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RESEARCH ARTICLE

Antioxidant and antimicrobial activities of the Mizo traditional medicinal plant, *Helicia excelsa* and chemical investigation on its bioactive metabolites

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Abstract

The study analysed the leaf extract of *Helicia excelsa* for the antioxidant, antifungal and antibacterial activities, as well as the chemical components. The plant extract contains appreciable amounts of total antioxidant (26.4 mg ascorbic acid equivalent/g), flavonoid (60 mg quercetin equivalent/g) and phenolic (11.7 mg gallic acid equivalent/g) compounds. The half-maximal inhibitory concentration (IC_{50}) against 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was 17.79± 0.64 µg/mL. It also showed concentration-dependent antioxidant activity by ferric reduction assay. It was effective against fungal species including *Fusarium solani*, *F. keratoplasticum*, *F. oxysporum* and *Pyricularia oryzae,* showing best activity against *P. oryzae.* It exerted antibacterial activity against both Gram-negative and Gram-positive species with highest efficacy against *Pseudomonas aeruginosa* and *Bacillus cereus.* The minimum inhibitory concentrations were 7.81 mg/mL for *B. cereus*, *B. subtilis*, *Klebsiella pneumoniae* and *Salmonella typhimurium*; while it was 15.63 mg/mL for *P. aeruginosa* and *Staphylococcus aureus.* MBC/MIC ratio showed that the plant extract exhibited bactericidal effects against *B. cereus*, *B. subtilis, P. aeruginosa* and *S. aureus* and bacteriostatic effects against *K. pneumoniae and S. typhimurium.* Alkaloids, flavonoids, phenols, carbohydrates, glycosides, saponins, proteins and amino acids and phytosterols were detected as the major secondary metabolites. 3-O-methyl-d-glucose was identified as the principle bioactive compound. The findings substantiate *H. excelsa* as an important medicinal plant that could be a potential source of pharmacologically useful molecules.

Keywords: antifungal; antibacterial; antioxidant; *Helicia excelsa*; medicinal plant

Introduction

Drug resistance has become a major challenge in modern medicine as it turns into the cause of increasing incidences of chronic diseases such as cancer, hypertension, diabetes and various medical conditions due to infectious pathogens. There is a renewed need for novel or improved drugs and several lines of evidence indicate that plants offer some of the most important biological activities related to the diseases and lead molecules for drug development. Medicinal plants have long been used in traditional healthcare systems throughout the course of human history. According to the World Health Organization, nearly 80 % of the global population depends on traditional medicine as first-line treatments for several diseases (1). Many botanical products have been documented to exhibit valuable biological and therapeutic potentials including antioxidant, antitumor, antimutagenic, antidiabetic, analgesic, antidementia, anti-inflammatory, anticancer, antimicrobial, antileishmanial and antimalarial properties (2). Plants as the major natural antioxidants are particularly valued for their association with reduced risks and amelioration of complicated diseases that are difficult to treat (3, 4).

The emergence of antimicrobial resistance has become one of the most serious medical issues and resulted in the increased difficulty in infection managements (5, 6). Pathogenic microorganisms have evolved various mechanisms to evade the effects of antimicrobial agents including enzymatic degradation of drugs, alteration of drug targets, efflux pump activation and biofilm formation (7). This growing phenomenon has rendered many conventional antibiotics ineffective, leading to prolonged illnesses, higher healthcare costs and increased mortality rates (8). As resistance continues to outpace the development of new drugs, there is a reinvigoration in the search for medicinal plants as sources of pharmaceutical compounds, particularly from well-established traditional systems (9, 10).

Plants of the genus *Helicia* (family Proteaceae) hold special significance in different traditional practices. Among the species, *H. nilagirica* is the most popularly used throughout different Asian cultures, variously for treating stomach ailments and cancer (11, 12). Helicid, a glycoside present in the seed, is established to be a powerful analgesic with hypnotic and

sedative effects and has a potential for clinical use in the treatment of different neurological conditions (13, 14). In contrast, *H. excelsa* (Roxb.) Blume, the leaves of which are also used in gastric problems and its seeds as an anti-convulsion agent in Mizo traditional medicine, is poorly understood and remains underrecognized in scientific research (15). Nothing is known about its chemical or biological properties that might be beneficial to health and disease management. Therefore, it is important to investigate on the pharmacological and chemical properties of the plant in view of its notable medicinal applications.

Materials and Methods

Sample collection and identification

The leaves of *H. excelsa* were collected from Aizawl, Mizoram, India, located between 23.7307° N and 92.7173° E. Herbaria of the flowers, leaves and fruits were identified and authenticated at the Botanical Survey of India, Eastern Regional Centre, Shillong, with identification number BSI/ERC/ Tech/2021-22/389. Additionally, they were compared with herbarium specimens at the Royal Botanic Gardens, Kew (catalogue numbers K000009323 and K001110658) and the Natural History Museum, London (catalogue numbers BM013822430 and BM000951124).

Sample preparation

The plant extract was prepared by a standard maceration process (16). The leaves were thoroughly dried and ground into small pieces. The dried samples were submerged in distilled water, continuously stirred and left to macerate at room temperature for seven days. The extract solution was filtered using Whatman No. 1 filter paper and then concentrated under reduced pressure in Buchi Rotavapor® R-100 (Flawil, Switzerland). The concentrated extract was then stored at 4 °C.

Total antioxidant content

The total antioxidant content of *H. excelsa* extract was assessed using the phosphomolybdenum reaction method (17). A series of concentrations, *viz.* 10, 20, 40, 60, 80 and 100 µg/mL, of both the sample and ascorbic acid, a standard reference, were prepared and 3 mL of a reagent solution (composed of 0.6 M sulphuric acid, 4 mM ammonium molybdate and 28 mM sodium phosphate) was added to each. The samples were then incubated at 95 °C for 90 min. After they were cooled down to room temperature and the absorbances were measured at the wavelength of 695 nm in a UV-visible spectrophotometer (Labtronics LT39, Haryana, India). A calibration curve was plotted for ascorbic acid and the total antioxidant content of the extract was calculated from this standard curve, expressed as milligrams of ascorbic acid equivalent per gram of the dried extract (mg AAE/g).

Total flavonoid content

The total flavonoid content was determined based on the reactions of flavonoids and aluminium chloride (18). The plant extract and standard quercetin were prepared at concentrations like those used in the total antioxidant assay. To each solution, 2 mL of distilled water, 3 mL of 5 % sodium nitrite solution, 0.3 mL of 10 % aluminium chloride solution and 2 mL of sodium hydroxide were sequentially added. The final volume was adjusted to 10 mL by adding distilled water. The reaction mixtures were then incubated at room temperature (25 ± 2°C) for 1 hr. The absorbance was measured at 510 nm. A calibration curve of quercetin was used to estimate the flavonoid content of the plant extract and expressed as milligrams of quercetin equivalent per gram of the dried extract (mg QE/g).

Total phenolic content

The total phenolic content was determined by Folin-Ciocalteu assay (19). A sample solution was prepared at a concentration of 100 µg/mL and standard gallic acid was prepared in varying concentrations as in other assays. To each test solution, 5 mL of Folin-Ciocalteu reagent and 4 mL of 0.7 M sodium carbonate solution were added. The reaction mixtures were then allowed to react at room temperature for 1 hr. After incubation, the absorbances were measured at 765 nm. A calibration curve of the gallic acid was used to determine the phenolic content of the extract, which was expressed as milligrams of gallic acid equivalent per gram of the dried extract (mg GAE/g).

DPPH-scavenging assay

The free radical-scavenging activity was evaluated using the DPPH (2, 2-diphenyl-1-picrylhydrazyl) scavenging reaction (20). A series of concentrations of the plant extract and a standard antioxidant, butylated hydroxytoluene (BHT) were prepared. 0.5 mL of freshly prepared DPPH was added to each sample and the mixtures were incubated in the dark for 30 min at 37 °C. The optical densities (ODs) were then measured at 517 nm. The percentage of DPPH inhibition was calculated from the absorbance values using the following formula:

$$\text{Scavenging activity (\%)} = [(\text{Control OD} - \text{Sample OD}) \div \text{Control OD}] \times 100$$

The DPPH scavenging activity was expressed as the half-maximal inhibitory concentration (IC_{50}), which was determined from nonlinear regression analysis (curve fitting).

Ferric-reducing antioxidant power (FRAP) assay

The free radical reducing power was determined using ferric reduction reaction, using ascorbic acid as the reference antioxidant (21). Both the plant extract and ascorbic acid were prepared in a series of concentrations from 10 to 100 µg/mL. To each sample, 2.5 mL of phosphate buffer (pH 6.6) and 2.5 mL of 1 % potassium ferricyanide solution were added. The mixtures were incubated at 50 °C for 30 min. 2.5 mL of 10 % trichloroacetic acid was added to terminate the reaction and the solutions were centrifuged at 3000 rpm for 10 min. 2.5 mL of the resulting supernatant of each sample was mixed with 2.5 mL of distilled water. Then, 0.5 mL of freshly prepared 0.1 % ferric chloride solution was added. Chemical reaction was allowed for 10 min. A blank sample was made from a mixture of equivalent amounts of distilled water, phosphate buffer and potassium ferricyanide. The ODs of each final reaction mixture was measured at 700 nm and adjusted to that of the blank sample.

Antifungal toximetric assay

The antifungal activity was evaluated against two phytopathogenic species, *Fusarium solani* (MTCC 350) and *Pyricularia oryzae* (MTCC 1477) and two human and animal pathogenic species, *Fusarium keratoplasticum* (ATCC 36031) and *Fusarium oxysporum* (MK209108). Fungal inhibition was assessed by the poison plate technique modified with potato-dextrose agar (PDA) culture method (22, 23). Plant extract was prepared at 10, 5, 2.5 and 1.25

mg/mL concentrations and thoroughly mixed with molten, sterilized PDA maintained at approximately 48 ± 2 °C. Plates containing 0.01 % fluconazole (v/v) served as the positive control and PDA without any treatment was used as the negative control. Mycelial plugs (6 mm in diameter) from actively growing margins of seven-day-old fungal cultures were aseptically inoculated at the centre of each treatment plate. All plates were incubated at 28 ± 2 °C. The radial growth of fungal colonies was measured daily for seven days using a dial calliper. The percentage inhibition of fungal growth due to treatment was calculated using the formula:

$$\text{Growth inhibition (\%)} = [(dc - dt) \div dc] \times 100$$

Where dc is the average mycelial growth in control and dt, the average mycelial growth in treatment.

Antibacterial activity

The antibacterial activity was evaluated by well diffusion method (24). Gram-positive bacteria such as *Bacillus cereus* (ATCC 13061), *Bacillus subtilis* (ATCC 11774), *Staphylococcus aureus* 1 (ATCC 700698), *Staphylococcus aureus* 2 (ATCC 11632) and Gram negative species such as *Klebsiella pneumoniae* (ATCC 10031), *Pseudomonas aeruginosa* (ATCC 10145) and *Salmonella typhimurium* (ATCC 51812) were used. Plant extract was prepared at 500, 250, 125 and 62. 5 mg/mL. Ciprofloxacin served as the positive control and dimethyl sulfoxide (DMSO) was used as the negative control. The sub-cultured samples were spread onto agar in separate Petri dishes. 6 mm wells were made on the agar into which bacteria were inoculated. The culture plates were incubated at 28 °C for 24 hr. The inhibition zones were determined from the areas of halos surrounding each well.

Minimum inhibitory concentration and minimum bactericidal concentration

The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were determined using a resazurin-based microdilution assay in a 96-well microtiter plate (25). A stock solution of the extract (250 mg/mL) was added to the first well of each column, followed by 150 µL of nutrient broth containing resazurin dye. The wells were diluted to create a series of concentrations up to the tenth well. 6 µL of the bacterial inoculum was added to each well. The 11^{th} and 12^{th} wells were used as positive (media with bacteria, no extract) and negative (media only, no bacteria or extract) controls, respectively. The plate was incubated at 37 ± 2 °C for 24 hr and MIC was determined by observing the colour change from blue (no growth) to pink (bacterial growth). MBC was determined as the lowest concentration of the extract that prevented any visible bacterial growth on the agar surface.

Phytochemical detection

The major secondary metabolites of *H. excelsa* extract were qualitatively determined following standard compound detection assays (26). Alkaloids were identified using Mayer's, Dragendorff's, Wagner's and Hager's tests; flavonoids by alkaline reagent, lead acetate, ferric chloride and Shinoda's tests; phenols by lead acetate, ferric chloride, potassium dichromate, iodine solution, ellagic acid and gelatin tests; carbohydrates by Molisch's, Benedict's, iodine and Fehling's tests; glycosides by Liebermann's, Salkowski's, Keller-Kiliani, Borntrager's and Legal's tests; saponins by froth and foam tests; proteins and amino acids by Biuret, Millon's, ninhydrin and xanthoproteic tests; and phytosterols by Salkowski and Liebermann-Burchard's tests.

Identification of bioactive compounds

Compound identification was carried out in a single quadrupole gas chromatography-mass spectrometry (GC-MS) system, TRACE™ 1300 ISQ™ LT (Thermo Scientific™, USA). The plant extract was prepared in a solution by dissolving it in acetonitrile (Sigma-Aldrich). A non-polar column TR-5MS (260F142P) having a dimension of 30 m × 0.25 mm × 0.25 µm and a film thickness of 0.25 µm was used. The optimal temperature for the injector port was 250 °C and the oven temperature was 70 °C which was raised incrementally up to 250 °C. The carrier gas, helium, was released at 1 mL/min. 1 µL of the sample was injected in a split mode at a ratio of 1:50. The electron ionization was set at 70 eV, while the temperatures for ion source and transfer line were set at 250 °C. The mass scanning was run for 27 min covering a spectral range of 10 to 1000 amu. The data generated by Thermo Scientific™ Xcalibur™ software were then identified from the chemical databases of Wiley Registry™ and the National Institute of Standards and Technology (U.S. Department of Commerce).

Statistical analysis

Data were expressed as the means ± standard errors of the means from three independent replicates (n = 3). Statistical differences were compared using Student's t-test and one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test in Prism GraphPad version 10.5.0 (Dotmatics, San Diego, US). A p value less than 0.05 was considered statistically significant.

Results

Total antioxidant content

The total antioxidant content of *H. excelsa* extract was determined from the calibration curve prepared of the standard ascorbic acid as shown in Fig. 1. The value of the total antioxidant was determined as 26.4±0.36 mg AAE/g.

Total flavonoid content

The total flavonoid content of *H. excelsa* extract was calculated from the standard graph value plotted for the standard quercetin as shown in Fig. 2. The value of the total flavonoid was found to be 60.0±7.64 mg QE/g.

Total phenolic content

The calibration curve of gallic acid at 765 nm was used for determining the total phenolic content of shown *H. excelsa* extract in Fig. 3. The total phenolic content was estimated as 11.7 ± 0.35 mg GAE/g.

Free radical-reducing power

Ferric-reducing antioxidant activity of *H. excelsa* extract and ascorbic acid is shown in Fig. 4. Ferric ion (Fe^{+3}) is a highly oxidant form of iron in potassium ferricyanide and was effectively reduced by the plant extract of the plant and ascorbic acid, both showing a steady increasing response as their concentrations were increased.

DPPH scavenging activity

The dose responsive curve for the scavenging activity of *H. excelsa* extract and BHT against DPPH radicals is shown in Fig. 5. Both the plant extract and standard antioxidant showed dose-dependent

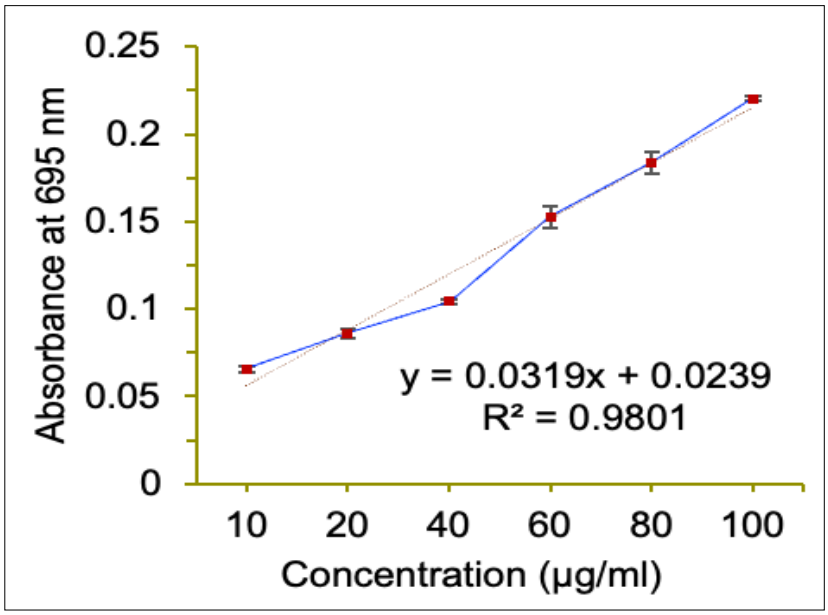

Fig. 1. Standard graph of ascorbic acid for calculation of the total antioxidant content of *H. excelsa* extract. The dotted line represents the linear regression. Values are expressed as means ± standard errors of the means (n = 3).

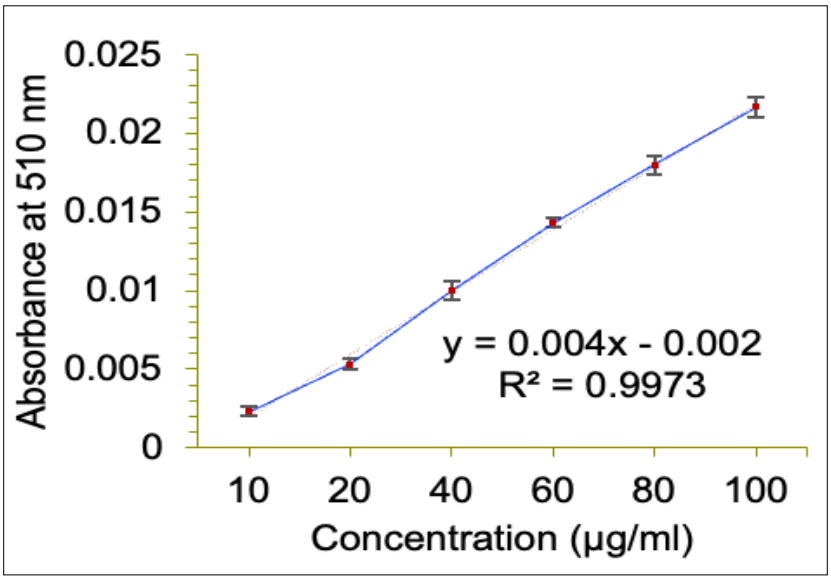

Fig. 2. Standard graph of quercetin for estimation of the total flavonoid content of *H. excelsa* extract. The dotted line represents the linear regression. Values are expressed as means ± standard errors of the means (n = 3).

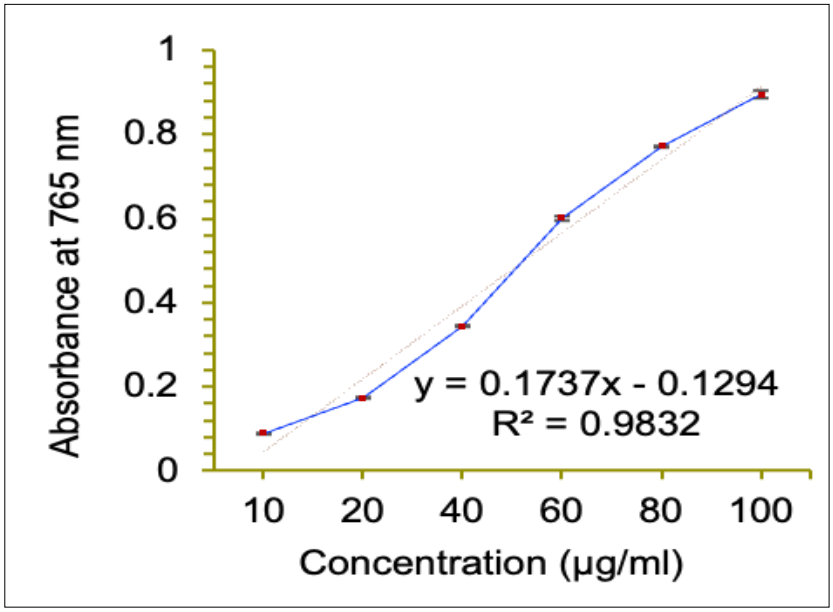

Fig. 3. Standard graph of gallic acid for the estimation of the total phenolic content of *H. excelsa* extract. The dotted line represents the linear regression. Values are expressed as means ± standard errors of the means (n = 3).

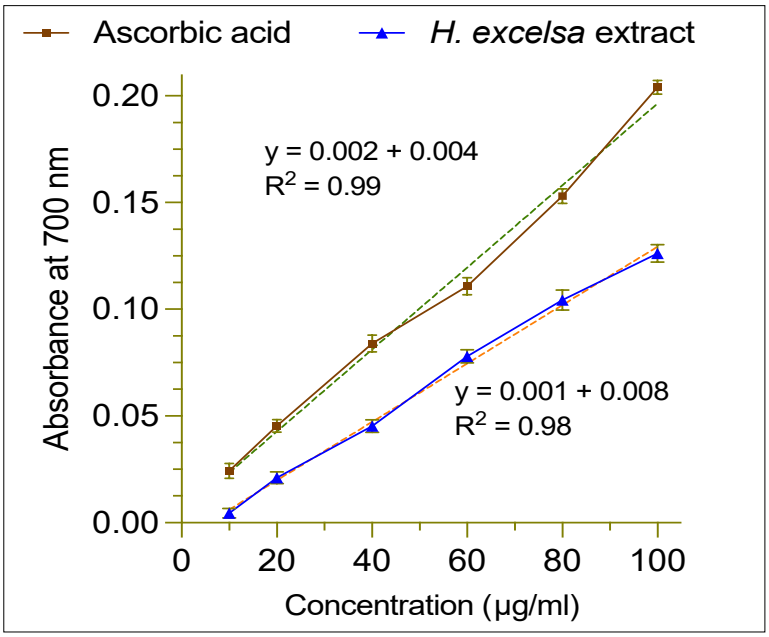

Fig. 4. Ferric reducing chemical reaction of *H. excelsa* extract and ascorbic acid. Values are expressed as means ± standard errors of the means (n = 3).

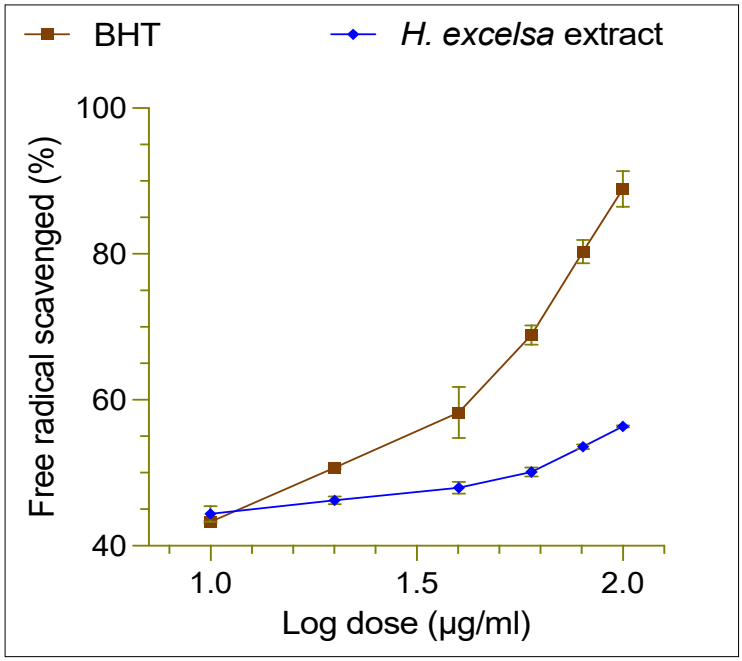

Fig. 5. Dose response curve (log_{10}) of DPPH radical scavenging activity between *H. excelsa* extract and butylated hydroxytoluene. Values are expressed as means ± standard errors of the means (n = 3).

activity, with the plant extract indicating higher log dose values than BHT. The concentration of antioxidant compound necessary to scavenge 50 % of free radicals, calculated as IC_{50}, was 17.79± 0.64 µg/mL for BHT and 41.65± 0.90 µg/mL for the extract. There was significant difference between the efficacy of the plant extract and BHT as illustrated in Fig. 6. It was found that the pure antioxidant compound was significantly (p < 0.0001) more powerful than the plant extract in scavenging DPPH.

Antifungal activity

Fungal growth was maintained and monitored using PDA culture method for seven days. The growth patterns under different experimental conditions are shown in Fig. 7 and the statistical comparison of the antifungal activity of *H. excelsa* extract and the fluconazole is summarized in Fig. 8. The extract showed significant efficacy, particularly against *P. oryzae*. At the highest concentration (i.e. 10 mg/mL), the plant extract inhibited *P. oryzae* growth by 62 ± 0.6 %, surpassing the inhibition by fluconazole at 57 ± 0.1 % inhibition. Lower concentrations of the plant extract, 5, 2.5 and 1.25 mg/mL also indicated substantial inhibitory effects, with inhibition scores of 58 ± 1.4 %, 51 ± 0.4 % and 49 ± 0.3 %, respectively. The plant extract also exhibited significant inhibitory activities against three *Fusarium* species causing inhibition of *F. oxysporum* by 34 ± 1 % and both *F. solani* and *F. keratoplasticum* by 26 ± 0.7 %.

Antibacterial activity

Comparison of the antibacterial activities of *H. excelsa* extract and ciprofloxacin is illustrated in Fig. 9. The plant extract showed significant inhibitory effects against all bacteria tested. It was most highly active against *B. cereus* and *P. aeruginosa* showing inhibition even at the lowest concentration, i.e. 62.5 mg/mL. It showed relatively weak activity at lower concentrations against *K. pneumoniae*, *S. aureus* strains 1 and 2 and *S. typhimurium*, upon which there were no inhibition at the lowest concentration. The overall effectiveness was in the order *P. aeruginosa* > *B. cereus*

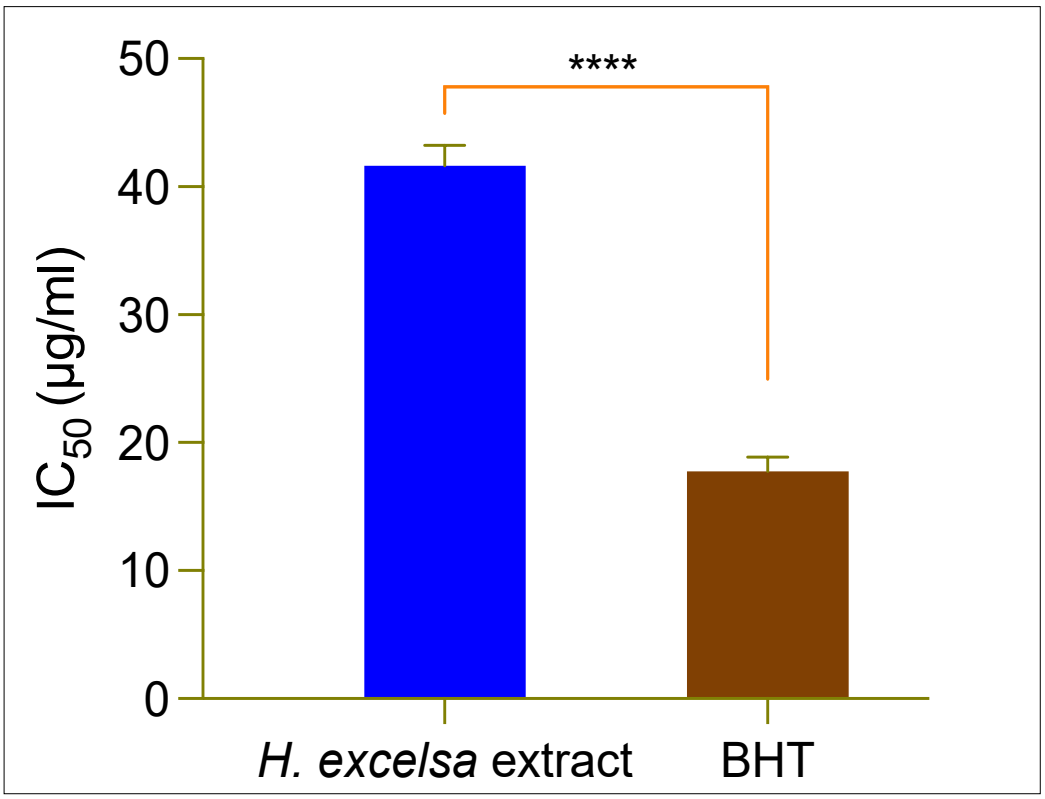

Fig. 6. Comparison of the IC_{50} of *H. excelsa* extract and butylated hydroxytoluene. Student's *t*-test indicated with **** = $p < 0.0001$.

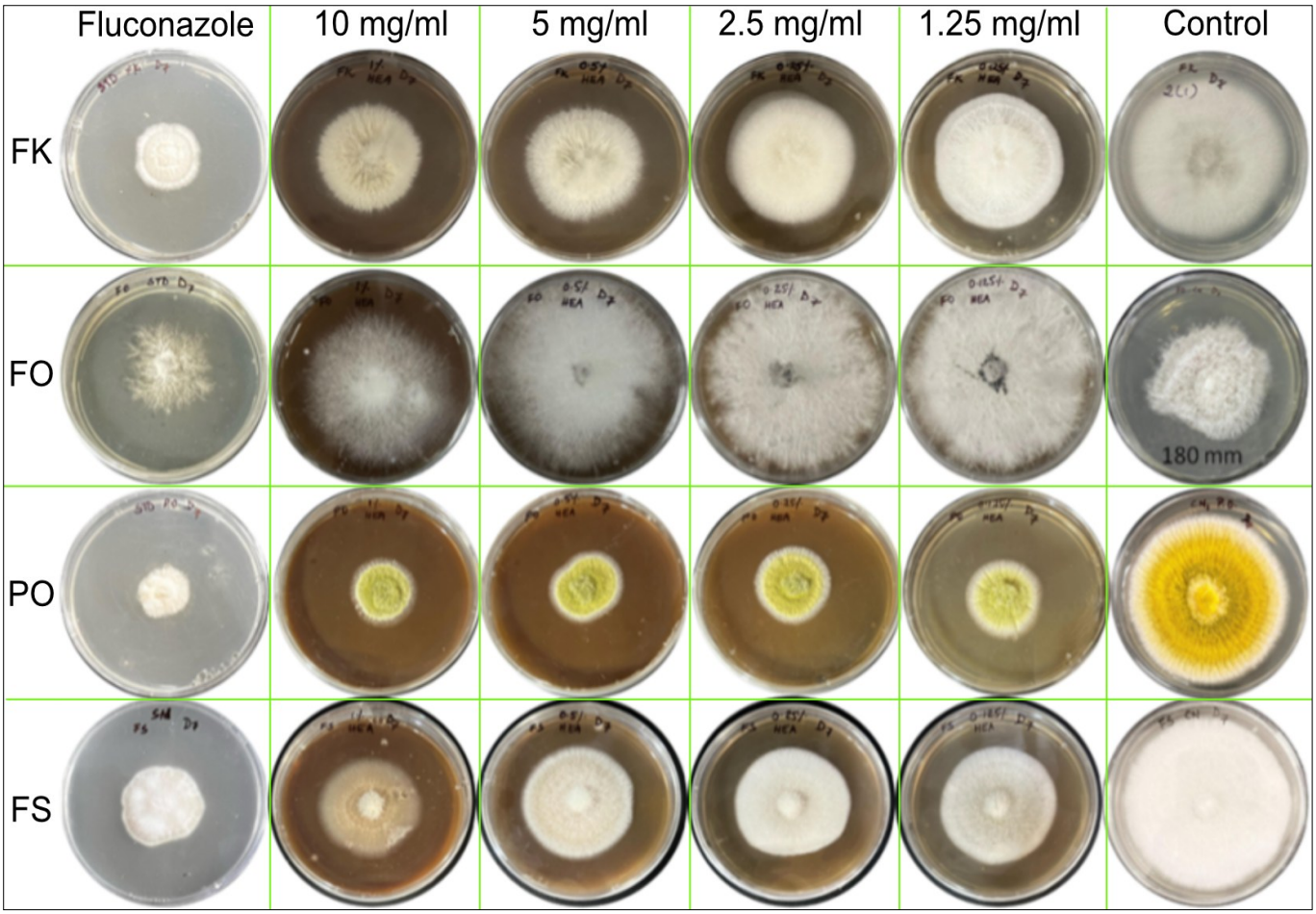

Fig. 7. Growth patterns of *Fusarium keratoplasticum* (FK), *Fusarium oxysporum* (FO), *Fusarium solani* (FS) and *Pyricularia oryzae* (PO) under control (negative) and treatments with *H. excelsa* extract and fluconazole (positive control) after seven-day continuous culture.

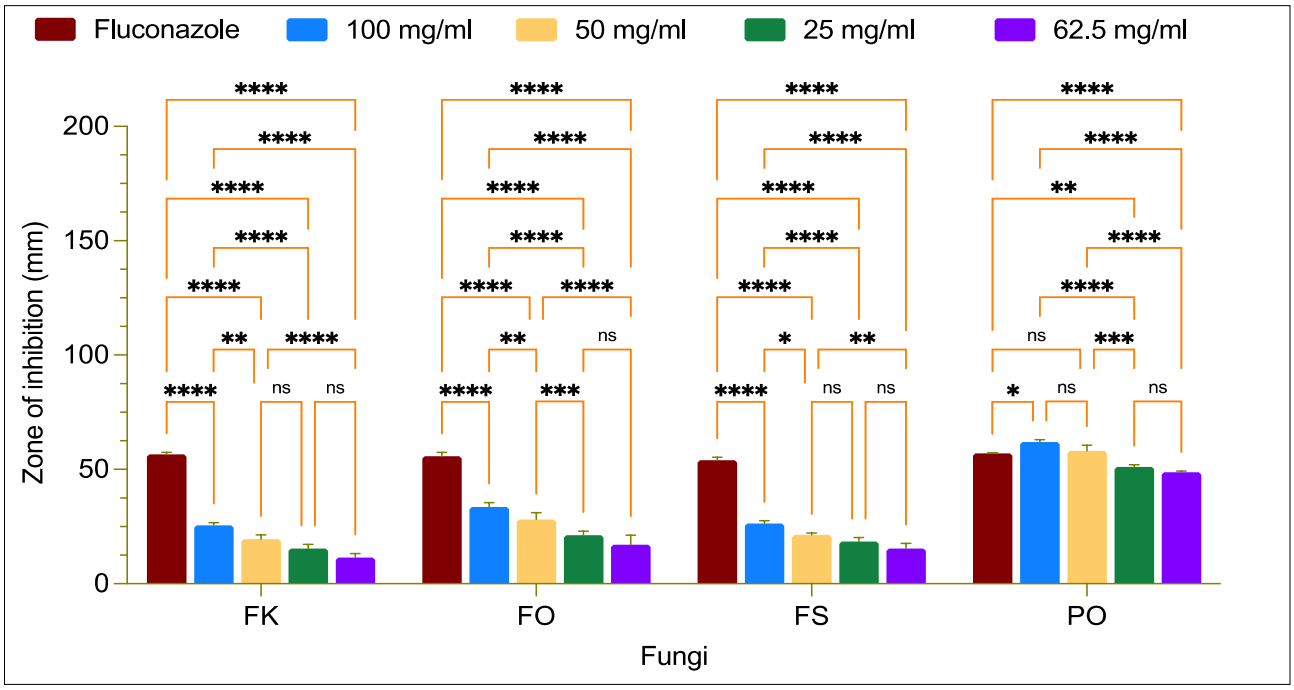

Fig. 8. Statistical comparison of the growth inhibition of *Fusarium keratoplasticum* (FK), *Fusarium oxysporum* (FO), *Fusarium solani* (FS) and *Pyricularia oryzae* (PO) after treatments with *H. excelsa* extract and fluconazole.

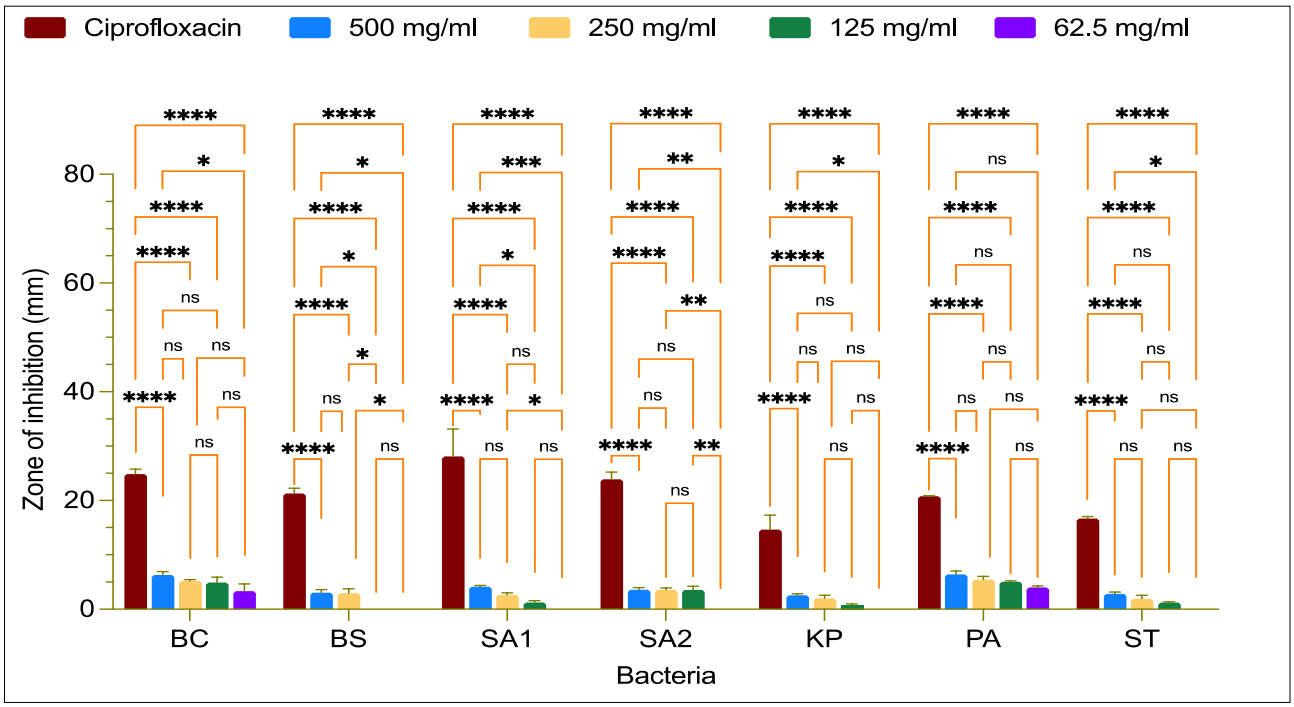

Fig. 9. Antibacterial activity indicated by inhibition zones of *H. excelsa* extract and ciprofloxacin against bacterial strains *Bacillus cereus* (BC), *B. subtilis* (BS), *Klebsiella pneumoniae* (KP), *Pseudomonas aeruginosa* (PA), *Staphylococcus aureus* strain 1 (SA1), *S. aureus* strain 2 (SA2) and *Salmonella typhimurium* (ST). ns = not significant ($p > 0.05$), * = $p < 0.05$, ** = $p < 0.002$, *** = $p \leq 0.0002$, **** = $p < 0.0001$.

> *S. aureus* strain 2 > *S. aureus* strain 1 > *B. subtilis* > *S. typhimurium* > *K. pneumoniae*. The minimum inhibitory concentration (MIC) are tabulated in Table 1. The MIC of *H. excelsa* extract was 7.813 mg/mL for *B. cereus*, *B. subtilis*, *K. pneumoniae* and *S. typhimurium*; while it was 15.63 mg/mL for *P. aeruginosa* and *S. aureus* strains 1 and 2. The MBC values were 15.63 mg/mL for *B. cereus*, *B. subtilis* and *S. aureus* strains 1 and 2; whereas it was 31.25 mg/mL for *K. pneumoniae, P. aeruginosa* and *S. typhimurium*. *S. aureus* strains 1 and 2 exhibited the highest bactericidal (killing) effects with an MBC/MIC ratio of 1. *B. cereus*, *B. subtilis and P. aeruginosa* showed moderate bactericidal effects at an MBC/MIC ratio of 2. In contrast, *K. pneumoniae* and *S. typhimurium* only exhibited bacteriostatic (growth inhibition without killing) effects at an MBC/MIC ratio > 4.

Secondary metabolites

The phytochemical compounds detected by qualitative analyses from the leaf extract of *H. excelsa* extract are shown in Table 2. The plant extract contained a variety of secondary metabolites including alkaloids, flavonoids, phenols, carbohydrates, glycosides, saponins, proteins and amino acids and phytosterols.

Bioactive compounds

The gas chromatogram of *H. excelsa* extract shown in Fig. 10 was used for identifying the specific bioactive components. Many compounds detected but with low abundance and probability

Table 1. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *H. excelsa* extract against different species of bacteria

Bacteria	Gram type	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC ratio
Bacillus cereus	Positive	7.81	15.63	2.001
Bacillus subtilis	Positive	7.81	15.63	2.001
Staphylococcus aureus 1	Positive	15.63	15.63	1
Staphylococcus aureus 2	Positive	15.63	15.63	1
Klebsiella pneumoniae	Negative	7.81	31.25	4.001
Pseudomonas aeruginosa	Negative	15.63	31.25	2
Salmonella typhimurium	Negative	7.813	31.25	4.001

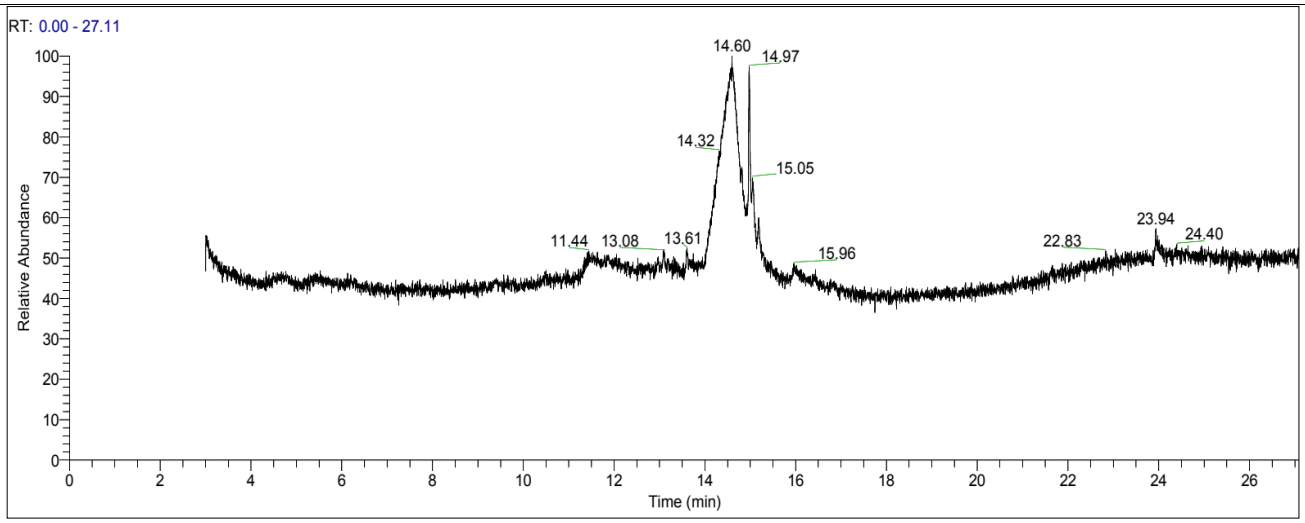

Fig. 10. Gas chromatogram of the leaf extract of *H. excelsa.*

Table 2. Secondary metabolites detected from the leaf extract of *H. excelsa*

Compounds	Biochemical test	Presence or absence
Alkaloid	1. Hager's test	+
	2. Wagner's test	+
	3. Mayer's test	+
	4. Dragendorff's test	+
Flavonoid	1. Alkaline test	+
	2. Lead acetate test	+
	3. Ferric chloride test	+
	4. Shinoda's test	+
Phenol	1. Lead acetate test	-
	2. Ferric chloride test	+
	3. Potassium dichromate test	-
	4. Iodine solution test	-
	5. Ellagic acid test	-
	6. Gelatin test	+
Carbohydrate	1. Molisch's test	+
	2. Benedict's test	+
	3. Iodine test	-
	4. Fehling's test	-
Glycoside	1. Liebermann's test	-
	2. Salkowski's test	-
	3. Keller-Kiliani's test	+
	4. Borntrager's test	-
	5. Legal's test	-
Saponin	1. Froth test	+
	2. Foam test	+
Proteins and amino acid	1. Biuret test	-
	2. Millon's tet	+
	3. Ninhydrin test	-
	4. Xanthoproteic acid test	+
Phytosterol	1. Salkowski test	+
	2. Liebermann-Burchard's test	+

Table 3. Compounds identified in the leaf extract of *H. excelsa* by GC-MS

Retention Time	Compound	Chemical formula	Molecular weight (g/mol)	Relative abundance (%)
11.44	1,4:3,6-Dianhydro-2,5-di-O-nitro-D-mannitol	$C_6H_8N_2O_8$	236	51.7
13.08	1-Acetoxy-p-menth-3-one	$C_{12}H_{20}O_3$	212	52.2
13.61	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	$C_{21}H_{38}O_2$	322	52.5
14.32	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194	76.9
14.60	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194	99.9
14.97	5-Hydroxy-4,4,6-trimethyl-7-oxabicyclo[4.1.0]heptan-2-one	$C_9H_{14}O_3$	170	98.1
15.05	3,7-Dimethyl-1-octen-3-ol	$C_{10}H_{20}O$	156	60.3
15.96	Desulphosinigrin	$C_{10}H_{17}NO_6S$	279	48.8
22.83	Hexamethyl-1,5-diethoxy trisiloxane	$C_{10}H_{28}O_4Si_3$	296	51.9
23.94	1-Di(tert-butyl)silyloxy-3,5-dimethylbenzene	$C_{16}H_{28}OSi$	264	57.8
24.40	Hexamethyl-1,5-diethoxy trisiloxane	$C_{10}H_{28}O_4Si_3$	296	51.1

were ruled out from the important components. As shown in Table 3, nine compounds were identified from 11 major peaks of the chromatogram, indicating that carbohydrate derivatives and ketones are the principal compounds of the plant extract. The major compound was a hexose derivative, 3-O-methyl-d-glucose ($C_7H_{14}O_6$) detected at two major peaks with relative abundance of 99.9 and 76.9 % respectively. A ketone, 5-hydroxy-4,4,6-trimethyl-7-oxabicyclo[4.1.0]heptan-2-one ($C_9H_{14}O_3$) was also detected with high abundance level at 98.1 %.

Discussion

Phytochemicals have long been foundational to modern medicine with an estimate of approximately 25 % of pharmaceutical drugs derived from plants (27). Free radicals, produced from normal cellular metabolic reactions, tend to accumulate as the endogenous antioxidant processes fail to cope up with their production, thereby leading to the development of the most complicated diseases including cancers, cardiovascular diseases, immune disruption, diabetes, neurodegenerative disorders and metabolic disorders (28, 29). Plants in our diet are the best supplementary antioxidant to combat cellular damages due to free radicals (30). Secondary metabolites of plants like alkaloids, terpenoids, flavonoids, tannins, phenols and glycosides, are all antioxidants that possess antimicrobial, anti-inflammatory and anticancer activities. Phenols constitute the major source of pharmaceutical compounds (50 %), followed by terpenoids (26 %) and alkaloids (6 %) (31). The substantial amounts of total antioxidants, flavonoids and phenols found in *H. excelsa* thereby underscores the importance of this medicinal plant. A similar group of secondary metabolites and flavonoid content (92 mg/g) was reported in the aqueous extract of *H. nilagirica* stem (32). A high amount of phenol (576 mg/g) was recorded for the ethanol extract of *H. robusta* leaves (33). The antioxidant capacity of *H. excelsa* was further shown by the free radical-reducing reactions, as a clear pattern of Fe^{3+} reduction to Fe^{2+} was observed in FRAP assay and that of DPPH scavenging reaction. *H. nilagirica* indicated higher DPPH scavenging activity with an IC_{50} of 3.92 µg/mL, but lower flavonoid content (56 mg/g) in comparison to *H. excelsa* (32).

Antimicrobials are often limited by their side effects and species specificity, making them useless in many conditions (34). Plants have been established to contain diverse compounds with potent antimicrobial activities (35). Our findings on the antibacterial and antifungal assays add *H. excelsa* to the repertoire of such medicinal plants with potential antimicrobial source. The poison plate method is regarded as the most sensitive assay available for antifungal activity (23). Using the technique, the plant extract was found to cause growth inhibition of all the tested fungal species in a concentration-dependent manner. Maximum activity was observed against *P. oryzae*, a causative agent of rice blast disease that is the cause of decreased annual rice production by 10 to 30 % globally (36). It also showed high activity against *F. oxysporum,* a devastating pathogen of fruits and vegetables (37).

H. excelsa extract was effective against all the Gram-positive and Gram-negative bacteria tested. It showed best activity against *B. cereus* (Gram positive) and *P. aeruginosa* (Gram negative). Antibiotics are mostly Gram-specific and are thus limited in their medical applications (38). *H. excelsa* is thus an interesting source of broad-spectrum antibacterial compound. The plant extract was most active against *B. cereus*, *B. subtilis*, *K. pneumoniae* and *S. typhimurium* with MIC at 7.813 mg/mL. *B. cereus, B. subtilis* and two strains of *S. aureus* showed MBC value of 15.625 mg/mL, while the Gram-negative species indicated 31.25 mg/mL. MIC/MBC ratio is considered to be an indication of bactericidal activity at ≤4 and of bacteriostatic effect at >4 (39). *H. excelsa* showed bactericidal activity against *B. cereus, B. subtilis, P. aeruginosa* and the two strains of *S. aureus*; while it showed bacteriostatic effect against *K. pneumoniae* and *S. typhimurium*. A closely related species, *H. robusta*, was reported to have antibacterial activities against different Gram-positive and Gram-negative bacteria including some of the species used in the present study. However, it showed weaker activity with comparatively poor MIC and MBC against all the bacteria used (33).

The different compounds in *H. excelsa* leaf identified by GC-MS are reported from other plants to have various biological activities such as antimicrobial, anti-parasitic, anti-inflammatory, anticancer, antioxidant and antiseptic (40-42). Two principal compounds were identified, 3-O-methyl-d-glucose and 5-hydroxy-4,4,6-trimethyl-7-oxabicyclo[4.1.0]heptan-2-one. The latter is rarely reported in plants, but a common component in different species of *Trichoderma* (43). On the other hand, 3-O-methyl-d-glucose is a normal compound in glucose metabolism in plants. It is one of the major bioactive compounds in *Bauhinia tomentosa* and is linked to the plant's antioxidant, anthelmintic, antibacterial and neuro-protective activities (44). It has been isolated as the major antioxidant constituent in the coat, seed and cotyledon of *Vigna mungo* (black gram) (45). It is also identified in the leaves of *Gymnema sylvestre* and the seed of *Eugenia jambolana* in which it is attributed to the anticancer, antifungal and antioxidant activities of the plants (46). Thus, the same compound detected in *H. excelsa* suggests the antimicrobial and antioxidant effects, expanding the known therapeutic potentials of the compound. However, there may be additional bioactive compounds present in the plant. Thus,

it is important to work out the details of the plant's chemical composition and pharmacological properties.

Conclusion

The study evaluates and substantiates some of the chemical and biological of *H. excelsa* used as a traditional medicine. The presence of total antioxidant, flavonoid and phenolic compounds indicated the health-beneficial property of the plant. DPPH scavenging and FRAP assays corroborated the antioxidant activity. The plant extract was effective against all the fungal species tested including *F. solani*, *F. keratoplasticum*, *F. oxysporum* and *P. oryzae*. It showed excellent activity against *P. oryzae*. The plant possesses broad-spectrum antimicrobial activity inhibiting both Gram-negative and Gram-positive bacteria. It showed strong activity particularly against *B. cereus* and *P. aeruginosa* even at the lowest concentration tested. The plant extract exhibited bactericidal effect against *B. cereus*, *B. subtilis*, *P. aeruginosa* and *S. aureus* strains, while bacteriostatic effects against *K. pneumoniae* and *S. typhimurium*. The extract was found to rich in a range of secondary metabolites including alkaloids, carbohydrates, flavonoids, glycosides, phenols, saponins, proteins and amino acids and phytosterols. The presence of 3-O-methyl-d-glucose as the major bioactive compound suggests that the plant can be a source of wide-ranging bioactive compounds. The data pose a challenge for further pharmacological studies.

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Authors' contributions

KL and PBL conceived the experiments. LT conducted the survey, specimen collection and all experiments. L and LL assisted in plant extraction and chemical analysis. MLC assisted in the antimicrobial tests. PBL executed GC-MS, KL analysed the data. LT prepared the first draft. KL acquire funds and facilities, finalised data interpretation and the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

References

1. Aware CB, Patil DN, Suryawanshi SS, Mali PR, Rane MR, Gurav RG, et al. Natural bioactive products as promising therapeutics: A review of natural product-based drug development. S Afr J Bot. 2022;151:512-28. https://doi.org/10.1016/j.sajb.2022.05.028
2. El-Saadony MT, Saad AM, Mohammed DM, Korma SA, Alshahrani MY, Ahmed AE, et al. Medicinal plants: bioactive compounds, biological activities, combating multidrug-resistant microorganisms and human health benefits-a comprehensive review. Front Immunol. 2025;16:1491777. https://doi.org/10.3389/fimmu.2025.1491777
3. Rysz J, Franczyk B, Rysz-Górzyńska M, Gluba-Brzózka A. Ageing, age-related cardiovascular risk and the beneficial role of natural components intake. Int J Mol Sci. 2021;23(1):183. https://doi.org/10.3390/ijms23010183
4. Riaz M, Khalid R, Afzal M, Anjum F, Fatima H, Zia S, et al. Phytobioactive compounds as therapeutic agents for human diseases: A review. Food Sci Nutr. 2023;11(6):2500-29. https://doi.org/10.1002/fsn3.3308
5. Ding D, Wang B, Zhang X, Zhang J, Zhang H, Liu X, et al. The spread of antibiotic resistance to humans and potential protection strategies. Ecotoxicol Environ Safety. 2023;254:114734. https://doi.org/10.1016/j.ecoenv.2023.114734
6. Aslam B, Khurshid M, Arshad MI, Muzammil S, Rasool M, Yasmeen N, et al. Antibiotic resistance: one health one world outlook. Front Cell Infect Microbiol. 2021;11:771510. https://doi.org/10.3389/fcimb.2021.771510
7. Darby EM, Trampari E, Siasat P, Gaya MS, Alav I, Webber MA, et al. Molecular mechanisms of antibiotic resistance revisited. Nat Rev Microbiol. 2023;21(5):280-95. https://doi.org/10.1038/s41579-022-00820-y
8. Tang KW, Millar BC, Moore JE. Antimicrobial resistance (AMR). Br J Biomed Sci. 2023;80:11387. https://doi.org/10.3389/bjbs.2023.11387
9. Guedes BN, Krambeck K, Durazzo A, Lucarini M, Santini A, Oliveira MB, et al. Natural antibiotics against antimicrobial resistance: sources and bioinspired delivery systems. Braz J Microbiol. 2024;55(3):2753-66. https://doi.org/10.1007/s42770-024-01410-1
10. Angelini P. Plant-derived antimicrobials and their crucial role in combating antimicrobial resistance. Antibiotics. 2024;13(8):746. https://doi.org/10.3390/antibiotics13080746
11. Meti MD, Xu Y, Xie J, Chen Y, Wu Z, Liu J, et al. Multi-spectroscopic studies on the interaction between traditional Chinese herb, helicid with pepsin. Mol Biol Rep. 2018;45:1637-46. https://doi.org/10.1007/s11033-018-4306-5
12. Jagetia GC, Zoremsiami J. Anticancer activity of Helicia nilagirica Bedd. in mice transplanted with Dalton's lymphoma. Int J Complement Altern Med. 2018;11(2):112-23. https://doi.org/10.15406/ijcam.2018.11.00380
13. Li XY, Qi WW, Zhang YX, Jiang SY, Yang B, Xiong L, et al. Helicid ameliorates learning and cognitive ability and activities cAMP/PKA/CREB signalling in chronic unpredictable mild stress rats. Biol Pharm Bull. 2019;42(7):1146-54. https://doi.org/10.1248/bpb.b19-00012
14. Liu Y, Cai Y, Bai X, Zhao X, Meng X, Zhang X, et al. A sensitive on-tissue chemical derivatization-mass spectrometry imaging method for the quantitative visualization of helicid in mice. Int J Mass Spectrom. 2023;488:117038. https://doi.org/10.1016/j.ijms.2023.117038
15. Sharma HK, Chhangte L, Dolui AK. Traditional medicinal plants in Mizoram, India. Fitoterapia. 2001;72(2):146-61. https://doi.org/10.1016/S0367-326X(00)00278-1
16. Azwanida NN. A review on the extraction methods use in medicinal plants, principle, strength and limitation. Med Aromat Plants. 2015;4(196):2167-0412.
17. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem. 1999;269(2):337-41. https://doi.org/10.1006/abio.1999.4019
18. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 1999;64(4):555-9. https://

doi.org/10.1016/S0308-8146(98)00102-2

19. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic. 1965;16(3):144-58. https://doi.org/10.5344/ajev.1965.16.3.144
20. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958;181:1199-200. https://doi.org/10.1038/1811199a0
21. Oyaizu M. Studies on products of browning reaction antioxidative activities of products of browning reaction prepared from glucosamine. Jpn J Nutr Diet. 1986;44(6):307-15. https://doi.org/10.5264/eiyogakuzashi.44.307
22. Grover RK, Moore JD. Toximetric studies of fungicides against the brown rot organisms, Sclerotinia fructicola and S. laxa. Phytopathology. 1962;52:876-9.
23. Erhonyota C, Edo GI, Onoharigho FO. Comparison of poison plate and agar well diffusion method determining the antifungal activity of protein fractions. Acta Ecol Sin. 2023;43(4):684-9. https://doi.org/10.1016/j.chnaes.2022.08.006
24. Devillers J, Steiman R, Seigle-Murandi F. The usefulness of the agar-well diffusion method for assessing chemical toxicity to bacteria and fungi. Chemosphere. 1989;19(10-11):1693-700. https://doi.org/10.1016/0045-6535(89)90512-2
25. Elshikh M, Ahmed S, Funston S, Dunlop P, McGaw M, Marchant R, et al. Resazurin-based 96-well plate microdilution method for the determination of minimum inhibitory concentration of biosurfactants. Biotechnol Lett. 2016;38:1015-19. https://doi.org/10.1007/s10529-016-2079-2
26. Evans WC, Trease GC. Trease and Evans' Pharmacognosy. 16th ed. London: Balliere Tindal; 2009:356, 378.
27. Newman DJ, Cragg GM. Natural products as sources of new drugs over the nearly four decades from 1981 to 2019. J Nat Prod. 2020;83 (3):770-803. https://doi.org/10.1021/acs.jnatprod.9b01285
28. Chaudhary P, Janmeda P, Docea AO, Yeskaliyeva B, Abdull Razis AF, Modu B, et al. Oxidative stress, free radicals and antioxidants: Potential crosstalk in the pathophysiology of human diseases. Front Chem. 2023;11:1158198. https://doi.org/10.3389/fchem.2023.1158198
29. Zhu L, Luo M, Zhang Y, Fang F, Li M, An F, et al. Free radical as a double-edged sword in disease: Deriving strategic opportunities for nanotherapeutics. Coord Chem Rev. 2023;475:214875. https://doi.org/10.1016/j.ccr.2022.214875
30. Nwozo OS, Effiong EM, Aja PM, Awuchi CG. Antioxidant, phytochemical and therapeutic properties of medicinal plants: A review. Int J Food Prop. 2023;26(1):359-88. https://doi.org/10.1080/10942912.2022.2157425
31. Porras G, Chassagne F, Lyles JT, Marquez L, Dettweiler M, Salam AM, et al. Ethnobotany and the role of plant natural products in antibiotic drug discovery. Chem Rev. 2020;121(6):3495-560. https://doi.org/10.1021/acs.chemrev.0c00922
32. Zoremsiami J, Jagetia GC. Phytochemical analysis and free radical scavenging activity of Helicia nilagirica *in-vitro*. Asian J Pharm Clin Res. 2014;7(5):246-9.
33. Mariani F, Tammachote R, Kusuma IW, Chavasiri W, Prasongsuk HP. Phenolic content and biological activities of ethanol extracts from medicinal plants in East Kalimantan, Indonesia. Sains Malays. 2021;50(8):2193-205. https://doi.org/10.17576/jsm-2021-5008-05
34. Tyers M, Wright GD. Drug combinations: a strategy to extend the life of antibiotics in the 21st century. Nat Rev Microbiol. 2019;17(3):141-55. https://doi.org/10.1038/s41579-018-0141-x
35. Li S, Jiang S, Jia W, Guo T, Wang F, Li J, et al. Natural antimicrobials from plants: Recent advances and future prospects. Food Chem. 2024;432:137231. https://doi.org/10.1016/j.foodchem.2023.137231
36. Law JW, Ser HL, Khan TM, Chuah LH, Pusparajah P, Chan KG, et al. The potential of Streptomyces as biocontrol agents against the rice blast fungus, Magnaporthe oryzae (*Pyricularia oryzae*). Front Microbiol. 2017;8:3. https://doi.org/10.3389/fmicb.2017.00003
37. Srivastava V, Patra K, Pai H, Aguilar-Pontes MV, Berasategui A, Kamble A, et al. Molecular dialogue during host manipulation by the vascular wilt fungus Fusarium oxysporum. Annu Rev Phytopathol. 2024;62(1):97-126. https://doi.org/10.1146/annurev-phyto-021722-034823
38. Richter MF, Hergenrother PJ. The challenge of converting Gram-positive-only compounds into broad-spectrum antibiotics. Ann N Y Acad Sci. 2019;1435(1):18-38. https://doi.org/10.1111/nyas.13598
39. Parvekar P, Palaskar J, Metgud S, Maria R, Dutta S. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of silver nanoparticles against Staphylococcus aureus. Biomat Investig Dent. 2020;7(1):105-9. https://doi.org/10.1080/26415275.2020.1796674
40. Jeon JH, Kim MG, Lee HS. Acaricidal activities of bicyclic monoterpene ketones from Artemisia iwayomogi against Dermatophagoides spp. Exp Appl Acarol. 2014;62:415-22. https://doi.org/10.1007/s10493-013-9739-x
41. Moradi KM, Khoradmehr A, Dehghani FA, Tavassoli A, Mohammad RF, Mohebbi G, et al. Induction of apoptosis by ethanol, methanol and ethyl acetate extracts from Cyperus rotundus leaf on *in vitro* human ovarian, cervix and breast cancer cell lines. Int J Fertil Steril. 2024;19(3):326-36.
42. Roslizawaty R, Gholib G, Rahmi N, Khairan K, Idroes R, Syafruddin S, et al. Identification, screening and analysis of secondary metabolite content in methanol extracts of ant nests plant tubers from Aceh, Indonesia. Biodiversitas. 2023;24(12):6934-41. https://doi.org/10.13057/biodiv/d241255
43. Chóez-Guaranda I, Espinoza-Lozano F, Reyes-Araujo D, Romero C, Manzano P, Galarza L, et al. Chemical characterization of Trichoderma spp. extracts with antifungal activity against cocoa pathogens. Molecules. 2023;28(7):3208. https://doi.org/10.3390/molecules28073208
44. Sathasivampillai SV, Sebastian PR, Varatharasan S. Medicinal values of a Saiva ritual plant - Bauhinia tomentosa L. Front Life Sci Relat Technol. 2020;1(2):63-8.
45. Singh PK, Kannan D, Gopinath SC, Raman P. Purification and characterization of 3-O-methyl-D-glucose from the seed coat of Vigna mungo (L.) Hepper. Proc Biochem. 2024;143:83-97. https://doi.org/10.1016/j.procbio.2024.04.021
46. Sindhuja G, Agnes AM. Combination of selected medicinal plants using ethanol extract by GC-MS analysis. Mass Spectrom Lett. 2025;16(1):22-30.

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