Antioxidant and DNA damage protective activities of selected endophytic actinobacteria isolated from *Harpagophytum procumbens*: A Kalahari desert-adapted plant

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Given that synthetic antioxidants are currently in use and have been demonstrated to have known negative side effects, it is important to look for natural antioxidant sources. Endophytic actinobacteria, especially those associated with medicinal plants, are one understudied source. The goal of this study was to investigate whether the secondary metabolites of endophytic actinobacteria isolated from *Harpagophytum procumbens*, commonly known as the Devil's claw, have antioxidant and DNA damage protective properties. *H. procumbens* secondary tubers were collected from the Khomas and Hardap regions of Namibia, yielding a total of 23 isolated actinobacteria. The identities of the actinobacterial isolates were determined by sequencing the 16S rRNA gene. This revealed 6 families affiliated to 7 genera that included the dominant *Streptomyces* genus (57%) whereas rare genera (*Agromyces, Nocardiosis, Rubrobacter, Patulibacter, Rhodococcus, Curtobacterium*) and 2 unidentified strains accounted for 43%. The antioxidant results revealed that the ethyl acetate extracts of *Streptomyces* sp. species strain A36, had the highest phenolic content of 1.993 ± 0.004 and total antioxidant activity of 0.258 ± 0.001. Meanwhile, extracts from *Streptomyces* spp. strain B12, had the highest radical scavenging activity of 95% at 0.1 mg·mL⁻¹ while B43, an unidentified actinobacterium, had the highest total reducing power of 0.849 ± 0.003. In addition, 61% of extracts showed the ability to protect DNA from damage when exposed to hydrogen peroxide. None of the samples contained terpenoids, while 13 (72%) contain alkaloids, 17 (94%) contain phenols and 8 (44%) contain flavonoids. The present study concludes that endophytic actinobacteria associated with *H. procumbens* are a potential source of bioactive compounds with pharmacological properties.

**Key words:** *Harpagophytum procumbens*, actinobacteria, antioxidants, DNA damage protection, endophytes, bioactive compounds

INTRODUCTION

Actinobacteria are a highly ubiquitous and diverse group of prokaryotes that produce a plethora of bioactive
secondary metabolites (Solecka et al., 2012; Mousumi and Dayanand, 2013; Janardhan et al., 2014). *Streptomyces*, the largest genus of actinobacteria, produce up to 73% of known bioactive metabolites, with the remaining 27% produced by rare actinobacteria (Mousumi and Dayanand, 2013). Antimicrobial, anticancer, immunosuppressive, antioxidant, and enzyme inhibitory properties are among the known bioactivities of these secondary metabolites (Savi et al., 2015; Manimaran and Kannabiran, 2017; Robl et al., 2019; Vu et al., 2019; Fadji and Babalola, 2020). Endophytic actinobacteria have also been demonstrated to be rich sources of bioactive natural compounds (Singh and Dubey, 2015). These grow intra- or intercellularly in plant tissues without harming the host (Dhayanithy et al., 2019), and confer a range of essential bioactivities to the host plant, such as promoting plant growth, eliciting pathogen defense responses, disease resistance, and acting as abiotic stress remediators (Sudha et al., 2016; Khare et al., 2018; Lata et al., 2018; Suarez-Moreno et al., 2019; Ansari et al., 2020; Fadji and Babalola, 2020).

Plant components and/or extracts have been used for therapeutic purposes since ancient times. To date, just a handful of research has been done on the relationship between medicinal plant bioactivities and the bioactivities of their endophytes (Passari et al., 2015; Singh and Dubey, 2018). Several investigations, however, have suggested that endophytic actinobacteria present in medicinal plants may produce secondary metabolites with distinct bioactivities than those produced by host plants (Shan et al., 2018). Due to their propensity to create uncomon products as a survival strategy under abiotic stressors, plants growing in harsh settings are a key target in the search for novel endophytic actinobacteria with improved bioactivities (Gos et al., 2017; Singh and Dubey, 2018). *Harpagophyllum procumbens*, often known as the devil’s claw, is a perennial herbaceous plant species indigenous to Namibia's Kalahari Desert. In Southern Africa, the plant is used for a variety of therapeutic purposes, particularly in Botswana, Namibia, South Africa, and Zimbabwe (Geogriev et al., 2010). It has been used to treat fever, malaria, indigestion, pain, arthritis, osteoarthritis, tendonitis, renal inflammation, heart illness, and blood ailments, and its anti-inflammatory qualities have also been demonstrated to help with rheumatic diseases (Abdulhussein et al., 2018; Vreju et al., 2019; Brendler, 2021; Hu et al., 2021). For ages, *H. procumbens* has been used to treat a variety of illnesses, most notably as an anti-inflammatory agent (Cole and Strohbach, 2007). Given the close link between inflammation and oxidative stress (Schaffer et al., 2013), it is logical to assume that medicinal plants with anti-inflammatory effects will also have antioxidant capabilities, making *H. procumbens* a good source of potential antioxidants.

Only a few studies, such as Geogriev et al. (2010), have looked at the antioxidant activity of *H. procumbens’* aerial parts, with promising results. While human bodies have the intrinsic potential to produce their own antioxidants to protect their DNA from the damaging effects of metabolic by-products such as reactive nitrogen species (RNS) and reactive oxygen species (ROS), this ability tends to deteriorate as they become older. DNA/RNA oxidation can occur in the absence of antioxidants, resulting in single or double-strand fragmentation or changes to the nitrogenous bases (Ore and Akinloye, 2019). Although cells have compensatory mechanisms to counteract the negative consequences of oxidative stress, DNA damage can still occur in some cases, leading to diseases such as cancer (Sabahi et al., 2018). As a result, there is a pressing need for more research into natural antioxidant sources, especially since synthetic antioxidants are frequently linked to harm (Golla and Bhimathati, 2014; Lee et al., 2014). This study was, therefore, aimed at assessing the enzymatic, antioxidant, and DNA protecting potential of the secondary metabolites of the endophytic actinobacteria present in *H. procumbens*, as an alternative source of natural antioxidants which are currently in short supply.

**MATERIALS AND METHODS**

**Plant sample collection**

Non-destructive methods were used to obtain six lateral *H. procumbens* secondary tubers from the Khomas (A: 22°51'34'' S, 17°6'35.5'' E) and Hardap (B: 22°37'4.7'' S, 17°5'44.7'' E) regions in Namibia in May 2018. Briefly, a spade was used to dig a hole around the main tubers and lateral tubers were carefully cut off, leaving the main tuber in the ground and closing the hole. In total, six tubers, three from each site, were collected and transported to the laboratory at the University of Namibia in sterile plastic bags where they were stored at -4°C until further processing. The plant was identified by the National Botanical Research Institute in Windhoek, Namibia.

**Isolation of actinobacteria strains**

Endophytic actinobacteria were isolated following the methods earlier described by Janardhan et al. (2014) where they isolated actinobacteria from the soil onto starch casein media, with modifications. Plant tubers were surface sterilised by washing with 70% (v/v) ethanol for 3 min a total of three times and then rinsed with distilled water three times and oven-dried at 35°C for 24 h. Following this, 5 g pieces of dried, surface sterilised secondary tubers were crushed into powder using a sterile mortar and pestle, and serially diluted from 10⁻¹ to 10⁻⁶ using sterile deionized water. One hundred microliters (100 µl) aliquots of each tuber dilution

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were then plated onto starch casein media agar (Genmed, Windhoek) supplemented with nystatin and cycloheximide at 10 mg·mL⁻¹ (Genmed, Windhoek) each to suppress the growth of bacteria and fungi, respectively. The plates were incubated at 30°C for a maximum of 28 days or until actinobacteria growth was observed. Once growth was observed, axenic cultures were obtained using the perpendicular streak method over three to four rounds of subculturing onto freshly prepared starch casein agar plates. Purified colonies of each axenic culture were then transferred into freshly prepared starch casein broth in 250 mL Erlenmeyer flasks. The broth cultures were incubated with shaking at 35°C and 10°g for 21 days to allow for the production and excretion of secondary metabolites. A total of 23 isolates were selected.

**DNA extraction and identification of actinobacteria isolates**

DNA was extracted and amplified using a method outlined by Parthasarathi et al. (2012). Broth cultures of the 23 isolates were centrifuged at 11000×g for 5 min and the supernatant was discarded of. This was repeated until there was a large enough pellet which was then washed 5 times. The washing step involved centrifuging the pellet at 11000×g for 5 min in double distilled water, each time discarding the supernatant. The DNA was then extracted from the cell pellet using a ZR Fungal/Bacterial DNA kit according to the manufacturer’s protocol with a slight adjustment to the vortexing time from 5 to 20 min due to the difficulty to disrupt actinobacteria cell walls. The DNA was then subjected to polymerase chain reaction (PCR) amplification targeting the 16S rRNA gene using the primers: 27F (5'-GAGTTTGTGATCCTGCTAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3'). PCR was done in 50 µl reaction volumes consisting of 2.0 µl each of the forward and reverse primers (each primer to a final concentration of 0.00001 mol·L⁻¹), 25 µl of PCR Master Mix (Inqaba Biotech, Pretoria, South Africa), 3 µl template DNA and 18 µl nuclease free water. The PCR cycling protocol was as follows: 95°C hot start for 4 min, followed by 30 cycles of 95°C for 55 s (denaturation), 52°C for 1 min (annealing), 72°C for 1.5 min (elongation), and a final elongation step at 72°C for 5 min. The amplified DNA was sent for sequencing at Inqaba Biotech (Pretoria, South Africa). The chromatograms were edited using Chromas lite and Biodit software. Consensus sequences were generated and aligned with similar 16S rRNA gene sequences retrieved from the nucleotide database EZBioCloud (https://www.ezbiocloud.net/) and NCBI (http://www.ncbi.nlm.nih.gov/BLAST/) to identify the closest match.

**Phylogenetic analysis**

Phylogenetic analysis was carried out according to the protocol outlined by Hall (2013). A Maximum Parsimony (MP) phylogenetic tree was constructed using generated consensus sequences and aligned using Mega X (https://www.megasoftware.net/) and ClustalX (http://www.clustal.org/clustal2/) and compared to sequences available in the GenBank database (http://www.ncbi.nlm.nih.gov/BLAST/). Gaps and missing data were eliminated (complete deletion option) (Kumar et al., 2018). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates were collapsed.

**Extraction of secondary metabolites**

The secondary metabolites were extracted as previously described by Khanna et al. (2011) with modifications. The broth cultures prepared during isolation were centrifuged at 20000×g for 10 min to sediment the cell mass. The supernatant portions were divided into aliquots, which were then separately mixed with different extraction solvents (ethyl acetate, chloroform, and methanol) in a 1:1 ratio (v/v), vigorously shaken by hand for 10 min followed by agitation on an orbital shaker at 10×g for 30 min and left to stand at 4°C for 12 h. After the incubation, the mixtures were vigorously shaken for 5 min and then left to stand at room temperature in a separating funnel until the solvent layer and aqueous layer were completely separated. The solvent portions were then placed into pre-weighed, sterile glass beakers and left to evaporate under a fume hood. The beakers were then re-weighed to determine the weight of the precipitates, and then re-dissolved using calculated volumes of the same solvent to produce stock solutions with a concentration of 10 mg·mL⁻¹. These were then placed in sterile microfuge tubes and stored at -20°C until use.

**Determination of total phenolics (TPC)**

The total phenolic content (TPC) for all 23 extracts in 3 different solvents of ethyl acetate, chloroform, and methanol, were estimated using the Folin-Ciocalteu's method with gallic acid as a standard and positive control, following a modified method of Gautham and Onkarappa (2013). The reagent was formed by mixing phosphotungstic acid and phosphomolybdic acid, which was reduced to a mixture of blue oxides of tungsten and molybdenum after oxidation of the phenols. This blue colour produced a maximum absorption at around 765 nm and was proportional to the total quantity of phenolic compounds originally present. Total phenolic content (TPC) was determined spectrophotometrically by combining 2.5 mL Folin-Ciocalteu reagent (diluted 1:10 v/v with distilled water) and 2 mL sodium carbonate solution (7.5% v/v) with 1 mL of 1 mg·mL⁻¹ solvent extract. The tubes were vortexed and left to stand for 90 min at ambient temperature. The absorbance was read spectrophotometrically against a blank at 765 nm and compared to a standard curve plotted using different concentrations of gallic acid (0-1000 µg·mL⁻¹). The TPC of the extract was expressed in terms of milligrams (mg) of gallic acid equivalents per gram (GAE·g⁻¹) of dry weight using the equation:

\[ C = cV/m \]

where c is the concentration of gallic acid as obtained from the calibration curve in mg·mL⁻¹, V is the volume of the extract in mL, and m is the mass of the extract in grams. The reactions were conducted in triplicate, and the results were averaged.

**Determination of total antioxidant activity (TAA)**

To determine the total antioxidant activity (TAA) of the extracts, 0.3 mL aliquots of 100 µg·mL⁻¹ solvent extracts were combined with 3 mL reagent solution of 0.6 mol·L⁻¹ sulphuric sodium phosphate with 0.00004 mol·L⁻¹ ammonium molybdate and incubated at 95°C for 90 min in a water bath. The absorbance of all the sample mixtures was measured at 695 nm against a gallic acid (100 µg·mL⁻¹) blank as the standard and positive control, as described by Janardhan et al. (2014). The absorbance of the extracts was expressed in GAE·g⁻¹ using the same formula as for TPC.

**Determination of DPPH radical scavenging activity**

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity was determined following the method described by Janardhan et al. (2014). Briefly, 2 mL of different concentrations (0.005 - 0.100 mg·mL⁻¹) of each extract and gallic acid standard as the positive control were placed into tubes containing 2 mL of 0.002% in
methanol. The tubes were then incubated in the dark at room temperature for 30 min and the optical density was measured at 517 nm. Percentage scavenging activity was calculated using the following equation:

\[ \% = \frac{(A - B)}{A} \times 100, \]

where A is the absorbance of DPPH control and B is the absorbance of DPPH in the presence of extract/standard.

The IC\textsubscript{50} was determined by producing standard curves for each extract. Each curve produces a line equation that was then used to determine the concentration of extracts where 50% of the free radicals would be scavenged:

\[ 50 = mx + c \]

where m is the gradient of the trendline, and c is the y-intercept, and calculating for x will give the IC\textsubscript{50}.

**Determination of total reducing power**

To determine total reducing power, solvent extracts and gallic acid standard and positive control at 0.100 mg·mL\textsuperscript{-1} in phosphate buffer were mixed with 1% potassium ferricyanide in equal volumes and incubated at 50°C for 20 min following a method previously described by Janardhan et al. (2014). After incubation, 2.5 mL of 10% trichloroacetic acid (TCA) was added to the mixture followed by centrifugation at 2800 g for 10 min. Following centrifugation, 2.5 mL of the upper layer of the mixture was added to 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride. Absorbance was then measured at 700 nm.

**Determination of DNA protecting potential**

DNA nicking was evaluated using hydrogen peroxide after coating the DNA with the extracts to determine whether the extracts have any DNA damage protection properties. To determine the ability of extracts to protect DNA from oxidation by hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}), in vitro tests were performed as described by Golla and Bhimathati (2014) and Shameem et al. (2015). Briefly, the reaction mixture, containing 3 µl of pUC19 plasmid DNA, 10 µl of Fenton’s reagent (0.00003 mol·L\textsuperscript{-1} H\textsubscript{2}O\textsubscript{2}, 0.00005 mol·L\textsuperscript{-1} ascorbic acid, and 0.00008 mol·L\textsuperscript{-1} FeCl\textsubscript{3}) and 5 µl of 50 µg·mL\textsuperscript{-1} of the extract of gallic acid standard, was incubated for 15 min at room temperature. The negative control was without addition of extracts or standards. The tubes were then incubated for 1 h at 37°C. The reaction was terminated by addition of 2 µl of loading dye and running the content on 0.8% agarose gel in TBE buffer at 100 V for 45 min. The gel was visualised under UV light.

**Thin layer chromatography screening of phytochemicals**

High performance liquid chromatography grade dichloromethane (DCM; Biodynamics, Windhoek, Namibia) was used in this research. The TLC mobile phase liquids, alkane mixture, and reference standards used for retention time comparison were of analytical grade. TLC plates were silica gel based (Merck silica gel 60 F254). All chemicals and materials were purchased from Sigma Aldrich (Germany), Biodynamics, and Genmed (Windhoek, Namibia).

For qualitative phytochemical analysis by TLC, 20 µl of each of 18 extracts were separately loaded on silica gel TLC plates together with solutions of positive controls. Ethyl acetate was used as a negative control. The TLC analyses were performed using different solvent systems. Each analysis was performed in triplicate.

For alkaloids, methanol and concentrated ammonia mixed in a ratio of 200:3 were used as the mobile phase for alkaloid detection. Dragendorff reagent was used as a spraying reagent to identify alkaloid compounds in the extracts (Suliman, 2018). The manifestation of an orange colour on the TLC plate indicated the presence of alkaloids in the samples. Quinine served as a positive control. For the detection of terpenoids, hexane and ethyl acetate (1:1) were used as the mobile phase using vanillin-sulfuric acid as a spraying reagent. The plates were heated at 120°C for 5 min (Suliman, 2018). A brown spot indicated the presence of terpenoids. β-sitosterol served as a positive control. The mobile phase used for flavonoids was n-butanol, acetic acid, and water (2:2:6), and all plates were visualized under UV light at 254 nm and 366 nm after drying (Sharma and Janmeda, 2017). Quercetin was used as a positive control. For phenols the mobile phase was hexane, ethyl acetate, and acetic acid (31:14:5) and viewed under UV light at 254 and 366 nm, using gallic acid as the positive control (Medic-Saric et al., 2004).

**Data analysis**

All photospectrometric determinations were done in triplicate. Normality tests were done using the Shapiro-Wilk test. Averages were compared to significant differences between standard of gallic acid. ANOVA was carried out using the statistical package SPSS 24 on all tests to determine if there were any significant differences between the different solvent extracts, and between the solvent extracts and the respective positive controls. Differences were deemed significant if the P-value was less than 0.05. A Pearson correlation was done between TPC and TAA to determine if there were any correlation between the results of these two tests.

**RESULTS**

**Isolation and identification of actinobacteria strains**

The IDs of the 23 isolates were designated in the lab before further identification as an A for those isolated from the Khomas region and a B for those isolated from the Hardap region. Numbers were designated in order of isolation. A total of 15 isolates were obtained from tubers obtained from the Khomas Region and 8 isolates from tubers from the Hardap Region. Table 1 shows the closest similarities of the 23 isolates with those from the EZBio Cloud and NCBI databases. Out of the 23 isolates, 13 had the highest similarity to Streptomyces species, 2 had the highest similarity to a Patulibacter and Rubrobacter species, 1 each had the highest similarity to a Nocardiopsis, Agromyces, Rhodococcus, and Curtobacterium species and 2 were unknown. A total of 7 genera were isolated.

The genus Streptomyces and non-Streptomyces genera were analysed in separate phylogenetic trees (Figures 1 and 2). The phylogenetic trees demonstrated that all endophytic actinobacteria were grouped into 7 different clades for actinobacteria, three for the Streptomyces genus tree in Figure 1 and four for the non-Streptomyces genera tree in Figure 2. In both Figures 1 and 2, most isolates did not cluster with the strains they showed high similarity to using BLASTn on NCBI or EZBioCloud.

Isolates A3, A7, and A57 in clade 1 clustered with S. misionensis, S. vellosus, and S. tendae with a bootstrap
value of 100%, isolates B12 and A36 in clade 2 clustered with *S. vietnamensis* with a bootstrap value of 94% and isolate B70 in clade 2 clustered with *S. griseoviridis* with a bootstrap value of 95%. Isolate A68 in clade 3 clustered with *S. laurentii* and not *S. virginiae* to which it showed the highest similarity after performing the BLASTn on NCBI, indicating that they might be novel species. These values indicate that the isolates are probably species of *Streptomyces*. The remaining isolates in clade 3 clustered together but not with any known strains. These isolates formed a separate monophyletic distinct cluster supported by a high bootstrap value (100%) with recently described *Streptomyces* genus but not associated with any of the type strains, suggesting they might be novel species.

The phylogenetic tree of rare actinobacteria is as shown in Figure 2. It indicated that rare actinobacteria were diversely distributed within 4 clusters. In the first clade, isolate A29 clustered...
Figure 1. Consensus of the most likely tree inferred using 16S rRNA sequences of endophytic actinobacteria associated with *H. procumbens*. The phylogenetic tree represents a Maximum Parsimony analysis of 13 isolates with their reference strains of *Streptomyces* spp.
Figure 2. Consensus of the most likely tree inferred using 16S rRNA sequences of endophytic actinobacteria associated with *H. procumbens*. The phylogenetic tree represents a Maximum Parsimony analysis of 10 isolates with their reference strains of non-*Streptomyces* spp.

with *A. flavus*, *A. subtropicus*, and *A. indicus* with a bootstrap value of 93% and is thus likely a species of *Agromyces*. Isolates A66 and B43 in clade 4 clustered with an unidentified actinobacterium strain with a bootstrap value of 52 and 97%, respectively. Isolate A38 in clade 2 clustered with an Actinomycetales bacterium and *N. dassonvillei* with a bootstrap value of 88% and thus might be a species of *Nocardiopsis*. The remaining isolates (A39, A67, B44, B24, B23, and A63) formed clade 3 and clustered together but not with other known strains or the strains they showed the highest similarity to when performing the BLASTn on NCBI, indicating that they might be novel species.

**Total phenolic content**

Ethyl acetate extracts contained significantly higher amounts of phenols than the extracts of the other solvents (*p = 0.000*). Extracts from isolate A36 ( *Streptomyces* spp.) in ethyl acetate had the highest phenolic content of 1158.65 mgGAE·g⁻¹ (milligram of gallic acid equivalents per gram), higher than that of the gallic acid standard (980.41 mg GAE·g⁻¹), followed by the extracts from isolates B12 ( *Streptomyces vietnamensis*) at 969.24 mg GAE·g⁻¹ and B44 ( *Curtobacterium pusillum*) at 962 mg GAE·g⁻¹. For chloroform extracts, isolate B44 ( *Curtobacterium pusillum*) had the highest phenolic
content (460.41 mg GAE·g⁻¹) while all methanol extracts were below 200 mg GAE·g⁻¹. Results for the 23 extracts in the three solvents are shown in Figure 3 compared to the gallic acid standard.

**Total antioxidant activity**

Again, the ethyl acetate extracts had the highest total antioxidant activity (p = 0.000) followed by chloroform, and lastly methanol. The ethyl acetate extracts from isolate A36 (*Streptomyces* spp.) had the highest TAA at 524.45 mg GAE·g⁻¹ followed by extracts from isolates B70 (*Streptomyces* spp.) at 343.52 mg GAE·g⁻¹. Both readings were higher than the gallic acid standard (301.96 mg GAE·g⁻¹). Extracts from isolate B44 (*Curtobacterium pusillum*) had the third highest TAA at 289.73 mg GAE·g⁻¹. The chloroform extracts from isolate B70 (*Streptomyces* spp.) exhibited the highest TAA at 191.93 mg GAE·g⁻¹ while the TAA of all methanol extracts were below 100 mg GAE·g⁻¹. A Pearson correlation was run between the results of TPC and TAA which found a positive correlation of 0.706 which was also seen in the scatter plot. Results for the 23 extracts in the three solvents are as shown in Figure 4 compared to the gallic acid standard.

**Free radical scavenging activity using DPPH assay**

Free radical scavenging activity for all 23 extracts in the three different solvents was determined at different concentrations. The results for the highest concentration of 0.1 mg·mL⁻¹ are as shown in Figure 5. The IC₅₀ was also calculated using the line equation of the curve produced by the different concentrations for each extract as shown in Figure 6.

The ethyl acetate extracts exhibited the highest radical scavenging activity (p = 0.000). Ethyl acetate extracts from isolate B12 (*Streptomyces vietnamensis*) performed the best, with a free radical scavenging activity of 95% at 0.1 mg·mL⁻¹ with an IC₅₀ of 0.014 9 mg·mL⁻¹ followed by extracts from isolate A65 (*Streptomyces flaveus*) (93%) (IC₅₀: 0.0149 mg·mL⁻¹), and A69 (*Streptomyces flaveus*) (92%) (IC₅₀: 0.0152 mg·mL⁻¹). For chloroform extracts, extracts from isolate B12 (*Streptomyces vietnamensis*) showed the highest free radical scavenging activity (73%) at 0.1 mg·mL⁻¹ with an IC₅₀ of 0.0689 mg·mL⁻¹ while all methanol extracts showed activity below 30%.

Free radical scavenging activity was observed to increase with...
Figure 4. Total antioxidant activity of each extract as milligrams of gallic acid equivalents per gram in three different solvents. All readings were statistically significant compared to the gallic acid standard and between treatments as determined by a post hoc Tukey test.

Figure 5. Free radical scavenging activity as a percentage compared to gallic acid of each extract in three different solvents. All readings were statistically significant compared to the gallic acid standard and between treatments as determined by a post hoc Tukey test.
concentration.

**Total reducing power**

Reducing power tended to increase with increasing extract concentration. Figure 7 shows the total reducing power for all 23 extracts compared to the gallic acid standard. The ethyl acetate extracts had the highest reducing power ($p = 0.000$). The ethyl acetate extracts with the highest activity were from isolates A7 (*Streptomyces misionensis*) ($0.849 \pm 0.002$) and B43 (unidentified actinobacteria) ($0.849 \pm 0.003$) followed by A66 (unidentified actinobacteria) ($0.843 \pm 0.001$). The gallic acid standard only had an activity of $0.402 \pm 0.002$. For chloroform, extracts from isolates B43 (unidentified actinobacteria) had the highest activity ($0.196 \pm 0.004$) while all methanol extracts were below $0.050$.

**DNA nicking assay**

Results were recorded according to the amount of DNA damage protection. After the DNA nicking assay, it was found that extracts from isolate B12 (*Streptomyces vietnamensis*), B44 (*Curtobacterium pusillum*), and A63 (*Patulibacter brassicae*) had the highest DNA damage protection ability against hydrogen peroxide, protecting both the linear and plasmid DNA from fragmentation. Extracts from isolates A7 (*Streptomyces misionensis*), A29 (*Agromyces flavus*), A36 (*Streptomyces spp.*), A66 (unidentified actinobacteria), A67 (*Rhodococcus agglutinans*), B43 (unidentified actinobacteria), and B70 (*Streptomyces spp.*) protected the linear DNA from fragmentation but not the plasmid DNA. Furthermore, extracts from isolates A68 (*Streptomyces virginiae*) and A57 (*Streptomyces misionensis*) had low DNA damage protection ability with fragmentation in both linear and plasmid DNA detected. The rest of the extracts had no DNA damage protection ability as no DNA was detected and as it had been completely degraded by the hydrogen peroxide.

**TLC phytochemical screens for terpenoids, alkaloids, phenols, and flavonoids**

None of the samples contained terpenoids as shown in Figure 8. It was found that all samples except B23 contain alkaloids as shown in Figure 9. Phenols were detected in all samples except for B23 as shown in Figure 10. Samples B12, B21, B24, B44, B70 A36 A63, and A69 contain flavonoids as shown in Figure 11. The Rf values of all samples are listed in Table 2.
**DISCUSSION**

This study shows that the secondary tubers of *H. procumbens* contained a diversity of endophytic actinobacteria endowed with various bioactivities. Endophytes, according to Rahman et al. (2017), are chemical synthesizers inside plants that are entirely or partially responsible for their hosts’ therapeutic characteristics. The number and kind of endophytic bacteria are mostly determined by the composition of plant tissue as well as the surrounding environment (Jaginakere et al., 2016). *Streptomyces* species were found to be the most common genus among the endophytic isolates, at 57% of the total actinobacterial species identified. This concurs with the findings of Qin et al. (2009), Jiang et al. (2018), and Shan et al. (2018) on endophytic actinobacteria isolated from a variety of medicinal plants. All of the taxa found in this study were
also detected in these studies, with the exception of *Agromyces, Rubrobacter*, and *Patulibacter* which have, however, been found as endophytes by Kaur et al. (2013), Jin et al. (2016), and Khan et al. (2017). While the rest of the isolates had similarity scores of 95% or higher, isolate A67 had a 91.2% similarity to *Rhodococcus agglutinans*, indicating that it could be a novel species. Also, isolates A66 and B43 had no strong resemblance to any recognised genera, a likely indication that they too could be novel actinobacteria genera.

The endophytic actinobacteria secondary metabolites of *H. procumbens* were subjected to four in vitro antioxidant tests and a DNA nicking experiment in the current study. Various investigations have indicated that *Streptomyces* spp. compounds have significant antioxidant and DNA damage protective capabilities (Tan et al., 2017; Rani et al., 2018; Kemung et al., 2020). Rare actinobacteria and their antioxidant and DNA damage prevention characteristics have only been studied in a few cases. Siddharth and Rai (2019) discovered that a *Nocardiopsis* spp. developed free radical scavenging chemicals 4-bromophenol, and bis (2-ethylhexyl) phthalate. No studies could be found on the other rare actinobacteria genera in regards to antioxidant and DNA damage protection activity.

The findings corroborate research-based evidence
pointing to medicinal plants as well as endophytic microbes as important natural sources of antioxidants (Jaginakere et al., 2016). In studies done by Zheng et al. (2016) and Danagoudar et al. (2017), endophytic bacterium Bacillus cereus SZ1 isolated from Artemisia annua L and the endophytic fungus Aspergillus austroafricanus CGJ-B3 isolated from Zingiber officinale, respectively, showed good antioxidant and DNA damage protection activities. However, apart from this study, no research has previously reported the isolation of

Table 2. Phytochemicals detected in the ethyl acetate extracts of endophytic actinobacteria isolated from H. procumbens.

| Sample | Terpenoids | Alkaloids | Phenols | Flavonoids |
|--------|------------|-----------|---------|------------|
| β-sitosterol | 0.32±0.02 | -         | -       | -          |
| Quinine | -          | 0.66±0.03 | -       | -          |
| Gallic acid | -        | -         | 0.73±0.02 | -          |
| Quercetin | -         | -         | -       | 0.39±0.02  |
| A3     | -          | -         | 0.90±0.02 | -          |
| A7     | -          | 0.83±0.02 and 0.72±0.04 | 0.90±0.02 | -          |
| A29    | -          | 0.83±0.03 and 0.80±0.02 | 0.90±0.03 | -          |
| A36    | -          | 0.83±0.04 | 0.90±0.02 | 0.79±0.02  |
| A38    | -          | 0.83±0.02 and 0.80±0.02 | 0.90±0.04 | -          |
| A63    | -          | 0.83±0.03 | 0.90±0.03 | 0.13±0.01  |
| A64    | -          | 0.74±0.03 | 0.90±0.02 | -          |
| A69    | -          | 0.83±0.04 and 0.72±0.03 | 0.90±0.04 | 0.82±0.03  |
| A70    | -          | 0.71±0.03 and 0.63±0.03 | 0.85±0.02 | -          |
| B12    | -          | 0.83±0.02 and 0.80±0.03 | 0.90±0.02 | 0.52±0.02  |
| B21    | -          | 0.83±0.04 | 0.90±0.03 | 0.82±0.04  |
| B23    | -          | -         | -       | -          |
| B24    | -          | -         | 0.87±0.03 | 0.82±0.03, 0.52±0.02 and 0.13±0.01 |
| B43    | -          | 0.83±0.02 and 0.68±0.03 | 0.90±0.02 | -          |
| B44    | -          | -         | 0.90±0.03 | 0.82±0.02, 0.52±0.02 and 0.13±0.01 |
| B69    | -          | 0.83±0.02 | 0.90±0.02 | -          |
| B70    | -          | 0.83±0.03 | 0.90±0.04 | 0.82±0.03, 0.52±0.03 and 0.13±0.01 |

Figure 11. TLC screen for flavonoids. A black spot is observed where a flavonoid is present (The positive control (+) is Quercetin and the negative control (-) is ethyl acetate).
endophytic actinobacteria from *H. procumbens*, nor the determination of their antioxidant and DNA protection activities. Nonetheless, in experimental conditions, *H. procumbens* ethyl acetate tissue extracts were noted to prevent brain lipid peroxidation generated by pro-oxidants, as well as combat a decrease in catalase activity and thiol levels, which the body needs to counteract oxidative stress (Schaffer et al., 2013).

Other plant extracts, such as *Desmostachya bipinnata* L. Staph's hydroalcoholic extracts also showed antioxidant and DNA damage protective properties (Golla et al., 2014). Similarly, we observed that endophytic actinobacteria isolates extracted in ethyl acetate exhibited the highest antioxidant activity, followed by chloroform and lastly methanol extracts. Because it is a hydrogen bond acceptor molecule, ethyl acetate is a potent organic solvent that can extract solutes that donate electrons more readily than solvents like chloroform (Siek, 1978). In a study published by Lahneche et al. (2019) discovered that the ethyl acetate extracts of *Centaurea sphaerocephala* L showed stronger antioxidant and DNA damage protection activity than the n-butanol extracts they examined. Because phenols are natural antioxidants, there was usually a correlation between TPC and TAA, which was validated in our study with a positive correlation of 0.706 using the Pearson correlation statistical test. Geogriev et al. (2010, 2012) observed that methanolic extracts of *H. procumbens*' aerial parts had higher DPPH radical scavenging activity than pure harpagoside, and that crude methanolic extracts from *H. procumbens* cell and hairy root cultures had about twice the ion-chelating capacity of commercial positive control, butylated hydroxianisole, respectively. As a result, ethyl acetate and methanol were found to be the most effective solvents for extracting secondary metabolites from organic sources. While research show that *H. procumbens* extract have antioxidant activity, multiple studies have also demonstrated that secondary metabolites of endophytic actinobacteria from various plants also have antioxidant activity (Golinska et al., 2015; Jaginakere et al., 2016; Nafis et al., 2018; Photolo et al., 2020). Our findings, together with those of Geogriev et al. (2010; 2012), suggest that due to the co-evolution between endophytes and their host plants, endophytic microorganisms have shaped a huge and largely untapped reservoir of bioactive compounds with high therapeutic potentials in their host plants (Strobel and Daisy, 2003; Qin et al., 2009; Matsumoto and Takahashi, 2017).

Aqueous *H. procumbens* extracts were found to attenuate the DNA-damaging effects of SnCl₂ on numerous *E. coli* strains in a study comparable to this one (Almeida et al., 2007). The extracts were thought to have achieved this effect through chelators of stannous ions preventing the generation of free radicals, or scavengers of free radicals protecting cells from oxidation, and/or lowering SnCl₂ cytotoxicity. However, whereas Almeida et al. (2007) report on the DNA damage protection activity of *H. procumbens* extracts, this study report on the DNA damage protection activity of *H. procumbens* endophytic actinobacteria. Thus, knowing that *H. procumbens* plant extract, as well as its endophytic actinobacteria, have the ability to produce antioxidants that protect DNA from degradation, it is possible that the endophytic actinobacteria from *H. procumbens* added to the plants' DNA damage protection properties.

Of the possible classes of compounds with possible therapeutic bioactivity, traces of alkaloids, phenols, and flavonoids were found in the secondary metabolites of the actinobacteria. Despite the fact that terpenoids have been linked to actinobacteria, according to Shirai et al. (2010), no terpenoids were detected in the extracts.

Phenols are natural antioxidants found in all plant materials, and have been noted for their antiallergic, anti-inflammatory, antidiabetic, antibacterial, and antiviral characteristics, and are known to help prevent diseases like cancer, heart disease, cataracts, and eye disorders (Huyut et al., 2017). According to Janardhan et al. (2014), actinobacteria have long been known for producing high phenolic content. Cao et al. (2019) discovered three novellavandulylated flavonoids from a sponge-derived *Streptomyces* sp. Flavonoids have a wide range of pharmacological effects, including antioxidant activity and the potential to activate human protective enzyme systems. They also aid in the defense of plants against microbial diseases, which could be one of the ways these endophytic actinobacteria assist the *H. procumbens* host plant (Kumar and Pandey, 2013). While flavonoids have previously been linked to actinobacteria, they are also common in plants (Kumar and Pandey, 2013). Alkaloids are a class of nitrogen-containing natural chemicals with a low molecular weight whose range of bioactivities includes emetic, anticholinergic, anticancer, diuretic, antiviral, and antibacterial effects (Alves de Almeida et al., 2017). Alkaloids associated with endophytic actinobacteria from *Fritillaria unibracteata* on the western Sichuan plateau were studied and their antibacterial potential established (Chen et al., 2017).

Thus, our findings affirm that endophytic actinobacteria from *H. procumbens*’ secondary tubers are a good source of antioxidants and DNA protecting compounds. This is significant, particularly in the pharmaceutical business, because cells occasionally require assistance in combating oxidative stress, which includes DNA damage. Since synthetic antioxidants are generally hazardous, discovering natural antioxidant sources is essential.

**CONFLICT OF INTERESTS**

The authors declare that no conflict of interest exists.

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REFERENCES

Abdulhussein A, Hattab Mutlag S, Khamees AH, Sahib H, Ghazi MF (2018). Evaluation of the antiangiogenic and antioxidant activity of *Harpagophytum procumbens* (devil’s claw). Drug Invention Today, 10(4):3542-3546.

Almeida MC, Soares SF, Abreu PRC, Jesus LM, Brito LC, Bernardo-Filho M (2007). Protective effects of an aqueous extract of *Harpagophytum procumbens* upon *Escherichia coli* strains submitted to the lethal action of stannous chloride. Cell and Molecular Biology (Noisy-le-Grand, France) 53(6):923-927.

Alves de Almeida AC, de-Faria FM, Dunder RJ, Manzo LP, Souza-Brito AR (2017). Root N. penicilis in pharmacological activity of alkaloids in animal colitis: potential use for inflammatory bowel disease. Evidence-based complementary and alternative medicine, 2017.

Ansari WA, Krishna R, Zeyad MT, Singh S, Yadav AK (2020). Antimicrobial activity and antioxidant activities of ethyl acetate and n-butanol extracts of *Harpagophytum procumbens* (devil’s claw). Minerva Medica 110:3542-3546.

Benda T (2021). From bush medicine to modern medicinal plants and their uses in the management of common diseases. Frontiers in Microbiology 8:1515.

Chen J, Ta XZ, Zeng H, Li Z, Wang F, Liu S (2017). Screening, identification and antimicrobial activity of alkali produced by endophytic actinomyces from Fritillaria unibracteata in western Sichuan plateau. Zhongguo Zhong yao za zhi = Zhongguo zhongyao yu huaxue yanjiu. 42(9):1378-1385.

Cole D, Strohbach M (2007). Population dynamics and sustainable harvesting of *Harpagophytum procumbens DC* (Devil’s Claw) in Namibia. Bin Skr 203-59. Available at: http://the-eism.elibrary/search/21458. Site visited: 29/11/2021.

Danagoudar A, Joshi CG, Kumar RS, Poyya J, Nivya T, Hulikere M (2017). Molecular profiling and antioxidant as well as anti-bacterial potential of polyphenol containing endophytic fungus *Aspergillus australicus* from *Centauria cyanus* (L). Mycology 8(1):28-38.

Dhayanithy K, Babalola OO (2020). Elucidating mechanisms of endophytes for the ethnobotanical management of medicinal plants. Biotechnology & Biotechnological Equipment. pp. 167-180.

Jaginakere V, Harischandrapa SN, Monnanda SN (2016). Actinomycete endophytes from the ethno medicinal plants of Southern India: Antioxidant activity and characterization studies. Journal of Biologically Active Products from Nature 6(2):166-172.

Janardhan A, Kumar AP, Viswanath B, Saigopal DVR, Narasimha G (2018). Production of bioactive compounds by actinomycetes and their antioxidant properties. Biotechnology Research International 8:1-8.

Jiang Z, Luo L, Huang D, Osterman IA, Tyrpin AP, Liu S, Lukyanov DA, Sergiev PV, Donsotova OA, Korshun VA, Li F, Sun C (2018). Diversity, novelty, and antibiotic activity of endophytic actinobacteria from mangrove plants in Beilin Estuary National Nature Reserve of Guangxi, China. Frontiers in Microbiology 9:868.

Jin D, Kong X, Li H, Luo L, Zhuang X, Zhuang G, Deng Y, Bai Z (2016). *Patulibacter brassicae* sp. nov., isolated from rhizosphere soil of Chinese cabbage (*Brassica campestris*). International Journal of Systematic and Evolutionary Microbiology 66(12):5056-5060.

Kaur C, Pananka AK, Bahl M, Singh S, Seth V, Kaur GS (2015). *Agromyces arachidis* sp. nov. isolated from a peanut (*Arachis hypogaea*) crop field. International Journal of Microbiology 1 pp. 1-6.

Kemung HM, Tan LT, Chan K, Ser H, Law JW, Lee L, Goh B (2020). Antioxidant activities of Streptomyces sp. strain MUSC 14 from mangrove forest soil in Malaysia. BioMed Research International 2020.

Khan A, Asaf S, Al-Rawahi A, Lee I, Al-Harrasi A (2017). Rhizospheric microbial communities associated with wild and cultivated frankincense producing *Boswellia sacra* tree. Plos One 12(10):e0186939.

Khanna MS, Solanki R, Lal R (2011). Selective isolation of rare actinomycetes producing novel antimicrobial compounds. International Journal of Advanced Biotechnology and Research 2(6):375-376.

Khare E, Mishra J, Arora N (2018). Multifaceted interactions between endophytes and plant: Developments and prospects. Frontiers in Microbiology 9:2732.

Kumar S, Pandey A (2013). Chemistry and biological activities of flavonoids: An overview. The Scientific World Journal pp. 1-16.

Kumar S, Stecher G, Li M, Knayz C, Tamura K (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 35(6):1547-1547.

Lahneche A, Boucheham R, Ozen T, Altun M, Boubekri N, Demirtas I, Bicha S, Bentamene A, Benayache F, Benayache S, Zama D (2019). In vitro antioxidant, DNA-damaged protection and anti-proliferative activities of ethyl acetate and n-butanol extracts of *Centaurea sphaerocephala* L. Annals of the Brazilian Academy of Sciences 91:1-11.

Lata R, Chowdhury S, Gond SK, White JF (2018). Induction of abiotic stress tolerance in plants by endophytic microbes. Letters in Applied Microbiology 66(4):268-276.

Lee D, Lee S, Choi B, Cheng J, Lee Y, Yang SH, Suh J (2014). Antioxidant activity and free radical scavenging activities of *Streptomyces* sp. strain MJM 10778. Asian Pacific Journal of Tropical Medicine 7(12):962-967.

Manimaran M, Kannabiran K (2017). Marine *Streptomyces* Sp. VITMK1 derived pyrrole [1, 2-A] pyrazine-1, 4-dione, hexahydro-3(2- methylylpropyl) and its free radical scavenging activity. The Open Bioactive Compounds Journal 5(1).
Matsumoto A, Takahashi Y (2017). Endophytic Actinomycetes: Promising Source of Novel Bioactive Compounds. Journal of Antibiotics 70(5):514-519.

Medic-Saric M, Jaspel I, Smocic-Bubalo A, Mornar A (2004). Optimization of chromatographic conditions in thin layer. Croatian Chemica Acta 77(1-2):361-366.

Mousumi D, Dayanand A (2013). Production and antioxidant attribute of L-Glutaminase from Streptomyces enisocasaeisl DMO-24. International Journal of Latest Research in Science and Technology 2(3):1-9.

Nalis A, Kasrati A, Azmani A, Ouhdouch Y, Hassani L (2018). Endophytic actinobacteria of medicinal plant Aloe Vera: Isolation, antimicrobial, antioxidant, cytotoxicity assays and taxonomic study. Asian Pacific Journal of Tropical Biomedicine 8(10):513.

Ore A, Akinloye O (2019). Oxidative stress and antioxidant biomarkers in clinical and experimental models of non-alcoholic fatty liver disease. Medicina 55(2):26.

Parthasarathi S, Sathy S, Bupesh G, Samy R, Mohan M, Kumar G, Manna N, Jan M, Baharun K (2012). Isolation and characterization of antimicrobial compound from marine Streptomyces hygroscopicus BDUS49. World Journal of Fish and Marine Sciences 4(3):268-277.

Passari A, Mishra VK, Saikia R, Gupta VK, Singh BP (2015). Isolation, abundance and phylogenetic affiliation of endophytic actinomycetes associated with medicinal plants and screening for their in vitro antimicrobial biosynthetic potential. Frontiers in Microbiology 6:273.

Photoliturgia; Marumengwana V, Sitole L, Tleu MG (2020). Antimicrobial and antioxidant properties of a bacterial endophyte, Methylobacterium radiotolerans MAMP 4754, isolated from Combretum erythrophyllum seeds. International Journal of Microbiology 2020:1-11.

Qin S, Chen H, Zhao G, Zhu W, Jiang C, Xu L, Li W (2009). Isolation, diversity, and antimicrobial activity of rare actinomycetes from medicinal plants of tropical rain forests in Xishuangbanna, China. Applied Microbial Environmental Microbiology 75(19):6176-6186.

Rahman L, Shinwari ZK, Iqar I, Rahman L, Tanveer F (2017). An assessment on the role of endophytic microbes in the therapeutic potential of Fagonia indica. Annals of Clinical Microbiology and Antimicrobials 16(1):1-12.

Rani R, Arora S, Kaur J, Manhas RK (2018). Phenolic compounds as antioxidants and chemopreventive drugs from Streptomyces cellulase strain TES17 isolated from rhizosphere of Camellia sinensis. BMC Complementary and Alternative Medicine 18(1):1-15.

Robi D, Mergei CM, Costa P, Pradella JG, Padilla G (2019). Endophytic actinomycetes as potential producers of hemilcellulases and related enzymes for plant biomass degradation. Brazilian Archives of Biology and Technology 62 p.

Sabahi Z, Soltani F, Moein M (2018). Insight into DNA protection ability of medicinal herbs and potential mechanisms in hydrogen peroxide damaged model. Asian Pacific Journal of Tropical Biomedicine 8(2):120-129.

Savi DC, Haminiuk CWI, Sora GTS, Adamsoki DM, Kenski J, Winniscofer SM, Gilienke C (2015). Antitumor, antioxidant and antimicrobial activities of secondary metabolites extracted by endophytic actinomycetes isolated from Vochysia divergens. International Journal of Pharmaceutical, Chemical and Biological Sciences 5(1):347-356.

Schaffer LF, Peroza LR, Boligon AA, Athayde ML, Alves SH, Fachineto R, Wagner C (2013). Harpagophytyrh procumbens Prevents Oxidative Stress and Loss of Cell Viability in vitro. Neurochemical Research 38(11):2256-2267.

Shameem N, Kamili AN, Ahmad M, Masoodi FA, Parray JA (2015). Radial scavenging potential and DNA damage protection of wild edible mushrooms of Kashmir Himalaya. Journal of the Saudi Society of Agricultural Sciences 16(4):314-321.

Shan W, Zhou Y, Liu H, Yu X (2018). Endophytic actinomycetes from tea plants (Camellia sinensis): Isolation, abundance, antimicrobial, and plant-growth-promoting activities. BioMed Research International 2018.

Sharma V, Jannmada P (2017). Extraction, isolation and identification of flavonoids from Euphorbia neriifolia leaves. Arabian Journal of Chemistry 10(4):509-514.

Shirai M, Okuda M, Motoshahi K, Imoto M, Furuhatka M, Matsuo Y, Katsuta A, Shizuri Y, Seto H (2010). Terpenoids produced by actinomycetes: isolation, structural elucidation and biosynthesis of new diterpenoid gilohemolones and B from Gloeotrichia gilohemoria YM28-088. The Journal of Antibiotics 63(5):245-250.

Siddharth S, Rai R (2019). Isolation and characterization of bioactive compounds with antibacterial, antioxidant and enzyme inhibitory activities from marine-derived rare actinobacteria, Nocardiosis sp. SCA21. Microbial Pathogenesis 137:10775.

Siek T (1978). Effective use of organic solvents to remove drugs from biologic specimens. Clinical Toxicology 13(2):205-230.

Singh R, Dubey A (2015). Endophytic actinomycetes as emerging source for therapeutic compounds. Ind Global Journal of Pharmaceutical Sciences 5(2):106-116.

Singh R, Dubey A (2018). Diversity and applications of endophytic actinobacteria of plants in special and other ecological niches. Frontiers in Microbiology 9:1767.

Solecka J, Zajko J, Postek M, Rajnisz A (2012). Biologically active secondary metabolites from actinomycetes. Central European Journal of Biology 7(3):373-390.

Strobel G, Daisy B (2003). Bioprospecting for microbial endophytes and their natural products. Microbiology and Molecular Biology Review 67(4):491-502.

Suarez-Moreno ZR, Vinchira-Villarrage DM, Vergara-Morales DI, Castellanos L, Ramos FA, Guanaccia C, Degassi G, Venturi V, Moreno-Sarmiento N (2019). Plant-growth promotion and biocontrol properties of three Streptomyces spp. isolates to control bacterial rice pathogens. Frontiers in Microbiology 10:290.

Sudha V, Govindaraj R, Baskar K, Al-Dhabi NA, Duraipandian V (2016). Biological properties of endophytic fungi. Brazilian Archives of Biology and Technology 59:1-7.

Sulliman M (2018). Preliminary phytochemical screening and thin layer chromatography analysis of Swietenia macrophylla king methanol extracts. Chemistry and Advanced Materials 3(1).

Tan LH, Chan K, Khan TM, Bukhari SI, Saokaew S, Duangjai A, Pusparajah P, Lee L, Goh B (2017). Streptomyces sp. MUM212 as a source of f.c. antioxidants with radical scavenging and metal chelating properties. Frontiers in Pharmacology 8:276.

Vreju FA, Ciurea PL, Rosu A, Chisalau BA, Pavanescu CD (2019). The effect of glucosamine, chondroitin and Harpagophytyrh procumbens on femoral hyaline cartilage thickness in patients with knee osteoarthritis– An MRI versus ultrasonography study. Journal of Mind and Medical Sciences 6(1):162-168.

Vu THN, Nguyen QH, Dinh TML, Quach NT, Khieu TN, Hoang H, Son C, Vu TT, Chu HH, Lee J, Kang H, Li W, Phi Q (2019). Endophytic actinomycetes associated with Cinnamomum cassia Presl in Hoa Binh province, Vietnam: Distribution, antimicrobial activity and, genetic features. Journal of General and Applied Microbiology 66(1):24-31.

Zheng L, Zhou T, Ma YJ, Wang JW, Zhang YQ (2016). Antioxidant and DNA damage protecting activity of exopolysaccharides from the endophytic bacterium Bacillus Cereus S21. Molecules 21(2):1-15.