TROPICAL LIFE SCIENCES RESEARCH

Comparison of Chemical Components and Biological Properties in Internal Tissue's *Dictyophora indusiata* Extracts: Effects of Temperature and Solvent Systems

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Highlights

- Optimised ultrasonication conditions significantly enhanced bioactive compound extraction from *D. indusiata*.
- Extracts demonstrated potent antioxidant and tyrosinase inhibitory activities with minimal cytotoxic effects on normal cell lines.
- Chemical profiling revealed multiple shared bioactive compounds, highlighting their potential for future in vivo studies and functional food applications.

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EARLY VIEW

Comparison of Chemical Components and Biological Properties in Internal Tissue's *Dictyophora indusiata* Extracts: Effects of Temperature and Solvent Systems

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Running head: Chemical and Biological Properties of Dictyophora

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Abstract: Noncommunicable diseases (NCDs) pose significant public health challenges and have substantial economic impacts. Bioactive compounds represent promising targets for reducing the risk of NCD progression. This study aimed to extract chemical constituents from the internal tissue of *D. indusiata* mushrooms using 35 kHz ultrasonication at varying temperatures and with different solvents, and to compare extract yield, antioxidant activity, tyrosinase inhibitory activity, and cytotoxicity in normal cell lines, including Vero cells and hepatocyte growth factor (HGF) cells. In addition, total phenolic, flavonoid, and terpenoid contents were quantified, and compound profiles were characterised using thin-layer chromatography (TLC) and gas chromatography–mass spectrometry (GC–MS). The findings indicate that deionised water at 80 °C was the most effective extraction solvent. The extracts designated L-A3-02-80W and O-A3-01-80W yielded the highest levels of phenolics, flavonoids, and terpenoids, and also exhibited superior antioxidant and antityrosinase activities compared with other extraction conditions. Moreover, both extracts demonstrated low cytotoxicity towards Vero and HGF cells. Polyphenolic and flavonoid constituents displayed strong antioxidant signals on the TLC plates. Eight identical compounds were

identified in both extracts, including tetradecanoic acid, decanoic acid silver(1+) salt, 2-azido-2,4,4,6,6-pentamethylheptane, trans-2,4-dimethylthiane, S,S-dioxide, trans-2-methyl-4-n-pentylthiane, S,S-dioxide, methyl 12,13-tetradecadienoate, 2-azido-2,4,4,6,6,8,8-heptamethylnonane, and 9-borabicyclo[3.3.1]nonane 9-(3-methoxycyclohexyl)oxy-. These findings provide preliminary evidence that extracts from the internal tissue of *D. indusiata* may represent promising candidates for further in vivo validation and formulation studies aimed at developing potential functional food applications.

Keywords: Antioxidant, Oxidative Damage, Bioactive Compound, Noncommunicable Diseases, *Dictyophora indusiate*

INTRODUCTION

Currently, approximately 41 million people die from Noncommunicable diseases (NCDs) each year, accounting for 71% of all global deaths. NCDs include heart disease, cancer, chronic respiratory diseases, and diabetes. NCDs are chronic conditions influenced by various intrinsic and extrinsic factors, including genetic, physiological, environmental, and lifestyle factors (World Health Organization, 2022).

Free radicals are another factor associated with developing non-communicable diseases (NCDs). Free radicals' origin is associated with two primary sources: endogenous (mitochondrial respiration, enzymatic reactions, immune cell activation) and exogenous sources (UV radiation, ionizing radiation, tobacco smoke, pollution, chemicals, and drugs). Increased free radicals lead to oxidative stress and damage (Mau *et al*. 2002 Chandimali *et al*. 2025).

Therefore, the body has mechanisms to regulate the levels of free radicals through various enzymes, such as catalase, superoxide dismutase (SOD), and glutathione peroxidase (GPx). Furthermore, when the number of free radicals exceeds the capacity of these enzymes, using antioxidants from natural food sources or synthetic unities is one approach humans use to help restore the balance of free radicals in the body (Chandimali et al., 2025). However, antioxidants from synthetic sources have several limitations, including the cost of synthetic antioxidants, their stability in the presence of external factors, and the side effects from synthetic compounds (Xu *et al*. 2021), including the accumulation of chemicals from the synthetic process.

Nutraceutical or functional foods refer to foods containing components beyond basic nutrients, demonstrating properties that enhance the body's biochemical functions. These include supporting the immune system, lowering blood sugar levels, reducing free radicals,

etc. For this reason, functional foods are considered an essential approach to help prevent and reduce the risk of developing non-communicable diseases (NCDs) (Damián *et al*. 2022).

Mushrooms are classified under the kingdom of fungi. The fungus that produces macroscopic fruiting bodies are commonly referred to as mushrooms (or higher fungi). Species from the phyla Ascomycota and Basidiomycota are primarily referred to by this somewhat erroneous term. We don't really know how many species there are in the kingdom of fungus. It was estimated to be approximately 1.5 million, while more recent estimates put the global figure between 2.2 and 3.8 million. Approximately 120,000 fungal species have been described thus far. Most "mushrooms" are members of the phylum Basidiomycota, which includes over 35,000 fungal species (Thu *et al*. 2020). There are many edible mushrooms that have widely reported various bioactive compounds that display biological properties such as antioxidants, immunomodulatory, antivirus, antivirus, antimicrobial, anti-hypertensive, anti-diabetic mellitus, anti-inflammatory, anti-tumor, anticancer, including cardiovascular disease, cancer, diabetes mellitus, and some other clinical diseases. Based on the information above, mushrooms are a potential raw material for developing functional and future foods (Contato & Conte-Junior 2025).

Bamboo mushrooms or lady mushrooms are the common names for the mushrooms of *Dictyophora* species. These lady mushrooms are classified in the Kingdom of Fungi, *Phallales* order, *Plallacea* family, and *Phallus* genus. Bamboo mushrooms can grow in areas such as the Americas, Africa, Australia, and Asia (Habtemariam 2019). *D. indusiata* is an edible bamboo mushroom that has been reported to have various components. Polysaccharides are the major components of the body. Simultaneously, the minor compounds are proteins, lipids, phenolics, flavonoids, terpenoids, alkaloids, allantoin, glycolic acid, and 5-(Hydroxymethyl)-2-furfural (Habtemariam 2019). Previous research has found that bioactive substances *in D. indusiata* extracts exhibit a wide range of biological activities, as observed in both *in vitro* and *in vivo*. These include antioxidant activity, antimicrobial effects, tyrosinase enzyme inhibition, anticancer properties, anti-inflammatory effects, immune system stimulation, tumor growth inhibition, cardiovascular protection, blood cholesterol reduction, and prevention of Alzheimer’s disease (Habtemariam 2019).

However, the composition study of compounds and their biological activity from the internal tissue of *D. indusiata* mushroom extracts that use different solvents and temperatures still lacks comprehensive research data.

Therefore, this research aims to extract chemical components in immature internal tissue of *D. indusiata* using different temperatures and solvents to compare yield, the antioxidant activity, inhibition of tyrosinase enzyme, and cytotoxicity in normal cells such as Hepatocyte Growth Factor (HGF) cells and Vero cells to compare the total phenolic, total flavonoid, and

total terpenoid contents from the extraction of the internal tissue of *D. indusiata* mushroom to examine the small bioactive compounds in the samples with high efficiency using GC/MS.

The results from this study are expected to lead to a process for preparing active compounds from the tissue of *D. indusiata* mushroom that is convenient, easy to control, and capable of extracting active compounds with antioxidant properties, anti-tyrosinase enzyme activity, and non-toxicity to normal cells. These data will allow the extract to be further studied at the animal trial level, in clinical research, and for future development in functional foods.

MATERIALS AND METHODS

Materials

2,2-diphenyl-1picrylhydrazyl (DPPH), 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), enzyme tyrosinase mushroom, and quercetin were purchased from Sigma Aldrich (Saint Louis, MO, USA), l-ascorbic acid was purchased from Ajax Finechem Pty Ltd (Carlton Rd, Auckland New Zealand), Kojic acid, 3,4-Dihydroxyl-L-phenylalanine (L-DOPA) were purchased from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), Folin-Ciocalteu's phenol reagent was purchased from V.W.R. International S.A.S (Fontenay-sous-Bois, France), gallic acid was purchased from E.M.D. Millipore Corporation (Billerica, MA, USA).

D. indusiata growth

The immature stage of *D. indusiata* was washed in sterile DI water three times and then soaked into 70%v/v ethanol. Cleaned immature stage *of D. indusiata* was placed on the plate and cut by a sterile scalpel into half. The middle part was transferred into a potato dextrose agar plate and then incubated at 30 °C for 7-14 days. The filament on potato dextrose agar was transferred into the mushroom nodules. Next, the filament was incubated in the mushroom dome at 90-95% humidity until the filament could succeed in growth. After that, the filament in mushroom nodules was cultivated in the Poy Jaem bamboo forest in the Ban Ko Rang village, Khao Chakan district, Sa Kaeo province, Thailand. This area is certified as an organic bamboo garden. The *D. indusiata* was cultured under 90-95% humidity and covered by the soil and bamboo leaf for 45 days. Next, the immature *D. indusiata* succeeded in representing for 8-14 days and then expressed to the mature stage within two hours.

Raw material collection and preparations

The immature stage of *D. indusiata* was collected in September 2024. The components of the immature stage of *D. indusiata* were separated into A1: peels, A2: Jelly, and A3: internal tissue (Figure 1). In this study, the A3 part was separated and cleaned with deionized water-dried three times. The A3 part was cut and frozen at -20 °C. The frozen internal tissue (A3) was homogenized using the blender. This homogenate was separated into two groups. The first group was dried using a hot air oven at 60°C for 24 h. It is named O-A3-01. The second group was lyophilized and called L-A3-02.

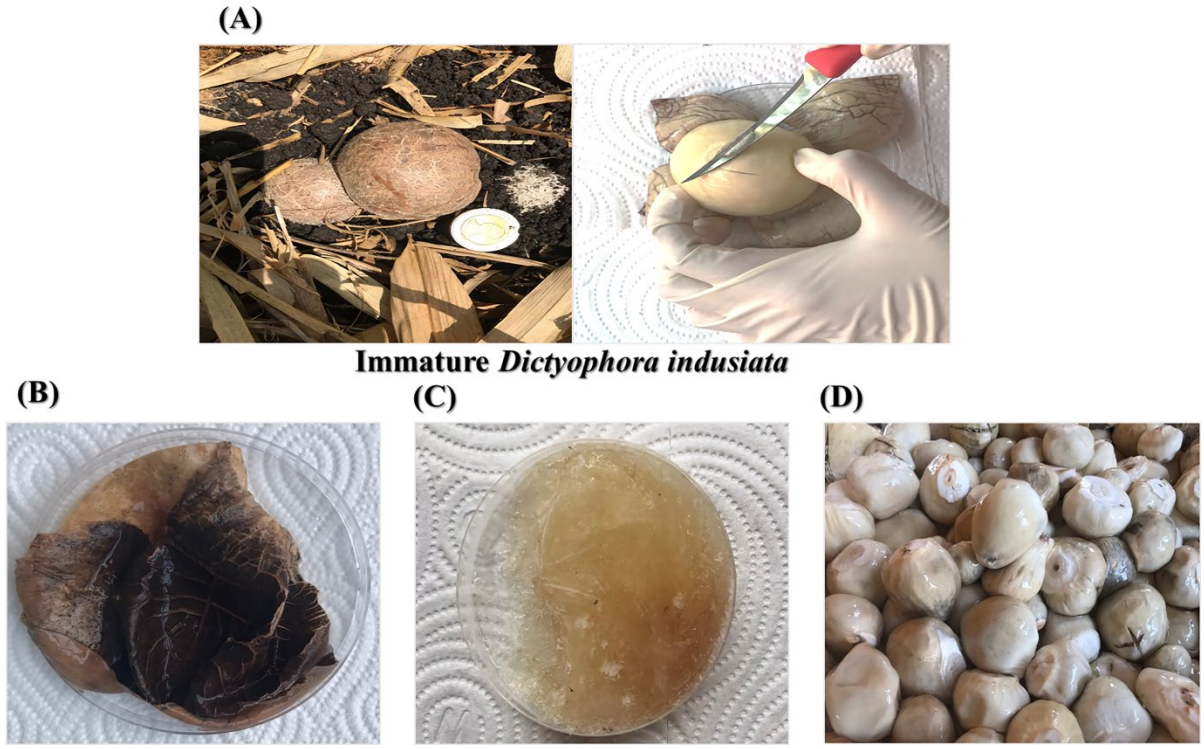

Figure 1: (A) Immature stage of D. Indusiata; (B) A1: Peels; (C) A2: Jelly; and (D) A3: Internal tissues.

Extraction process

This extraction procedure was performed in two different major factors the first factor was temperature (4 °C and 80 °C), and the second was the extracted solvents (Deionized water, methanol, and ethanol). The dried raw materials L-A3-02 and O-A3-01 were balanced for 10.0 g and placed into extracted chambers. Deionized water (DI), methanol, and ethanol were incubated at 4 °C and 80 °C before use. These solvents were subjected to the extracted

chambers with the ratio of 1:20 (w/v) and then incubated in different temperatures (4 °C and 80 °C) for 1 h. After that, chambers were placed in an ultrasonic machine according to the temperature conditions and then extracted continuously by 35 kHz of ultrasonic for one hour. Then, the supernatant of each condition was collected using 160 mm of the mesh. The precipitants were extracted and repeated under the same conditions two times. The supernatant of three repeated extractions was combined. The clear supernatant was prepared using centrifugation at 5,000 rpm for 5 min. The IDs of each extract were named according to the different temperatures and extracted solvents. For example, L-A3-02-4W, L-A3-02-4ME, and L-A3-02-4ET called the extracts that performed at 4°C and used water (W), methanol (ME), and ethanol (ET) as the solvent, respectively. The supernatant that used water as the solvent was dried by a freezing dryer, while the methanol and ethanol conditions were concentrated by evaporating. All extracts were balanced, calculated the % yields, and then stored at -20 °C.

DPPH radical scavenging assay

The antioxidant activity of bioactive agents in each extract was investigated according to the mini-modified method as previously described by Patathananone *et al*. (2019). All extracts were dissolved and prepared in different concentrations using two flow dilution techniques. L-ascorbic acid was prepared to be used as a positive control for antioxidants. The reaction was performed in the 96-well plate. In the order of test condition, 190 µL of 0.1014 mM DPPH radical solution (0.1014 mM of DPPH in ethanol) was placed into each well. The 10 µL of different concentrations for each extract were incubated with the DPPH radical solution, mixed gently, and incubated at 37 °C in the dark for 1 h. The absorbance values were measured at 515 nm using the microplate reader. The percentage of DPPH radical scavenging activity was analyzed as previously described by Patathananone *et al*. (2019).

Ferric reducing antioxidant power (FRAP) assay

The total antioxidants in foods have been investigated wildly by FRAP assay (Kelman *et al*. 2012). Iron [III]-2,4,6-tripyridyl-S-triazine (Fe^{3+}—TPTZ) is reduced to ferrous-tripyridyl triazine (Fe^{2+}—TPTZ) by setting with an antioxidant chemical in the FRAP assay reaction. This reaction produces a striking blue hue. For the initial step, 1.0 mL of 10 mM of 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) in 40 mM of hydrochloric acid was mixed with 1 mL of 20 mM ferric chloride and 10.0 mL of 300 mM acetate buffer (pH 3.6), according to the ratio 10:1:1 (v/v), and then incubated at 37 °C for 1 min. The working solution of freshly FRAP reagent was diluted by 40 mM of hydrochloric acid. The reaction was performed by mixing 2.6 mL of FRAP

reagent, 100 µL of sample extracts, and 300 µL of deionized water. In addition, α-tocopherol was used as positive control. The reaction of each tube was incubated at 37 °C for 30 min. The absorbance values were measured using a UV-Vis spectrophotometer at a wavelength of 593 nm. Furthermore, ferrous sulphate ($FeSO_4$) with concentrations of 0.07875, 0.1575, 0.315, 0.629, and 1.258 mM was used to prepare a standard curve. The value of antioxidant content can be calculated using the FRAP value equation in mM Fe^{2+}/g extract using the formula value y = 5.1142x + 1.2293, R^2 = 0.9973.

Anti-tyrosinase activity assay

Tyrosinase is an enzyme that greatly affects the levels of melanin synthesis. Tyrosinase inhibition is very important in reducing melanin levels and treating various skin and neurodegenerative diseases. The anti-tyrosinase test was carried out by weighing a sample of 0.01 g and dissolved in 1 mL of deionized water. Then, the sample solution was filtered through a 0.45 nm filter membrane, and the sample was diluted with a double dilution system using 50 mM phosphate buffer pH 6.8 until the solution reached six-fold dilutions. The experimental procedure was analyzed on 96 microplates (96-well plates). In the initiation step, 100 µL of 250 Units/mL mushroom tyrosinase was incubated with 10 µL of different concentrations of the extract samples. Then, 70 µL of 50 mM phosphate buffer pH 6.8 was added and mixed gently. The reaction was incubated at 37 °C for 10 min. Furthermore, 20 µL of 20 mM L-Dopa in DMSO was placed, mixed, and incubated at 37 °C for 30 min. Kojic acid was used as a positive control, and anti-tyrosinase activity was investigated using the same method. The absorbance values were measured at a wavelength of 495 nm. The percentage of anti-tyrosinase activity was analyzed as previously described by Patathananone *et al*. (2019).

Determination of cytotoxic effect in Hepatocyte growth factor (HGF) cells and Vero cells

Based on the safety of the cosmetic ingredient, the cytotoxic effect of bioactive agents in the extracts should be clarified. Fibroblast ("HGF") and Vero cells were used as the target cells to study the cytotoxic effect of O-A3-01-80W and L-A3-02-80W extracts. In a sterile tissue culture plate containing 5.0 mL complete-DMEM media, HGF. and Vero cells were cultured (DMEM supplemented with 10% fetal bovine serum and 1.0% penicillin-streptomycin). The cell culture plate was incubated at the temperature of 37 °C with 5% CO_2 and 95% air, as described previously by Patathananone *et al*. (2019). Cells were treated with 2 mL of trypsin-EDTA and incubated at 37 °C for 2 min after confluent growth of approximately 90% of the culture plate area. The suspended cells were collected using a centrifuge at 906xg for 5 min. Furthermore,

2 mL DMEM was used to resuspend both cell pellets. An automated cell counter was used to count cells after straining 10 µL of suspended cells with 10 µL of 1X trypan blue (Bio-Rad). The 100 µL of suspended survival cells (2 x 10^5 cells/mL) were seeded into each well of the 96-well plate and incubated for 24 h under the same conditions. The cells were then incubated at the temperature of 37 °C for 24 or 48 h after being treated with various final concentrations of the samples. The recent medium was removed. Herein, the survival HGF and Vero cells were strained by 1X Presto-Blue and then incubated for two hours. Then, the reactions were measured at the excitation filter for 560 nm and emission at 590 nm (Thermo Scientific, Sweden). The percentage of cell survival was calculated according to the formula (see below).

$$\% \text{ cell survival} = [\frac{Test}{Control}] \times 100$$

Test = Fluorescence intensity of samples

Control = Fluorescence intensity of samples

Total phenolic content

Phenolics are bioactive compounds found in many plants that have many health benefits. Identification of phenolic content was carried out to determine the total phenolic content in mushrooms by modifying the Folin-Ciocalteu method carried out by (Aryal *et al*. 2019). The gallic acid standard curve equation is y = 9.3779x + 0.096, R^2 = 0.9946. It was used to calculate the total phenolic content in the extracted samples. Data are presented in mg GAE/g dry extract.

Total flavonoid content

The total content of flavonoids is part of the phenolic, which has biological activity associated with biopharmaceutical compounds. The total flavonoid content was determined using a colorimetric method involving modified aluminum chloride ($AlCl_3$) (Baba *et al*. 20115). The total flavonoid content was calculated as (mg Quercetin (QE)/g dry extract) using the equation y = 1.1305x + 0.0407, R^2 = 0.9991.

Total terpenoid content

Terpenoids are compounds found in fungi. The total terpenoid content of the extract was determined by Truong *et al*. (2021). The method uses menthol, a standard terpenoid compound. The chloroform solution of as much as 2 mL was added to 10 mg/mL of the sample.

Then, the sample mixture was shaken until evenly mixed and left for 3 min. After that, 200 µL of concentrated sulfuric acid (H_2SO_4) was added to the mixture, and the solution was then incubated at 37 °C for 30 min. During the incubation process, a reddish-brown precipitate will form in the solution. The supernatant was then carefully poured without disturbing the precipitation, and then 3 mL of absolute methanol was added to the precipitate and shaken until well mixed. A UV-Vis spectrophotometer was used to measure the absorbance at 538 nm. The standard curve equation for menthol is shown as $y = 0.4269x + 0.091$, $R^2 = 0.9986$. It was used to calculate the total terpenoid content in the extracted samples. Data are presented in mg MT/g extract.

GC-MS analysis

Gas chromatographic analysis was performed using an Agilent 7890B GC system equipped with an HP-5 MS capillary column (25 m × 250 µm × 0.25 µm). Helium (99.999% purity) was used as the carrier gas at a constant flow rate of 1.2 mL/min. The injector temperature was set at 250 °C and the injection was carried out in splitless mode for 0.75 min. The MSD transfer line temperature was maintained at 250 °C. The GC oven temperature program was as follows: the initial temperature was set at 60 °C and held for 3 min, followed by a ramp to 80 °C at 1 °C/min with no hold time. The temperature was then increased to 120 °C at a rate of 3 °C/min, and subsequently ramped to 240 °C at 4 °C/min, giving a total runtime of 66.33 min. Mass spectrometric detection was carried out using an Agilent 5977B MSD operating in electron ionization (EI) mode at a fixed ionization energy of 70 eV. The mass spectrometer was operated in scan mode with a mass range of m/z 45–500. The ion source temperature was set at 230 °C and the quadrupole temperature was maintained at 150 °C. Mass spectral profiles obtained from the GC–MS analysis were processed using Mass Hunter software (Agilent Technologies, Inc.). The software automatically matched the acquired mass spectra with reference spectra contained in its integrated library database. Compound identification was based on the similarity score and probability of match generated by the library search algorithm. The spectral library used for compound identification was the NIST17.L database, which provided reference information for comparison and confirmation of analyte identities (Kunu *et al*. (2025).

Separation of phytochemicals using thin-layer chromatography

The extract from the internal tissue of *D. indusiata* was separated on TLC plates using a mobile phase consisting of n-butanol, acetic acid, and water (4:1:1, v/v/v). Following development, the TLC plates were dried and examined under ultraviolet light at 254 and 365 nm to observe

compound migration. In addition, the plates were stained with 2% w/v vanillin–sulphuric reagent. Antioxidant activity was assessed using the bioautography technique, in which the developed plates were sprayed with 0.04% w/v DPPH reagent and subsequently analysed for colour changes and retention factor (Rf) values, as described by Patathananone *et al* (2019).

Statistical data analysis

Statistical analysis was conducted by investigating the ANOVA variance test to see if there was a difference in the value concentration of the sample test using Duncan's at an alpha subset rate of 5%. The value concentrations are analyzed using the software.

RESULTS

Extract Yield

The study on the extraction of the internal tissue of *D. indusiata* (A3) compares the effects of three factors—namely, (1) the method used to prepare the raw material, (2) the solvent, and (3) the extraction temperature—on the extract yield is shown in Table 1. The data indicated that these factors have an impact on the extract yield. The L-A3-02-80W extract exhibited the highest yield, at 58.57 ± 1.17%. The high extraction temperature showed a % yield higher than the low temperature. Additionally, the extract that used water as the solvent exhibited a % yield higher than methanol and ethanol.

Table 1: The extract yield, FRAP values including IC_{50} dose of antioxidant using DPPH assay, and antityrosinase.

ID Samples	Extraction yields (%)	FRAP value (mM Fe^{2+}/g)	IC_{50} dose (mg/mL)	
			Antioxidant	Antityrosinase
L-A3-02-4W	36.05 ± 1.42^{a}	122.74 ± 0.21^{b}	0.58	0.79
L-A3-02-4ME	27.25 ± 0.85^{b}	79.26 ± 0.42^{f}	0.62	0.87
L-A3-02-4ET	26.05 ± 1.42^{b}	59.25 ± 0.24^{j}	0.64	0.99
O-A3-01-4W	37.83 ± 0.85^{j}	145.22 ± 0.21^{a}	0.38	0.77
O-A3-01-4ME	12.63 ± 0.12^{c}	105.01 ± 0.12^{e}	0.61	0.83
O-A3-01-4ET	10.31 ± 0.08^{i}	80.70 ± 0.21^{i}	0.62	0.89
L-A3-02-80W	58.57 ± 1.17^{h}	269.58 ± 0.21^{d}	0.29	0.29
L-A3-02-80ME	47.13 ± 2.19^{g}	237.06 ± 0.31^{h}	0.48	0.49
L-A3-02-80ET	20.15 ± 0.24^{d}	216.33± 0.12^{l}	0.63	0.55
O-A3-01-80W	41.78 ± 2.83^{f}	276.62 ± 0.91^{c}	0.25	0.69

O-A3-01-80ME	15.68 ± 0.06^{e}	243.71 ± 0.46^{g}	0.44	0.77
O-A3-01-80ET	12.31 ± 0.09^{c}	244.68 ± 0.12^{k}	0.61	0.87

Antioxidant properties

The results of the study on the antioxidant activity of internal tissue of *D. indusiata* extracts under various conditions, as determined by the DPPH radical scavenging assay, are presented in Figure 2(A–D). The results indicate that all extract conditions exhibited antioxidant properties, albeit with varying levels of efficacy. The IC_{50} dose is represented in Table 1. The extract O-A3-01-80W exhibited an IC_{50} value of 0.25 mg/mL, which was lower than that of the other conditions. Water as the extraction solvent at a temperature of 80°C demonstrated more effective antioxidant properties than extracts obtained using methanol and ethanol as solvents. This data is consistent with the FRAP values, indicating that water yielded higher FRAP values than methanol and ethanol as solvents. Moreover, the FRAP values at the extraction temperature of 80°C were higher than those obtained at 4°C across all solvent conditions.

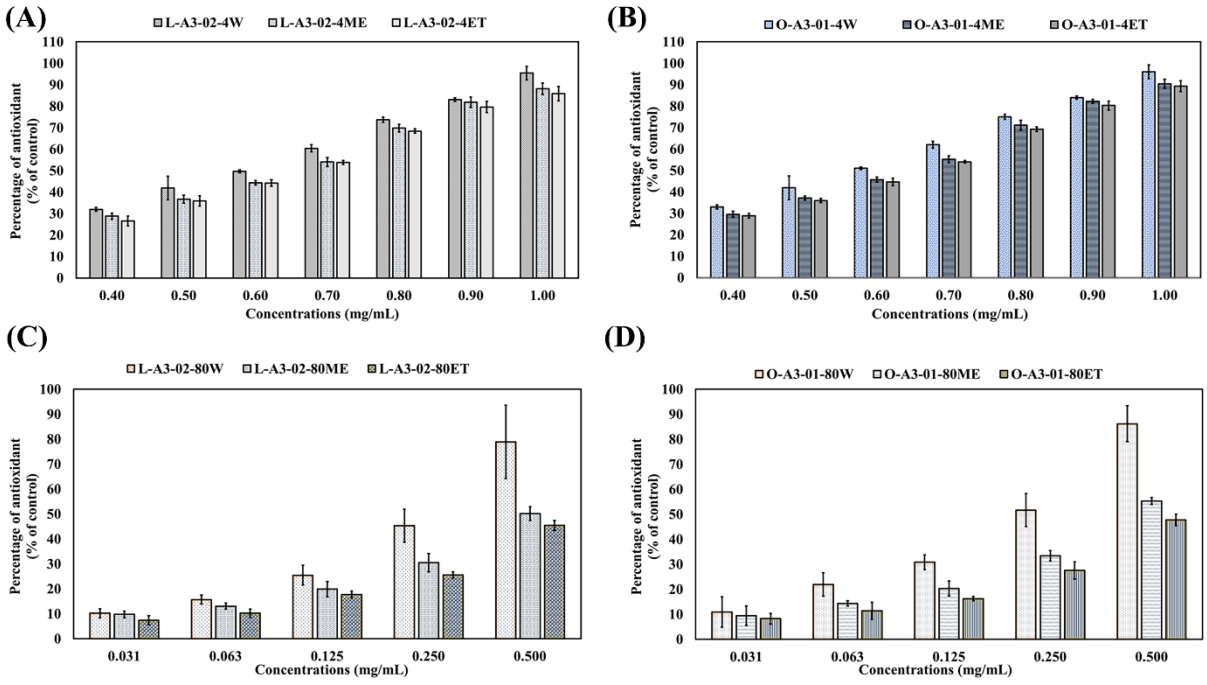

Figure 2: DPPH radical scavenging activity (A) the treatment extracts as L-A3-02-4W, L-A3-02-4ME, L-A3-02-4ET; (B) O-A3-01-4W, O-A3-01-4ME, O-A3-01-4ET); (C) L-A3-02-80W, L-A3-02-80ME, L-A3-02-80ET; (D) O-A3-01-80W, O-A3-01-80ME, O-A3-01-80ET. IC50 value of L-ascorbic acid represented at 0.19 mg/mL.

Anti-tyrosinase activity

The anti-tyrosinase property of bioactive agents is a goal target in the development of cosmetic and functional foods. In this study, the internal tissue of *D. indusiata* extracts exhibited the potential for anti-tyrosinase activity. The results are shown in Figure 3. In addition, IC_{50} values of each extracted condition are shown in Table 1. Kojic acid is the positive agent that exhibited the IC_{50} value at 0.34 mg/mL. The data indicated that the raw material preparation method, solvent, and extraction temperature affected the anti-tyrosinase property. L-A3-02-80W represented the IC_{50} dose at 0.29 mg/mL lower than other extracted conditions, including kojic acid. The 80°C water of extraction presented an IC_{50} value of less than 4°C. Additionally, water conditions exhibited the IC_{50} dose smaller than methanol and ethanol for both temperatures.

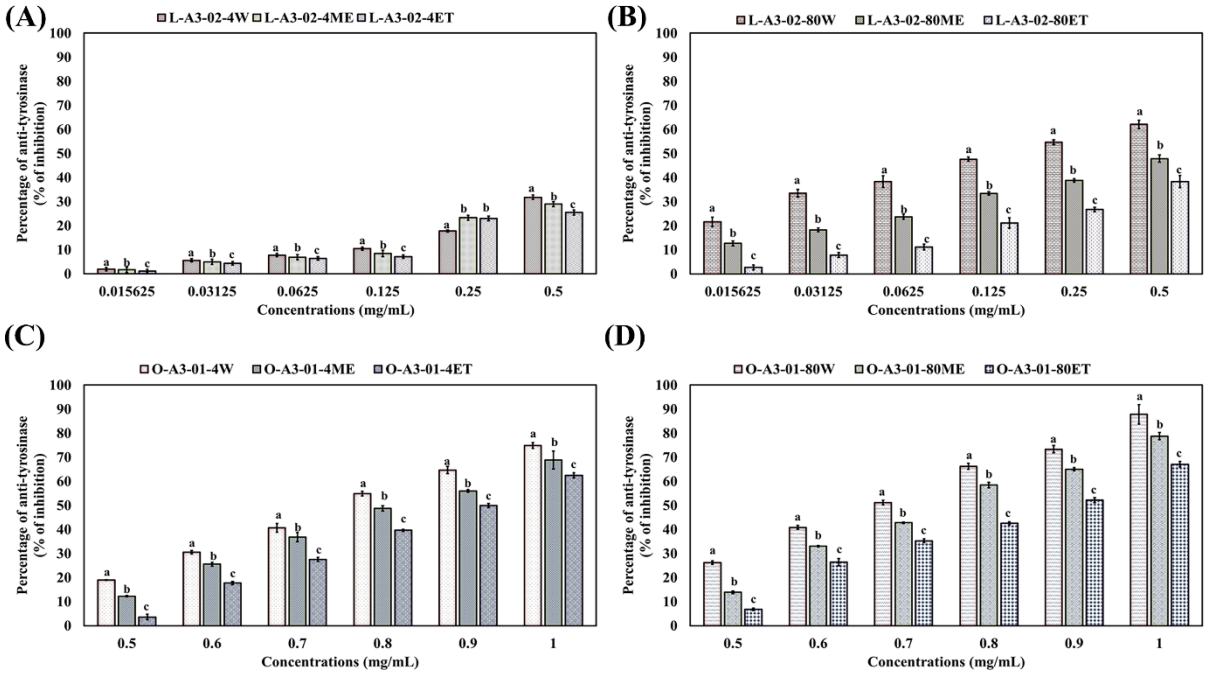

Figure 3: Anti-tyrosinase activity of the extracts (A) L-A3-02-4W, L-A3-02-4ME, L-A3-02-4ET; (B) L-A3-02-80W, L-A3-02-80ME, L-A3-02-80ET; (C) O-A3-01-4W, O-A3-01-4ME, O-A3-01-4ET; (D) O-A3-01-80W, O-A3-01-80ME, O-A3-01-80ET. IC50 value of kojic acid represented at 0.34 mg/mL.

Total phenolic, flavonoid, and terpenoid contents

The effects of solvents and temperatures on the total phenolic, flavonoid, and terpenoid contents in the extracts are presented in Table 2. The findings indicate that the solvent

pronounced affected the variation in the extracts' phenolic, flavonoid, and terpenoid contents. Water was found to be the solvent with the highest total content of all three compound groups compared to methanol and ethanol at both temperatures. O-A3-01-80W is the extract with higher levels of phenolic, flavonoid, and terpenoid contents than the other conditions. The amount of compounds found in the sample extracted with water at 80°C may be correlated with its antioxidant activity and the inhibition of tyrosinase enzyme activity.

Table 2: Total phenolic, flavonoid, and terpenoid contents of each extract.

ID Samples	Total phenolic (mg GAE/g)	Total flavonoid (mg QE/g)	Total terpenoid (mg MT/g)
L-A3-02-4W	31.46 ± 0.11^{b}	21.14 ± 0.05^{b}	103.08 ± 1.05^{b}
L-A3-02-4ME	30.53 ± 0.22^{e}	18.57 ± 0.05^{f}	95.77 ± 0.40^{f}
L-A3-02-4ET	27.37 ± 0.22^{g}	16.83 ± 0.09^{j}	89.37 ± 1.05^{g}
O-A3-01-4W	34.69 ± 0.16^{a}	23.64 ± 0.15^{a}	120.68 ± 0.68^{a}
O-A3-01-4ME	32.52 ± 0.11^{d}	21.37 ± 0.18^{e}	110.62 ± 1.05^{c}
O-A3-01-4ET	30.57 ± 0.16^{e}	19.60 ± 0.14^{i}	98.51 ± 1.43^{b}
L-A3-02-80W	32.56 ± 0.16^{d}	22.08 ± 0.05^{d}	121.14 ± 1.05^{d}
L-A3-02-80ME	31.00 ± 0.22^{f}	20.40 ± 0.14^{h}	104.22 ± 2.06^{b}
L-A3-02-80ET	28.43 ± 0.27^{h}	18.69 ± 0.17^{f}	92.80 ± 0.40^{h}
O-A3-01-80W	36.86 ± 0.12^{c}	24.65 ± 0.05^{c}	124.11 ± 0.69^{c}
O-A3-01-80ME	34.83 ± 0.16^{a}	22.91 ± 0.14^{g}	118.62 ± 1.81^{a}
O-A3-01-80ET	32.84 ± 0.28^{d}	20.40 ± 0.14^{h}	110.85 ± 1.05^{e}

GAE: gallic acid equivalent; QE: quercetin equivalent values followed by different superscript letters (a–l) in the same column are significantly different ($p < 0.05$)

MT: menthol equivalent; values followed by different superscript letters (a–l) in the same column are significantly different ($p < 0.05$)

Cytotoxic effect of L-A3-02-80W, O-A3-01-80W on HGF and Vero cells

The extracts L-A3-02-80W and O-A3-01-80W that represented the potential of antioxidant and anti-tyrosinase activities determined the cytotoxic effect on Vero cells and HGF cells. The results are exhibited in Fig. 4 (A-D). The percentage of cell viability of both cells presented higher than 90% for all treatment concentrations. These data indicated that the bioactive agents showed very low cytotoxic effects on Vero cells and HGF cells. This advantage promoted the development of these extracts in functional foods, including other healthcare products.

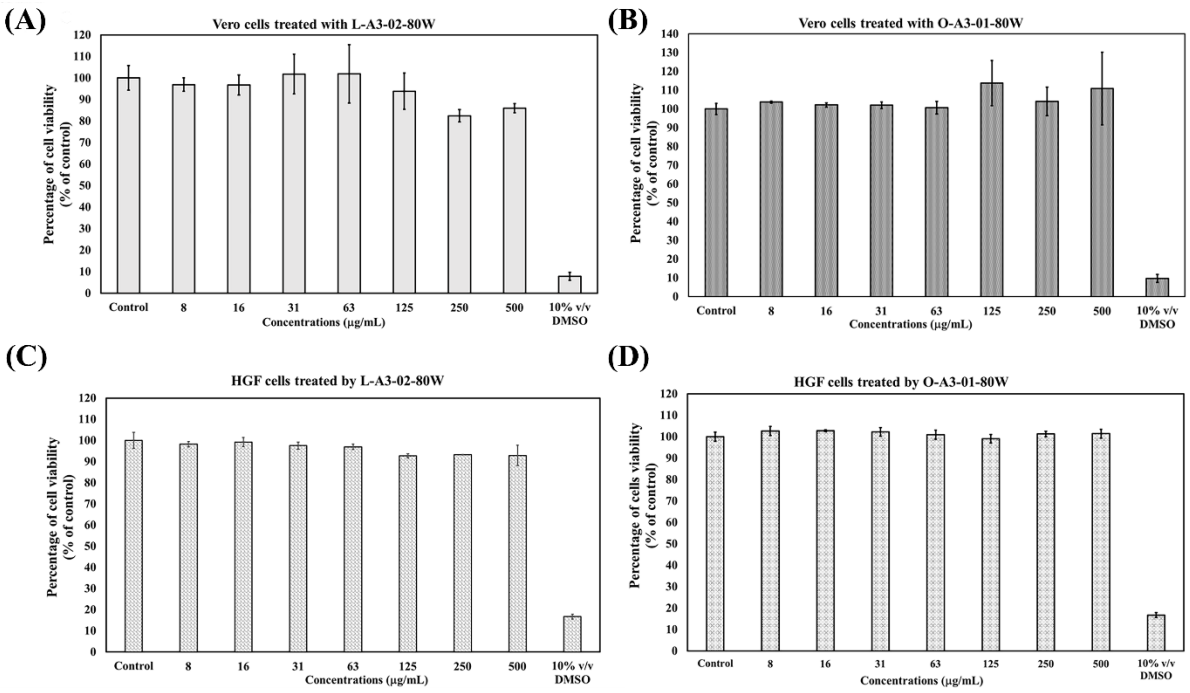

Figure 4. Cytotoxic effect of biological components in the extracts of D. indusiata internal tissue. (A) and (B) Vero cells treated by L-A3-02-80W and O-A3-01-80W, respectively; (C) and (D) HGF cells treated with L-A3-02-80W and O-A3-01-80W, respectively.

GC-MS data

The extracts L-A3-02-80W and O-A3-01-80W demonstrated potential antioxidant and anti-tyrosinase activities. Therefore, both samples were subjected to GC–MS analysis to identify their bioactive constituents. The GC–MS chromatographic profiles are presented in Figure 5. The mass spectra of the detected compounds were analysed and are summarised in Table 3. The analysis of both extracts revealed approximately ten detectable compounds. Among these, eight compounds were found in both samples, namely: tetradecanoic acid, decanoic acid silver(1+) salt, 2-azido-2,4,4,6,6-pentamethylheptane, trans-2,4-dimethylthiane, S,S-dioxide, trans-2-methyl-4-n-pentylthiane, S,S-dioxide, methyl 12,13-tetradecadienoate, 2-azido-2,4,4,6,6,8,8-heptamethylnonane, and 9-borabicyclo[3.3.1]nonane 9-(3-methoxycyclohexyl)oxy-. The presence of similar compounds in both extracts suggests that the extraction and analytical procedures were stable and reproducible. However, certain compounds were unique to each extract. Borane diethyl (decyloxy)- and hexadecanoic acid methyl ester were detected exclusively in L-A3-02-80W, whereas O-(n-butyl) S-[2-(diethylamino)ethyl]-methanephosphonothioate, 2-{[1-(3-methoxypropyl)-1H-1,2,3-triazol-4-yl]methyl}-4-[methyl(propyl)amino]isothiazolidine 1,1-dioxide, and n-hexadecanoic acid were found only in O-A3-01-80W. The compounds identified in the extracts represent small-

molecule constituents that may provide useful information for exploring possible associations with the biological activities observed in this study. Nevertheless, the mass spectral similarity scores ranged between 50–80%, indicating that the identified compounds can be considered tentative matches with structures previously reported in mass spectral libraries. Confirmation of the complete and accurate structures, with similarity scores ≥ 85%, would require more specific and definitive analytical techniques.

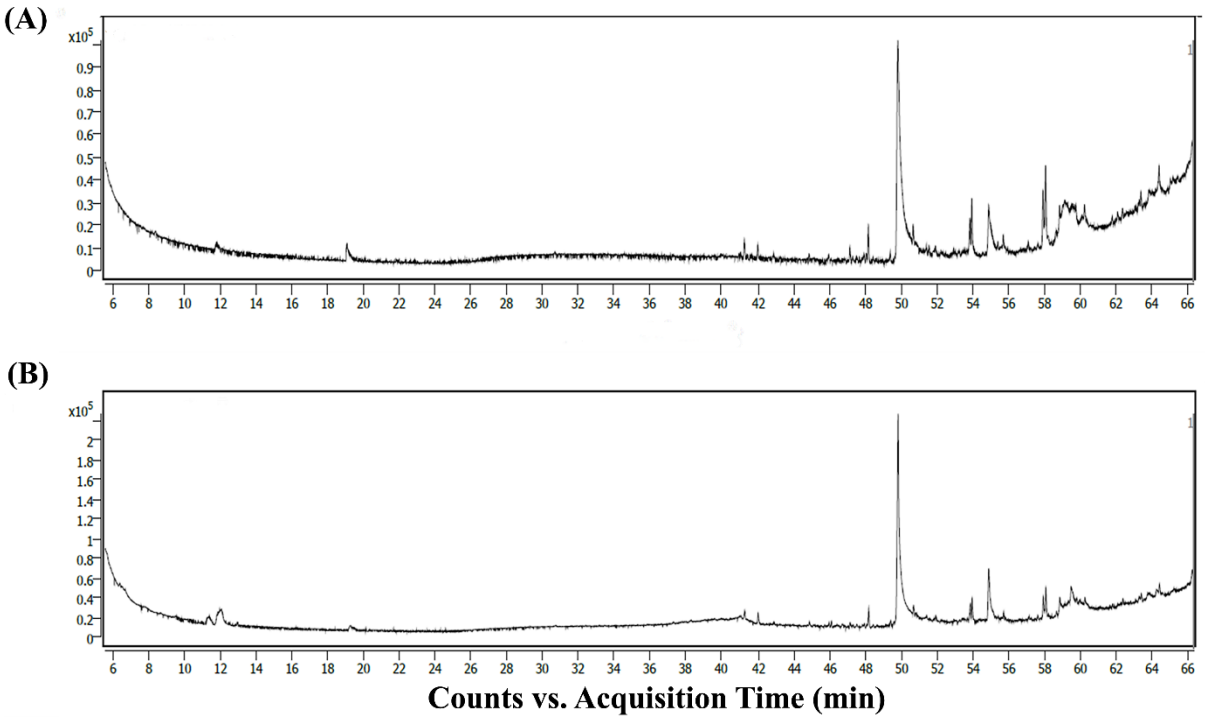

Figure 5: GC-MS chromatogram of extract (A) L-A3-02-80W and (B) O-A3-01-80W.

Table 3: Chemical compound identified in the L-A3-02-80W and O-A3-01-80W extracts by GC-MS analysis.

No	Bioactive agents	RT (min)	%Area	Formula	Exact Mass	Biological properties
L-A3-02-80W						
1	Borane diethyl (decyloxy)-	48.179	4.36	C_{14} H_{31} B O	226.21	Mechanism of action neuroprotection. (Niemuth et al., 2016)

No	Bioactive agents	RT (min)	%Area	Formula	Exact Mass	Biological properties
2	Tetradecanoic acid	49.830	100.00	C_{14} H_{28} O_2	228.37	Antioxidant activity, Antimicrobial activity, α-Amylase and α-Glucosidase inhibitor (Types 2 Diabetes Mellitus). (Lakshmanasenthil et al., 2018).
3	Decanoic acid,silver(1+) salt	50.684 54.894	1.58 18.98	C_{10} H_{19} Ag O_2	279.13	Antioxidant activity, Antibacterial activity, Antiviral activity, Anti-inflammatory, Anti-diabetes. (Kaushik et al., 2020)
4	2-Azido-2,4,4,6,6-pentamethyl heptane	53.850	6.08	C_{12} H_{25} N_3	211.35	Antitumor, Anti-inflammatory. (Addai et al., 2022)
5	Hexadecanoic acid, methyl ester	53.950	8.33	C_{17} H_{34} O_2	270.45	Antioxidant activity, Anti-inflammatory, Anti-cancer. (Pan et al., 2019)
6	trans-2,4-Dimethylthiane, S,S-dioxide	55.711	2.63	C_7H_{14} O_2S	130.25	Antimicrobial activity (Deka and Jha, 2019)
7	trans-2-methyl-4-n-pentylthiane, S,S-dioxide	57.111 58.070 59.155 59.591 60.115 60.235 62.091 63.291 63.386 64.403 65.032 65.179	1.04 11.41 14.31 1.56 1.59 3.73 1.11 1.49 1.90 4.33 1.00 1.61	$C_{11}H_{22}O_2S$	218.35	Anti-inflammatory, Antimicrobial. (Selvakumar et al., 2015 Hassan et al., 2021)

No	Bioactive agents	RT (min)	%Area	Formula	Exact Mass	Biological properties
8	Methyl 12,13-tetradecadienoate	57.929	8.58	$C_{15}H_{26}O_2$	238.37	Control human brain tissue. (Hammond et al., 2020)
9	2-Azido-2,4,4,6,6,8,8-heptamethyl nonane	58.851	2.85	$C_{16}H_{33}N_3$	267.45	Antitumor, Anti-inflammatory. (Addai et al., 2022)
10	9-Borabicyclo [3.3.1] nonane, 9-(3-methoxycyclohexyl)oxy-	66.243	2.51	C_{15} $H_{27}BO_2$	250.21	ND
O-A3-01-80W						
1	O-(n-Butyl) S-[2-(diethylamino)ethyl]-methanephosphonothioate	41.270	1.41	$C_{11}H_{26}$ NO_2PS	267.36	Anti-cholinesterase. (Watson et al., 2015)
2	2-{[1-(3-Methoxypropyl)-1H-1,2,3-triazol-4-yl]methyl}-4-[methyl(propyl)amino]iso thiazolidine 1,1-dioxide	48.189	3.24	C_{14} $H_{27}N_5O_3S$	345.50	ND
3	Tetradecanoic acid	49.830	100.00	$C_{14}H_{28}O_2$	228.37	Antioxidant activity, Antimicrobial activity, α-Amylase and α-Glucosidase inhibitor (Types 2 Diabetes Mellitus). (Lakshmanasenthil et al., 2018).
4	Decanoic acid,silver(1+)salt	50.690 53.956	1.61 3.89	C_{10} $H_{19}AgO_2$	279.13	Antioxidant activity, Antibacterial activity, Antiviral activity, Anti-inflammatory, Anti-diabetes. (Kaushik et al., 2020)
5	2-Azido-2,4,4,6,6-pentamethyl heptane	53.856	3.03	C_{12} H_{25} N_3	211.35	Antitumor, Anti-inflammatory.(Addai et al., 2022)

No	Bioactive agents	RT (min)	%Area	Formula	Exact Mass	Biological properties
6	n-Hexadecanoic acid	54.889	20.87	C_{16} H_{32} O_2	256.42	Antioxidant activity, Anti-Inflammatory, Antioxidant, Antimicrobial. (Premathilaka et al., 2016)
7	trans-2-methyl-4-n-pentylthiane, S,S-dioxide	55.722 58.071 58.486 58.946 63.884	1.19 6.09 2.71 1.09 3.55	C_{11} H_{22} O_2 S	218.35	Anti-inflammatory, Antimicrobial. (Selvakumar et al., 2015 Hassan et al., 2021)
8	Methyl 12,13-tetradecadienoate	57.924	4.20	C_{15} H_{26} O_2	238.37	Control human brain tissue. (Hammond et al., 2020)
9	2-Azido-2,4,4,6,6,8,8-heptamethylnonane	59.491 60.246 63.396	5.71 1.42 1.23	$C_{16}H_{33}N_3$	267.45	Antitumor, Anti-inflammatory. (Addai et al., 2022)
10	9-Borabicyclo [3.3.1] nonane, 9-(3-methoxycyclohexyl)oxy-	65.205	1.17	C_{15} $H_{27}BO_2$	250.21	ND

Thin-layer chromatography

The separation profile of the extract from the internal tissue of *D. indusiata* obtained using thin-layer chromatography (TLC) is presented in Figure 6 (A–D). Figures 6(A) and 6(B) illustrate the appearance of compound spots under UV light at 254 nm and 365 nm, respectively. Figure 6(C) displays the spots visualised following staining with 2% w/v vanillin–sulphuric reagent, whereas Figure 6(D) shows the antioxidant-active spots on the TLC plate after spraying with 0.04% w/v DPPH reagent. The resulting chromatographic data were processed, and the R_f values and corresponding spot colours are summarised in Table 4. Compounds exhibiting UV absorption at 254 nm appeared at Rf values of 0.30, 0.38, 0.48, 0.60, 0.75, and 0.93, indicating the presence of aromatic rings or conjugated double-bond systems. In addition, the fluorescence observed under UV 365 nm at R_f 0.08, 0.20, 0.30, 0.38, 0.48, 0.60, and 0.75 suggests that these compounds contain extended conjugation or multiple aromatic rings, typical of flavonoids, coumarins, and related groups. Interestingly, staining with 2% w/v

vanillin–sulphuric reagent produced brown spots at R_f 0.10, 0.18, and 0.60, which may indicate the presence of alkaloids. Dark green spots at R_f 0.23, 0.30, and 0.38 were also observed. The compound with an R_f value of 0.48 exhibited a yellow spot, characteristic of polyphenolic compounds, flavonoids, or coumarins. Spots at Rf 0.75 and 0.93 showed purple to greenish-purple hues, suggesting the presence of triterpenoids, steroids, or saponins. Most notably, antioxidant activity was detected at R_f 0.30, 0.38, 0.48, and 0.55, where yellow spots appeared against a purple background after DPPH spraying. This finding indicates that polyphenolic compounds, including flavonoids and coumarins, are likely the major contributors to the strong antioxidant activity observed in the extract from the internal tissue of *D. indusiata*.

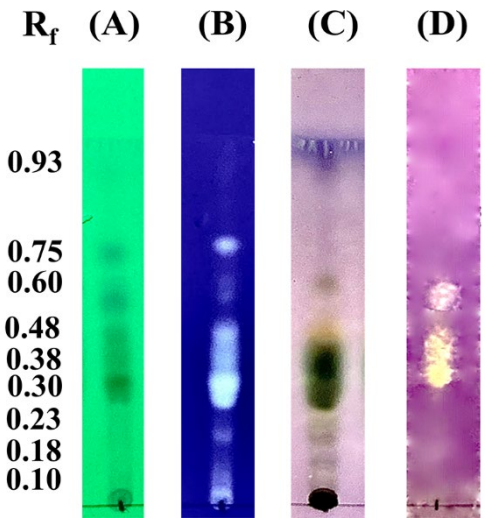

Figure 6: Thin layer chromatography. (A) The absorbant spots at 254 nm; (B) The fluorescent spots under UV 365 nm; (C) The spots of compounds that stained by 2% w/v vanillin–sulphuric; (D) The bioautography of antioxidant activity that detected using 0.04% w/v DPPH solution.

DISCUSSION

Mushrooms are considered a natural resource that has been extensively studied and developed for human use, particularly in food, functional foods, and future innovations. *D. indusiata* is another mushroom species that has gained popularity and is widely utilised in East Asia, mainly in China. Previous reports have shown that *D. indusiata* contains high levels of polysaccharides, proteins, and a diverse range of bioactive compounds that exhibit medicinal properties *in vitro* and animal models. Conspicuously, extracts from the fruiting body have been reported to possess anti-inflammatory, anticancer, and immunomodulatory activities, among others (Habtemariam 2019, Nazir *et al*. 2021)

This study highlights that the bioactivities, as well as the levels of phenolic, flavonoid, and terpenoid compounds, are directly influenced by the choice of solvent and extraction temperature when using ultrasonic-assisted extraction at a frequency of 35 kHz for 60 minutes, repeated three times. It was found that sample drying under cold conditions, hot water extraction, and extraction at 80°C were the most effective conditions impacting both the bioactivity and the concentration of bioactive compounds in the extracts from the internal tissue of *D. indusiata*. The extraction method employed in this study resulted in a high extraction yield, utilised a safe solvent, and involved equipment based on relatively simple technology.

Nazir *et al*. (2021) investigated extracts from the immature (egg) stage of *D. indusiata*, dividing the samples into three parts: peel and green mixture (PGW), core (CW), and whole mushroom (WW), using water as the extraction solvent. Their study reported 21.11%, 51.48%, and 10.15% yields for PGW, CW, and WW, respectively. In contrast, the present study, which focused on extracting bioactive compounds from the internal tissue of *D. indusiata* using hot water at 80 °C, yielded the highest extraction percentage at 58.57 ± 1.17%. This result is notably higher than the yields reported by Nazir *et al*. (2021). Furthermore, it was found that the extraction process in the current study influenced the levels of total phenolics and flavonoids, with the hot water extract containing higher amounts of these compounds compared to those reported in Nazir *et al*. (2021).

A previous study by Oyetayo *et al*. (2009) investigated the extraction of bioactive compounds from the footing body of *D. indusiata* using an aqueous extraction method at 95–100°C for 2 hours. They found that the extract exhibited antioxidant activity—including DPPH• scavenging, reducing power, hydroxyl radical scavenging, and superoxide anion scavenging—at a 2 mg/mL concentration. Additionally, Mau *et al*. (2002) reported on the antioxidant properties of a methanolic extract from the footing body of *D. indusiata*. The findings from the present study indicate that the aqueous extract demonstrated effective antioxidant activity, with a lower IC_{50} value compared to that reported by Oyetayo *et al*. (2009). The particular interest is that the polyphenolic and flavonoid constituents detected in the extract of the internal tissue of *D. indusiata* exhibited clear antioxidant activity on the TLC plate.

The compound groups identified in this study through GC–MS analysis correspond to several previously reported substances known for their antioxidant properties, including tetradecanoic acid, decanoic acid silver(I) salt, hexadecanoic acid methyl ester, and n-hexadecanoic acid (Table 3). Furthermore, certain compounds detected in the L-A3-02-80W and O-A3-01-80W extracts also exhibit additional biological activities, such as antitumor, antibacterial, and anti-inflammatory effects. However, some compounds identified in this study

have not yet been reported to possess any biological activities. These unidentified activities may therefore be associated with the bioactivity observed in the present investigation.

Myristic acid (C14:0) is directly involved in post-translational protein changes and mechanisms that control important metabolic processes in the human body. It was reported that moderate myristic acid consumption improves long-chain omega-3 fatty acids levels in plasma phospholipids, which could exert improvement of cardiovascular health parameters in humans (Ruiz-Núñez *et al*. 2016). Another research by the same group reported that the consumption of myristic acid from dairy fat increased HDL cholesterol and decreased triacyl glycerides levels, while no changes in LDL cholesterol were observed (Speziali *et al*. 2018). Additional immunomodulatory functions are exerted by myristic acid through the increase of a specific protein involved in the activation of macrophages in murine with high levels of myristic acid intake. It might display antimicrobial activity and be expressed as an inhibitor of the α-amylase and α-glucosidase (Lakshmanasenthil *et al*. 2018). This molecule might decrease the risk incidence of type 2 diabetes mellitus. Therefore, the compounds in L-A3-02-80W and O-A3-01-80W that expressed various biological activities might reduce the deceleration of biomolecular degradation processes associated with ageing or some NCDs.

Moreover, in this study, tyrosinase inhibitory activity was also observed in the extract L-A3-02-80W, which exhibited an IC_{50} value of 0.29 mg/mL—lower than that of kojic acid. This result is consistent with the report by Theeraraksakul *et al*. (2023), who demonstrated tyrosinase inhibition in mushroom extracts. Notably, the ten low-molecular-weight compounds identified in L-A3-02-80W have not previously been associated with tyrosinase inhibitory activity. Thus, the present findings provide the first evidence suggesting that certain constituents in L-A3-02-80W possess effective tyrosinase-inhibiting properties. Furthermore, the high levels of phenolics/polyphenolics, flavonoids, and terpenoids detected in L-A3-02-80W and O-A3-01-80W may contribute to their tyrosinase inhibitory activity. This observation is in agreement with previous studies reporting that phenolics/polyphenolics, flavonoids, and terpenoids exhibit a wide range of pharmacological properties (Alseekh *et al*., 2020), including antioxidant and anti-tyrosinase activities (Albuquerque *et al*., 2021).

Previous reports explained that terpenoids are phytochemical compounds that show biological properties such as antioxidant, anti-tumor, anti-inflammatory, antibacterial, anti-viral, and anti-malarial. Moreover, there are types of terpenoids that can increase transdermal absorption, prevent cardiovascular disease, and express hypoglycemic activity (Yang *et al*. 2020). In addition, terpenoids showed cholinesterase inhibitor activity, which can be developed as a drug for Alzheimer's disease (Min *et al*. 2022). Therefore, the antioxidant and anti-tyrosinase properties of *D. indusiata* extract might be associated with the representation of these compounds.

The tyrosinase inhibition potential of these mushroom extracts suggests possible applications in various beauty and health products, particularly skin-whitening formulations or products aimed at reducing hyperpigmentation. Mushroom extracts may serve as alternatives to hydroquinone, which is known for its high toxicity, or kojic acid, which can cause adverse effects (Owolabi *et al*. 2020). They may also represent a more cost-effective substitute for the relatively expensive alpha arbutin. In addition, the risk and symptoms of neurodegenerative diseases such as Parkinson's or Alzheimer's diseases (Min *et al*. 2022) may also be overcome using tyrosinase inhibitors contained in *D. indusiata* extract.

Furthermore, L-A3-02-80W and O-A3-01-80W were evaluated for cytotoxicity using Vero cells and HGF cells, which represent normal kidney and liver cell lines, respectively. The results demonstrated that, at a concentration of 0.5 mg/mL, both cell types exhibited greater than 80% viability, indicating that the extracts possess low cytotoxicity. At concentrations close to the IC_{50} values obtained from the antioxidant and anti-tyrosinase assays, only minimal toxicity towards normal cells was observed. Therefore, the IC_{50} values for cytotoxicity are likely to be higher than 0.5 mg/mL, further supporting the safety of these extracts under the tested conditions.

CONCLUSIONS

The findings of this study demonstrate that the internal tissue of *Dictyophora indusiata* possesses notable antioxidant and anti-tyrosinase activities in vitro, together with minimal cytotoxicity towards Vero and HGF cells. GC–MS analysis identified approximately ten low-molecular-weight compounds in the extracts, several of which have been previously associated with biological activities relevant to the outcomes observed in this work. Polyphenolics/flavonoids are the compounds that exhibit antioxidant properties on TLC. The consistency of compound profiles between extraction batches further supports the reliability of the extraction and analytical procedures employed.

Overall, the results indicate that the internal tissue of *D. indusiata* represents a promising candidate for development as a functional food ingredient. The extraction method used in this study offers key advantages, including safety, low cost, and operational simplicity, suggesting its potential suitability for future applications within the food industry. Further research, particularly involving in vivo studies and compound-specific bioactivity verification, is recommended to strengthen the evidence for commercial and nutritional utilisation of this mushroom tissue.

CONFLICT OF INTEREST

The author(s) declares no conflict of interest.

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AUTHORS' CONTRIBUTIONS

Supawadee Patathananone: Conceptualization, Visualization, Funding acquisition, Writing – original draft.
Sebrian Yusbani: Investigation, Methodology.
Orathai Peethong: Investigation, Methodology.
Wuttisak Kunu: Formal analysis, Validation, Writing – review & editing.

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