ARTICLE IN PRESS

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergikafkas.org
E-ISSN: 1309-2251

Kafkas Univ Vet Fak Derg
xx (x): xx, 2025
DOI: 10.9775/kvfd.2025.34347

RESEARCH ARTICLE

Evaluating the Multifunctional Therapeutic Potential of Phycocyanin: Antidiabetic, Antioxidant, Anticancer and Antimicrobial Effects on Metabolic, Oxidative, and Histopathological Parameters in Dithizone-Induced Diabetic Rats

Manal Abduallah ALDUWISH [1] Nahed S. ALHARTHI [2] Asmaa Ali ALHARBI [3]
Mody ALBALAWI [4] Amnah OBIDAN [4] Eman A. BEYARI [5] Hawazen K. AL-GHEFFARI [5]
Amani Osman SHAKAK [6,7] Aminah ALLOHIBI [7] Layla A. ALMUTAIRI [8 (*)]
Suad Hamdan ALMASOUDI [9] Rabah N. ALSULAMI [10] Amin A. AL-DOAISS [11]
Abdelghafar Mohamed ABU-ELSAOUD [12]

[1] Department of Biology, College of Science and Humanities in Al-Kharj, Prince Sattam Bin Abdulaziz University, Alkarj 11942, SAUDI ARABIA
[2] Department of Medical Laboratory, College of Applied Medical Sciences in Al-Kharj, Prince Sattam Bin Abdulaziz University, Al-Kharj, 11942, SAUDI ARABIA
[3] Department of Biochemistry, Faculty of Science, King Abdulaziz University, P.O. Box: 80200, Jeddah 21589, SAUDI ARABIA
[4] Department of Biochemistry, Faculty of Science, University of Tabuk, Tabuk,71491, SAUDI ARABIA
[5] Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah, SAUDI ARABIA
[6] Biological Sciences Department, College of Science & Arts, King Abdulaziz University, Rabigh 21911, SAUDI ARABIA
[7] Faculty of Medical Laboratory Sciences, University of Shendi, Shendi P.O. Box 142, SUDAN
[8] Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, SAUDI ARABIA
[9] Department of Biology, College of Sciences, Umm Al-Qura University, Makkah 21955, SAUDI ARABIA
[10] Department of Biology, College of Applied Sciences, Umm Al-Qura University, SAUDI ARABIA
[11] Biology Department, College of Science, King Khalid University, P.O. Box 9004, Abha 61413, SAUDI ARABIA
[12] Department of Biology, College of Science, Imam Mohammad Ibn Saud Islamic University (IMSIU), Riyadh, 11623, SAUDI ARABIA

(*) **Corresponding author:**
Layla A. Almutairi
Phone: +966 50 021 2732
Fax: +966 50 021 2732
E-mail: laAlmutairy@pnu.edu.sa

How to cite this article?

Alduwish MA, Alharthi NS, Alharbi AA, Albalawi M, Obidan A, Beyari EA, Al-Gheffari HK, Shakak AO, Allohibi A, Almutairi LA, Almasoudi SH, Alsulami RN, Al-Doaiss AA, Abu-Elsaoud AM: Evaluating the multifunctional therapeutic potential of phycocyanin: Antidiabetic, antioxidant, anticancer and antimicrobial effects on metabolic, oxidative, and histopathological parameters in dithizone-induced diabetic rats. *Kafkas Univ Vet Fak Derg*, 2025 (Article in Press).
DOI: 10.9775/kvfd.2025.34347

Article ID: KVFD-2025-34347
Received: 03.05.2025
Accepted: 12.08.2025
Published Online: 20.08.2025

Abstract

Dithizone, a heavy metal chelator, induces diabetes in animals to model human diabetes. This study evaluates the effects of a phycocyanin extract (PE) from *Spirulina platensis* on growth, blood indices, oxidative status, gut microbiota, and tissue histology in dithizone-challenged rats, while also assessing its antidiabetic, antioxidant, anticancer, antimicrobial, and antiviral properties against HSV-1 and influenza A (H1N1) viruses. PE is rich in active compounds, notably C-Phycocyanin (1.2 mg/g). The extract demonstrated robust antioxidant activity by scavenging 92% of DPPH radicals, inhibited breast cancer cell line growth by 81%, and suppressed pathogenic microbes. It also reduced α-glucosidase and α-amylase activity by 75% and 80%. Antiviral assays indicated dose-dependent inhibition of HSV-1 (65% reduction in plaque formation, IC50 = 42.5 µg/mL) and H1N1 (58% viral load reduction, IC50 = 50.3 µg/mL), with suppression of viral entry, neuraminidase activity, and upregulation of antiviral genes (IFN-α and MX1). *In vivo*, 200 rats were divided into control, dithizone, PE-treated, and dithizone + PE groups for 30 days. PE treatment resulted in improved glucose control, lower HbA1c, enhanced insulin sensitivity, reduced malondialdehyde, increased glutathione, and favorable changes in gut bacteria. Gene expression of proinflammatory cytokines and precancerous markers (BCL-2, Nrf-2, OH, β-actin, IL-1β, TNF-α, BAX, and Casp-3) was significantly reduced. Histological examination showed improved pancreatic and hepatic structure, and overall growth performance and blood profiles were enhanced. Collectively, PE mitigates metabolic disturbances from dithizone and shows promise as an antiviral agent, supporting its potential in functional foods and nutraceuticals.

Keywords: *Phycocyanin, Spirulina platensis,* Antidiabetic activity, Antioxidant activity, Anticancer, Antimicrobial, Antiviral, Dithizone-induced diabetic rats, Gut microbiota, Nutraceuticals

Introduction

With the global prevalence of chronic diseases like diabetes mellitus rising, there is increasing scientific emphasis on alternative and complementary therapies that offer beneficial effects across multiple pathological domains with minimal side effects. Diabetes mellitus, particularly, is a metabolic disorder marked by chronic hyperglycemia and is well known for its far-reaching consequences, triggering oxidative stress, tissue degeneration, dyslipidemia, chronic inflammation, heightened susceptibility to infections, and a higher incidence of certain cancers [1]. The dithizone-induced diabetic rat model has been widely utilized to simulate key aspects of human diabetes, including β-cell dysfunction, oxidative injury, and organ pathology, thereby providing a robust platform to evaluate interventions with both systemic and organ-specific effects [2].

Phycocyanin, a vibrant blue pigment-protein complex predominantly extracted from cyanobacteria such as *Spirulina platensis*, has emerged as a highly promising natural compound due to its diverse bioactive properties, notably its antidiabetic, antioxidant, anticancer, and antimicrobial activities [3,4]. Antidiabetic effects of phycocyanin have been demonstrated in several animal models, where phycocyanin supplementation results in significant reductions in fasting blood glucose and HbA1c levels, while also improving lipid profiles by lowering triglycerides, total cholesterol, and LDL cholesterol, and raising HDL cholesterol [5,6]. These metabolic improvements are often attributed to the restoration of pancreatic β-cell integrity, enhancement of endogenous insulin secretion, and activation of cellular signaling pathways such as AMPK and Akt, which collectively modulate gluconeogenesis, lipogenesis, and insulin responsiveness at a molecular level [7,8]. Phycocyanin not only plays a role in metabolism but is also a strong antioxidant. Its chemical structure allows it to neutralize harmful reactive oxygen species (ROS). By doing so, phycocyanin helps lower cell damage seen as reduced levels of malondialdehyde (MDA) and protein carbonylation. It also boosts the body's own antioxidants, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, making cells more resistant to oxidative stress [9,10]. Importantly, these protective mechanisms are mediated by upregulation of the Nrf2 pathway, conferring robust cellular defense against oxidative and inflammatory insults in tissues commonly affected by diabetes, such as the liver, kidney, and pancreas [10].

The anticancer potential of phycocyanin further distinguishes it from single-action natural compounds. Multiple studies have shown its ability to induce apoptosis, autophagy, and cell-cycle arrest in a range of tumor cell lines, while suppressing metastasis and angiogenesis by downregulating matrix metalloproteinases and vascular endothelial growth factors [11]. Notably, phycocyanin demonstrates selective cytotoxicity, effectively targeting malignant cells with minimal toxicity toward normal tissues, and shows promise as an adjunct to conventional chemotherapeutics in preclinical models. In the context of diabetes, where the risk for carcinogenesis is elevated due to persistent inflammation and impaired immune surveillance, phycocyanin's anticancer effects are especially relevant, potentially addressing both primary and secondary disease mechanisms [12].

Another layer of phycocyanin's medicinal versatility is reflected in its broad-spectrum antimicrobial and immunomodulatory activities. The heightened infection risk observed in diabetic individuals is partly offset by phycocyanin's demonstrated ability to disrupt the membranes of pathogenic bacteria, inhibit biofilm formation, and modulate immune cell function, including the enhancement of lymphocyte proliferation, antibody production, and natural killer cell activity [13]. These actions contribute not only to the direct suppression of pathogen growth but also to faster recovery and reduced inflammatory complications in diabetic hosts. Animal studies, including those utilizing streptozin-induced diabetic rats have further validated phycocyanin's capacity to preserve histopathological integrity, protecting pancreatic islet architecture, reducing hepatic steatosis, and ameliorating renal and hepatic oxidative lesions [14].

The main gap lies in previous studies' reliance on broad or less specific disease models, fragmented evaluation of phycocyanin's therapeutic effects, and limited mechanistic clarity. The current study addresses these by utilizing the dithizone-induced diabetic rat, delivering comprehensive, mechanistic, and translational insights that advance phycocyanin research. Therefore, the objectives of this study are to comprehensively evaluate the medicinal potential of a phycocyanin extract derived from *Spirulina platensis* in mitigating dithizone-induced diabetes in rats. Specifically, the study aims to assess the impact of the extract on growth performance, metabolic and hematological parameters, oxidative stress markers, gut microbiota composition, and tissue histopathology. Furthermore, it investigates the extract's antidiabetic, antioxidant, anticancer, antimicrobial, and antiviral effects -particularly against HSV-1 and H1N1 viruses- alongside profiling its major phenolic compounds. Through these multifaceted approaches, the research seeks to elucidate the broad-spectrum health benefits and underlying mechanisms of phycocyanin extract in a relevant diabetes model.

Materials and Methods

Ethical Approval

The animal study has been reviewed and approved by ZU-IACUC committee. was performed in accordance with the guidelines of the Egyptian Research Ethics Committee

and the guidelines specified in the Guide for the Care and Use of Laboratory Animals (2024). Ethical code number ZU-IACUC/2/F/497/2024. Written informed consent was obtained from the owners for the participation of their animals in this study.

Spirulina platensis Extract Preparation

A pure culture of *Spirulina platensis* was recovered and grown using the Zarrouk medium created by Zarrouk in 1966. The composition of the Zarrouk medium included 1 g NaCl, 16.8 g $NaHCO_3$, 2.5 g $NaNO_3$, 0.5 g K_2HPO_4, 1 g K_2SO_4, 0.2 g $MgSO_4.7H_2O$, 0.04 g $CaCl_2.2H_2O$, 0.01 g $FeSO^4.7H_2O$, and 0.08 g EDTA per liter of water. Using a 1M KOH solution, the medium's pH was brought to 9.5. To initiate a fresh *Spirulina platensis* culture, 10 mL of a 5-day-old culture was added to a 250 mL amount of Zarrouk's media in 500 mL screw bottles. The bottles were placed in an environment with a constant temperature of 25± 2°C and exposed to continuous light from a 36W white fluorescent lamp with an intensity of 600-800 lux for ten days. The *Spirulina platensis* pure culture was successfully obtained through the effective streaking method on Zarrouk's media. Single culture from this strain was isolated using the streaking technique on Zarrouk's medium to produce a pure culture of *Spirulina platensis* [15]. The plates were carefully stored in an environment with a temperature of 25°C and a constant light exposure of 600 lux. Once the colonies were obtained, they were carefully collected and inspected under a microscope. Zarrouk's medium was then used to preserve the *Spirulina platensis* cells on slants.

Phycocyanin Extraction and Purification Process

To extract phycocyanin, wet biomass of *Spirulina platensis* was steeped in distilled water at a 1:25 (w/v) ratio for 24 h. Afterward, the mixture was centrifuged at 10.000 x g for 15 min at 4°C to remove cellular debris; the supernatant, containing the crude extract, was collected while the precipitate was discarded. The *Spirulina platensis* crude was adjusted to pH 7 using KOH solution (1N). The resulted crude extract was precipitated using various concentrations of ammonium sulphate (10, 15, and 40%). The efficiency of these saturation levels was then compared to the 25% ammonium sulfate method. The resulting solutions were left undisturbed for 2 h before undergoing centrifugation at 12.000 x g for 30 min. The blue precipitate obtained was subsequently dissolved in a 0.005 M Na-phosphate buffer (pH 7.0) to yield a clear solution, following the methodology described in Kamble et al.[16].

Chemical Composition of Phycocyanin Extract

The PE carbohydrate, protein, fat, moisture, fiber, and ash contents were examined using the methodology specified in AOAC [17]. Amino acid analysis is a technique that utilizes ion exchange liquid chromatography. It is widely used in various domains to determine the qualitative and quantitative composition of substances accurately. Within biochrom systems, this fundamental concept has been enhanced to provide automated, rapid, and highly sensitive tests [18], known as classical amino acid analysis.

Phenolic Compounds Profile by LC-MS

Sample Preparation

Phenolic compounds were extracted from freeze-dried *phycocyanin* (100 mg) using 70% methanol (MeOH) in an ultrasonic bath (40 kHz, 30 min, 25°C). The extract was centrifuged (10.000 x g, 15 min, 4°C), filtered through a 0.22 µm PTFE syringe filter, and concentrated under a nitrogen stream at 40°C. The residue was reconstituted in 1 mL LC-MS grade methanol before analysis. To ensure accuracy, syringic acid-d_4 was used as an internal standard (IS), and recovery tests were performed by spiking samples with known phenolic standards. Solvent blanks were run between samples to prevent carryover contamination [19].

Chromatographic separation was performed on a C18 reversed-phase column (e.g., Zorbax Eclipse Plus, 2.1 x 100 mm, 1.8 µm) with a binary mobile phase consisting of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile. A gradient elution was applied as follows: 5% B (0-2 min), increased to 50% B (2-15 min), then to 95% B (15-20 min), held for 2 min (20-22 min), and finally re-equilibrated at 5% B (22-25 min). The flow rate was maintained at 0.3 mL/min, with a column temperature of 35°C and an injection volume of 5 µL.

Mass spectrometric detection was conducted using electrospray ionization (ESI) in negative mode with the following parameters and Full-scan data (m/z 50-1000) were acquired alongside targeted MS/MS for compound confirmation [20,21]. LC-MS data were processed using Agilent MassHunter, Thermo Xcalibur, or Skyline, with metabolite identification supported by METLIN, mzCloud, and GNPS databases.

Biological Activities of *Spirulina* Extract

Antidiabetic Activity

The α-glucosidase inhibitory activity of PE was calorimetrically assessed. Incubation of α-glucosidase with PE solutions (100, 150, 200, 250, and 300 µg/mL) was performed for 10 min at 37°C and pH 6.9. Subsequently, 50 µL of a 1 mM p-nitrophenyl-α-D-glucopyranoside solution (in phosphate buffer) was added. The reaction was terminated after a 10-min reaction period, and the optical density (OD) was measured at 405 nm. These values were then used in the equation [22].

$$\%\ \alpha - glucosidase\ inhibition\ activity = \frac{OD\ control - OD\ sample}{OD\ control} x100 \quad (1)$$

Inhibitory activity against α-amylase was evaluated using the methodology described by Nair et al.[23]. with minor adjustments. The process involved combining 50 µL of various PE concentrations with 1 mg/mL acarbose and 500 µL of α-amylase in a pH 6.8 phosphate buffer. This initial mixture was allowed to incubate for 10 min at room temperature. Subsequently, 500 µL of soluble starch was introduced, and the incubation continued under the same parameters. To terminate the reaction, 1 mL of 96 mM 3,5-dinitrosalicylic acid was added, followed by 5 min of steam heating and cooling to 25°C. The optical density was then determined at 540 nm, and these measurements were utilized in the relevant equation to ascertain the inhibitory activity.

$$\%\ \alpha - amylase\ inhibition\ activity = \frac{OD\ control - OD\ sample}{OD\ control} x100 \quad (2)$$

Antioxidant Activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity of PE was assessed as per Alsubhi et al.[24]. A reaction between 0.5 mL of ethanolic DPPH and 1 mL of PE was incubated for 30 min in the dark, and the absorbance at 517 nm was measured spectrophotometrically. The IC50 value reflects the minimum concentration required to scavenge 50% of the DPPH radical [25]. The percentage of DPPH scavenging activity was calculated using the formula:

$$\%\ DPPH\ scavenging\ activity = \frac{Abs\ control - Abs\ sample}{Abs\ control} x100 \quad (3)$$

Antimicrobial Activity

The antibacterial properties of PE were tested against *Salmonella typhi, Bacillus cereus, Escherichia coli,* and *Staphylococcus aureus*. Microbial strains were preserved at 4°C by subculturing on nutrient agar slants. The antibacterial potential of the PE was evaluated using the agar well-disc diffusion method as per [25]. After adding 50 mL of melted Muller-Hinton agar (MHA) to plates, a loopful of bacterial inoculum was evenly distributed across each plate's surface. Each plate was punctured with 8 mm wells, into which 6 mm discs soaked with 50 µL PE at levels of 100, 150, and 200 µg/mL were added. Negative control wells contained discs with water. MHA plates were then incubated for 24-48 h at 37°C. The diameters of inhibition zones (in mm) were measured to indicate antibacterial activity [26].

The antifungal potential of PE concentrations (100, 150, 200, 250, and 300 µg/mL) was tested against pathogenic fungi: *Fusarium oxysporium, Fusarium oxysporium, Fusarium solai,* and *Aspergills niger.* Antifungal activity of PE was quantified via a disc diffusion assay. Sabouraud dextrose agar plates were uniformly inoculated with fungal suspensions. Sterile paper discs, pre-loaded with varying concentrations of PE, were subsequently positioned on the inoculated media. Plates were incubated at 28°C for a duration of seven days, after which the diameters of the observed inhibition zones were recorded in millimeters [27].

Anticancer Activity

Cell Culture: MCF-7 breast carcinoma cells, acquired from Nawah Scientific Inc. (Mokatam, Cairo, Egypt), were cultured in DMEM media. This media was enriched with 100 mg/mL streptomycin, 100 units/mL penicillin, and 10% heat-inactivated fetal bovine serum. Cells were kept at 37°C in a humidified atmosphere with 5% (v/v) CO_2 [28].

Cytotoxicity Assay: The Sulforhodamine B (SRB) assay was used to determine cell viability. Briefly, $5x10^3$ cells/well were seeded in 96-well plates and incubated for 24 h. Cells were then treated with varying concentrations of drugs for 72 h. Following fixation with 10% trichloroacetic acid (TCA) and washing, cells were stained with 0.4% SRB for 10 min. After washing with 1% acetic acid and drying, protein-bound SRB was dissolved with 10 mM TRIS, and absorbance was measured at 540 nm using a microplate reader [29].

$$\text{Cell survival rate (\%)} = \frac{\text{OD sample} - \text{OD blank}}{\text{OD control} - \text{OD blank}} \text{x } 100 \quad (2)$$

Animal Behavior and Design Methodology

A total of 200 Sprague-Dawley white male albino rats from the Serum and Vaccine Center, Dokki, Giza, were used in the study, with an average weight of 150±10 g. Before the experiment, the rats were housed in cages that maintained a controlled temperature of 22-24°C and a 12-h light/12-h dark lighting schedule. This housing condition was maintained for at least 7 days. The rats were divided into five groups, each consisting of 40 (10 x 4). Subsequently, each group was assigned a distinct food item to consume for 28 days, according to the following distribution: Group I received a routine (basal) diet only. Group II received a diet and was injected intraperitoneally with 100 mg/kg b.w. dithizone (dissolved in olive oil) for 7 days. Group III received a routine (basal) diet supplemented with 100 mg/kg body weight of *PE* powder throughout the experimental period at the same time. Group IV received a routine (basal) diet mixed with 300 mg/kg body weight of PE powder. Group V received a mix of 300 mg/kg body weight of PE and 100 mg/kg of dithizone.

Biochemical Parameters

After the experiment, all rats were anesthetized using an R550 Multioutput Laboratory Small Animal Anesthesia Machine. This machine is designed for simultaneously anesthetizing 1-5 small animals, including rats, mice, cats, and rabbits. Each anesthesia channel can be controlled independently, and the gas flow to the induction box can

be adjusted separately within a range of 0-2.0 L/min., and blood samples were obtained from the hepatic portal vein to assess the biochemical variables. The blood samples were placed into heparin tubes to analyze the biochemical characteristics. The samples were thereafter centrifugated at 3.000 revolutions per minute for 15 min to isolate the serum. The levels of serum total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-c) were measured using enzymatic colorimetric techniques [30]. The VLDL-c concentration, measured in milligrams per deciliter, is calculated by dividing the triglyceride level by 5 [31]. The LDL-c was calculated using the following equation: The formula for calculating LDL cholesterol (LDL-c) in milligrams per deciliter (mg/dL) is as follows: LDL-c = Total cholesterol (TC) – [HDL cholesterol (HDLc) + Very low-density lipoprotein cholesterol (VLDL-c)] [32]. The AST, ALT, AST/ALT ratio, and ALP were quantified using the methods outlined in reference [33]. The liver was extracted, and its tissues were washed in a chilled 0.9% saline solution (by weight or volume). The tissues were then quantified and stored at a temperature of -70°C. The levels of malondialdehyde (MDA) and the activities of superoxide dismutase (SOD), glutathione (GSH), and catalase (CAT) were estimated, following the steps described in reference [34]. Total antioxidant capacity (TAC) was also measured [35].

Liver Histopathology

The liver was preserved using 10% neutral buffered formalin immediately after removal from the animals. The fixed tissues were thereafter subjected to known histological tests [36].

Gene Expression

RNA extraction was performed using rat liver. The RNA globules were dissolved in diethylpyrocarbonate (DEPC)-treated water. The RNA concentration was measured using spectrophotometry at an optical density (OD) ratio of 260/280 [37]. The semiquantitative reverse transcription PCR used 3 µg of RNA. This process included subjecting the plate to denaturation in PCR thermocycler (Bio-Rad T100TM) at 70°C for 5 min.

Additionally, 0.5 nanograms of oligo (dT) primers were used. Two microliters of 10X RT buffer, two microliters of 10 mM dNTP, and one microliter of 100 M reverse transcriptase were mixed to make cDNA. The mixture was incubated at 42°C for 1 h and then heated at 70°C for 10 min to assure deactivation of the enzyme. To evaluate gene expression levels, densitometry was used to quantify mRNA expression, with β-actin mRNA serving as a reference standard *(Table 1)*. The 2-ΔΔCT method was used to measure the expression levels of these genes using real-time PCR. The endogenous reference gene, actin, was used to standardize the analyzed genes. The CT values were used to examine the alterations in gene density and mRNA expression using a comparative approach.

Statistical Analysis

The data were analyzed using the SPSS program (Version 17.0). The data were reported as the mean±SE. Following a one-way ANOVA, Fisher's least significant difference (LSD) test was used to compare

Table 1. *The primer sequence used for quantitative real-time PCR in liver rats with dithizone-induced toxicity*

Gene	Entry Number	Product Size (bp)	Sequence
β-actin	NM_007393.4	140 bp	CCAGCCTTCCTTCTTGGGTA
			CAATGCCTGGGTACATGGTG
Bcl2	NM_009740	154 bp	AGCCTGAGAGCAACCCAAT
			AGCGACGAGAGAAGTCATCC
HO-1	NM_010442.1	125 bp	CGCCTCCAGAGTTTCCGCAT
			GACGCTCCATCACCGGACTG
Nrf2	NM_010902.3	139 bp	CGCCTGGGTTCAGTGACTCG
			AGCACTGTGCCCTTGAGCTG
IL-1β	NM_007393	304 bp	TAAGGCCAACCGTGAAAAG
			GTACGACCAGAGGCATACAG
TNF-α	NM_000594.3	229 bp	AGGCAATAGGTTTTGAGGGCCAT
			TCCTCCCTGCTCCGATTCCG
BAX	NM_001291428	108 bp	GGTTGTCGCCCTTTTCTA
			CGGAGGAAGTCCAATGTC
Casp-3	NM_004346.3	834 bp	GGAAGCGAATCAATGGACTCTGG
			GCATCGACATCTGTACCAGACC

the data. The statistical significance criterion was set at a p-value of ≤0.05.

Results

Chemical Composition of Phycocyanin Extract

The proximate composition analysis of the phycocyanin extract, conducted on a dry weight basis from three replicates, reveals its notable nutritional profile *(Table 2)*. With a minimal moisture content of 3.0 g/100 g, the extract is primarily dry, contributing to its concentrated nature. It stands out as a significant protein source, comprising 65.0 g/100 g of crude protein, of which a remarkable 50.0 g/100 g is attributed specifically to phycocyanin. This high phycocyanin content underscores the efficacy of the extraction process. Additionally, the extract contains 20.0 g/100 g of total carbohydrates, with 8.5 g/100 g being dietary fiber, suggesting potential digestive benefits. Conversely, the lipid content is considerably low at 2.5 g/100 g, indicating a low-fat profile. The ash content, representing minerals, is 4.0 g/100 g. Overall, the phycocyanin extract provides a substantial energy content of 360 kcal, predominantly from its rich protein and carbohydrate composition, positioning it as a valuable ingredient for various applications requiring high protein and specific bioactive compounds.

Table 3 presents a detailed amino acid profile of *PE*, quantified in grams per 100 grams of protein. The analysis of the phycocyanin extract's amino acid profile, based on three replicates, indicates a high-quality protein source due to the presence of all essential amino acids. glutamic acid and aspartic acid are the most prevalent, at 112.4 mg/g protein and 98.2 mg/g protein respectively, playing crucial roles in protein structure and function. Notably, branched-chain amino acids (BCAAs) such as leucine (87.3 mg/g protein), valine (58.2 mg/g protein), and Isoleucine (39.6 mg/g protein) are well-represented, which is advantageous for muscle protein synthesis and recovery. Other essential amino acids like lysine (49.5 mg/g protein), threonine (42.1 mg/g protein), and phenylalanine (36.8 mg/g protein), along with sulfur-containing methionine (12.4 mg/g protein) and cysteine (8.3 mg/g protein), and the serotonin precursor tryptophan (14.2 mg/g protein), further contribute to the extract's comprehensive nutritional value. This complete and balanced amino acid composition suggests the phycocyanin extract's potential for use in nutritional supplements, functional foods, and other applications where a robust and readily available amino acid supply is desired.

Phenolic Compounds Profile of Phycocyanin Extract

Table 4 presents a comprehensive profile of phenolic compounds identified in *Spirulina* extract using LC-MS/MS in negative ionization mode. he LC-MS/MS analysis of the phycocyanin extract, conducted in negative ionization mode, provides a detailed elucidation of its complex phytochemical composition, indicating a rich array of bioactive compounds. Expectedly, C-Phycocyanin subunit (1200.5 µg/g) and Allophycocyanin subunit (980.2 µg/g) are the most abundant constituents, consistent with the extract's primary nature. The fragmentation ions for C-Phycocyanin subunit are observed at m/z 1124, 843, 560, 397, while for Allophycocyanin subunit, they are 1320, 1056, 788, 512. A significant concentration of

Table 2. *Proximate composition of phycocyanin extract (per 100 g dry weight ± Standard Deviation)*

Parameter	Content (g/100 g)
Moisture	3.0±0.5
Crude protein	65.0±5.0
Phycocyanin (of total protein)	50.0±4.0
Total carbohydrates	20.0±3.0
Dietary fiber	8.5±1.5
Total lipids	2.5±0.5
Ash	4.0±0.8
Energy Content	360±20 kcal

n = 3; data are presented as mean ± SD. Different lowercase letters in the same column indicate significant variation at P<0.05

Table 3. *Amino Acid Profile of phycocyanin extract (g/100 g ± Standard Deviation)*

Amino Acid	Abbreviation	Content (mg/g protein)
Aspartic acid	Asp (D)	98.2±5.1
Glutamic acid	Glu (E)	112.4±6.3
Serine	Ser (S)	45.6±3.2
Glycine	Gly (G)	62.8±4.0
Histidine	His (H)	18.5±1.5
Arginine	Arg (R)	54.3±3.8
Threonine	Thr (T)	42.1±2.9
Alanine	Ala (A)	76.5±4.5
Proline	Pro (P)	33.7±2.1
Tyrosine	Tyr (Y)	28.9±.8
Valine	Val (V)	58.2±3.6
Methionine	Met (M)	12.4±1.0
Cysteine	Cys (C)	8.3±0.7
Isoleucine	Ile (I)	39.6± 2.4
Leucine	Leu (L)	87.3± 5.2
Phenylalanine	Phe (F)	36.8± 2.3
Lysine	Lys (K)	49.5± 3.1
Tryptophan	Trp (W)	14.2± 1.2

n = 3; data are presented as mean ± SD. Different lowercase letters in the same column indicate significant variation at P<0.05

Table 4. *Phenolic compounds identified in phycocyanin extract by LC-MS/MS in negative ionization mode*

Compound Name	Molecular Weight (g/mol)	Content (µg/g)	Fragmentation Ions (m/z)
C-Phycocyanin subunit	~28,000	1200.5	1124, 843, 560, 397
Allophycocyanin subunit	~30,500	980.2	1320, 1056, 788, 512
Phycocyanobilin	586.6	450.8	586, 299, 253, 165
Linolenic acid	278.4	320.3	277, 205, 149, 95
β-Carotene	536.9	210.7	536, 445, 321, 177
Pheophytin a	871.2	185.4	870, 592, 533, 461
Astaxanthin	596.8	150.9	595, 479, 401, 255
Zeaxanthin	568.9	132.6	567, 430, 375, 221
Chlorophyll a derivative	614.6	98.3	613, 555, 487, 329
Scytonemin	544.6	75.2	543, 468, 325, 211
Gallic acid	170.1	68.5	169, 125, 97, 79
Caffeic acid	180.2	55.7	179, 135, 107, 89
Ferulic acid	194.2	42.3	193, 149, 134, 117
Quercetin	302.2	38.9	301, 179, 151, 121
Kaempferol	286.2	29.6	285, 229, 185, 117
Apigenin	270.2	24.1	269, 225, 181, 117
Myricetin	318.2	18.7	317, 179, 137, 109
Indole-3-carboxylic acid	161.2	15.4	160, 116, 89, 71
p-Coumaric acid	164.2	12.8	163, 119, 93, 65
Sinapic acid	224.2	9.5	223, 178, 149, 121

Phycocyanobilin (450.8 µg/g) is also detected, presenting fragmentation ions at m/z 586, 299, 253, 165; this chromophore is crucial for the extract's antioxidant and anti-inflammatory properties. Beyond these core components, the extract contains an impressive profile of additional beneficial compounds. Notably, the essential fatty acid Linolenic acid (320.3 µg/g), with fragmentation ions at m/z 277, 205, 149, 95, is present. A strong suite of carotenoid antioxidants includes β-Carotene (210.7 µg/g) (fragmentation ions: m/z 536, 445, 321, 177), Astaxanthin (150.9 µg/g) (fragmentation ions: m/z 595, 479, 401, 255), and Zeaxanthin (132.6 µg/g) (fragmentation ions: m/z 567, 430, 375, 221), all recognized for their protective roles against oxidative stress and specific health benefits. Further enhancing the extract's complexity are chlorophyll derivatives like Pheophytin a (185.4 µg/g) and a Chlorophyll a derivative (98.3 µg/g), as well as the unique UV-absorbing compound Scytonemin (75.2 µg/g). A diverse group of phenolic acids, including Gallic acid (68.5 µg/g) with fragmentation ions at m/z 169, 125, 97, 79, Caffeic acid (55.7 µg/g), and Ferulic acid (42.3 µg/g), contribute significantly to the extract's antioxidant capacity. Prominent flavonoids such as Quercetin (38.9 µg/g) (fragmentation ions: m/z 301, 179, 151, 121), Kaempferol (29.6 µg/g), Apigenin (24.1 µg/g), and Myricetin (18.7 µg/g) further enrich the extract's biological activity, offering broad-spectrum antioxidant, anti-inflammatory, and potentially anti-carcinogenic effects.

Biological Activities of Phycocyanin Extract

Antioxidant Activity

Fig. 1 demonstrates the DPPH free radical scavenging activity of PE at concentrations ranging from 100 to 300 µg/mL, alongside that of the antioxidant

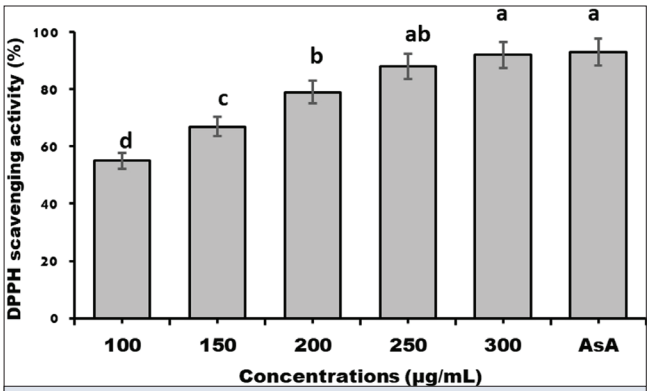

Fig 1. Antioxidant activity of *phycocyanin extract* against DPPH free radicals compared to ascorbic acid. Different lowercase letters above the columns indicate significant variation at P<0.05

standard Ascorbic acid (AsA). *Fig. 1* illustrates a clear concentration-dependent relationship for Spirulina extract, with scavenging activity increasing from approximately 55% at 100 µg/mL to around 67% at 150 µg/mL, further rising to about 80% at 200 µg/mL. The highest concentrations of PE, 250 µg/mL and 300 µg/mL, exhibited potent scavenging activities of approximately 90-92%, statistically comparable to Ascorbic acid (93%). Statistical analysis revealed significant differences in scavenging activity between the lower concentrations of PE (100 and 150 µg/mL) and the higher concentrations (250 and 300 µg/mL) and Ascorbic acid. The IC50 value, representing the concentration required for 50% DPPH scavenging, can be inferred from the graph to be 120 µg/mL for phycocyanin extract, highlighting its antioxidant potential. Ascorbic acid, with its high scavenging activity even at a concentration lower than the tested range, would have a considerably lower IC50 value, indicating its superior antioxidant potency compared to the tested concentrations of phycocyanin extract.

Anticancer Activity

The effect of PE on the viability of MCF-7 breast cancer cells was investigated through both microscopy and quantitative analysis *(Fig. 2)*. The microscopic images illustrate the decreasing density and altered morphology of MCF-7 cells with increasing PE concentrations, with control cells appearing dense and healthy, while those treated with higher PE concentrations and doxorubicin show a sparse population with signs of cell damage *(Fig. 2-A,B,C,D)*. The quantitative data, presented as a bar graph, reveals a clear dose-dependent inhibition of MCF-7 cell viability by PE. Starting with 62% viability at 100 µg/mL, the percentage of viable cells progressively decreased with increasing PE concentrations, reaching a low of 20% at 300 µg/mL *(Fig. 2-E)*. This significant reduction in viability at the highest PE concentration was comparable to the effect observed with the known chemotherapy drug doxorubicin. Statistical analysis confirmed the significant differences in cell viability across the increasing PE concentrations, highlighting the potent anti-proliferative activity of *Spirulina* extract against MCF-7 breast cancer cells in a dose-dependent manner, suggesting its potential as an anti-cancer agent.

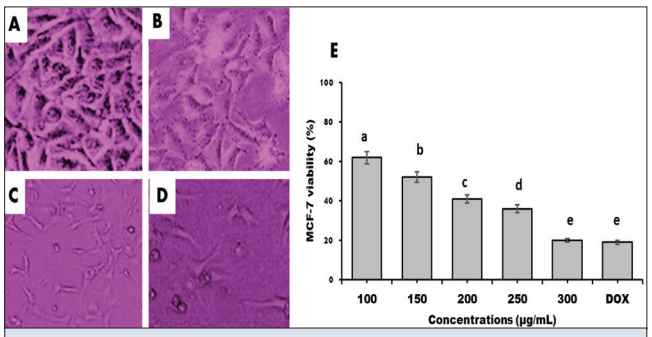

Fig 2. Microscopic images (A, B, C, D) of the effect of *phycocyanin* extract on the breast cancer cell lines compared to doxorubicin (DOX). (E) the effect of PE concentration on inhibition of the viability of MCF-7 cancer cells. Different lowercase letters above the columns indicate significant variation at P<0.05

Antidiabetic Activity

Fig. 3 illustrates the inhibitory effects PE at varying concentrations (100-300 µg/mL) on the activities of α-glucosidase (α-glu) and α-amylase (α-amyl), key

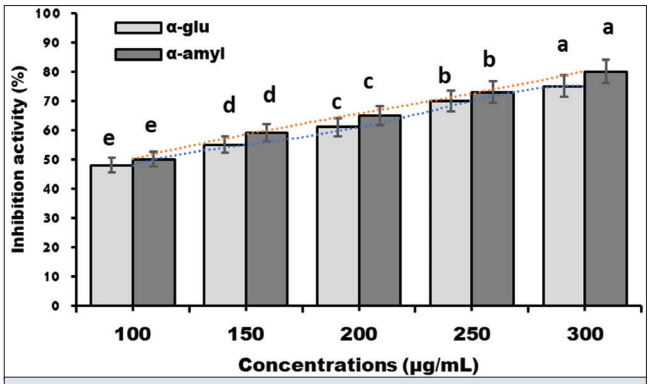

Fig 3. The inhibitory effect of PE concentrations on the activity of α-amylase and α-glucosidase. Different lowercase letters above the columns indicate significant variation at P<0.05

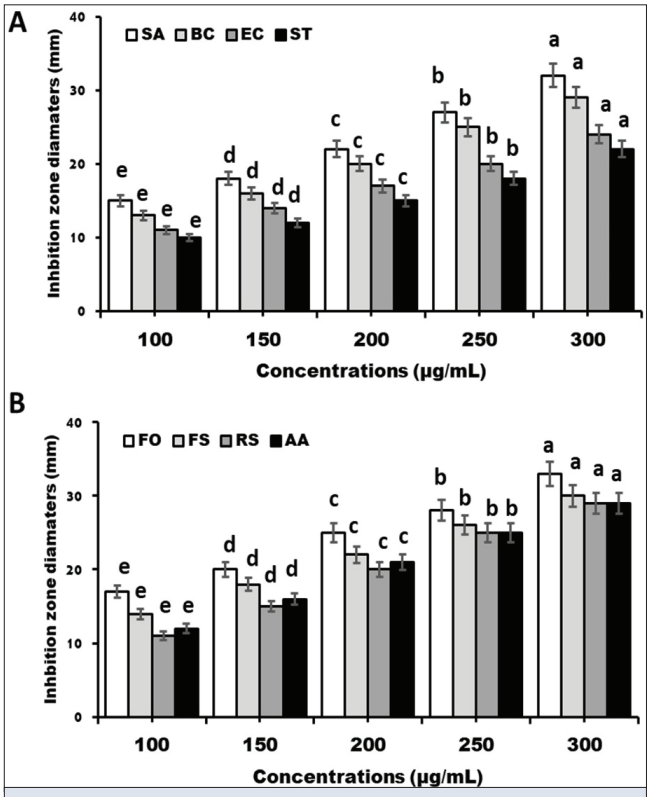

Fig 4. (A) Antibacterial activity of PE against pathogenic bacteria, *Staphylococcus aureus* (SA), *Bacillus cereus* (BC), *Escherichia coli* (EC), and *Salmonella typhi* (ST). (B) pathogenic fungi, *Fusarium oxysporium* (FO), *Fusarium solani* (FS), *Rhizoctonia solani* (RS), and *Alternaria alternata* (AA). Different lowercase letters on the columns indicate significant variation at P<0.05

enzymes in carbohydrate metabolism relevant to diabetes management. For α-glucosidase, the inhibition activity increases from approximately 50% at 100 µg/mL to around 75% at 300 µg/mL, representing a relative increase of 50% in inhibition over this concentration range. Similarly, α-amylase inhibition starts at roughly 52% at 100 µg/mL and rises to about 80% at 300 µg/mL, showing a relative increase of approximately 54% in inhibition across the tested concentrations. Notably, the percentage of α-amylase inhibition is slightly higher at each concentration than that of α-glucosidase. Statistical analysis, denoted by the letters above the bars, confirms significant increases in inhibitory activities for both enzymes as the PE concentration rises. These findings suggest that Spirulina extract exhibits a dose-dependent ability to inhibit both α-glucosidase and α-amylase, with a slightly more pronounced inhibitory effect on α-amylase, indicating its potential as a natural agent for managing postprandial hyperglycemia in diabetes by hindering carbohydrate digestion and absorption.

Antimicrobial Activity

Fig. 4 presents the antimicrobial activity of PE at concentrations ranging from 100 to 300 µg/mL, measured by the diameter of the inhibition zone (mm) against several pathogenic microorganisms. *Fig. 4-A* illustrates the antibacterial activity of PE against four bacterial species: *S. aureus, B. cereus, E. coli,* and *S. typhi*. The results show a general trend of increasing inhibition zone diameters with increasing PE concentration for all tested bacteria. *S. aureus* consistently exhibited the largest inhibition zones across all concentrations, reaching approximately 32 mm at 300 µg/mL. *B. cereus* also showed significant inhibition, with the zone diameter increasing to 30 mm at the highest concentration. *E. coli* and *S. typhi* displayed moderate susceptibility to PE, with inhibition zones reaching approximately 24 mm and 22 mm, respectively, at 300 µg/mL. Statistical analysis, indicated by the letters above the bars, reveals significant differences in the inhibition zone diameters across the different PE concentrations for each bacterium, generally showing increased inhibition with higher concentrations.

Fig. 4-B demonstrates the antifungal activity of PE against four pathogenic fungal species: *Fusarium oxysporium, Fusarium solani, Rhizoctonia solani,* and *Alternaria alternata*. Similar to the antibacterial effects, the antifungal activity generally increased with higher PE concentrations. *F. oxysporium* showed the most sensitivity to PE, with the inhibition zone reaching approximately 30 mm at 300 µg/mL. *F. solani* also exhibited substantial inhibition, with the zone diameter increasing to around 29 mm at the highest concentration. *R. solani* and *A. alternata* displayed moderate sensitivity, with inhibition zones reaching approximately 25 mm and 28 mm, respectively, at 300 µg/mL. Statistical analysis again indicates significant differences in the inhibition zone diameters across the different PE concentrations for each fungus, with higher concentrations generally leading to larger inhibition zones.

Phycocyanin extract demonstrates both antibacterial and antifungal activities against the tested panel of pathogenic microorganisms in a concentration-dependent manner. The degree of susceptibility varied among the different species, with *S. aureus* and *F. oxysporium* showing the highest sensitivity to PE among the bacteria and fungi tested, respectively. These findings suggest the potential of PE as a natural source of antimicrobial and antifungal compounds.

Antiviral Activity

Fig. 5 clearly demonstrates the dose-dependent antiviral efficacy of the PE against both Herpes Simplex Virus type 1 (HSV-1) and Influenza A virus subtype H1N1. A consistent trend of increasing viral plaque reduction is observed for both viruses as the concentration of the substance rises from 50 µg/mL to 300 µg/mL. Notably, the extract consistently exhibits greater effectiveness against HSV-1 across all concentrations, achieving a peak reduction approaching 90% at 300 µg/mL, compared to approximately 78-79% for H1N1 at the same concentration.

Fig. 6-A,B meticulously illustrate the phycocyanin extract's dose-dependent capacity to upregulate the relative expression of critical antiviral genes: Interferon-alpha (IFN-α) and Myxovirus resistance protein 1 (MX1). A clear and statistically significant increase in the fold change of both IFN-α and MX1 expression is observed as the extract's concentration rises from 50 µg/mL to 300 µg/mL, unequivocally demonstrating its ability to stimulate these key components of the host's innate antiviral defense. The concomitant upregulation of IFN-α, a primary type I interferon, and MX1, a downstream interferon-stimulated

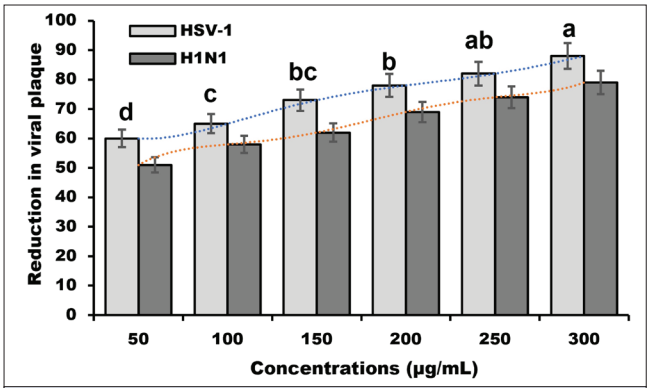

Fig 5. Dose-Dependent Antiviral Efficacy of *phycocyanin* extract Against HSV-1 and H1N1 Evaluated by Reduction in Viral Plaque Formation. Different lowercase letters on the column indicate significant variation at P<0.05

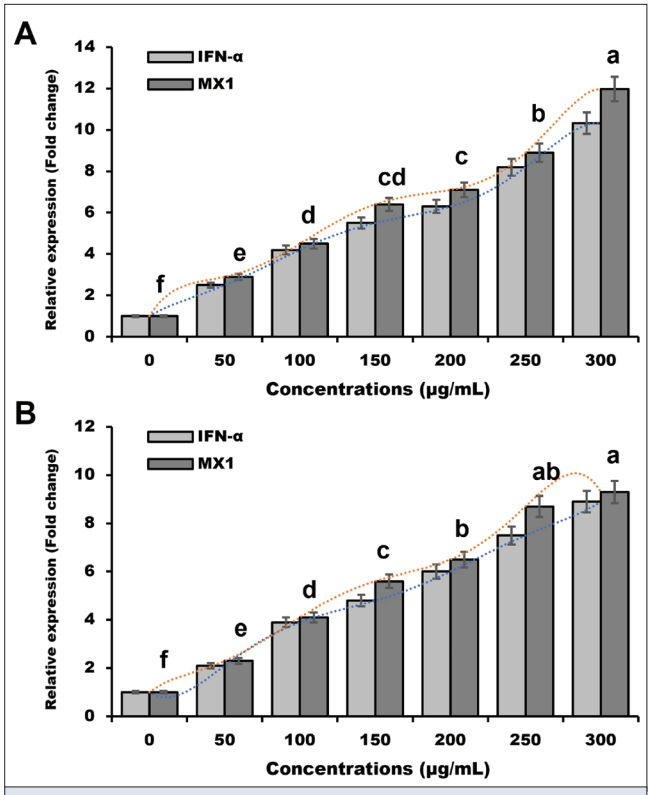

Fig 6. Dose-Dependent Upregulation of IFN-α and MX1 Gene Expression Induced by *phycocyanin* extract in a Concentration-Gradient Manner. Different lowercase letters on the column indicate significant variation at P<0.05

gene, strongly suggests that the phycocyanin extract activates the interferon signaling pathway, leading to the production of essential antiviral effector molecules. While both panels show this robust induction, Panel A consistently exhibits a higher magnitude of gene expression, with IFN-α reaching approximately a 10-fold change and MX1 nearly a 12-fold change at 300 µg/mL, compared to approximately a 9-fold change for both in Panel B. These differences might stem from variations in experimental conditions or cell types, but the underlying immunostimulatory effect remains evident.

The findings showed the considerable antiviral activities of PE and that is clear in IC50 where PE reduced 50% of viral plaque at 42.5 and 50 µg/mL for HSV-1 and H1N1, respectively. Also, the CC50 of PE was 110 and 142 µg/mL for HSV-1 and H1N1, respectively.

Blood Biochemistry

Table 5 reveals a significant impact of dietary PE on various serum biochemical parameters in rats compared to the Positive Control (PC) group, which likely represents a state of induced dysfunction.

Regarding liver function, the PC group exhibited elevated liver enzymes. However, PE treatment at 100 mg/kg resulted in a 65.4% decrease in Aspartate transaminase

Table 5. *The influence of dietary phycocyanin extract on the serum biochemical parameters of rats*

Serum Biochemistry		Phycocyanin Extract Treatments (mg/kg)					P-value
		NC	PC	100	300	PC+PE	
Liver and kidney functions	AST (U/L)	105c	295a	102c	98d	112b	<0.0001
	ALT (U/L)	28b	58a	25c	22d	26c	<0.0001
	ALP (U/L)	115c	190a	105d	100d	125b	<0.0001
	AST/ALT ratio	3.75d	5.07a	4.01c	4.5b	4.3b	0.89
Lipid profile	Total cholesterol (mg/dL)	85d	288a	92c	81d	132b	<0.0001
	Triglycerides (mg/dL)	78d	195a	81c	76d	111b	<0.0001
	HDL (mg/dL)	56b	39c	66a	68a	54b	<0.0001
	LDL (mg/dL)	18d	35a	25c	22c	28b	<0.0001
	VLDL (mg/dL)	17c	38a	25b	21bc	25b	<0.0001
Oxidative stress	GSH (ng/mL)	2.1b	0.56d	2.9b	4.1a	1.8c	<0.0001
	SOD (U/mL)	42.3b	17.5d	48.3b	51.3a	36c	<0.0001
	CAT (ng/mL)	1.2b	0.41d	1.5ab	1.7a	0.87c	<0.0001
	MDA (nmol/mL)	3.5bc	18.5a	2.1c	1.5d	5.5b	<0.0001
	TAC (ng/mL)	1.45c	0.30e	1.8b	2.2a	1.22d	<0.0001
Diabetes markers	Glucose (mg/dL)	89bc	455a	91b	85c	97b	<0.0001
	HbA1c (%)	5.6c	14.3a	5.5c	5.2c	7.5b	<0.0001
	Insulin (µU/L)	16.6av	1.2c	17.5a	16.9ab	9.8b	<0.0001

n = 3; data are presented as mean ± SD. Groups sharing the same lowercase letter within a row/column are not statistically different, while those with different letters show a statistically significant difference (P<0.05); PE: Phycocyanin extract

Table 6. The influence of dietary phycocyanin extract on proinflammatory cytokines and cancerous genes (mean ±SD)

Proinflammatory Cytokines/ Apoptosis Genes (Fold change)	Phycocyanin Extract Treatments (mg/kg)					P-value
	NC	PC	100	300	PC+PE	
BCL-2	1.0±0.0d	9.1±0.9a	1.2±0.5c	1.1±0.2cd	2.1±0.6b	<0.0001
Nrf-2	1.1±0.2d	7.5±0.5a	1.25±0.6c	1.15±.1cd	1.5±0.5b	<0.0001
OH	1.2±0.6d	8.2±0.8a	1.3±0.2c	1.2±0.2cd	1.9±0.8b	<0.0001
B-actin	1.0±0.0d	10.5±0.3a	1.36±0.3c	1.18±0.3cd	2.5 ±.6b	<0.0001
IL-1β	1.00±0.09d	8.30±0.61a	1.20±0.11c	1.10±0.10cd	2.00±0.18b	<0.0001
TNF-α	1.05±0.07d	9.00±0.77a	1.24±0.13c	1.16±0.09cd	2.15±0.21b	<0.0001
BAX	1.00±0.08d	2.80±0.20a	1.30±0.10c	1.10±0.09cd	1.80±0.16b	<0.01
Casp-3	1.00±0.09d	3.10±0.23a	1.22±0.12c	1.13±0.10cd	1.95±0.17b	<0.01

n = 3; data are presented as mean ± SD. Groups sharing the same lowercase letter within a row/column are not statistically different, while those with different letters show a statistically significant difference (P<0.05)

(AST), 300 mg/kg led to a 66.8% decrease, and the PC+PE group showed a 62.0% decrease in AST levels compared to the PC group. Similarly, Alanine Aminotransferase (ALT) levels decreased by 56.9%, 62.1%, and 55.2% in the 100 mg/kg, 300 mg/kg, and PC+PE groups. Alkaline Phosphatase (ALP) levels also saw substantial reductions of 47.4% in both the 100 mg/kg and 300 mg/kg PE groups, and 34.2% in the PC+PE group. The AST/ALT ratio showed a 19.5% increase in the 100 mg/kg group but decreased by 11.2% and 14.2% in the 300 mg/kg and PC+PE groups, respectively.

In terms of the lipid profile, the PC group displayed dyslipidemia. PE treatment at 100 mg/kg led to a 68.1% reduction in total cholesterol, while 300 mg/kg resulted in a 71.9% decrease, and PC+SPE showed a 54.2% decrease. Triglyceride levels decreased by 58.5%, 60.5%, and 43.1% in the respective SPE treatment groups. Notably, HDL increased by 69.2% with 100 mg/kg PE, 74.4% with 300 mg/kg PE, and 38.5% in the PC+PE group. The detrimental LDL cholesterol showed dramatic reductions of 87.8%, 89.3%, and 84.4%, while VLDL decreased by 55.3%, 44.7%, and 34.2% in the 100 mg/kg, 300 mg/kg, and PC+PE groups compared to the PC group *(Table 5)*.

Regarding oxidative stress, the PC group showed signs of increased oxidative damage. PE treatment at 100 mg/kg led to a remarkable 417.9% increase in GSH levels, while 300 mg/kg resulted in a 632.1% increase, and PC+PE showed a 221.4% increase. Similarly, SOD activity increased by 176.0%, 193.1%, and 105.7%, and CAT activity increased by 317.1%, 361.0%, and 112.2% in the respective PE treatment groups. Conversely, MDA, a marker of lipid peroxidation, decreased significantly by 88.6%, 91.9%, and 70.3%. Total antioxidant capacity (TAC) also showed substantial increases of 500.0%, 633.3%, and 306.7% in the 100 mg/kg, 300 mg/kg, and PC+PE groups compared to the PC group.

Concerning diabetes markers, the PC group exhibited hyperglycemia and impaired glucose control. PE treatment at 100 mg/kg resulted in an 80.7% decrease in glucose levels, while 300 mg/kg led to an 81.3% decrease, and PC+PE showed a 78.7% decrease. HbA1c levels also decreased significantly by 61.5%, 63.6%, and 47.6% in the respective PE treatment groups. Notably, insulin levels showed dramatic increases of 1375.0% with 100 mg/kg PE, 1358.3% with 300 mg/kg PE, and 733.3% in the PC+PE group compared to the PC group *(Table 5)*.

In summary, the PE-treated groups demonstrated substantial improvements across all measured parameters compared to the Positive Control group. The percentage changes highlight the considerable protective and healing potential of PE in mitigating liver dysfunction, dyslipidemia, oxidative stress, and hyperglycemia in this animal model. While the PE-only groups often showed more pronounced effects, the PC+PE group also exhibited significant improvements, suggesting that PE can offer benefits even in the presence of the inducing agent.

Proinflammatory Cytokines

The influence of dietary PE on pro-inflammatory cytokines revealed a consistent pattern of reduction across all measured markers compared to the Positive Control (PC) group *(Table 6)*. Specifically, Bcl-2 levels decreased substantially by 86.8% and 87.9% in the 100 mg/kg (9.1 PC vs 1.2 at 100) and 300 mg/kg PE-treated groups (9.1 PC vs 1.1 at 300), respectively, with a notable 76.9% reduction in the PC+PE group (9.1 PC vs 2.1 at PC+PE). Similarly, Nrf-2 levels saw significant decreases of 83.3% (7.5 PC vs 1.25 at 100), 84.7% (7.5 PC vs 1.15 at 300), and 80.0% (7.5 PC vs 1.5 at PC+PE) in the corresponding treatment groups. The levels of OH also exhibited marked reductions of 84.1% (8.2 PC vs 1.3 at 100), 85.4% (8.2 PC vs 1.2 at 300), and 76.8% (8.2 PC vs 1.9 at PC+PE) in the 100 mg/kg, 300 mg/kg, and PC+PE

groups, respectively. The most pronounced decreases were observed in β-actin levels, with 87.0% (10.5 PC vs 1.36 at 100), 88.8% (10.5 PC vs 1.18 at 300), and 76.2% (10.5 PC vs 2.5 at PC+PE) reductions in the respective PE-treated groups. Moving to pro-inflammatory cytokines, IL-1β levels significantly decreased by 85.5% (8.30 PC vs 1.20 at 100), 86.7% (8.30 PC vs 1.10 at 300), and 75.9% (8.30 PC vs 2.00 at PC+PE) in the 100 mg/kg, 300 mg/kg, and PC+PE groups, respectively. TNF-α levels also showed substantial reductions of 86.2% (9.00 PC vs 1.24 at 100), 87.1% (9.00 PC vs 1.16 at 300), and 76.1% (9.00 PC vs 2.15 at PC+PE) across these same groups. Furthermore, the extract demonstrated an influence on apoptosis-related genes. BAX levels decreased by 53.6% (2.80 PC vs 1.30 at 100), 60.7% (2.80 PC vs 1.10 at 300), and 35.7% (2.80 PC vs 1.80 at PC+PE) in the 100 mg/kg, 300 mg/kg, and PC+PE groups, respectively. Similarly, Casp-3 levels were reduced by 60.6% (3.10 PC vs 1.22 at 100), 63.5% (3.10 PC vs 1.13 at 300), and 37.1% (3.10 PC vs 1.95 at PC+PE) in the corresponding treatment groups. PE administration led to significant down-regulation of these pro-inflammatory markers and also mitigated the upregulation of BAX and Casp-3, suggesting a potent anti-inflammatory and potentially anti-apoptotic or protective effect in this experimental model. The consistent and substantial percentage decreases across all cytokines and treatment dosages underscore the potential of PE as an anti-inflammatory agent, even when administered in conjunction with the inducing agent in the PC+PE group. However, the PE-only groups generally exhibited slightly more pronounced effects. The p-values of <0.0001 or <0.01 for all parameters further confirm the high statistical significance of these observed effects.

Histology of Liver Tissue

Histological examination of rat liver tissue stained with Hematoxylin and Eosin (H&E) reveals distinct architectural features across the experimental groups. The control group *(Fig. 7-A)* exhibits a normal hepatic structure with well-organized hepatocyte cords radiating from central veins, clear sinusoids, and structurally sound portal triads. In contrast, the dithizone-challenged group *(Fig. 7-B)* displays significant liver damage, characterized by disorganization of hepatic cords, swollen hepatocytes potentially exhibiting vacuolation, and marked inflammation with an increased presence of inflammatory cells around structurally abnormal or congested portal triads. The phycocyanin-treated group *(Fig. 7-C)*, receiving only phycocyanin, shows a liver morphology comparable to the control group, indicating no apparent adverse effects of phycocyanin alone on the liver tissue. Notably, the group treated with both dithizone and phycocyanin extract *(Fig. 7-D)* demonstrates a clear attenuation of the dithizone-induced liver damage.

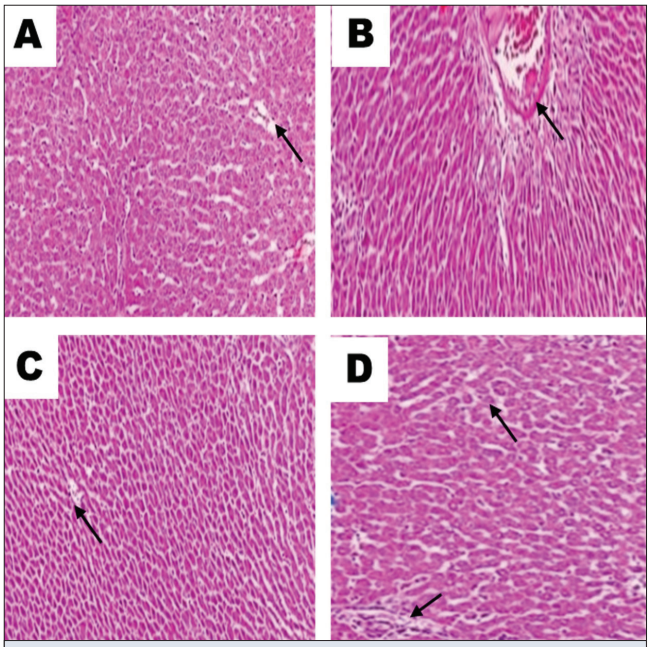

Fig 7. presents representative photomicrographs of rat liver tissue stained with Hematoxylin and Eosin (H&E) from different experimental groups. (A) Control Group, showcasing normal hepatic architecture with well-organized hepatocyte cords, clear sinusoids, and a structurally intact portal triad *(black arrow)*, (B) Dithizone-Challenged Group, exhibiting significant liver damage characterized by disarrayed hepatic cords, cellular swelling (hepatocyte ballooning), inflammatory cell infiltration, and a congested or structurally altered portal triad *(black arrow)*, (C) represents the *phycocyanin*-treated Group (PE alone), displaying liver tissue with a normal histological appearance, similar to the control group, with well-preserved hepatic architecture and a normal-looking portal triad *(black arrow)*, indicating no adverse effects of *phycocyanin* extract alone, (D) Dithizone + *phycocyanin* extract (PE) Group, revealing a noticeable improvement in liver morphology compared to the dithizone-challenged group, with less cellular disorganization, reduced inflammation, and a relatively better-preserved portal triad *(black arrows)*, suggesting the protective effect of *Phycocyanin* extract against dithizone-induced liver injury

While some signs of cellular disorganization and mild inflammation may still be present, the severity of the pathological changes is markedly reduced compared to the dithizone-challenged group, suggesting a protective effect of phycocyanin extract against chemical liver injury by preserving hepatic architecture and reducing cellular damage and inflammation.

Discussion

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia, with significant implications for oxidative stress, tissue damage, and elevated risks for secondary complications such as cancer, infections, and organ degeneration [38]. Dithizone-induced diabetes in animal models, especially rats, serves as a relevant and reproducible system to investigate drugs with multifaceted medicinal effects [39]. Among natural compounds, phycocyanin, a blue pigment-protein complex found in cyanobacteria such as *Spirulina*, has

drawn considerable attention due to its pronounced antioxidant, antidiabetic, anticancer, and antimicrobial activities [40].

Phycocyanin has demonstrated significant antidiabetic effects in various experimental models, including dithizone- and high-fat-diet-induced diabetic rodents. Its ability to lower fasting blood glucose (FBG) levels and improve glucose tolerance is attributed to several mechanisms. Recent *in vivo* studies show that administration of phycocyanin alleviates hyperglycemia, enhances glucose clearance during glucose tolerance tests, and improves pancreatic β-cell function as indicated by increased fasting insulin levels and improved HOMA-β indices in diabetic rodents [41,42]. These effects are mediated through the activation of AKT and AMPK signaling pathways in hepatic tissue, bolstering both insulin signaling and cellular glucose uptake [41]. Notably, the hypoglycemic action of phycocyanin, while progressive, is safe and maintains FBG within normal physiological ranges compared to control treatments with standard antidiabetic drugs such as metformin [41]. Diabetes is often accompanied by dyslipidemia and hepatic dysfunction, exacerbating the risk of cardiovascular complications. Phycocyanin treatment in diabetic rats leads to marked improvements in serum biochemical profiles, including reductions in triglycerides (TG), total cholesterol (TC), and liver transaminases (AST and ALT), while promoting healthy HDL cholesterol levels. Restoration of normal lipid metabolism is paralleled by suppressed hepatic steatosis and improved liver architecture, reflecting enhanced metabolic regulation. Collectively, these improvements not only mitigate diabetic complications but also reduce oxidative stress by limiting substrates for lipid peroxidation [43,44].

One of phycocyanin's most profound biological activities is its capacity to counteract oxidative stress, a major pathophysiological factor in diabetes and its complications. Dithizone-induced diabetic rats exhibit excessive formation of reactive oxygen species (ROS), leading to lipid peroxidation, protein glycation, and DNA damage. Supplementation with phycocyanin enhances endogenous antioxidant capacity, as evidenced by elevated superoxide dismutase (SOD) activity and reductions in malondialdehyde (MDA) levels-key biomarkers of oxidative injury. Furthermore, phycocyanin's chromophore, phycocyanobilin, acts as a potent free radical scavenger, directly neutralizing ROS and thus stabilizing cellular redox homeostasis [14,43,45].

Several intersecting pathways explain phycocyanin's antioxidant mechanisms, where phycocyanin upregulates nuclear factor erythroid 2-related factor 2 (Nrf2)-dependent transcription of antioxidant genes, as well as directly stimulating enzymatic antioxidants such as catalase and glutathione peroxidase. This regulatory effect minimizes oxidative assaults on vital organs like the pancreas and liver, preserving their architecture and preventing necrosis or fibrosis [46,47].

Experimental and preclinical research has illuminated phycocyanin's anticancer properties. Mechanistically, phycocyanin arrests cancer cell proliferation through blockage of cell-cycle progression, triggers apoptosis and autophagy in neoplastic cells, and downregulates pro-angiogenic factors like VEGF [48]. In rodent models, phycocyanin administration not only reduces the incidence and volume of chemically-induced tumors (notably colon cancer in conjunction with diabetic models) but also suppresses metastasis by decreasing matrix metalloproteinases (MMPs) and hypoxia-inducible factor-1α (HIF-1α). Importantly, these anticancer activities are achieved with minimal toxicity to normal tissues, highlighting phycocyanin's selectivity for tumor cells.

Diabetes is a known risk factor for cancer, partly through persistent oxidative stress, chronic inflammation, and impaired immune surveillance. By ameliorating hyperglycemia, modulating immune response, and reducing oxidative damage, phycocyanin may decrease the promotional effect of diabetes on tumorigenesis [41,42].

Phycocyanin exhibits notable antimicrobial activity against various pathogenic bacteria, fungi, and viruses, which is particularly valuable given the elevated infection risk in diabetic individuals. Recent studies document its ability to disrupt bacterial cell membranes, inhibit biofilm formation, and neutralize bacterial toxins. These effects are effective against both Gram-positive and Gram-negative strains, making phycocyanin a potential candidate for managing diabetic infections, including those resistant to standard antibiotics [14].

Underlying the antimicrobial efficacy of phycocyanin is its broader immunomodulatory effect. Phycocyanin enhances lymphocyte proliferation, stimulates antibody production, and boosts natural killer cell activity. These responses are associated with quicker resolution of infections and reduced inflammation at infection sites, thereby improving recovery and preventing chronic complications in diabetic rats [49].

Dithizone-induced diabetes primarily damages pancreatic β-cells, resulting in islet cell degeneration, necrosis, and inflammatory infiltration. Histological investigations reveal that phycocyanin administration preserves islet architecture, limits infiltration by inflammatory cells, and facilitates regeneration of β-cells. This protective effect coincides with the improvement in insulin secretion and normalization of glucose homeostasis [39]. Diabetes induces hepatic steatosis and renal tubular

degeneration. Phycocyanin reconstructs normal liver and kidney morphology in diabetic rats, preventing fatty infiltration, sinusoidal dilatation, and fibrotic changes. Its antioxidant action underlies the reduction of hepatic lipid peroxidation, while improved serum markers (reduced ALT, AST, and creatinine) corroborate these findings at the functional level [14,45,49].

Microscopic observation of treated diabetic rats shows attenuation of oxidative lesions, restoration of normal cellular ultrastructure, and reduction of apoptotic cell death in multiple organs. These changes indicate that phycocyanin not only prevents but may also reverse diabetes-related tissue injury. By improving insulin sensitivity and lipid profiles, phycocyanin reorients whole-organism metabolism toward a healthier state, countering hyperphagia, polydipsia, weight loss, and fatigue commonly observed in diabetic rats. Its comprehensive effect on both glucose and lipid metabolism distinguishes it from single-action antidiabetic agents [41,42].

Oxidative stress serves as a nexus linking metabolic dysregulation, tissue injury, infection susceptibility, and carcinogenesis in diabetic models. Phycocyanin's multifactorial properties create a positive feedback loop amelioration of oxidative stress reduces secondary tissue damage and cancer risk, which further supports metabolic control and immune competence. Although most recent studies have utilized high-fat-diet and streptozotocin-induced models, available data strongly suggest these effects extend to dithizone-induced diabetes due to the similarity in pathological mechanisms: loss of insulin-producing β-cells, heightened oxidative stress, and secondary organ involvement [42,49]. Phycocyanin's robust antioxidant, antihyperglycemic, and tissue-protective qualities make it a promising agent for experimental validation in dithizone-induced diabetic rats.

Phycocyanin possesses an array of therapeutic effects antidiabetic, antioxidant, anticancer, and antimicrobial that operate synergistically to alleviate the metabolic, oxidative, and histopathological complications of diabetes. Its mechanisms include improving glycemic control, restoring lipid metabolism, enhancing endogenous antioxidant responses, inducing apoptosis in cancer cells, protecting tissues against oxidative and inflammatory damage, and reducing infection risks through direct antimicrobial actions and immune modulation. Future research should focus on translating these capabilities into clinical settings, precisely mapping its molecular targets in various diabetic and cancer models, and optimizing its delivery for maximal efficacy. Nevertheless, phycocyanin stands as a powerful phytochemical, with promising multifunctional therapeutic potential in the management of diabetes and associated complications

Declarations

Availability of Data and Materials: The datasets used and/or analyzed during the current study are available from the corresponding author (L. A. Almutairi) on reasonable request.

Competing Interests: The authors declared that there is no conflict of interest.

Acknowledgment: The authors gratefully acknowledge Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2025R457), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia. The authors extend their appreciation to the Deanship of Research and Graduate Studies at King Khalid University for funding this work through Large Research Project under grant number RGP2/178/46.

Funding: Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2025R457), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia. Deanship of Research and Graduate Studies at King Khalid University for funding this work through Large Research Project under grant number RGP2/178/46.

Declaration of Generative Artificial Intelligence (AI): The author declare that the article tables and figures were not written or created by AI and AI-assisted technologies.

Author Contributions: Conceptualization, MAA, NSA, AMA, AAA, MA, AO, EAB, HKAG, AOS, LAA, SHA, and RNA, formal analysis, HKAG, AOS, LAA, SHA, AAA, and RNA, investigation, MAA, NSA, AAA, MA, AO, and EAB, data curation, HKAG, AOS, LAA, SHA, and RNA, writing original draft preparation, MAA, NSA, AAA, MA, AAA, AO, and EAB, writing final manuscript and editing, HKAG, AOS, LAA, SHA, and RNA, visualization and methodology, MAA, NSA, AAA, MA, AO, EAB, HKAG, AAA, AOS, LAA, SHA, and RNA. All authors have read and agreed to the published version of the manuscript.

References

1. Morya S, Kumar Chattu V, Khalid W, Zubair Khalid M, Siddeeg A: Potential protein phycocyanin: An overview on its properties, extraction, and utilization. *Int J Food Prop,* 26, 3160-3176, 2023. DOI: 10.1080/10942912.2023.2271686

2. García-Gómez C, Aguirre-Cavazos DE, Chávez-Montes A, Ballesteros-Torres JM, Orozco-Flores AA, Reyna-Martínez R, Torres-Hernández ÁD, González-Meza GM, Castillo-Hernández SL, Gloria-Garza MA, Kačániová M, Ireneusz-Kluz M, Elizondo-Luevano JH: Phycobilins versatile pigments with wide-ranging applications: Exploring their uses, biological activities, extraction methods and future perspectives. *Mar Drugs,* 23 (5):201, 2025. DOI: 10.3390/md23050201

3. Chavda V, Patel S: Hyperglycaemic metabolic complications of ischemic brain: Current Therapeutics, anti-diabetics and stem cell therapy. *CNS Neurol Disord Drug Targets,* 22, 832-856, 2023. DOI: 10.2174/1871527321666220609200852

4. Qamar F, Sultana S, Sharma M: Animal models for induction of diabetes and its complications. *J Diabetes Metab Disord,* 22, 1021-1028, 2023. DOI: 10.1007/s40200-023-01277-3

5. Ou Y, Lin L, Yang X, Pan Q, Cheng X: Antidiabetic potential of phycocyanin: Effects on KKAy mice. *Pharm Biol,* 51, 539-544, 2013. DOI: 10.3109/13880209.2012.747545

6. Prabakaran G, Sampathkumar P, Kavisri M, Moovendhan M: Extraction and characterization of phycocyanin from *Spirulina platensis* and evaluation of its anticancer, antidiabetic and antiinflammatory effect. *Int J Biol Macromol,* 153, 256-263, 2020. DOI: 10.1016/j.ijbiomac.2020.03.009

7. Munawaroh HSH, Gumilar GG, Nurjanah F, Yuliani G, Aisyah S,

Kurnia D, Wulandari AP, Kurniawan I, Ningrum A, Koyande AK, Show PL: *In-vitro* molecular docking analysis of microalgae extracted phycocyanin as an anti-diabetic candidate. *Biochem Eng J,* 161:107666, 2020. DOI: 10.1016/j.bej.2020.107666

8. Ziyaei K, Abdi F, Mokhtari M, Daneshmehr MA, Ataie Z: Phycocyanin as a nature-inspired antidiabetic agent: A systematic review. *Phytomedicine,* 119:154964, 2023. DOI: 10.1016/j.phymed.2023.154964

9. Gabr GA, El-Sayed SM, Hikal MS: Antioxidant activities of phycocyanin: A bioactive compound from *Spirulina platensis*. *J Pharm Res Int,* 32, 73-85, 2020. DOI: 10.9734/jpri/2020/v32i230407

10. Liu R, Qin S, Li W: Phycocyanin: Anti-inflammatory effect and mechanism. *Biomed Pharmacother,* 153:113362, 2022. DOI: 10.1016/j.biopha.2022.113362

11. Braune S, Krüger-Genge A, Kammerer S, Jung F, Küpper JH: Phycocyanin from *Arthrospira platensis* as potential anti-cancer drug: Review of *in vitro* and *in vivo* studies. *Life,* 11 (2):91, 2021. DOI: 10.3390/life11020091

12. Ravi M, Tentu S, Baskar G, Rohan Prasad S, Raghavan S, Jayaprakash P, Jeyakanthan J, Rayala SK, Venkatraman G: Molecular mechanism of anti-cancer activity of phycocyanin in triple-negative breast cancer cells. *BMC Cancer,* 15:768, 2015. DOI: 10.1186/s12885-015-1784-x

13. Bougatef H, Hadrich F, Gazbar M, Sila A, Chamkha M, Bougatef A: Development of a novel method for the extraction of phycocyanin pigment from *Spirulina platensis* and assessment of its antioxidant, antimicrobial, and anticancer activities. *Biomass Convers Biorefin,* 15, 8001-8013, 2025. DOI: 10.1007/s13399-024-05540-2

14. Husain A, Khanam A, Alouffi S, Shahab U, Alharazi T, Maarfi F, Khan S, Hasan Z, Akasha R, Farooqui A, Ahmad S: C-phycocyanin from cyanobacteria: A therapeutic journey from antioxidant defence to diabetes management and beyond. *Phytochem Rev,* 2024:1-19, 2024. DOI: 10.1007/s11101-024-10045-x

15. Jiang L, Yu S, Chen H, Pei H: Enhanced phycocyanin production from *Spirulina subsalsa* via freshwater and marine cultivation with optimized light source and temperature. *Bioresour. Technol,* 378:129009, 2023. DOI: 10.1016/j.biortech.2023.129009

16. Kamble SP, Gaikar RB, Padalia RB, Shinde KD: Extraction and purification of C-phycocyanin from dry *Spirulina* powder and evaluating its antioxidant, anticoagulation and prevention of DNA damage activity. *J Appl Pharm Sci,* 3, 149-153, 2013.

17. AOAC: Official Methods of Analysis. 18th ed., Association of Official Analytical Chemists, Arlington, VA., USA, 2012.

18. Pearson D: The Chemical Analysis of Food. 6th ed., 504-530, Church Hill, Livingstone, Edinburgh, 1981.

19. Hajiyeva S, Cankilic MY, Kilic V, Gorgulu S, Patrignani F, Lanciotti R: An insight into the potential antioxidant, anticancer and antimicrobial activities of *Geitlerinema* sp. C-phycocyanin extracts. *Algal Res,* 85:103836, 2025. DOI: 10.1016/j.algal.2024.103836

20. Shrivastava A, Gupta VB: Methods for the determination of limit of detection and limit of quantitation of the analytical methods. *Chron Young Sci,* 2 (1): 21-25, 2011. DOI: 10.4103/2229-5186.79345

21. Alkhudaydi HMS, Muriuki EN, Spencer JP: Determination of the polyphenol composition of raspberry leaf using LC-MS/MS. *Molecules*, 30 (4):970, 2025. DOI: 10.3390/molecules30040970

22. Carneiro AdA, Sinoti SBP, de Freitas MM, Simeoni LA, Fagg CW, Magalhães PdO, Silveira D, Fonseca-Bazzo YM: Hydroethanolic extract of *Morus nigra* L. leaves: A dual PPAR-α/γ agonist with anti-inflammatory properties in lipopolysaccharide-stimulated RAW 264.7. *Plants,* 11 (22):3147, 2022. DOI: 10.3390/plants11223147

23. Nair SS, Kavrekar V, Mishra A: *In vitro* studies on alpha amylase and alpha glucosidase inhibitory activities of selected plant extracts. *Eur J Exp Biol,* 3, 128-132, 2013.

24. Alsubhi NH, Al-Quwaie DA, Alrefaei GI, Alharbi M, Binothman N, Aljadani M, Saad A: Pomegranate pomace extract with antioxidant, anticancer, antimicrobial, and antiviral activity enhances the quality of strawberry-yogurt smoothie. *Bioengineering,* 9 (12):735, 2022. DOI: 10.3390/bioengineering9120735

25. Alowaiesh BF, Alhaithloul HAS, Saad AM, Hassanin AA: Green biogenic of silver nanoparticles using polyphenolic extract of olive leaf wastes with focus on their anticancer and antimicrobial activities. *Plants,* 12 (6):1410, 2023. DOI: 10.3390/plants12061410

26. Sayed-Ahmed ETA, Salah KBH, El-Mekkawy RM, Rabie NA, Ashkan MF, Alamoudi SA, Alruhaili MH, Al Jaouni SK, Almuhayawi MS, Selim S, Saad AM, Namir M: The preservative action of protein hydrolysates from legume seed waste on fresh meat steak at 4°C: Limiting unwanted microbial and chemical fluctuations. *Polymers,* 14 (15):3188, 2022. DOI: 10.3390/polym14153188

27. El-Saadony MT, Saad AM, Najjar AA, Alzahrani SO, Alkhatib FM, Shafi ME, Selem E, Desoky EM, Fouda SEE, El-Tahan AM, Hassan MAA: The use of biological selenium nanoparticles to suppress *Triticum aestivum* L. crown and root rot diseases induced by *Fusarium* species and improve yield under drought and heat stress. *Saudi J Biol Sci,* 28, 4461-4471, 2021. DOI: 10.1016/j.sjbs.2021.04.043

28. Rubinstein LV, Shoemaker RH, Paull KD, Simon RM, Tosini S, Skehan P, Scudiero DA, Monks A, Boyd MR: Comparison of *in vitro* anticancer-drug-screening data generated with a tetrazolium assay versus a protein assay against a diverse panel of human tumor cell lines. *J Natl Cancer Inst,* 82, 1113-1117, 1990. DOI: 10.1093/jnci/82.13.1113

29. Allam RM, Al-Abd AM, Khedr A, Sharaf OA, Nofal SM, Khalifa AE, Mosli HA, Abdel-Naim AB: Fingolimod interrupts the cross talk between estrogen metabolism and sphingolipid metabolism within prostate cancer cells. *Toxicol Lett,* 291, 77-85, 2018. DOI: 10.1016/j.toxlet.2018.04.008

30. Chu SY, Jung JH, Park MJ, Kim SH: Risk assessment of metabolic syndrome in adolescents using the triglyceride/high-density lipoprotein cholesterol ratio and the total cholesterol/high-density lipoprotein cholesterol ratio. *Ann Pediatr Endocrinol Metab,* 24, 41-48, 2019. DOI: 10.6065/apem.2019.24.1.41

31. Bijland S, Pieterman EJ, Maas AC, van der Hoorn JW, van Erk MJ, van Klinken JB, Havekes LM, van Dijk KW, Princen HM, Rensen PC: Fenofibrate increases very low density lipoprotein triglyceride production despite reducing plasma triglyceride levels in APOE* 3-Leiden. CETP mice. *J Biol Chem,* 285, 25168-25175, 2010. DOI: 10.1074/jbc.M110.123992

32. Sampson M, Ling C, Sun Q, Harb R, Ashmaig M, Warnick R, Sethi A, Fleming JK, Otvos JD, Meeusen JW, Delaney SR, Jaffe AS, Shamburek R, Amar M, Remaley AT: A new equation for calculation of low-density lipoprotein cholesterol in patients with normolipidemia and/or hypertriglyceridemia. *JAMA Cardiol,* 5, 540-548, 2020. DOI: 10.1001/jamacardio.2020.0013

33. Cheng CH, Chu CY, Chen HL, Lin IT, Wu CH, Lee YK, Hu PJ, Bair MJ: Subgroup analysis of the predictive ability of aspartate aminotransferase to platelet ratio index (APRI) and fibrosis-4 (FIB-4) for assessing hepatic fibrosis among patients with chronic hepatitis C. *J Microbiol Immunol Infect,* 53, 542-549, 2020. DOI: 10.1016/j.jmii.2019.09.002

34. Alatawi FS, Faridi UA, Alatawi MS: Effect of treatment with vitamin D plus calcium on oxidative stress in streptozotocin-induced diabetic rats. *Saudi Pharm J,* 26, 1208-1213, 2018. DOI: 10.1016/j.jsps.2018.07.012

35. Pappas A, Tsiokanos A, Fatouros IG, Poulios A, Kouretas D, Goutzourelas N, Giakas G, Jamurtas AZ: The effects of *Spirulina* supplementation on redox status and performance following a muscle damaging protocol. *Int J Mol Sci,* 22:3559, 2021. DOI: 10.3390/ijms22073559

36. Chen JC, Fang C, Zheng RH, Chen ML, Kim DH, Lee YH, Bailey C, Wang KJ, Lee JS, Bo J: Environmentally relevant concentrations of microplastics modulated the immune response and swimming activity, and impaired the development of marine medaka *Oryzias melastigma* larvae. *Ecotoxicol Environ Saf,* 241:113843, 2022. DOI: 10.1016/j.ecoenv.2022.113843

37. Saif GB, Khan IA: Association of genetic variants of the vitamin D receptor gene with vitiligo in a tertiary care center in a Saudi population: A case-control study. *Ann Saudi Med,* 42, 96-106, 2022. DOI: 10.5144/0256-4947.2022.96

38. González P, Lozano P, Ros G, Solano F: Hyperglycemia and oxidative stress: an integral, updated and critical overview of their metabolic interconnections. *Int J Mol Sci,* 24:9352, 2023. DOI: 10.3390/ijms24119352

39. Algul S, Ozcelik O: Comprehensive review of animal models in diabetes research using chemical agents. *Lab Anim,* 59, 356-363, 2025. DOI:

10.1177/00236772241296199

40. Papadaki S, Tricha N, Panagiotopoulou M, Krokida M: Innovative bioactive products with medicinal value from microalgae and their overall process optimization through the implementation of life cycle analysis - An overview. *Mar Drugs,* 22:152, 2024. DOI: 10.3390/md22040152

41. Hao S, Li F, Li Q, Yang Q, Zhang W: Phycocyanin protects against high glucose high fat diet induced diabetes in mice and participates in AKT and AMPK signaling. *Foods,* 11 (20):3183, 2022. DOI: 10.3390/foods11203183

42. Husain A, Alouffi S, Khanam A, Akasha R, Farooqui A, Ahmad S: Therapeutic efficacy of natural product 'C-phycocyanin'in alleviating streptozotocin-induced diabetes via the inhibition of glycation reaction in rats. *Int J Mol Sci,* 23:14235, 2022. DOI: 10.3390/ijms232214235

43. Bahrini I, Alamoudi M, Alazami M, Alrashdi J, Alouche N: Protective effects of C-Phycocyanin from *Spirulina platensis* against pancreatic inflammation, lymphocyte infiltration, impaired carbohydrate digestion, and glucose metabolism dysregulation in diabetic rats. *J Agric Food Res,* 2025:101951, 2025. DOI: 10.1016/j.jafr.2025.101951

44. Biryulina N, Sidorova YS, Zorin S, Petrov N, Guseva G, Mazo V, Kochetkova A: Evaluation of the combined effect of *Arthrospira platensis* biomass phycyanin concentrate and soy protein on male Wistar rats fed a high-fat diet with added cholesterol. *Vopr Pitan,* 94 (2): 73-84, 2025. DOI: 10.33029/0042-8833-2025-94-2-73-84

45. Zheng J, Inoguchi T, Sasaki S, Maeda Y, McCarty MF, Fujii M, Ikeda N, Kobayashi K, Sonoda N, Takayanagi R: Phycocyanin and phycocyanobilin from *Spirulina platensis* protect against diabetic nephropathy by inhibiting oxidative stress. *Am J Physiol Regul Integr Comp Physiol,* 304 (2): 110-120, 2013. DOI: 10.1152/ajpregu.00648.2011

46. Xu F, Yang F, Qiu Y, Wang C, Zou Q, Wang L, Li X, Jin M, Liu K, Zhang S, Zhang Y, Li B: The alleviative effect of C-phycocyanin peptides against TNBS-induced inflammatory bowel disease in zebrafish via the MAPK/Nrf2 signaling pathways. *Fish Shellfish Immunol,* 145:109351, 2024. DOI: 10.1016/j.fsi.2023.109351

47. Puengpan S, Phetrungnapha A, Sattayakawee S, Tunsophon S: Phycocyanin attenuates skeletal muscle damage and fatigue via modulation of Nrf2 and IRS-1/AKT/mTOR pathway in exercise-induced oxidative stress in rats. *PLoS One,* 19:e0310138, 2024. DOI: 10.1371/journal.pone.0310138

48.Jayanti DAPIS, Abimanyu IGAM, Azzamudin H: *Spirulina platensis's* phycocyanobilin as an antiangiogenesis by inhibiting VEGFR2-VEGFA pathway in breast cancer: *In silico* study. *J Smart Bioprospe Technol,* 2 (3): 87-91, 2021. DOI: 10.21776/ub.jsmartech.2021.002.03.87

49. Hoseini F, Hoseini S, Fazilati M, Ebrahimie E, Hoseini F, Choopani A: Evaluation of C-phycocyanin effects with drug purity on the immune system through its effect on interferon-gamma (INF-γ). *Int J Med Rev,* 8, 188-193, 2021. DOI: 10.30491/ijmr.2021.270381.1181