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Metabolite profiling, antioxidant, antibacterial, and carbohydrate hydrolyzing enzyme inhibition activities of *Drymaria cordata*

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ABSTRACT

Introduction: *Drymaria cordata* is used traditionally against hyperglycemia. In this research the methanol (DCM), hexane (DCH), and water (DCW) extracts of *D. cordata* were investigated for their metabolite profiling, antioxidant, antibacterial, and carbohydrate hydrolyzing enzyme inhibitory activities.

Methods: The antidiabetic activities of the extracts were investigated using the α-amylase and α-glucosidase (carbohydrate-hydrolyzing enzymes) inhibition assays and yeast glucose uptake assays. Antibacterial investigation of *D. cordata* extracts was done against methicillin-resistant *Staphylococcus aureus* (MRSA) and β-lactam-resistant *Escherichia coli*. The zone of inhibition and minimum inhibitory concentration (MIC) were observed.

Results: GC-MS metabolite profiling revealed the presence of stearyl aldehyde, henicosanal, glycidyl palmitate, eicosane, phytol, octacosanal, and neophytadiene. The DCM extract had a higher phenolic (168.19 ± 3.34 mg gallic acid equivalent/g), flavonoid (843 ±11.55 mg rutin equivalents/g), and ferric reducing potential (556.083 ± 6.51 mg ascorbic acid equivalent/g) than the DCH and DCW extracts. Also, DCM showed its greatest scavenging activity with a minimum IC50 value using the ABTS assay. DCM extract had the highest zone of inhibition and lowest MIC value against *E. coli* and *S. aureus*. Carbohydrate-hydrolyzing enzymes were inhibited, with DCM extract having minimum IC50 values of 714.66 µg/ml and 508.94 µg/ml. Yeast glucose uptake assays confirmed the highest efficacy of DCM extract for glucose uptake by yeast cells.

Conclusion: *Drymaria cordata*, especially DCM, has the potential to be considered an effective phytopharmaceutical drug for the treatment of oxidative stress, bacterial infections, and type 2 diabetes.

Implication for health policy/practice/research/medical education:
Our findings show the inhibition of carbohydrate hydrolyzing enzymes using *Drymaria cordata* extracts, which might be a promising candidate for the treatment of diabetes.

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Introduction

Pharmacological investigations encompass an intriguing arc of traditional knowledge from the indigenous community. The present pharmacopeia manufactures about 25% of the herbal and synthetic drugs using bioactive compounds from medicinal plants. Secondary metabolites have specific properties that are evolutionarily optimized to perform various metabolic functions, such as binding target proteins and other biomolecules. Plant-based herbal medicines and their derived products are well-known for having fewer side effects. Medicinal plants are known for having secondary metabolites such as alkaloids, cardiac glycosides, phenols, flavonoids, terpenes, and terpenoids. Bioactive compounds have hormone-like actions that help fight several diseases, such as hyperglycemia, hypertension, cancer, oxidative stress,

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and bacterial infections (1,2).

Diabetes mellitus is characterized by abnormal blood glucose levels resulting from insulin dysfunction (3). According to estimates, 463 million (9.3%) people across the globe had diabetes in 2019, and its percentage is expected to rise to 10.2%, i.e., 578 million, and 10.9%, i.e., 700 million, by 2030 and 2040, respectively (4). Diabetes is associated with several fatal consequences, such as kidney dysfunction, lactic acidosis, organ failure, and liver problems (5). In India, 67% of diabetic patients use naturopathy or Ayurveda. The ethanol extract of a shirishadi polyherbal drug (*Albizia lebbeck, Cyprus rotundus, Solanum xanthocarpum*) was investigated and confirmed the inhibition of amylase and glycosidase enzymes (6). Flavonoids of *Citrus reticulata* exhibit antioxidant, anticancer, anti-inflammatory, antifungal, antibacterial, and antioxidant activities (7). Polymethoxyflavones are potential biologically active compounds found in the peel of *C. reticulata* linked to a variety of biological activities, including the inhibition of sterol regulatory element protein binding, reduction of nitric oxide production, and antiproliferative effects on tumor cancer cells (8). Similarly, α-amylase and α-glucosidase inhibitory activity of *C. reticulata* peel extracts was investigated to control diabetes (9).

The prevalence of bacterial infections is a worldwide health concern. Some bacteria, such as *Staphylococcus aureus* and *Escherichia coli*, cause various human infections in specific organs. For instance, *S. aureus* occurs within the skin and mucous membranes and causes infections of the soft tissues. Also, it spreads easily through the bloodstream and can cause various infections, like lung infections, heart valve infections, and abdominal and urinary tract infections. Excessive use and abuse of antibiotics are the key factors that cause antibiotic resistance (10). The use of antibiotics in the treatment of COVID-19 has elevated the risk of antimicrobial resistance, making this a global concern (10).

Plant-based secondary metabolites are also known to have antioxidant properties. Antioxidants are well-known for their ability to scavenge free radicals such as peroxide, hydroperoxide, and lipid peroxyl radicals. The accumulation of free radicals in the body causes a condition known as oxidative stress. Oxidative stress influences the pathogenesis of diabetes, asthma, stroke, cancer, infertility, cardiovascular disorders, neurodegenerative disorders, and urolithiasis (11). Diabetes metabolism involves the production of free radicals by glucose oxidation, protein glycation, and the succeeding breakdown of glycated proteins. A large number of free radicals in combination with low antioxidant levels of enzymes induce enzyme deactivation resulting in damage to cells (12). Bacterial pathogens are able to circumvent oxidative stress conditions and colonise the host cell. Numerous microbes, including *S. aureus*, can persist facultatively within human cells under stress conditions (13). The advent of type 2 diabetes mellitus (T2DM), antibiotic resistance, and free radical toxicity has sparked a rebirth of research into the potential benefits of bioactive substances, as well as their equivalent safety and therapeutic efficacy.

Zebrafish embryos were used to evaluate the toxicity of *Drymaria cordata* methanol leaf extract. Microscopically, defects in the morphology and development of male and female zebrafish embryos were analysed, and the fifty percent lethal dose (LC50) of methanol extract was established. Several evident developmental abnormalities, including diminished pigmentation, heart rate, spinal curvature, oedema, and an underdeveloped yolk sac, as well as a slower rate of hatching and growth, were noticed after embryos were exposed to methanol extract (14).

Drymaria cordata (Linn.) Willd is a medicinal herb from the family Caryophyllaceae. It has antitussive, anti-inflammatory, and anxiolytic biological activities. Our aim is to investigate the metabolite profiling, antioxidant, antibacterial, and carbohydrate hydrolyzing enzymatic inhibitory properties of *D. cordata*. For the first time, a detailed comparative study of *D. cordata* was done to depict the bioactive components present in different solvent extracts that are responsible for therapeutic activities such as diabetes, bacterial disorders, and antioxidant nature.

Material and Methods

Collection and process of extraction

Drymaria cordata specimens were procured from Machi (24.530417, 94.213295), situated in the Chandel district, state of Manipur, India. The herbarium specimen was submitted to the Manipur University Museum of Plants (MUMP), Manipur University, Imphal, Manipur, India and received the accession number, i.e., 001037 MUMP. The field collection was carried out in accordance with the approved norms published by the National Medicinal Plant Board, AYUSH, Government of India. A Soxhlet apparatus was used to obtain crude plant extracts. The whole plant specimens were air dried for 10 days in a shaded location (27 °C), and the dried whole plant samples were powdered. After that, samples were extracted using three solvents: methanol, hexane, and water, using the Soxhlet extraction method. The viscous semisolid crude extracts were obtained using a rotary vacuum evaporator. The crude extracts were kept at 4 °C in dark containers for further use.

Metabolite profiling

Metabolite profiling was done using ultra-violet (UV), Fourier-transform infrared spectroscopy (FTIR) and gas chromatography mass spectrometry (GCMS) analysis of methanol (DCM), hexane (DCH), and water (DCW) extracts of *D. cordata*. A DLAB UV-1000 spectrophotometer was employed to conduct UV-visible spectroscopy in the 300-700 nm regions. FTIR

(PerkinElmer FTIR Spectrometer) was used for the study of plant extracts, i.e. DCM, DCH, and DCW using a frequency that ranged from 4000 to 400 cm^{-1}. GCMS analysis was done for the screening of metabolites present in crude extract using GCMS-QP2010 Plus. A GCMS QP2010 plus was provided with a thermal desorption system (TD 20) with ionisation of electrons and a constant flow rate of helium (1.21 mL/min). The analysis of metabolites was done using the mass spectral libraries (National Institute of Standards and Technology and the WILEY 8 libraries).

Antioxidant activities

Quantification of total phenolic and flavonoid contents

The total phenolic content (TPC) of extracts from *Drymaria cordata* was determined using the Folin-Ciocalteu technique (15). As a standard, gallic acid was used, and the absorbance was observed at 765 nm. The TPC was determined in the term of gallic acid (mg) equivalent per gram of plant extract (mg GAE/g extract). The colorimetric methodology of aluminium chloride was employed for estimating the total flavonoid content (16). Rutin was used as a standard. A spectrophotometer was used for the measurement of absorbance at 506 nm. TFC was calculated in milligrams of rutin equivalent per grams of plant extract.

ABTS free radical scavenging assay

The production of $ABTS^{\bullet+}$ cationic radical was done by the reaction of 7 mM ABTS (9.5 mL) with 100 mM potassium persulfate (245 µL). Dilution of the $ABTS^{\bullet+}$ reaction mixture was done using 0.1 M of potassium phosphate (pH 7.4). The wavelength of 734 nm was used for taking absorbance values. The reaction solutions were generated by mixing 2.90 mL of ABTS with 100 µL of extract or standard (25-1000 µg/mL). Ascorbic acid was taken as a standard solution. For control test solution, 100 µL of methanol was used instead of the sample (17). The activity of ABTS to scavenge free radicals was measured using the following equation:

$$\text{Percentage (\%) of antioxidant activity} = [(Abs_c - Abs_s)/Abs_c] \times 100$$

Abs_c and Abs_s represent the absorbance of the control and sample, accordingly.

Ferric reducing antioxidant power (FRAP) assay

The Benzie and Strain methodology was employed for determining the FRAP (18). TPTZ solution was prepared using 40 mM HCl and 30 mM of acetate buffer with a pH of 3.6. The reaction mixture contained 10 mM of TPTZ solution mixed with a 20 mM solution of ferric chloride. After that, for 15 minutes, the reaction solution was maintained at 37 °C. Further, FRAP reagent (2.85 mL) was mixed with 150 µL of plant samples or standard (25-1000 µg/mL). The value of the absorbance was determined at 593 nm following 30 minutes of the incubation process in darkness. The standard graph was generated utilizing ascorbic acid. The FRAP values were determined using ascorbic acid equivalents per grams of plant extract.

Antibacterial activity

The Kirby-Bauer disc diffusion susceptibility experiment was used to assess the antibacterial attributes of *D. cordata* extracts. Bacterial pathogens were obtained using the MTCC (Microbial Type Culture Collection and Gene Bank) located in Chandigarh, Punjab, India. Methicillin-resistant *S. aureus* (MTCC 11949) and β-lactams-resistant *E. coli* (MTCC 1652) bacterial strains were used. At 37 °C, bacterial strains were cultivated for 12 hours using Luria-Bertani broth culture media. Bacterial culture media were centrifuged for ten minutes at 10 000 rpm. Gentamycin was used as a positive control. An inoculum of 20 µL having bacterial concentration of 10^5 CFU mL^{-1} was pipetted out and poured onto petri plates. Using a sterilised glass spreader, we spread the cultures around the petri dish. A sterile, clean, clear paper disc (5 mm) was used for the disc diffusion experiment. The sterile discs were submerged with plant extract concentrations ranging from 25 to 1000 µg/mL. After that, agar petri plates were wrapped using parafilm, and maintained at 37 °C for 10-16 hours. Values regarding the minimum inhibitory concentration (MIC) and zone of inhibition (ZOI) were determined in triplicate.

In vitro antidiabetic effect

Inhibition of α-amylase enzyme

Sodium phosphate buffer (0.02 M) rendered with 0.006 M NaCl and pH 6.9, was used to produce a 0.5 mg/mL solution of α-amylase. Plant extracts (500 µL) with the concentrations ranging from 25 to 1000 µg/mL were mixed and incubated for 15 minutes at 27 °C. Sodium phosphate buffer (0.2 M) was used for the preparation of 1% starch solution. After mixing 1 mL of the starch solution, the reaction was maintained at room temperature for 15 minutes. Dinitro-salicylic acid (DNSA) (1 mL) was used to stifle the reaction, and the mixture was allowed to stand for 10 minutes at 90 °C. Water (15 mL) was used for the further dilution of reaction mixtures. The measurement of absorbance was carried out using a wavelength of 540 nm. Acarbose served as an accepted standard drug reference for the experiment (19). IC50 values were also determined. The percentage of α-amylase inhibition was calculated using the following formula:

$$\text{Percentage Inhibition (\%)} = [(Abs_c - Abs_s)/(Abs_c)] \times 100$$

Whereas Abs_c represents for the reaction solution devoid of extracts or standard and Abs_s for the reaction solution containing both extracts and standard.

Mode of α-amylase inhibition

The extract exhibiting a low IC50 value was used to determine the mode of α-amylase inhibition. In one set of test tubes, plant extract (250 µL) was combined with 250 µL of prepared solution of α-amylase. The mixture solution underwent a pre-incubation period of ten minutes at 25 °C. Similarly, another set of test tubes with 250 µL of α-amylase solution and 250 µL of phosphate buffer having pH 6.9 were prepared and incubated. After mixing 250 mL of starch solution with concentrations ranging from 0.5 to 5 mg/mL into both sets of test tubes, the reaction was allowed to kept at 25 °C for 10 minutes. Finally, DNSA (500 µL) was used for the reaction inhibition, and the solution was heated for 5 minutes. The maltose standard calibration curve was permuted to the velocities of reaction and used to quantify the reducing sugars. Using (1/V vs 1/S), a double reciprocal graph was generated, where (V) stands for the reaction's velocity and (S) for the substrate's concentration. The analysis of double reciprocal plot (Lineweaver-Burk) was done by Michaelis-Menten kinetics (20).

Inhibition of α-glucosidase enzyme

The inhibition of α-glucosidase enzyme was estimated using a methodology developed by Kim et al (21). A 0.01 mg/mL solution of α-glucosidase was prepared and incubated with 50 µL of sample (plant/standard) solutions for 10 minutes at 37 °C. The PNPG (4-nitrophenyl-β-D-glucopyranoside) (3 mM) substrate was prepared using phosphate buffer (20 mM) having a pH of 6.9. Following pre-incubation, 50 µL of PNPG was used for further reaction mixture and incubated for 20 minutes at 37 °C. For reaction termination, about 2 mL of 0.1M Na_2CO_3 was mixed to test tube solution. For the control test of tube's reaction, 50 µL of buffer solution was added in place of the sample. Acarbose served as an accepted standard drug reference for the experiment. A measurement of absorbance was carried out using a wavelength of 405 nm. The percentage inhibition of α-glucosidase was measured with the following formula

$$\text{Inhibition (\%)} = [(Abs_c - Abs_s)/(Abs_c)] \times 100$$

Where Abs_c denotes the reaction devoid of extracts or standard and Abs_s depict the solution mixture using extract or standard solution.

Mode of α-glucosidase inhibition

The extract exhibiting the lowest IC50 value was used to evaluate the manner of α-glucosidase inhibition. In the first set of test tubes, 100 µL of α-glucosidase was incubated with 50 µL of extract for ten minutes at 25 °C.In the second set of test tubes, 100 µL of α-glucosidase enzyme had been incubated with 50 µL of phosphate buffer solution (pH 6.9). To start the reaction, 50 µL of PNPG (0.5-5.0 mg/mL) substrate was mixed into the pair of test tubes and kept at 25 °C for 10 minutes. After that, Na_2CO_3 (500 µL) was used to inhibit the process of reaction. The paranitrophenol standard calibration curve was used for the quantification of reducing sugars. A double reciprocal graph was plotted between reaction velocity *(V)* and substrate concentration (*S*). Michaelis-Menten kinetics was employed to determine the double reciprocal plot (Lineweaver-Burk) (20).

Yeast uptake glucose assay

The yeast glucose uptake assay was carried out using the technique published by Shehzadi et al (22) with some modifications. Yeast cells (*Saccharomyces cerevisiae*) were submerged in distilled water in order to obtain a 10% (v/v) suspension, and subjected to repetitive centrifugation (3000 g) for a period of 5 minutes. Different concentrations of glucose, i.e., 5mM, 10mM, and 25mM were mixed into 100 µL of plant samples (25–1000 µg/mL) and kept in incubation at 37 °C for 10 minutes. To begin the reaction, yeast suspension (100 µL) was incorporated and reaction solution was shaken strenuously for 20 seconds and kept in incubation at 37 °C for 60 minutes. The reaction solution was allowed to be centrifuged at 2000 rpm. To end the reaction, DNSA was incorporated into the reaction solution and heated at 85 °C for 5 minutes. Using a spectrophotometer, the quantity of supernatant glucose was observed at wavelength of 540 nm. The percentage (%) uptake of glucose by yeast cell was measured with the following formula:

Percentage increase in uptake of glucose = [(Abs. of control– Abs. of sample) / (Abs. of control)] × 100

Statistical analysis

All the experimental data were done in triplicates. Obtained data values were depicted as mean ± standard error means (SEM). The level of significance ($P \leq 0.05$) was determined using a one-way ANOVA with Duncan multiple comparison tests.

Results

Metabolite profiling

The UV-Vis profile of all three extracts revealed a decreasing trend of absorption from 300 to 450 nm, indicating the presence of flavonoids, flavonoids derivatives, alkaloids, phenolic compounds, tannins, and carotenoids (Figure 1). Figure 1A represents the UV-Vis spectroscopy of DCM extract that shows a major peak of chlorophyll at 660 nm. Figure 1B shows the UV-Vis profile of DCH, which indicates the presence of two small peaks, i.e., 502 and 533 nm, and confirms the presence of terpenoids (23). Other peaks at 666 nm provide confirmation of chlorophyll compounds (24). Figure 1C represents the absorption spectra of DCW extract, which do not indicate any specific absorption peak.

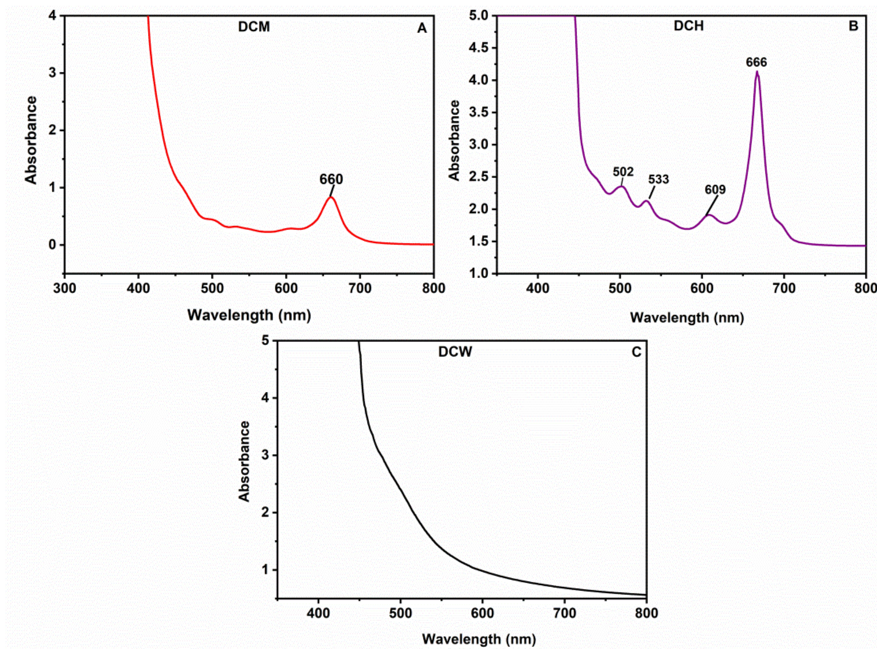

Figure 1. UV-visible spectrum analysis of *Drymaria cordata* extracts A: Methanol extract (DCM) B: Hexane extract (DCH) C: Water extract (DCW).

The FT-IR spectrum of the extracts was analysed and presented in Figure 2. Plant extracts showed a characteristic peak at 3697 cm^{-1} and confirmed the O-H bending that depicts the presence of alcohol (Figure 2A). The sharp absorption peaks at 3357, 3352, and 3344 cm^{-1} revealed the presence of secondary amines, and the stretch of N-H band was indicated (Figure 2A, B, and C). Two peaks at 2930 and 2917 cm^{-1} revealed the presence of the methylene group (Figure 2A and B). The N-H stretch was observed at 2342 and 2361 cm^{-1} depicting the presence of amino-related components (Figure 2A and B). The observed peak at 1728 cm^{-1} represented the aromatic ring (Figure 2A). The observed peaks at 1608 and 1636 cm^{-1} revealed the presence of amines and nitrates (Figure 2A, B and C). The O-H stretch confirmed the presence of phenol at 1369 and 1374 cm^{-1} peaks (Figure 2A and B). The peaks obtained at 1259 cm^{-1} indicated CN stretch showing the presence of an amine group (Figure 2B).

GC-MS analysis was done to investigate the presence of metabolites in the crude extract of *D. cordata*, i.e., DCM, DCH, and DCW. The chromatogram reveals the presence of 36 compounds in hexane extract (DCH), followed by 35 bioactive compounds in methanol extract (DCM), and 15 compounds in aqueous extract (DCW). The GCMS chromatogram of DCH extract revealed several bioactive compounds, including the most dominant compounds such as stearyl aldehyde (23%), henicosanal (10%), glycidyl palmitate (4%), eicosane (4%), phytol (3.3%), eicosanal (3%), octacosanal (3%), and neophytadiene (2%) (Supplementary file 1, Table S1). The GCMS analysis of DCM extract revealed prevailing compounds such as hexanedioic acid (22%), nonadecadiene (10%), hexadecanoic acid (12%), hexadecen-1-ol (8%), and octadecadienoic acid (7%), which are responsible for several therapeutic activities (Supplementary file 1, Table S2). The presence of hexadecanoic acid (8.18%), hexanedioic acid (6.78%), hexadecen-1-ol (6.18%), neophytadiene (4.75%), octadecadienoic acid (2.73%), and nonadecadienoic acid (3.85%) was demonstrated by GCMS metabolite profiling of water extract (DCW) (Supplementary file 1, Table S3).

Antioxidant activities

Quantification of total phenolic and flavonoid contents

Total phenolic concentrations of *D. cordata* extracts were expressed as milligram of gallic acid equivalents per gram of plant extract. The experimental data were obtained using a calibration curve of gallic acid, i.e., $y = 0.0007x + 0.1846$, $R^2 = 0.9412$.

The DCM extract had the highest phenolic contents (168.19 ± 3.34 mg GAE/g) than the DCH and DCW extracts (133.43 ± 2.97 milligrams of GAE/g and 99.62 ± 3.34 mg GAE/g, respectively). The total flavonoid content of *D. cordata* extracts was expressed in mg of rutin equivalents per gram of the extract. The results of experiments were observed using a calibration curve ($y = 0.0001x + 0.048$, $R^2 = 0.94$) of rutin. The DCH had the highest flavonoid content (909.66 ± 8.82 mg RE/g) than the DCM and DCW extracts (843 ± 11.55 mg RE/g and 653 ± 5.77 mg RE/g, respectively).

ABTS free radical scavenging assay

The ABTS is the most widely used approach for determining the free radical scavenging activity of plant

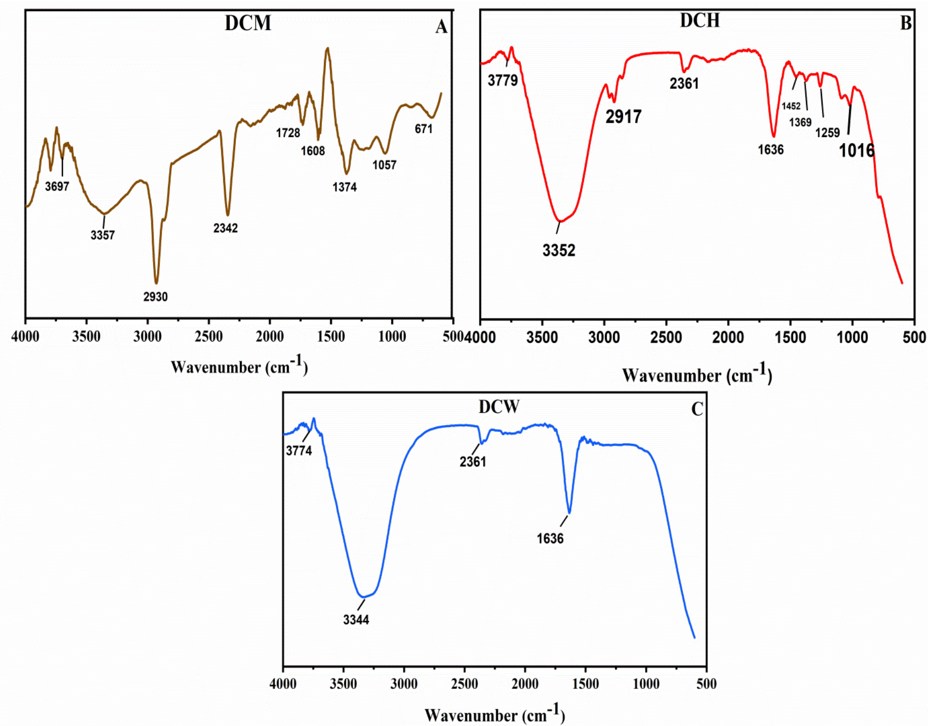

Figure 2. FTIR spectrum of *Drymaria cordata* (A): Methanol extract (DCM); (B): Hexane (DCH) extract; (C): Water (DCW) extract.

extracts and their food products. In a dose-dependent manner, all plant extracts demonstrated good scavenging capacity. The maximum scavenging activity was observed using DCM extract, i.e., 59.33 ± 0.34 %, followed by DCW and DCH (49.81 ± 0.21 and 43.52 ± 0.31 %, respectively) extracts at 1000 µg/mL. An ABTS radical scavenging potency with a minimum IC50 value was recorded for DCM (599.31 µg/mL), followed by DCW and DCH (916.07 and 1720.10 µg/mL, respectively) (Figure 3). The percentage scavenging activity and IC50 values of the extracts were compared with those of the ascorbic acid used as a standard.

Ferric reducing antioxidant power (FRAP) assay

The antioxidant power of DCM, DCH, and DCW extracts was determined using the conversion of ferric ions (Fe^{3+}) to ferrous (Fe^{2+}) ions. The conversion of ferric ions to ferrous ions results in the formation of a ferrous tripyridyltriazine complex, i.e., Fe^{2+}-TPTZ (blue-coloured complex). The yellow test solution in the FRAP assay shifted to blue depending on the reduction capability of extracts or compounds. The calibration curve of ascorbic acid (standard) was used to quantify the ferric reducing potential in the form of milligram of ascorbic acid equivalent per gram of plant extract (mg AAE/g).

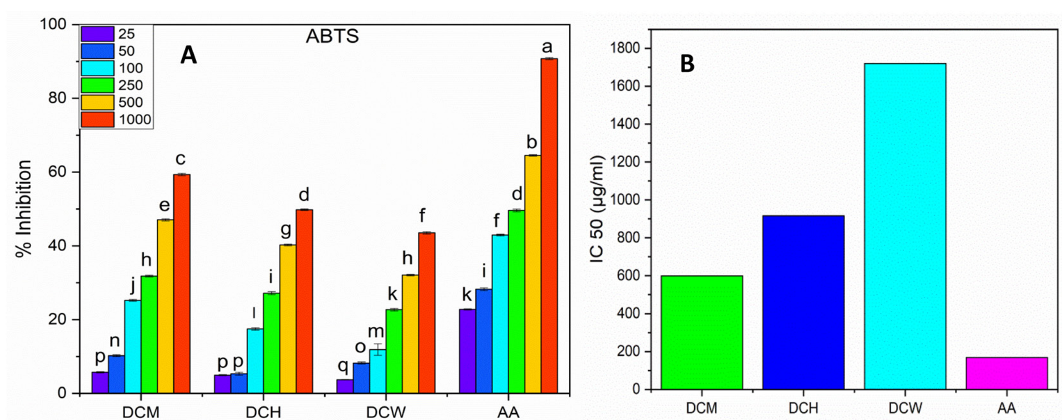

Figure 3 Antioxidant activity of *Drymaria cordata* extracts, i.e., methanol (DCM), hexane (DCH) and water (DCW) extracts. **(A)** ABTS ((2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) free radical scavenging activity, **(B)** IC50 values of *D. cordata* extracts. Different letters on columns indicate significantly different ($P < 0.05$).

The calibration curve (y = 0.0004x + 0.0589, R^2 = 0.957) of ascorbic acid (25–1000 µg/mL) was used to derive the results. The DCM had the highest ferric reducing antioxidant power (556.083 ± 6.51 mg AAE/g) compared to the DCH and DCW extracts (501.92 ± 4.41 and 486.083 ± 6.29 mg AAE/g, respectively).

Antibacterial activity

Drymaria cordata extracts (DCM, DCH, and DCW) were tested using pathogenic bacteria, i.e., *S. aureus* and *E. coli*. The inhibition zone was observed and measured in triplicate. The zones of inhibition were presented in the form of mean ± SEM. ANOVA and Duncan's tests were used to illustrate the statistically significant differences, i.e., $P \leq 0.05$. Figure 4 demonstrates the antibacterial potential plant extracts, i.e., DCM, DCH, and DCW. Our findings showed that all DCM, DCH, and DCW extracts were beneficial in reducing bacterial growth. DCM was the efficient extract against *E. coli* strain at various doses (25-1000 µg/mL) in retarding microbial growth and had a ZOI of 18.33 ± 0.16 mm, followed by DCW and DCH (12.5 ± 0.28 and 10.667 ± 0.33 mm) at 1000 µg/mL. Experiments were carried out to assess their MIC against both pathogenic bacterial strains. The highest antibacterial potency against *E. coli* with a minimum MIC value was recorded for DCM (50 ± 2.89 µg/mL), followed by DCW and DCH (100 ± 2.89 and 125 ± 2.9 µg /mL, respectively).

Figure 4A demonstrates the antibacterial activity against *S. aureus* by DCM, DCH, and DCW extracts. DCM was the most effective extract against *S. aureus* in retarding microbial growth and had a ZOI of 16 ± 0.29 mm, followed by DCW and DCH (11.5 ± 0.29 and 11.83 ± 0.167 mm) at 1000 µL, respectively. Experiments were carried out to assess their MIC against the *S. aureus* bacterial strains. The most efficient antibacterial efficacy against the *S. aureus* with a minimum MIC value was recorded for DCM (45 ± 2.89 µg/mL), followed by DCW and DCH extracts (95± 2.89 and 100 ± 2.89 µg/mL, respectively) (Figure 4C).

Antidiabetic activities

Inhibition of α-amylase enzyme

The antidiabetic potential of the *D. cordata* extracts was investigated in terms of the percentage inhibition of alpha-amylase and compared with that of acarbose as a standard drug. DCM, DCH, and DCW extracts exhibited enzyme inhibition activity in a dose-dependent manner and the percentage inhibition surge considerably with incresed concentrations (25-1000 µg/mL). The IC50 values were calculated by plotting the percentage inhibition of amylase concentration of extracts.DCM was the most efficient extract for α-amylase inhibition and had an inhibition percentage of 66.66 ± 0.09%, followed by DCW and DCH (61.65±0.13% and 59.68±0.13%) at 1000 µg/mL, respectively (Figure 5A). The IC50 values of DCM,

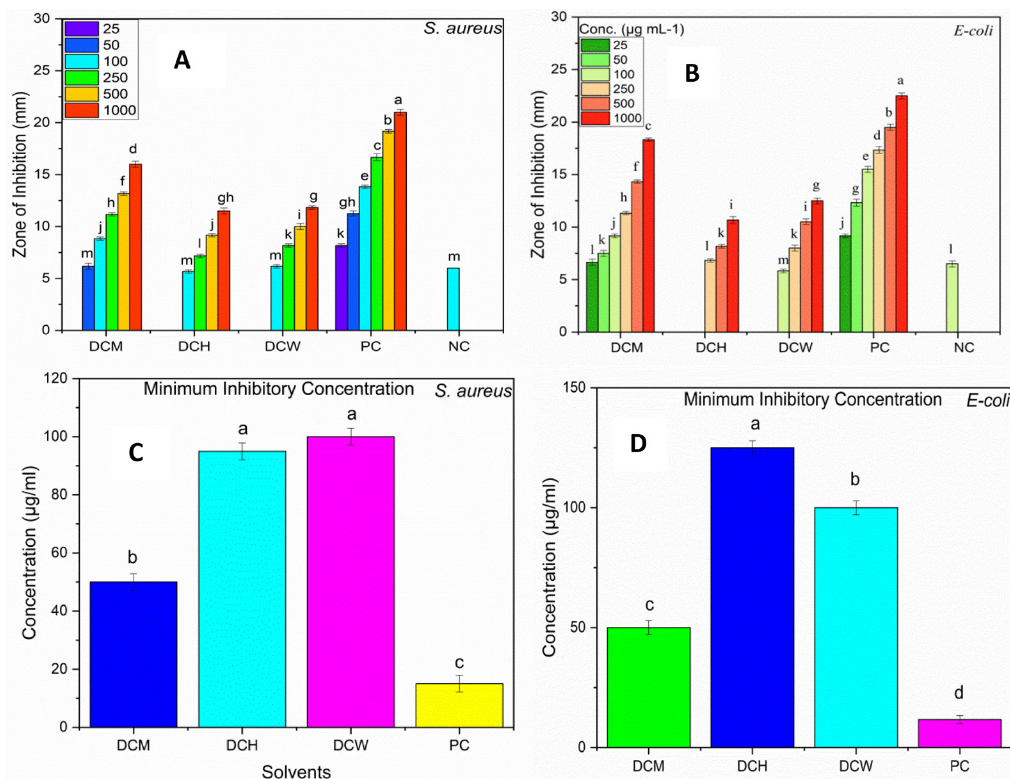

Figure 4. Antibacterial potential of *Drymaria cordata* extracts, i.e., methanol (DCM), hexane (DCH) and water (DCW) extracts. (A-B): Zone of inhibition (ZOI), (C-D): Minimum inhibitory concentration (MIC) against *Staphylococcus aureus* and *E-coli* bacterial strains.Different letters on columns indicate significantly different ($P < 0.05$).

DCH and DCW extract were found to be 714.66 µg/mL, 780.44 µg/mL and 808.12 µg/mL, respectively (Figure 5B). A Michaelis-Menten plot was derived between reaction velocities (V) and substrate concentration (S). For each substrate concentration, the reaction velocities are measured and shown in a Michaelis-Menten plot. The resulting curve looks like a hyperbola, where reaction velocity rises quickly at first but finally reaches its maximum level. Since DCM inhibits the enzyme more than the control, the values of reaction velocity were lower in comparison to the control (Figure 5C). The Lineweaver-Burk graph was used to represent the mode of inhibition using α-amylase activity, and it revealed that the DCM extract presented to show the non-competitive enzyme inhibition (Km increased, Vmax decreased) of α-amylase enzyme (Figure 5D).

Inhibition of α-glucosidase enzyme

DCM, DCH, and DCW extracts exhibited α-glucosidase enzyme inhibition activity in a dose dependent manner, and the percentage inhibition surged considerably with higher doses of the extract (25–1000 µg/mL). DCM was the most effective extract for the inhibition of α-glucosidase and had an inhibition percentage of 65.46 ± 0.14% followed by DCW and DCH (60.85 ± 0.11and 57.44 ± 0.18%, respectively) at 1000 µg/mL (Figure 6A). The IC_{50} values of methanol, hexane, and aqueous were found to be 508.94 µg/mL, 594.21 µg/mL, and 653.87 µg/mL, respectively (Figure 6B). A Michaelis-Menten plot was drawn between reaction velocities (V) and substrate concentration (S). The extract (DCM) inhibited enzymes more than the control, and the reaction velocities were lower in comparison to the control (Figure 6C). The Lineweaver-Burk plot was employed to identify the mode of inhibition of the α-glucosidase enzyme activity by the methanol extract (DCM) and depicted the non-competitive enzyme inhibition (Km increased, Vmax decreased) (Figure 6D).

Glucose uptake assay

Glucose uptake by yeast cells was investigated to determine the effect of various bioactive compounds present in plant extracts (DCM, DCH, and DCW) on hyperglycemia. For in vitro investigation, three glucose concentrations, i.e., 5, 10, and 25 mM were used along with extract concentrations (25–1000 µg /mL) to examine transport across glucose yeast cells (Figure 7). After a certain time period, the supernatant glucose revealed the quantity absorbed by yeast cells. At 5 millimolar of glucose, the uptake capacity of the yeast cells using DCM extract at 1000 µg/mL was 60.52 ± 0.12%, followed by DCH and DCW (56.21 ± 0.11 and 54.47 ± 0.12 µg/mL, respectively) (Figure 7A). At 10 millimolar glucose, the yeast glucose uptake capacity using DCM extract at 1000 µg/mL was 56.07 ± 0.18 %, followed by DCH and DCW (48.61 ± 0.42 and 45.31 ± 0.11, respectively) (Figure 7B).

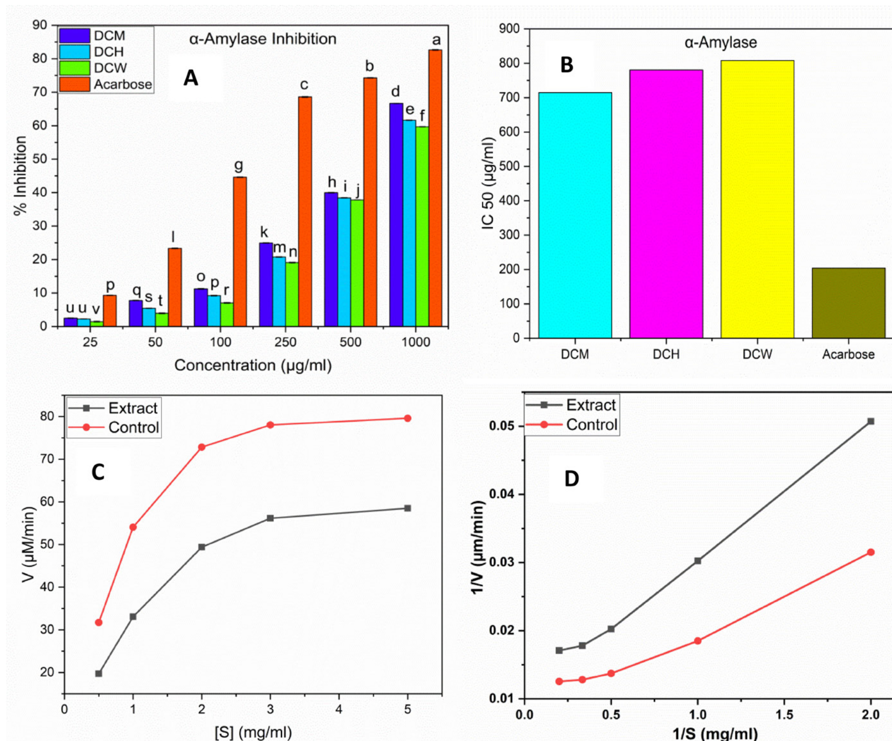

Figure 5. Inhibition potential and mode of inhibition using α-amylase. A: α-amylase; B: IC50 values; C: Michaelis-Menten plot; D: Lineweaver-Burk plot. DCH, DCM, and DCW represent respectively the methanol, hexane, and water extracts of *Drymaria cordata*. Different letters on columns indicate significantly different ($P < 0.05$).

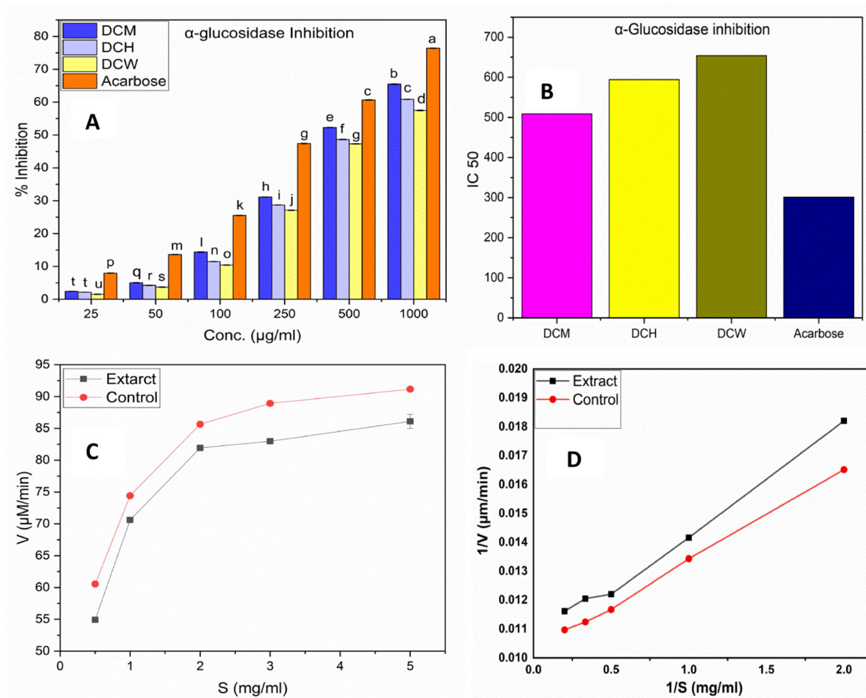

Figure 6. Inhibition potential and mode of inhibition of α-glucosidase enzyme A: Percentage inhibition (%) of α-glucosidase enzyme using DCM, DCH, and DCW extracts B: IC50 values; C: Michaelis-Menten plot; D: Lineweaver-Burk plot. DCH, DCM, and DCW represent respectively the methanol, hexane, and water extracts of *Drymaria cordata*. Different letters on columns indicate significantly different ($P < 0.05$).

At 25 millimolar glucose, the glucose uptake capacity at 1000 µg/mL for DCM extract was 50.4 ± 0.11%, followed by DCH and DCW (48.03 ± 0.11 and 43.75 ± 0.08 µg/mL, respectively) (Figure 7C).

Discussion

The present investigation was done to evaluate the comparative antioxidant, antibacterial, and antidiabetic activities of DCM, DCH, and DCW extracts. Our findings stemmed from total phenols, flavonoids, ABTS, and FRAP investigations and demonstrated that DCM extract had a higher antioxidant capacity than the other two extracts. The major phytochemical compounds with antioxidant capabilities found in plants are phenolics and flavonoids. Phenolic and flavonoid compounds have redox potential, which is responsible for the antioxidant activity of the plant extract (25). The extraction processes and the types of used solvents are accountable for the isolation of the bioactive compounds (26). Furthermore, bioactive compounds might be either polar or non-polar. Phenolic compounds are known for having hydroxyl groups, which allow their better solubility in polar organic solvents (27). Hence, methanol was chosen as the extraction solvent. Phenol and flavonoid compounds are the principal antioxidant components that have electron-donating hydroxyl groups, and their total quantity is directly related to the antioxidant capacity of plant extracts. Phenolic and flavonoid compounds are used for the prevention of oxidative stress and its related disorders. The presence of carotenoids, sugars, ascorbic acid, genetic diversity, geographical variance, and extraction procedures affect the number of phenolics and flavonoids (28). The capacity for reduction is related to the presence of reductones, which are antioxidant components that begin the process of splitting the chain of free radical mechanisms. ABTS is a free radical used to assess the in vitro antioxidant activity of plant extracts. Free radicals have an affinity for key biological elements such as cell membrane, proteins, and DNA of a cell. Free radicals interact with antioxidants present in extracts, which gradually neutralise them before occurring further harm. Our findings from the ABTS and FRAP scavenging assays are consistent with the result of high phenolic and flavonoid concentrations and confirm the better scavenging ability of DCM extract than DCH and DCW extracts. *D. cordata* extract has the capacity to donate electrons to free radicals and neutralise them to minimise oxidative damage to the cell.

Similarly, bacterial infections cause oxidative damage as a result of altered metabolic pathways. Bacterial toxicity has also been linked to organ damage and an increased risk of malignancy (13). In this regard, an antibacterial investigation was also done to check the antibacterial efficacy of *D. cordata* extracts. *D. cordata* extracts were also tested against *S. aureus* and *E. coli* bacterial strains. Our result shows similarity with the previous findings that the zone of inhibition was directly proportional to extract concentration. The values of MIC obtained using the methanol extracts (DCM) were generally lower than those

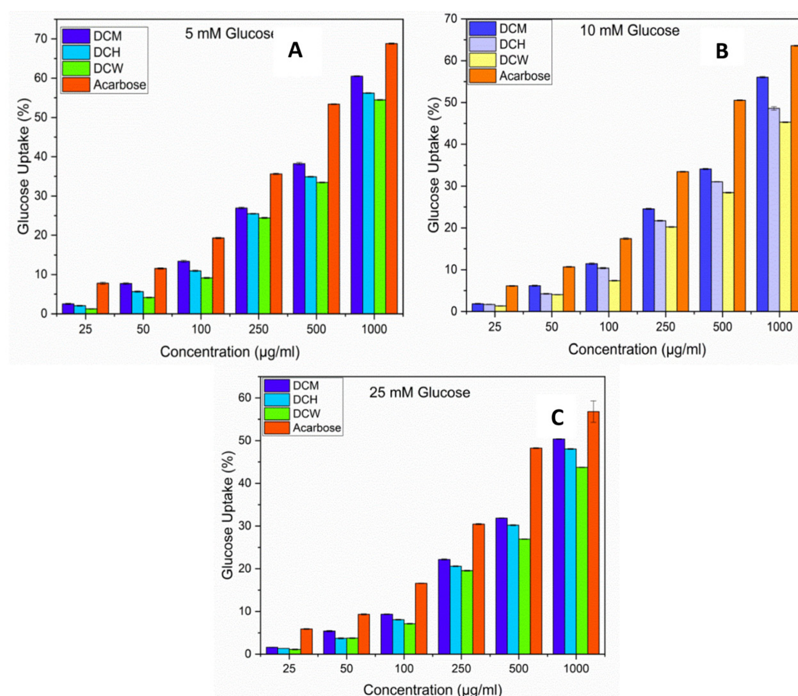

Figure 7. Effect of *Drymaria cordata* extracts on the absorption of glucose by yeast cells. A. 5 mM, B. 10 mM, and C. 25 mM of glucose concentration. DCH, DCM, and DCW represent respectively the methanol, hexane, and water extracts of *Drymaria cordata.*

of the hexane and aqueous extracts. So, it can be concluded that DCM extract has a better capacity to inhibit bacterial growth. The alcoholic extracts had stronger antibacterial activity than water and hexane extracts. Furthermore, it has been documented that phenols and their derivatives, fatty acid esters, terpenes, and other chemical compounds present in alcoholic extracts can affect several target sites in bacterial cells (29). Gentamicin was used as a standard drug for positive control, and methanol, hexane, and water were used as control solvents. GCMS analysis also demonstrated the list of bioactive compounds such as henicosanal, neophytadiene, eicosanal, and phytol, which are antimicrobial in nature. Gram-positive bacteria are more vulnerable to bioactive compounds present in plant extracts than gram-negative bacteria. Synthetic medications are also commonly adulterated and have negative effects, making them not only expensive but also ineffective for treating diseases. As a result, new infection-fighting therapeutics is needed to combat microbial illnesses (30). Our findings are consistent with prior research on the antioxidant and antibacterial properties of *Mespilus germanica* leaves methanolic extract. Our findings are consistent with prior research on the antioxidant and antibacterial properties of *M. germanica* methanol extract of leaves (31).

Hyperglycemia-induced tissue damage is exacerbated by oxidative stress, which contributes to the early development of T2DM. There is a surge of scientific interest at present in plant-based treatments capable of reversing oxidative stress and primary hyperglycemia (32). Alpha-amylase is a calcium metalloenzyme that breaks down starch glycosidic bonds in order to create simpler oligosaccharides or disaccharides. This enzyme is found in simpler microorganisms, plants, and mammals and is responsible for hydrolyzing -1,4-glucosidic linkages in amylose, amylopectin, and glycogen. Alpha-Glucosidase is present on the brush membrane of the small intestine. The enzyme delivers molecules of glucose throughout the bloodstream via carbohydrate metabolism (33). The antidiabetic activities of *D. cordata* extracts were investigated using the enzymes (α-amylase and glucosidase) inhibition and glucose uptake assays, and the obtained results were outstanding. In the preceding results, the *D. cordata* extract's ability to bind α-glucosidase was shown to be superior to that of amylase and can diminish or postpone diabetes mellitus. Our result shows that DCM extract has the best efficacy to act as a hydrolyzing enzyme inhibitor in comparison to DCH and DCW extracts, and it may be advised for diabetes therapy. The GC-MS data indicated a plethora of physiologically active chemicals with biological and pharmacological functions. According to the GCMS analysis, many antidiabetic compounds were present in the crude extract of the plant, such as eicosane, phytol, hexadecanoic acid, nonadecane, neophytadiene, and dodecane.

Literature shows that bioactive compounds are responsible for specific biological activities. Stearyl aldehyde is known for its anti-inflammatory and anti-apoptotic effects (34); henicosanal is used for its antibacterial activity and antidandruff efficacy (35);

glycidyl palmitate revealed its therapeutic potential as an anti-staphylococcal agent; eicosane is depicted as a wound healing, antifungal, and antidiabetic agent (36); phytol, is known for its antidiabetic, antimicrobial, and antioxidant activities (37); eicosanal and octacosanal are both known for antibacterial activities. Neophytadiene shows its pharmacological efficacy against bacterial disorders, diabetes, skin disease, and rheumatism (38). Hexadecanoic acid is widely known for antidiabetic and hypocholesterolemic activities (39). Octadecadienoic acid confirms its bioactive potential for antidiabetic and anticancer activities (40). Nonadecadiene and hexanedioic acid are known for antimicrobial and immunity booster agents. Hexadecen-1-ol is known for its efficacy against microbes and diabetes (34). The existence of bioactive components in extracts governs pharmacological activity. The presence of major bioactive compounds confirms the therapeutic potential of *D. cordata* such as anti-microbial, antioxidants, and diabetes. It was concluded from GCMS analysis that *D. cordata* extracts contained a high concentration of bioactive compounds with therapeutic efficacy that may be employed to discover and develop new drugs.

Enzyme inhibitors promote the delayed absorption of glucose after eating food. The usual limits are maintained by α-amylase and glucosidase inhibition in the circulation (41). Mode of α-amylase and glucosidase inhibition was also evaluated using methanol extract (DCM). For both the α-amylase and glucosidase enzymes, a double reciprocal graph was plotted using their respective standards (maltose and paranitrophenol) calibration curves. The Michaelis-Menten plot showed a hyperbola representing the initial rise in reaction velocity with an increase in substrate concentrations; it shows that DCM extract had the ability to limit the reaction velocity of carbohydrate hydrolysing enzymes (α-amylase and glucosidase). The Lineweaver-Burk plot (also known as the double reciprocal plot) is a graphical illustration of the Michaelis-Menten equations for the kinetics of enzymes. Enzyme kinetic investigations revealed non-competitive inhibitory mechanisms against the amylase and glucosidase enzymes, respectively. In non-competitive, the apparent value of V_{max} is decreased and that of K_m usually increased. The carbohydrate hydrolysing enzyme inhibitory potential of *Morinda lucida* leaf extracts showed a similar finding to our results (20). Postprandial hyperglycemia can be regulated by limiting the digestibility of carbohydrates, which is related to the inhibition of hydrolyzing enzymes (α-amylase and glucosidase) and is considered as a feasible preventative treatment for T2DM (42). Similar results were shown by DCM extract using the yeast glucose uptake assay. It was observed that the glucose uptake percentage by yeast cells surges with increasing concentrations of the extract but decreases with increasing concentrations of glucose (5-25 milli molar). *Cassia nemophila* pod (EECNP) ethanol extract and *Ajuga parviflora* extract were evaluated by yeast glucose uptake assay, which showed a similarity with our observations (5,43). The results also showed that the extracts could facilitate glucose transport across the yeast cell membrane, and it was recognized that the diffusion process helped glucose diffuse across the yeast membrane. The findings also suggest that the extracts can aid in the transport of glucose through the yeast cells using the diffusion process. The presence of *D. cordata* extracts allows yeast cells to absorb glucose more readily, which may result from both facilitated diffusion and higher glucose metabolism. Furthermore, the process of glucose uptake in yeast cells may differ from that occurs in other eukaryotes or human body cells. Glucose transport across the yeast membrane may utilise facilitated diffusion instead of the intervention of a phosphotransferase system of enzymes or an unknown alternative method. Several parameters influence the glucose movement across the cell membrane of yeast cell. Such factors include the amount of glucose and its metabolism within the cell. Glucose uptake will be preferred if interior sugar is converted into other metabolites (43). It will undoubtedly be fascinating to investigate the function of extracts (DCM, DCH, and DCW) *in vivo* experiments. This may aid in enhancing the uptake of glucose by adipose and muscular tissues. At last, it was confirmed that *D. cordata* was capable of binding and transporting glucose across the yeast cell membrane for further glucose metabolism.

Conclusion

The extract of *D. cordata* was reported to have a high number of bioactive compounds. The beneficial effects of its bioactive components and probably their synergistic effects are the primary rationale for therapeutic values. The methanol extract (DCM) of *D. cordata* is the most effective one against oxidative stress, pathogenic bacteria, and T2DM. This study also showed that carbohydrate hydrolysing enzymes (alpha amylase and glucosidase) inhibition of *D. cordata* is one of the strategies through which this plant reveals its antidiabetic potential. Based on the observed results, it is possible to recommend this plant as an excellent alternative for anti-oxidant, anti-bacterial, and anti-diabetic drugs.

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Methodology: Atul Arya, Suresh Kumar.

Supervision: Suresh Kumar.
Validation: Suresh Kumar.
Visualization: Dolly Kain.
Writing–original draft: Atul Arya, Dolly Kain.
Writing–review & editing: Suresh Kumar, Dolly Kain, Vandana, Khaling Mikawlrawng.

Conflict of interests

Authors declare no conflict of interest.

Ethical considerations

The authors of this article have strictly adhered to all ethical issues such as copyright infringement, data creation, duplicate publication, and redundancies. According to the authors, no animal or human subjects have been utilised in the present investigation.

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Supplementary files

Supplementary file 1 contains Tables S1-S3 and Figures S1-S4.

References

1. Arya A, Kumar S, Paul R, Suryavanshi A, Kain D, Sahoo RN. Ethnopharmacological survey of indigenous medicinal plants of Palampur, Himachal Pradesh in north-western Himalaya, India. Adv Tradit Med. 2023;23(1):169-212. doi: 10.1007/s13596-021-00607-1.
2. Arya A, Kumar S, Suryavanshi A, Kain D, Vandana. Biosynthesis of metallic nanoparticles from medicinal plants: a review. J Nanosci Technol. 2019;5(5):827-31. doi: 10.30799/jnst.277.19050502.
3. Suryavanshi A, Kumar S, Kain D, Arya A. Chemical composition, antioxidant and enzyme inhibitory properties of *Ajuga parviflora* Benth. Biocatal Agric Biotechnol. 2021;37:102191. doi: 10.1016/j.bcab.2021.102191.
4. Bindu J, Narendhirakannan RT. Role of medicinal plants in the management of diabetes mellitus: a review. 3 Biotech. 2019;9(1):4. doi: 10.1007/s13205-018-1528-0.
5. Suryavanshi A, Kumar S, Kain D, Arya A. In vitro antidiabetic, antioxidant activities and chemical composition of *Ajuga parviflora* Benth. shoot. J Herbmed Pharmacol. 2022;11(1):131-9. doi: 10.34172/jhp.2022.15.
6. Kajaria D, Ranjana, Tripathi J, Tripathi YB, Tiwari S. In-vitro α amylase and glycosidase inhibitory effect of ethanolic extract of antiasthmatic drug - Shirishadi. J Adv Pharm Technol Res. 2013;4(4):206-9. doi: 10.4103/2231-4040.121415.
7. Wang Y, Qian J, Cao J, Wang D, Liu C, Yang R, et al. Antioxidant capacity, anticancer ability and flavonoids composition of 35 citrus (*Citrus reticulata* Blanco) varieties. Molecules. 2017;22(7):1114. doi: 10.3390/molecules22071114.
8. Duan L, Dou LL, Yu KY, Guo L, Bai-Zhong C, Li P, et al. Polymethoxyflavones in peel of *Citrus reticulata* 'Chachi' and their biological activities. Food Chem. 2017;234:254-61. doi: 10.1016/j.foodchem.2017.05.018.
9. Ghauri S, Raza SQ, Imran M, Saeed S, Rashid M, Naseer R. Assessment of α-amylase and α-glucosidase inhibitory potential of *Citrus reticulata* peel extracts in hyperglycemic/hypoglycemic rats. 3 Biotech. 2021;11(4):167. doi: 10.1007/s13205-021-02717-8.
10. Ahanger AM, Kumar S, Kain D, Arya A, Vandana. Nanoscale zeolitic imidazolate framework–8 encapsulates crude extract of *Ajuga bracteosa* wall ex. Benth and enhanced their antibacterial efficiency. Inorg Chem Commun. 2021;133:108940. doi: 10.1016/j.inoche.2021.108940.
11. Kain D, Kumar S. Synthesis and characterization of chitosan nanoparticles of *Achillea millefolium* L. and their activities. F1000Res. 2020;9:1297. doi: 10.12688/f1000research.26446.1.
12. Mahdi-Pour B, Jothy SL, Latha LY, Chen Y, Sasidharan S. Antioxidant activity of methanol extracts of different parts of *Lantana camara*. Asian Pac J Trop Biomed. 2012;2(12):960-5. doi: 10.1016/s2221-1691(13)60007-6.
13. Mourenza Á, Gil JA, Mateos LM, Letek M. Oxidative stress-generating antimicrobials, a novel strategy to overcome antibacterial resistance. Antioxidants (Basel). 2020;9(5):361. doi: 10.3390/antiox9050361.
14. Patra S, Bala A, Sharma N, Haldar PK. Developmental toxicity assessment of *Drymaria cordata* (Linn.) willd using zebrafish embryo. Curr Drug Saf. 2023;18(2):214-23. doi: 10.2174/1574886317666220514153858.
15. Aryal S, Baniya MK, Danekhu K, Kunwar P, Gurung R, Koirala N. Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. Plants (Basel). 2019;8(4):96. doi: 10.3390/plants8040096.
16. Ahmed D, Fatima K, Saeed R. Analysis of phenolic and flavonoid contents, and the anti-oxidative potential and lipid peroxidation inhibitory activity of methanolic extract of *Carissa opaca* roots and its fractions in different solvents. Antioxidants (Basel). 2014;3(4):671-83. doi: 10.3390/antiox3040671.
17. Xiao F, Xu T, Lu B, Liu R. Guidelines for antioxidant assays for food components. Food Front. 2020;1(1):60-9. doi: 10.1002/fft2.10.
18. González-Palma I, Escalona-Buendía HB, Ponce-Alquicira E, Téllez-Téllez M, Gupta VK, Díaz-Godínez G, et al. Evaluation of the antioxidant activity of aqueous and methanol extracts of *Pleurotus ostreatus* in different growth stages. Front Microbiol. 2016;7:1099. doi: 10.3389/fmicb.2016.01099.
19. Ranilla LG, Kwon YI, Genovese MI, Lajolo FM, Shetty K. Antidiabetes and antihypertension potential of commonly consumed carbohydrate sweeteners using in vitro models. J Med Food. 2008;11(2):337-48. doi: 10.1089/jmf.2007.689.
20. Kazeem MI, Adamson JO, Ogunwande IA. Modes of inhibition of α-amylase and α-glucosidase by aqueous extract of *Morinda lucida* Benth leaf. Biomed Res Int. 2013;2013:527570. doi: 10.1155/2013/527570.
21. Kim YM, Jeong YK, Wang MH, Lee WY, Rhee HI. Inhibitory effect of pine extract on alpha-glucosidase activity and postprandial hyperglycemia. Nutrition. 2005;21(6):756-61. doi: 10.1016/j.nut.2004.10.014.
22. Shehzadi N, Hussain K, Bukhari NI, Islam M, Khan MT, Salman M, et al. Hypoglycemic, hepatoprotective and molecular docking studies of 5-[(4-chlorophenoxy) methyl]-1,3,4-oxadiazole-2-thiol. Bangladesh J Pharmacol. 2018;13(2):149-56. doi: 10.3329/bjp.v13i2.35514.
23. Patle TK, Shrivas K, Kurrey R, Upadhyay S, Jangde R, Chauhan R. Phytochemical screening and determination

of phenolics and flavonoids in *Dillenia pentagyna* using UV-vis and FTIR spectroscopy. Spectrochim Acta A Mol Biomol Spectrosc. 2020;242:118717. doi: 10.1016/j.saa.2020.118717.

24. Mabasa XE, Mathomu LM, Madala NE, Musie EM, Sigidi MT. Molecular spectroscopic (FTIR and UV-Vis) and hyphenated chromatographic (UHPLC-qTOF-MS) analysis and in vitro bioactivities of the *Momordica balsamina* leaf extract. Biochem Res Int. 2021;2021:2854217. doi: 10.1155/2021/2854217.
25. Singh H, Kumar S, Arya A. Ethno-dermatological relevance of medicinal plants from the Indian Himalayan region and its implications on cosmeceuticals: a review. J Drug Res Ayurvedic Sci. 2023;8(2):97-112. doi: 10.4103/jdras.jdras_140_22.
26. Sahoo RN, Kumar S, Suryavanshi A, Kain D, Arya A, Chaudhry B. Venerated and medicinal aspects of plants used in India: an ethnobotanical review. J Drug Res Ayurvedic Sci. 2021;6(3):128-40. doi: 10.4103/jdras.jdras_15_21.
27. Kain D, Kumar S, Vandana, Suryavanshi A, Arya A. Metabolic characterization, antioxidant capacity, and in vitro antiurolithiatic potential of *Achillea millefolium* L. J Young Pharm. 2021;13(3):211-6.
28. Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: an overview. Medicines (Basel). 2018;5(3):93. doi: 10.3390/medicines5030093.
29. Gonelimali FD, Lin J, Miao W, Xuan J, Charles F, Chen M, et al. Antimicrobial properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms. Front Microbiol. 2018;9:1639. doi: 10.3389/fmicb.2018.01639.
30. Ahanger AM, Kumar S, Arya A, Suryavanshi A, Kain D, Vandana. Synthesis and encapsulation of *Ajuga parviflora* extract with zeolitic imidazolate framework-8 and their therapeutic action against G+ and G- drug-resistant bacteria. ACS Omega. 2022;7(2):1671-81. doi: 10.1021/acsomega.1c03984.
31. Safari M, Ahmady-Asbchin S. Evaluation of antioxidant and antibacterial activities of methanolic extract of medlar (*Mespilus germanica* L.) leaves. Biotechnol Biotechnol Equip. 2019;33(1):372-8. doi: 10.1080/13102818.2019.1577701.
32. Jangid R, Jain S, Sharma MK, Chatterjee S. In vitro antioxidant and antidiabetic activity of ethanolic extract of *Prosopis* species growing in Rajasthan, India. Vegetos. 2023;36(1):62-9. doi: 10.1007/s42535-022-00487-z.
33. Saleem F, Kanwal, Mohammed Khan K, Chigurupati S, Andriani Y, Solangi M, et al. Dicyanoanilines as potential and dual inhibitors of α-amylase and α-glucosidase enzymes: synthesis, characterization, in vitro, in silico, and kinetics studies. Arab J Chem. 2022;15(3):103651. doi: 10.1016/j.arabjc.2021.103651.
34. Dinesh Kumar G, Karthik M, Rajakumar R. GC-MS analysis of bioactive compounds from ethanolic leaves extract of *Eichhornia crassipes* (Mart) Solms. and their pharmacological activities. Pharma Innov J. 2018;7(8):459-62.
35. Kafle S, Basnyat RC, Rajbhandari M. Phytochemical analysis, antioxidant, antimicrobial activities and GC-MS profiling of *Drymaria diandra* Blume. Sci World. 2021;14(14):90-8. doi: 10.3126/sw.v14i14.35000.
36. Kumar P, Sati SC. Chemical composition, antioxidant and antimicrobial activities of Himalayan *Fraxinus micrantha* Lingelsh leaf extract. Nat Prod Res. 2021;35(20):3519-23. doi: 10.1080/14786419.2019.1710706.
37. Ismail GA, Gheda SF, Abo-Shady AM, Abdel-Karim OH. In vitro potential activity of some seaweeds as antioxidants and inhibitors of diabetic enzymes. Food Sci Technol. 2019;40(3):681-91. doi: 10.1590/fst.15619.
38. Khusairi Azemi A, Mokhtar SS, Ghulam Rasool AH. *Clinacanthus nutans*: its potential against diabetic vascular diseases. Braz J Pharm Sci. 2020;56(3):e18838. doi: 10.1590/s2175-97902020000118838.
39. Pinto MEA, Araújo SG, Morais MI, Sá NP, Lima CM, Rosa CA, et al. Antifungal and antioxidant activity of fatty acid methyl esters from vegetable oils. An Acad Bras Cienc. 2017;89(3):1671-81. doi: 10.1590/0001-3765201720160908.
40. Yang D, Chen X, Liu X, Han N, Liu Z, Li S, et al. Antioxidant and α-glucosidase inhibitory activities guided isolation and identification of components from mango seed kernel. Oxid Med Cell Longev. 2020;2020:8858578. doi: 10.1155/2020/8858578.
41. El Hachlafi N, Benkhaira N, Al-Mijalli SH, Mrabti HN, Abdnim R, Abdallah EM, et al. Phytochemical analysis and evaluation of antimicrobial, antioxidant, and antidiabetic activities of essential oils from Moroccan medicinal plants: *Mentha suaveolens*, *Lavandula stoechas*, and *Ammi visnaga*. Biomed Pharmacother. 2023;164:114937. doi: 10.1016/j.biopha.2023.114937.
42. Gong L, Feng D, Wang T, Ren Y, Liu Y, Wang J. Inhibitors of α-amylase and α-glucosidase: potential linkage for whole cereal foods on prevention of hyperglycemia. Food Sci Nutr. 2020;8(12):6320-37. doi: 10.1002/fsn3.1987.
43. Rehman G, Hamayun M, Iqbal A, Ul Islam S, Arshad S, Zaman K, et al. In vitro antidiabetic effects and antioxidant potential of *Cassia nemophila* Pods. Biomed Res Int. 2018;2018:1824790. doi: 10.1155/2018/1824790.