that all peaks possessed inhibitory potential evincing 50.23, 66.56, 57.32, 51.29 and 54.23% AG inhibitory activity, respectively. All the isolated peaks exhibited lesser activity than their respective fractions, indicating a synergistic effect of the inhibitory compounds.

Identification of active compounds

Biochemical analysis was carried out to study the nature of compounds in AF1 and AF2. In AF1, two peaks P3 and P5 were found to be phenolic while in AF2, all the collected peaks were observed to be phenolic in nature as they stained with Fast Blue B and $FeCl_3$. It was observed that AF2 contained more phenolic content than AF1. HPLC based identification of phenolics was performed and on comparison with standards, the peaks P3 and P5 of AF1 showed retention times similar to that of ellagic acid and kaempferol at 15.753 and 17.502 min, respectively (Online Resource 2). The peak P2 in case of AF2 showed similar retention time (14.56 min) as that of rutin. Using the standard addition protocol, 10 µl of standard ellagic acid, kaempferol and rutin were added in respective peaks and occurrence of spiking indicated that the compounds could be ellagic acid, kaempferol and rutin. To validate the results, AGI activity of standard ellagic acid, kaempferol and rutin was also determined. It was observed to be 72.54%, 66.34% and 74.34% for ellagic acid, kaempferol and rutin respectively.

Peak P2 of AF1 (Fig. 5a) was subjected to mass spectroscopy and infrared spectroscopy analysis for identification (Fig. 5b, c). Analysis of IR data: 1721.43 (C=O of ester), 1650.34 (C=C), 1641.64 (C=O of amide), 1452.93 ($–CH_3$ Bending), 1069.52 (C–O), 1044.46 (C–N amines) and 898.67 (C=C out of plane) showed the presence of esteretic and amide carbonyls, aromatic double bonds, methyl and amines. The presence of a sharp signal at m/z 601.13 in MS analysis, suggests that the mass of the compound could be 600.

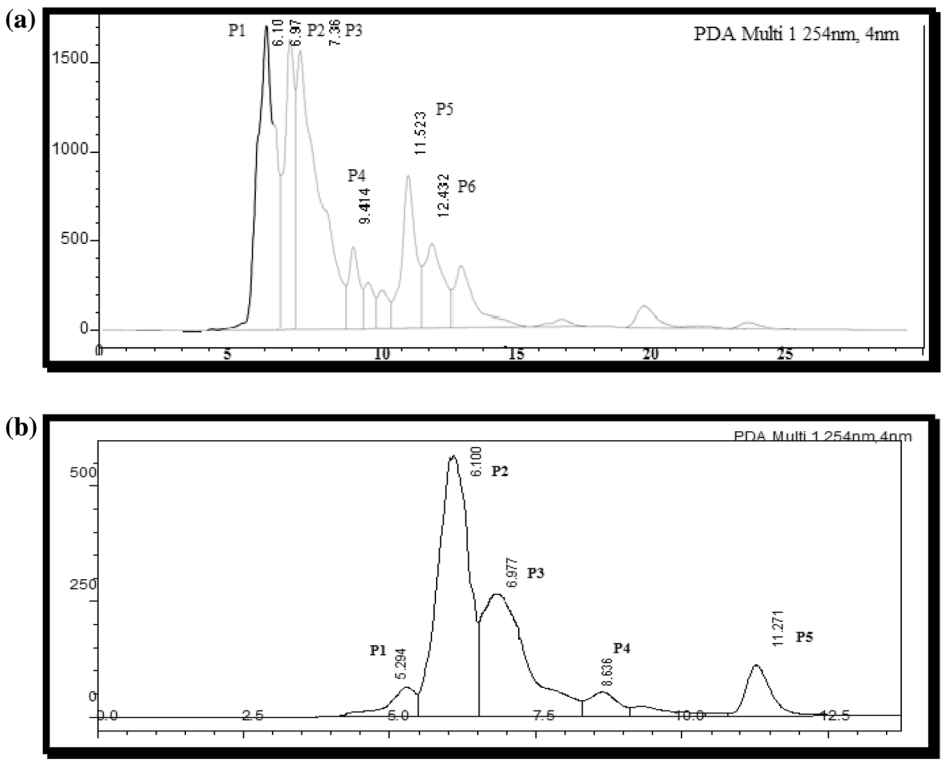

Fig. 4 Semi preparatory HPLC chromatgram of **a** AF1 and **b** AF2 of *A. destruens* AKL-3

Safety analysis

Mutagenicity testing

Both the active fractions AF1 and AF2 were checked for their mutagenicity using Ames mutagenicity test. Revertant colonies ranging from 5 to 20 were obtained in bacteria incubated with active fractions and negative control whereas, hundreds of revertant colonies were observed in the positive control (sodium azide) (Fig. 6a–d). The results indicated the non-mutagenic nature of the active fractions.

Cytotoxicity of the active fractions AF1 and AF2 was tested at a concentration of 120 µg ml^{-1}, using MTT toxicity assay on CHO cell line. At this concentration, the active fractions were found to be non-cytotoxic with 90.2 and 86.64% cell viability as compared to 46% cell viability for DOX.

Discussion

In the present study, active fractions AF1 and AF1 obtained from an endophytic fungus *A. destruens* AKL-3 were evaluated for their antimicrobial potential. Though, number of reports are available on antiviral and antifungal activities of AGIs [2–5], they have not been much explored for their antibacterial potential. Patients with DM type 2 are more prone to infectious diseases as compared to the healthy individuals [6, 7]. Therefore, for the management of diabetes, AGIs possessing antimicrobial activity would be better therapeutic agents. It was observed that AF1 showed antagonistic activity against all the tested microbes while AF2 showed activity only against *S. aureus, V. cholerae*, *S. enterica* and *C. albicans*. Another factor that increases the pathogenicity of bacterial infections in diabetic patients is biofilm formations, which delay the healing of wounds as well as contribute to antibiotic resistance [8].Therefore, effect of AF1 and AF2 of *A. destruens* AKL-3 was studied on biofilm formation and dispersion of pathogens. Active fraction AF1 significantly inhibited the biofilm formation as well as caused the dispersion of preformed biofilm. Enzymes glycosyltransferases along with glycosidases play a key role in biofilm synthesis by initiating or elongating carbohydrate chains during polysaccharide biosynthesis through the transfer of activated mono or oligosaccharide residue to an existing acceptor molecule, forming a glycosidic bond [23]. There is a possibility that a compound inhibiting α-glucosidase enzyme might have inhibitory activity against these enzymes also. 1-deoxynojirimycin (DJM), a potent AGI was found to inhibit the biofilm formation by *Streptococcus mutans* [24]. DJM is a sugar analogue and acts as competitive inhibitor of glycosyltransferases by competing with sugar molecules. Ceftezole is a β-lactam antibiotic having α-glucosidase inhibitory potential [25]. Cefazolin, a structurally related compound of ceftezole was found to be effective in aggregative stages of adherence and in elongation process of biofilm

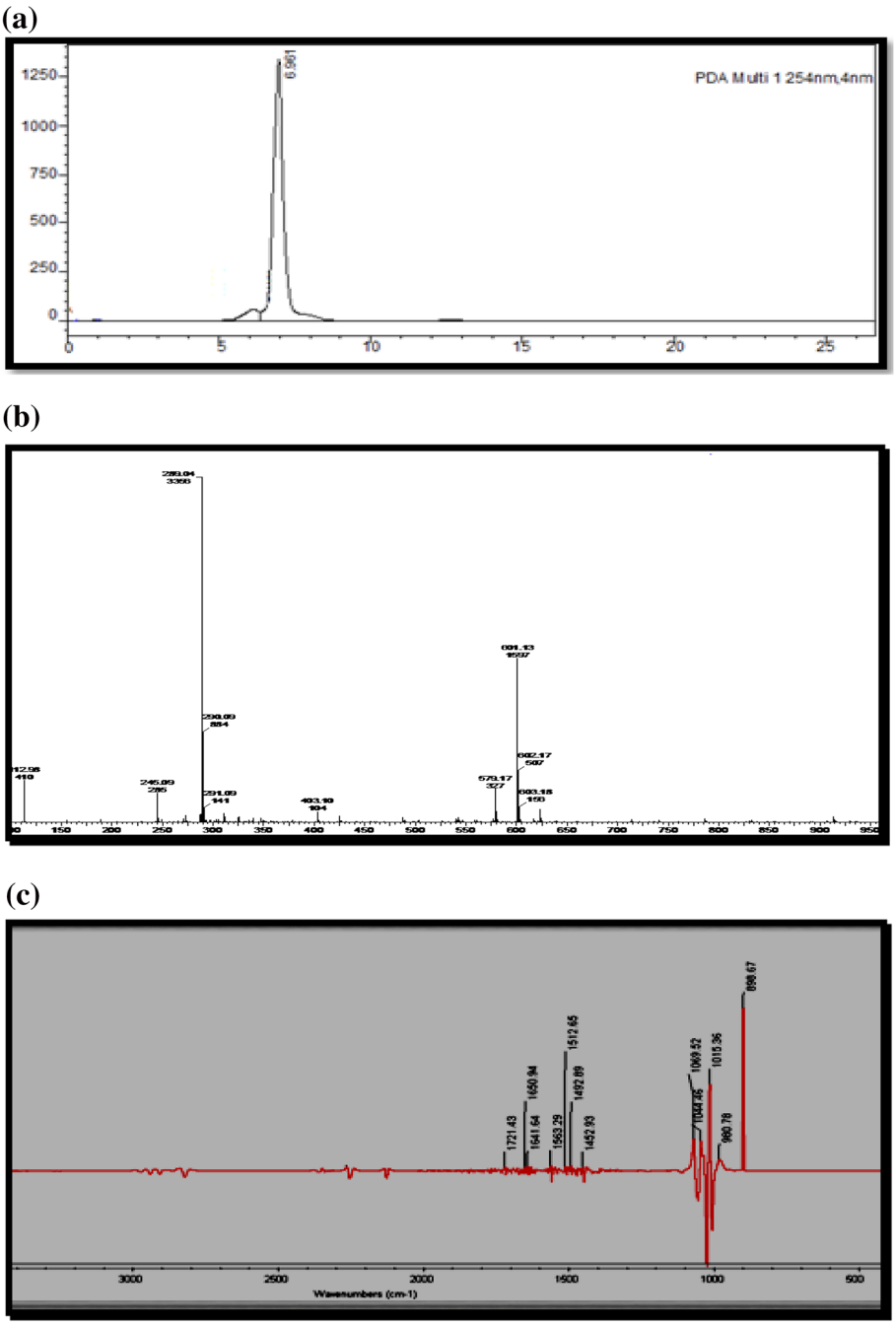

Fig. 5 **a** HPLC chromatogram, **b** MS spectrum, **c** FTIR spectrum of peak P2 from AF1 of *A. destruens* AKL-3

formation [26]. Upon biochemical characterization both fractions were found to contain high amount of phenolics. Phenolics such as chlorogenic acid have been documented for α-glucosidase inhibition and also possess antimicrobial potential by increasing the permeability of outer and plasma membrane which results in leakage of nucleotides [14, 27]. The inhibitory as well as disruptive ability of ellagic acid has also been reported on biofilms of *S. aureus*, *E. coli* and *C. albicans* [28].Yamada et al. (1999) [29] and Al-Majmaieet al. (2019) [30] have documented the antimicrobial potential of kaempferol and rutin. The active fractions of *A. destruens* AKL-3 were studied for their thermostability and were found to be thermostable. Both the fractions revealed the presence of phenolics. A survey of literature revealed that various phenolic compounds obtained from natural resources can be highly stable under extreme conditions of pH and temperature [31, 32]. The active fractions were also determined to be non- mutagenic and non-cytotoxic, further enhancing their potential applicability as therapeutics.

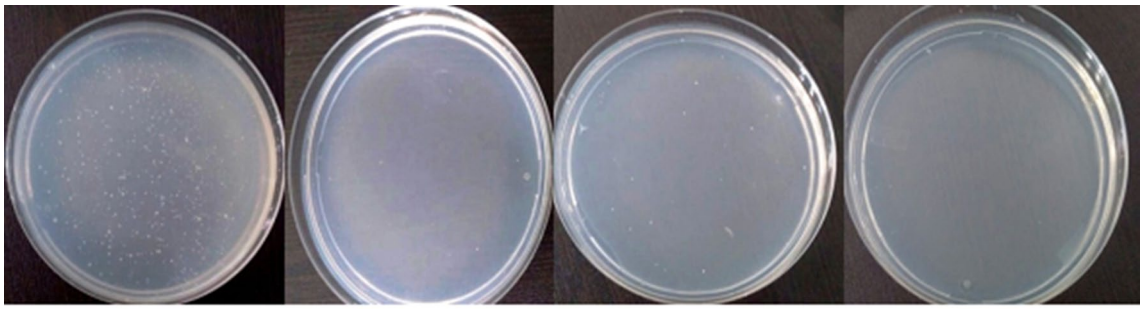

Fig. 6 Colony counts for mutagenicity testing in Ames test. **a** Positive control, **b** AF1, **c** AF2 from *A. destruens* AKL-3, **d** negative control

Conclusion

It can be concluded that the bioactive components from *A. destruens* AKL-3 can be developed as antidiabetic agents with amtimicrobial activity.

Funding Authors acknowledge the grant of fellowship under UPE (University with Potential for Excellence) scheme of University Grants Commission and Council of Scientific and Industrial Research (CSIR), New Delhi, India for providing financial assistance.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

References

1. Purich DL (2010) Enzyme kinetics: catalysis and control: a reference of theory and best-practice methods. Elsevier, Amsterdam
2. Asano N (2003) Glycosidase inhibitors: update and perspectives on practical use. Glycobiology 13(10):93R–104R
3. Kim SK, Nho HJ (2004) Isolation and characterization of α-glucosidase inhibitor from the fungus *Ganoderma lucidum*. J Microbiol 42(3):223–227
4. Kajimoto T, Node M (2009) Inhibitors against glycosidases as medicines. Curr Top Med Chem 9(1):13–33
5. Alonzi DS, Scott KA, Dwek RA, Zitzmann N (2017) Iminosugar antivirals: the therapeutic sweet spot. Biochem Soc Trans 45(2):571–582
6. Geerlings SE, Hoepelman AI (1999) Immune dysfunction in patients with diabetes mellitus (DM). FEMS Immunol Med Microbiol 26(3–4):256–265
7. Muller LMAJ, Gorter KJ, Hak E, Goudzwaard WL, Schellevis FG, Hoepelman AIM, Rutten GEHM (2005) Increased risk of common infections in patients with type 1 and type 2 diabetes mellitus. Clin Infect Dis 41(3):281–288
8. Neut D, Tijdens-Creusen EJ, Bulstra SK, Van der Mei HC, Busscher HJ (2011) Biofilms in chronic diabetic foot ulcers—a study of 2 cases. Acta Orthop 82(3):383–385
9. Jalgaonwala RE, Mohite BV, Mahajan RT (2017) A review: natural products from plant associated endophytic fungi. J Microbiol Biotechnol Res 1(2):21–32
10. Strobel GA, Daisy B (2003) Bioprospecting for microbial endophytes and their natural products. Microbiol Mol Biol Rev 67:491–502
11. Wibowo M, Prachyawarakorn V, Aree T, Wiyakrutta S, Mahidol C, Ruchirawat S, Kittakoop P (2014) Tricyclic and spirobicyclic norsesquiterpenes from the endophytic fungus *Pseudolagarobasidium acaciicola*. Eur J Org Chem 19:3976–3980
12. Wibowo M, Prachyawarakorn V, Aree T, Mahidol C, Ruchirawat S, Kittakoop P (2016) Cytotoxic sesquiterpenes from the endophytic fungus *Pseudolagarobasidium acaciicola*. Phytochemistry 122:126–138
13. Masi M, Maddau L, Linaldeddu BT, Scanu B, Evidente A, Cimmino A (2018) Bioactive metabolites from pathogenic and endophytic fungi of forest trees. Curr Med Chem 25(2):208–252
14. Singh B, Kaur T, Kaur S, Manhas RK, Kaur A (2016) Insecticidal potential of an endophytic *Cladosporium velox* against *Spodoptera litura* mediated through inhibition of alpha glycosidases. Pest Biochem Physiol 131:46–52
15. Singh B, Kaur A (2016) Antidiabetic potential of a peptide isolated from an endophytic *Aspergillus awamori*. J Appl Microbiol 120:301–311
16. Kaur J, Kaur R, Dutta R, Kaur S, Kaur A (2018) Exploration of insecticidal potential of an alpha glucosidase enzyme inhibitor from an endophytic *Exophiala spinifera*. J Appl Microbiol 125(5):1455–1465
17. Kaur J, Sharma A, Sharma M, Manhas RK, Kaur S, Kaur A (2019) Effect of α-glycosidase inhibitors from endophytic fungus *Alternaria destruens* on survival and development of insect pest *Spodoptera litura* Fab. and fungal phytopathogens. Sci Rep 9(1):1–13
18. Kaur S, Sharma P (2015) Protease-sensitive inhibitory activity of cell-free supernatant of *Lactobacillus crispatus* 156 synergizes with ciprofloxacin, moxifloxacin and streptomycin against *Pseudomonas aeruginosa*: an in vitro study. Probiot Antimicro 7(2):172–180
19. Djordjevic D, Wiedmann M, McLandsborough LA (2002) Microtiter plate assay for assessment of *Listeria monocytogenes* biofilm formation. Appl Environ Microbiol 68(6):2950–2958
20. Waksmundzka HM, Sherma J, Kowalska T (2008) Thin layer chromatography in phytochemistry. CRC Press, Boca Raton
21. Maron DM, Ames BN (1983) Revised method for *Salmonella* mutagenicity test. Mutat Res 113(3–4):173–215
22. Ciapetti G, Cenni E, Pratelli L, Pizzoferrato A (1993) In vitro evaluation of cell/biomaterial interaction by MTT assay. Biomaterials 14(5):359–364

23. Rehm BH (2010) Bacterial polymers: biosynthesis, modifications and applications. Nat Rev Microbiol 8(8):578
24. Islam B, Khan SN, Haque I, Alam M, Mushfiq M, Khan AU (2008) Novel anti-adherence activity of mulberry leaves: inhibition of *Streptococcus mutans* biofilm by 1-deoxynojirimycin isolated from *Morus alba*. J Antimicrob Chemother 62(4):751–757
25. Lee DS, Lee JM, Kim SU, Chang KT, Lee SH (2007) Ceftezole, a cephem antibiotic, is an α-glucosidase inhibitor with in vivo anti-diabetic activity. Int J Mol Med 20(3):379–383
26. Rupp ME, Hamer KE (1998) Effect of sub inhibitory concentrations of vancomycin, cefazolin, ofloxacin, L-ofloxacin and D-ofloxacin on adherence to intravascular catheters and biofilm formation by *Staphylococcus epidermidis*. J Antimicrob Chemother 41(2):155–161
27. Lou Z, Wang H, Zhu S, Ma C, Wang Z (2011) Antibacterial activity and mechanism of action of chlorogenic acid. J Food Sci 76(6):M398–M403
28. Bakkiyaraj D, Nandhini JR, Malath B, Pandian SK (2013) The anti-biofilm potential of pomegranate (*Punica granatum* L.) extract against human bacterial and fungal pathogens. Biofouling 29(8):929–937
29. Yamada Y, Yamamoto A, Yoneda N, Nakatani N (1999) Identification of kaempferol from the leaves of *Diospyros kaki* and its antimicrobial activity against *Streptococcus mutans*. Biocontrol Sci 4(2):97–100
30. Al-Majmaie S, Nahar L, Sharples GP, Wadi K, Sarker SD (2019) Isolation and antimicrobial activity of rutin and its derivatives from *Ruta chalepensis* (Rutaceae) growing in Iraq. Rec Nat Prod 13(1):64–70
31. Friedman M, Jurgens HS (2000) Effect of pH on the stability of plant phenolic compounds. J Agric Food Chem 48(6):2101–2110
32. Arabshahi DS, Devi DV, Urooj A (2007) Evaluation of antioxidant activity of some plant extract and their heat, pH and storage stability. Food Chem 100(3):1100–1105