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Research article

Profile and *in silico* analysis of metabolite compounds of the endophytic fungus *Alternaria alternata* K-10 from *Drymoglossum piloselloides* as antioxidants and antibacterials

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ABSTRACT

Endophytic fungi are known for producing secondary metabolites with valuable biological activities, including antiviral, anticancer, antibacterial, and antioxidant properties. This study aims to evaluate an endophytic fungus from Dragon Scales leaves (*Drymoglossum piloselloides*) and analyze its metabolites as antioxidants and antibacterials. In this study, an endophytic fungus was isolated from the leaves of Dragon Scales (*D. piloselloides*) and identified using molecular analysis of the Internal Transcribed Spacer (ITS) ribosomal RNA locus. The fungus was authenticated as *Alternaria alternata* strain K-10. Crude extracts were obtained using *n*-hexane and ethyl acetate and analyzed via GC-MS Shimadzu-QP 2010 Ultra with NIST spectral library. Antibacterial activity was observed against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa* using the paper disc method, showing inhibition zones of 8.7–9.3 mm and 8.8–9.4 mm for ethyl acetate and *n*-hexane extracts, respectively. Ethyl acetate and *n*-hexane extracts exhibited strong antioxidant potential against 2,2-diphenyl-1-picrylhydrazil (DPPH) radical (IC_{50} values of 50.99 μg mL^{-1} and 74.44 μg mL^{-1}, respectively). GC–MS analysis revealed 40 compounds in both extracts, some of which, including 2-ethylhexyl ester benzoic acid, benzo-b-dihydropyran-6-hydroxy-4-4-5-7-8-pentamethyl, diethyl phthalate, and octadecanoic acid, were identified through *in silico* analysis and found to possess antioxidant properties. These

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findings hold implications for potential applications of the plant and its biological constituent to be developed as lead compounds in the medical sector.

1. Introduction

Drymoglossum piloselloides (local name, *sisik naga*) plant of the Polypodiaceae family is a small epiphytic fern is common in the wild of many Asian countries. The plant has unique characteristics and potential as a traditional medicinal plant containing polyphenols, essential oils, steroids, flavonoids, sugar, and tannins. Throughout history, humans have utilized plants for the treatment of diseases. Scientific research has empirically demonstrated the efficacy of therapy using plants, attributed to the vast diversity of specific metabolites or phytochemicals such as alkaloids, terpenoids, and phenolic compounds. Plant-based medicinal products could serve as a sustainable alternative to mitigate the use of synthetic drugs. However, stringent legal controls are necessary to prevent toxic effects or exploitation of plant species through comprehensive scientific studies. Further research is essential to verify the therapeutic properties of medicinal plants and assess the efficacy of specific plant species in disease treatment. Plants identified with efficacy in traditional medicine are potential candidates for advancing subsequent ethnopharmacological studies. The findings of such research will provide robust support for the utilization of plants in modern medicine [1], *D. piloselloides* plant also plays its role as an antibacterial agent for pathogens such as *S. aureus* and *Salmonella enteritidis* which often infect the skin and digestive system [2]. For safety and environmental reasons, compared to synthetic compounds, plant extracts become preferable choice [3,4]. The secondary metabolites of *A. alternata* have been of interest due to their potential as alternative drugs and their effects on various biological activities [5–8].

In recent times, there has been a shift in attention regarding the usual utilization of medicinal plants to their microbial associates or microbiomes as the promising bioproducers of secondary metabolites. This transition has prompted the exploration of endophytic fungi residing within medicinal plants for potential antioxidant and antibacterial agents, opening up an intriguing avenue for bioprospecting. Among the plant-associated microorganisms, endophytic fungi stand out, garnering significant interest in contemporary drug discovery initiatives. This is primarily due to the intricate and diverse chemicals following its complexity of their natural products, which remain beyond the realm of synthetic replication [9,10]. Endophytic fungi represent a category of microorganisms engaged in symbiosis with the tissues of healthy plants, without inflicting deleterious effects upon said plant tissues [11]. Through an evolutionary process of endosymbiosis, these fungi have co-living with their host plants (co-evolution), facilitating the exchange of genetic material between endophytes and their plant hosts [12,13]. The capacity of endophytic fungi to synthesize similar secondary metabolites as their host plants underscores their potential significance as candidates for the development of novel pharmaceutical agents in the future [14–18].

Within the plant tissues of *D. piloselloides*, *A. alternata* synthesizes metabolites aimed at protecting the host plant against an array of biotic and abiotic stressors. Concurrently, *A. alternata* derives nutrients from the host plant [19,20]. One of the interesting aspects of the endophytic fungus *A. alternata* is the producing a potential of the bioactive compounds. Several recent studies revealed that the extract of the fungus produces some compounds with antimicrobial, anti-inflammatory, and antioxidant activities. The metabolite profiles of the crude ethyl acetate extract of *A. alternata* from QTOF-HRLCMS analysis which are antioxidant characterized are avenalumic acid ($C_{11}H_{10}O_3$), gambiriin A3 ($C_{30}H_{28}O_{12}$), 5-*O*-feruloylquinic acid ($C_{17}H_{20}O_9$), pinobanksin ($C_{15}H_{12}O_5$), okanin ($C_{15}H_{12}O_6$), and isoathyriol ($C_{14}H_{10}O_6$) [21–26]. Antioxidant activity is important for protecting body cells from oxidative damage caused by free radicals. Thus, consuming antioxidant compounds can help people to avoid disease and can improve body health. The endophytic fungal metabolites of *A. alternata* have essential benefits for developing new applications as raw materials for medicines, health supplements, and beauty products. The compounds are natural alternatives to overcome oxidative stress conditions. The antimicrobial properties of the metabolites in the ethyl acetate extract of *A. alternata* are kigelinone ($C_{14}H_{10}O_5$), kaempferol 3-*O*-β-D-galactoside ($C_{21}H_{20}O_{11}$) [27,28]. Due to their antibacterial and antifungal properties, these compounds can protect humans from various infectious diseases. In this study, the endophytic fungus *A. alternata* was successfully isolated from the leaves of *D. piloselloides* which grows in Bogor, West Java, Indonesia.

Research on the activity of metabolites from the *D. piloselloides* plant as antimicrobials has been conducted by Somchit et al. [2], and the results show inhibitory activity against the indicator bacteria. Gram-positive bacteria exhibited greater susceptibility to *D. piloselloides* extract compared to Gram-negative bacteria. This inhibitory action is attributed to the phytochemical content in the extracts of *D. piloselloides*, primarily comprising sterols/triterpenes, phenols, flavonoids, and tannins [29]. The antibacterial potential of *A. alternata* isolated from the leaves of *Eremophila longifolia* has been conducted by Caruso et al. [30]. Testing using the Gram-positive bacterium *S. aureus*, which is a major pathogen associated with Antimicrobial Resistance (AMR), revealed that the etil asetat extract from *A. alternata* inhibits the bacterial strain *S. aureus*. Several tests were carried out on bacteria of *B. subtilis*, *E. coli*, *P. aureoginosa*, and *S. aureus* using paper-disc method. The identification of compound was analyzed by using GC-MS. Potential test upon *A. alternata* as an antioxidant was carried out using DPPH radicals and *in silico* studies. The aim of this study is to investigate the potential health benefits of metabolites produced by *A. alternata*, particularly their role as antibacterial and antioxidant agents. This research aims to bridge the existing gap in knowledge by employing *in silico* studies of *A. alternata* metabolites. Through molecular modeling techniques, the study seeks to predict and identify compounds with the potential to act as antioxidants.

2. Materials and methods

2.1. Materials

Microorganisms: *Alternaria alternata, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis,* and *Staphylococcus aureus.* Media: Potato Dextrose Agar (PDA, Merck), Bacto agar (Merck), Mueller Hinton Agar (Himedia), Nutrient Agar (Himedia) dan Nutrient Broth (Himedia). Reagents: Deionized water, Tween 20 (Merck), Ethanol (Merck), sodium hypochlorite (Merck), Methanol (Merck), Streptomycin sulfate (Sigma-Aldrich), PHYTOPure nucleon reagent (Amersham LIFE SCIENCE). Nuclease-Free Water (Himedia), Primer Forward ITS 5 (Macrogen), Primer Reverse ITS 4 (Macrogen), GoTaq Green Master Mix 2X (Promega), Whatman filter paper No. 1 (Sigma-Aldrich), Filter paper for antibacterial test (Oxoid), *n*-Hexane (Merck), Ethyl acetate (Merck), dimethyl sulfoxide (Sigma-Aldrich), 2,2-diphenyl-1-picrylhydrazyl (Sigma-Aldrich). Instruments: Analytic balance (AND GR-200, GF-2000), Laminar air flow (Sanyo), shaker incubator (Yamato SA320 and IC602), oven (Jisico), Centrifuge (Tomy MX-305), Autoclave (Tomy SX-300), Rotary evaporator (IKA RV10), Microscope (Interscience 4000), Magnetic strirrer (Ayela RCH-3), Varioskan Lux (Thermoscientific), GCMS (Shimadzu-QP 2010 Ultra), PCR (Thermo Scientific Arktik), DNA sequencer (ABI PRISM 3130 Genetic Analyzer).

2.2. Collection and preparation of plant

Healthy dragon scales plants (*D. piloselloides*) were collected from Bogor-West Java, Indonesia (6°38′13.48"S, 106°48′59.55"E), and their endophytic fungi were isolated. The leaves and stems, which have been reduced to small portions (±1 cm), were sterilized based on the modified Petrini and Dreyfuss [31]. The plant parts used as samples were rinsed with running water, tween 20, ethanol (70%), and sodium hypochloride (1:3). Subsequently, the plant parts were rinsed with 70% ethanol and sterile distilled water; then dried. Pieces of plant parts (±1 cm) were aseptically inoculated on Petri dish containing Potato Dextrose Agar (PDA) medium supplemented with streptomycin. Furthermore, the fragments of plant parts were incubated at 28 ± 1 °C. The growing of endophytic fungi were transferred to slanting PDA media in tubes and stored in refrigerator (4 °C) until further experimentation.

2.3. Identification of endophytic fungi based on ITS region

The fungal specimen isolated from *D. piloselloides* was identified molecularly based on the partial genetic sequence at the ITS ribosomal RNA fungi. The DNA isolation process began by culturing *A. alternata* in Potato Dextrose Broth (PDB, Oxoid) medium and incubating it for five days. The biomass (mycelium) of *A. alternata* was harvested and mechanically disrupted. The protocol for DNA extraction followed the technical procedure given by PHYTOPure nucleon reagent (Amersham LIFE SCIENCE). The DNA precipitate that had been dried (aerated) was dissolved with nuclease-free water (NFW). PCR amplification on ITS using 1 μL DNA template was added with 9.5 μL NFW, 1 μL Primer Forward ITS 5: 5`–GGA AGT AAA AGT CGT AAC AAG G–3′ and 1 μL Primer Reverse ITS 4: 5`– TCC TCC GCT TAT TGA TAT GC–3′, 12.5 μL of Go Taq Green Master Mix 2x was added. The amplification process consisted of the following parameters on a thermal cycler: the reaction mixture was preheated for 5 min at 94 °C, denaturation for 30 s at 94 °C, annealing for 30 s at 50 °C, extension for 60 s at 72 °C, and final extension for 5 min at 72 °C. The PCR products were visualized using a UV transilluminator with a 1% agarose gel electrophoresis.

The purification of PCR products was carried out using the polyethylene glycol (PEG) precipitation [32] followed by sequencing clean-up. The sequencing cycle outcomes were further subjected to purification through the ethanol purification method. The analysis of nitrogen base sequence readings was performed employing an automated DNA sequencer, namely the ABI PRISM 3130 Genetic Analyzer by Applied Biosystems. Following the sequencing, the acquired data was subjected to trimming and assembly via the utilization of the BioEdit program, accessible at http://www.mbio.ncsu.edu/BioEdit/bioedit.html. Subsequent to this step, to ascertain the taxon or species that exhibited the greatest homology and closest molecular resemblance, the assembled sequence data underwent a BLAST search against genomic data previously registered at NCBI, accessible at http://www.ncbi.nlm.nih.gov/BLAST/. PCR product purification was carried out using polyethylene glycol (PEG) precipitation method [32] and was followed by cle sequencing. The results of the sequencing cycle were purified again with the ethanol purification method. Analysis of nitrogen base sequence readings using an automated DNA sequencer (ABI PRISM 3130 Genetic Analyzer) (Applied Biosystems). The results of sequencing data was then trimmed and assembled using the BioEdit program (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). Afterwards, to determine the taxon or species having homology the largest and closest molecular similarity, the assembled sequence data were BLAST with genomic data that had been registered at NCBI.

2.4. Culture and extraction of A. alternata with ethyl acetate and n-hexane

2.4.1. Preparation of crude extract

Active-growing *A. alternata* isolate were inoculated into eight Erlenmeyer containers containing 300 mL of PDB (24 g/L) medium, then incubated at 25 °C for ten days [33] on an incubator shaker at 200 rpm. Each fungal culture was filtered to separate the liquid from the mycelia by vacuum filtration through Whatman filter paper No. 1. The supernatant obtained were then extracted with *n*-hexane and ethyl acetate in equal volumes (1:1) and repeated until the extract was perfect. Finally, they were concentrated with a rotary evaporator. The crude extract was stored at −20 °C for antimicrobial and antioxidant tests.

2.5. Antibacterial test

2.5.1. Microorganism

The indicator microorganisms used to test the antibacterial activity in this study included two types of Gram-negative bacteria, namely *E. coli* and *P. aeruginosa,* and two types of Gram-positive bacteria, namely *B. subtilis* and *S. aureus*. The indicator strains were cultured in nutrient broth medium at 37 °C for 24 h and maintained on NA medium.

2.5.2. Antibacterial testing with paper disc method [34,35]

Bacterial culture with Optical Density 0.5 (λ600 nm) was pipetted 100 μL into warm Mueller Hinton (Himedia), then homogenized. The soft agar media was poured evenly over the Mueller Hinton media (1.5% agar) in a Petri dish and allowed to stand until it solidified. Furthermore, paper discs are placed on the surface of the solid media. As much as 20 μL of positive control and negative control solutions, ethyl acetate extract test solution, and *A. alternata n*-hexane, each was pipetted onto a paper disc. Subsequently, the Petri dish was incubated at 37 °C for 24–48 h, and the diameter of the inhibition zone (DIZ) was measured.

$$\mathrm{DIZ} = \frac{(Dv - Dc) + (Dh - Dc)}{2}$$

Dv = Vertical diameter of the inhibition zone.
Dh = Horizontal diameter of the inhibition zone.
Dc = Diameter of disc paper.

2.6. Antioxidant test

The assessment of antioxidant activity in the crude extract of *A. alternata* was conducted at various concentrations through the utilization of the DPPH (2,2-diphenyl-1-picrylhydrazyl) method [36–38] with minor adjustments. A range of concentrations for the crude extracts (20, 40, 60, 80, 100, 120 μg/mL) were employed to assess their capability in scavenging DPPH radicals. The monitoring of the DPPH reaction was carried out for a duration of 30 min at 517 nm, utilizing the Varioscan LUX instrument by Thermoscientific. The determination of the antioxidant activity, both in the standard and the extracts, was calculated in terms of DPPH scavenging activity (%).

$$DPPH\ Scavenging\ activity\ (\%) = \frac{(absorbance\ control - absorbance\ sample\ )}{(absorbance\ control)} x100\%$$

2.7. GC–MS analysis

Crude ethyl acetate and *n*-hexane extracts derived from *A. alternata* were introduced into the GC–MS instrument for the purpose of identifying metabolite compounds. The GC–MS analysis was conducted utilizing the Shimadzu GC-MS-QP 2010 Ultra Instrument and the NIST 17 version of the computer mass spectral library. The stationary phase employed was Rtx-5MS, a 30-m long column with a diameter of 0.25 mm, containing 5% diphenyl and 95% dimethyl polysiloxane. The carrier gas, ultrahigh purity helium, was maintained at a pressure of 40.7 kPa. The injection volume was set at 1 μL, with an injector temperature of 200 °C, ion source temperature of 200 °C, and interface temperature of 230 °C. The analysis was conducted in split mode. The temperature of the column was programmed to initiate at 50 °C and then incrementally raised to 100 °C at a rate of 7 °C per minute, where it was held for 3 min. Subsequently, the temperature was elevated to 200 °C at a rate of 10 °C per minute and maintained for 2 min. The final column temperature was set at 270 °C, with a rate of increase at 5 °C per minute. The ion source temperature and the interface temperature for the MS detector were both set at 230 °C and 250 °C, respectively. The MS detector employed both SCAN and SIM methods, with an injection volume of 1.0 mL. The spectra of the unidentified component were cross-referenced against the data available in the GC–MS library.

2.8. In silico study of A. alternata metabolites as an antioxidant

Docking studies using Autodock VINA software (https://vina.scripps.edu/). The SOD crystal structure with an obtained resolution of 2.30 Å from the Protein Data Bank (PDB: 1CB4). (https://www.rcsb.org/structure/1CB4). Ligand structures were extracted from the PubChem database in SDF format and converted to PDB format using Open Babel GUI 3.1.1. (https://openbabel.org/). Prepare the protein and ligand using Autodock Tools (ADT) before binding the ligand complex to the target. Targeted docking was initiated within docking sites or grid box with the dimensions of X: 40.91, Y:61.77, Z:42.02 Å, with a grid spacing of 0.375 Å, centered on X:16.17, Y:69.88, Z:15.33 Å [39]. The polar hydrogen atoms of the removed water molecules are added to the amino acid residues and all the protein atoms are assigned a gas-tiger charge. Proteins in PDBQT format were used as input to the docking program. Perform ligand docking simulations for each compound, and display docking results using PyMOL-2.5.5 (https://pymol.org/2/) and LigPlot (https://www.ebi.ac.uk/thornton-srv/software/LigPlus/) to obtain information about binding interactions and 3D-visualize the docking process.

2.8.1. In silico toxicology analysis

Predicting compound toxicity traditionally involves resource-intensive animal models. However, the adoption of computer-based toxicity assessments offers a viable alternative to costly and time-consuming methods, eliminating the need for animal testing. In this context, four compounds (PubChem ID 30541, 613266, 6781, 5282750) were assessed for their toxicity using ProTox-II (https://tox-new.charite.de/protox_II/index.php?site=compound_input), potentially expediting toxicity analysis while minimizing ethical and resource concerns associated with animal experimentation.

2.8.2. Druglikeness and pharmacokinetics of lead compounds

Pharmacokinetics analysis was conducted online through the Swiss ADME website (http://www.swissadme.ch/index.php) by inputting the SMILES code of the compounds obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/). The SMILES codes of the four compounds (PubChem ID 30541, 613266, 6781, 5282750) were inserted, and the "run" option was selected to obtain predictive data results. Pharmacokinetic property analysis based on ADME encompassed parameters such as GI absorption, BBB permeability, and Log Kp. Furthermore, druglikeness analysis based on ADME was conducted to ascertain Lipinski's rule criteria and bioavailability scores.

2.9. Statistical analysis

The antibacterial test data was statistically tested by two-way analysis of variance (ANOVA) with three replication, followed by Duncan's Multiple Range Test (DMRT). This statistical analysis was performed in Microsoft Excel 2021.

3. Results

3.1. Morphology of A. alternata on PDA plates

The fungus, *A. alternata* was successfully isolated from *D. piloselloides* plants (Fig. 1A). Aerial hyphae developed by the fungal strain *A. alternata* were observed on colonies displaying a greyish-white hue, which subsequently transitioned to an olive-green to black coloration when cultivated on PDA medium (refer to Fig. 1B). Within the PDA culture, the growth manifested in elongated chains of dark brown conidiophores, which gave rise to asexual spores referred to as conidiospores (conidia).

3.2. Identification of endophytic fungi based on ITS region

Identification of fungal isolates was carried out molecularly based on partial genetic analysis at the Internal Transcribed Spacer (ITS) ribosomal RNA of the fungi. The results showed that the endophytic fungi isolate from *D. piloselloides* had a very close resemblance to *A. alternata* strain KU20017.1 (Accession No. MT487794.1) using 18S rRNA gene sequence with 100% homology of the endophyte isolate.

3.3. Phylogenetic analysis based on ITS region sequences

The evolutionary relationships among taxa were inferred using the Neighbor-Joining method [40]. The optimal tree was made with the bootstrap test (1000 replicates), the percentage of replicate trees associated with taxa clustered together is shown below the branches [41]. The branch lengths were the same units as those of the evolutionary distances used to infer the phylogenetic tree. Kimura 2-parameter method was used to compute the evolutionary distances [42]. This analysis involved eleven strains from the NCBI

Fig. 1. (A) *Drymoglossum piloselloides* plant, (B) colony of *A. alternata* growing on PDA for 6 days at 28 °C.

database. Data Coverage less than 95% of the site was eliminated. For alignment gaps lower than 5%, missing data and ambiguous bases were allowed at any position (partial deletion option). The phylogenetic tree was conducted in MEGA version 11 [43].

3.4. A. alternata extract

The results of weighing the culture extract of the fungus *A. alternata* grown in 300 mL of PDB media were: 1.705 ± 0.123 g of ethyl acetate extract and 1.6025 ± 0.046 g of *n*-hexane extract.

3.5. Antibacterial activity of A. alternata extract

Antibacterial testing was carried out using the paper disc method. The test results for *A. alternata* extract are listed in Table 1. The ethyl acetate extract of *A. alternata* showed inhibitory activity against the four test bacteria, namely *E. coli*, *P. aeruginosa*, *B. subtilis*, and *S. aureus*, while the *n*-hexane extract of *A. alternata* showed zones of inhibition against the three test bacteria, namely *E. coli*, *P. aeruginosa*, and *S. aureus* and there was no inhibitory activity against *B. subtilis* bacteria. The antibacterial activity exhibit a significant mean value dependent on the tested bacterial species ($F(3, 1) = 147.6$, $P = 0.000$) and the type of extract ($F(3, 1) = 139.67$, $P = 0.000$) (Table 2). The results of the two-way ANOVA demonstrated that both factors significantly influenced the generated antibacterial values ($F(3, 1) = 148.18$, $P = 0.000$). These findings indicate that the activity spectrum of the extract is broad and possesses an optimal concentration dependent on the bacterial species under testing.

3.6. Antioxidant activity of A. alternata extract

Antioxidant activity test used compound 1,1-diphenyl-2-picrylhydrazyl (DPPH) reagent. Its activity was measured as the decrease in the absorption of the DPPH solution to the sample at the maximum observed wavelength (λ 515 nm) and was calculated as % inhibition. The correlation between the concentration of *A. alternata n*-hexane extract and the inhibition percentage of DPPH radical activity is shown in Fig. 4a and the ethyl acetate extract in Fig. 4b.

The value of the correlation coefficient indicated a linear correlation between the concentration of *A. alternata* extract and visible light absorption. The value of a good correlation coefficient was close to 1. In this experiment, the correlation coefficient value for the ethyl acetate extract was 0.9923, and for the *n*-hexane extract was 0.9917. It shows that the increase in extract concentration was directly proportional to the increase in visible light absorption. From the calculation results, the IC_{50} value of the ethyl acetate extract was 50.99 ppm, and the *n*-hexane extract IC_{50} value was 74.44 ppm.

3.7. Gas chromatography-mass spectrometry analysis

Gas chromatography has wide applications for the separation and analysis of mixtures of several components. This method proves effective for detecting compounds even when present in exceedingly low concentrations. Consequently, it becomes possible to identify secondary metabolites synthesized by microorganisms, and this identification is substantiated through the generation of chromatograms and corresponding mass spectra. The process of identifying individual peaks within the chromatogram involved comparing the mass spectrum (MS spectrum) of each peak against the Wiley database, thus facilitating the determination of the specific compound type [44]. Metabolite compounds in the culture extract of *A. alternata* fungi have been successfully detected by GC-MS analysis. The results of the GC-MS chromatogram of the *n*-hexane extract of *A. alternata* indicated that there were ten compounds contained, as shown in Fig. 5.

Several compounds that have a peak area greater than 0.8% are listed in Table 3. The results of the GC-MS chromatogram of the ethyl acetate extract of *A. alternata* showed that there were 30 compounds contained, as shown in Fig. 6. Several compounds with peak areas greater than 0.6% are listed in Table 4. Several compounds contained in the ethyl acetate extract and *n*-hexane extract from *A. alternata* are antioxidants.

Table 1
Average inhibition zone of *A. alternata* endophytic fungus extract.

Indicator bacteria	Average inhibition zone diameter (mm)	
	Ethyl acetate extract [(A)]	N-hexane extract [(B)]
Eschericia coli [(a)]	8.7833 ± 0.1607	8.8167 ± 0.2081
Pseudomonas aeruginosa [(a)]	9.0333 ± 0.1258	9.1000 ± 0.5000
Bacillus subtilis [(b)]	9.0833 ± 0.3329	0 ± 0
Staphylococcus aureus [(a)]	9.3333 ± 0.3055	9.4300 ± 1.0750

Data are means ± SD of three replicates.
The sample extract and the Indicator bacterial followed by the same letters are not significantly different.
(Sig. 0.05).

Table 2
Two-way of Anova with replication.

Source of Variation	SS	df	MS	F	P-value	**F crit. (α 0.05)**
Bacteria	93.84364583	3	31.28121528	147.64	0.000	3.238872
Extract solvent	29.59260417	1	29.59260417	139.6701	0.000	4.493998
Interaction	94.19114583	3	31.39704861	148.1867	0.000	3.238872
Error	3.39	16	0.211875			

>Contig-K10 (5'-3')
AACAAGGTCTCCGTAGGTGAACCTGCGGAGGGATCATTACACAAATATGAAGGCGGGCTGGAATCTCT
CGGGGTTACAGCCTTGCTGAATTATTCACCCTTGTCTTTTGCGTACTTCTTGTTTCCTTGGTGGGTTCGC
CCACCACTAGGACAAACATAAACCTTTTGTAATTGCAATCAGCGTCAGTAACAAATTAATAATTACAA
CTTTCAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAGTGTGA
ATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCAAAGGGCATG
CCTGTTCGAGCGTCATTTGTACCCTCAAGCTTTGCTTGGTGTTGGGCGTCTTGTCTCTAGCTTTGCTGGA
GACTCGCCTTAAAGTAATTGGCAGCCGGCCTACTGGTTTCGGAGCGCAGCACAAGTCGCACTCTCTAT
CAGCAAAGGTCTAGCATCCATTAAGCCTTTTTTCAACTTTGACCTCGGATCAGGTAGGGATACCCGCT
GAACTTAAGCATATC

Fig. 2. The DNA sequence at ITS region of the endophytic fungi isolated from the *Drymoglossum piloselloides* plant was alignment by the Bio-Edit program.

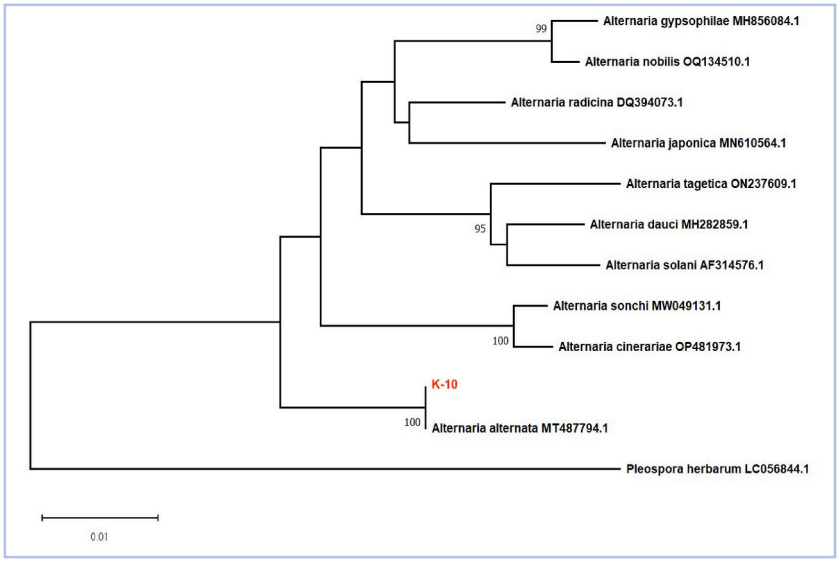


Fig. 3. Phylogenetic relationship of *A. alternata* strain with eleven closest strains, based on ITS-5 and ITS-4 DNA sequences.

3.8. In silico study of active compounds as antioxidants

In this study, 25 secondary metabolites detected by GC-MS analysis of *A. alternata* were computationally tested. Methylphenidate was used as a positive control to predict the efficacy of compounds as antioxidants [45]. The structure of the enzyme used to recognize the compound's potential as an antioxidant is that of superoxide dismutase (PDB:1CB4) (Fig. 7).

3.9. Computational toxicology analysis

Toxicology testing is imperative as it helps ascertain whether a molecule exhibits any adverse effects on humans, animals, or the environment prior to its use as a potential medicine or drug candidate. Hence, toxicity prediction marks the initial stage in selecting a molecule for medicinal purposes. Four compounds (2-Ethylhexyl 4-(dimethylamino) benzoate, Benzo-b-dihydropyran-6-hydroxy-4-4-5-7-8-pentamethyl, Diethyl Phthalate, Octadecenoic acid) were chosen through the widely used web-based servers, ProTox-II (Table 7). The outcomes indicate that all four compounds displayed 'no' results concerning hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, cytotoxicity, Aryl hydrocarbon Receptor (AhR), Androgen Receptor (AR), and Heat shock factor response

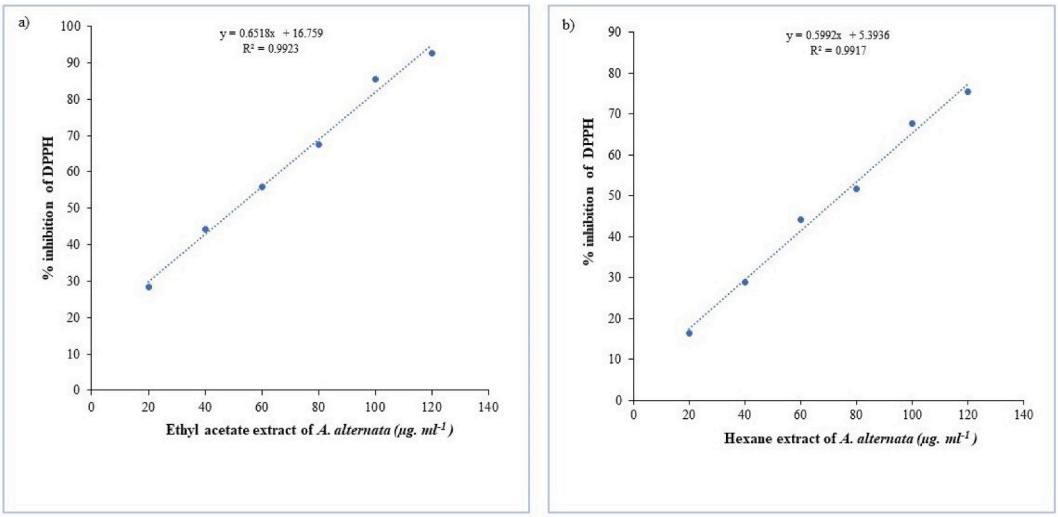


Fig. 4. The correlation between the concentrations of the ethyl acetate extract (A) and the hexane extract (B) of *A. alternata* to the % inhibition of DPPH radical.

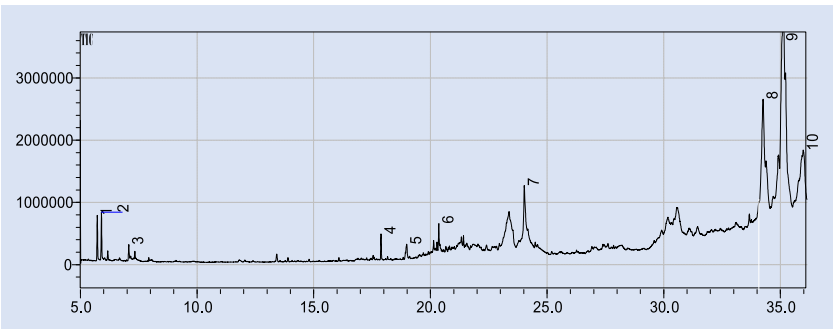


Fig. 5. GC-MS chromatogram of the *n*-hexane extract from *A. alternata*.

Table 3
Identified compounds from GC-MS chromatogram of the *n*-hexane extract of *A. alternata*.

Retention time	Area	Area (%)	Compound name
5.722	1286418	2.06	Silane, ethenyl trimethyl-
5.900	1340195	2.15	Pentanoic acid, 4-methyl-2-oxo-, methyl ester
7.073	515068	0.83	Benzene, 1,3-dichloro-
17.882	732502	1.17	Phenol, 2,4-bis(1,1-dimethyl ethyl)-
18.984	731998	1.17	Diethyl Phthalate
20.359	659885	1.06	Pentadecanal-
24.023	3233696	5.18	l-(+)-Ascorbic acid 2,6-dihexadecanoate
34.258	15337082	24.57	.gamma.-Sitosterol
35.104	30924713	49.53	9,19-Cyclolanost-23-ene-3,25-diol, (3.beta.,23E)-
35.984	7665325	12.28	17-(1,5-Dimethyl-3-phenylthiohex-4-enyl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclope

element.

3.10. Computational ADME properties and pharmacokinetics prediction studies

3.10.1. Pharmacokinetics

The pharmacokinetic properties of the designed compounds, as presented in Table 8, encompass gastro-intestinal absorption (GIA), blood-brain barrier (BBB) permeability, and log Kp. Among the four compounds (2-Ethylhexyl 4-(dimethylamino) benzoate, Benzo-b-

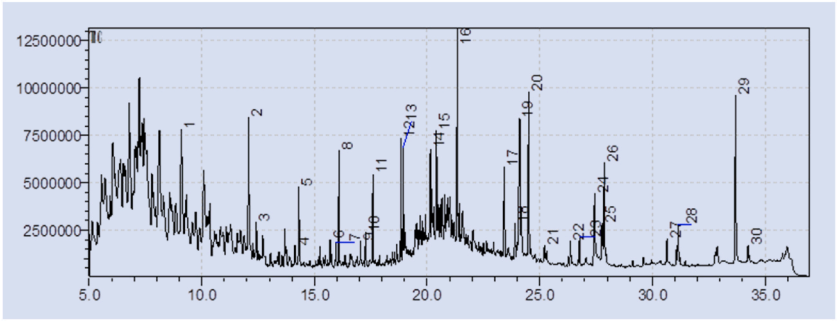


Fig. 6. GC-MS chromatogram of the ethyl acetate extract from *A. alternata*.

Table 4
Identified compounds from GC-MS chromatogram of the ethyl acetate extract of *A. alternata*.

Retention time	Area	Area (%)	Compound name
9.092	21260622	8.29	Undecane
12.076	16778134	6.54	Dodecane
12.413	4621163	1.80	Undecane, 2,6-dimethyl-
14.129	1829513	0.71	2-Pyrazoline, 3-ethyl-1-isopropyl-
14.302	7294425	2.84	Tridecane
15.691	2143025	0.84	Hexadecane, 2,6,10,14-tetramethyl-
15.951	2066453	0.81	1-Tetradecene
16.081	10364114	4.04	Tetradecane
17.038	2215972	0.86	Heptadecane, 2,6,10,14-tetramethyl-
17.269	2981206	1.16	Cyclodecane
17.590	7252606	2.83	Pentadecane
18.838	10126097	3.95	Cetene
18.927	7496187	2.92	Hexadecane
20.144	6162758	2.40	Heptadecane
20.412	10278484	4.01	Benzoic acid, 2-ethylhexyl ester
21.342	21266131	8.29	E−15-Heptadecenal
23.424	10319065	4.02	Hexadecanoic acid, methyl ester
23.902	4163634	1.62	Silane, diethylhexyloxy(3-methylbutoxy)-
24.113	19537633	7.62	l-(+)-Ascorbic acid 2,6-dihexadecanoate
24.510	21047048	8.20	Octadecyl trifluoroacetate
25.209	1606062	0.63	Benzo[*b*]dihydropyran, 6-hydroxy-4,4,5,7,8-pentamethyl-
26.361	3018999	1.18	Methyl *cis*-vaccenate
26.773	3106817	1.21	Methyl stearate
27.439	11946343	4.66	Octadecanoic acid
27.767	2684959	1.05	Hexadecanoic acid, butyl ester
27.862	11072976	4.32	Eicosyl trifluoroacetate
30.644	3106808	1.21	Oleic acid, butyl ester
31.134	3779810	1.47	Docosyl trifluoroacetate
33.687	25460995	9.91	Diisooctyl phthalate
34.242	1569711	0.61	1-Heptacosanol

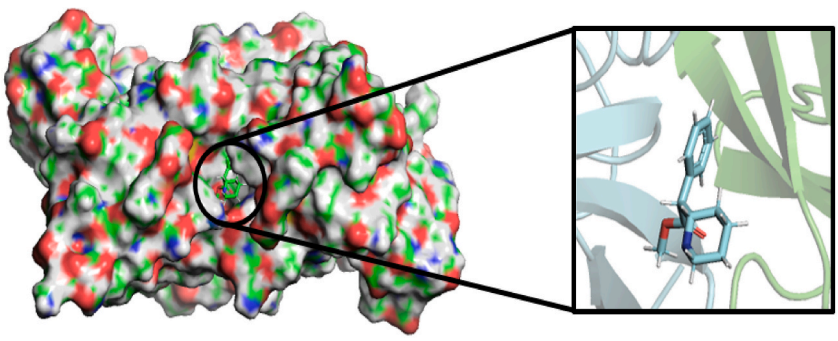


Fig. 7. Three-dimensional visualization of the interaction of methylphenidate with 1CB4 protein.

Table 5
Receptor-ligand binding energy value and interaction.

Compound name	*ΔG* (Kcal/mol)	Hydrogen bond (A)	Hydrophobic bond (A)
1-3-dichloro-benzene	−4.3	-	Asn51(A) Val146(A) Gly145(A) Val146(B)
2-4-bis(1-1-dimethyl ethyl)-phenol	−0.5	-	Cys6(A) Val7(A) Cys144(A) Gly49(A) Asn51(B) Cys6(B) Val7(B) Gly49(B) Gly145(B) Val146(A)
2-6-dimethyl-undecane	−4.7	-	Val7(A) Asn51(A) Val146(A) Val7(B) gly49(B) Asn51(B) Val146(B)
2-ethylhexyl-ester-Benzoic-acid	−5.9	Asn51(A)	Val7(A) Val146(A) Val7(B) Gly49(B) Asn51(B) Val146(B)
3-ethyl-1-isopropyl-2-Pyrazoline	−4.8	Val146(B)	Val7(A) Asn51(A) Gly145(A) Val146(A) Val7(B) Asn51(B)
9–19-Cyclolanost-23-ene-3-25-diol-(3-beta-23E)	10.5	Val7(A) Val146(B) Val7(B) Gly49(B)	Val5(A) Cys6(A) Gly49(A) Asn51(A) Gly145(A) Val146(A) Val5(B) Cys6(B) Lys9(B) Asn51(B) Thr52(B) Cys144(B) Gly145(B) Gly148(B)
Ascorbic-acid-2-6-dihexadecanoate	−4.3	Asn51(A) Val146(A) Val146(B)	Val5(A) Cys6(A) Val7(A) Lys9(A) Gly49(A) Vys144(A) Gly145(A) Cys6(B) Val7(B) Val5(B) Lys9(B) Gly49(B) Asp50(B) Asn51(B) Cys144(B) Gly145(B)
Benzo-b-dihydropyran-6-hydroxy-4-4-5-7-8-pentamethyl	−5.9	Val7(A) Cys144(A)	Val7(B) Leu8(A) Lys9(A) Asn51(A) Gly145(A) Lys9(B) Asn51(B) Cys144(B) Gly145(B) Val146(B)
Butyl-ester-oleic-acid	−5.3	Asn51(B) Val146(B)	Val7(A) Lys9(A) Asn51(A) Cys144(A) Gly145(A) Val146(A) Val7(B) Lys9(B) Gly145(B)
Cyclodecane	−5.2	-	Val7(A) Lys9(A) Asn51(A) Cys144(A) Gly145(A) Val146(A) Asn51(B) Val146 (B)
Diethyl-Phthalate	−6.2	Asn51(A) Val146(A) Val146(B)	Val7(A) Lys9(B) Gly49(A) Gly145(A) Val5(B) Cys6(B) Val7(B) Gly145(B)
Docosyl-trifluoroacetate	−5.6	Val7(B)	Val7(A) Lys9(A) Asn51(A) Cys144(A) Gly145(A) Val146(A) Lys9(B) Asn51(B) Val146(B)
Dodocane	−4.5	-	Val7(A) Lys9(A) Asn51(A) Cys144(A) Gly145(A) Val146(A) Val7(B) Asn51(B) Val146(B)
Eicosyl-trifluoroacetate	−5.4	Val146(B)	Val7(A) Lys9(A) Asn51(A) Cys144(A) Gly145(A) Val7(B) Lys9(B) Asn51(B) Val146(B)
Ethenyltrimethyl_Silane	−2.8	-	Asn51(A) Gly145(A) Val146(A) Val7(B) Val146(B)
Gamma-Sitosterol	3.3	Gly49(B)	Val5(A) Cys6(A) Val7(A) Asp50(A) Asn51(A) Gly145(A) Val146(A) Gy148(A) Val5(B) Cys6(B) Val7(B) Lys9(B) Asp50(B) Asn51(B) Gly145(B) Val146(B) Gly148(B)
Hexadecanoic-acid-butyl-ester	−5.4	Val146(A)	Val5(A) Cys6(A) Val7(A) Asn51(A) Gly145(A) Val146(A) Val7(B) Gly49(B) Asp50(B) Asn51(B) Val146(B)
Methyl-cis-vaccenate	−5.6	Val7(A) Asn51(B)	Val5(A) Cys6(A) Gly49(A) Asn51(A) Val146(A) Val5(B) Cys6(B) Val7(B) Gly49(B) Val146(B)
Methyl-stearate	−5.6	Val7(A)	Lys9(A) Asn51(A) Val146(A) Cys6(B) Val7(B) Gly49(B) Asn51(B) Val146(B)
Octadecanoic-acid	−5.9	Val5(A) Val146(A) Gly49(B)	Val7(A) Gly49(A) Asn51(A) Val5(B) Cys6(B) Val7(B) Asn51(B) Val146(B)
Octadecyl-trifluoroacetate	−5.5	-	Val5(A) Cys6(A) Val7(A) Lys9(A) Asn51(A) Cys144(A) Gly145(A) Val146(A) Val7(B) Lys9(B) Gly49(B) Asn51(B) Gly145(B) Val146(B)
Pentanoic-acid-4-methyl-2-oxo-methyl-ester	−4.5	Val146(A) Val146(B)	Val7(A) Asn51(A) Gly145(A) Val7(B) Asn51(B) Gly145(B)
Silane-diethylhexyloxy(3-methylbutoxy)	−4.5	-	Val7(A) Lys9(A) Asn51(A) Gly145(A) Val146(A) Val7(B) Gly49(B) Asn51(B) Gly145(B) Val146(B)
2-6-10–14-tetramethyl-Hexadecane	−4.8	-	Val7(A) Lys9(A) Gly49(A) Asn51(A) Cys144(A) Val146(A) Val5(B) Cys6(B) Val7(B) Asn51(B) Cys144(B) Gly145(B) Val146(B) Gly148(B)
Undecane	−4.3	-	Val7(A) Asn51(A) Val146(A) Val7(B) Asn51(B) Val146(B)

dihydropyran-6-hydroxy-4-4-5-7-8-pentamethyl, Diethyl Phthalate, Octadecenoic acid), it is evident from Table 8 that they exhibit high gastrointestinal absorption. Additionally, in terms of skin permeation, a more negative value of log Kp indicates a superior permeation ability (logKp < -2.5 cm/s). Considering the skin permeant ability results provided for these compounds in Tables 7 and it is inferred that these compounds can effectively penetrate the skin, as all of their permeant abilities are < −2.5 cm/s.

3.10.2. Druglikeness

As indicated by the results presented in Table 8, the physicochemical assessments of all the designed compounds adhere to Lipinski's rule of five [46], evident from their score value of 'YES,' and a bioavailability score of ≥0.55.

4. Discussion

This study focused on efforts to explore the benefits of *A. alternata,* which is a producer of various secondary metabolites that can be applied in various fields, including agriculture, medicine, and ecology. Some of the compounds it produces can be used in the production of pharmaceuticals, chemicals, and other biological materials. Ecologically, these fungi play a role in the cycle of nutrients in certain ecosystems. These fungi can also play a role in the decomposition of organic matter and interact with other organisms in the food chain. *A. alternata* belongs to the Ascomycota group of fungi and the ordo of Pleosporales. These fungi have unique morphological characteristics because it has fruiting bodies (conidia), which are generally cylindrical or flat in shape with tapered/pointed ends. These conidia usually have quite thick walls with varying colors, ranging from black and dark brown to green (Fig. 1b). The hyphae are

Table 6
Docking score of the four Ethyl Acetate Extract compounds from *A. alternata*.

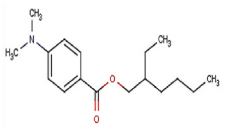
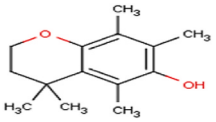
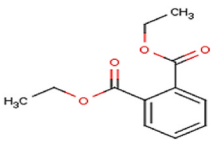
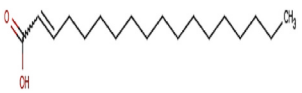
PubChem ID	Chemical name	Molecular formula	Chemical structure	MW	Binding affinity (Kcal/mol)
30541	2-Ethylhexyl 4-(dimethylamino) benzoate	$C_{17}H_{27}NO_2$		277.4 g/ mol	−5.8
613266	Benzo-b-dihydropyran-6-hydroxy-4-4-5-7-8-pentamethyl	$C_{14}H_{20}O_2$		220.31 g/ mol	−5.9
6781	Diethyl Phthalate	$C_6H_4(COOC_2H_5)$		222.24 g/ mol	−6.2
5282750	Octadecenoic acid	$C_{18}H_{34}O_2$		282.5 g/ mol	−5.9

Table 7
List of the selected compounds possessing toxicity features.

Endpoint	Target	PubChem ID 30541	PubChem ID 613266	PubChem ID 6781	PubChem ID 5282750
Tissue harmfulness	Hepatotoxicity	No	No	No	Yes
Toxicity endpoints	Carcinogenicity	No	No	No	No
	Immunotoxicity	No	No	No	No
	Mutagenicity	No	No	No	No
	Cytotoxicity	No	No	No	No
	Toxicity (class)	5	5	6	4
Tox21-Nuclear receptor signaling pathways	Aryl hydrocarbon Receptor (AhR)	No	No	No	No
	Androgen Receptor (AR)	No	No	No	No
Tox21-Stress response pathway	Heat shock factor response element	No	No	No	No

Table 8
List of the chosen compounds having pharmacokinetics and druglikeness features.

Endpoint	Target/Indicator	PubChem ID 30541	PubChem ID 613266	PubChem ID 6781	PubChem ID 5282750
Descriptor	Molecular Weight	277.408	220.31	222.24	282.468
Pharmacokinetics	GI absorption	High	High	High	High
	BBB permeant	Yes	Yes	Yes	No
	Log K_p (skin permeation: cm/s)	−4.42	−4.87	−5.94	−2.28
Druglikeness	Lipinski	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation	Yes; 0 violation
	Ghose	Yes	Yes	Yes	No; 1 violation: WLOGP>5.6
	Veber	Yes	Yes	Yes	No; 1 violation: Rotors>10
	Bioavailability score	0.55	0.55	0.55	0.85

branched and dark in color. One of the characteristics of these fungi can be seen in their ability to produce pigments and secondary metabolites. The pigment gives color to the colonies, whose role is to protect the skin from sunlight and play a role in competition with other microorganisms.

Molecular identification of endophytic fungi is important in understanding the diversity of microorganisms in plants and their potential to provide benefits related to human health. In this study, primers Forward ITS5 and Reverse ITS4 [47,48] were used to amplify ITS DNA fragments in endophytic fungi isolated from *D. piloselloides*. Based on the results of agarose electrophoresis analysis, it was found that there were DNA fragments that matched the size expected from the amplification of the ITS area (Fig. 2). DNA

sequences were analyzed and compared with a genetic database containing ITS sequences from various known fungi (Fig. 3). The results of DNA sequence analysis showed the suitability of the ITS sequence from *A. alternata* species. Further studies are needed to investigate the genetic diversity of *A. alternata* and to confirm the accuracy of the identification using other primer sets.

In 2011, WHO reported that as many as 55 million people died worldwide due to communicable diseases [49]. It was related to the difficulty of handling diseases caused by microbes so that the disease becomes resistant. The increase in the number of types of bacteria that were resistant to drugs and disinfectants resulted in an increase in morbidity and mortality [50]. As an effort to suppress the condition, experts were looking for natural metabolite compounds that are antimicrobial. This study used some indicator pathogenic bacteria *E. coli, P. aeruginosa, B. subtilis,* and *S. aureus* to test the antibacterial activity of the extract of the fungi *A. alternata*. The test results are listed in Table 1, which shows that the *n*-hexane and ethyl acetate extracts of *A. alternata* formed a zone of inhibition against the growth of the four tested bacteria in the range of 8783 ± 0.161 mm to 9430 ± 1075 mm. However, the *n*-hexane extract of *A. alternata* did not show any zone of inhibition against *B. subtilis*. The results indicated that the type of solvent used for the extraction of *A. alternata* metabolites affected the effectiveness of the extract as an antimicrobial. The formation of inhibition zones against pathogenic bacteria indicated that *A. alternata* extract had the potential as an antibacterial. Our results supports the previous reports, which used *A. alternata* extracts isolated from different host plants and had antibacterial activity [51–54].

The spectrum of activity of the ethyl acetate extract of *A. alternata* is broader in comparison to the *n*-hexane extract. This indicates that ethyl acetate extract possesses a higher concentration of bioactive compounds that play a direct or indirect role in influencing the inhibition zone. The bioactivity of secondary metabolites is linked to their capability to impact various bacterial processes, including cell wall synthesis, cell membrane depolarization, protein synthesis inhibition, nucleic acid synthesis inhibition, and disruption of metabolic pathways [55]. The results of this study demonstrate that the choice of solvent can influence the antimicrobial properties of the extract. Another study on extracts of *A. tenuissima*, a species closely related to *A. alternata*, showed various biological activities, including entomotoxic properties. Prior studies have also reported that the etil asetat extract from *A. alternata* is non-toxic and safe for use [56].

A. alternata was reported to have potential as a source of antioxidant compounds. The ethyl acetate extract of *A. alternata* had higher antioxidant activity than the *n*-hexane extract. Therefore, it can be concluded that the IC_{50} of the ethyl acetate extract was 50.99 μg/mL, while the IC_{50} of the *n*-hexane extract was 74.44 μg/mL with the inhibition percentage shown in Fig. 4. These results were in line with other studies, which proved that some metabolites produced by *A. alternata* isolated from *Coffea arabica* L. were found to have antioxidant properties with an IC_{50} value of 86.7 μg/mL when tested with DPPH free radicals [57].

The IC_{50} value represents the concentration at which the inhibition of DPPH free radical activity reaches 50%. A lower IC_{50} value indicates a higher level of antioxidant activity within the sample [58,59]. The results of these experiments indicated that the solvent used in the extraction could affect the antioxidant activity of a sample. In this case, the ethyl acetate extract of *A. alternata* had higher antioxidant activity than the *n*-hexane extract. This shows that antioxidant compounds dissolved in ethyl acetate were more effective in inhibiting DPPH free radical activity than compounds that dissolved in *n*-hexane. This experiment showed that both the ethyl acetate extract and the *n*-hexane extract of *A. alternata* were classified as having strong activity. Therefore, *A. alternata* metabolites have the potential for the development of antioxidant compounds. These results could be the basis for further research involving the characterization of the antioxidant compounds contained in the *n*-hexane and ethyl acetate extracts of *A. alternata*. Furthermore, experiments can be carried out to identify these compounds and understand their mechanism of action in inhibiting free radical activity.

The extraction process of *A. alternata* metabolites using *n*-hexane resulted from the extraction of most of the nonpolar substances (Fig. 5). Furthermore, the metabolites were extracted with a more polar compound, namely ethyl acetate (Fig. 6). Metabolite analysis using GC MS and data were aligned with the MS database. Antimicrobial and antioxidant activities of secondary metabolites have been observed using *n*-hexane extracts, as evident from the GC-MS analysis results (Table 3). The experiment reveals that compounds extracted from *A. alternata* using ethyl acetate are more diverse, comprising a total of 30 different compounds. Meanwhile, extraction using *n*-hexane yields only 10 compounds. These findings indicate that the active compounds in the endophytic fungus *A. alternata* are predominantly semi-polar rather than polar in nature.

Benzene, 1,3-dichloro-, hexadecanoic acid, pentadecanal-, l-(+)-ascorbic acid 2,6-dihexadecanoate, gamma-sitosterol, cyclolanost-23-ene-3,25-diol, (3.beta.,23E), undecane, undecane, 2,6-dimethyl-, hexadecane, 2,6,10,14-tetramethyl-, 1-tetradecene, tetradecane, cetene, heptadecane, methyl ester, methyl stearate, octadecanoic acid, diisooctyl phthalate, all of which have been reported to exert antioxidative activities [60–76]. Previously, E−15-heptadecenal, eicosyl trifluoroacetate, and 1-heptacosanol have been reported to exhibit solely antimicrobial activities [77–80]. Conversely, dodecane, tridecane, cyclodecane, pentadecane, octadecyl trifluoroacetate, and butyl ester have been reported to possess both antioxidant and antimicrobial activities [81–86].

The specific compounds obtained from the extract and their potential for application in the medical field required further analysis and identification. The presence of a higher number of compounds need not necessarily indicate greater medicinal potency. Molecular docking was a key tool that could provide insight into the identification of potential mechanisms of action exhibited by biologically active molecules *in vitro*. In this context, molecular docking was used to find as many protein targets as possible that could be related to (if possible, to interpret) the antioxidant activity observed *in vitro*. An *in silico*-based approach was used to investigate whether certain compounds could inhibit certain proteins involved in biological redox processes. The target protein is SOD (superoxide dismutase) [87]. SOD is an enzyme in which copper and zinc ions are bound together and mature through an intramolecular disulfide bond. SOD was evaluated under two different conditions depending on the presence of copper and zinc metals in the environment. The initial configuration was Cu_2Zn_2-SOD, comprising human SOD with copper and zinc ions, while an alternate variant, referred to as apo-SOD, lacking any metal ions. Cu and Zn ions in the form of Cu_2Zn_2-SOD played an important role in the catalytic activity of enzymes. Besides, zinc was able to retain a secondary structure despite the reduced disulfide bonds due to the fact that zinc tended to bind to SOD. On the other hand, the protein apo-SOD was structurally more stable compared to the Cu_2Zn_2-SOD structure. Furthermore, Cu_2Zn2-SOD

exhibited high thermal stability (~98 °C) due to intact disulfide bonds occurring within the SOD structure with Cu and Zn, whereas Apo-SOD exhibited low thermal stability with a melting point of 52 °C [88,89]. The Analysis showed that the binding energy (ΔG) of the methylphenidate compound was −5.8 kcal/mol forming a hydrogen-bonding interaction with residue Val146(B) with an interaction length of 3.25 Å, the interactions Val7(A), Lys9(A), Asn51(A), Val146(A), Val7(B), Lys9(B), Asn51(B), Gly145(B). A complete visualization of 2D interactions between ligands and proteins can be seen in Fig. 8(a–d).

Results of molecular docking simulations performed on 25 compounds (Table 5) showed that there were four active compounds that gave good results (Fig. 9(a–d)), specifically lower affinity ratios than methylphenidate (Fig. 7). The compounds were 2-ethylhexyl ester benzoic acid (ΔG: 5.9 kcal/mol), benzo-b-dihydropyran-6-hydroxy-4-4-5-7-8-pentamethyl (ΔG: 5.9 kcal/mol), diethyl phthalate (ΔG: 6.2 kcal/mol) and octadecanoic acid (ΔG: 5.9 kcal/mol) (Table 6). The results predicted that the four compounds have antioxidant potential. The results were consistent with laboratory *in vitro* studies, and got positive results on the DPPH analysis. *In silico* studies could evaluate the antioxidant activity of *A. alternata* metabolites [90]. It involves analyzing the ability of these compounds to scavenge free radicals and protect cells from oxidative damage. In our study, we screened four compounds for their human non-toxicity as potential drug candidates using the ProTox-II (tox-new.charite.de), aligning with similar findings reported by previous studies [91]. ProTox-II predicts the toxicities of small molecules, a crucial aspect in the drug development process. The results presented in Table 7 indicate that the four active compounds produced by *A. alternata* K-10 exhibit no significant toxicity. These findings align with previous research, which established the acute non-toxicity of Physcion to humans [92]. Subsequently, Swiss ADME was employed to evaluate the Pharmacokinetics and Druglikeness of these compounds (Table 8). The ADME properties of drugs play a crucial role in determining their fate within the body [93]. Drugs intended for oral administration should be effectively absorbed in the gastrointestinal tract to achieve optimal pharmacokinetics. All four active compounds derived from *A. alternata* K-10 met the criteria for gastrointestinal absorption and blood-brain barrier (BBB) permeability, suggesting their suitability for both oral and injectable drug formulations. The BBB is particularly important in limiting the penetration of drugs into the central nervous system (CNS) [94]. The

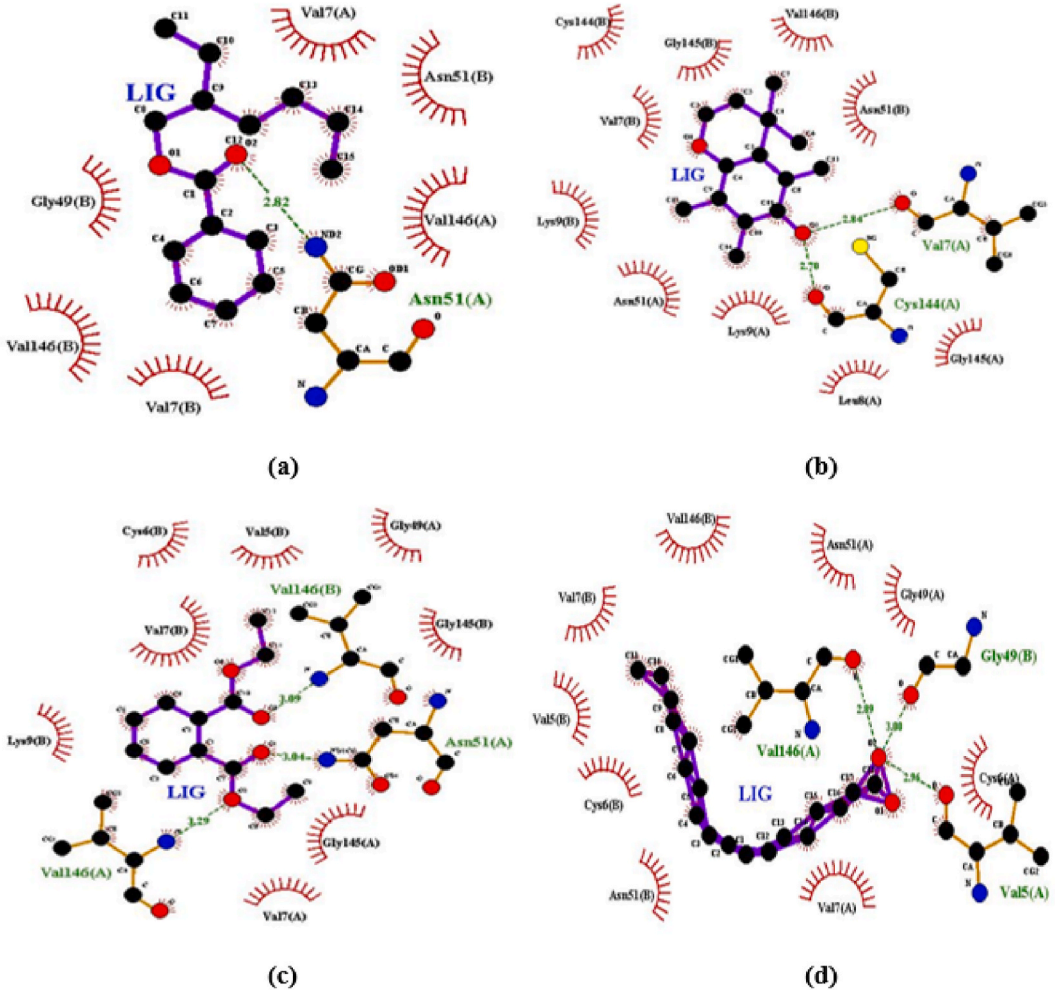


Fig. 8. Two-dimensional (2D) visualization of ligand interaction analysis (A) 2-ethylhexyl ester of benzoic acid (B), benzo-b-dihydropyran-6-hydroxy-4-4-5-7-8-pentamethyl (C), diethyl phthalate, and (D) octadecanoic acid with 1CB4.

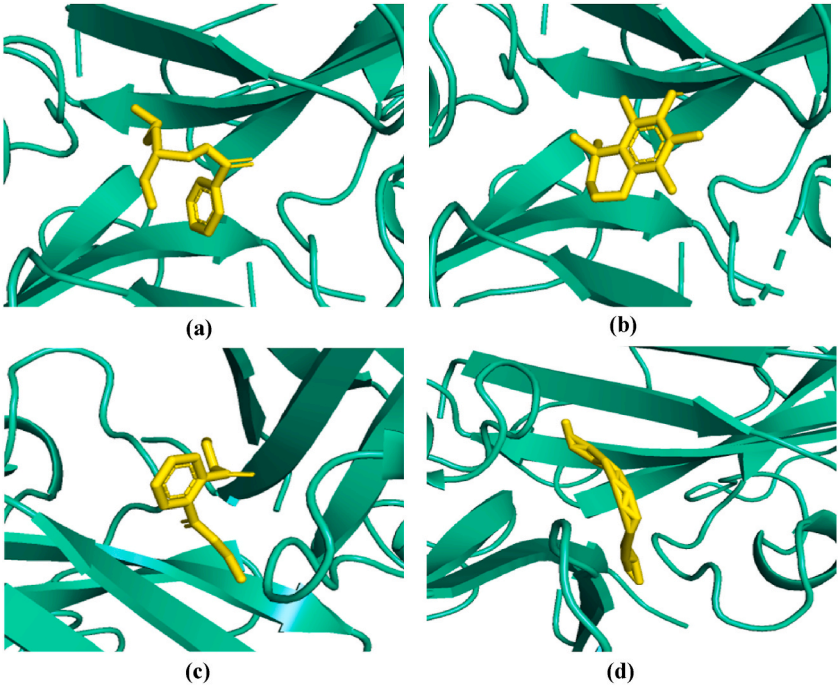


Fig. 9. Three-dimensional (3D) visualization of the ligand and 1CB4 receptor complex (A) 2-ethylhexyl benzoic acid ester (B) benzo-b-dihydropyran-6-hydroxy-4-4-5-7-8-pentamethyl (C) diethyl phthalate (D) interaction of octadecanoic acid with the 1CB4 receptor.

four compounds (2-ethylhexyl 4-(dimethylamino) benzoate, benzo-b-dihydropyran-6-hydroxy-4-4-5-7-8-pentamethyl, diethyl phthalate, and octadecenoic acid) exhibited log (Kp) values of (−4.42, −4.87, −5.94, and −2.28 cm/s). According to Daina and Zoete [95], who discussed the prediction of the skin permeability coefficient (Kp) of a compound, a higher negative log Kp value indicates lower skin permeability for the compound.

Furthermore, drug-likeness analysis, based on Lipinski's rule, Ghose, and Veber rules, was conducted and the results are presented in Table 8. All four active compounds met the requirements of Lipinski's rule of five, as well as the Ghose and Veber rules. These findings suggest that the tested compounds, namely 2-ethylhexyl 4-(dimethylamino) benzoate, benzo-b-dihydropyran-6-hydroxy-4-4-5-7-8-pentamethyl, diethyl phthalate, and octadecenoic acid, have the potential to be developed into drugs, specifically orally active drugs, with a bioavailability score of ≥0.55%.

According to established standards [96,97], a compound is considered to be well-absorbed in the body if its bioavailability value is ≥ 0.55%. Among the three active compounds, three exhibited 0.55%, and one active compound from *A. alternata* K-10 exhibited 0.85% oral bioavailability, suggesting its suitability for use as an oral medication. This implies that all four compounds are likely to be well-absorbed through the membranes into the systemic circulation, thereby achieving high bioavailability. Bioavailability's significance lies in its ability to reveal the time required for a drug to exert a therapeutic effect and the extent to which it can be absorbed by the body. The Swiss ADME property analysis unequivocally demonstrated that the four selected compounds possess attributes suitable for drug candidacy. These findings underscore the potential therapeutic applications of *A. alternata* metabolites with antioxidant properties.

5. Conclusions

A thorough investigation, both *in vitro* and *in silico*, was conducted to comprehensively explore the advantages and potential applications of secondary metabolite compounds derived from *A. alternata*. *In vitro* studies reveal Extracts from *A. alternata* exhibit significant antibacterial activity against pathogenic bacteria such as *E. coli, P. aeruginosa, B. subtilis*, and *S. aureus.* The antioxidant activity of the extract evaluated using the DPPH method showed promising results with a low IC_{50} value. *In silico* studies, including molecular docking, reveal that bioactive compounds from *A. alternata* have good molecular interactions as antioxidant compounds; in addition, based on computational toxicology and pharmacology evaluations, they show little side effects and also the suitability of the compounds to be used as drug formulations. The combination of experimental and computational approaches provides a strong understanding of the potential applications of *A. alternata* in medicine and other fields. Further development of the resulting compound can be achieved by combining it with other compounds or chemical auxiliaries to obtain better drug composition combinations in the future. Additionally, exploring the compatibility of *A. alternata* as an endophyte and testing other plant growth-promoting traits may reveal its broader contributions.

Data availability

Data will be made available on request.

CRediT authorship contribution statement

Kusmiati Kusmiati: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Asrul Fanani:** Writing – review & editing, Investigation, Data curation. **Arif Nurkanto:** Writing – review & editing, Resources, Data curation. **Ismu Purnaningsih:** Writing – review & editing, Validation, Data curation. **Jendri Mamangkey:** Writing – review & editing, Writing – original draft, Validation, Methodology, Data curation. **Indriati Ramadhani:** Writing – review & editing, Validation, Investigation. **Dian Alfian Nurcahyanto:** Writing – review & editing, Validation, Investigation. **Partomuan Simanjuntak:** Writing – review & editing, Validation, Methodology. **Fifi Afiati:** Writing – review & editing, Validation. **Herman Irawan:** Validation, Methodology, Investigation. **Ade Lia Puteri:** Writing – review & editing, Validation. **Muhammad Farrel Ewaldo:** Writing – review & editing, Methodology, Data curation. **Ario Betha Juanssilfero:** Writing – review & editing, Validation, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] C. Torres-León, F.R. Rebolledo Ramírez, J.A. Aguirre-Joya, et al., Medicinal plants used by rural communities in the arid zone of Viesca and Parras Coahuila in northeast Mexico, Saudi Pharmaceut. J. 31 (2023) 21–28, https://doi.org/10.1016/j.jsps.2022.11.003.
[2] M.N. Somchit, In vitro anti-fungal and anti-bacterial activity of *Drymoglossum piloselloides* L. Presl. against several fungi responsible for Athlete's foot and common pathogenic bacteria, Afr. J. Microbiol. Res. 5 (21) (2011), https://doi.org/10.5897/ajmr11.719, 2011.
[3] K. Jabeen, A. Javaid, Antifungal activity of *Syzygium cumini* against Ascochyta rabiei-the cause of chickpea blight, Nat. Prod. Res. 24 (12) (2010) 1158–1167, https://doi.org/10.1080/14786410902941154.
[4] M. Abid, S. Chohan, M.A. Mehmood, S. Naz, S.A. Hasan Naqvi, Antifungal potential of indigenous medicinal plants against Myrothecium leaf spot of bitter Gourd (*Momordica charantia* L.), Braz. Arch. Biol. Technol. (2017), https://doi.org/10.1590/1678-4324-2017160395.
[5] J.B.L. Tan, Y.Y. Lim, Critical analysis of current methods for assessing the in vitro antioxidant and antibacterial activity of plant extracts, Food Chem. 172 (2015) 814–822, https://doi.org/10.1016/j.foodchem.2014.09.141.
[6] S. Latif, G. Chiapusio, L.A. Weston, Allelopathy and the role of allelochemicals in plant defence, Adv. Bot. Res. (2017), https://doi.org/10.1016/bs.abr.2016.12.001.
[7] A. Zeid, Efficacy of leaf extracts of neem (*Azadirachta indica*) and chinaberry (*Melia azedrach*) against early blight and wilt diseases of tomato, Aust J Basic Appl Sci 2 (3) (2008) 763–772.
[8] Q. Kanwal, I. Hussain, H. Latif Siddiqui, A. Javaid, Antifungal activity of flavonoids isolated from mango (*Mangifera indica* L.) leaves, Nat. Prod. Res. 24 (20) (2010) 1907–1914, https://doi.org/10.1080/14786419.2010.488628.
[9] G. Strobel, B. Daisy, Bioprospecting for microbial endophytes and their natural products, Microbiol. Mol. Biol. Rev. 67 (4) (2003) 491–502, https://doi.org/10.1128/mmbr.67.4.491-502.2003.
[10] A. Nath, S.R. Joshi, Bioprospection of endophytic fungi associated with ethnoveterinary plants for novel metabolites, in: Fungi Bio-Prospects in Sustainable Agriculture, Environment and Nano-Technology, Academic Press, Massachusetts, United States, 2021, pp. 375–406.
[11] D. Dutta, K.C. Puzari, R. Gogoi, P. Dutta, Endophytes: exploitation as a tool in plant protection, Braz. Arch. Biol. Technol. 57 (2014) 621–629, https://doi.org/10.1590/s1516-8913201402043.
[12] F.Y. Lim, J.F. Sanchez, C.C. Wang, N.P. Keller, Toward awakening cryptic secondary metabolite gene clusters in filamentous fungi, Methods Enzymol. 517 (2012) 303–324, https://doi.org/10.1016/B978-0-12-404634-4.00015-2.
[13] F.M. Carroll, Sources, in: A Good and Wise Measure, University of Toronto Press, Toronto, Canada, 2017, pp. 411–430.
[14] K.R.V. Kurapati, V.S. Atluri, T. Samikkannu, G. Garcia, M.P. Nair, Natural products as anti-HIV agents and role in HIV-associated neurocognitive disorders (HAND): a brief overview, Front. Microbiol. 6 (2016) 1444, https://doi.org/10.3389/fmicb.2015.01444.
[15] J. Mamangkey, L.W. Mendes, M.T. Sibero, P.E. Nadapdap, R.V. Siregar, Bioprospecting for bacterial endophytes associated with zingiberaceae family rhizomes in sibolangit forest, North Sumatera, Int. J. Serv. Technol. Manag. 1 (1) (2020) 26–36, https://doi.org/10.46729/ijstm.v1i1.7.
[16] J. Mamangkey, A. Hartanto, C.G.P. Rumahorbo, Biodiversity and biopharmaceuticals of endophytic fungi associated with medicinal plants and therapeutic activity: case studies of Lamiaceae Crops, Acta Microbiol. Bulg. 39 (2) (2023) 140–146.
[17] J. Mamangkey, L. Pardosi, R.S. Wahyuningtyas, Aktivitas mikrobiologis endofit Dari ekstrak daun binahong (*Anredera cordifolia* (ten.) Steenis), J. Pro-Life 9 (2022) 376–386.
[18] J. Mamangkey, L.W. Mendes, A.Z. Mustopa, A. Hartanto, Endophytic aspergilli and Penicilli species from medicinal plants: a focus on antimicrobial and multi-drug resistant pathogens inhibitory activity, Biotechnologia 105 (1) (2024).
[19] R.N. Kharwar, V.C. Verma, G. Strobel, D. Ezra, The endophytic fungal complex of *Catharanthus roseus* (L.) G. Don, Curr. Sci. 95 (2) (2008) 228–233.
[20] S.K. Gond, V.C. Verma, A. Mishra, A. Kumar, R.N. Kharwar, Role of fungal endophytes in plant protection, in: Management of Fungal Plant Pathogens, 2010, https://doi.org/10.1079/9781845936037.0183.
[21] E. Decker, G. Beecher, J. Slavin, H.E. Miller, L.F. Marquart, Whole grains as a source of antioxidants, Cereal Foods World 47 (8) (2002) 370–373.

[22] M.J. Kassim, M.H. Hussin, A. Achmad, N.H. Dahon, T.K. Suan, H.S. Hamdan, Determination of total phenol, condensed tannin and flavonoid contents and antioxidant, Majalah Farmasi Indonesia 22 (1) (2011) 50–59.

[23] M.F. Matei, R. Jaiswal, N. Kuhnert, Investigating the chemical changes of chlorogenic acids during coffee brewing: conjugate addition of water to the olefinic moiety of chlorogenic acids and their quinides, J. Agric. Food Chem. 60 (49) (2012) 12105–12115, https://doi.org/10.1021/jf3028599.

[24] Y.Z. Zheng, G. Deng, D.F. Chen, Q. Liang, R. Guo, Z.M. Fu, Theoretical studies on the antioxidant activity of pinobanksin and its ester derivatives: effects of the chain length and solvent, Food Chem. 240 (2018) 323–329, https://doi.org/10.1016/j.foodchem.2017.07.133.

[25] M.M. Kabanda, S. Gbashi, N.E. Madala, Proportional coexistence of okanin chalcone glycoside and okanin flavanone glycoside in *Bidens pilosa* leaves and theoretical investigation on the antioxidant properties of their aglycones, Free Radic. Res. 55 (1) (2021) 53–70, https://doi.org/10.1080/10715762.2020.1859107.

[26] U.F. Umoh, P.S. Thomas, E.E. Essien, J.E. Okokon, M. De Leo, K.K. Ajibesin, G. Flamini, O.A. Eseyin, Isolation and characterization of bioactive xanthones from *Hippocratea africana* (Willd.)Loes.ex engl. (Celastraceae), J. Ethnopharmacol. 280 (2021) 114031, https://doi.org/10.1016/j.jep.2021.114031.

[27] O.A. Binutu, K.E. Adesogan, J.I. Okogun, Antibacterial and antifungal compounds from *Kigelia pinnata*, Planta Med. 62 (4) (1996) 352–353, https://doi.org/10.1055/s-2006-957900.

[28] I. Orhan, E. Küpeli, S. Terzioğlu, E. Yesilada, Bioassay-guided isolation of kaempferol-3-O-β-d-galactoside with anti-inflammatory and antinociceptive activity from the aerial part of *Calluna vulgaris* L, J. Ethnopharmacol. 114 (1) (2007) 32–37, https://doi.org/10.1016/j.jep.2007.06.017.

[29] S. Dalimartha, *Atlas tumbuhan obat Indonesia* (atlas of medicinal plants of Indonesia), in: PT Pustaka Pembangunans, Swadaya Nusantara, Jakarta, 2008, p. 89.

[30] D.J. Caruso, E.A. Palombo, S.E. Moulton, P.J. Duggan, B. Zaferanloo, Antibacterial and antibiofilm activity of endophytic *Alternaria sp.* isolated from *Eremophila longifolia*, Antibiotics 12 (2023) 1459, https://doi.org/10.3390/antibiotics12091459.

[31] O. Petrini, M. Dreyfuss, Endophytische Pilze in epiphytischen Araceae, Bromeliaceae und Orchidaceae, Sydowia 34 (1981) 135–145.

[32] A. Hiraishi, Y. Kamagata, K. Nakamura, Polymerase chain reaction amplification and restriction fragment length polymorphism analysis of 16S rRNA genes from methanogens, J. Ferment. Bioeng. 79 (6) (1995), https://doi.org/10.1016/0922-338X(95)94742-A.

[33] S.L. Gagana, B.E. Kumaraswamy, M.B. Shivanna, Diversity, anti-bacterial and antioxidant activities of the fungal endophytes asso-ciated with *Schleichera oleosa* (Lour.) Merr, South Afr. J. Bot. 134 (2020) 369–381, https://doi.org/10.1016/j.sajb.2020.06.012.

[34] S. Vasanthakumar, G. Dineshkumar, K. Jayaseelan, Phytochemical screening, GC-MS analysis and antibacterial evaluation of ethanolic leaves extract of *Avicennia marina*, J. Drug Deliv. Therapeut. 9 (4-A) (2019) 145–150, https://doi.org/10.22270/jddt.v9i4-a.3431.

[35] Wojdyło, A., Oszmiański, J CR. Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chem.. 105(3): 940-949. doi:10.1016/j.foodchem.2007.04.038.

[36] S. Dudonné, X. Vitrac, P. Coutière, M. Woillez, Mérillon, R. Czemerys, Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays, J. Agric. Food Chem. 57 (5) (2009) 1768–1774, https://doi.org/10.1021/jf803011r.

[37] D. Huang, B. Ou, R.L. Prior, The chemistry behind antioxidant capacity assays, J. Agric. Food Chem. 53 (6) (2005) 1841–1856, https://doi.org/10.1021/jf030723c. PMID: 15769103.

[38] W. Brand-Williams, M.E. Cuvelier, C. Berset, Use of a free radical method to evaluate antioxidant activity, Food Sci Technol − LEB. 28 (1995) 25–30, https://doi.org/10.1016/S0023-6438(95)80008-5.

[39] B. Hatai, S.K. Banerjee, Molecular docking interaction between superoxide dismutase (receptor) and phytochemicals (ligand) from *Heliotropium indicum* linn for detection of potential phytoconstituents: new drug design for releasing oxidative stress condition/inflammation of osteoar, J. Pharmacogn. Phytochem. 8 (2) (2019) 1700–1706.

[40] N. Saitou, M. Nei, The neighbor-joining method: a new method for reconstructing phylogenetic trees, Mol. Biol. Evol. 4 (1987) 406–425.

[41] J. Felsenstein, Confidence limits on phylogenies, Evolution 39 (1985) 783–791.

[42] M. Kimura, A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences, J. Mol. Evol. 16 (1980) 111–120.

[43] K. Tamura, G. Stecher, S. Kumar, MEGA11: molecular evolutionary genetics analysis version 11, Mol. Biol. Evol. 38 (7) (2021) 3022–3027, https://doi.org/10.1093/molbev/msab120.

[44] A.F. Al-Rubaye, I.H. Hameed, M.J. Kadhim, A review: uses of gas chromatography-mass spectrometry (GC-MS) technique for analysis of bioactive natural compounds of some plants, Int J Toxicol Pharmacol Res 9 (1) (2017) 81–85.

[45] Gomes, M. Karin, G. Cecília, Inácio, S. Samira, Valvassori, Z. Gislaine, Réus, Carina R. Boeck, Felipe Dal-Pizzol and JQ. Superoxide production after acute and chronic treatment with methylphenidate in young and adult rats, Neurosci. Lett. 465 (1) (2009) 95–98.

[46] C.A. Lipinski, Rule of five in 2015 and beyond: target and ligand structural limitations, ligand chemistry structure and drug discovery project decisions, Adv. Drug Deliv. Rev. 101 (2016) 34–41, https://doi.org/10.1016/j.addr.2016.04.029.

[47] A. Mohammadi, S. Bahramikia, Molecular identification and genetic variation of *Alternaria* species isolated from tomatoes using ITS1 sequencing and inter simple sequence repeat methods, Curr Med Mycol 5 (2) (2019) 1–8, https://doi.org/10.18502/cmm.5.2.1154.

[48] M. Kordalewska, A. Brillowska-Dąbrowska, T. Jagielski, B. Dworecka-Kaszak, PCR and real-time PCR assays to detect fungi of *Alternaria alternata* species, Acta Biochim. Pol. 62 (4) (2015) 707–712, https://doi.org/10.18388/abp.2015_1112.

[49] R.Y.A. Elghaffar, B.H. Amin, A.H. Hashem, A.E. Sehim, Promising endophytic *Alternaria alternata* from leaves of *Ziziphus spina-christi*: phytochemical analyses, antimicrobial and antioxidant activities, Appl. Biochem. Biotechnol. 194 (9) (2022) 3984–4001, https://doi.org/10.1007/s12010-022-03959-9.

[50] H. Miladi, T. Zmantar, Y. Chaabouni, K. Fedhila, A. Bakhrouf, K. Mandouani, K. Chaieb, Antibacterial and efflux pump inhibitors of thymol and carvacrol against food-borne pathogens, Microb. Pathog. 99 (2016) 95–100, https://doi.org/10.1016/j.micpath.2016.08.008.

[51] Nabavi, S.M., Marchese, A., Izadi, M., Curti, V., Daglia, M., Ns. Plants belonging to the genus Thymus as antibacterial agents: from farm to pharmacy. Food Chem.. 173:339-347. doi: 10.1016/j.foodchem.2014.10.042.

[52] S.K. Nagar, S.C. Meena, A. Chattopadhyay, R.K. Meena, Antimicrobial action of hexane, methanolic and ethyl acetate extract of *Cinnamomum tamala* Linn. against some plant pathogenic fungi, Green Farming 4 (1) (2013) 87–91.

[53] P. Privatus, J.D. Maethya, F.N. Mpenda, Antimicrobial activity of crude extract of endophytic fungi isolated from leaves of *Kigelia africana* and *Phyllanthus niruri* collected from Dar es Salaam Tanzania, J Biosci Biotechnol 11 (1) (2022) 47–55.

[54] Q. Liu, X. Meng, Y. Li, C.N. Zhao, G.Y. Tang, H.B. Li, Antibacterial and antifungal activities of spices, Int. J. Mol. Sci. 18 (6) (2017) 1283, https://doi.org/10.3390/ijms18061283.

[55] W.C. Reygaert, An overview of the antimicrobial resistance mechanisms of bacteria, AIMS Microbiol 4 (3) (2018) 482–501, https://doi.org/10.3934/microbiol.2018.3.482.

[56] D. Salimova, A. Dalinova, V. Dubovik, I. Senderskiy, E. Stepanycheva, O. Tomilova, Q. Hu, A. Berestetskiy, Entomotoxic activity of the extracts from the fungus, *Alternaria tenuissima* and its major metabolite, tenuazonic acid, J Fungi (Basel). 7 (9) (2021) 774, https://doi.org/10.3390/jof7090774.

[57] S. Chatterjee, R. Ghosh, N.C. Mandal, Production of bioactive compounds with bactericidal and antioxidant potential by endophytic fungus *Alternaria alternata* AE1 isolated from Azadirachta indica A, Juss. PLoS One 14 (4) (2019) e0214744, https://doi.org/10.1371/journal.pone.0214744.

[58] S. Zhao, J. Li, J. Liu, S. Xiao, S. Yang, J. Mei, M. Ren, S. Wu, H. Zhang, X. Yang, Secondary metabolites of *Alternaria*: a comprehensive review of chemical diversity and pharmacological properties, Front. Microbiol. 13 (2022) 1085666, https://doi.org/10.3389/fmicb.2022.1085666.

[59] R. Song, J. Wang, L. Sun, Y. Zhang, Z. Ren, B. Zhao, H. Lu, The study of metabolites from fermentation culture of *Alternaria oxytropis*, BMC Microbiol. 19 (1) (2019) 35, https://doi.org/10.1186/s12866-019-1408-8.

[60] R. Batubara, B. Wirjosentono, A.H. Siregar, U. Harahap, Tamrin, Chemical compounds and antioxidant potential in hot water extract of cultivated agarwood (*Aquilaria malaccensis*) lamk leaves, IOP Conf. Ser. Earth Environ. Sci. 830 (2021) 012070, https://doi.org/10.1088/1755-1315/830/1/012070.

[61] G. Rajeswari, M. Murugan, V.R. Mohan, J. Pharmaceut. Biomed. Sci. 29 (2013) 818–824.

[62] Y. Gao, H. Rao, L.J. Mao, Q.L. Ma, Chemical composition, antioxidant, antibacterial and cytotoxic activities of essential oil of *Leontopodium leontopodioides* (Willd.) Beauverd, Nat. Prod. Res. 33 (4) (2019) 612–615, https://doi.org/10.1080/14786419.2017.1402310.
[63] Z. Zhou, J. Tang, X. Song, Chemical composition of the essential oil of *Ligularia hodgsonii* and free radical scavenging activity of the oil and crude extracts, Nat. Prod. Commun. 9 (10) (2014) 1511–1514. PMID: 25522549.
[64] R. Gomathi, N. Anusuya, S.A. Manian, Dietary antioxidant supplementation of Jamaican cherries (*Muntingia calabura* L.) attenuates inflammatory related disorders, Food Sci. Biotechnol. 22 (2013) 787–794, https://doi.org/10.1007/s10068-013-0146-1.
[65] J.P. Mehta, P.H. Parmar, S.H. Vadia, M.K. Patel, C.B. Tripathi, In-vitro antioxidant and in-vivo anti-inflammatory activities of aerial parts of Cassia species, Arab, J. Chem. 10 (2) (2017) 1654–1662, https://doi.org/10.1016/j.arabjc.2013.06.010.
[66] N.A.R. Tahir, H.A. Azeez, K.A. Muhammad, S.A. Faqe, D.A. Omer, Exploring of bioactive compounds in essential oil acquired from the stem and root derivatives of *Hypericum triquetrifolium* callus cultures, Nat. Prod. Res. 33 (10) (2019) 1504–1508, https://doi.org/10.1080/14786419.2017.1419228.
[67] S.S. El-Hawary, F.M. Hammouda, W.A. Tawfik, H.A. Kassem, K.A. Abdelshafeek, S.S. El-Shamy, Investigation of some chemical constituents, cytotoxicity and antioxidant activities of *Beta vulgaris* var. *altissima* cultivated in Egypt, Rasayan J Chem 10 (4) (2017) 1391–1401, https://doi.org/10.7324/rjc.2017.1041936.
[68] M.I. Tleubayeva, R.M. Abdullabekova, U.M. Datkhayev, M.Y. Ishmuratova, M.B. Alimzhanova, K.K. Kozhanova, A.M. Seitaliyeva, K.S. Zhakipbekov, Z. B. Iskakova, E.A. Serikbayeva, E.V. Flisyuk, Investigation of CO2 extract of *Portulaca oleracea* for antioxidant activity from raw material cultivated in Kazakhstan, Int J Biomater 22 (2022) 6478977, https://doi.org/10.1155/2022/6478977.
[69] V. Gautam, S.K. Kohli, S. Arora, R. Bhardwaj, M. Kazi, A. Ahmad, M. Raish, M.A. Ganaie, P. Ahmad, Antioxidant and antimutagenic activities of different fractions from the leaves of *Rhododendron arboreum* Sm. And their GC-MS profiling, Molecules 23 (9) (2018) 2239, https://doi.org/10.3390/molecules23092239.
[70] T.S. Rangnathrao, P. Shanmugasundaram, Antioxidant and Hepatoprotective activity of *Ehretia laevis* Roxb against paracetamol induced acute Hepatotoxicityin wistar rats, Res. J. Pharm. Technol. 12 (12) (2019) 6143–6148, https://doi.org/10.5958/0974-360X.2019.01067.9.
[71] S. Tonisi, K. Okaiyeto, H. Hoppe, L.V. Mabinya, U.U. Nwodo, A.I. Okoh, Chemical constituents, antioxidant and cytotoxicity properties of *Leonotis leonurus* used in the folklore management of neurological disorders in the Eastern Cape, South Africa, 3 Biotech 10 (3) (2020) 141, https://doi.org/10.1007/s13205-020-2126-5.
[72] D. Sivaraj, R.J. Butcher, J.P. Jasinski, S. Sathyanarayanan, R. Ponnusamy, S.P. Sasidharan, K. Muniyandi, P. Thangaraj, K. Arunachalam, Antioxidant and antivenom potential of an essential oil, 4-(2-Oxo-propyl)-cyclopentane-1,3-dione, and allantoin derived from the polyherbal combination of *Aristolochia indica* L. And *Piper nigrum* L, Evid Based Complement Alternat Med 2022 (2022) 4797884, https://doi.org/10.1155/2022/4797884.
[73] K. Mazumder, A. Nabila, A. Aktar, A. Farahnaky, Bioactive variability and in vitro and in vivo antioxidant activity of unprocessed and processed flour of nine cultivars of Australian lupin species: a comprehensive substantiation, Antioxidants 9 (4) (2020) 282, https://doi.org/10.3390/antiox9040282.
[74] L.C.D. Santos, L.S. Azevedo, E.P. de Siqueira, A.H.F. Castro, L.A.R. dos Santos Lima, Chemical characterization, antioxidant activity, and cytotoxicity of fatty acids methyl esters from *Handroanthus impetiginosus* (Mart. ex DC.) Mattos (Bignoniaceae) seeds, Nat. Prod. Res. (2023), https://doi.org/10.1080/14786419.2023.2179624.
[75] Z. Wang, C.L. Liang, G.M. Li, C.Y. Yu, M. Yin, Stearic acid protects primary cultured cortical neurons against oxidative stress, Acta Pharmacol. Sin. 28 (3) (2007) 315–326, https://doi.org/10.1111/j.1745-7254.2007.00512.x.
[76] L.S. Ngidi, C.I. Nxumalo, J.S. Shandu, T.S. Maliehe, K. Rene, Antioxidant, anti-quorum sensing and cytotoxic properties of the endophytic *Pseudomonas aeruginosa* CP043328.1 's extract, Phcog. J. 13 (2) (2021) 332–340.
[77] N.A. Supardy, D. Ibrahim, S.F. Sulaiman, N.A. Zakaria, Inhibition of *Klebsiella pneumoniae* ATCC 13883 cells by hexane extract of *Halimeda discoidea* (Decaisne) and the identification of its potential bioactive compounds, J. Microbiol. Biotechnol. 22 (2012) 872–881, https://doi.org/10.4014/jmb.1111.11053.
[78] S.K. Niazi, D.S. Basavarajappa, S.H. Kumaraswamy, A. Bepari, H. Hiremath, S.K. Nagaraja, M. Rudrappa, A. Hugar, M.A.W. Cordero, S. Nayaka, GC-MS based characterization, antibacterial, antifungal and anti-oncogenic activity of ethyl acetate extract of *Aspergillus niger* strain AK-6 isolated from rhizospheric soil, Curr. Issues Mol. Biol. 45 (5) (2023) 3733–3756, https://doi.org/10.3390/cimb45050241.
[79] B. Chakraborty, R.S. Kumar, A.I. Almansour, P. Gunasekaran, S. Nayaka, Bioprospection and secondary metabolites profiling of marine *Streptomyces levis* strain KS46, Saudi J. Biol. Sci. 29 (2) (2022) 667–679, https://doi.org/10.1016/j.sjbs.2021.11.055.
[80] E. Sánchez-Hernández, L. Buzón-Durán, B. Lorenzo-Vidal, J. Martín-Gil, P. Martín-Ramos, Physicochemical characterization and antimicrobial activity against *Erwinia amylovora, Erwinia vitivora*, and *Diplodia seriata* of a light purple *Hibiscus syriacus*, L. Cultivar. Plants (Basel). 10 (9) (2021) 1876, https://doi.org/10.3390/plants10091876.
[81] J. Wang, H. Liu, H. Gao, J. Zhao, L. Zhou, J. Han, Z. Yu, F. Yang, Antimicrobial and antioxidant activities of the flower essential oil of *Halimodendron halodendron*, Nat. Prod. Commun. 6 (11) (2011) 1749–1753.
[82] R.L. Shrestha, N. Timilsina, Antioxidant and antimicrobial activity and GC-MS analysis of extract of *Rumex nepalensis* Spreng, J. Pharm. Innov. 6 (9) (2017) 155–158.
[83] A.I. Foudah, M.H. Alqarni, A. Alam, M. Ayman Salkini, E.O. Ibnouf Ahmed, H.S. Yusufoglu, Evaluation of the composition and in vitro antimicrobial, antioxidant, and anti-inflammatory activities of Cilantro (*Coriandrum sativum* L. leaves) cultivated in Saudi Arabia (Al-Kharj), Saudi J. Biol. Sci. 28 (6) (2021) 3461–3468, https://doi.org/10.1016/j.sjbs.2021.03.011.
[84] I. Ashraf, M. Zubair, K. Rizwan, N. Rasool, M. Jamil, S.A. Khan, R.B. Tareen, V.U. Ahmad, A. Mahmood, M. Riaz, M. Zia-Ul-Haq, H.Z.E. Jaafar, Chemical composition, antioxidant and antimicrobial potential of essential oils from different parts of *Daphne mucronata* Royle, Chem. Cent. J. 12 (1) (2018) 135, https://doi.org/10.1186/s13065-018-0495-1.
[85] T. Rhetso, R. Shubharani, M.S. Roopa, V. Sivaram, Chemical constituents, antioxidant, and antimicrobial activity of *Allium chinense* G. Don, Futur J Pharm Sci 6 (2020) 102, https://doi.org/10.1186/s43094-020-00100-7.
[86] B. Zieniuk, C.J. Ononamadu, K. Jasińska, K. Wierzchowska, A. Fabiszewska, Lipase-catalyzed synthesis, antioxidant activity, antimicrobial properties and molecular docking studies of butyl dihydrocaffeate, Molecules 27 (15) (2022) 5024, https://doi.org/10.3390/molecules27155024.
[87] H. Kiziltas, A.C. Goren, S.H. Alwasel, İ. Gulcin, Sahlep (*Dactylorhiza osmanica*): phytochemical analyses by LC-HRMS, molecular docking, antioxidant activity, and enzyme inhibition profiles, Molecules 27 (20) (2022) 6907, https://doi.org/10.3390/molecules27206907.
[88] F. Gür, B. Gür, B. Erkayman, Z. Halıcı, A. Karakoç, Investigation of serum and brain superoxide dismutase levels depending on atomoxetine used in attention-deficit/hyperactivity disorder treatment: a combination of in vivo and molecular docking studies, Bioorg. Chem. 105 (2020) 104435, https://doi.org/10.1016/j.bioorg.2020.104435.
[89] Y. Furukawa, I. Anzai, S. Akiyama, M. Imai, F.J. Cruz, T. Saio, K. Nagasawa, T. Nomura, K. Ishimori, Conformational disorder of the most immature Cu, Zn-superoxide dismutase leading to amyotrophic lateral sclerosis, J. Biol. Chem. 291 (8) (2016) 4144–4155, https://doi.org/10.1074/jbc.M115.683763.
[90] H.T. Khazaal, M.T. Khazaal, A.S. Abdel-Razek, A.A. Hamed, H.Y. Ebrahim, R.R. Ibrahim, M. Bishr, Y.E. Mansour, R.A. El Dib, H.S.M. Soliman, Antimicrobial, antiproliferative activities and molecular docking of metabolites from *Alternaria alternata*, Amb. Express 13 (1) (2023) 68, https://doi.org/10.1186/s13568-023-01568-1.
[91] P. Banerjee, A.O. Eckert, A.K. Schrey, R. Preissner, ProTox-II: a webserver for the prediction of toxicity of chemicals, Nucleic Acids Res. 46 (2018) W257, https://doi.org/10.1093/NAR/GKY318.
[92] R.N. Roy, S. Laskar, S.K. Sen, Dibutyl phthalate, the bioactive compound produced by *Streptomyces albidoflavus* 321.2, Microbiol. Res. 161 (2) (2006) 121–126, https://doi.org/10.1016/j.micres.2005.06.007.
[93] H.K. Shin, Y.M. Kang, K.T. No, Predicting ADME properties of chemicals, in: J. Leszczynski (Ed.), Handbook of Computational Chemistry, Springer, Dordrecht, 2016, https://doi.org/10.1007/978-94-007-6169-8_59-1.
[94] N.J. Abbott, Prediction of blood-brain barrier permeation in drug discovery from in vivo, in vitro and in silico models, Drug Discov. Today Technol. 1 (4) (2004) 407–416, https://doi.org/10.1016/j.ddtec.2004.11.014.

[95] A. Daina, V. Zoete, A BOILED-egg to predict gastrointestinal absorption and brain penetration of small molecules, ChemMedChem 11 (11) (2016) 1117–1121, https://doi.org/10.1002/cmdc.201600182.
[96] T. Narko, B. Permana, R. Prasetiawati, D. Soni, F. dan Khairiyah, Molecular docking study of bulb of bawang dayak (*Eleutherine palmifolia* (L) Merr) compound as anti servical cancer, J. Ilm. Farm. Bahari 8 (2) (2017) 1–14.
[97] A. Daina, O. Michielin, V. Zoete, SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules, Sci. Rep. 7 (42717) (2017) 1–13, https://doi.org/10.1038/srep42717.