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Glavni urednik/Editor-in-chief
Matevž Likar, Slovenija / Slovenia, matevz.likar@bf.uni-lj.si

Odgovorna urednica/Managing editor
Anita Jemec Kokalj, Slovenija / Slovenia, anita.jemec@bf.uni-lj.si

Oblikovanje/Design
Ajda Fortuna

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Original Research

Saffron (*Crocus sativus* L.) based protection against Aflatoxin B1 induced haematological and organ damages in rats

Hayat Ashi [1,2], Enas A. Hamed [3], Bassem Refaat [4], Shakir Idris [4], Latifa Khayyat [1,2], Tasahil S. Albishi [1,2], Leena A. Neyaz [1,2], Outour Tariq Alami [1,2], Fatimah Al-Rahmani [5], Shirin Aashi [6], Abdulaziz A. Alamri [6], Ghazi H. Abduljawad [6], Ayman A. Alobaidi [6], Fahad A. Alburberry [6], Saleh H. Alsalhi [6], Rayyan M. Wali [6], Khaled Elbanna [1,2,7], Hussein H. Abulreesh [1,2*]

Abstract

Saffron is well-known for its anti-apoptotic, anti-inflammatory, and antioxidant properties. Saffron's nutritional and medicinal properties support its numerous uses as a flavouring and herbal remedy. This study investigated the protective efficacy of saffron administration against aflatoxin B1 (AFB1)-induced toxicity in adult male Wistar albino rats during an experimental period of 21 days. Aflatoxin B1 (AFB1) is a common mycotoxin of soils and foodstuffs. Thirty-two rats were divided into four groups (Control group, AFB1 group, Saffron group, and AFB1+ Saffron group), and their body weights were measured on days 1, 7, 14, and 21. Blood samples were collected on the 21st day for haematological and biochemical studies (testosterone, kidney and liver function tests, and oxidative stress markers). Tissue samples from testes, liver, and kidney were subjected to histological examinations. The results depicted a significant decrease in the body weights after 7, 14, and 21 days of Saffron, AFB1, and AFB1+ Saffron treatments in comparison to control. Haematological investigations showed that basophils, platelets, monocytes, lymphocytes, and eosinophils greatly increased compared to the control group, whereas neutrophils and eosinophils dramatically decreased. There was a significant rise in the serum levels of uric acid, creatinine, aspartate transaminase, alkaline phosphatase, nitric oxide, and malondialdehyde. Contrarily, testosterone levels notably reduced in AFB1-

1 Department of Biology, Faculty of Science, Umm Al-Qura University, Makkah 21955, Saudi Arabia

2 Research Laboratories Unit, Faculty of Science, Umm Al-Qura University, Makkah 21955, Saudi Arabia

3 Department of Medical Physiology, Faculty of Medicine, Assiut University, Assiut, Egypt

4 Department of Clinical Laboratory Sciences, Faculty of Applied Medical Sciences, Umm Al-Qura University, Makkah 21955, Saudi Arabia

5 Department of Chemistry, Faculty of Science, Umm Al-Qura University, Makkah 21955, Saudi Arabia

6 College of Pharmacy, King Abdulaziz University, Jeddah, Saudi Arabia

7 Department of Agricultural Microbiology, Faculty of Agriculture, Fayoum University, Fayoum 63514, Egypt

administered rats as compared to controls. AFB1 group exhibited several histological modifications in testes, liver, and kidney tissues. Oxidative stress biomarkers, testosterone, kidney and liver functions, and haematological parameters of the AFB1+ Saffron group remained similar to the control group. Kidney and liver tissues of Saffron-treated rats also displayed normal structure similar to the control group, which confirmed its protective efficacy against AFB1-induced toxicity. Saffron's bioactive components and antioxidant and pharmacological properties might have contributed to its promising anti-AFB1-toxicity potential.

Keywords

Aflatoxin B1, Antioxidant, Hemotoxicity, Hepatotoxicity, Nephrotoxicity, Saffron, Testosterone, Oxidative stress.

* **Corresponding author:**
E-mail address: hhabulreesh@uqu.edu.sa

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Žafran (*Crocus sativus* L.) kot zaščita pred poškodbami organov in hematološkimi spremembami pri podganah zaradi aflatoksina B1

Izvleček

Žafran je znan po svojih anti-apoptotičnih, protivnetnih in antioksidativnih lastnostih. Prehranske in zdravilne lastnosti žafrana podpirajo njegovo številno uporabo kot začimbe in zeliščnega zdravila. V tej študiji je bila raziskana zaščitna učinkovitost žafrana pred delovanjem aflatoksina B1 (AFB1), pri odraslih samcih podgan Wistar albino v poskusnem obdobju 21 dni. Aflatoksin B1 (AFB1) je pogost mikotoksin v tleh in živilih. V poskusu smo 32 podgan razdelili v štiri skupine (kontrolna skupina, skupina z AFB1, skupina z žafranom in skupina z AFB1 + žafran). Njihovo rast smo spremljali preko telesne teže 1., 7., 14. in 21. dan. Ob zaključku poskus smo 21. dan odvzeli vzorce za hematološke in biokemične analize (testosteron, testi delovanja ledvic in jeter ter markerji oksidativnega stresa). Vzorci tkiva semenčic, jeter in ledvic so bili predmet histoloških preiskav. Rezultati so pokazali znatno zmanjšanje telesne teže po 7, 14 in 21 dneh zdravljenja z žafranom, AFB1 in AFB1+ žafranom v primerjavi s kontrolo. Hematološke preiskave so pokazale, da so se bazofili, trombociti, monociti, limfociti in eozinofili močno povečali v primerjavi s kontrolno skupino, medtem ko so se nevtrofilci in eozinofilci močno zmanjšali. V serumu so se znatno povečale vrednosti sečne kisline, kreatinina, aspartatne transaminaze, alkalne fosfataze, dušikovega oksida in malondialdehida. Nasprotno pa se je raven testosterona pri podganah, ki so prejemale AFB1, v primerjavi s kontrolami opazno zmanjšala. Pri skupini z AFB1 so se pokazale številne histološke spremembe v testisih, jetrih in ledvicah. Biomarkerji oksidativnega stresa, testosteron, delovanje ledvic in jeter ter hematološki parametri skupine z AFB1+ žafranom so ostali podobni kontrolni skupini. Tudi ledvična in jetrna tkiva podgan, zdravljenih z žafranom, so imela normalno strukturo, podobno kontrolni skupini, kar je potrdilo njegovo zaščitno učinkovitost pred toksičnostjo, povzročeno z AFB1. Bioaktivne sestavine žafrana ter njegove antioksidativne in farmakološke lastnosti so morda prispevale k njegovemu obetavnemu potencialu proti toksičnosti AFB1.

Ključne besede

Aflatoksin B1, antioksidanti, hemotoksičnost, hepatotoksičnost, nefrotoksičnost, žafran, testosteron, oksidativni stres

Introduction

Mycotoxin pollution has emerged as a global phenomenon in recent years. Hazardous aflatoxins (AF) of *Aspergillus parasiticus* and *A. flavus* can infect agricultural produce during harvesting, storage, and processing. Airborne particulate matter can also spread aflatoxin during the storage and processing of contaminated cereal crop products, which increases the aflatoxin exposure risk in animals and humans. Aflatoxin can also be found in food products such as milk, eggs, and ruminant meat that are fed on contaminated diets (Rushing and Selim, 2019). A. parasiticus produces four types of aflatoxins (B1, B2, G1, and G2), whereas A. flavus produces two types of aflatoxins (B1 and B2) (Mtimet et al., 2015).

Aflatoxin B1 (AFB1) is highly detrimental that can cause growth retardation, mutagenesis, and carcinogenesis (Shabeer et al., 2022). The genotoxicity of AFB1 can also impair the testes, kidneys, heart, and liver. However, its toxicity mechanisms require further elucidation (Dai et al., 2017). Aflatoxins' purity in the foodstuff could reveal the mechanism of its successful growth in target organs. For instance, in the liver as a primary target, the toxin purity might also affect heart cells, kidneys, and lungs (Karmanov et al., 2021).

Crocus sativus L., a perennial herb of the Iridaceae family, is grown in various countries such as Mexico, China, France, Morocco, Iran, Turkey, Azerbaijan, Egypt, Greece, Spain, India, and Italy. There are three primary saffron constituents such as (a) crocin, the main colouring agent (mono- and diglycosyl esters of a polyene dicarboxylic acid known as crocetin), (b) glycoside picrocrocin, the safranal precursor, adds bitter taste, and (c) safranal, a monoterpene aldehyde (deglycosylated picrocrocin), that produces distinctive saffron aroma (Rezaee and Hosseinzadeh, 2013). The dried stigma of the C. sativus plant (saffron) is a commonly used spice and culinary colouring agent (Rezaee and Hosseinzadeh, 2013). Recent pharmacological studies have revealed that saffron and its bioactive components can reduce male erectile dysfunction and possess antioxidant, anti-inflammatory, and antinociceptive characteristics (Hosseinzadeh and Shariaty, 2007). This study elaborated on the protective effects of oral saffron extract administration (21 days) against hazardous impacts of AFB1 on the haematological parameters, liver, kidneys, and testes of adult male Wistar albino rats.

Materials and Methods

Animals

Thirty-two healthy adult male Wistar albino rats (180–200g, 7-8 weeks old) were obtained from King Fahd Medical Research Centre, King Abdulaziz University, Jeddah, Saudi Arabia. The rats were kept in clean, sterile polypropylene cages and exposed to a 12-hour light/dark cycle during the experimental period (4 weeks). To acclimatize, the rats were housed in air-conditioned rooms (21-23°C and 60-65% humidity) for one week before the initiation of experimental procedures. The rats had free access to a basic chow diet and tap water. The experiment protocol was approved by the Ethical Committee of King Fahd Medical Research Centre, Jeddah, Saudi Arabia (Approval # 163-19). ARRIVE guidelines were followed to carefully handle the experimental rats.

Reference fungal isolate

Aflatoxin-producing *Aspergillus flavus* CYA reference strain (AUMC 9779) was provided by Professor Ahmed Y. Abdelmalek, Moubasher Mycological Centre, Assiut University, Egypt. The isolate was inoculated on Potato Dextrose agar (PDA) slants and stored at five °C for short-period preservation (10-20 days) and -80°C (cryogenic temperature) for long-term preservation (more than six months).

Local fungal isolate

To obtain a local Saudi Arabian isolate, a loopful of spores was scrapped off from mouldy bread and cultured on PDA plates. PDA was prepared by adding freshly prepared potato extract (4.0g), glucose (20 g) (BDH Chemicals Ltd, England), and agar (20 g) (MOLEQULE-ON, New Zealand) to distilled water (1000 ml). The mixture was boiled to dissolve the agar, sterilized, and poured into plates. The isolate was also retrieved in parallel by using Sabouraud dextrose agar (SDA) (HiMedia, India). Both media (PDA and SDA) were incubated for 5 to 7 days at 25±2 °C (Fakruddin et al., 2015; Ashi et al., 2023 a, b). Then, a cork pourer was used to inoculate the fungal disk on *Aspergillus* differential agar [tryptone (15 g), yeast extract (10 g), ferric citrate (0.5 g), agar (15 g), and distilled water (1000 ml)]. The media was boiled and autoclaved before usage (Sreekanth et al., 2011; Ashi et al., 2023a, b).

Maintaining and storage of the isolates

The long-duration storage of isolates was carried out by culturing A. flavus isolate on SDA plates and incubating (25 ± 2 °C) for 5 to 7 days. Then, A. flavus colonies were inoculated into a sterile glycerol solution (15%) in sterile microfuge tubes (250 µl). These tubes were preserved at -80 °C to maintain fungal spores' vitality (Nielsen and Smedsgaard, 2003).

Preparation of broth media

A 500 ml of Sabouraud dextrose broth (SDB) (HiMedia) was prepared by following the manufacturer's guidelines. SDB aliquots (150 ml) were autoclaved (121 °C, 15 min, and 1.5 atmospheric pressure) in 250 ml flasks. The flasks were kept in the refrigerator until utilized.

Production, extraction, and determination of aflatoxin from *A. flavus*

A. flavus isolate was cultivated (25±2°C) in SDB under aerobic and shaking conditions for 5 to 14 days. Then, a sterile funnel was used to filter the culture through sterile filter papers (MN 615 - ∅ 150 mm) into a flask. The filtrates were transferred to sterile conical tubes (15ml) (Plastilab) and left overnight at cryogenic -80°C to weaken the fungal cell walls. It helped in better solvent penetration into the cell for secondary products' extraction (Saldan et al., 2018; Ashi et al., 2023a, b). The contents were transferred to a methanol-containing (15 ml) hydrophilic bottle and subjected to ultrasonic vibration for 30 min (Nielsen and Smedsgaard, 2003; Ashi et al., 2023a, b). Then, it was placed in an orbital shaker (100 rpm, 30 min), and the step was repeated several times. Finally, the extract was filtered through micropore (Bedford, USA) filter paper in a sterile glass funnel. The filtrate was evaporated under a nitrogen flow. This concentrated extract was dissolved in methanol (1.5 ml) and subjected to Gas Chromatography (GC) (Shimadzu, Kyoto, Japan) and high-performance liquid chromatography (HPLC) (Shimadzu, Kyoto, Japan) (Bertrand et al., 2013; Ashi et al., 2023a, b). Aflatoxin presence was confirmed by comparing with standard crude AFB1 extract (Ashi et al., 2023a, b).

Confirmation of aflatoxin production

A. flavus-based aflatoxin production was quickly confirmed according to the Ammonia vapour method, which turned toxin-secreting colonies to pink on PDA and SDA culture plates. Ammonia solution (25%) was prepared by adding ammonia (25 ml) into distilled water (75 ml). This solution (0.2 ml) was added to the Petri dish followed by incubation (24 hours, 25°C), which yielded a red colour at the bottom of fungal colonies (Saito and Machida, 1999; Ashi et al., 2023a, b).

Preparation of saffron extract

Dried saffron threads were purchased from Afghanistan Saffron Co. (Herat, Afghanistan). Saffron extract was prepared by adding saffron (90 mg) to distilled water (200 ml) at 80°C. The solution was left for five minutes and filtered.

Experimental design

Thirty-two adult male Wistar rats were randomly divided into four groups of eight rats. The first group was orally administered a basal diet, which served as the negative control. The second group was orally administered with saffron extract (80 mg/kg) for 21 days and fed on the basal diet (Mashmoul et al., 2016). The third group was orally administered with Aflatoxin B1 (1 mg/ kg) for 21 days and fed on the basal diet (Rotimi et al., 2019). The fourth group was orally administered with aflatoxin + saffron extract (AFB1 1 mg/ kg + Saffron 80 mg/kg) for 21 days and fed on the basal diet (Türk et al., 2008).

Biological and biochemical studies

Rats' body weight was measured on the 1st, 7th, 14th, and 21st days using a digital scale. After the completion of the experimental period, the rats were anaesthetized (isoflurane) and sacrificed by cervical dislocation. Blood samples (6 ml) were collected following the procedure for orbital sinus blood samples collection (Parasuraman et al. 2010) in two tubes (a plain tube and an EDTA (ethylenediaminetetraacetic acid-containing tube). Briefly, the animals were scruffed with the thumb and forefinger of the non-dominant hand and the skin around the eye was pulled taut. A capillary was inserted into the medial canthus of the eye around 30 30-degree angles to the nose; with slight thumb pressure, the capillary entered the plexus/sinus, allowing the blood to flow into the tube (Parasuraman et al. 2010). Blood samples in plain tubes were centrifuged (6000xg, four °C) for 10 minutes. The serum was aliquoted and immediately frozen at -80°C until further analysis. Sera were used to analyze

liver functions [albumin, alkaline phosphatase (ALP), and aspartate transferase (AST)], kidney functions [urea and uric acid, creatinine using rat-specific ELISA kits (Bender Med-Systems GmbH, Wien, Austria) following the manufacturer's procedure, oxidative stress biomarkers [nitric oxide (NO), and malondialdehyde (MDA)] were determined using colourimetric nitric oxide assay kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and rat malondialdehyde ELISA kit (Antibodies.com, Cambridge, UK) according to the manufacturers' instruction. Testosterone hormone was determined using a testosterone rat/mouse ELISA kit (Rocky Mountain Diagnostics Inc., Colorado Springs, USA) as described in the manufacturers' guidelines. Blood samples in EDTA tubes were examined for the complete blood count (CBC), haemoglobin, red blood cells (RBCs), platelets, mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), white blood cells (WBCs), neutrophils, monocyte, lymphocyte, basophil, and eosinophil using Dymin DH36 Auto Hematology Analyzer (Wuhan Aliroad Medical Equipment Co. Ltd., Wuhan, China).

Histological examinations

The rats were sacrificed at the end of the experimental period. The abdomen and pelvis were dissected, whereas the testes, kidneys, and liver were excised, followed by cleaning with distilled water. A neutral formalin solution (10%) was initially used to fix the specimens, which were subjected to gradual ethanol (50–100%) dehydration and xylene-based cleansing, followed by paraffin embedding. Sections of 5mm thickness were cut and stained with eosin and hematoxylin. An experienced histopathologist examined the organ slices under a light microscope to assess the tissue alterations.

Determination of total phenolic and flavonoid contents in saffron

A standard HPLC system (maximum pressure < 400 bar) (Shimadzu, Kyoto, Japan) was employed to determine phenolic compounds in saffron. The procedure of Manchón et al. (2010) was followed to select the mobile phases, which revealed a much lower system back pressure as compared to other solvents such as methanol. It assisted in rapid analysis with a standard HPLC system. UV spectra were recorded between 210 and 800 nm, whereas chromatograms were registered at 280 and 380 nm.

Statistical Analysis

The experimental data was expressed as mean ± standard error of means (SEM) or mean ± standard deviation (SD). SPSS version 22 (Statistical Package for Social Sciences, IBM Corp., USA) was used for the data analysis. Shapiro-Wilk test revealed the normal value distributions. One-way ANOVA was performed to examine the data variance, whereas the means of the different groups were compared through Tukey's test at a significance level of P <0.05.

Results

Total phenolic and flavonoid contents

Table 1 presents the saffron contents. The total phenolics were calculated according to the methodology of Folin-Ciocalteu. Saffron (*C. sativus*) samples exhibited high content of phenolics, anthocyanins, and flavonoids. The presence of phenolics and anthocyanins was noted to be higher than flavonoids (Table 1 and Figure 1).

Identification of phenolic, flavonoid, and anthocyanin compounds in saffron

The compounds such as gallic acid, kaempferol-3-o-glucoside, gentisitic acid, syringic acid, catechol, and vanillin were identified in saffron samples (Table 2). The identified compounds presented a maximum absorption within a range of 272–380nm. Molecular formula and chemical structures of major identified compounds are shown in Table 2 and Fig. 2.

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The purple-coloured DPPH can exist as a stable free radical at an absorbance wavelength of 520 nm. The interaction of antioxidants converts DPPH to a non-radical form, and the alteration of colour from purple to yellow confirms the antioxidant activity. The acceptance of electrons reduces the absorbance of DPPH with the conversion into a non-radical form. Fig. 3 exhibits the alterations in DPPH radical scavenging impacts of saffron and Beautlated hydroxy anisol (BHA) at various concentrations (0.1, 0.25, 0.50, and 0.75 mg/ml). The results depicted a higher DPPH radical scavenging efficiency of saffron than BHA.

Table 1. Total phenolic, flavonoid, and anthocyanin compounds in saffron samples (mean ±SD).

Tabela 1. Skupni fenoli, flavonoidi in antociani v vzorcih žafrana (povprečje ±SD).

Sample	Phenolic Content (mg gallic acid EQ/100g DW)	Flavonoid Content (mg catechine EQ/100g DW)	Anthocyanin content (mg cyanidin EQ/ 100g DW)
Saffron (0.25 mg)	1144.1 ± 93.45	104 ± 43.84	1109.11±87.37
Saffron (0.50 mg)	1353 ± 135.22	326.09±75.70	1435.09±152.24
Saffron (0.75 mg)	1876 ± 246.28	657±98.32	1690.11±387.62

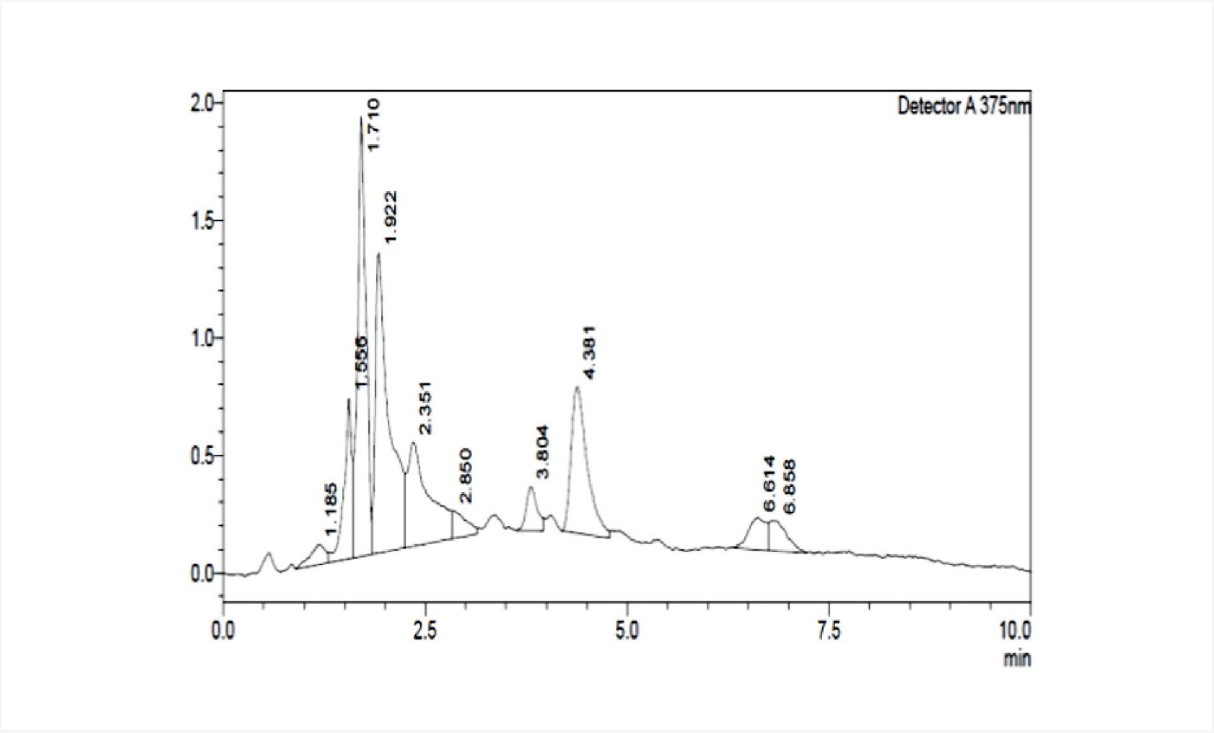

Figure 1. Identified compounds in saffron.

Slika 1. Identificirane snovi v žafranu.

Table 2. HPLC-MS-ESI based detection of major saffron compounds.

Tabela 2. HPLC-MS-ESI detekcija poglavitnih snovi v žafranu.

No.	Assignment compounds	(min)	UV data	$[M-H]^-$	Molecular formula
1	Gallic acid	1.71	380	169.9	$C_7H_6O_5$
2	Kaempferol-3-o-Glucoside	1.92	380	447.4	$C_{21}H_{19}O_{11}$
3	Gentisitic acid	2.35	380	154.12	$C_7H_6O_4$
4	Syringic acid	3.80	380	198.17	$C_9H_{10}O_5$
5	Catechol	4.38	380	110.11	$C_6H_6O_2$
6	Vanillin	6.61	380	152.14	$C_8H_8O_3$

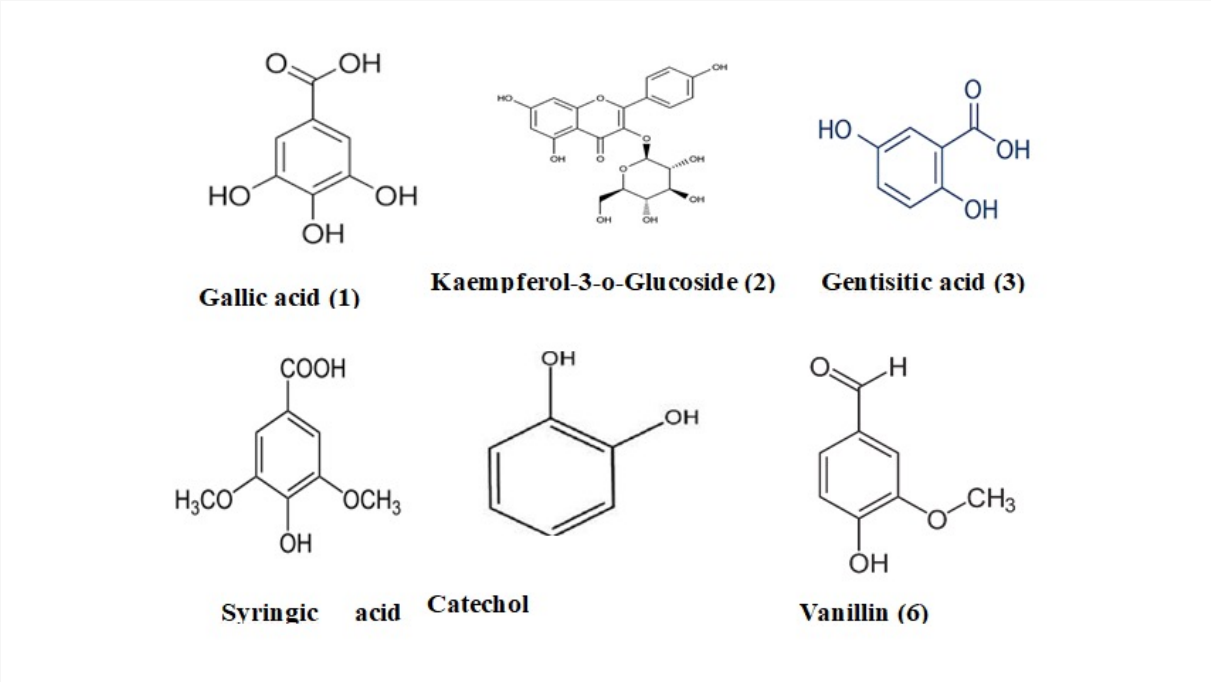

Figure 2. Chemical structures of major identified compounds in saffron.

Slika 2. Kemijska struktura pogavitnih identificiranih snovih v žafranu.

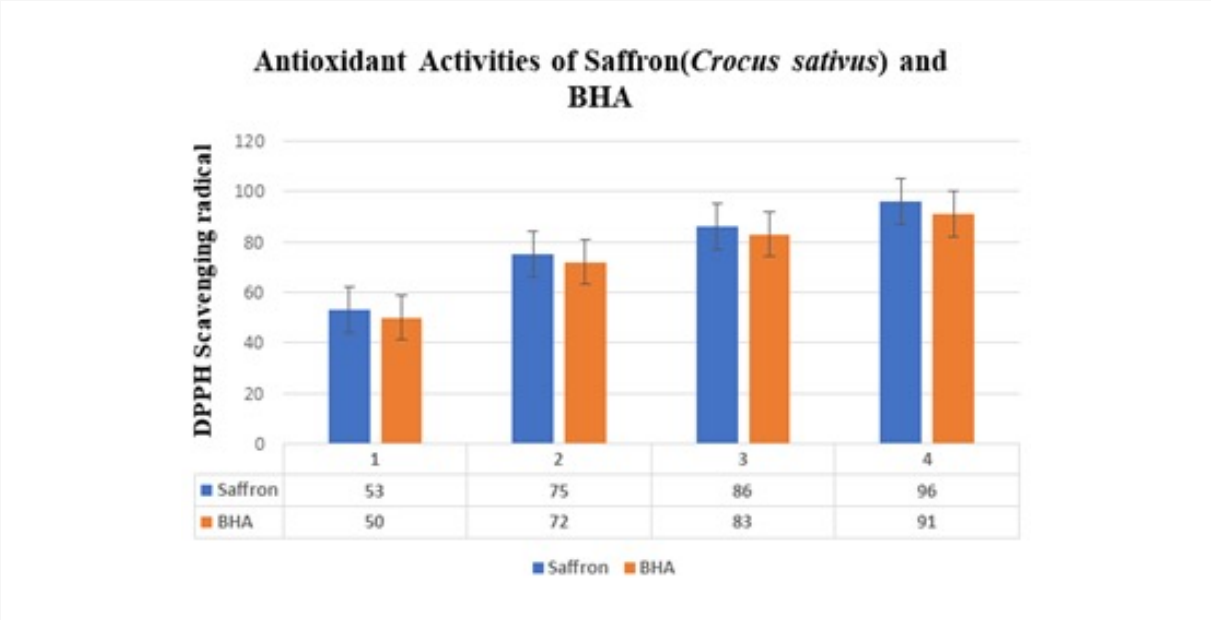

Figure 3. DPPH radical scavenging activity of saffron and BHA at 0.1, 0.25, 0.50, and 0.75 mg/ml. Each value represents mean ± SD of triplicate (n = 3) measurements. Means were compared using an unpaired t-test (* = $p < 0.05$, ns = non-significant).

Slika 3. Aktivnost DPPH v ekstrktu žafrana in BHA pri 0,1, 0,25, 0,50 in 0,75 mg/ml. Vsaka vrednost predstavlja povprečje ± SD meritev v treh ponovitvah (n = 3). Srednje vrednosti so bile primerjane z neparnim t-testom (* = $p < 0,05$, ns = nepomembno).

Animal weight

Table 3 reveals the impacts of Saffron, AFB1, and AFB1 + Saffron treatments on rat's body weight on the 1st, 7th, 14th, and 21st days. The body weight significantly decreased after 7, 14, and 21 days of treatment with Saffron ($P < 0.010$), AFB1 ($P < 0.0001$), and AFB1 + Saffron ($P < 0.0001$) in comparison to the control group. The highest rise in total body weight from 1st to the 21st day was noted in controls (9.97%) followed by the AFB1 group (4.34%), AFB1 + Saffron group (1.14%), and Saffron group (0.99%).

Haematological parameters

The results depicted insignificant changes in RBCs, MCV, MCH, and MCHC counts and haemoglobin content among Saffron, AFB1, and AFB1+ Saffron groups as compared to the control group. Meanwhile, platelet count was significantly increased in the AFB1 group than in the control ($P <0.010$), Saffron ($P <0.010$), and AFB1 + Saffron ($P <0.050$) groups. AFB1 group presented a significant rise in lymphocytes, monocytes, and basophil count in comparison to the control and Saffron groups ($P <0.001$). Contrarily, neutrophil and eosinophil counts significantly decreased in the AFB1 group as compared to the control and Saffron groups ($P <0.001$). AFB1 with Saffron combination improved neutrophil, lymphocytes, and eosinophil counts in comparison to the AFB1 group ($P <0.001$). However, neutrophil count was significantly reduced in the AFB1 group than in the control group ($P <0.001$), whereas a significant rise was noted in monocytes and basophils as compared to the control group ($P <0.001$ and $P <0.010$). Table 4 demonstrates the protective efficacy of saffron against blood toxicity.

Table 3. Rat body weight in response to Saffron, AFB1, and AFB1 + Saffron administrations after 7, 14, and 21 days of treatment.

Tabela 3. Telesna teža podgan glede na dodatek žafrana, AFB1 oz. AFB1 + žafran po 7, 14 in 21 dneh od tretiranja.

Variables	Control	Saffron	AFB1	AFB1 + Saffron
Body weight on 1st day (grams)	286.00±3.49	291.75±1.53	282.50±5.90	284.50±8.92
Body weight on 7th day (grams)	300.00±4.71	290.00±7.56†**	246.25±6.78†***	255.00±5.35†***
Body weight on 14thday (grams)	313.25±4.05	285.00±7.62†**	279.25±1.73†***	263.75±5.57†***
Body weight on 21st days (grams)	314.50±5.04	294.63±2.14 †**	294.75±3.64†**	287.75±4.08 †***
The ratio of the total increase in body weight (%)	9.97%	0.99%	4.34%	1.14%

Data is expressed as mean ± SEM. †: Significance versus control. *: $P <0.050$, **: $P <0.010$, ***: $P <0.0001$. The ratio of increase in total body weight (%) = Final body weight – initial body weight/ initial body weight X 100.

Table 4. Impact of Saffron, AFB1, and AFB1+ Saffron administrations (21 days) on rats' complete blood count (CBC).

Tabela 4. Vpliv žafrana, AFB1 in AFB1 + žafrana na krvne parametre po 21. dneh.

Variables	Control	Saffron	AFB1	AFB1 + Saffron
RBCs (X106/µL)	8.62±0.18	8.21±0.10	8.5±0.07	8.76±0.16
Hemoglobin (g/dL)	15.81±0.16	15.71±0.20	16.38±0.12	16.15±0.66
MCV (fL)	51.89±0.85	54.24±0.56	54.49±0.75	54.36±1.36
MCH (pg/dL)	18.49±0.39	19.04±0.27	19.18±0.13	18.51±0.33
MCHC (g/dL)	35.61±0.34	35.15±0.56	35.23±0.35	34.05±0.30
Platelets ($X10^3$/µL)	752.38±60.79	721.75±45.59†**	823.75±62.61‡**	799.63±43.62†*
WBCs ($X10^3$/µL)	10.15±1.32	10.70±0.52	10.82±1.23	10.93±1.13
Neutrophil ($X10^3$/µL)	12.88±0.17	12.65±0.16†***	6.59±0.06‡***	11.14±0.13‡***,†**
Lymphocytic ($X10^3$/µL)	8.03±2.04	8.60±3.79†***	9.41±0.54‡***	8.96±1.68†***
Monocyte ($X10^3$/µL)	1.50±0.05	1.45±0.08†***	8.26±2.11‡***	3.58±2.61†***
Eosinophil ($X10^3$/µL)	0.56±0.33	0.53±0.57	0.30±0.02‡***	0.84±0.22†***
Basophil ($X10^3$/µL)	0.72±0.11	0.25±0.04	2.21±0.23‡***	1.64±0.21‡**

Data is expressed as mean ± SEM.†: Significance versus Aflatoxin B1 ‡: Significance versus control; *: $P <0.050$; **: $P <0.010$; ***: $P <0.001$.

Kidney function test

A significant decrease was noted in the creatinine serum level of AFB1 + Saffron treated group than in the control and AFB1 treated groups (P <0.0001 and P <0.050). Serum levels of uric acid significantly increased in the AFB1 group as compared to the control and AFB1 + Saffron groups (P <0.0001). Contrarily, albumin serum levels significantly decreased in the Saffron, AFB1, and AFB1 + Saffron groups in comparison to the control group (P <0.050). The results depicted a significant rise in AST serum levels of the AFB1 group than in the control and AFB1 + Saffron groups (P <0.0001) (Table 5).

Serum testosterone levels and oxidative stress markers

The level of testosterone hormone was measured in serum to assess the testes' function. AFB1 and AFB1 + Saffron treatments caused a significant reduction in testosterone serum levels as compared to the control group (P <0.0001). Contrarily, a significant rise was noted in MDA and NO serum levels of the AFB1-treated group in comparison to the control and AFB1 + Saffron groups (P <0.0001) (Table 6).

Protective effects of saffron against aflatoxin-induced organ damage

The negative control and Saffron-treated rats presented normal renal histology (Fig. 4A, Fig. 4B). AFB1 administration caused drastic glomerular and tubular damage (Fig. 4C). It also induced severe hepatic injuries characterized by marked congestion, leucocytic infiltration, enlarged central veins, and large numbers of apoptotic/necrotic cells in comparison to the negative control and Saffron-treated groups (Fig. 5A, Fig. 5B, Fig. 5C). Moreover, AFB1 administered rats exhibited significant testicular damage that was characterized by interstitial degeneration, atrophy, and seminiferous tubules' disintegration with reduced number of cells (germ, Leydig, and Sertoli) as compared to the negative control and Saffron-administered groups (Fig. 6A, Fig. 6B, Fig. 6C). The co-administration of saffron and AFB1 effectively mitigated the renal (Fig. 4D), hepatic (Fig. 5D), and testicular (Fig. 6D) toxicities.

Table 5. Impact of Saffron, AFB1, and AFB1 + Saffron administrations (21 days) on kidney and liver functions of albino rats.

Tabela 5. Vpliv žafrana, AFB1 in AFB1 + žafrana na funkcije ledvic in jeter albino podgan po 21. dneh.

Variables	Control	Saffron	AFB1	AFB1 + Saffron
Kidney function tests				
Urea (mmol/L)	9.24±0.65	7.08±0.44	7.14±0.49	7.83±0.75
Creatinine (µmol/L)	48.23±1.77	50.18±2.17	44.43±0.74	37.19±1.41‡***,‡*
Uric acid (µmol/L)	57.30±7.00	52.53±3.04	117.76±7.32†***	67.93±3.33†***
Liver function tests				
Albumin (g/L)	38.63±0.46	32.75±1.11‡*	33.13±1.92‡*	32.38±1.49‡*
AST (U/L)	94.00±5.71	95.25±6.47	169.75±9.57‡***	94.38±7.20†***
ALP (U/L)	130.38±6.07	139.63±5.18	143.38±8.74	141.13±4.65

Data is expressed as mean ± SEM. †: Significance versus Aflatoxin B1 ‡: Significance versus control; *: P <0.050; **: P <0.010. ***: P <0.001.

Table 6. Impact of Saffron, AFB1, and AFB1 + Saffron administrations (21 days) on testosterone hormone and oxidative stress markers in male rats.

Tabela 6. Vpliv žafrana, AFB1 in AFB1 + žafrana na testosteron in markerje oksidativnega stresa pri podganjih samcih 21. dan po tretiranju.

Variables	Control	Saffron	AFB1	AFB1 + Saffron
Testosterone (nmol/L)	15.57±1.13	9.97±1.19	2.68±0.33‡***	3.80±00.18‡***
Oxidative stress markers				
MDA (µmol/L)	0.31±0.02	0.33±0.03	1.72±0.11‡***	0.39±0.06†***
NO (µmol/L)	27.75±0.41	28.38±1.05	83.00±4.06†***	28.38±0.73†***

Data is expressed as mean ±SEM. †: Significance versus Aflatoxin B1 ‡: Significance versus control; ***: P <0.001.

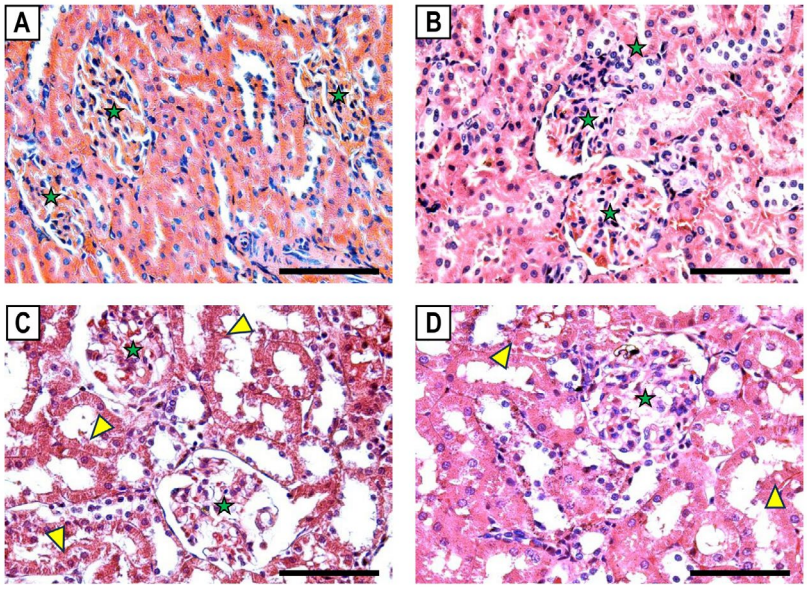

Figure 4. H&E staining of renal tissue sections of (A) negative control, (B) Saffron, (C) AFB1, and (D) AFB1 + Saffron groups (40× objective; scale bar = 10 µm; green star = glomerulus; yellow arrowhead = tubular damage).

Slika 4. H&E barvanje ledvičnega tkiva (A) negativne kontrole in tretmaja z (B) žafranom, (C) AFB1 ter (D) AFB1 + žafran (40x objektiv; črta označuje 10µm; zelena zvezda = glomerul; rumena puščica = poškodbe tubularnega tkiva).

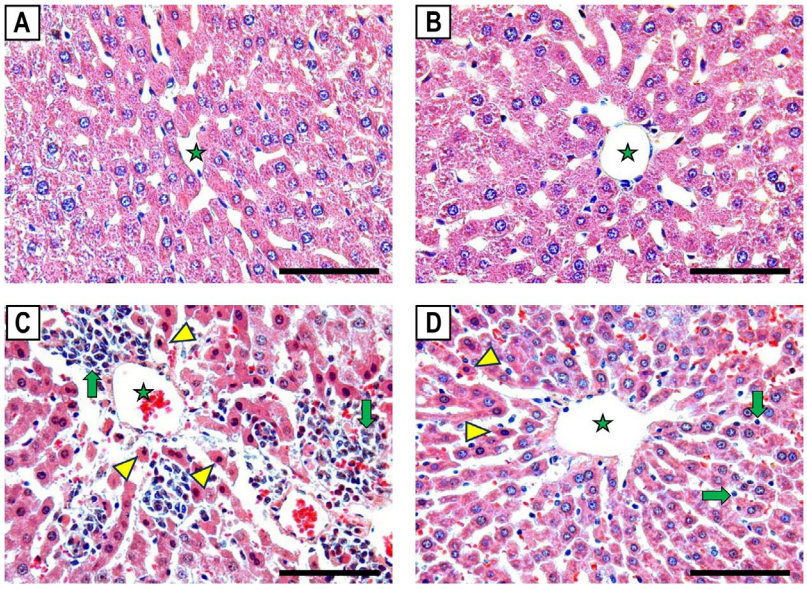

Figure 5. H&E staining of hepatic tissue sections of (A) negative control, (B) Saffron, (C) AFB1, and (D) AFB1 + Saffron groups (40× objective; scale bar = 10 µm; green star = central vein; green arrow = lymphocytic infiltration; yellow arrowhead = apoptotic/necrotic cells).

Slika 5. H&E barvanje jetrnega tkiva (A) negativne kontrole in tretmaja z (B) žafranom, (C) AFB1 ter (D) AFB1 + žafran (40x objektiv; črta označuje 10µm; zelena zvezda = osrednjo veno; zelena puščica = infiltracija limfocitov; umena puščica = apoptotične/nekrotične celice).

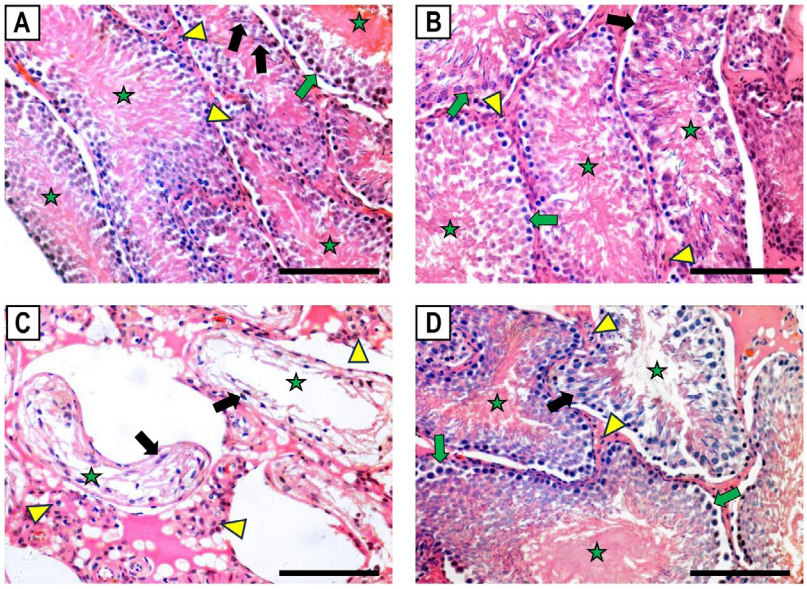

Figure 6. H&E staining of testicular tissue sections of (A) negative control, (B) Saffron, (C) AFB1, and (D) AFB1 + Saffron groups (40× objective; scale bar = 10 µm; green star = seminiferous tubules; green arrow = spermatogonia; black arrow = Sertoli cells; yellow arrowhead = Leydig cells).

Slika 6. H&E barvanje tkiva testisov (A) negativne kontrole in tretmaja z (B) žafranom, (C) AFB1 ter (D) AFB1 + žafran (40x objektiv; črta označuje 10µm; zelena zvezda = tubuli seminiferi; zelena puščica = spermatogonija; črna puščica = Sertolijeve celice; rumena puščica = Leydigove celice).

Discussion

Highly toxic aflatoxins are widely detected in human food and animal feed. The toxic impacts of aflatoxins can be mitigated by the use of medicinal plants. This study investigated the protective effect of the saffron extract on AFB1 toxicity in haematological parameters and multiple organs of male Wistar albino rats. Moreover, the protective efficacy of oral Saffron administration (21 days) against AFB1-induced toxicity was also elaborated. The data demonstrated a significant weight reduction in the AFB1-treated group after 7 and 14 days compared to the control group. There was a 4.34% increase in the total body weight ratio of the AFB1 group from the 1st to the 21st day. A significant body weight reduction has been reported in AFB1-treated (10, 20, or 50 mg/kg) rats (Supriya et al., 2014). Ahamad et al. (2015) reported duration and dose-dependent reduction in body weight in response to long-term AFB1 administration. Khaled and Thalij (2021) revealed that feeding AFB1-contaminated corn for 21 days decreased rats' body weight and ratio of weight gain.

During this study, the Saffron and AFB1+ Saffron groups experienced a significant decrease in body weight after 7, 14, and 21 days of treatment compared to the control group. However, there was a rise in the total body weight ratio from the 1st to 21st day in the Saffron (0.99%) and AFB1 + Saffron (1.14%) groups. Multiple studies have reported decreased rat appetite in response to ethanolic saffron stigma extract treatment, which resulted in significantly reduced body weight (Noorbala et al., 2005; Akhoundzadeh et al., 2008). Hariri et al. (2010) stated that crocin and safranal administrations remained unable to protect or suppress diazinon intake-related weight loss (Hariri et al., 2010). Thomson et al. (2009) reported insignificantly different rat body weights after four weeks of feeding on saffron water (3 mg/L) in comparison to the control group (Thomson et al., 2009).

The current results demonstrated insignificant haematological changes (WBCs, RBCs, haemoglobin content, MCV, MCHC, and MCH) in AFB1-treated as compared to the control group. A significant alleviation was noted in neutrophil and eosinophil counts of the AFB1 group, whereas platelet, lymphocytes, monocytes, and basophil counts were significantly higher in the AFB1 group than in the control group. A high count of lymphocytes and monocytes represented the

AFB1-associated inflammation and toxicity, which stimulated the body's immune responses. Husain et al. (2014) have also reported reduced RBC count and haemoglobin content and high WBC count in AFB1-contaminated diet-fed (1mg/kg) rats. Another study revealed that AFB1 feeding decreased the RBC count and haemoglobin content while simultaneously increasing the WBCs in laboratory animals (Ramamurthy and Rajakumar, 2016). Khaled and Thalij (2021) also reported a significant reduction in blood parameters (haemoglobin contents and RBC count) of AFB1-fed rats (39.5 µg/kg), whereas only insignificant differences were found at lower AFB1 concentrations (32.7, 37.9, and 29.0 µg/kg). They noted significantly increased WBCs in AFB1-fed rats (39.5, 32.7, 37.9, and 29.0 µg/kg). A low RBC count in AFB1-treated rats could be associated with anaemia because of the down-regulation of liver and kidney-secreted erythropoietin hormone activity. Similarly, a low erythrocyte volume is caused by defective heme-biosynthesis in bone marrow or a low erythropoietin formation rate, which results in lower haemoglobin levels. Contrarily, higher WBC count (mainly neutrophils) could be attributed to the cellular inflammatory response. The varying results of the current study from previous reports studies might be due to different AFB1 doses, treatment periods, and animal types.

The haematological parameters of the Saffron group remained similar to the control group during the experimental period. The AFB1 group experienced a significant rise in platelet, lymphocyte, monocyte, and basophil counts, whereas a significant reduction was noted in neutrophil and eosinophil counts compared to the Saffron group. These findings are in line with a previous study, which revealed that the injection of saffron aqueous extract (50, 100, and 200 mg/kg) thrice a week for four weeks didn't affect rats' CBC (Moallem et al., 2014). The combination of AFB1 + Saffron improved the platelet, monocyte, lymphocyte, and basophil counts as compared to the AFB1-treated group. The neutrophil count significantly decreased; however, a significant rise was noted in monocytes and basophils in the AFB1 + Saffron group compared to the control group. Babaei et al. (2014) reported that i.p injection of saffron petal extract (75, 150, 225, and 450 mg/kg) for 14 days did not alter the haemoglobin content, RBCs, MCH, MHCH, and MCV. However, a significantly increased WBC count indicated the immunomodulatory potential of saffron petals. The present study also indicated the beneficial impacts of saffron in protecting adult male rats' blood against AFB1-induced toxicity.

AFBI toxicity mainly affects the kidneys and liver (Yilmaz et al., 2018). The results depicted a significant rise in liver enzyme activities and renal parameters, whereas albumin was significantly reduced in the AFB1-treated group as compared to the control group. Kheir Eldin et al. (2008) confirmed a considerable increase in ALP, AST, and ALT serum levels in AFB1-administered rats (250 mg/kg/day for two weeks), which indicated the liver cells' dysfunction. Several studies have reported similarly high levels of ALP, AST, ALT, bilirubin, urea, and creatinine in AFB1-administered rats in comparison to controls (Rotimi et al., 2019; Khaled and Thalij, 2021; Karaca et al., 2021). The reason for the increase in liver enzyme activity can be because of the liver exposure to AFB1-induced damage to hepatocytes and increased membrane permeability that stimulates the release of liver enzymes into the bloodstream leading to high serum levels (Wang et al., 2020). The degeneration of the biliary system and hepatic tissues is indicated by higher levels of transaminases, bilirubin, and alkaline phosphatase (Owumi et al., 2019).

The results of this study demonstrated improved liver and kidney functions in AFB1 + Saffron-administered rats than in the AFB1 group. Moreover, uric acid, AST, creatinine, and albumin serum levels remained similar to the control group, which confirmed the protective efficacy of saffron against AFB1-induced hepato-renal toxicity. A study evaluated the hepatoprotective effects of saffron extract against acetaminophen toxicity in male Wistar rats. Saffron lowered AST, ALT, and bilirubin levels and significantly increased total protein and albumin (Omidi et al., 2014). Saffron's crocin and crocetin content contribute to its protective role against AFB1-induced DNA damage and hepatotoxicity by reducing hepatic injury markers [γ-GGT, ALP, AST, and ALT)] and enhancing hepatic glutathione peroxidase (GPX), glutathione S transferase (GST), and glutathione (GSH) in animal models (Giaccio, 2004). Anlin et al. (2000) reported the curative efficacy of saffron extract against carbon tetrachloride (CCl4) and alcohol-linked liver toxicities in rats. Shati and Alamri (2010) reported reduced aluminium (AlCl3)-induced hepatotoxicity in response to saffron treatment with significantly improved lipid peroxidation and liver biochemical markers (γ-GGT, triglycerides, cholesterol, ALT, AST, and ALP levels) (Shati and Alamri, 2010). Saffron's hepatoprotective effects against CCl-4-related liver damage could be due to (a) hepatic cell membrane fixation, (b) radical scavenging and antioxidant properties, and (c) alleviated CCl-4 metabolic activity via cytochrome

P450 inhibition (Iranshahi et al., 2011). The crocin content of saffron is also well known for protection against kidney damage (Yuan et al., 2014; Altinoz et al., 2015; Yarijani et al., 2017). Mahmoudzadeh et al. (2017) demonstrated that saffron extract treatment effectively reduced ischemia/reperfusion (I/R)-associated acute kidney injury.

This study detected the presence of gallic acid, vanillin, kaempferol-3-o-glucoside, syringic acid, gentisitic acid, and catechol in saffron. Esmaeili et al. (2011) have reported similar compounds in saffron through thin-layer chromatography (TLC). Moreover, Pandita (2021) has established phenolics-based antioxidant properties of saffron, which confirms their protection impacts against toxicity. Multiple studies have attributed the pharmacological and biological activities of saffron extract to its bioactive ingredients. Crocin is considered the main component that contributes to its pharmacological activity. Other saffron metabolites (terpenes, anthocyanins, flavonoids, and carotenoids) are also known for their pharmacological properties such as hypolipidemic, antioxidant, satiety enhancer, anti-inflammatory, hypoglycemic, antitumor, antihypertensive, neuroprotective, antidepressant, anti-diabetic, and antianxiety (Bolhassani, 2018; Lahmass et al., 2021). During this study, AFB1 administration (21 days) significantly enhanced the NO and MDA serum levels compared to the control, which are associated with continuous free radical production and incapacitated protection against antioxidants. Similarly, a study has linked the AFB1-related enhanced MDA and NO levels with DNA damage and mitochondrial dysfunction (Karaca et al., 2021).

The phenolic compounds of saffron (gallic acid, syringic acid, kaempferol-3-o-glucoside, and gentisitic acid) detected during this study might be responsible for the antioxidant, anti-inflammatory, and anti-toxicity properties. Mirhadi et al. (2020) have also reported bioactive components in saffron (Safranal, Crocin, Picrocrocin, and Crocetin) and their antioxidant properties. During the current study, AFB1+ Saffron treatment significantly mitigated the oxidative stress biomarkers, NO, and MDA as compared to the AFB1 group. NO and MDA levels appeared to be similar to the control group, which confirmed saffron's protection against AFB1-induced oxidative stress. Daryoush and Yousef (2012) stated that the ethanolic extract of saffron reduced lipid peroxidation and improved the antioxidant enzyme activities (CAT, SOD, and GSH) in cisplatin-treated rats' liver (Daryoush and Yousef, 2012). Pan et al. (2013) suggested that saffron regulates protein oxidation to reduce hepatic injury. Koul and Abraham (2017) demonstrated saffron-associated reduced lipid peroxidation with a concomitant rise in antioxidants (TAC, GSH, GST, and GPX) (Koul and Abraham, 2017). Harchegani et al. (2019) revealed that the administration of high saffron extract concentration (1mg/kg) for eight weeks alleviated the hepatic injury and MDA level with significantly increased TAC level (Harchegani et al., 2019). Giaccio (2004) reported that crocetin in saffron protects against AFB1-related oxidative damage and hepatotoxicity with increased GST levels in rats.

The results of this study depicted a significant reduction in testosterone serum levels in the AFB1-treated group in comparison to the control group, which confirmed the AFB1 toxicity on testes. Testosterone, produced in Leydig cells, is crucial for testicular function and spermatogenesis. Supriya et al. (2014) reported that rat exposure to different AFB1 concentrations (10, 20, or 50 mg/kg) considerably alleviated serum testosterone levels. Thus, AFB1 can disrupt testicular testosterone production, which facilitates sperm development in adult males (Abdel-Aziem et al., 2011; Adedara et al., 2014). Another study has reported enhanced cholesterol levels in AFB1-treated mice testes, which could be due to partially impaired steroidogenesis or partial cholesterol utilization (Verma and Nair, 2002). During the study, testosterone serum level was also significantly reduced in the AFB1+ Saffron group compared to the control group, which indicated that the saffron was unable to protect it from AFB1 toxicity.

The histopathological examinations of the liver, kidneys, and testes revealed several changes in the AFB1-treated group as compared to the normal control group. AFB1-induced hepatic injuries were characterized by amalgamated and hypertrophied hepatocytes with granular vacuolated cytoplasm, deeply stained pyknotic nuclei, and cell necrosis. The central vein and hepatic sinusoids appeared congested, whereas the portal area was dilated by the infiltration of immune and Kupffer cells. Several studies have reported AFB1-associated histopathological alterations in hepatocytes with massive cell necrosis, vacuolar degeneration, sinusoidal endothelium damage, invasion of Disse space with hyperactive immune and Kupffer cells (Ali et al., 2022; Li et al., 2022). Rotimi et al. (2019) also noticed that AFB1 toxicity led to hepatic sinusoid and vesicular (micro and macro) degeneration, proliferation of bile ductules, inflammation of the central vein, and infiltration of inflammatory cells.

The histopathology of the kidneys revealed several AFB1-induced abnormalities, such as glomerular capil-

laries' congestion and atrophy within Bowman's capsule, renal tubules' congestion, bleeding, and capsular wall degeneration, leading to wider spaces between capsule and glomerulus. The epithelial cell lining of the renal tubule appeared swollen with narrow tubular lumen and fine granular cytoplasm, whereas other renal tubules presented blood congestion and damaged walls. Yilmaz et al. (2018) reported AFB1-associated serious kidney damage, including vacuolization, renal cell necrosis, and exfoliation. Popescu et al. (2022) noticed glomerular tufts atrophy and modified Bowman's capsule in AFB1-treated animals. Inflammatory cell aggregation was also noted between tubules and glomeruli, along with the focal congested areas in the medullary region of blood vessels, whereas collagen proliferation mainly occurred at tubular injury sites (Popescu et al., 2022). Li et al. (2011) also demonstrated AFB1 toxicity in mouse kidneys, which involved downstream apoptosis factors (cleaved Caspase-3, Bax, and Bcl-2) and upstream regulator proline dehydrogenase (PRODH).

During the current study, the histological examination of testes displayed severe damage and a complete absence of spermatozoa, spermatogonia, spermatids, primary spermatocytes, and secondary spermatocytes in the AFB1-treated group. Moreover, seminiferous tubules' necrosis and atrophy were characterized by Leydig cell degeneration, deep staining of pyknotic nuclei, and thickened basement membrane. Kudayer et al. (2019) have reported excessive testicular cell vacuolation and spermatogenesis suppression after AFB1 administration for seven days (Kudayer et al., 2019). Another study has reported AFB1-induced necrosis and degeneration of seminiferous tubules' epithelium lining, changes in sperm mitochondria, and outer dense fibre extrusion (Faisal et al., 2008). Multiple studies have reported a significant reduction in testes index (sperm quality and concentration) and changes in spermatozoa production and testicular function after AFB1 treatments (Abu El-Saad and Mahmoud, 2009; Cao et al., 2017). The mechanism of AFB1 cytotoxicity involves either direct toxicity or oxidative stress on biological macromolecules (Towner et al., 2002). Due to the presence of highly enriched polyunsaturated fatty acid, the spermatozoa and testes are prone to oxidative stress. Oxidative stress reduces sperm quality, which is a widely accepted AFB1-toxicity to testes (Abdel-Aziem et al., 2011).

Sakr et al. (2014) described that saffron extract ameliorated sperm count, abnormalities, and testicular destruction caused by valproate treatment (Sakr et al., 2014). Asadi et al. (2014) also reported improved semen parameters (sperm motility, concentration, and viability in cauda epididymis) in cadmium-exposed rats after saffron treatment. Heidary et al. (2008) further elaborated that saffron intake in non-smoking infertile males suffering from oligospermia could enhance the sperms' average number and motility (Heidary et al., 2008). Modaresi et al. (2008) reported that saffron administration (100 mg/kg) for 20 days could elevate the follicle-stimulating hormone, testosterone, and luteinizing hormone levels in mice (Modaresi et al., 2008).

Conclusions

This study establishes the protective impacts of saffron and its bioactive components on blood parameters, liver, kidneys, and testes against AFB1-induced toxicity. Thus, saffron can effectively serve as a natural food additive and novel pharmacological compound for the alleviation of oxidative stress. However, further studies are necessary to evaluate the efficacy of saffron in protecting against toxins and related oxidative stress. Moreover, other beneficial components of saffron should also be investigated to improve toxins-related health issues.

Author Contributions

Research designing, H.A., L.K., K.E., H.H.A.; Conducting experiments, H.A., O.T.A., F.A.-R., S.A., A.A.Ala., G.H.A., A.A.Alo., F.A.A., S.H.A., R.M.W.; Data curation and analysis, L.K., E.A.H., B.R., S.I., T.S.A.; Writing-first draft, H.A., H.H.A.; Writing-final draft and editing, H.H.A., L.A.N. All authors have read and agreed to the published version of the manuscript.

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Ethical Approval

The experiment protocol was approved by the Ethical Committee of King Fahd Medical Research Centre, Jeddah, Saudi Arabia (Approval # 163-19). The care and handling of experimental animals were performed according to ARRIVE guidelines for the care and use of laboratory animals.

Conflicts of Interest

The authors declare no conflict of interest.

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ABS

Original Research

Study of the Effects of Bioactive Compounds of Cyanobacterium *Desmonostoc alborizicum* on Pathogenic Fungi of Wheat

Bahareh Nowruzi [1*], Mahdieh Salehi [2], Ali Talebi [2]

Abstract

Wheat, as one of the most economically important crops, constitutes a major part of the human diet. One of the major challenges in wheat preservation is combating various pests, including fungi, with different pesticides. Chemical pesticides cause toxicity in agricultural fields, leading to a growing inclination towards the use of biopesticides. These biopesticides not only possess antimicrobial properties but also aid in the growth and development of crops. In this context, cyanobacteria's bioactive compounds are considered potential biopesticide candidates. Therefore, the aim of this study is to observe the antifungal effect of bioactive compounds from the cyanobacterium *Desmonostoc alborizicum* on pathogenic fungi affecting wheat. To achieve this, we cultivated the cyanobacterial strain *Desmonostoc alborizicum* for 14 days, then applied the cyanobacterial extract to wheat plants infected with *Alternaria alternata*, *Fusarium oxysporum*, *Aspergillus terreus*, and *Phytophthora nicotianae* var. We then evaluated the activity of antioxidant enzymes and performed the MTT assay on 4T1 cells. The results showed that *Aspergillus terreus* exhibited the highest resistance, while *Fusarium oxysporum* showed the highest sensitivity to *Desmonostoc alborizicum's* cyanobacterial extract. The enzymes guaiacol peroxidase, superoxide dismutase, catalase, and glutathione peroxidase activity significantly decreased ($p<0.05$) in infected plants that were treated with the cyanobacterial extract. This shows that the treatments effectively reduced stress and improved the immune response. Therefore, the results of this study suggest that the use of *Desmonostoc alborizicum* extract can be effective as an antifungal agent in protecting wheat and other agricultural crops.

Keywords

Antifungal activity, *Desmonostoc alborizicum*, wheat, bioactive compounds

1 Associate professor, Department of Biotechnology, Faculty of Converging Sciences and Technologies, Islamic Azad University, Science and Research Branch, Tehran, Iran

2 Master's student, Department of Biotechnology, Faculty of Converging Sciences and Technologies, Islamic Azad University, Science and Research Branch, Tehran, Iran

*** Corresponding author:**
E-mail address: bahareh.nowruzi@srbiau.ac.ir

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Študija učinkov bioaktivnih spojin cianobakterije *Desmonostoc alborizicum* na patogene glive pšenice

Izvleček

Pšenica kot ena od gospodarsko najpomembnejših poljščin predstavlja glavni del človeške prehrane. Eden glavnih izzivov pri ohranjanju pšenice je boj proti različnim škodljivcem, vključno z glivami, z različnimi pesticidi. Kemični pesticidi se nalagajo na kmetijskih poljih, zato se vse bolj nagibamo k uporabi biopesticidov. Ti biopesticidi nimajo le protimikrobnih lastnosti, temveč pomagajo tudi pri rasti in razvoju pridelkov. V tem okviru se bioaktivne spojine cianobakterij štejejo za potencialne kandidate za biopesticide. Cilj naše študije je raziskati protiglivni učinek bioaktivnih spojin iz cianobakterije *Desmonostoc alborizicum* na patogene glive, ki inficirajo pšenico. V ta namen smo 14 dni gojili cianobakterijski sev *Desmonostoc alborizicum*, nato pa uporabili njegov izvleček na rastlinah pšenice, okužene z *Alternaria alternata*, *Fusarium oxysporum*, *Aspergillus terreus* in *Phytophthora nicotianae*. Nato smo ocenili aktivnost antioksidativnih encimov in izvedli test MTT na celicah 4T1. Rezultati so pokazali, da je *Aspergillus terreus* pokazal največjo odpornost, *Fusarium oxysporum* pa največjo občutljivost na izvleček cianobakterij *Desmonostoc alborizicum*. Aktivnost encimov gvajakol peroksidaze, superoksid dismutaze, katalaze in glutation peroksidaze se je pri okuženih rastlinah, ki so bile zdravljene z izvlečkom cianobakterij, znatno zmanjšala ($p<0,05$). To kaže, da je zdravljenje učinkovito zmanjšalo stres in izboljšalo obrambni odziv. Rezultati te študije kažejo, da je uporaba izvlečka *Desmonostoc alborizicum* lahko učinkovita kot protiglivno sredstvo pri zaščiti pšenice in drugih kmetijskih pridelkov.

Ključne besede

protiglivna aktivnost, *Desmonostoc alborizicum*, pšenica, bioaktivne snovi

Introduction

Wheat (*Triticum L.*) is one of the most important agricultural and economic crops. Protecting agricultural products, especially wheat, from biotic and abiotic factors is crucial for improving agricultural productivity. Some plants have their own defence and resistance mechanisms; however, to achieve long-term productivity, external protective agents must be applied to food crops (Gonçalves, 2021) .To maximize productivity and produce high-quality crops, most producers use mineral fertilizers and pesticides. Consequently, the excessive use of chemicals in the agricultural industry for disease control and pest management has led to environmental pollution. Additionally, concerns about the harms of these chemicals, propagated by competitors of chemical pesticide producers, have meaningfully changed public perception towards the use of chemical pesticides in agriculture. Public concern about the use of pesticides as a preventive measure against pests and diseases has increased interest in using biopesticide alternatives against plant pathogens (Kumar et al., 2022). Biopesticides typically possess antimicrobial, antioxidant, antiviral, or antifungal properties and not only protect plants from pathogenic organisms but also enhance crop growth (Gonçalves, 2021).

Since the 1980s, researchers have recognized fungi as a major pathogenic factor, particularly in immunodeficiency diseases and other serious conditions. Antifungal drugs have limitations in terms of cost and side effects. Therefore, researchers are exploring biodiversity to search for new pioneer compounds with minimal or no toxicity (Gonçalves, 2021).

Alternaria alternata is recognized as a serious pathogen of wheat, causing substantial damage to a wide range of crops. *Alternaria* spp. has over 275 species, with *A. alternata* being the predominant species in most soils and plant tissues. It has a global distribution and attacks cereals, ornamental plants, oil plants, vegetables such as broccoli, eggplant, pepper, carrot, potato, tomato, bean, and fruits such as citrus, apple, berry, and peach (Dixon and Dixon, 1981). *Alternaria* causes disease by creating spots on leaves and green parts of plants, reducing photo-

synthesis (Woudenberg et al., 2015). This fungus produces cellulase and pectin methyl galacturonase (PMG) enzymes, breaking down the cell wall and producing alternariol, which kills the host cells, absorbs the nutrients it needs, and proliferates on the host. Pathotypes of *A. alternata* have specific and limited host ranges due to their ability to produce host-specific toxins (Templeton, 2013). Some isolates of this fungus are non-pathogenic, growing as saprophytes or endophytes on the surface and within plant tissues.

Fusarium oxysporum is another filamentous ascomycete fungus that causes wilting, blight, and rot in many horticultural, field, ornamental, and forest crops in both agricultural and natural ecosystems (Woudenberg et al., 2013). *Fusarium* also produces a diverse array of toxic secondary metabolites (mycotoxins), such as trichothecenes and fumonisins, which can contaminate agricultural products and render them unsuitable for food or feed (Ma et al., 2013).

Unlike other *Aspergillus species*, *Aspergillus terreus* has the potential to cause pathogenicity and contamination spread. Under laboratory conditions, A. *terreus* species produce numerous secondary metabolites and mycotoxins; however, the in vivo production of these substances during invasive growth remains poorly studied (Gower et al., 2010; Ma et al., 2013). Furthermore, A. terreus produces a variety of mycotoxins, including citrinin, patulin, citreoviridin, terretonin, and gliotoxin (Lass-Flörl et al., 2021).

Phytophthora nicotianae is a principal genus of plant pathogens within oomycetes, with a host range spanning over 72 plant genera. This fungus causes devastating plant diseases worldwide, leading to the rotting of roots, fruits, leaves, and collars. It is ranked among the top 10 major oomycete pathogens due to its scientific and economic importance. This pathogen induces symptoms such as leaf yellowing, stem cankers, reduced growth, and plant death. It also causes stem and root rot, resulting in water deficiency symptoms in affected plants such as tomato, tobacco, avocado, cotton, some ornamental plants, and trees (Quintana et al., 2017).

In this context, bioactive compounds present in cyanobacteria are considered the most promising candidates against plant pathogenic fungi. Indeed, due to their wide range of bioactive compounds, including phenolic compounds, polysaccharides, phytohormone-like substances, and proteins, the use of these microorganisms (or their extracts) can provide sufficient protection for crops against biotic and abiotic stressors. Additionally, they can be regarded as plant growth promoters (Khalifa et al., 2021).

The antifungal compounds produced by cyanobacteria belonging to Stigonematales, Nostocales, and Oscillatoriales include fischerellin A, hapalindole, carazostatin, phytoalexin, tolytoxin, cryptophycins, toyocamycin, nostocyclamide, and nostodione (Feller et al., 2018). Studies have demonstrated the antifungal activity of various cyanobacterial strains. For instance, the cyclic depsipeptide Lyngbyabellin B isolated from *L. majuscula* shows toxicity against *Candida albicans* (Lam and Lee, 2012). Extracts such as the ethanolic extract of *Phormidium corium*, methanolic extract of Lyngbya martensiana, and diethyl ether extract of Microcystis aeruginosa exhibit antifungal properties. *Oscillatoria* laetevirens, Chroococcus *minor*, and *Microcystis aeruginosa* also show antifungal activity against *C. albicans*. Furthermore, the crude methanolic extract of *Aphanothece bullosa* demonstrates stronger antifungal activity compared to *Lyngbya aestuarii* and crude extracts of other freshwater cyanobacteria like *Anabaena*, *Nostoc*, Aphanocapsa, *Synechocystis*, and *Synechococcus* (Farid et al., 2019). In addition to these important properties, the biomass production of cyanobacteria can be highly beneficial compared to other biological sources (Chiaiese et al., 2018). Due to their biological activities, cyanobacteria produce a wide range of metabolites that can serve as biofertilizers, biostimulants, or biopesticides in agriculture (Gonçalves, 2021).

Desmonostoc species are an important source of bioactive compounds and belong to the Nostocaceae family, which contains a variety of bioactive substances such as carotenoids, triterpenoids, amino acids, phenolics, sulfates, polysaccharides, phycocyanin's, and poly-unsaturated fatty acids. These components may have bactericidal, antioxidant, and antimicrobial activity (Hrouzek et al., 2013). A microcystin-producing strain of *Desmonostoc alborizicum* was isolated from a water source system in Iran (Nowruzi et al., 2023), and this strain could also have useful antifungal properties. Since *Triticum* L. is one of the important crops in the agricultural industry, this study was conducted with the aim of investigating the antifungal properties of the cyanobacterium *Desmonostoc alborizicum* extract on some pathogenic fungi of the wheat plant. To our knowledge, this is the first scientific report on the non-hazardous use of the cyanobacterium *Desmonostoc alborizicum* extract and the monitoring of its antifungal activity against pathogenic plant fungi.

Materials and Methods

Materials

All the chemicals and reagents used in this study were purchased from Sigma-Aldrich (USA).

Cultivation of Desmonostoc alborizicum Cyanobacteria

Initially, the strain Desmonostoc alborizicum was obtained from the cyanobacteria culture collection of the Alborz Herbarium, Department of Biology, Islamic Azad University. The purity and axenic nature of the cultures were confirmed. Purified samples were cultured in liquid BG110 medium in a growth chamber at 28°C under continuous fluorescent light with an intensity of 300 $\mu E/m^2/s^{-1}$ for 14 days (Figure 1) (Nowruzi et al., 2022).

Inoculum Preparation of Cyanobacterial Extract

Dry biomass of cyanobacteria (10 g) was processed in a homogenizer with a mixture of methanol (1:1) for 24 h at 4 °C. The crude extracts were centrifuged at 10,000 rpm for 15 min. The supernatant was collected and concentrated. The residue was separately re-extracted from the pellet using methanol (1:1 v/v). A portion of the concentrated cyanobacterial extract was dissolved in dimethyl sulfoxide (DMSO) and tested for biological activity (Figure 2) (Prasannabalaji et al., 2017; Seifi et al., 2024).

Cultivation Wheat

Initially, wheat seeds were sterilized with 0.1% mercury chloride ($HgCl_2$) for 10 min and then placed in an incubator at 28 °C for 48 h. Germinated seeds were sown individually in ten plastic pots (one plant per pot) containing Hoagland's solution and placed in a phytotron at 23 °C, with relative humidity of 75-70 %, light intensity of 130-110 $\mu E/m^2/s^{-1}$ and a photoperiod of 15 h light and 9 h dark to encourage sprouting.

Healthy germinated seeds were selected, and three seeds were planted in each pot. Experiments were conducted in a greenhouse with temperatures ranging from 30-25 °C during the day and 16 °C at night, with a 12-h light and 12-h dark cycle. Pots were irrigated every two days (Nowruzi and Hashemizaveh, 2024).

Contamination of plants with selected fungi

Before planting the seeds, the culture medium was contaminated with spore suspension. In this way, the spores were removed from the 1-week culture of the selected

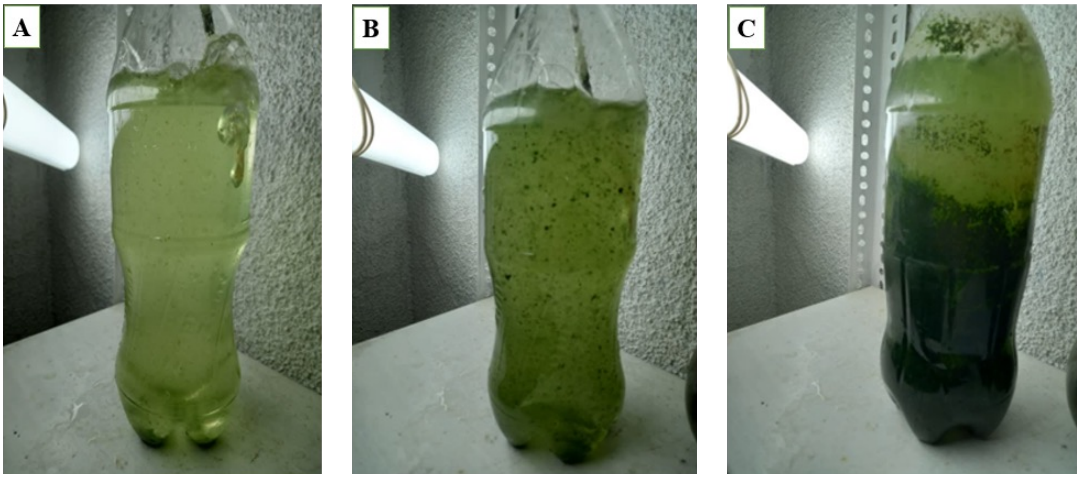

Figure 1. Cultivation stages of the Desmonostoc alborizicum cyanobacteria strain on days 0 (A), 7 (B), and 14 (C).

Slika 1. Faze gojenja cianobakterij Desmonostoc alborizicum na dan 0 (A), 7 (B) in 14 (C).

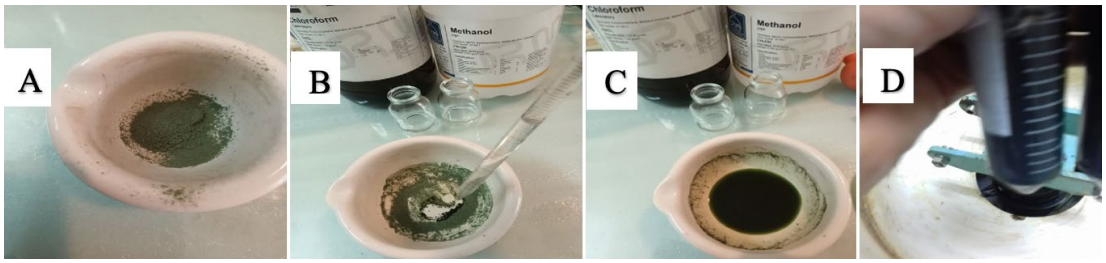

Figure 2. Separation and extraction of *Desmonostoc alborizicum* cyanobacteria extract. A) Biomass extraction, B) Addition of chloroform and methanol, C) Extracted extract, D) Centrifugation and extract extraction.

Slika 2. Ločevanje in ekstrakcija ekstrakta cianobakterij *Desmonostoc alborizicum*. A) ekstrakcija biomase, B) dodajanje kloroforma in metanola, C) ekstrahirani ekstrakt, D) Centrifugiranje in ekstrakcija ekstrakta.

fungi on the potato dextrose agar (PDA) culture medium containing chloramphenicol (0.1% concentration) in a completely sterile environment and the concentration of the suspension was adjusted to 10^5 spores per mm. Sampling and preparation were repeated three times (for three plant samples). The plates were incubated at 27 °C for five days. Then, spores were freshly harvested and resuspended in 10 ml of sterile distilled water containing 0.05 % Tween 80. Then, 50 ml of spore suspension was added to each of the pots, which had a diameter of 15 cm and mixed with the substrate (Alwathnani and Perveen, 2012).

Inoculation with Cyanobacterial Extract

In the V3 stage (second week of growth, plants have three visible collared leaves), wheat plants were sprayed with a 1 ml solution of *Desmonostoc alborizicum* extract at a concentration of 0.3 %. Subsequently, the wheat plants were divided into ten equal groups (Figure 3, A to D).

Evaluation tests were conducted on the same day as inoculation with cyanobacterial extract (day 14) (Figure 4, G to J) and on day 20 in the ten groups of treated plants with fungi and cyanobacterial extract (Figure 5, A to E).

Table 1. 10 equal groups of wheat plants in the experiment.

Tabela 1. Poskusne skupine pšenic in tretmaji.

Group	Description
Group A:	Wheat plants without contamination and without cyanobacterial extract
Group B:	Wheat plants inoculated with cyanobacterial extract
Group C:	Wheat plants inoculated with *Alternaria alternata* fungus
Group D:	Wheat plants inoculated with *Fusarium oxysporum* fungus
Group E:	Wheat plants inoculated with *Aspergillus terreus* fungus
Group F:	Wheat plants inoculated with *Phytophthora nicotianae* fungus
Group G:	Wheat plants inoculated with *Alternaria alternata* fungus and cyanobacterial extract
Group H:	Wheat plants inoculated with *Fusarium oxysporum* fungus and cyanobacterial extract
Group I:	Wheat plants inoculated with *Aspergillus terreus* fungus and cyanobacterial extract
Group J:	Wheat plants inoculated with *Phytophthora nicotianae* fungus and cyanobacterial extract

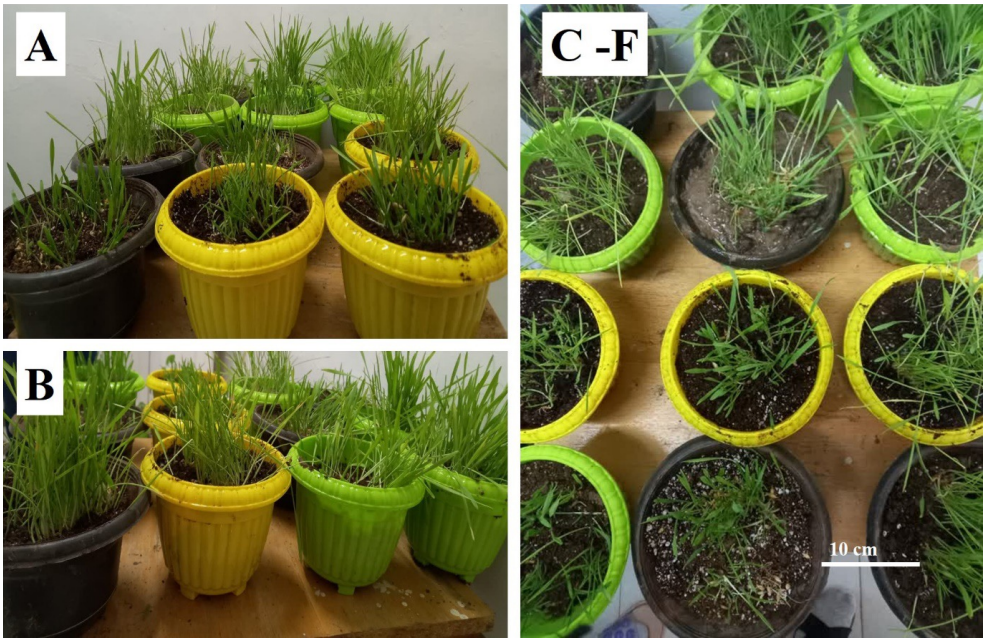

Figure 3. A) Non-infected wheat plants without fungal contamination and cyanobacterial extract, B) Wheat plants inoculated with cyanobacterial extract, C-F) Wheat plants inoculated with fungi, Bar, 10 cm.

Slika 3. A) neokužene rastline pšenice brez glivične okužbe in izvlečka cianobakterij, B) rastline pšenice, inokulirane z izvlečkom cianobakterij, C-F) rastline pšenice, inokulirane z glivami, Bar, 10 cm.

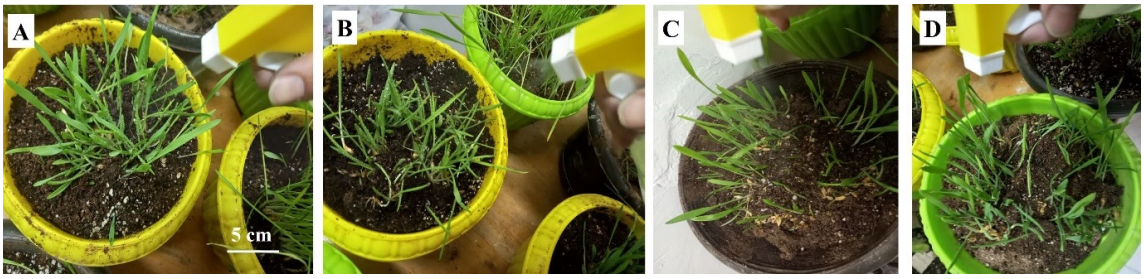

Figure 4. Inoculation of cyanobacterial extract into wheat plants inoculated with A) *Alternaria alternata*, B) *Fusarium oxysporum*, C) *Aspergillus terreus*, and D) *Phytophthora nicotianae* var. on day 14, Bar, 5 cm.

Slika 4. Tretiranje rastlin pšenice z izvlečkom cianobakterij. Inokulacija z A) *Alternaria alternata*, B) *Fusarium oxysporum*, C) *Aspergillus terreus* in D) *Phytophthora nicotianae* var. 14. dan, Bar, 5 cm.

Figure 5. Inoculation of wheat plants inoculated with A) *Alternaria alternata*, B) *Fusarium oxysporum*, C) *Aspergillus terreus*, D) *Phytophthora nicotianae* var. E) cyanobacterial extract without fungal contamination on day 14, Bar, 5 cm.

Slika 5. Inokulacija rastlin pšenice z A) *Alternaria alternata*, B) *Fusarium oxysporum*, C) *Aspergillus terreus*, D) *Phytophthora nicotianae* var. E) izvlečkom cianobakterij brez glive 14. dan, Bar, 5 cm.

Antifungal Activity of Cyanobacterial Extract

The fungi investigated in this study included *Fusarium oxysporum, Alternaria alternata, Phytophthora nicotianae*, and *Aspergillus terreus*. The fungi were surface cultured on potato dextrose agar medium. After cultivation, they were incubated at 25 °C for seven days. To determine the antifungal activity, methanol extract, exopolysaccharide suspension, and methanol alone at concentrations of 1, 5, 10, and 20 mg/ml were used. The radial growth of fungi was recorded from day 1 to day 5, and the percentage of inhibition by the cyanobacterial methanol extract compared to the control was calculated using the formula below (Nowruzi et al., 2023).

I= (C–T/C) ×100
I: Percentage inhibition of fungal growth in plates treated with cyanobacterial extract.
C: Control percentage.
T: Radial growth of fungi in plates treated with cyanobacterial extract.

Measurement of Cytotoxicity

Cell cytotoxicity was assessed using the MTT assay (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) on 4T1 cells. This method is a mitochondrial metabolic test that relies on mitochondrial succinate dehydrogenase to reduce tetrazolium salt in living cells. In this method, 100 µl of culture medium containing 104 cells was placed in each well of a 96-well plate. After 24 hours of incubation, concentrations of 0.01, 0.1, 1, 10, and 50 (µg/ml) of the extract were added to the cells and incubated for 24, 48, and 72 h. After the mentioned incubation periods, 20 µl of MTT solution at a concentration of 5 mg/ml was added to each well and incubated in the dark for an additional 4 h. After the required time, the MTT-containing medium was carefully removed, and 200 µl of acidified isopropanol were added to each well to dissolve the purple formazan crystals. After 15 min of incubation at room temperature, the absorbance of each well was measured using an ELISA reader (Meizheng, PerkinElmer company, HF4500) at a wavelength of 570 nm, with a reference wavelength of 690 nm. The results were reported as cell viability percentage and IC50 (the concentration that inhibits cell growth by 50%) based on the concentration curve ((µg/ml). Each experiment was repeated three times for better accuracy, and cell viability was calculated and reported using the following formula (Mai et al., 2017).

Cell viability (%) = (Absorbance of test / Absorbance of control)×100

Measurement of Total Protein Content

For this purpose, the Bradford method was used. The absorbance of samples was measured at 585 nm, and the total protein content in each plant sample was calculated

(µg/g) using a standard curve and statistical data analysis (Khramtsov et al., 2021).

Determination of enzyme activity

To measure the activity of guaiacol peroxidase enzyme, the reaction solution consisted of 50 µl of enzyme extract, 350 µl of 100 mM phosphate buffer, 350 µl of 10 mM pyrogallol ($C_6H_3(OH)_3$), and 1 ml of 70 mM hydrogen peroxide (H_2O_2). The absorbance at 470 nm wavelength was recorded using a spectrophotometer (Shimadzu, UV-1900i). The enzyme peroxidase activity was calculated as µlmol of H_2O_2 decomposed (mg/min) (Fijalkowski and Kwarciak-Kozlowska, 2020).

The activity of SOD was measured spectrophotometrically (Shimadzu, UV-1900i) by assessing its ability to inhibit the photochemical reduction of NBT (nitroblue tetrazolium) in an aqueous solution. The reaction mixture consisted of 70 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 mM NBT, 1.0 mM EDTA (Ethylenediaminetetraacetic acid), enzyme extract, and 2 mM riboflavin. The mixture was initiated under a 30-watt fluorescent lamp at a distance of 30 cm above the test tube and continued for 15 min. The measurement was performed using visible light spectrophotometry (Shimadzu, UV-1900i). Finally, the amount of enzyme required to inhibit the reduction of NBT by 70% was expressed as U/min/mg/protein activity (Ściskalska et al., 2020).

The activity of CAT was determined by measuring the decomposition of hydrogen peroxide. The reaction mixture contained 100 mM phosphate buffer (pH 7.0), 3% hydrogen peroxide, and enzyme extract. Absorbance was measured at 290 nm (Lin et al., 2022).

To measure GPx activity, the reaction mixture consisted of 5.0 µl containing 0.4 M sodium phosphate buffer (pH 7.0), 10 mM sodium azide (NaN_3), 4 mM reduced glutathione, 5 mM hydrogen peroxide, and enzyme extract. The reaction was incubated for 0, 30, 60, and 90 s and then terminated with 10% TCA (trichloroacetic acid) followed by centrifugation. After that, 2 ml of supernatant was mixed with 3 ml of phosphate buffer and 1 ml of DTNB reagent (0.04% DTNB in 1% sodium citrate). Absorbance was measured at 412 nm wavelength using a spectrophotometer (Shimadzu, UV-1900i) (Wu et al., 2021).

Statistical Analysis

Statistical analyses of the data from each experiment were performed using SPSS software (version 24). All data were obtained from the results of three repetitions. Significant differences between measured factors were determined using a one-way analysis of variance (ANOVA) with a confidence level of 95%. Post-hoc comparisons of means were conducted using Tukey's test, and the results of the comparisons were visualized in graphs using Excel software.

Results

Results of the antifungal activity of cyanobacteria extract

The results of the antifungal activity of *Desmonostoc alborizicum* cyanobacterial extract against *Fusarium oxysporum*, *Alternaria alternata*, *Phytophthora nicotianae* and *Aspergillus terreus* are presented in Table 2. The *Desmonostoc alborizicum* cyanobacterial extract showed statistically significant differences against the tested pathogens ($p < 0.05$). It was found that *Aspergillus terreus* had the highest resistance to the *Desmonostoc alborizicum* cyanobacterial extract, with a growth inhibition zone diameter of 66.6 mm ($p < 0.05$). *Fusarium oxysporum* exhibited the highest sensitivity to *Desmonostoc alborizicum* cyanobacterial extract ($p < 0.05$) (Table 2) (Figure 6).

Table 2. Mean growth inhibition zone diameter (in mm) of *Desmonostoc alborizicum* Cyanobacterial Extract.

Tabela 2. Povprečni premer cone inhibicije rasti (v mm) izvlečka *Desmonostoc alborizicum* Cyanobacterial Extract.

Pathogens under investigation	Diameter of inhibition zone (mm)
Alternaria alternata	7.33 ± 0.47 [b]
Fusarium oxysporum	8.66 ± 0.47 [a]
Aspergillus terreus	6.66 ± 0.47 [c]
Phytophthora nicotianae	7.66 ± 0.47 [ab]

*Different letters (a, b, c) indicate significant differences among means ($p < 0.05$).

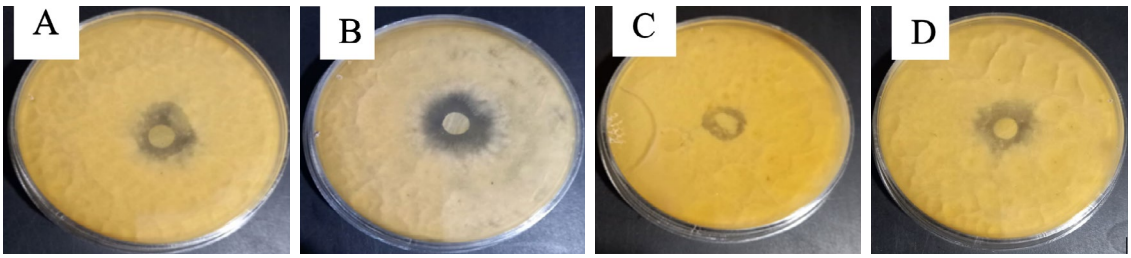

Figure 6. Growth inhibition zone diameter of *Desmonostoc alborizicum* cyanobacterial extract against A) *Alternaria alternata*, B) *Fusarium oxysporum*, C) *Aspergillus terreus*, D) *Phytophthora nicotianae* var.

Slika 6. Premer cone inhibicije rasti ekstrakta cianobakterij *Desmonostoc alborizicum* proti A) *Alternaria alternata* B) *Fusarium oxysporum* C) *Aspergillus terreus* D) *Phytophthora nicotianae* var.

Cytotoxicity Results

Table 3 displays the confirmed growth inhibition results of 4T1 cells measured using UV. It was found that cell death is directly related to the concentration of Desmonostoc alborizicum cyanobacterial extract ($P<0.05$) during the toxicity test. According to the results, an increase in the concentration of Desmonostoc alborizicum cyanobacterial extract led to an increase in the percentage of growth inhibition in the examined cells ($P<0.05$).

Table 3. Viability Percentage of 4T1 Cells at Different Concentrations of *Desmonostoc alborizicum* cyanobacterial Extract.

Tabela 3. Odstotek vitalnosti celic 4T1 pri različnih koncentracijah ekstrakta cianobakterije *Desmonostoc alborizicum*.

Concentration (ppm)	Viability (%)
0.00	100.00 ± 0.00 a
0.19	100.00 ± 0.00 a
0.39	100.00 ± 0.00 a
0.78	100.00 ± 0.00 a
1.56	99.83 ± 0.09 b
3.12	99.20 ± 0.06 c
6.25	99.36 ± 0.09 d
12.50	99.06 ± 0.23 e
25	94.40 ± 0.13 f
50	92.83 ± 0.03 g
100	89.86 ± 0.12 h
200	85.76 ± 0.09 i

*Lowercase letters indicate significant differences at the 0.05 level.

Enzymatic activity results

The results on zero-day revealed no statistically significant difference in the activity levels of guaiacol peroxidase, superoxide dismutase, catalase, and glutathione peroxidase enzymes among different treatments ($p > 0.05$). This indicates the absence of stress and low levels of superoxide radicals in the examined plants. After 20 days of fungal inoculation and extract treatment, a significant difference in the activity of these enzymes was observed in the wheat samples under study ($p<0.05$). The lowest enzyme activity levels were reported in wheat samples treated with cyanobacteria extract ($p<0.05$). Pathogenic fungal inoculation significantly increased all four enzyme activity levels in the samples ($p<0.05$), indicating stress imposition and increased superoxide radical levels in the wheat plants under study. The highest enzyme activity was observed in wheat plants inoculated with Alternaria alternata and Fusarium oxysporum fungi ($p<0.05$). Wheat plants that were infected with pathogenic fungi and cyanobacteria extract were treated at the same time. This significantly decreased the activity of all four enzymes in the plants ($p<0.05$), showing that these treatments effectively reduced stress and strengthened the immune systems of the plants (Tables 4 to 7) (Figures 7 to 10).

Table 4. Average results of guaiacol peroxidase enzyme activity (unit/mg pr).

Tabela 4. Povprečni rezultati encimske aktivnosti gvajakol peroksidaze (enota/mg pr).

Pathogens under investigation	Day 0	Day 20
Uninfected wheat plants and cyanobacterial extract (A)	219.79 ± 4.24 [a]	235.21 ± 6.58 [i]
Wheat plants inoculated with cyanobacterial extract (B)	219.49 ± 7.23 [a]	243.80 ± 1.97 [h]
Wheat plants inoculated with *Alternaria alternata* (C)	220.61 ± 3.62 [a]	420.55 ± 2.48 [a]
Wheat plants inoculated with *Fusarium oxysporum* (D)	223.58 ± 3.39 [a]	385.49 ± 5.78 [b]
Wheat plants inoculated with *Aspergillus terreus* (E)	221.24 ± 3.47 [a]	347.66 ± 1.55 [d]
Wheat plants inoculated with *Phytophthora nicotianae* (F)	213.11 ± 5.77 [a]	278.67 ± 3.53 [f]
Wheat plants inoculated with *Alternaria alternata* and cyanobacterial extract (G)	219.85 ± 2.86 [a]	363.35 ± 1.29 [c]
Wheat plants inoculated with *Fusarium oxysporum* and cyanobacterial extract (H)	222.05 ± 2.00 [a]	305.79 ± 3.64 [e]
Wheat plants inoculated with *Aspergillus terreus* and cyanobacterial extract (I)	219.20 ± 2.44 [a]	282.74 ± 7.03 [f]
Wheat plants inoculated with *Phytophthora nicotianae* and cyanobacterial extract (J)	216.07 ± 6.10 [a]	263.50 ± 1.61 [g]

*Different lowercase letters indicate significant differences at the 0.05 level.

Table 5. Average results of superoxide dismutase enzyme activity (unit/mgPr).

Tabela 5. Povprečni rezultati aktivnosti encima superoksid dismutaza (enota/mgPr).

Pathogens under investigation	Day 0	Day 20
Uninfected wheat plants and cyanobacterial extract (A)	91.09 ± 0.48 [a]	96.37 ± 1.37 [g]
Wheat plants inoculated with cyanobacterial extract (B)	91.91 ± 0.77 [a]	94.33 ± 2.08 [g]
Wheat plants inoculated with *Alternaria alternata* (C)	91.10 ± 0.32 [a]	159.41 ± 1.20 [a]
Wheat plants inoculated with *Fusarium oxysporum* (D)	90.74 ± 1.37 [a]	155.12 ± 6.70 [a]
Wheat plants inoculated with *Aspergillus terreus* (E)	90.98 ± 1.39 [a]	148.13 ± 3.14 [b]
Wheat plants inoculated with *Phytophthora nicotianae* (F)	90.52 ± 1.74 [a]	124.27 ± 2.46 [e]
Wheat plants inoculated with *Alternaria alternata* and cyanobacterial extract (G)	91.34 ± 0.79 [a]	147.14 ± 1.18 [b]
Wheat plants inoculated with *Fusarium oxysporum* and cyanobacterial extract (H)	91.29 ± 1.61 [a]	138.10 ± 0.79 [c]
Wheat plants inoculated with *Aspergillus terreus* and cyanobacterial extract (I)	91.44 ± 1.11 [a]	130.02 ± 2.58 [d]
Wheat plants inoculated with *Phytophthora nicotianae* and cyanobacterial extract (J)	92.11 ± 0.54 [a]	110.50 ± 2.10 [f]

Table 6. Average results of catalase enzyme activity (unit/mgPr).

Tabela 6. Povprečni rezultati encimske aktivnosti katalaze (enota/mgPr).

Pathogens under investigation	Day 0	Day 20
Uninfected wheat plants and cyanobacterial extract (A)	18.68 ± 1.43 [a]	24.14 ± 0.73 [fg]
Wheat plants inoculated with cyanobacterial extract (B)	18.66 ± 1.66 [a]	22.87 ± 1.21 [g]
Wheat plants inoculated with *Alternaria alternata* (C)	18.99 ± 1.56 [a]	57.19 ± 1.08 [a]
Wheat plants inoculated with *Fusarium oxysporum* (D)	17.56 ± 1.47 [a]	53.18 ± 0.74 [b]
Wheat plants inoculated with *Aspergillus terreus* (E)	20.07 ± 1.57 [a]	41.21 ± 1.57 [c]
Wheat plants inoculated with *Phytophthora nicotianae* (F)	19.39 ± 2.91 [a]	27.22 ± 2.51 [ef]
Wheat plants inoculated with *Alternaria alternata* and cyanobacterial extract (G)	19.68 ± 0.81 [a]	38.74 ± 2.88 [cd]
Wheat plants inoculated with *Fusarium oxysporum* and cyanobacterial extract (H)	19.56 ± 2.51 [a]	35.65 ± 1.06 [d]
Wheat plants inoculated with *Aspergillus terreus* and cyanobacterial extract (I)	19.50 ± 2.55 [a]	30.19 ± 4.37 [e]
Wheat plants inoculated with *Phytophthora nicotianae* and cyanobacterial extract (J)	18.96 ± 1.90 [a]	25.61 ± 2.47 [fg]

Table 7. Average results of glutathione peroxidase enzyme activity (unit/mgPr).

Tabela 7. Povprečni rezultati encimske aktivnosti glutation peroksidaze (enota/mgPr).

Pathogens under investigation	Day 0	Day 20
Uninfected wheat plants and cyanobacterial extract (A)	2013.18 ± 3.55 [a]	220.80 ± 4.34 [h]
Wheat plants inoculated with cyanobacterial extract (B)	213.03 ± 2.80 [a]	217.51 ± 4.14 [h]
Wheat plants inoculated with *Alternaria alternata* (C)	213.62 ± 4.57 [a]	373.91 ± 2.75 [a]
Wheat plants inoculated with *Fusarium oxysporum* (D)	210.88 ± 1.68 [a]	350.94 ± 1.15 [b]
Wheat plants inoculated with *Aspergillus terreus* (E)	213.88 ± 2.02 [a]	310.13 ± 2.30 [d]
Wheat plants inoculated with *Phytophthora nicotianae* (F)	213.26 ± 3.59 [a]	267.42 ± 3.18 [f]
Wheat plants inoculated with *Alternaria alternata* and cyanobacterial extract (G)	213.73 ± 2.00 [a]	338.88 ± 5.74 [c]
Wheat plants inoculated with *Fusarium oxysporum* and cyanobacterial extract (H)	212.52 ± 2.02 [a]	296.56 ± 5.76 [e]
Wheat plants inoculated with *Aspergillus terreus* and cyanobacterial extract (I)	212.02 ± 2.58 [a]	269.51 ± 3.23 [f]
Wheat plants inoculated with *Phytophthora nicotianae* and cyanobacterial extract (J)	213.03 ± 2.80 [a]	246.32 ± 1.33 [g]

Discussion

The extract of *Desmonostoc alborizicum* cyanobacteria demonstrated a significant difference in the studied pathogens and effectively reduced the growth of the examined fungi in this study. According to the results, *Aspergillus terreus* showed the highest resistance to *Desmonostoc alborizicum* cyanobacterial extract. Conversely, *Fusarium oxysporum* exhibited the highest sensitivity to *Desmonostoc alborizicum* cyanobacterial extract. Among the various activities of cyanobacteria, their efficacy against the growth of pathogenic fungal colonies of different plant species is noteworthy. Various studies have identified antifungal compounds such as Nostofungicidine, amino-6-hydroxy stearic acid, Microviridins, Nostopeptides, and *Nostoc* sp. cyanobacterial extracts (Nowruzi and Porzani, 2021). Additionally, among the compounds synthesized by cyanobacteria, chitosanase homologs, endoglucanase, and benzoic acid were identified, and their presence was associated with activity against fungi (Righini and Roberti, 2019).

Ismail and Ismail, 2011, investigated the antifungal activity of certain fungal species (*Gliocladium deliquescens*, *G. virens*, *Trichoderma hamatum*, and *T. harzianum*) and cyanobacteria against Rhizoctonia solani, the causal agent of soybean root rot. They demonstrated that Trichoderma harzianum was the most effective fungal antagonist, while among cyanobacteria, *Nostoc entophytum* exhibited higher antifungal activity compared to *Nostoc muscorum*. The inhibitory effect was found to be dependent on the type of biological agent (Ismail and Ismail, 2011). Tiwari and Sharma 2013, explored the antifungal activity of *Anabaena variabilis* against plant pathogens. They observed that extracts derived from *A. variabilis* were capable of reducing the growth and development of pathogenic fungal strains *Aspergillus niger* and *Rhizopus stolonifer*. They attributed this antifungal effect to the presence of cyclic peptides, alkaloids, and lipopolysaccharides (Tiwari and Sharma, 2013).

Additionally, Shishido et al. (2015) investigated the antifungal properties of cyanobacterial compounds. They identified the production of antifungal glycolipopeptides hassallidins in strains *Anabaena* spp. BIR JV1 and HAN7/1 and in *Nostoc* spp. 6 sf Calc and CENA2019. These researchers reported that all strains producing antifungal compounds belonged to the cyanobacterial orders Nostocales or Stigonematales (Shishido et al., 2015). Petrova et al. (2020) investigated the antifungal properties of the cyanobacteria *Arthronema africanum* Lukavsky and

Nostoc commune Vaucher against nine bacterial strains (2 Gram-positive and 7 Gram-negative), as well as the fungal strain *Candida albicans*. They demonstrated that the aqueous extract obtained from the *N. commune* biomass was highly effective against many of the tested microorganisms. However, the present study prepared the cyanobacterial extract and evaluated its antifungal activity using a methanol (1:1) solvent extract (Petrova et al., 2020).

Ismail et al. (2021) investigated the effect of cyanobacterial extract on improving maize tolerance to cadmium stress. They demonstrated that the application of cyanobacterial extract significantly enhanced maize growth and reduced cadmium accumulation. Their results indicated that the imbalance between free radicals and cadmium antioxidants significantly increased the ratio of GSH/GSSG, glutathione reductase, superoxide dismutase, and catalase. However, specific activities of ascorbate peroxidase and guaiacol peroxidase were reduced. These findings conflicted with the results obtained in this study, where the activities of guaiacol peroxidase, superoxide dismutase, catalase, and glutathione peroxidase were significantly reduced with the addition of cyanobacterial extract during fungal stress, demonstrating the effective role of bioactive compounds in cyanobacteria against fungi (Ismail et al., 2021).

Additionally, Hamed et al. (2020) explored the physiological and biochemical responses of two cyanobacterial species, *Anabaena laxa* and *Nostoc muscorum*, to R-metalaxyl toxicity. They demonstrated that *A. laxa* induced the production of phenolic compounds, flavonoids, tocopherols, and glutathione, as well as the levels of peroxidase, glutathione peroxidase, glutathione reductase, and glutathione transferases, to mitigate R-metalaxyl toxicity. In contrast, *N. muscorum* showed significant induction of antioxidants, limiting the activities of enzymes like ascorbate peroxidase, catalase, and dehydroascorbate reductase (Hamed et al., 2020).

Priya et al. (2015) demonstrated the use of the cyanobacterial strain *Calothrix elenkenii* in flooded rice fields, showing that cyanobacterial extract led to increased expression levels of certain plant defence enzymes (Priya et al., 2015). Similarly, Gaafar et al. (2022) showed in wheat plants that induction of antioxidant defence enzymes such as SOD (Superoxide Dismutase), CAT (Catalase), GPX (Glutathione Peroxidase), GST (Glutathione S-Transferase), and non-enzymatic molecules like GSH (Glutathione) increased with treatment of *Arthrospira platensis* extract (Gaafar et al., 2022).

Mutale-Joan et al. (2021) investigated the extract of cyanobacteria, including *Dunaliella salina*, *Chlorella ellipsoidea*, *Aphanothece* sp. and *Arthrospira maxima* on the tolerance of potato plants to environmental stress factors. They demonstrated that lipid peroxidation in leaves, induced by oxidative stress ROS (Reactive Oxygen Species), significantly decreased with increased activities of CAT (Catalase) and SOD (Superoxide Dismutase) in plants treated with cyanobacterial extracts. These extracts also led to a considerable reduction in fatty acid contents, indicating the conversion of fatty acids into other lipid forms, such as alkanes, which are crucial in the synthesis of plant cuticular wax under hydric stress (Mutale-Joan et al., 2021). These results are consistent with findings from Silva et al. (2019), who showed that plants produce ROS as a strong defence response to pathogen infection. However, the accumulation of ROS in plant tissues can damage cells and promote infection by necrotrophic pathogens. Antioxidant enzymes like APX (Ascorbate Peroxidase), CAT, POX (Peroxidase), and SOD protect cells from oxidative damage during infection by pathogens (Silva et al., 2019).

Quan et al. (2008) reported that plants produce reactive oxygen species (ROS), such as H2O2, as a strong defence response to pathogen infection. However, the accumulation of ROS in plant tissues leads to oxidative stress, which can damage cells and promote infection by necrotrophic pathogens. Antioxidant enzymes like APX, CAT, POX, and SOD protect cells from oxidative damage during infection by pathogens (Quan et al., 2008).

Additionally, Mai et al. (2017) demonstrated that inoculating wheat plants with an extract from the cyanobacterium *Nostoc* sp., containing various enzymatic and non-enzymatic antioxidants, enhanced the plant's compatibility to counterbalance between free radicals and antioxidants. This contributed to increasing the plant's resistance and protection against oxidative imbalance, thereby aiding in wheat's resilience (Mai et al., 2017).

Conclusion

In conclusion, considering the biological risks associated with chemical pesticides, this study aimed to investigate the effect of *Desmonostoc alborizicum* cyanobacterial extract on reducing the pathogenicity of wheat fungal pathogens. The results showed that *Aspergillus terreus* was the most resistant to the cyanobacterial extract, with

a halo diameter of 66.6 mm. On the other hand, *Fusarium oxysporum* was the most sensitive to the *Desmonostoc alborizicum* cyanobacterial extract. Cell toxicity assays revealed a direct relationship between the concentration of *Desmonostoc alborizicum* cyanobacterial extract and cell death in 4T1 cells, with increasing extract concentration leading to higher growth inhibition of 4T1 cells. The enzymatic activity of wheat plants, specifically guaiacol peroxidase, superoxide dismutase, catalase, and glutathione peroxidase, showed no significant differences on the first day. However, wheat plant treatment with pathogenic fungi induced plant stress, resulting in increased enzyme activity during the 20-day period post-inoculation. *Alternaria alternata* inoculated wheat plants showed the highest enzyme activity, followed by *Fusarium oxysporum*. Wheat plants infected with fungi treated with *Desmonostoc alborizicum* cyanobacterial extract showed a significant reduction in enzyme activity, indicating effective stress control in these treatments. These findings strongly suggest that *Desmonostoc alborizicum* cyanobacterial extract could be used as a fungicide in agricultural products.

Author Contributions

Conceptualization, B. N.; methodology, M. S., A. T.; software, B. N.; validation, B. N.; formal analysis, B. N.; investigation, M. S., A. T.; resources, B. N.; data curation, B. N.; writing—original draft preparation, M. S., A. T.; writing—review and editing, B. N.; visualization, B. N.; supervision, B. N.; project administration, B. N.; funding acquisition, B. N. All authors have read and agreed to the published version of the manuscript.

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Original Research

Adulticidal activity of essential oils of *Ageratum conyzoides* L., *Hyptis suaveolens* L., *Ocimum basilicum* L. and their synergistic effects against anopheles mosquitoes

Tunde Ayobami Owolabi [1*], Destiny Sakpana [1], Jude Obodo-Elue [1], Duke Odiase [1], Happiness Anusonwu [1], Mennor Maryann Ogoh [2], James Danga [3]

Abstract

This study investigated the insecticidal efficacy of essential oils (EOs) extracted from *Ageratum conyzoides*, *Hyptis suaveolens*, and *Ocimum basilicum* against female *Anopheles* mosquitoes, aiming to explore their potential as alternatives to synthetic insecticides amidst rising resistance issues. *EOs* were obtained through steam distillation from freshly harvested plant aerial parts, and their chemical compositions were analyzed using gas chromatography-mass spectrometry. The results revealed significant variations in chemical profiles among the oils, with precocene I dominating in *A. Conyzoides*, eucalyptol in *H. suaveolens*, and estragole in *O. basilicum*. Thin layer chromatography analyses revealed various components with rf values ranging from 0.25 – 0.93. Mosquito bioassay demonstrated varying knockdown effects across the oils, with *H. suavolens* achieving 77.5% knockdown within six minutes of the observation period. None of the oils or their combinations reached susceptibility status (98–100% mortality), indicating prevalent resistance among the mosquito population in the study area. The combination of *A. conyzoides* and *H. suavolens* essential oil gave the highest percentage mortality (70%) at the least time (9 minutes), this is a suggestion of synergistic activity. Despite resistance challenges, this study highlights the promise of botanical insecticides in sustainable mosquito control and underscores the ongoing need for innovation and adaptation in vector management strategies.

Keywords

Natural insecticides; Essential oil; Mosquitoes; Gas Chromatography-Mass Spectrometry; Thin-Layer Chromatography; Chromatograms

1 Department of Pharmacognosy, Dora Akunyili College of Pharmacy, Igbinedion University, Okada, Edo State, Nigeria

2 Department of Biochemistry, Faculty of Life Sciences, Ambrose Alli University, Ekpoma, Edo State, Nigeria

3 Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand

*** Corresponding author:**
E-mail address: owolabitunde1@gmail.com

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Adulticidna aktivnost eteričnih olj vrst *Ageratum conyzoides* L., *Hyptis suaveolens* L., *Ocimum basilicum* L. in njihovi sinergijski učinki proti komarjem anopheles

Izvleček

V tej študiji je bila raziskana insekticidna učinkovitost eteričnih olj (EO), pridobljenih iz rastlinskih vrtst *Ageratum conyzoides*, *Hyptis suaveolens* in *Ocimum basilicum*, na samice komarjev *Anopheles*, da bi raziskali njihov potencial kot alternativo sintetičnim insekticidom zaradi naraščajočih težav z odpornostjo komarjev nanje. EO smo pridobili s parno destilacijo iz sveže nabranih nadzemnih delov rastlin, njihovo kemično sestavo pa smo analizirali s plinsko kromatografijo in masno spektrometrijo. Rezultati so razkrili precejšnje razlike v kemijskih profilih med olji, pri čemer je v *A. Conyzoides* prevladoval prekocen I, v *H. suaveolens* evkaliptol, v *O. basilicum* pa estragol. Analize s tankoslojno kromatografijo so pokazale različne sestavine z vrednostmi RF od 0,25 do 0,93. Biološki poskus s komarji je pokazal različne učinke pri vseh oljih, pri čemer je olje *H. suavolens* v šestih minutah opazovanja doseglo 77,5-odstotni učinek. Nobeno od olj ali njihovih kombinacij ni doseglo statusa občutljivosti (98-100-odstotna smrtnost), kar kaže na prevladujočo odpornost med populacijo komarjev na preučevanem območju. Kombinacija eteričnega olja *A. conyzoides* in *H. suavolens* je dala najvišji odstotek smrtnosti (70 %) v najkrajšem času (9 minut), kar kaže na sinergijsko delovanje. Kljub izzivom glede odpornosti ta študija poudarja obetavnost botaničnih insekticidov pri trajnostnem nadzoru komarjev in poudarja stalno potrebo po inovacijah in prilagajanju strategij za obvladovanje vektorjev.

Ključne besede

Naravni insekticidi; eterično olje; komarji; plinska kromatografija - masna spektrometrija; tankoslojna kromatografija; kromatogrami.

Introduction

Malaria and other mosquito-borne illnesses are particularly endemic to areas with warm temperatures and stagnant waters, which serve as a perfect breeding habitat for mosquitoes. Extremely impacted are tropical and subtropical regions in Africa, Southeast Asia, and portions of Latin America. In addition to providing ideal conditions for the reproduction of these vectors, crowded cities with poor sanitation and few medical resources put residents at risk of malaria (Nabatanzi et al., 2022). Because it can result in low productivity and lost incomes, the impact extends beyond human health to a state's or nation's economy. Efforts to address mosquito-related issues, including exploring natural pest control methods, are crucial in these vulnerable regions to alleviate the burden on public health and the local economy (Gallup and Sachs, 2001).

Poisonous chemical mixtures called synthetic insecticides are designed to kill, repel, or stop any kind of insect or pest. But synthetic pesticides have a host of dangerous side effects that extend well beyond their intended objectives (Meier, Rouhier and Hillyer, 2022). In addition to losing their effectiveness in controlling insect pests, the majority of these pesticides are also extremely dangerous to animals, humans, pollinators, and other non-target insects as well as their neurological and reproductive systems (Pierre et al., 2018). Most researchers suggested natural products from plants as good pesticide alternatives to conventional synthetic pesticides (Culicidae, 2017).

Plants are essential resources for both human (and animal) and environmental health. The benefits plants bring to our daily lives cannot be overstated; these benefits include oxygen production by photosynthesis, ecological preservation (in terms of food production, nutrient cycling, and habitat development), and aesthetic value. Plants serve an important function in sustaining our planet's ecosystems. Biodiversity is known to have a key role in ecosystem functioning, and so may favorably impact on the delivery of ecosystem services that benefit society (Veiga et al., 2020; Stuart Chapin, 2023). The very many compositions of plants enable them to provide these numerous benefits, of these compositions are the essential oils (EO) that are obtained

from plants through hydro-distillation or other processes (Ouedrhiri et al., 2017). EOs are extracted from numerous sections of a plant, including flowers, seeds, bark, roots, and leaves (Mahajan et al., 2021). They have numerous biological actions, including antibacterial, antioxidant as well as insecticidal effects (Joudeh and Luqman, 2022). Research on essential oils as mosquitocides has witnessed great focus due to the increasing resistance of mosquitoes to synthetic insecticides and the desire for more eco-friendly alternatives. Several studies have demonstrated the effectiveness of various essential oils in repelling and killing of mosquitoes (Isman, 2017).

According to some reports (Zibaee and Khorram 2015; Ailli et al., 2023), good number of plants that possess EO can be used as pesticides or repellents. Many EOs have been implicated as good candidates in mosquito control, the best of such EOs are *H. suaveolens*, *O. basilicum*, and *Ageratum conyzoides*. These EOs have been independently confirmed to have mosquitocidal properties (Pintong et al., 2020; Peniche et al., 2022), however, there have been reports (Goulart et al., 2022; Raj et al., 2020; Santos et al., 2021) of resistance owing to over-usage and other biological factors such as adaptation and mutations in mosquitoes. Given the abundance of data on the EOs of these plants' ability to control mosquitoes on their own, it is worthwhile to investigate the synergistic characteristics for a possibly better long-term mosquito control strategy. Furthermore, all of the studies that have indicated the synergistic action of various essential oil combinations against insects, in general, have not explicitly focused on the usage of the combinations of the plants chosen for this study.

The objective of this study was to investigate the adulticidal potential of several combinations of *H. suaveolens*, *O. basilicum* and *Ageratum conyzoides* EOs against female adults of *Anopheles* mosquitoes.

Materials and Methods

Plant collection and identification

In or around November 2023, the aerial portions (leaves, stems, and flowers) of *A. conyzoides*, *O. basilicum*, and *H. suaveolens* were collected from Okada settlements in

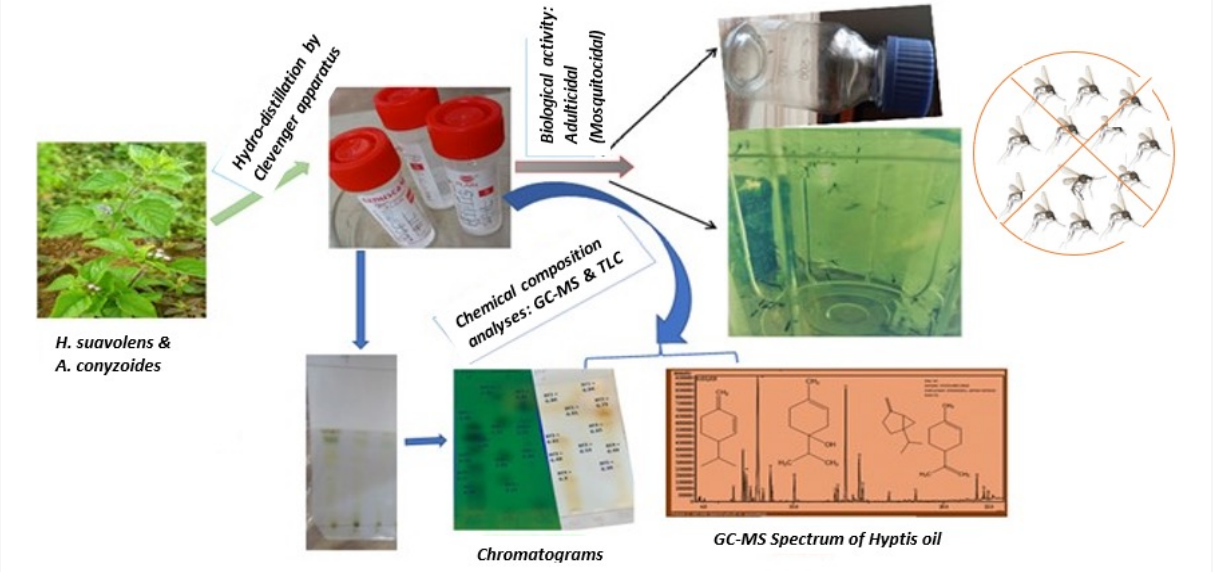

Figure 1. Flowchart of the methodologies

Slika 1. Potek analiz.

Ovia-Northeast LGA, Edo State, Nigeria. The plants were identified and authenticated in the Dora Akunyili College of Pharmacy Herbarium, Igbinedion University, Okada, a voucher specimen was deposited in the herbarium, and the herbarium number (IUO/11/015; IUO/16/96; IUO/21/352) were given.

Extraction of the essential oils

The fresh aerial parts of the plants (250 g) of the selected plants were separately extracted using a distillation apparatus consisting of an electric heating mantle, a 2000 mL flat-bottomed flask, a Clevenger, a condenser, and a chiller. The flask was filled with 250 g fresh plant material and 1200 mL of distilled water was added, the flask was then heated by an electric heating mantle to about 100 °C for at least 3h until completion of distillation, after which no more EO could be obtained. The EO was collected as distillate (a mixture of essential oil and water). This was transferred to a glass-separating funnel and the essential oil separated from the water based on density. The percentage yield of the EOs extracted were calculated using the formula;

$$\%\text{Yield} = \frac{\text{Volume of extracted Oil}}{\text{Weight of original plant material}} \times 100$$

Thin Layer Chromatography (TLC) Analysis

The TLC was performed on an analytical pre-coated TLC plate (Silica gel, 60 F254, Sigma Aldrich, Germany). Sampling of the individual EOs was done with the aid of micro-capillary tubes on the TLC plate and developed in TLC tank (Shandon Southern T.L.C Chromatank, Unikit) developed in n-hexane - ethyl acetate (1:1) mobile phase. The developed plates were sprayed with anisaldehyde-sulfuric acid reagent, the resultant chromatograms were dried in an oven at 105°C for 5 minutes (Owolabi, Amodu and Danga, 2023).

Observation of TLC Separation

Chromatograms were examined using a 254 nm UV light (ZF-1, Niusiwen UV lamp, China), treated with iodine vapor, and subsequently observed under visible light (Owolabi et al., 2022).

Recording Chromatograms

Fluorescent and non-fluorescent images under UV light were captured using a digital camera (Redmi 13C, Rear 50 MP, 5P lens, f/1.8) (Owolabi et al., 2022).

Gas Chromatography-Mass Spectroscopy (GC-MS)

The EOs were subjected to GC-MS analysis on a GC-MS instrument (SHIMADZU, JAPAN QP2010) with Elite – DB-5M column and the GC-MS solution version 2.53 software.

Essential oil combinations

Table 1. Individual EOs and their combinations.

Tabela 1. Posamezni EO in njihove kombinacije.

EOs and their combinations	Plant	Code	Formulation
Individual Eos	*A. conyzoides*	ACEO	10% *A. conyzoides* EO + 90% ethyl alcohol
	H. suavolens	HSEO	10% *H. suavolens* EO + 90% ethyl alcohol
	O. basillicum	OBEO	10% *O. basillicum* EO + 90% ethyl alcohol
Combinations		FM1	10% *A. conyzoides* + 10% *H. suavolens* + 80% ethyl alcohol
		FM2	10% *A. conyzoides* + 10% *O. basillicum* + 80% ethyl alcohol
		FM3	5% *A. conyzoides* + 5% *H. suavolens* + 5% *O. basillicum* + 75% ethyl alcohol
	Positive control	LDT	10% LDT + 90% ethyl alcohol
	Negative control	ETAL	100% Ethyl alcohol

EO-Essential oil, ACEO- A. conyzoides essential oil, HSEO- *H. suavolens* essential oil, OBEO- O. basillicum essential oil, FM1- Formula 1, FM2- Formula 2, FM3- Formula 3

Mosquito Rearing

The eggs of the Anopheles Mosquitoes were collected within the Okada communities. The mosquito colony was kept under standard laboratory conditions and photoperiod of 12-h light and 12-h dark. The eggs of the mosquito were brought to hatch in a plastic tray containing 1000 mL of clean water. The larvae were fed with fish food pellets for 12–15 days until they pupated. The pupae were not fed with any food. One hundred new pupae were collected in a 300-ml plastic cup containing 200 ml of clean water, transferred into an insect cage (the size of 30 × 30 × 30 cm3), and left lying until developed into adults. Mosquito adults were provided with 5% glucose solution as food, soaked in cotton sheets. Two-day-old female adults (not yet fed with blood meal) were collected as subjects for a World Health Organization (WHO, 2018) susceptibility test.

World Health Organization Susceptibility Test

Knockdown and mortality tests against Anopheles mosquito were performed using the World Health Organization (WHO 2018) Susceptibility Test. Five mosquitoes were taken to the Zoology laboratory for identification. Twenty-five 2-day-old female mosquitoes (not yet fed with blood meal) were exposed to 2 mL of each formulation, which was dropped onto a filter paper (Whatman® No.1) the size of 12 × 15 cm2 for 1 hr in a treatment tube (44 mm in diameter and 125 mm in length) then transferred to a non-treatment tube. The knockdown rate was recorded at 30 mins, and the mortality rate was recorded at 1 hr after exposure. Each treatment was performed in three replicates. Ten percent (w/v) Lambdacyhalothrin and 90% v/v ethyl alcohol were used as positive control and negative control, respectively. The criterion for knockdown and mortality was no movement of any of the mosquito bodies. The distinction between knockdown and mortality was that knockdown was an occurrence recorded at 30 min after exposure while mortality was an occurrence recorded at 1 hr after exposure. Knockdown (KR%) and mortality rates (MR%) were calculated by the following formula:

$$\text{Knock down Rate (KR\%)} = \frac{NK}{NT} \times 100$$

$$\text{Mortality Rate (MR\%)} = \frac{ND}{NT} \times 100$$

where NK is the total number of knocked-down adults; ND is the total number of dead adults, and NT is the total number of treated adults. The means of these rates were analyzed and compared by analysis of variance (ANOVA) Susceptibility levels were classified according to WHO criteria: Susceptible (S) means 98–100% of mosquito mortality, Possible Resistant (PR) means 80–97% of mosquito mortality, and Resistant (R) means less than 80% of mosquito/housefly mortality.

Results

Percentage yield of Essential oil from the three selected plants

The quantity of essential oil obtained from several rounds of extractions (steam distillation) of total of 2kg freshly collected plants for the individual selected plants are presented in Table 2 below. An average quantity of 0.4-0.5, 0.18-0.2, 1.0-1.1 mLs were separately extracted equivalent to average yields of 0.16, 0.08, and 0.44% respectively for A. conyzoides, H. suaveolens , and O. balsilicum. The colors range from clear white, dense white, and pale yellowish liquids.

Chemical compositions of the evaluated Essential Oils

GC-MS studies on the chemical constituents of *A. conyzoides*, *H. suaveolens*, and *O. basilicum* oils revealed the

Table 2. Physical characteristics and percentage yield of EOs extracted from three plant species.

Tabela 2. Fizikalne lastnosti in odstotni izkoristek EO, pridobljenih iz treh rastlinskih vrst.

Plant	Family	Common name	Part used	Colour	Yield (%)
A. conyzoides	Asteraceae	Goat weed	Aerial part	white	0.16
H. suaveolens	Lamiaceae	Mosquito plant	Aerial part	white	0.08
O. balsilicum	Lamiaceae	Sweat basil, Curry leaf	Aerial part	yellow	0.44

presence of 17, 18, and 21 compounds, constituting 97.88, 91.1 and 97.35% of all the compositions, respectively (Table 3 – 5).

Results of GC-MS analysis of essential oil from *H. suaveolens*

From the mass spectra of H. suaveolens obtained by GC-MS analysis it showed that the essential oil from the plant contains eighteen (18) compounds (Table 3). The most abundant compound is Eucalyptol with a peak area of 33.2% and a retention time of 6.978. This is followed by Bicyclo [7.2.0] undec-4-ene4,11,11-trimethyl-8-methylene, with a peak area of 14.20% and retention time of 14.032, while 1,3,6,10-Cyclotetradecatetraene, 3,7,11-trimethyl-14-(1-methylethyl) is the least abundant compound with a peak of 0.62% and a retention time of 21.998. Table 3.2 shows the breakdown of the entire components in the essential oil from *H. suaveolens*.

Results of GC-MS analysis of essential oil from *Ocimum basilicum*

From the mass spectra of *O. basilicum* obtained by GC-MS analysis it showed that the essential oil from the plant contains twenty-one (21) compounds (Table 4). The most abundant compound is Estragole with a peak area of 51.9 and retention time of 27.21. This is followed by β-Linalool with a peak area of 14.1% and retention time of 24.79, while Sabinene is the least abundant compound with a peak of 0.16% and a retention time of 20.87. Table 4 shows the breakdown of the entire components in the essential oil from *O. balsilicum*.

Table 3. GC-MS Analysis of volatile oil composition of *H. suaveolens.*

Tabela 3. GC-MS analiza sestave hlapnih olj *H. suaveolens*.

S/N	Retention time	Compounds	% Concentration
1	6.978	Eucalyptol	33.2
2	6.646	Bicyclo[3.1.0]hexane	6.71
3	8.586	Bicyclo[2.2.1]heptan-2-one, 1,3,3-trimethyl	3.01
4	6.735	Beta-Pinene	2.17
5	7.147	Alpha- Phellandrene	2.01
6	10.071	3-Cyclohexen-1-ol,4- methyl-1-(1-methylethyl)	3.05
7	7.997	Bicyclo[3.1.1]hept-2-ene	1.72
8	8.478	Cyclohexene, 4-methyl-3 -(1-methylethylidene	5.02
9	12.766	Copaene	1.52
10	14.032	Bicyclo [7.2.0]undec-4-ene4,11,11-trimethyl-8- methylene,[IR(IR,4Z,9S)]	14.20
11	14.386	1,6-Cyclodecadiene,1- methyl-5-methylene-8-(1- methylethyl),[s-(E,E)]	6.31
12	22.684	7-Isopropyl-1,1,4a trimethyl1,2,3,4,4a,9,10,10a-0ctahydrophenanthrene	1.08
13	14.617	gamma-Elemene	2.29
14	16.362	1H-3a,7 Methanoazulene, octahydro-1,4,9,9- tetramthyl	1.13
15	12.592	Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)	2.99
16	21.998	1,3,6,10-Cyclotetradecatetraene, 3,7,11-trimethyl-14-(1- methylethyl)	0.62
17	18.149	Bergamotol,Z-alpha-trans	1.30
18	22.223	Phenanthrene,7-ethenyl- 1,2,3,4,4a,4b,5,6,7,8,8a,9-dodecahydro-1,1,4b, tetramethyl-,[4aS-(4a.alpha.,4b. beta.,7.alpha.,8a.alpha.)]	2.81
		Unidentified	8.9
		Total	100

Table 4. GC-MS Analysis of volatile oil composition of *O. basilicum*.

Tabela 4. GC-MS analiza sestave hlapnih olj *O. basilicum*.

S/No	Retention time	Compounds	% Concentration
1	22.67	o-Cymene	0.37
2	22.9	Cineole	5
3	23.36	β-Ocimene	0.4
4	20.87	Sabinene	0.16
5	24.55	Fenchone	0.68
6	24.79	β-Linalool	14.1
7	22.81	D-Limonene	0.92
8	28.15	Chavicol	0.25
9	27.21	Estragole	51.9
10	31.52	Caryophyllene	1.85
11	23.70	γ-Terpinene	0.31
12	31.81	β-Farnesene	0.18
13	32.65	Germacrene D	0.4
14	26.00	α-Camphor	1.08
15	26.47	α-Terpineol	0.31
16	26.72	4-Terpineol	2.95
17	27.00	Terpineol	1.09
18	30.19	Eugenol	3.72
19	31.62	α-Bergamotene	3.1
20	32.14	Humulene	0.66
21	32.96	β-Bisabolene	3.58
		Unidentified	2.65
		Total	100

Results of GC-MS analysis of essential oil from *A. conyzoides*

The spectra of A. conyzoides obtained by GC-MS analysis it showed that the essential oil from the plant contains seventeen (17) compounds (Table 5). The most abundant and major compound is Precocene I with a peak area of 91.69, and retention time of 8.65, followed by β-Caryophyllene with a peak area of 2.52% and retention time of 13.65, while D-limonene is the least abundant compound with a peak of 0.12% and a retention time of 5.94. Table 5 shows the breakdown of the entire components in the essential oil from *A. Conyzoides*.

Results of Knockdown and mortality efficacy of EOs on Anopheles mosquitoes

All the EOs, and combinations from the selected plants showed mosquitocidal activity against adult mosquitoes except *A. conyzoides* which only had a slight knockdown effect but no lethal effect was observed at the end of 60 minutes of observation of mosquitocidal effect, all the mosquitoes in different groups except those in the control group were not active. In the first and second cages positive and negative controls, exhibited 100 and 0% knockdown and mortality, while, the 3rd to the 8th cages represent the treated group of individuals and combinations of various

Table 5. GC-MS Analysis of the volatile composition of *A. conyzoides*.

Tabela 5. Analiza hlapni sestavin *A. conyzoides* z GC-MS.

S/N	RT (min.)	Compounds	% Concentration
1	4.25	Camphene	0.42
2	4.83	4-carene	0.32
3	5.32	γ-terpinene	0.20
4	5.58	α-pinene	0.07
5	5.84	bornyl isoformate	0.19
6	5.94	D-limonene	0.12
7	6.30	Bornyl acetate	0.5
8	6.93	Germacrène D	0.45
9	7.29	Thymol	0.24
10	7.73	β-cubebene	0.06
11	8.65	Precocene I	91.69
12	9.03	2,2'-ethylidene bis (5-methylfurane)	0.18
13	12.03	bicyclogermacrene	0.26
14	12.18	α-Caryophyleène	0.13
15	12.71	copaene	0.22
16	13.64	β-Caryophyllene	2.52
17	15.24	caryophyllene oxide	0.33
		Unidentified	2.1
		Total	99.98

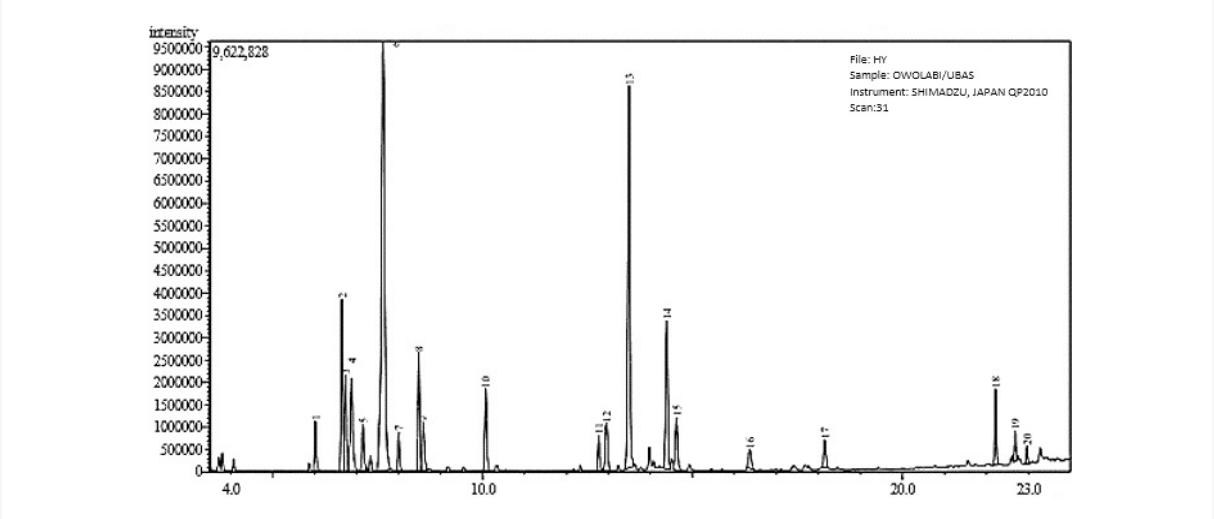

Figure 2. GC-MS Spectrum of *H. suavolen*.

Slika 2. GC-MS spekter *H. suavolen*.

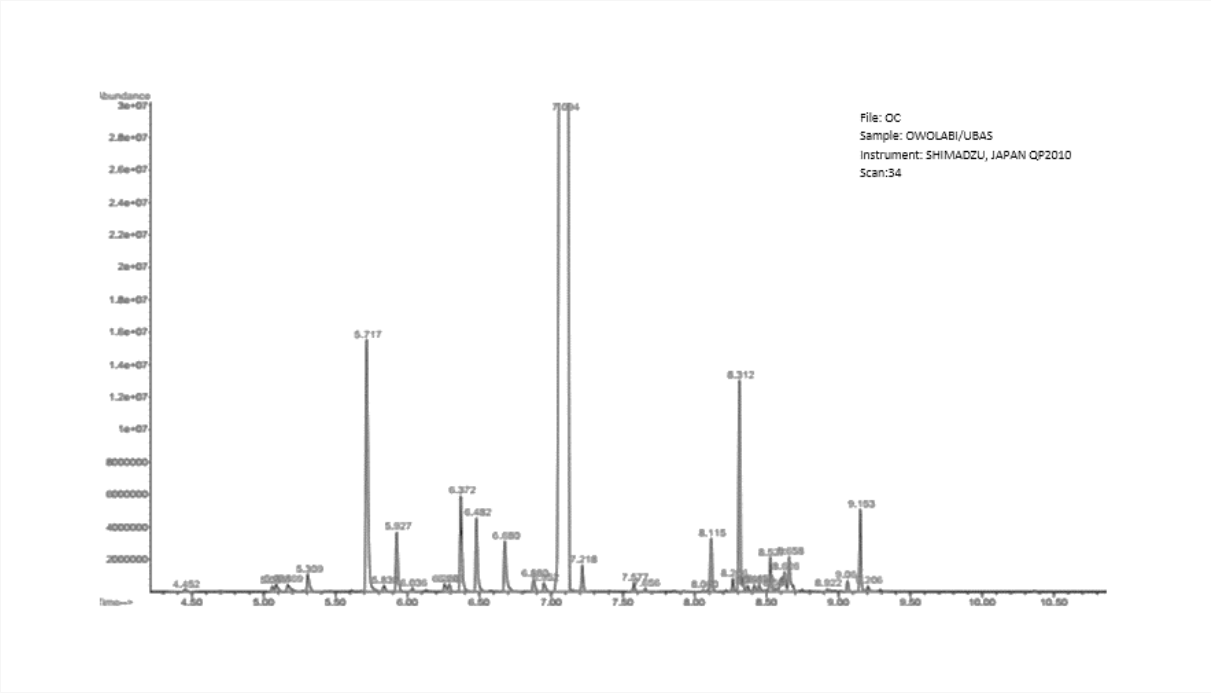

Figure 3. GC-MS Spectrum of *O. balsilicum*.

Slika 3. GC-MS spekter *O. balsilicum*.

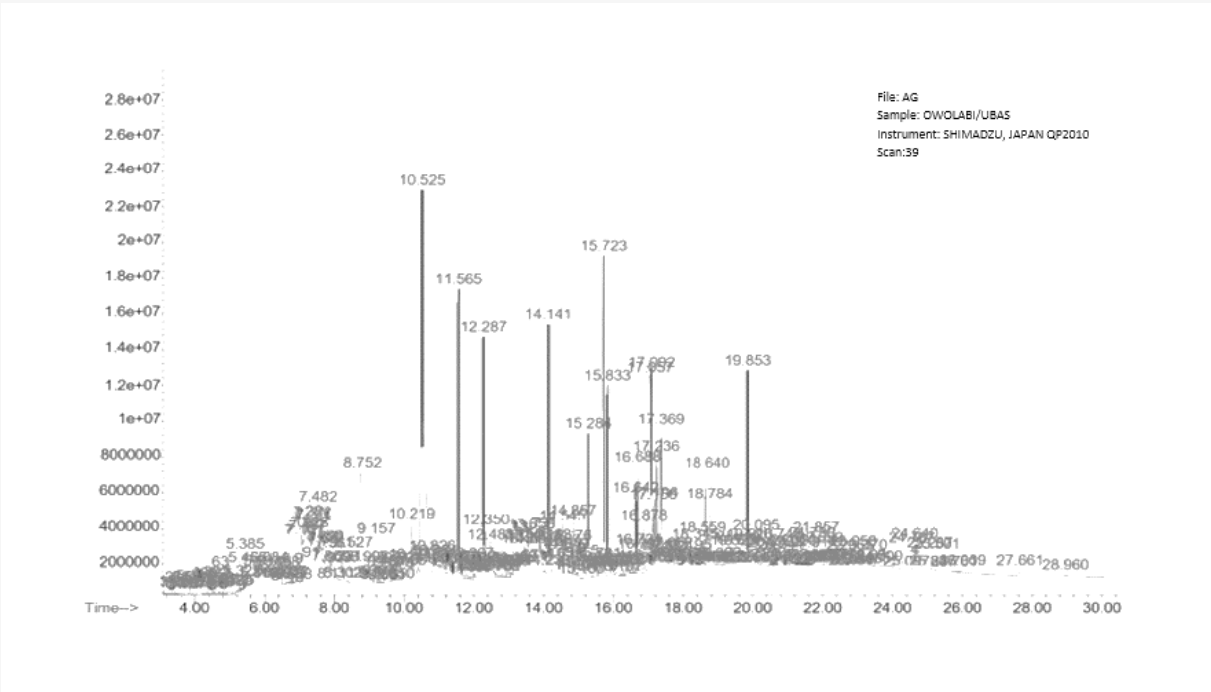

Figure 4. GC-MS Spectrum of *A. conizoides*.

Slika 4. GC-MS spekter *A. conizoides*.

oils (ACEO, HSEO, OBEO, FM1, FM2, FM3) 0, 77.5 ± 0.75, 25 ± 0.66, 70 ± 0.66, 60 ± 0.866, 60 ± 0.66% were knockdown at ∞, 6, 15, 6, 8, 8 minutes respectively, while the remaining percentages were observed to be perching far away from the filter paper treated with the EOs were placed.

The mortality trend as presented in table 3.5 above showed that the treated groups (ACEO, HSEO, OBEO, FM1, FM2, FM3) have 0, 12 ± 0.5, 30 ± 0.57, 70 ± 0.74, 60 ± 0.67, 67 ± 0.37% mortality at 0, 28, 21, 9, 12, 10 minutes respectively. The most lethal EO was the combination of *A. conyzoides* and *H. suaveolens* (FM1) with a percentage mortality of 70.0%, however, the exhibited lethal activities of all the tested oils and combinations are lesser than that of the positive control which was 100%. Table 3.5 summarises the mosquitocidal potential of the essential oil.

Table 6. Percentage knockdown rates (KR) and knockdown time (KT) of individual *EOs* and their combinations against female anopheles mosquitoes at 30 min after exposure.

Tabela 6. Odstotna stopnja knockdowna (KR) in čas knockdowna (KT) posameznih *EO* in njihovih kombinacij proti samicam komarjev anopheles 30 minut po izpostavitvi.

Treatment code	KR%	KT (min)
LDT	100kd	3
ETAL	0kd	0
ACEO	0kd	∞
HSEO	77.5±0.75	6
OBEO	25±0.66	15
FM1	70±0.66	6
FM2	60±0.866	8
FM3	60±0.66	8

LDT: Lambdacyhalothrin, ETAL: Ethyl alcohol, ACEO: *A. conyzoides* essential oil, HSEO: *H. suaveolens* essential oil, OBEO: *O. basilicum* essential oil, FM1: Formula 1, FM2: Formula 2, FM3: Formula 3

Table 7. Percentage mortality rates (MR), mortality time (MT) and susceptibility status (S) of individual essential oil and their combinations against females anopheles mosquito at 30 mins after exposure.

Tabela 7. Odstotki smrtnosti (MR), čas smrtnosti (MT) in status občutljivosti (S) posameznih eteričnih olj in njihovih kombinacij proti samicam komarja anopheles v 30 minutah po izpostavljenosti.

Treatment code	MR%	Status	MT (mins)
LDT	100d	S	3
ETAL	0d	R	0
ACEO	0d	R	0
HSEO	12±0.5	R	28
OBEO	30±0.57	R	21
FM1	70±0.74	R	9
FM2	60±0.67	R	12
FM3	67±0.37	R	10

S = Susceptible is defined as 98–100% mortality, PR = Possible Resistant is defined as 80–97% mortality, R = Resistant is defined as < 80% mortality.

Results of Thin Layer Chromatography

After the TLC plates were viewed under ultraviolet light (254nm) for fluorescent constituents, the dried chromatographic plates were subjected to universal chemical derivatizations, anisaldehyde-Sulfuric acid being a general spraying reagent for terpenes.

Discussion

LDT is an insecticide in broad-spectrum organochlorines insecticides, which are a large class of structurally very diverse. Organochlorines are used worldwide to control virtually all arthropods of agricultural and medical importance. However, the need to reduce the use of conventional synthetics and develop alternatives is now urgent due to the deleterious effect of applying synthetic insecticides, particularly regarding developing and widespread mosquito resistance as well as the impact on long-term health and the environment (Jayaraj, Megha & Sreedev, 2016). In addition to protecting the environment and human health, the benefits of botanical insecticides are, for example, high selectivity, worldwide availability, and convenient production and application, which make them more attractive candidates for use in mosquito control management (Mansour et al., 2012), however, some previously justified plants with insecticidal properties are gradually losing their potency to resistance by the target insects due to several factors (van et al., 2021). In this study, apart from GC-MS analysis for illustrating the chemical profiles of effective EOs, evaluation of the adulticidal activity of EOs and their combinations for possible increasing effectiveness were undertaken against female Anopheles mosquitoes.

The results of EOs evaluated for adulticidal activity against female Anopheles mosquitoes in Tables 6 and 7 showed that all of the EOs exhibited knockdown and lethal effects at considerable period, but none of the individual oils or their combination was susceptible to the female Anopheles mosquitoes. Although, apart from A. conyzoi-

Table 8. RF values of chromatograms of *A. conyzoides*, *H. suavolens*, and *O. basilicum* EOs in different conditions.

Tabela 8. RF vrednosti kromatogramov EO *A. conyzoides*, *H. suavolens* in *O. basilicum* v različnih pogojih

Sample	Components	254nm		Anisaldehyde-Sulfuric acid	
		Color	Rf (cm)	Color	Rf (cm)
ACEO	1	Black	0.69	Light brown	0.86
	2	Black	0.61	Light brown	0.61
	3	Black	0.48	Light brown	0.48
	4	Black	0.31	Light brown	0.3
	5	Black	0.19	-	-
HSEO	1	Black	0.88	Light brown	0.81
	2	Black	0.71	Light brown	0.53
	3	Black	0.44	-	-
	4	Black	0.25	-	-
OBEO	1	Black	0.81	Light brown	0.94
	2	Black	0.71	Light brown	0.75
	3	Black	0.49	Light brown	0.65
	4	-	-	Light brown	0.49
	5	-	-	Light brown	0.36

ACEO = *A. Conyzoides* essential oil
HSEO = *H. suavolens* essential oil
OBEO = *O. basilicum* essential oil

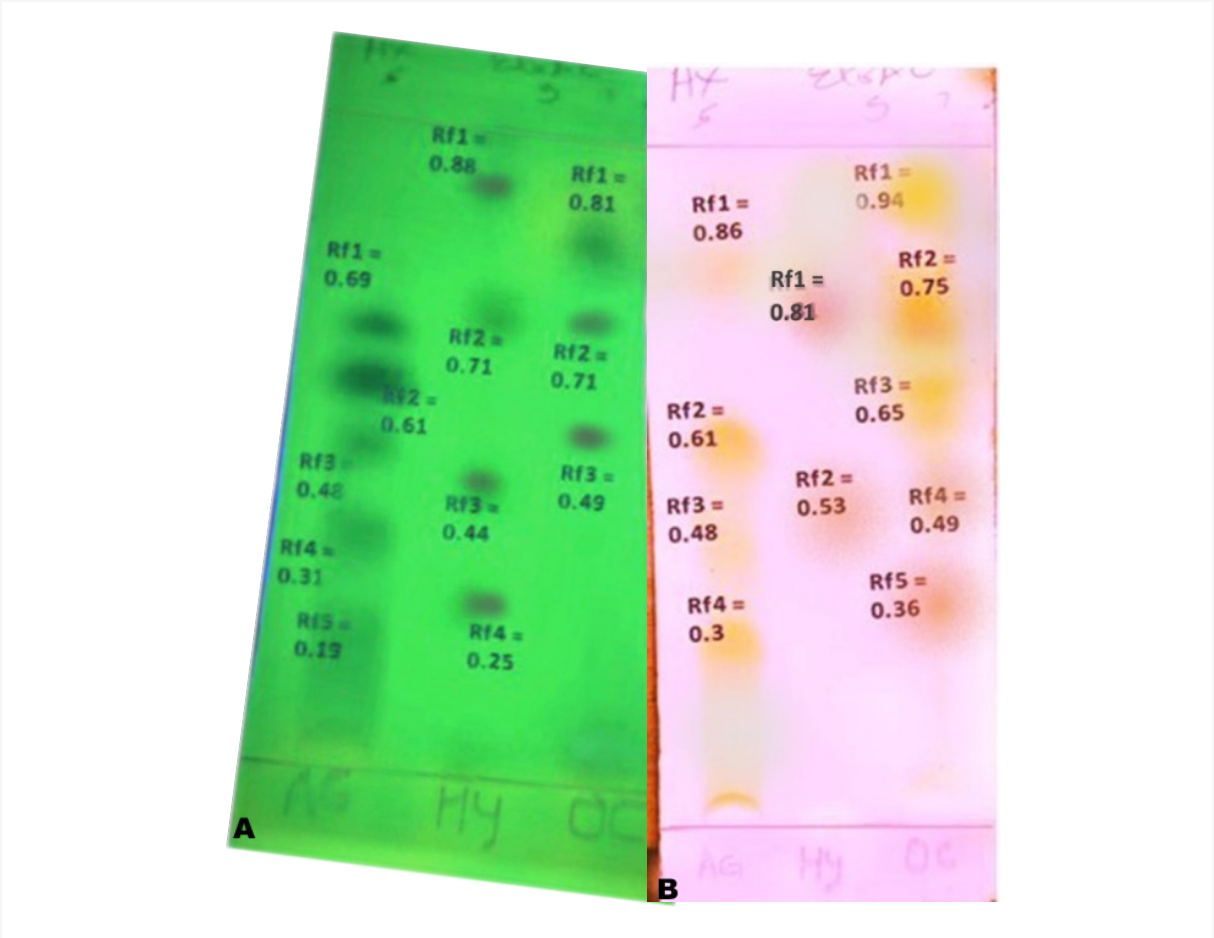

Figure 5. Chromatograms of chloroform fraction of Portulaca oleracea; Adsorbent – Silica gel GF254, Solvent systems: n-hexane:Ethylacetate (1:1), (a) Viewed under Ultra Violet light at 254 nm (b) Viewed under day light after spraying with Anisaldehyde-Sulfuric acid. AG = *A. conyzoides*, Hy = *H. suavolens*, OC = *O. basilicum*.

Slika 5. Kromatogrami kloroformske frakcije Portulaca oleracea; adsorbent - Silica gel GF254, sistemi topil: n-heksan:etilacetat (1:1), (a) gledano pod ultravijolično svetlobo pri 254 nm (b) gledano pod dnevno svetlobo po pršenju z anizaldehidno žvepleno kislino. AG = *A. conyzoides*, Hy = *H. suavolens*, OC = *O. basilicum*

des oil, all other oil proved to have effects but are resistant perhaps due to long usage, overuse, or low concentration. However, it was clearly shown in this study that there is a synergistic effect between the EOs of H. suaveolens and A. conyzoides, where the combination produced better activities than the individual oil. GC-MS characterization showed Eucalyptol (33.2%), Estragole (51.9%), and Precocene I (91.69%) for H. suaveolens, O. balsilicum, and A. conyzoides oils respectively. These chemicals have demonstrated several biological activities as documented by many researchers (Intirach et al., 2012). Some researchers have reported estragole of O. basilicumto be between 88.6%), (Imade and Ayinde, 2022). These quantitative and qualitative variances in oil content could be attributable to geographical, meteorological, and soil circumstances, as well as the plant's maturity during harvest time (Karalija et al., 2022). Thin-layer chromatography is a widely accepted, fast technique for separating a mixture of compounds. The usage of TLC in quality control as well as in the standardization of raw materials is of high importance (Agli et al., 2012; Wangrawa et al., 2015; Zibaee and Khorram, 2015). Its excellence in the evaluation of terpenes and sesquiterpenes which are the major compositions of EOs has been established by many researchers (Pyka et al., 2022; Pietraś et al., 2022). TLC can also be utilized to compare different EOs samples, helping to assess their quality and

authenticity. By comparing the Rf values of components, one can determine the similarity between samples (Basak et al., 2018). In this study, TLC was used to demonstrate the presence of terpenes in the studied EOs and also as a standardization tool. From the TLC results it can be concluded that the major constituents of the evaluated volatile oils are terpenes and alcohols which were shown after the chromatograms were sprayed with anisaldehyde-sulfuric acid. Terpenes are known to have high insecticidal properties in several literature (Pietraś, Skibiński, & Trebacz, Gumieniczek, 2012). The insecticidal activity observed in this research could be attributed to the identified terpenes from TLC and GC-MS analyses. The insecticidal activities of Eos have associated with several mechanisms of action that disrupt insect physiology and behavior such as nervous system disruption (Isman, 2017), respiratory toxicity (Zhu et al., 2010), they can also interfere with metabolic pathways, particularly lipid metabolism and lead to energy depletion in insects (El-Sayed et al., 2016).

Conclusions

This study investigated the insecticidal properties of EOs extracted from Agerantum conizoides, H. suaveolens, and O. basilicum against female Anopheles mosquitoes. The research showed that the mosquitoes of the study areas have developed some kind of resistance to the tested individual essential oil, but combinations of A. conyzoides and H. suavolens are synergistically potent against mosquitoes. Future research directions include exploring synergistic effects with other insecticides, conducting field trials to assess real-world efficacy, and investigating mechanisms of resistance to optimize botanical insecticide use.

Author Contributions

Conceptualization, O.T. methodology, O.T.; software, O.M., D.J.; investigation, O.T., S.D.; resources, S.D., O.-E.J., O.D., A.H.; data curation, O.T., D.J.; writing—original draft preparation, S.D.; writing—review and editing, O.M.; visualization, O.T., D.J.; supervision, O.T. All authors have read and agreed to the published version of the manuscript.

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Data Availability

Other data are available and can be accessed by mailing the corresponding author via owolabitunde1@gmail.com.

Conflicts of Interest

The authors declare no conflict of interest.

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ABS

Original Research

Evaluation of the in vitro toxicity and anti-inflammatory activity of the methanolic extract of the leaves of *Pistacia lentiscus* L. harvested from northwestern Algeria

Bourroubey Bachir [1,2*], Chelli Nadia [1], Tir Touil Aicha [1], Meddah Boumediene [1], Bettouati Abdelkader [3], Berkane Ibrahim [3]

Abstract

Medicinal and aromatic plants have been used for thousands of years for their therapeutic properties, to treat various ailments and to maintain health. This study was carried out to test the in vitro toxicity and anti-inflammatory activity of the methanolic extract of the leaves of *Pistacia lentiscus* L., native to the northwestern region of Algeria. Toxicity was studied in vitro by the hemolysis test and anti-hemolytic activity on human red blood cells, while anti-inflammatory activity was assessed in vitro by the protein denaturation method. The study of toxicity by hemolysis of red blood cells showed a high rate of hemolysis at a dose of 200 µg/mL, with a hemolytic percentage of around 88.64%, while a high rate of anti-hemolysis was observed at a dose of 25 µg/mL, with an inhibition percentage of around 83.17%. Evaluation of anti-inflammatory activity revealed a high percentage of inhibition of ovalbumin denaturation, reaching 63.53% at the 2000 µg/mL dose, while the lowest percentage was obtained at the 3500 µg\mL concentration with 10.16%. This extract has a toxic substance but does not exceed the toxicity threshold, which achieves significant anti-inflammatory activity in dose-dependent manner.

Keywords

anti-inflammatory activity, anti-hemolytic activity, *Pistacia lentiscus*, methanolic extract, toxicity

1 Laboratory of Bioconversion, Microbiological Engineering and Health Safety, Faculty of Natural and Life Sciences, University Mustapha Stambouli of Mascara, 29000, Algeria

2 Ain Tadles Hospital in Mostaganem, 27000, Algeria

3 Faculty of Sciences and Technology, Ahmed Zabana University, Relizane, 48000, Algeria

*** Corresponding author:**
E-mail address: bbmosta2911@yahoo.fr

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Vrednotenje in vitro toksičnosti in protivnetnega delovanja metanolnega izvlečka listov *Pistacia lentiscus* L., pridelanih v severozahodni Alžiriji.

Izvleček

Zdravilne in aromatične rastline se že tisočletja uporabljajo zaradi svojih terapevtskih lastnosti za zdravljenje različnih bolezni in ohranjanje zdravja. Ta študija je bila izvedena za testiranje in vitro toksičnosti in protivnetnega delovanja metanolnega izvlečka listov *Pistacia lentiscus* L., ki izvira iz severozahodne regije Alžirije. Toksičnost smo preučevali in vitro s testom hemolize in antihemolitičnim delovanjem na človeških rdečih krvničkah, protivnetno delovanje pa smo ocenili in vitro z metodo denaturacije beljakovin. Študija toksičnosti s hemolizo eritrocitov je pokazala visoko stopnjo hemolize pri odmerku 200 µg/ml s približno 88,64-odstotnim deležem hemolize, medtem ko je bila pri odmerku 25 µg/ml opažena visoka stopnja antihemolize s približno 83,17-odstotnim deležem inhibicije. Ocena protivnetnega delovanja je pokazala visok odstotek inhibicije denaturacije ovalbumina, ki je pri odmerku 2000 µg/mL dosegel 63,53 %, medtem ko je bil najnižji odstotek dosežen pri koncentraciji 3500 µg/mL z 10,16 %. Ta izvleček vsebuje toksično snov, ki pa ne presega praga toksičnosti, pri čemer doseže pomembno protivnetno aktivnost, ki je odvisna od doze.

Ključne besede

protivnetna aktivnost, antihemolitična aktivnost, *Pistacia lentiscus*, metanolni izvleček, toksičnost

Introduction

Given the ineffectiveness of chemical drugs and their impact on human health, especially in people with chronic diseases, scientists have focused on the use of medicinal plants, relying on ancestral knowledge, to treat chronic diseases such as diabetes that require long-term treatment and traditional pharmacopoeias resorted to treatment (Salmerón-Manzano et al., 2020; Süntar, 2020; Alhazmi et al., 2021). *Pistacia lentiscus* L., called “Darw”, a tree about 3 meters high, is widespread in northern Africa, especially in Algeria and Morocco (Cherbal et al., 2022; Bourroubey et al., 2023). This plant is a traditional medicinal plant; It has been used for a long time and is classified as antidiabetic, anti-inflammatory, antimicrobial, antioxidant, antiulcer, anticancer and antitoxin in general (Milia et al., 2021; Drioiche et al., 2023; Zitouni et al., 2023). In Algeria, people use different parts of the plant in traditional medicine in various ways, such as as an anti-diarrhea remedy and as an ingredient in livestock feed. However, it is not only a traditional remedy and an aromatic plant but also a powerful herbal product with various biological properties (Bourroubey et al., 2023).

Anti-inflammation and pain relief are probably the most common therapeutic indications in traditional Chinese medicines and popular prescriptions (Jiang et al., 2022; Li et al., 2023). Modern life brings with it a range of environmental toxicants, including acute and chronic exposures, which lead to many diseases. Many plants and animals have specific mechanisms enabling them to survive such toxic exposures. In addition, one of the biological characteristics of a healthy person is an acute inflammation reaction that is not excessive and stops on its own (Enyoh et al., 2020; Ali et al., 2021; Wiszniewska, 2021). On the other hand, excessive inflammation or even ongoing chronic inflammatory action always leads to disease, particularly non-communicable diseases associated with ageing. Chronic inflammation is linked to numerous diseases, such as cardiovascular disease, type 2 diabetes, chronic obstructive pulmonary disease, arthritis and Alzheimer's disease. It is, therefore, of the utmost importance to understand the mechanisms by which inflammation is regulated and to develop inflammation regulators to preserve health. Interestingly, many anti-inflammatory drugs and inflammation regulators are derived from natural products (Khan and Hegde, 2020; Tini et al., 2020; Leszek et al., 2021)

In addition, some exogenous or endogenous toxicants can be used in the development of anti-inflammatory drugs, and some well-known anti-inflammatory drugs exert anti-in-

flammatory effects while exhibiting toxic properties (Bindu et al., 2020; Nunes et al., 2020; Thiruchenthooran et al., 2023)

For this, this study aims to explore and understand the toxicological profiles of the methanolic extract of *Pistacia lentiscus* leaves through a hemolysis test and anti-inflammatory activity for the maintenance of a biologically safe mode. The main aim of this study is to evaluate the toxicological safety of using *P. lentiscus* in foods as well as in the treatment of wounds, diabetes, diarrhoea and other medical conditions. This study aims to investigate the effectiveness of leaf extracts from *P. lentiscus* L. in mitigating hemolysis and inflammation, which are critical factors in various pathological conditions. Despite previous studies highlighting the plant's bioactive compounds, such as flavonoids and phenolic acids, the specific mechanisms and efficacy of these extracts in reducing hemolytic activity and inflammatory responses remain inadequately explored. Therefore, this research seeks to address the knowledge gap by elucidating the pharmacological effects of the methanolic extract of *Pistacia lentiscus* L. leaves through *in vitro* tests, ultimately contributing to the understanding of its potential therapeutic applications. This work was adopted to determine whether the extract should be used in physiological mechanisms related to the inhibition of inflammation without a toxic effect.

Materials and method

Pistacia lentiscus (Figure 1) was identified by a botanist in the Department of Biology at Mascara University. A referenced specimen, AN00001, was introduced in our university's WAMAP-base of the Laboratory of Bioconversion, Microbiological Engineering and Health Safety (LBGMSS). The leaves of the *Pistacia lentiscus* plant were harvested during the last 15 days of April 2022 in the Yannaro region, located in Masra, Mostaganem, Algeria, characterized by moderate humidity and temperature.

The methanolic extraction of *Pistacia lentiscus* leaves was carried out using the maceration technique after drying under amber and grinding. 90% pure methanol was used (volume/weight). The final extract was recovered by rota-evaporation. The phytochemical characteristics of the extract have already been published (Elez et al., 2020; Sabrina et al., 2020; Bourroubey et al., 2023).

Hemolytic effect of *P. lentiscus* methanolic extract

A 6 mL voluntary blood sample was taken in a heparinized tube, and no anti-inflammatory treatment or medication was administered for two weeks.

Figure 1. Leaves of the *Pistacia lentiscus* L. plant.

Slika 1. Listi rastline *Pistacia lentiscus* L.

The blood used was collected in heparinized tubes from a single healthy donor. After centrifugation at 3,000 rpm for 10 min, the supernatant was removed, and the pellet was washed twice with PBS solution for a second centrifugation under the same conditions, suspended again with 1mL of PBS (D'Aquila et al., 2021). This is how the erythrocyte suspension was prepared.

The methanolic extract was weighed and dissolved in PBS to produce a range of four initial concentrations (25 µg /mL, 50 µg /mL, 100 µg /mL and 200 µg /mL).

The test for the hemolytic effect of the studied plant was performed using the modified method of Islam et al. 2022. Add 0.5 ml of PBS to 1 ml of each concentration of the previously prepared methanolic extract. After thorough mixing, incubation was carried out for 30 minutes at 37°C. Immediately afterwards, samples were centrifuged at 3000 rpm for 10 minutes. BPS was used as a control in place of the methanolic extract. The optical density (OD) of the isolated supernatant was read (at 540 nm using a spectrophotometer). The percentage hemolysis of different extract concentrations was calculated. SDS (sodium dodecyl sulfate) at a concentration of 1% was used as a control to ensure total lysis of erythrocytes (Madakka et al., 2021; Islam et al., 2022; Zhan et al., 2023).

% Hem = (ODt/ODc) * 100

Where %Hem is the hemolytic effect, ODt is the absorbance of the test, and ODc is the control absorbance (100% hemolysis with SDS).

Anti-hemolytic activity of *P. lentiscus* methanolic extract

The anti-hemolytic activity in vitro of methanolic extract of P. lentiscus leaves was performed using the modified method of Islam et al., 2022. The procedures were executed similarly to those in the hemolysis assay, with the addition of H_2O_2 to induce hemolysis. A fresh human blood sample was centrifuged at 3000 rpm for 10 minutes to isolate erythrocytes from the plasma. The erythrocytes were subsequently washed three times with PBS, using centrifugation at 3000 rpm for 10 minutes at 4 °C, with the supernatant discarded after each wash. The erythrocytes were then diluted with PBS to create a 5% suspension. To 1 ml of this erythrocyte suspension, 50 µl of various concentrations of P. lentiscus leaf extracts (25 µg/mL, 50 µg /mL, 100 µg /mL and 200 µg /mL) were added. The resulting mixture was incubated for 20 minutes, followed by the addition of 350 µl of H_2O_2. The incubation continued at 37°C for one and 30 min, after which the tubes were centrifuged again at 3000 rpm for 10 minutes at 4°C. The optical density (OD) was subsequently assessed at a wavelength of 540 nm. BPS was used as a control in place of the methanolic extract. The anti-hemolytic levels were determined using the following equation (Madakka et al., 2021; Islam et al., 2022):

% Anti-Hem = [(ODc-DOt)/ODc]*100

Where %Anti-Hem is the hemolytic effect inhibition rate, ODt is the absorbance of the test, and ODc is the control absorbance (100% hemolysis with H_2O_2).

Study of anti-inflammatory activity *in vitro*

In this study, the ovalbumin denaturation model was used to assess the anti-inflammatory activity of the methanolic extract of *Pistacia lentiscus* L. Tissue protein denaturation is a known consequence of inflammatory and arthritic diseases, which can lead to the production of autoantigens (Williams et al., 2008).

The principle of this technique is based on the ability of the plant extract to reduce the thermal denaturation of ovalbumin, a reference protein chosen for its stability during the anti-inflammatory process (Bouhlali et al., 2016). Evaluation of anti-inflammatory activity was performed according to the documented protocol using low-concentration bovine ovalbumin solution (Chandra et al., 2012; Das et al., 2022).

A volume of 1 mL of 2% ovalbumin solution was added to 1 mL of *P. lentiscus* methanolic extract solution at different concentrations (1000, 1500, 2000, 2500, 3000 and 3500 µg /mL), while the control was prepared by replacing the extract with distilled water. Aspirin was used as standard, and tubes were incubated at 72°C for 5 minutes. Readings were taken at 660 nm. Percentage inhibition of protein denaturation was calculated (Kar et al., 2012):

% Anti-inf =[(ODt-ODpc/ODtc]* 100

Where ODt is the Optical Density of the test, ODpc is the Optical Density of the product control solution, ODtc is the Optical Density of the test control solution, and % Anti-inf is the percentage of inhibition (anti-inflammatory). Control represents 100% denatured protein, and results are compared with aspirin.

Statistical study

Variations in concentrations of methanolic extract of *Pistacia lentiscus*, as well as conventional medicinal product (aspirin), were analyzed for statistical significance on the basis of a triple replication of all experiments. An analysis of variance (ANOVA available in IBM SPSS statistics version 25) was used, and all data were presented as means ± standard deviation (SD). A p-value of 0.05 was set as the threshold for determining statistical significance.

Results and discussion

Evolution of the haemolytic and anti-haemolytic effect in vitro of *Pistacia lentiscus* L. from the Mesra region (Mostaganem)

In this section, cytotoxicity is monitored by the leakage of intracellular haemoglobin from human red blood cells. Figure 02 shows the evolution of the hemolytic effect (by absorbance) in PBS buffer medium (pH 7.4) containing an erythrocyte suspension, incubated at 37°C, and in the presence of different concentrations (25 µg /mL, 50 µg / mL, 100 µg /mL and 200 µg /mL,), of methanolic extract of *Pistacia lentiscus* L. leaves.

According to the results obtained, we recorded increases in absorbance (hemolysis rate) during incubation of isolated erythrocytes in PBS (pH 7.4). Similarly, we noted that absorbances also increased as a function of concentration (25, 50, 100 and 200 µg /mL).

Figure 2 shows the rate of hemolysis, by percentage (%), in PBS buffer medium (pH 7.4) containing an erythrocyte suspension, incubated at 37°C, in the presence of different concentrations (25 µg /mL, 50 µg ug/L, 100 µg /mL and 200 µg /mL) of methanolic extract of *Pistacia lentiscus* L.

The results (Fig. 2) show very low hemolysis rates of 16.64% and 23.02% for the 25 and 50 µg /mL, respectively. There was no significant difference between these two concentrations. High rates of 41.9% and 88.64% were noted at 100 and 200 µg /mL, with a significant difference ($P<0.05$).

The hemolytic effect of medicinal plant leaves has been studied in various scientific research. According to Guo-Xiang L et Zai-Qun L (2007), the hemolysis test carried out showed that all four species (*Asteriscus graveolens, Cymbopogon schoenanthus, Panicum turgidum* and *Pitur-*

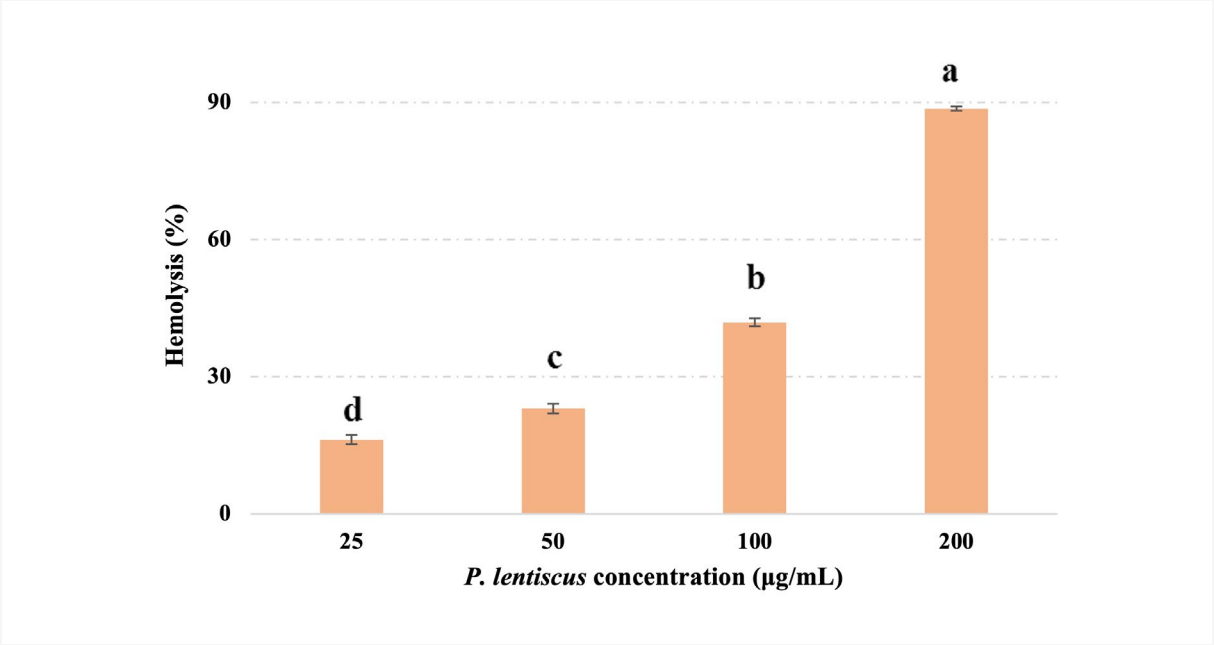

Figure 2. Hemolysis rate (%) for different extract concentrations. Different letters depict statistically significant difference at P<0.05.

Slika 2. Stopnja hemolize (%) pri različnih koncentracijah izvlečka. Različne črke označujejo statistično značilno razliko pri P<0,05.

anthos scoparius) exhibit a weak hemolytic effect. However, *Cymbopogon schoenanthus* and *Panicum turgidum* extracts can be slightly hemolytic at higher concentrations than our plant (Tanaka and Kashiwada, 2021).

Hemolysis is the destruction of red blood cells and can be caused by natural or synthetic substances. This activity can be beneficial in certain medical conditions, but it can also be toxic if used inappropriately.

According to the results shown in Figure 3, we noted very low antihemolytic rates of the order of 10.5% and 57.5% for 200 ug/mL and 100 ug/mL, respectively) with a significant difference (P<0.05). This rate is moderately increased at 77.43% and 83.17% for 50 and 25 ug/mL with a significant difference (P<0.05).

Hemolysis, defined as the destruction of red blood cells (RBCs), can be attributed to a variety of factors, including oxidative stress, autoimmune disorders, and infections. Oxidative stress results in the elevated generation of reactive oxygen species (ROS), which can compromise cell membrane integrity and ultimately lead to hemolysis. Recent research indicates that phytochemicals, specifically flavonoids, polyphenols, and tannins, exhibit anti-hemolytic properties, potentially reducing oxidative damage (Purba and Paengkoum, 2022; Cavalcanti et al., 2024). Flavonoids, such as quercetin and kaempferol, protect erythrocytes by stabilizing cell membranes and reducing lipid peroxidation. They also inhibit pro-inflammatory pathways that contribute to hemolysis (Berger, 2022). Research has shown that eating flavonoid-rich foods is associated with reduced markers of hemolysis in conditions such as diabetes and cardiovascular disease (Caro-Ordieres et al., 2020; Kejík et al., 2021). As concentration increases, so does toxicity. The high hemolysis rate can be explained by the depletion of toxic substances in the extract (Abdeddaim et al., 2021). In contrast, a more recent study examined the hemolytic effect of different extracts of *Pistacia lentiscus* L., including those prepared from leaves, fruit and resin, as well as aqueous and ethanolic extracts. The results showed that all the extracts tested had low to moderate hemolytic activity but that the leaf extract had the lowest hemolytic activity of all the extracts tested (Abdeddaim et al., 2021).

On the other hand, polyphenols, including resveratrol and catechins, exert protective effects on RBCs by

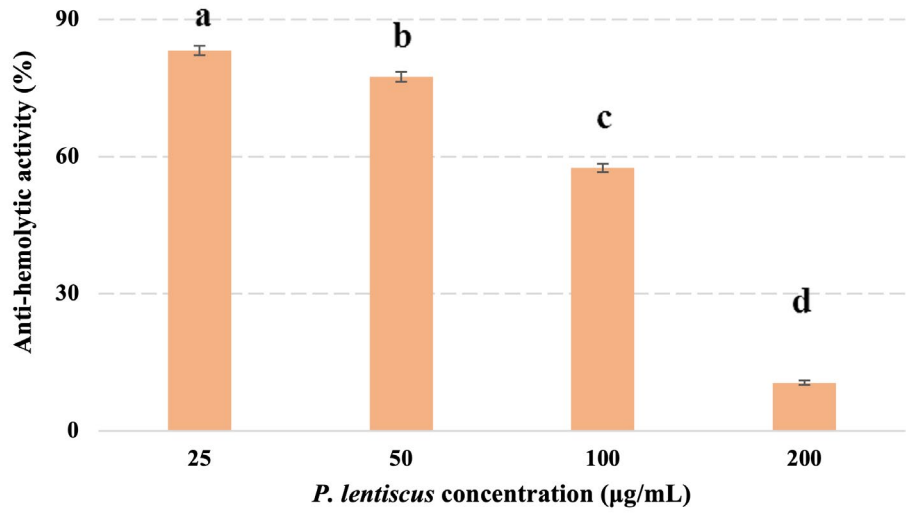

Figure 3. Percentage inhibition of hemolysis (%) for different concentrations of methanolic extract. Different letters depict statistically significant difference at P<0.05.

Slika 3. Odstotna inhibicija hemolize (%) za različne koncentracije metanolnega izvlečka. Različne črke označujejo statistično značilno razliko pri P<0,05.

enhancing the antioxidant defence system and reducing oxidative stress. They can modulate signalling pathways that influence erythrocyte survival (Checkouri et al., 2020; Tedesco et al., 2021). Rich sources of polyphenols include berries, green tea, dark chocolate, and red wine. Their consumption has been linked to improved erythrocyte integrity in various studies (Ooi et al., 2022; Buljeta et al., 2023). In other studies, tannins exhibit anti-hemolytic effects by interacting with membrane proteins, thereby promoting membrane stabilization. Their astringent properties may also play a role in limiting hemolytic activity by reducing oxidative damage (Olchowik-Grabarek et al., 2020; Purba and Paengkoum, 2022; Olchowik-Grabarek et al., 2023). Studies have indicated that tannin-rich foods can reduce indicators of hemolysis *in vitro* and *in vivo*, suggesting a protective role against hemolysis (Bharadwaj et al., 2021; Fraga-Corral et al., 2021; Benouali et al., 2023).

Since the studied extract has been recognized to be rich in several metabolites such as polyphenols, tannins and flavonoids (Bourroubey et al., 2023), our results indicate that *Pistacia lentiscus* leaf extract has moderate hemolytic activity, which varies according to the concentration.

Results of *in vitro* evaluation of the anti-inflammatory activity of methanolic extract of *Pistacia lentiscus* L. leaves (by ovalbumin denaturation)

The results of this study, shown in Figure 4, reveal the percentage inhibition of ovalbumin denaturation by the methanolic extract of *Pistacia lentiscus*. The highest inhibition percentage, 66.53%, was recorded at 2000 µg/mL with ($P<0.05$), followed by 61.16% at 1500 µg/mL. On the other hand, the lowest percentage was obtained at 3000 µg/mL and 3500 µg/mL with 28.58% and 10.16% respectively (with a significant difference ($P<0.05$)) (Fig. 4) compared to the control (Aspirin) which gave inhibition percentages of around 42.91% at a concentration of 100 µg/mL and 90.81%, 91.39%, 93.39 for doses 200, 250 and 300 µg/mL respectively where no significant difference was recorded ($P<0.05$), (Fig. 5).

Bovine serum albumin (BSA) denaturation is a common method used to assess the anti-inflammatory activity of plant extracts, including *Pistacia lentiscus* leaf extract. The anti-inflammatory activity of *Pistacia lentiscus* extract was assessed using BSA denaturation. The results showed sig-

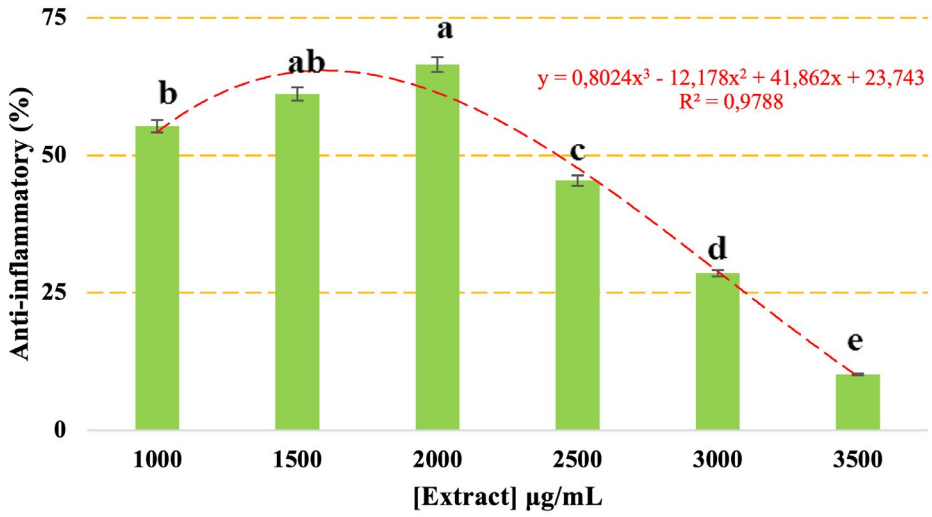

Figure 4. Percentage inhibition of ovalbumin denaturation by different concentrations of methanolic extract of *Pistacia lentiscus*. Different letters depict statistically significant difference at $P<0.05$.

Slika 4. Odstotna inhibicija denaturacije ovalbumina z različnimi koncentracijami metanolnega izvlečka *Pistacia lentiscus*. (Različne črke označujejo statistično značilno razliko pri $P<0,05$.

nificant anti-inflammatory activity, preventing heat-induced BSA denaturation (Boubaker et al., 2009) and the presence of hydrogen peroxide and oxidizing agents (Jahanban-Esfahlan et al., 2017).

The anti-inflammatory activity of different *Pistacia lentiscus* extracts using BSA denaturation as an in vitro model shows maximum inhibitory activity at a concentration of 100 µg/mL, but this activity decreased at higher concentrations. These findings are in line with our own. Also, authors have suggested that this could be due to a receptor saturation effect or competition with other compounds present in the extract at higher concentrations (Boukeloua, 2012; Bouzenna et al., 2016). They suggest that the anti-inflammatory activity of the extract could be linked to its antioxidant activity. These results suggest that the concentration of active compounds may play an important role in their biological activity, including their anti-inflammatory activity. *Pistacia lentiscus* extract is able to control self-antigen production by inhibiting protein denaturation. The denaturation-inhibiting activity of ovalbumin may be attributed to the presence of various bioactive compounds, such as flavonoids and tannins (Zam et al., 2020; Tebbi et al., 2024). These *in vitro* studies suggest that *Pistacia lentiscus* leaf extract may have anti-inflammatory potential thanks to its ability to prevent BSA denaturation. However, further in vivo studies are required to confirm these results.

Conclusion

Aromatic medicinal plants have played an important role in human health and well-being for thousands of years. They offer a natural alternative to pharmaceutical drugs, and many plant species have demonstrated beneficial therapeutic properties. The hemolytic effect of Pistacia lentiscus L. leaves showed a dose-dependent activity, both hemolytic and anti-hemolytic. This indicates that the higher the dose, the greater the hemolytic activity, while lower concentrations show stronger anti-hemolytic activity. The anti-inflammatory activity of the methanolic extract of Pistacia lentiscus L. leaves also showed a dose-dependent activity. The higher the dose, the greater the denaturation of proteins, while lower concentrations led to greater inhibition of denaturation. Pistacia lentiscus L. is a plant rich in bioactive substances, offering promising potential in the fields of anti-hemolysis and anti-inflammatory activity. Where in vitro studies have demonstrated its beneficial effects, which can have positive effects on human health.

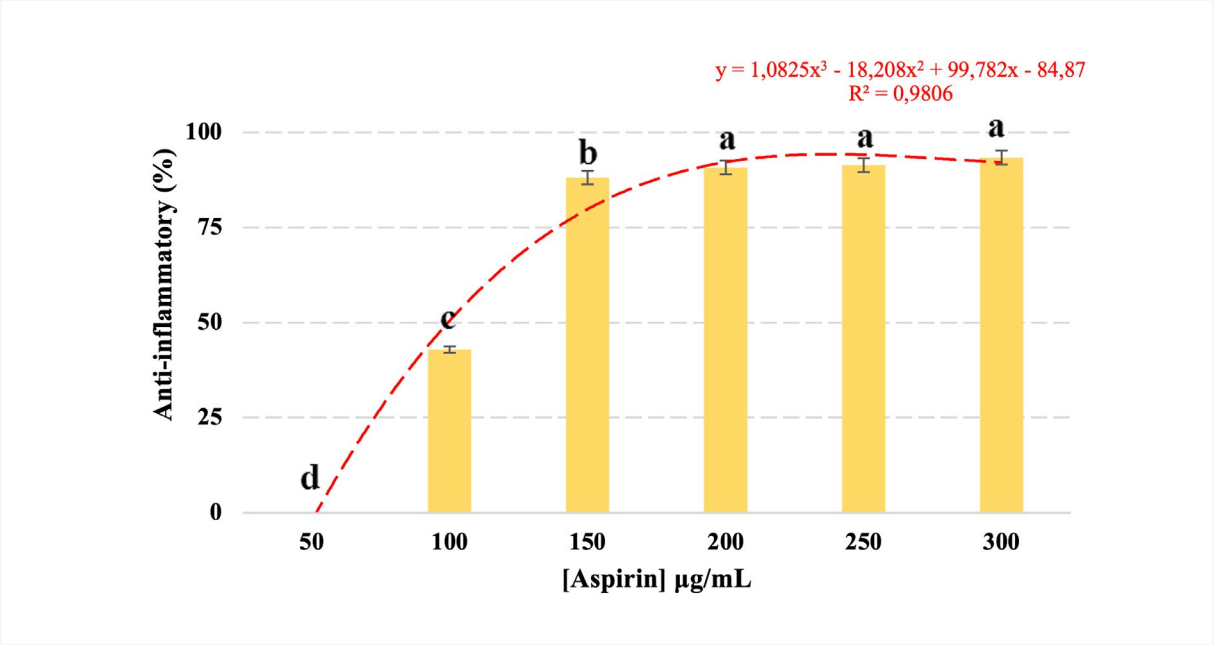

Figure 5. Percentage inhibition of ovalbumin denaturation by aspirin. Different letters depict statistically significant difference at P<0.05.

Slika 5. Odstotek inhibicije denaturacije ovalbumina z aspirinom. Različne črke označujejo statistično značilno razliko pri P<0,05.

Author Contributions

Conceptualization, B.B. and C.N.; methodology, B.B. and C.N.; software, B.B.; validation, B.B., C.N., B.A. and B.I.; formal analysis, B.A.; investigation, C.N. and B.I.; data curation, B.B., C.N., B.A., T.T.A., M.B., and B.I.; writing original draft preparation, B.B., C.N., B.A., T.T.A., M.B., and B.I.; writing review and editing, B.B., C.N., B.A., T.T.A., M.B., and B.I.; visualization, C.N; supervision, T.T.A.; project administration, M.B. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

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ABS

Original Research

Assessment of Genomic Integrity of *Vitex negundo L.*, An Important Indian Medicinal Plant, Using RAPD Markers

Shweta Chaudhary [1], Gunjan Garg [1], Alok Bharadwaj [2*]

Abstract

Vitex negundo is an Indian medicinal plant containing steroids, flavonoids, lignans and terpenoids that can be used as a precursor for commercial production. An efficient marker system such as Random Amplified Polymorphic DNA (RAPD) was used to assess the genetic integrity of *V. negundo*. The straightforward method RAPD can be used to evaluate genomic integrity because it uses a small amount of DNA for PCR amplification. Six out of thirteen RAPD primers generated 150 distinct bands, of which 31 were polymorphic, with an average of 5.16 polymorphic bands per primer. A maximum of up to 32 fragments were amplified, and an average of 25 per primer, and the amplicons varied in size between 100 and 2000bp. The percentage of polymorphism ranges from 12.9 to 22.5, with an average of 16.6. The PIC values ranged from 0.11 to 0.63 for RAPD primers. The study pointed out that RAPD markers evaluate the genetic fidelity in *Vitex negundo*. The UPGMA cluster analysis grouped all in vitro raised plantlets treated with different growth regulators such as BAP, DPU, TDZ, and mT. The principal component analysis also substantiates this clustering pattern. Thus, the phylogenetic relationship and a high genetic variation revealed in the present study could provide baseline data for the conservation and improvement of this plant in future. Also, the molecular marker identified in this study will be helpful in the authentication of this species to prevent adulteration in herbal medicine.

Keywords

Genetic integrity, Molecular marker, RAPD, *Vitex negundo*, Polymorphism, plant growth regulators, clustering analysis

1 School of Biotechnology, Gautam Buddha University, Greater Noida 201312, Uttar Pradesh, India.

2 Department of Biotechnology, GLA University, Mathura 281406, Uttar Pradesh, India

*** Corresponding author:**
E-mail address:
shwetachaudhary2022@gmail.com

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Ocena genomske celovitosti *Vitex negundo* L., pomembne indijske zdravilne rastline, z uporabo označevalcev RAPD

Izvleček

Vitex negundo je indijska zdravilna rastlina, ki vsebuje steroide, flavonoide, lignane in terpenoide, ki se lahko uporabljajo kot predhodnik za komercialno proizvodnjo. Za oceno genetske celovitosti *V. negundo* je bil uporabljen učinkovit markerski sistem Random Amplified Polymorphic DNA (RAPD). Preprosta metoda RAPD se lahko uporablja za ovrednotenje genomske celovitosti, ker uporablja majhno količino DNA za pomnoževanje PCR. Šest od trinajstih primerjev RAPD je ustvarilo 150 različnih pasov, od katerih je bilo 31 polimorfnih, s povprečno 5,16 polimorfnimi pasovi na začetni oligonukleotid. Pomnoženih je bilo največ do 32 fragmentov, v povprečju pa 25 na začetni oligonukleotid, velikosti pomnožkov pa so se gibale med 100 in 2000 bp. Odstotek polimorfizma se giblje od 12,9 do 22,5, v povprečju 16,6. Vrednosti PIC so se gibale od 0,11 do 0,63 za primerje RAPD. Študija je poudarila, da markerji RAPD ocenjujejo genetsko zvestobo pri *V. negundo*. Analiza grozdov UPGMA je združila vse rastline, vzgojene *in vitro*, tretirane z različnimi regulatorji rasti, kot so BAP, DPU, TDZ in mT. Analiza glavnih komponent tudi utemeljuje ta vzorec združevanja v gruče. Tako bi lahko filogenetski odnos in velika genetska variacija, razkrita v tej študiji, zagotovila osnovne podatke za ohranjanje in izboljšanje te rastline v prihodnosti. Molekularni marker, identificiran v tej študiji, bo prav tako v pomoč pri avtentifikaciji te vrste, da se prepreči ponarejanje v zeliščni medicini.

Ključne besede

Genetska celovitost, molekularni marker, RAPD, *Vitex negundo*, polimorfizem, rastlinski rastni regulatorji, analiza grozdenja

Introduction

Vitex negundo L., also known as "Nishinda", is a woody, fragrant, and therapeutic shrub that is a member of the Verbenaceae family (Koirala et al., 2020). In India, this plant grows haphazardly and is frequently utilised as a hedge. The plant is erect, thin, and ranges in height from 2 to 5 metres. It can be found in China, Madagascar, Ceylon, the Philippines, India, Afghanistan, and Tropical Africa (Manokari, Priyadharshini, and Shekhawat 2021). The leaves have five leaflets in a palmate arrangement, which are lanceolate 4-10 cm long, hairy beneath and pointed at both ends. There are lots of bluish-purple blossoms. The fruit is spherical, about 4 mm in diameter, black when ripe, and succulent (Tawfeeq et al., 2023). According to a phytochemical analysis of this plant, the leaves contained the following compounds: 5,3-dihydroxy-3,6,7,4-tetramethoxyllavone, hydroxyl-3,6,7,3,4-penta methoxy flavone, monoterpenes agnuside, flavonoids-casticin, chryso-sphenol and vitexin, flavonoids (vitexicarpin) (Alfarabi et al. 2022). *V. negundo* leaves have antibacterial, antitumor, astringent, febrifuge, sedative, tonic, and vermifuge properties (Vigneswari et al., 2023). Insecticidal action is observed in leaf extracts of this plant (Edwin and Jacob 2017). New leaves are burned with grass, which works as a fumigant against mosquitoes. (Alfarabi et al. 2022). According to Duke and Ayensu (1985), the fruit is also used to cure rheumatic problems, coughs, angina, colds, and other ailments. The root has febrifuge, expectorant, and tonic properties (Goswami and Roy, 2023). Medicinal plants are of great interest to researchers in the field of biotechnology as most of the drug industries depend, in part, on plants for the production of pharmaceutical compounds (Noor et al., 2022). Techniques for in vitro culture provide a practical means of conserving the germplasm and mass-multiplying uncommon, endangered, fragrant, and therapeutic plants (Mishra et al., 2022). Ever-increasing interest in in vitro culture techniques has been applied not only for the multiplication of several rare species of great importance but also for cloning elite types of plants on a larger scale. In vitro techniques are effectively utilised for germplasm conservation of rare, endangered, aromatic and medicinal (Priyanka et

al. 2021). Interspecific and intraspecific components make up biodiversity (Samanta et al., 2023). Because ecologists have been conducting diversity assessments for a long time, they are frequently restricted to species surveys and ignore the intra-specific components of diversity (Bublyk et al., 2020). Genetic variety is necessary for natural populations to continue as evolutionarily viable units capable of long-term adaptation to changing conditions (Iosefa et al. 2016). It is anticipated that geographic isolation will have a major impact on the population's genetic structure (Peng et al., 2021). The long-term evolutionary history of the species (distance changes, habitat fragmentation, and population isolation), mutation, genetic drift, mating system, gene flow, and selection are some of the processes whose interactions are reflected in the genetic structure of plant populations (Zhang et al. 2019).

The pharmaceutical industry uses medicinal plants as a major source of raw materials. Approximately 92% of the medicinal plants that are harvested destructively from the wild to make traditional medicines are used by industries. If biodiversity is not managed sustainably, there is a clear risk to the genetic stocks and diversity of medicinal plants (Savitikadi et al., 2020). In Indian traditional medical systems, *Vitex negundo* L. has long been employed. However, the preservation of this plant species has received little to no attention up to this point. Therefore, the goal of the current investigation was to ascertain the genetic integrity between the parent plant and treated explants that had varying concentrations of *V. negundo* plant growth regulators (Zavinon et al., 2020). Genetic variations are accumulated by geographically separated populations as they adjust to varying environmental conditions (Singer et al. 2021). Due to the impact of numerous environmental factors, genetic fidelity study based on morphological and biochemical criteria has many limitations. Because molecular markers are independent of environmental factors, they are useful for genetic diversity studies. In order to evaluate the genetic diversity of species from different phytogeographical regions, the RAPD technique has been effectively applied (Bi et al. 2021). The method was frequently used to estimate genetic links between and within species (Boomibalagan et al., 2021).

Maintaining genetic integrity is critical for conserving medicinal qualities and adaptability of *V. negundo*, as genetic fidelity ensures the stability of therapeutic compounds and allows for consistent biotechnological applications. A genetic resource management strategy for such species needs to be based on research data examining the extent of genetic differentiation within and between populations and on understanding the processes maintaining this variation. Random Amplified Polymorphic DNA(RAPD) is a simple technique that requires a small amount of DNA for PCR amplification and can be used for genotoxicity assessment (Srinivasan et al., 2021).

There could be variations in the final DNA profiles because of band shifts, absent bands, or the emergence of new bands. These bands are assessed to assess genetic dissimilarities or similarities (Rohela et al. 2019). Furthermore, their potential to serve as the foundation for novel biomarker assays for the identification of DNA damage and mutations in the living tissues of bacteria, plants, and animals is suggested by their use in surveying genomic DNA to detect different kinds of DNA destruction and mutations (example-rearrangements, point mutations, small insert or deletion of DNA, and ploidy changes) (Dang et al. 2022). In this paper, we evaluated the use of RAPD to detect genetic integrity and gene flow of the *V. negundo*, which was conducted when explants were treated with different plant growth regulators.

The aim of the present communication was (1) to standardize a reproducible protocol, which can be used at a commercial scale, for mass propagation using nodal explants derived from the mother plant and (2) to evaluate the genetic homogeneity among the generated plants by adopting molecular technique.

Materials and Methods

Plant material and extraction of genomic DNA

We gather samples of *V. negundo* from the GBU campus herbal garden as well as through in vitro plantlets that have been treated with various concentrations of plant growth regulators and are kept in a plant tissue culture facility. All explants (nodal segments) were cultured in phytajars on a medium containing Murashige and Skoog (MS) salts, vitamins and 3% (w/v) sucrose. Depending upon the experiments, MS medium was variously supplemented with growth regulators such as Benzyl amino purine (BAP), diphenyl urea (DPU), thidizuron (TDZ) and meta-topolin (mT) in various concentrations and combinations (Razani et al. 2020). The media were gelled with 0.8% (w/v) bacteriological grade agar, and its pH was adjusted to 5.8 using 1 N

NaOH before autoclaving at 121°C for 15 min. The cultures were maintained in a culture room illuminated by two cool white fluorescent lamps with a maintained temperature of 26±2°C (Fig. 1). For the purpose of assessing its genetic integrity, a total of six accessions were taken from the parent plant along with in vitro plantlets. (Table 1)

The Cetyl trimethylammonium bromide (CTAB) method described by Doyle and Doyle (1990) was followed with slight modification for genomic DNA isolation and purification from in vitro raised plantlets and a field-grown mother plant. The extracted DNA was air-dried and dissolved in 100 µl of sterile mQ water and tested for purity (A260/280 ratio) on a UV visible spectrophotometer and for size, purity and integrity in 0.8% (w/v) agarose gel at 60 V for 60 min (Fig. 2).

High molecular weight genomic DNA was found in the callus of the TDZ-treated samples and the juvenile leaf tissues of all the collected samples. Ethidium bromide staining was used to assess the purity of the isolated DNA and a UV spectrum photometer set at 260 nm was used to determine its quantity.

Table 1. Six accessions for assessing genetic integrity.

Tabela 1. Šest akcesij za oceno genske celovitosti.

Accessions label	Name of Accessions label
C1	Mother plant
C2	BAP
C3	DPU
C4	Mt
V5	TDZ
V6	Callus of TDZ

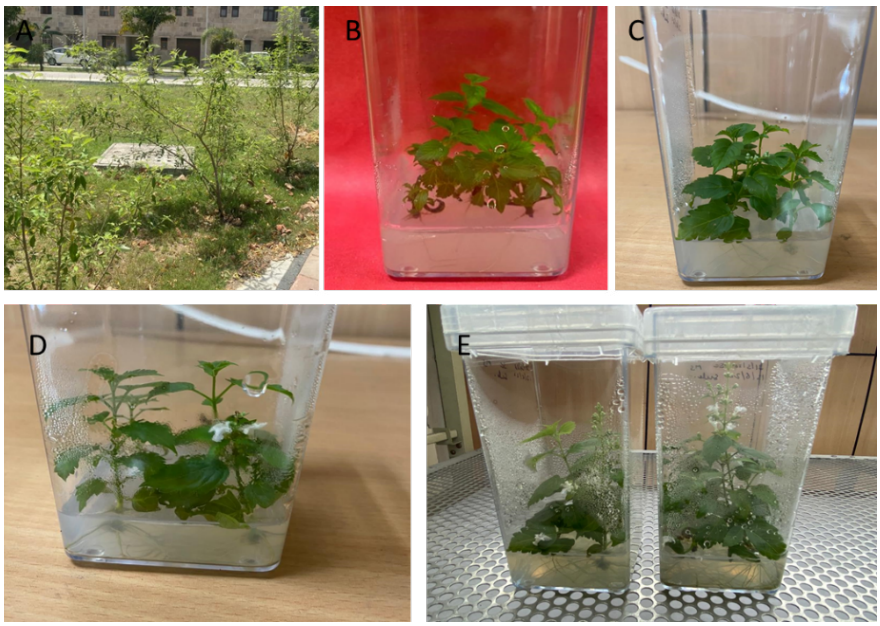

Figure 1. Samples of *V. negundo* a) Mother plant b) BAP c) DPU d) mT e) TDZ

Slika 1. Vzorci *V. negundo* a) Matična rastlina b) BAP c) DPU d) mT e) TDZ

RAPD evaluation

Genomic DNA was diluted to 50 ng/µl in order to facilitate amplification using specially designed random primers for RAPD analysis. One unit of Taq DNA polymerase, 50 ng of template DNA, 200 µM of each DNTP, 1X Taq buffer, 1.5 mM MgCl2, and 100 pmol primer were all included in the 2.5µl reaction mixture. PCR reactions are conducted in an Eppendorf thermal cycler, which is configured to denature for five minutes at 94°C, then anneal for one minute at 37°C, extend for two minutes at 72°C and repeat for 42 cycles. At the conclusion of the reaction, a final extension allowed was seven minutes at 72°C.

Gel separation

The 2% agarose gel in 1X TAE solution was used to resolve the amplified products, which were then stained with ethidium bromide, visualised, and recorded using a gel documentation system. Only primers that produced distinct and repeatable bands were taken into consideration for the final analysis after each experiment was run three times.

Data interpretation

Band presence in the RAPD profiles was graphically evaluated as "1" and absence as "0". Fuzzy bands were discarded, and only distinct, clear bands were scored. Using the formula PIC=1-∑ (i-1)^ k≈Pi2, the polymorphism information content (PIC) values for the RAPD primers were determined. Pi represents the frequency of the ith allele using the k primer. (Farahzadi et al. 2020). Pairwise similarity matrices (Jaccard 1908) were produced using Jaccard's similarity coefficient and the SimQual format for qualitative data (Tang et al. 2021). which was derived from the NTSYS-pc version 2.1 (Numerical Taxonomy and Multivariant Analysis System) (Kizilgeci et al. 2022). Using the Unweighted Pair Group Method with Arithmetic Average (UPGMA) and the SAHN module of NTSYS-pc, a dendrogram was created based on the similarity matrix (Khan et al. 2022).

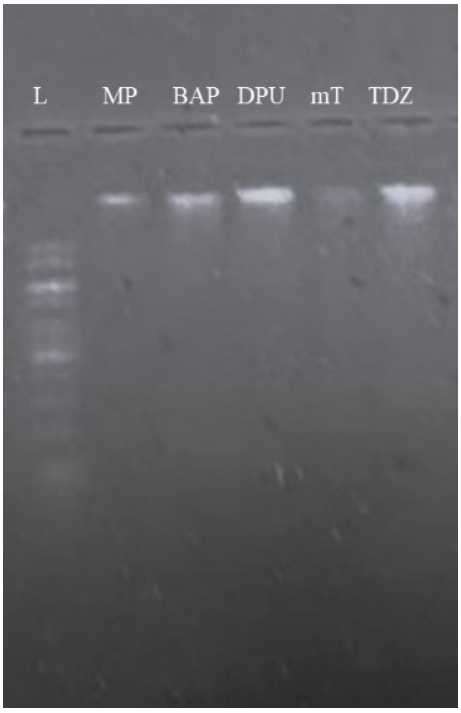

Figure 2. Image of DNA extraction on agarose gel.

Slika 2. Slika ekstrakcije DNK na agaroznem gelu.

Result and Discussion

RAPD Evaluation

Thirteen random primers were initially screened, with six primers producing banding patterns that could be visually scored, and these were selected for further analysis. Using RAPD analysis with these six primers, a total of 150 bands were successfully amplified, averaging 25 bands per primer. The amplified bands spanned sizes ranging from 100 to 2000 bp across all accessions. Out of these 150 bands, 31 were polymorphic, with a mean of 5.16 polymorphic bands per primer. The polymorphism percentage varied among primers, with OPX-20, OPX-17, and OPX-15 achieving a maximum of 100% polymorphism, whereas OPB-01 showed the lowest polymorphism percentage (Table 2, Fig. 1). The Polymorphism Information Content (PIC) values ranged from 0.11 (OPX-17) to 0.63 (OPX-20), with an average PIC of 0.27, highlighting the effectiveness of RAPD primers in detecting polymorphism among the selected accessions.

A dendrogram was constructed using the binary RAPD primer data and UPGMA clustering. The clustering represented six distinct samples of *Vitex negundo*, labelled as accessions C1, C2, C3, C4, V5, and V6. These accessions include plants grown in vitro and treated with various plant growth regulators such as BAP, DPU, TDZ, and mT, along

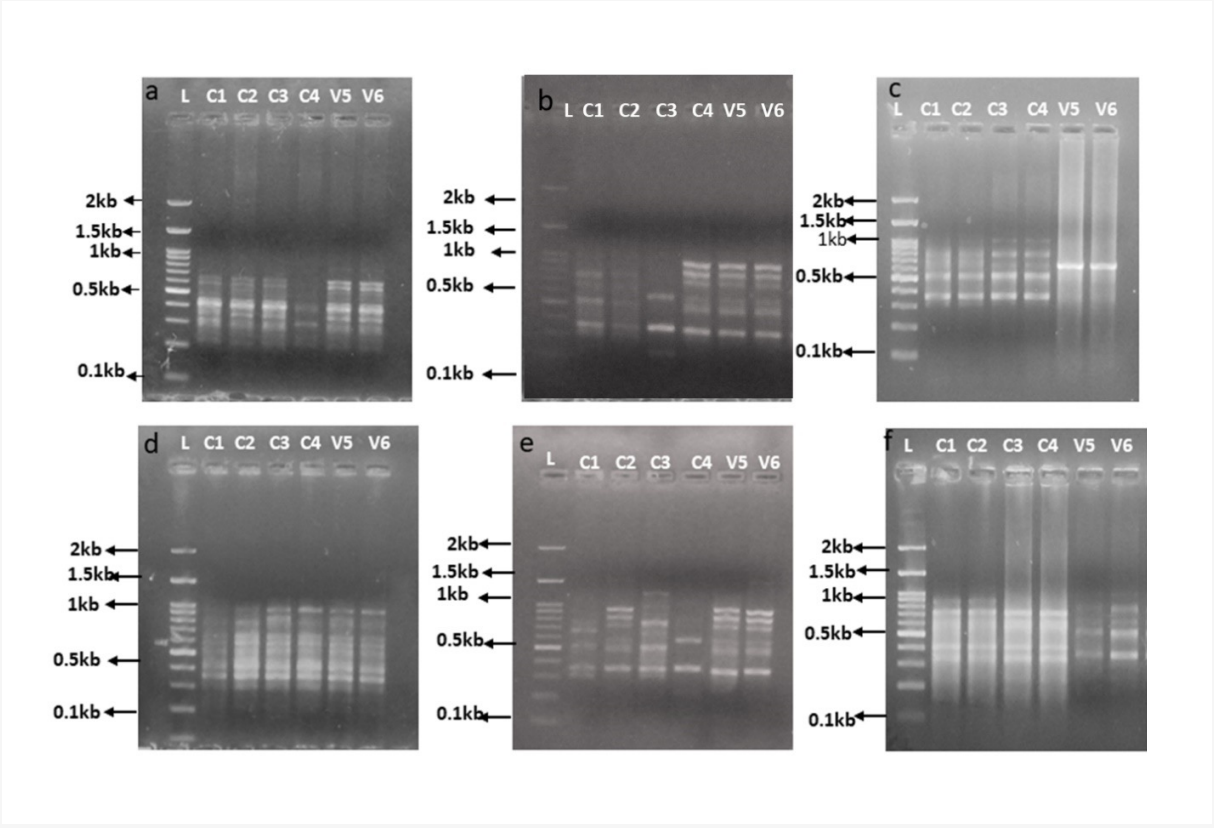

Figure 3. RAPD profiles of mother plant and *in vitro* raised plantlets of *V. negundo*. Banding pattern attained from a OPB-02, b OPX-17, c OPX-07, d OPB-012, e OPX-15, f OPX-20. L: DNA ladder, C1: DNA banding profile of mother plant, C2, C3, C4, V5: DNA banding profile of in vitro raised plantlets from nodes treated with different PGRs (BAP, DPU, Mt), V6: DNA banding profile of in vitro raised callus from node treated with TDZ.

Slika 3. Profili RAPD matične rastline in *in vitro* vzgojenih rastlin *V. negundo*. Profil pridobljen iz OPB-02, b OPX-17, c OPX-07, d OPB-012, e OPX-15, f OPX-20. L: DNA lestev, C1: profil DNA pasov matične rastline, C2, C3, C4, V5: profil DNA pasov in vitro vzgojenih sadik iz vozlišč, obdelanih z različnimi PGR (BAP, DPU, Mt), V6: profil DNA pasov in vitro dvignjen kalus iz vozla, zdravljenega s TDZ.

Table 2. The amplification pattern, percentage of polymorphism and PIC value of *V. negundo* accessions analysed by using 6 RAPD primers..

Tabela 2. Vzorec pomnoževanja, odstotek polimorfizma in vrednost PIC akcesij *V. negundo*, analiziranih z uporabo 6 primerjev RAPD.

Primers	Primer sequences (5'-3')	Size range of amplicons bp	Total no. of bands	Total no. of polymorphic bands	Total no. of polymorphic bands	% of polymorphism	PIC value
OPX-20	GGACCCTTAC	220-800	20	1	4	12.9	0.63
OPX-07	TGGCAACGCA	350-920	18	0	5	16.1	0.27
OPX-17	CAGACAAGCC	120-900	30	1	6	19.3	0.11
OPX-15	CTACTGGGAC	300-900	28	0	7	22.5	0.19
OPB-02	TGATCCCTGG	300-620	24	1	5	16.1	0.27
OPB-012	CCTTGACGCA	370-900	30	2	4	12.9	0.55

with one mother plant. Pairwise similarity matrix values, calculated with Jaccard's coefficient, ranged from 0.51 to 0.86. Specific accessions include C1 (mother plant), C2 (BAP-treated in vitro plant), C3 (DPU-treated sample), C4 (mT-treated sample), V5 (TDZ-treated in vitro plant), and V6 (callus sample). The dendrogram produced by the UPGMA clustering method is shown in Fig. 4, and it reflects the genetic relationships among the different accessions treated with various growth regulators.

RAPD method reproducibility

Genetic relationship in *Vitex negundo* L. in different in vitro plantlets treated with different growth regulators has been carried out using RAPD markers. Genetic variation in in vitro plantlets treated with different growth regulators is measured by the heterozygosity or the degree of polymorphism. For the conservation of a species, genetic variability is of the utmost importance to preserve. Genetic variability among all samples is important to maintain since it represents the 'blueprint' for all the living things on earth. The result obtained was analysed, and the dendrogram was obtained. In order to confirm the true-to-type nature, Random Amplified Polymorphic DNA (RAPD) analysis was carried out in a selected micro-propagated plant of *Vitex negundo*. Of the thirteen selected primers, six primers generated well-resolved and reproducible banding patterns.

RAPD Method Reproducibility

The genetic relationships within *Vitex negundo* accessions treated with different growth regulators were examined using RAPD markers. Genetic variation among the in vitro plantlets, as influenced by treatment with different growth regulators, was measured by heterozygosity and the degree of polymorphism. Genetic variability is critical to conserving species diversity, as it preserves the foundational genetic blueprint for living organisms. The generated dendrogram (Fig. 4) provides a reliable assessment of genetic relationships across *V. negundo* accessions, confirming the true-to-type nature of these samples through Random Amplified Polymorphic DNA (RAPD) analysis. Of the thirteen primers tested, six produced well-resolved and reproducible banding patterns.

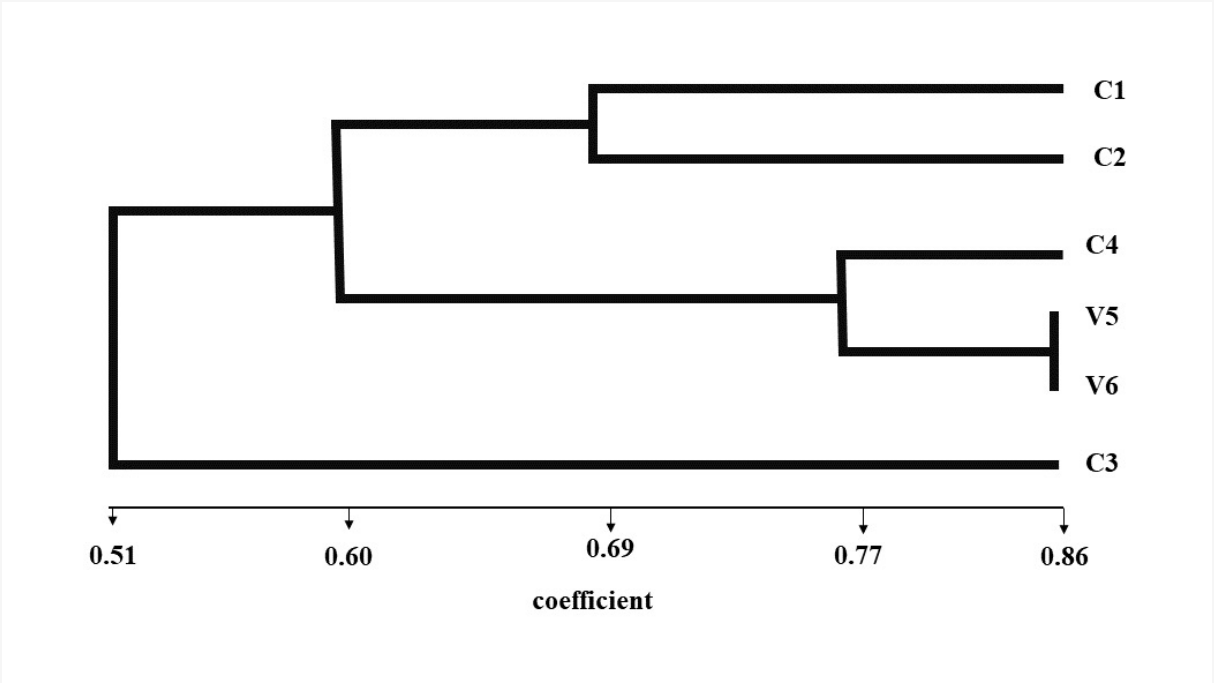

Figure 4. Dendogram obtained by UPGMA cluster analysis based on Jaccard's coefficient of 6 *Vitex negundo* accessions using RAPD data.

Slika 4. Dendogram, pridobljen z analizo grozdov UPGMA na podlagi Jaccardovega koeficienta 6 akcesij *Vitex negundo* z uporabo podatkov RAPD.

Genetic Integrity and Utility of RAPD Markers

The use of RAPD as a DNA fingerprinting technique effectively revealed the degree of polymorphism among six *V. negundo* accessions (Geetha and Siril 2022). Six RAPD primers yielded a total of thirteen polymorphic markers, with an average polymorphism percentage of 16.6%, which suggests a high level of genetic fidelity within the *V. negundo* accessions. The mean PIC value of 0.33, as noted by Powell et al. (1996), serves as an indicator of the usefulness of RAPD markers in detecting polymorphism across these taxa (Li et al. 2021). RAPD analysis captures a broad genetic picture by sampling from a substantial portion of the genome (Thakur et al. 2019). The clustering analysis groups the accessions according to their genetic similarity, as indicated by UPGMA analysis based on RAPD data (Seredin et al., 2022).

The dendrogram obtained from RAPD analysis of 150 PCR products demonstrates the high genetic integrity of *V. negundo* accessions. This finding is significant for selecting parent plants in breeding programs, as it supports the creation of populations useful for genome mapping and related genetic studies.

Conclusions

The molecular marker technique was helpful in determining high levels of genetic integrity and in estimating the genetic relationships between mother plant V. negundo accessions and in vitro-raised plantlets treated with various plant growth regulators. According to the study, RAPD markers are only slightly useful for determining the genetic diversity of V. negundo. The results of this work suggest that V. negundo in vitro plantlets treated with various growth regulators are an excellent source of genetic variety and that the identified molecular markers may be better utilised for the conservation of germplasm and genetic enhancement of this species.

Conflict of Interest

The authors declare that there is no conflict of interest.

Author Contributions

Conceptualization, S.C. and G.G.; Data collection, A.B.; Analysis and interpretation of results, A.B.; writing original draft, review, & editing, S.C. and G.G.

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Review

Vpliv prehrane na ustni mikrobiom in parodontalno zdravje

Tina Robič, DMD [1*]

Izvleček

Parodontalna bolezen je kronična vnetna bolezen, ki prizadene podporna tkiva zob in lahko vodi do izgube zob, če ni ustrezno zdravljena. Glavni vzrok za nastanek parodontalne bolezni je neravnovesje v ustnem mikrobiomu, kompleksni skupnosti mikroorganizmov, ki naseljujejo ustno votlino. Porušenje tega ravnovesja lahko povzroči razraščanje patogenih bakterij, ki sprožijo vnetni odziv dlesni. Prehrana ima pomemben vpliv tudi na sestavo in raznolikost ustnega mikrobioma, ter lahko tako bistveno vpliva na zdravje dlesni in splošno ustno zdravje. Vključitev protivnetnih hranil, kot so omega-3 maščobne kisline, antioksidanti, vitamin D, polifenoli ter zmanjšanje vnosa sladkorjev lahko pomaga pri obvladovanju in preprečevanju parodontalne bolezni. Terapevtska uporaba probiotikov, kot so bifidobakterije in laktobacili, predstavlja nov koncept v zobozdravstvu. Raziskave kažejo, da lahko redna uporaba probiotičnih dopolnil ali živil, bogatih s probiotiki, kot so jogurti in fermentirana hrana, podpira vzdrževanje uravnoteženega ustnega mikrobioma. Probiotiki pri parodontalni bolezni delujejo tako, da zavirajo rast patogenih bakterij, zmanjšujejo vnetne odzive v ustni votlini ter spodbujajo imunski sistem za boljšo obrambo pred okužbami. Poleg tega spodbujajo proliferacijo fibroblastov in tako podpirajo celjenje tkiv. Nadzor prehrane ter vnos vitaminskih dodatkov in probiotikov, lahko skupaj z dobro ustno higieno in rednimi obiski zobozdravnika znatno izboljša stanje dlesni in prepreči napredovanje parodontalne bolezni.

Ključne besede

Parodontalna bolezen; Hranila; Prehrana; Imunski odziv; Ustni mikrobiom; Probiotiki

1 Zobozdravstvo diamant d.o.o., 1000 Ljubljana, Slovenia

*** Corresponding author:**
E-mail address: tina@robic.si

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The Impact of Diet on Oral Microbiome and Periodontal Health

Abstract

Periodontal disease is a chronic inflammatory condition that affects the supporting tissues of the teeth and can lead to tooth loss if not properly treated. The main cause of periodontal disease is an imbalance in the oral microbiome, the complex community of microorganisms inhabiting the oral cavity. Disruption of this balance can lead to the overgrowth of pathogenic bacteria that trigger an inflammatory response in the gums. Diet also has a significant impact on the composition and diversity of the oral microbiome, and thus can greatly influence gum health and overall oral health. Including anti-inflammatory nutrients such as omega-3 fatty acids, antioxidants, vitamin D, polyphenols in the diet and reducing sugar intake can help manage and prevent periodontal disease. The therapeutic use of probiotics, such as bifidobacteria and lactobacilli, represents a new concept in dentistry. Research shows that regular use of probiotic supplements or probiotic-rich foods, such as yogurts and fermented foods, supports the maintenance of a balanced oral microbiome. Probiotics in periodontal disease work by inhibiting the growth of pathogenic bacteria, reducing inflammatory responses in the oral cavity, and stimulating the immune system for better defense against infections. Additionally, they promote the proliferation of fibroblasts, thereby supporting tissue healing. Diet control, intake of vitamin supplements, and probiotics, combined with good oral hygiene and regular dental visits, can significantly improve gum health and prevent the progression of periodontal disease.

Keywords

Periodontal disease; Nutrients; Diet; Immune response; Oral microbiome; Probiotics

Uvod

Parodontalna bolezen je kronična vnetna bolezen, ki prizadene podporna tkiva zob (slika 1). Začne se z gingivitisom, vnetjem dlesni, ki nastane zaradi z bakterijami bogatega zobnega biofilma. Če gingivitis ni ustrezno zdravljen, lahko napreduje v parodontitis, kjer vnetje povzroči razgradnjo kosti in tkiv, ki podpirajo zobe. Simptomi parodontalne bolezni vključujejo rdeče, otečene in krvaveče dlesni, slab zadah in umik dlesni. Parodontalna bolezen je pomemben vzrok za izgubo zob in je povezana s sistemskimi boleznimi, kot so diabetes in bolezni srca in ožilja (Isola idr., 2022). Na parodontalno zdravje vplivajo številni dejavniki, kot so ustna higiena, genetski in epigenetski dejavniki, sistemsko zdravje ter prehrana (Najeeb idr., 2016). Glavni vzrok za nastanek parodontalne bolezni je neravnovesje v ustnem mikrobiomu, kompleksni skupnosti mikroorganizmov, ki naseljujejo ustno votlino. Ustna votlina gosti drugi najbogatejši in raznovrstnejši mikrobiom v človeškem telesu takoj za prebavnim traktom. V zdravem ustnem mikrobiomu prevladujejo koristne bakterije, ki pomagajo pri prebavi hrane, vzdrževanju imunskega sistema in zaščiti pred patogeni. Trenutno velja, da človeški ustni mikrobiom sestavlja več kot 250 vrst, vključno s patogeni, kot so *Treponema denticola*, *Porphyromonas gingivalis*, *Tannerella forsythia* in *Aggregatibacter actinomycetemcomitans*, ki so povezani z etiologijo parodontalne bolezni (Lenartova idr., 2021). Vnetne spremembe v ustni votlini povzročajo neravnovesje mikrobioma, kar vodi do povečane rasti teh parodontopatogenih bakterij. Povzročitelji parodontalne bolezni sproščajo toksine, ki uničujejo tkivo dlesni in kosti, kar povzroča vnetje in pospešuje napredovanje bolezni. Za preprečevanje in zdravljenje parodontalne bolezni je ključno vzdrževanje zdravega ustnega mikrobioma. To vključuje redno ustno higieno, kot je ščetkanje zob, uporaba zobne nitke in antiseptičnih ustnih vod, ter zdravo prehrano, bogato z antioksidanti in probiotiki.

Pomen prehrane v okviru ustnega zdravja se običajno povezuje z lokalnimi učinki hrane in pijače v ustni votlini, ko ostanki niso odstranjeni s ščetkanjem. Vendar prehrana predstavlja tudi vir hranil, ki se po procesu prebave prek krvnega obtoka prenašajo v tkiva in organe ustne votline

ter preostale dele telesa. V zadnjih letih so raziskave pokazale, da ima prehrana pomembno vlogo pri preprečevanju in obvladovanju parodontalne bolezni. Hranila delimo na dve vrsti: mikrohranila in makrohranila. Mikrohranila so hranila, ki jih telo potrebuje v majhnih količinah, vendar so kljub temu ključnega pomena za zdravje in pravilno delovanje organizma. Mikrohranila vključujejo vitamine in minerale, ki sodelujejo v številnih biokemičnih procesih v telesu. Makrohranila so hranila, ki jih telo potrebuje v večjih količinah za zagotavljanje energije, rast in vzdrževanje telesnih funkcij. Obstajajo tri glavne vrste makrohranil: ogljikovi hidrati, beljakovine in maščobe.

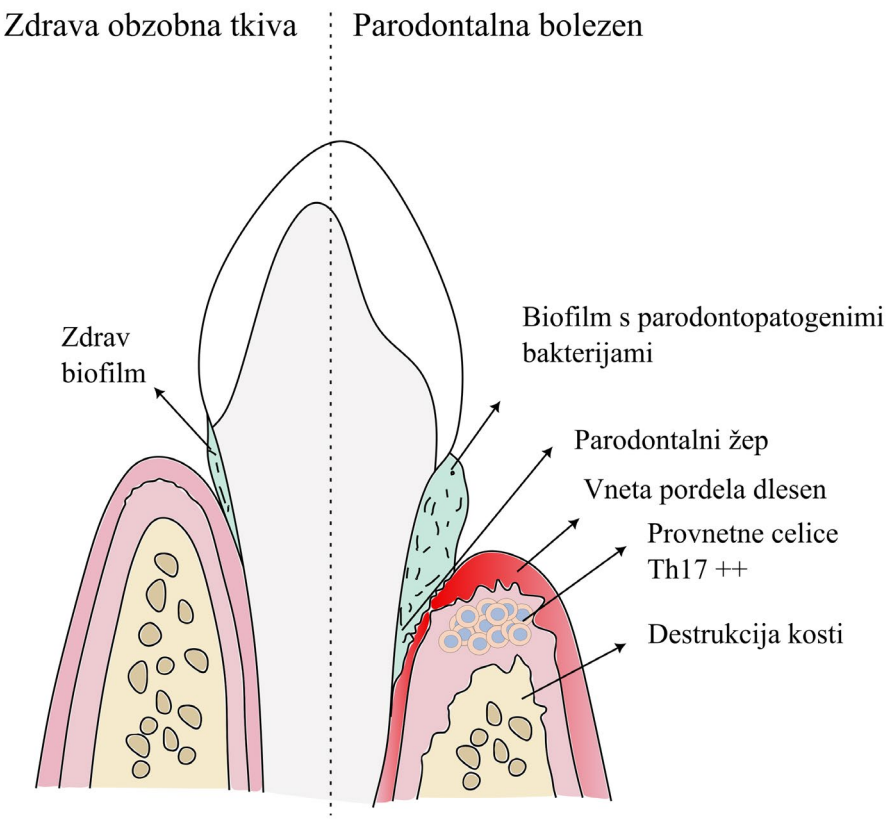
Članek podrobno obravnava kompleksen vpliv hranil na razvoj in zdravljenje parodontalne bolezni ter poudarja vlogo probiotikov pri uravnavanju ustnega mikrobioma.

Pregled hranil

Ogljikovi hidrati

Pogosto uživanje ogljikovih hidratov, povezano z redkim in neustreznim ščetkanjem, je glavni dejavnik pri nastanku zobnih oblog in zobnega kamna na površinah zobnih kron in korenin. Zobne obloge so biofilm glikoproteinov, mucina in bakterij, ki se prilepijo na površine v ustni votlini. Če se obloga ne odstrani z zob, se v nekaj dneh mineralizira in tvori zobni kamen. Porozni zobni kamen zagotavlja površino za naselitev parodontalnih patogenov, vključno s Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia in Treponema denticola.

Vpliv ogljikovih hidratov na parodontalno bolezen pa



Slika 1. Shematski prikaz zdravih obzobnih tkiv in parodontalno prizadetih obzobnih tkiv

Figure 1. Schematic representation of healthy periodontal tissues and periodontally affected tissues.

se odraža tudi v njihovem sistemskem delovanju. Znano je, da sladkorna bolezen predstavlja tveganje za nastanek parodontitisa, vendar tudi hrana z visokim glikemičnim indeksom, samostojno brez prisotnosti sladkorne bolezni, spodbuja razvoj vnetja v parodontalnih tkivih (Woelber & Tennert, 2020). Raziskave, izvedene na študentih dentalne medicine in ustnih higienikih, so pokazale, da je povečano uživanje sladkorja spremljalo tudi povečano krvavenje dlesni. Ob predpostavki, da ima ta skupina visoko znanje in veščine o pravilnem vzdrževanju zobne higiene, je bil vpliv zobnega biofilma na razvoj vnetja izključen (Hujoel & Lingström, 2017). Zanimivo raziskavo so izvedli Baumgartner in sodelavci, v kateri je bilo 10 udeležencev 4 tedne na prehrani t.i. kamene dobe, ki izključuje rafinirane izdelke, predvsem ogljikove hidrate, ob tem pa preiskovanci niso vzdrževali ustne higiene. Pokazalo se je, da je kljub povečanim ravnem zobnih oblog prišlo do zmanjšanja krvavenja ob sondiranju (angl. Bleeding On Probing, BOP) in globine sondiranja (angl. Probing Depth, PD), ob pojavu nekaj novih bakterijskih vrst subgingivalno, ki niso povezane z nastankom parodontitisa (Hujoel & Lingström, 2017).

Med ogljikovimi hidrati je treba omeniti tudi kompleksne ogljikove hidrate, kot so polnozrnata žita, sadje, zelenjava in stročnice, ki ugodno vplivajo na zdravje dlesni zaradi visoke vsebnosti vlaknin in nizke vsebnosti preprostih sladkorjev. Povečan vnos hrane, bogate z vlakninami, zmanjšuje tveganje za razvoj parodontitisa, kar se razlaga s pozitivnim vplivom takšne hrane na glikemični indeks. Vlaknine namreč stabilizirajo raven sladkorja v krvi, kar pomaga zmanjševati vnetja v telesu, vključno z vnetji v ustni votlini (Martinon idr., 2021). Žvečenje živil z visoko vsebnostjo vlaknin pomaga tudi pri mehanskem odstranjevanju zobnih oblog. Sveža in minimalno predelana živila ohranijo več hranil, ki so koristna za dlesni, medtem ko predelani ogljikovi hidrati, kot so beli kruh, testenine in rafinirana žita, pogosto vsebujejo dodane sladkorje, ki povečujejo tveganje za gingivitis. Več preprostih sladkorjev pomeni več hranil za škodljive bakterije, ki povzročajo karies in vnetje dlesni.

Proteini

Današnji življenjski slog vključuje povečan prehranski vnos mesa in mesnih izdelkov. Vloga proteinov pri začetku sistemskega vnetja in nastanku parodontitisa ni povsem jasna. Obstajajo domneve, da so za to odgovorni proteini živalskega izvora, medtem ko imajo proteini rastlinskega izvora nasproten učinek (Woelber & Tennert, 2020).

Staufenbiel in sodelavci so primerjali skupino 100 vegetarijancev z enakim številom udeležencev kontrolne skupine. Skupina vegetarijancev je imela nižje vrednosti PD in BOP, možni razlogi za to pa so poleg vegetarijanske prehrane tudi višja raven izobrazbe, bolj zdrave življenjske navade, boljša ustna higiena in rednejši zobozdravstveni pregledi (Woelber & Tennert, 2020). Po drugi strani pomanjkanje proteinov lahko resno vpliva na parodontalno zdravje in otežuje hitro izmenjavo celic gingivalnega epitela. Pri še večjih pomanjkanjih lahko pride do nastanka kwashiorkorja, sistemske bolezni, pri kateri sta prisotna izguba zob in parodontalne lezije (Hujoel & Lingström, 2017).

Maščobe

Nasičene maščobne kisline, trans-maščobne kisline in omega-6 maščobne kisline delujejo kot promotorji vnetja. Iwasaki in sodelavci so izvedli raziskavo z 264 Japonci in ugotovili, da je bil pri nekadilcih, ki so uživali večje količine teh maščobnih kislin, število mest s klinično izgubo parodontalnega pripoja (angl. clinical attachment loss, CAL) od 3 mm in več bistveno višje (Iwasaki idr., 2011).

Po drugi strani pa omega-3 maščobne kisline zmanjšujejo produkcijo vnetnih mediatorjev, kar lahko pomaga pri zmanjšanju simptomov parodontalne bolezni. Uživanje dodatkov omega-3 maščobnih kislin v kombinaciji s standardnimi parodontalnimi terapijami je pokazalo obetavne rezultate pri izboljšanju izidov parodontalnega zdravja (Van Ravensteijn idr., 2022). Pri zmanjševanju sistemskega in parodontalnega vnetja lahko pripomorejo tudi metaboliti omega-3 maščobnih kislin. To sta npr. eikozapentaenska (EPA) in dokozaheksaenska kislina (DHA), ki ju vnašamo s hrano, vendar lahko delno nastaneta tudi v telesu iz omega-3 maščobnih kislin. Tej pretvorbi pomaga hkrati zmanjšan vnos omega-6 maščobnih kislin. V raziskavah so potrdili pozitivne učinke uživanja dodatkov EPA in DHA na zmanjšanje znakov parodontalne bolezni in obnovo parodontalnega pripoja (Kruse idr., 2020). V 6-mesečni raziskavi so El-Sharkawy in sodelavci spremljali tudi koncentracijo ustnih matriksnih metaloproteinaz in RANKL (angl. Receptor Activator of Nuclear Factor κB Ligand), ki sodelujejo pri destrukciji parodontalnih tkiv, in ugotovili, da je prišlo do njihovega znatnega zmanjšanja. Ti podatki govorijo v prid uporabi dodatkov EPA in DHA v podporni terapiji parodontitisa (Kruse idr., 2020).

Antioksidanti

Antioksidanti so molekule, ki ščitijo celice pred oksidativnim stresom, ki je povezan z vnetjem in celičnimi poškodbami. Prisotni so v številnih hranilih in vključujejo vitamine (npr. vitamin C, vitamin E), minerale (npr. selen, cink) in rastlinske spojine (npr. karotenoidi, polifenoli). Najdbe podpirajo, da prehranski vnos antioksidantov pomaga pri izboljšanju zdravja dlesni, morda delno z izboljšanjem delovanja mitohondrijev (Cao idr., 2024). Antioksidanti ne le zmanjšujejo oksidativni stres v ustni votlini, ampak tudi pomagajo pri obnavljanju poškodovanih tkiv dlesni.

Likopen je močan antioksidant iz skupine karotenoidov, ki daje rdečo barvo nekateri zelenjavi in sadju. Chandra in sodelavci so izvedli raziskavo s 50 kadilci in 50 nekadilci. Vsem preiskovancem je bilo opravljeno subgingivalno odstranjevanje trdih in mehkih zobnih oblog. Obe skupini sta bili razdeljeni na kontrolno in poskusno skupino, ki je bila podporno lokalno obravnavana z 2 % likopen gelom. V poskusni skupini je prišlo do znatnega povečanja kliničnega pripoja. Likopen se je izkazal kot koristen dodatek prehrani pri preprečevanju in terapiji parodontitisa (Chandra idr., 2012).

Vitamin C je izjemno pomembno mikrohranilo za ohranjanje parodontalnega zdravja, še posebej pri osebah, ki kadijo (Dommisch idr., 2018). Chapple in sodelavci so pokazali, da je bila prevalenca hudega parodontitisa znatno višja pri osebah s serumskimi ravnmi vitamina C pod 8,52 mmol/L v primerjavi z osebami z višjimi koncentracijami vitamina C in da je 6-tedenska uporaba ustnih vod z vitaminom C pri osebah z gingivitisom privedla do znatnega zmanjšanja BOP (Chapple idr., 2007). Shimabukuro in sodelavci so izvedli randomizirano raziskavo, ki je pokazala, da zobna pasta z vitaminom C in magnezijem vodi do znatnega zmanjšanja gingivitisa (Shimabukuro idr., 2015), možen vzrok je zmanjšanje vnetja gingivalnih fibroblastov, ki ga povzročajo reaktivne kisikove vrste. Vitamin C je še posebej pomemben za zdravje dlesni, saj spodbuja tudi produkcijo kolagena, ki je bistven za obnovo tkiv. Pomanjkanje vitamina C vodi do nastanka skorbuta, bolezni, za katero so značilne nehotene podkožne krvavitve in krvavitve iz dlesni, majavost in izguba zob. Vitamin C lahko enostavno zaužijemo skozi različna živila. Staudte in sodelavci so ugotovili, da je uživanje grenivke, ki je bogata z vitaminom C, izboljšalo BOP pri bolnikih s kroničnim parodontitisom (Staudte idr., 2005).

Obstajajo šibki dokazi o vplivu vitamina E na parodontitis, vendar nekaj raziskav nakazuje na koristnost suplementacije z vitaminom E v okviru začetne terapije (Dommisch idr., 2018).

Čeprav je vitamin A poznan po svoji vlogi antioksidanta, se zdi, da nima izrazite vloge pri povečanju tveganja za obolevnost s parodontitisom (Dommisch idr., 2018). Prav tako se zdi, da nadomeščanje vitamina A ne pomaga pri terapiji parodontitisa.

Ustna voda iz rastline manuke (*Leptospermum scoparium*) , ki vsebuje sestavine, ki so bogat vir vitamina C in drugih antioksidantov (npr. lutein, alfa-linolenska kislina, omega-3 maščobne kisline), je enako učinkovita kot ustna voda z klorheksidinom (CHX) pri zmanjšanju kliničnih znakov parodontalne bolezni. CHX je znan po svojih izrazitih antimikrobnih lastnostih, ki učinkovito zmanjšujejo število bakterij v ustih, še posebej v parodontalnih žepkih, kjer se bakterije kopičijo in lahko povzročijo vnetje dlesni. Manuka ustna voda je zanesljiva alternativa ustni vodi, ki vsebuje CHX, hkrati pa ima manj neželenih učinkov povezanih z dolgotrajno uporabo CHX (Abullais idr., 2022).

Druga mikrohranila

Raziskave o pomembnosti magnezija, železa, cinka, kalija, kalcija, bakra, mangana in selena za zdravje obzobnih tkiv kažejo različne rezultate (Dommisch idr., 2018). Ta mikrohranila imajo pomembno vlogo v različnih kemijskih procesih v telesu in s tem vzdržujejo homeostazo. Čeprav je iz tega enostavno sklepati, da vplivajo tudi na zdravje parodontalnih tkiv, so potrebne še dobro zasnovane klinične študije, da bi jasno opredelili njihov pomen.

Vitamin D pomaga pri absorpciji kalcija, ki je ključen za močne in zdrave kosti, vključno z zobmi in podpornimi strukturami. Raziskave so pokazale, da pomanjkanje vitamina D lahko prispeva k večjemu tveganju za parodontalno bolezen. Petletna raziskava na 1904 udeležencih je pokazala, da z vsakim zvišanjem serumske koncentracije 25-hidroksi vitamina D za 10 mikroL/L tveganje za izgubo zob zaradi parodontitisa pade za 13 % (Dommisch idr., 2018). Metaanaliza, ki so jo izvedli Shah M in sodelavci je pokazala linearno povezavo med vitaminom D in parodontalnim zdravjem. Vitamin D poleg vpliva na presnovo kosti, deluje tudi kot protivnetno sredstvo in omogoča proizvodnjo protimikrobnih peptidov, ki pomagajo ohranjati ustno zdravje (Shah idr., 2023).

Pomanjkanje vitaminov B kompleksa vodi do zmanjšane odpornosti proti bakterijskim okužbam (Dommisch

idr., 2018). Zong in sodelavci so ugotovili, da imajo osebe z nižjimi serumski koncentracijami vitamina B12 večje tveganje za nastanek parodontitisa, kar potrjuje potrebo po njegovem nadomeščanju pri veganih (Zong idr., 2016). Sistematično jemanje folne kisline (vitamina B9) se je izkazalo za koristno za nosečnice pri nadzoru gingivitisa, podoben učinek pa je imela tudi njegova lokalna uporaba v ustnih vodah (Pack & Thomson, 1980). Kljub obetavnim rezultatom je treba potencial vitaminov B kompleksa še dodatno raziskati.

Nove raziskave kažejo, da bi prehranski dodatki, zlasti multi-nutrienti, lahko služili kot dopolnilna terapija za izboljšanje rezultatov zdravljenja parodontalne bolezni. Pacienti, vključeni v študijo (McSorley, 2024) , so bili naključno razporejeni, da prejmejo komercialno dostopno multi-hranilno prehransko dopolnilo (ki vsebuje: vitamin C, vitamin E, cink, selen, alfa-lipojsko kislino, izvleček brusnic, izvleček grozdnih pečk in koencim Q10) ali placebo, ki so ga jemali 2 meseca, sočasno s terapijo higienske faze brez kirurškega posega. Avtorji so zaključili, da je dodatek večhranilnega prehranskega dopolnila k ne-kirurški parodontalni terapiji za bolnike, ki se zdravijo zaradi parodontalne bolezni III. in IV. stopnje (tabela 1), povzročil večje zmanjšanje globine sondiranja (PD) in krvavenja ob sondiranju (BOP) v primerjavi s skupino, ki je jemala placebo ob nekirurški parodontalni terapiji.

Probiotiki

Probiotiki so živi mikroorganizmi, ki imajo koristne učinke na zdravje gostitelja. Probiotiki delujejo na več načinov, da bi izboljšali zdravje dlesni in pomagali pri zdravljenju parodontalne bolezni (slika 2).

Probiotiki tekmujejo s patogenimi bakterijami za prostor in hranila v ustni votlini, s čimer zmanjšujejo možnosti za kolonizacijo škodljivih bakterij, ki so povezane s parodontalno boleznijo. Spodbujajo tudi rast koristnih bakterij, kar pomaga ohranjati zdravo ravnovesje ustnega mikrobioma. Nekateri probiotiki proizvajajo antimikrobne snovi, kot so bakteriocini in organske kisline, ki lahko zavirajo rast patogenih bakterij, kot so *Porphyromonas gingivalis*, *Treponema denticola* in *Tannerella forsythia*, ki so glavni povzročitelji parodontalne bolezni. Poleg tega probiotiki lahko pomagajo pri krepitvi epitelijske pregrade v dlesni, kar zmanjšuje prodiranje patogenih bakterij in toksinov v globlja tkiva, ter zmanjšujejo aktivnost škodljivih encimov, kot so proteaze, ki jih proizvajajo patogeni. Probiotiki modulirajo imunski odziv, tako da spodbujajo dendritične celice k diferenciaciji T celic v T regulatorne celice, katere nato pomagajo nadzorovati in zmanjševati vnetje. Probiotiki spodbujajo tudi proliferacijo fibroblastov, ki pospešujejo celjenje tkiv. Vsi ti mehanizmi pomagajo pri zmanjševanju vnetja in poškodbe tkiv, ki so značilne za parodontalno bolezen (Roy idr., 2024).

Vključitev probiotikov v režim ustne higiene in prehrane predstavlja obetaven terapevtski pristop k celostnemu zdravljenju parodontalne bolezni (Shirbhate idr., 2023).

Pri parodontalni terapiji se uporabljajo probiotični sevi, kot so *Lactobacillus reuteri*, *Lactobacillus rhamnosus*, *Bifidobacterium* spp. in *Streptococcus salivarius*, ki dokazano zmanjšujejo vnetje, nabiranje zobnih oblog in škodljivih bakterij. Ti probiotiki se uživajo preko oralnih dodatkov, pastil, žvečilnih gumijev in topikalnih aplikacij, kot so ustne vodice, geli in spreji. Učinkoviti odmerki običajno segajo od 106 do 109 CFU (angl. colony forming units) na dan, kratkotrajna in dolgoročna uporaba pa prinašata pomembne koristi za parodontalno zdravje. Probiotike je mogoče sinergistično kombinirati z običajnimi terapijami, kot so luščenje in glajenje korenin in antibiotiki, da se pospeši celjenje in obnovi mikrobno ravnovesje, kar jih naredi dragocen dodatek k celoviti parodontalni oskrbi.

Tabela 1. Stopnje parodontalne bolezni

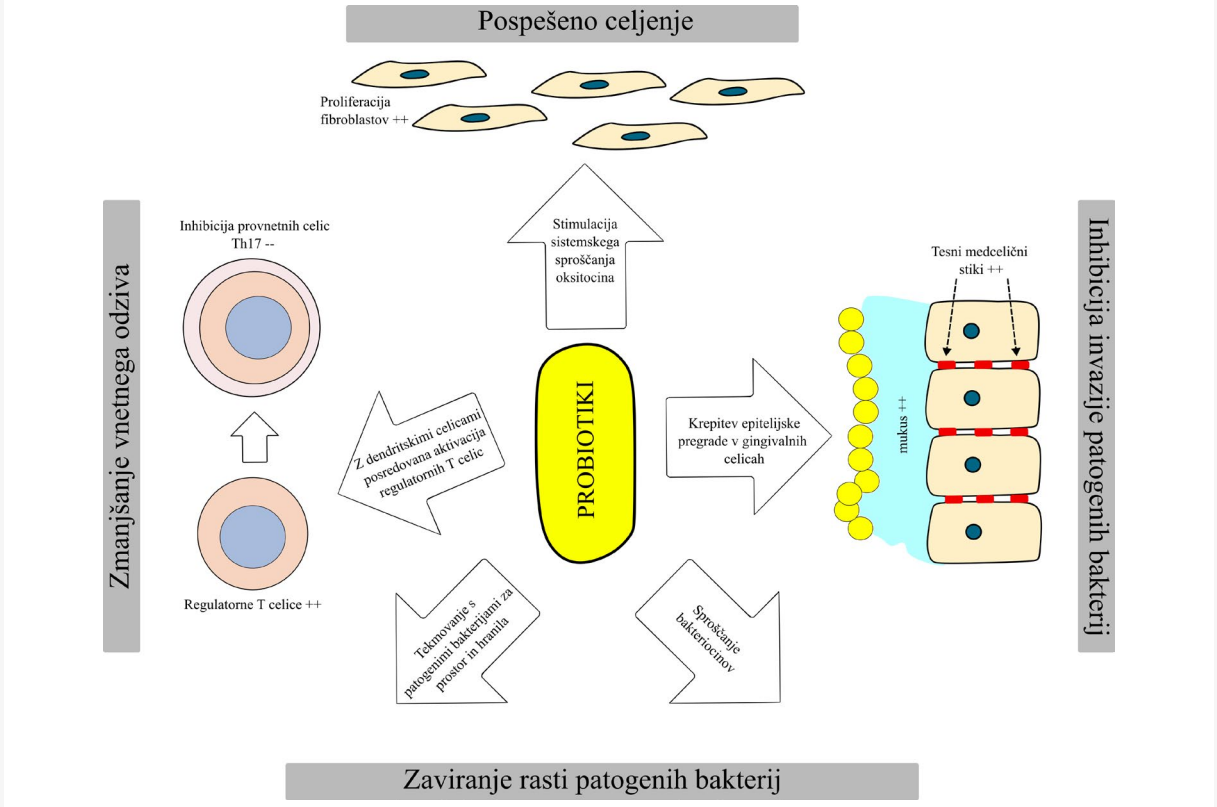
Table 1. Stages of periodontal disease

Stopnja	Značilnosti
I (blaga)	Manjša izguba alveolarne kosti (manj kot 15 %), z manjšo izgubo kliničnega pripoja. Globina žepov ≤ 4 mm. Ni izgube zob.
II (zmerna)	Zmerna izguba alveolarne kosti (do srednje tretjine korenine), z zmerno izgubo kliničnega pripoja. Globina žepov ≤ 5 mm. Ni izgube zob.
III (huda)	Huda izguba alveolarne kosti (do srednje tretjine korenine in naprej), z obsežno izgubo kliničnega pripoja. Globina žepov ≥ 6 mm, izguba zob zaradi parodontitisa je možna (≤ 4 zob).
IV (napredovala)	Zelo huda izguba kosti (do srednje tretjine korenine in naprej), s potrebo po kompleksnem zdravljenju in podpornih terapijah. Globina žepov ≥ 6 mm, izguba zob zaradi parodontitisa (≥ 5 zob), znatna majavost zob.

Shimazaki in sodelavci so v svoji raziskavi (Shimazaki idr., 2008), ki je vključevala vprašalnik o uživanju mlečnih izdelkov, odkrili pozitiven učinek fermentiranih mlečnih izdelkov, kot je jogurt, na zdravje parodontalnih tkiv.

Eden najbolj raziskanih probiotičnih sevov za parodontalno zdravje je *Lactobacillus reuteri*. V randomiziranem poskusu je bila ocenjena učinkovitost probiotičnih pastil, ki vsebujejo Lactobacillus reuteri, pri zdravljenju kroničnega parodontitisa. Bolniki, ki so prejeli luščenje in glajenje korenin skupaj s probiotičnimi pastili, so pokazali pomembne izboljšave kliničnih parametrov, kot so globina sondiranja (PD) in klinična izguba parodontalnega pripoja (CAL) v primerjavi s kontrolno skupino, ki je prejela luščenje in glajenje korenin s placebom. Poleg tega je skupina, ki je jemala probiotike imela nižje ravni vnetnih citokinov (IL-1β, TNF-α) v gingivalni sulkusni tekočini in zmanjšano prisotnost parodontopatogenov. Študija je sklenila, da so probiotične pastile učinkovito dopolnilo konvencionalni parodontalni terapiji, saj izboljšujejo klinične izide in zmanjšujejo vnetje pri bolnikih s kroničnim parodontitisom (Alshareef idr., 2020; Teughels idr., 2013).

Raziskave na temo kimčija, tradicionalne fermentirane zelenjavne jedi, kažejo, da lahko njegova vključitev v prehrano ponudi potencialne koristi za zdravje parodontalnih tkiv. *Lactobacillus curvatus*, probiotični sev mlečnokislinskih bakterij, ki ga pogosto najdemo v kimčiju, z zmanjševanjem proizvodnje provnetnih citokinov in modulacijo imunskega odziva v parodontalnih tkivih deluje protivnetno. *Lactobacillus curvatus* deluje tudi protimikrobno proti parodontopatogenom, kot je *Por-*



Slika 2. Shematski prikaz mehanizmov delovanja probiotikov v gostitelju na lokalni in sistemski ravni.

Figure 2. Schematic representation of mechanisms used by probiotics to interfere with their host locally and on a systemic level.

phyromonas gingivalis. Poleg tega študije nakazujejo, da lahko ta probiotik spodbuja regeneracijo tkiv in modulira imunski odziv gostitelja, kar dodatno prispeva k zdravju parodontalnih tkiv (Choi idr., 2021).

Kombuča, fermentirana čajna pijača, postaja vse bolj priljubljena tudi na naših trgih. Podobno kot jogurt in kimči, kombuča vsebuje več vrst probiotikov, najpogostejši so bakterije iz rodov *Lactobacillus* in *Bifidobacterium*, ter kvasovke *Saccharomyces*. Te koristne bakterije pomagajo vzdrževati uravnotežen ustni mikrobiom, kar lahko zmanjša rast škodljivih bakterij, ki povzročajo vnetje. Kljub temu je pomembno omeniti, da je kombuča kisla, kar lahko potencialno poškoduje zobno sklenino. Zato je priporočljivo uživati kombučo v zmernih količinah in po pitju sprati usta z vodo (Selvaraj & Gurumurthy, 2022).

Razprava

Sodobne raziskave vedno bolj poudarjajo pomen uravnotežene prehrane in ustreznega vnosa specifičnih hranil za preprečevanje in obvladovanje parodontalne bolezni. Omega-3 maščobne kisline, vlaknine, antioksidanti, vitamin D, vitamin C, kalcij, polifenoli in probiotiki so hranila, ki so pokazala obetavne rezultate pri zmanjševanju vnetja, uravnoteženju ustnega mikrobioma in krepitvi imunske odpornosti. Vendar pa lahko različni načini prehranjevanja vplivajo na razpoložljivost teh hranil.

Vegani lahko naletijo na izzive pri ohranjanju zdravja dlesni, predvsem zaradi možnosti pomanjkanja določenih hranil, ki so ključna za zdravje ustne votline. Ker vegani ne uživajo mlečnih izdelkov, ki so pogosto obogateni z vitaminom D, je pomembno, da se redno izpostavljajo sončni svetlobi in jemljejo dodatke vitamina D, saj je ta ključen za absorpcijo kalcija in zdravje kosti. Vegani morajo poskrbeti tudi za zadosten vnos kalcija skozi temno zeleno listnato zelenjavo, sezamova semena, tofu in obogatene rastlinske napitke, saj je kalcij pomemben za močne zobe in kosti.

Probiotiki, ki jih najdemo v fermentiranih živilih, kot so jogurt in kefir, lahko pomagajo uravnotežiti ustni mikrobiom in zmanjšati tveganje za razvoj in napredovanje parodontalne bolezni. Vključitev probiotičnih dopolnil v režim ustne higiene in prehrane je obetaven pristop k celovitemu zdravljenju parodontalne bolezni (Guo idr., 2023). Ker so jogurti in kefir živalskega izvora, lahko vegani dobijo probiotike iz fermentiranih rastlinskih živil, kot so kislo zelje, tempeh, kimči in kombuča.

Koencim Q10 je antioksidant, ki ščiti celice pred oksidativnim stresom in pomaga pri obnavljanju tkiv. Najdemo ga v mesu in ribah, zato vegani težje zagotovijo zadostne količine koencima Q10, razen če uživajo dodatke ali živila, kot so špinača in brokoli. Omega-3 maščobne kisline imajo protivnetne lastnosti in so pomembne za zdravje dlesni. Glavni vir teh maščobnih kislin so mastne ribe, zato morajo vegani iskati alternative, kot so lanena semena, chia semena in orehi.

V zahodnem svetu se zaradi neuravnotežene in visoko predelane prehrane še vedno pojavljajo bolezni zaradi pomanjkanja določenih hranil. Socioekonomski dejavniki, kot so nizki dohodki, lahko omejijo dostop do svežega sadja in zelenjave, kar povečuje tveganje za pomanjkanje vitamina C. Vitamin C je ključnega pomena za proizvodnjo kolagena in zdravje dlesni. Pomanjkanje tega vitamina lahko povzroči skorbut, stanje, ki se redko pojavlja v sodobnem svetu, vendar se še vedno pojavlja v nekaterih zahodnih državah. Starejši ljudje so bolj dovzetni za pomanjkanje hranil zaradi slabšega apetita, omejenih finančnih sredstev in težav z zobmi ter prebavili. Ključno je ozaveščanje javnosti o pomenu uravnotežene prehrane z zadostno količino vitamina C za preprečevanje te bolezni.

Po drugi strani zahodni način prehranjevanja, ki vključuje visok vnos enostavnih ogljikovih hidratov, škroba in nasičenih maščobnih kislin, negativno vpliva na parodontalno zdravje, saj spodbuja vzpostavitev mikrookolja s kislim pH-jem in s tem rast patogenih bakterij. Prehrana, bogata z ogljikovimi hidrati, povzroča tudi sistemsko vnetje, ki poleg bolezni, kot so diabetes mellitus, koronarne srčne bolezni in gastrointestinalne bolezni, povečuje tveganje za nastanek parodontitisa (Woelber idr., 2016). Za optimalno zdravje dlesni je ključnega pomena uravnotežena prehrana, ki vključuje vnos zadostne količine osnovnih hranil, sadja in zelenjave ter izogibanje preprostim sladkorjem in predelanim živilom. Tako lahko podpremo zdravje ustnega mikrobioma, zmanjšamo vnetje in s tem tveganje za parodontalno bolezen.

Zaključek

Parodontalna bolezen je kompleksna in multifaktorska bolezen, ki zahteva celovit pristop k njenemu zdravljenju in preprečevanju. Ustrezna prehrana, bogata s hranili, ki podpirajo zdravje dlesni, lahko skupaj z dobro ustno higieno in rednimi obiski zobozdravnika znatno izboljša stanje

dlesni in prepreči napredovanje parodontalne bolezni. Nadaljnje študije so potrebne za natančnejše razumevanje specifičnih mehanizmov, preko katerih hranila vplivajo na mikrobiom in zdravje dlesni, kar bo omogočilo razvoj še učinkovitejših terapevtskih in preventivnih strategij za izboljšanje ustnega zdravja.

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Data Availability

No new data were created.

Conflicts of Interest

The author declares no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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ABS

Review

Unique Characteristics of Adipocytes in Metabolic Health: Insights and Implications

Jeetendra Kumar Gupta [1], Yati Sharma [1], Nitin Wahi [2], Krishan Kumar [3*]

Abstract

Adipocytes secrete a wide range of bioactive peptides known as adipokines. These factors play a crucial role in the complex network of metabolic control that connects adipose tissue activity with systemic metabolic balance. The intricacy of adipokine signalling networks and their relationships with other bodily metabolic regulators is also highlighted in this study. Although adipokines are necessary for healthy metabolism, the pathophysiology of various metabolic illnesses, such as obesity, type 2 diabetes, and cardiovascular diseases, are associated with their dysregulation. We explored the effects of changes in adipokine function and levels that affect various disorders, providing a detailed picture of the pathophysiology. This review also delves into the potential of adipokine as a metabolic illness biomarker, including its prognostic and diagnostic potential. Moreover, it assesses the potential therapeutic benefits of regulating adipokine activity and explores present and upcoming approaches to target these molecules to enhance metabolic health. Overall, This study emphasizes the many functions of adipokines in several metabolic processes, such as insulin sensitivity, lipid metabolism, glucose regulation, and inflammation. Additionally, it offers a comprehensive and insightful understanding of adipokines, emphasizing their pivotal role in metabolic well-being and disease.

Keywords

Adipokines, Adiponectin, Insulin resistance, Leptin, Obesity

1 Institute of Pharmaceutical Research, GLA University, Mathura, Uttar Pradesh, India

2 Department of Bioinformatics, Pathfinder Research Training Foundation, Gr. Noida, India

3 Department of Chemistry, Indian Institute of Technology Delhi, Hauz Khas, New Delhi, India

*** Corresponding author:**
E-mail address: kjakhad21@gmail.com

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Edinstvene značilnosti adipocitov v presnovnem zdravju: vpogledi in posledice

Izvleček

Adipociti izločajo široko paleto bioaktivnih peptidov, znanih kot adipokini. Ti igrajo ključno vlogo v kompleksni mreži presnovnega nadzora, ki povezuje aktivnost maščobnega tkiva s sistemskim presnovnim ravnovesjem. V študiji je poudarjena zapletenost adipokinskih signalnih omrežij in njihovih odnosov z drugimi telesnimi presnovnimi regulatorji. Čeprav so adipokini potrebni za zdravo presnovo, je patofiziologija različnih presnovnih bolezni, kot so debelost, sladkorna bolezen tipa 2 in kardiovaskularne bolezni, povezana s slabšo regulacijo aktivnosti adipokinov. Raziskali smo, kako spremembe v funkcijah in ravni adipokinov vplivajo na različne motnje, s čimer smo ponudili podrobno sliko patofiziologije. Pregled se poglobi tudi v potencial adipokinov kot biomarkerjev presnovnih bolezni, vključno z njihovim prognostičnim in diagnostičnim potencialom. Pregled literature nam poleg tega omogoča oceniti potencialne terapevtske koristi uravnavanja aktivnosti adipokinov in raziskuje sedanje in prihajajoče pristope za izboljšanje presnovnega zdravja, ki temeljijo na uravnavanju nivojev teh molekul. Ta pregled poudarja številne funkcije adipokinov v več presnovnih procesih, kot so občutljivost na insulin, presnova lipidov, regulacija glukoze in vnetje. Poleg tega ponuja celovito in pronicljivo razumevanje adipokinov, s poudarkom na njihovi ključni vlogi pri presnovi in boleznih.

Ključne besede

adipokini, adiponektin, inzulinska rezistenca, leptin, debelost

Introduction

Adipose tissue is a morphologically dynamic tissue essential for maintaining health and homeostasis. Adipocytes are cells found in tissue and play a crucial role in storing and managing energy in the form of fat. These cells are essential for maintaining energy balance, regulating body temperature, and safeguarding organs (Figure 1). Adipose tissue consists of a combination of adipocytes, blood vessels, and other cell types. Adipocytes store energy as triglycerides and release them when the body requires it. Additionally, they produce hormones (Machado et al., 2022). Signaling molecules called adipokine affect metabolism, appetite, and inflammation (Figure 2a). There are multiple factors, e.g., overnutrition, metabolic syndrome, and genetic factors that may lead to adipose tissue accumulation. Disruption of the function of adipocytes can contribute to obesity and related metabolic disorders (Kirichenko et al., 2022). According to Wang et al. (2014), There are different types of adipocytes. Each has specific functions related to energy storage and expenditure. Gaining an understanding of adipocyte biology is essential for addressing issues related to obesity and metabolic health problems, as well as identifying potential therapeutic targets for intervention (Wang et al., 2014).

The most common white adipocytes store most of our energy and supply it on demand, especially when required. On the other hand, brown adipocytes specialize in generating heat through burning fat, a process called thermogenesis. These versatile cells help regulate energy expenditure and can play a role in fighting obesity. Together, these various types of adipocytes work to balance the body's energy levels carefully. Adipocytes synthesize and release a range of biologically active molecules called adipokine (Ladoux et al., 2021).

As mentioned earlier, the adipocytes of the body release several signalling molecules collectively called adipokine, including leptin, adiponectin, resistin, angiotensinogen, and cytokines (IL-6, TNF-α). Imbalance may lead to inflammation, insulin resistance, and atherosclerosis (Figure 2a and Figure 4).

Leptin is an adipokine that controls hunger and energy expenditure in the body. It is frequently called the satiety hormone because of its vital function in alerting the brain when the body has sufficient fat and energy reserves (Clemente-Suárez et al., 2023). Another significant adipokine with anti-inflammatory and insulin-sensitizing properties is adiponectin. It is usually regarded as advantageous for metabolic health and aids in controlling glucose metabolism

(Choi et al., 2020). Resistin is another adipokine. Although its precise function in humans is still debated, resistin was previously believed to be linked to insulin resistance. It might have a pro-inflammatory effect (Jamaluddin et al., 2012). Angiotensinogen is produced by adipose tissue but is not generally categorized as an adipokine. It is a precursor of the hormone angiotensin, which controls fluid and blood pressure (Than et al. 2017). Adipose tissue can also secrete several pro-inflammatory cytokines, including interleukin-6 (IL-6) and Tumor necrosis factor-alpha (TNF-α) (Al-Mansoori et al., 2022). These cytokines may be involved in low grade inflammation associated with metabolic diseases and obesity (Figure 2b). As this field of study develops, novel adipokine and their roles are also being identified (Ellulu et al., 2017).

Role of Adipocytes in Maintaining Energy Balance and Glucose Homeostasis

Understanding obesity and metabolic disorders like type 2 diabetes requires the recognition of adipocytes (fat cells) and their critical role in preserving energy and controlling glucose homeostasis (de Oliveira et al., 2016). In addition to being metabolic stores, these cells also play a role in immune responses, blood pressure regulation, homeostasis, bone mass, thyroid, and reproductive functions. Peptide hormone synthesis and release play a significant role in the regulation of their regulatory functions (Wang et al., 2022).

When glucose levels are low, adipocytes release fatty acids into the bloodstream, which most organs use as fuel. These fats can be stored more efficiently and have a higher energy density than carbohydrates. Most of the body's energy reserves are found in adipose tissue for energy balance. Obesity is not directly correlated with the number of fat cells; instead, the size of fat cells and their functions in energy control are essential factors. By controlling food intake and energy expenditure through both endocrine and non-endocrine systems, adipose tissue is crucial in integrating the body's energy demands (Sears et al., 2015).

Leptin, the first adipokine to be identified, is essential for controlling obesity. Leptin is a hormone that is almost entirely secreted by adipocytes. It suppresses appetite and increases energy expenditure by storing triglycerides and free cholesterol (Obradovic et al., 2021). The efficacy of leptin is demonstrated by the obesity observed in humans and animals with mutations in leptin or its receptor. In partic-

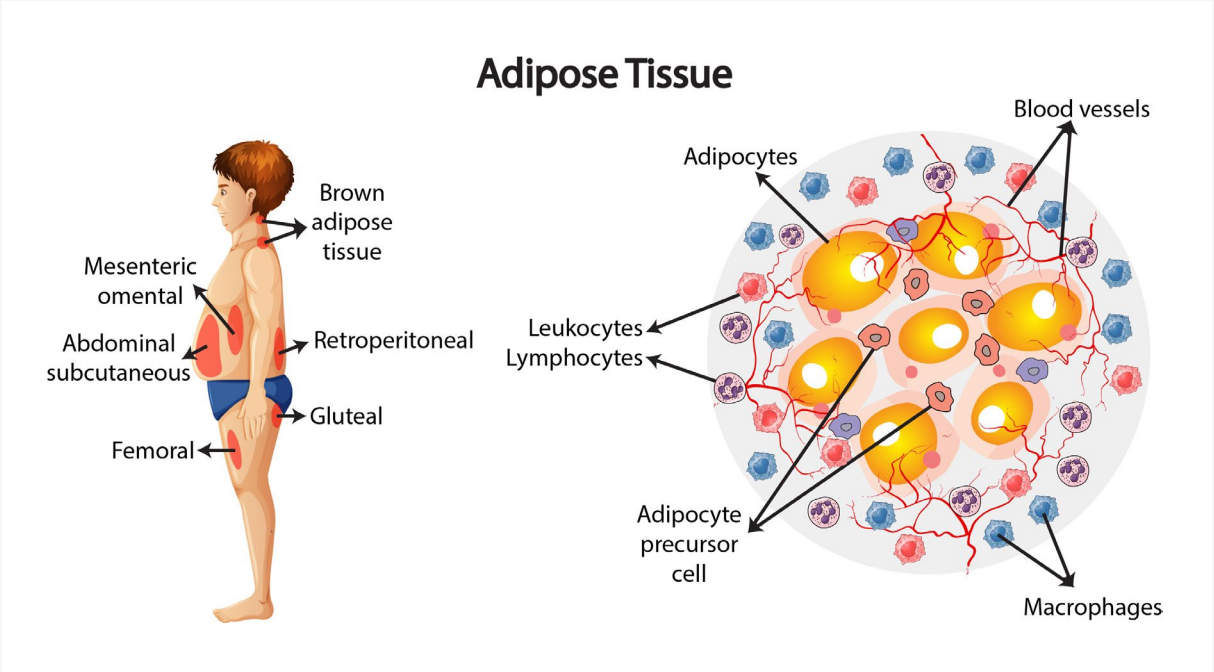

Figure 1. Distribution of adipose tissue in the human body and their microenvironments.

Slika 1. Razporeditev maščobnih tkiv v človeškem telesu in njihova mikrookolja.

ular areas of the hypothalamus, the central nervous system is the primary mechanism by which leptin affects hunger and energy expenditure (Figure 3). Adipose tissue has a more subtle but significant impact on glucose metabolism. Although muscle absorbs most of the glucose after meals, it only makes up around 10-15%; changes in adiposity significantly impact glucose regulation (Fazakerley et al., 2019). Excess or insufficient fat content is linked to hyperglycemia and insulin resistance. Adipocytes influence glucose balance through many endocrine and non-endocrine mechanisms (Wondmkun, 2020). Adipocyte-secreted non-esterified fatty acids (NEFAs) affect glucose homeostasis in several ways. They increase hepatic glucose production while decreasing adipocyte and muscle glucose uptake. As a nutritional supply during fasting, NEFAs direct the energy expenditure toward burning fat instead of glucose, which is necessary for neurons and red blood cells. Insulin influences NEFA levels, and increases in NEFA levels can aggravate insulin resistance, generating a vicious cycle. Long-term exposure to NEFAs can also affect pancreatic β-cell glucose sensing and insulin secretion (Wilcox, 2005). Pathologically, adipokine imbalances are associated with obesity and metabolic disorders, contributing to insulin resistance, chronic inflammation, and increased cardiovascular risk. This dysregulation can also lead to obesity-related health complications (Figure 2a).

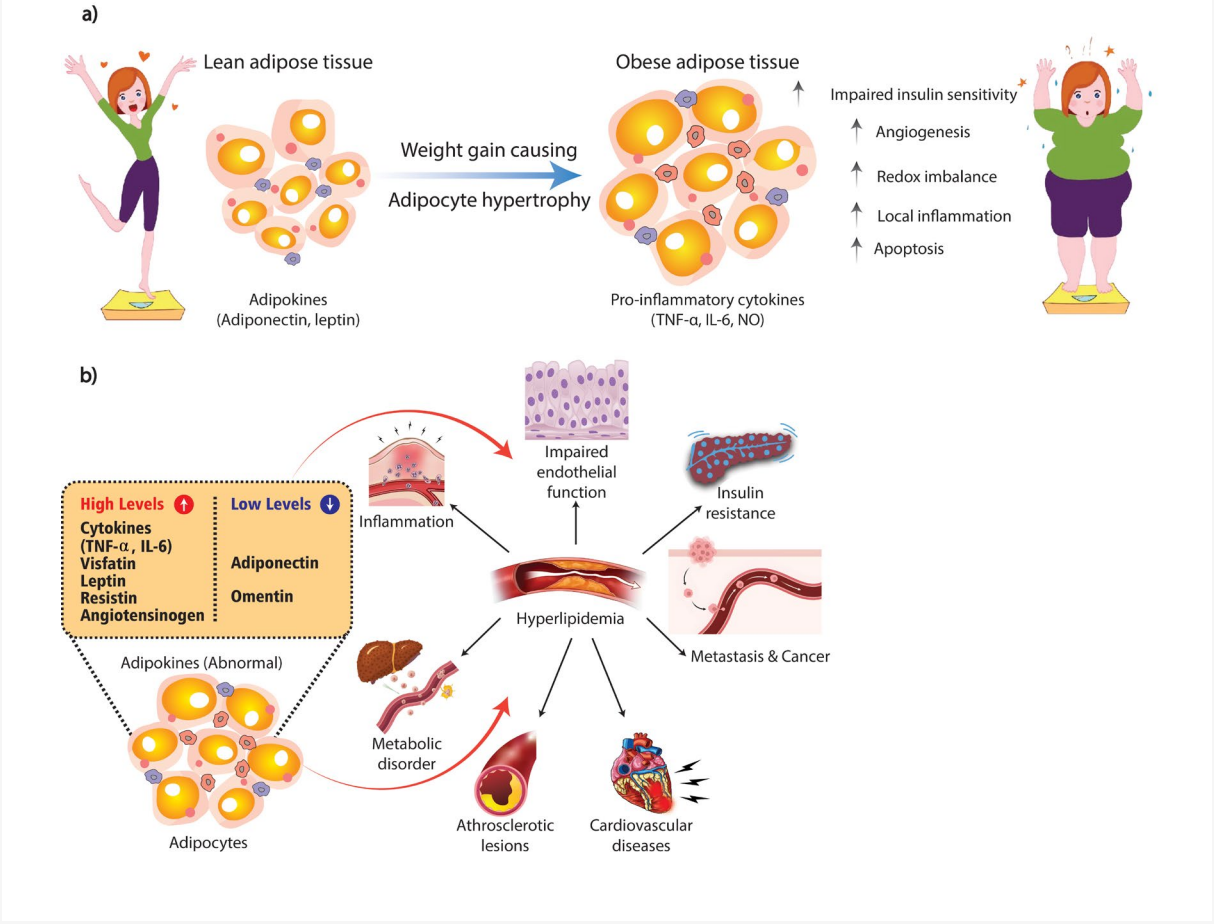

Figure 2. Consequences of impaired adipokine a) On weight gain, there is a complete shift in signalling molecules; obese adipose tissues are shown to secrete pro-inflammatory cytokines. b) These pro-inflammatory cytokines show different spectra of events in various body tissues and functions.

Slika 2. Posledice oslabljene funkcije adipokinov a) Pri povečanju telesne teže pride do popolnega premika signalnih molekul; debela maščobna tkiva dokazano izločajo pro-vnetne citokine. b) Ti vnetni citokini kažejo različne spektre dogajanja na različna telesna tkiva in funkcije.

Leptin metabolic functions, obesity, and resistance

Often referred to as the satiety hormone, leptin is an essential hormone that controls body weight via energy balance. Since its discovery in 1994, leptin has emerged as a critical player in our understanding of the complex processes underlying obesity and the metabolic processes of the body (Picó et al., 2022). Adipose tissue is the primary source of leptin production and secretion. The body's fat content is directly correlated with its bloodstream levels. The primary function of leptin is to send messages to the brain, especially the hypothalamus, which controls hunger and energy expenditure. Leptin levels rise in situations where fat stores are plentiful. This treatment suppresses appetite and increases energy expenditure, which helps maintain body weight within a healthy range. On the other hand, decreased fat stores cause a drop in leptin levels, which increases appetite and reduces energy expenditure (Klok et al., 2007).

Many obese people experience leptin resistance, in which the brain cannot process leptin signals correctly, even in the presence of elevated levels of the hormone in the blood. Due to this resistance, the body experiences a paradoxical state of starvation, which causes it to become hungrier and use less energy (Izquierdo et al., 2019). Several triggers cause the emergence of leptin resistance. Important roles of genetic susceptibility, chronic inflammation, and specific dietary variables. Elevations in free fatty acid levels in the bloodstream, which are frequently observed in obese individuals, can disrupt leptin signalling pathways. Moreover, breakdown of the blood-brain barrier may hinder leptin absorption into the hypothalamus. Obesity is caused by a vicious cycle (Figure 4) involving higher fat mass levels and the inability to respond to leptin signals. This is caused by leptin resistance. Resistance inhibits the expected decrease in hunger and increase in energy expenditure (Gruzdeva et al., 2019).

The intricate relationship between insulin and other hormones, such as ghrelin, affects energy metabolism and appetite regulation, whereas ghrelin increases hunger and counteracts the effects of leptin. These hormones are frequently dysregulated in obesity, thereby complicating metabolic processes that lead to weight gain (Chabot et al., 2014). To combat obesity, Understanding leptin resistance and related metabolic processes is essential. In the future, leptin-resistance-targeting therapeutics may be viable approaches to treating obesity and its associated metabolic disorders (Schwartz et al., 2017).

Pathophysiological significance of adiponectin

Another adipokine, adiponectin, is crucial in controlling insulin sensitivity, lipid metabolism, and glucose levels. AdipoR1 and AdipoR2 are the two central receptors that mediate anti-inflammatory, anti-fibrotic, and antioxidant effects. Discovered in 1995, adiponectin—also referred to as ACRP30, AdipoQ, apM1, or GBP28. The adiponectin structure comprises 244 amino acids in humans (28 kDa) and 247 amino acids in mice (30 kDa) (Achari and Jain, 2017). The constituent parts of this protein include a collagenous domain, variable region, signal transducer, and globular domain at the C-terminus. Three separate multimeric forms exist in plasma, each with unique biological characteristics and possibly distinct tissue targets (Begum et al., 2023). Interestingly, adiponectin and obesity have the opposite relationship: weight loss increases adiponectin levels in the blood, which is linked to better insulin sensitivity. The integral membrane proteins AdipoR1 and AdipoR2 receptors are expressed in various organs and have variable affinities to different forms of adiponectin. They are essential for maintaining insulin sensitivity and carbohydrate homeostasis (Khoramipour et al., 2021). Adiponectin regulates insulin sensitivity and glucose metabolism. It has an inverse relationship with insulin resistance in metabolic syndrome, obesity, and type 2 diabetes (T2DM). It regulates the operation of organs such as the liver and skeletal muscles, improves insulin sensitivity, and modifies glucose and lipid metabolism (Chakraborti, 2015).

Adiponectin impacts the cardiovascular system beyond carbohydrate metabolism, especially in atherosclerosis. It affects the behavior of macrophages, endothelial cells, and monocytes, thereby affecting atherosclerotic lesions. Additionally, adiponectin levels have been shown to have a significant sexual dimorphism in their plasma levels, making them a viable biomarker for evaluating cardiovascular risk (Hui et al., 2012). Adiponectin levels are markedly changed in patients with this disease. Research indicates that reduced synthesis of this agent is associated with the pathophysiology of insulin resistance and type 2 diabetes and plays a critical role in sensitizing insulin action. Clinical research supports the notion that elevated adiponectin levels are associated with a lower incidence of type 2 diabetes.

Additionally, adiponectin is involved in cancer; it has been shown to suppress tumor growth and spread, induce apoptosis in cancer cells, and influence the course of certain malignancies, such as endometrium, ovarian, thyroid, and prostate cancers (Bocian-Jastrzębska et al., 2023). It plays an essential role in several physiological and pathological processes. Its interactions with specific receptors exert protective effects against cancer, atherosclerosis, inflammation, and insulin resistance. Adiponectin is a prospective target for therapeutic approaches against metabolic illnesses because of its broad influence (Dalamaga et al., 2012). The leptin-to-adiponectin ratio is pivotal in health and disease. Imbalances result in obesity and are linked to insulin resistance, diabetes, and cardiovascular diseases, highlighting their crucial role in metabolic and cardiovascular health (Figure 3).

The Adiponectin-Leptin Ratio as a potential indicator of cardiometabolic risk

The Adiponectin-Leptin Ratio explores the growing significance of adiponectin and leptin as biomarkers in the medical domain, specifically for assessing the risks associated with obesity and related cardiometabolic processes. This ratio is becoming more widely acknowledged as a vital sign of dysregulation of adipose tissue function in obesity (Frühbeck et al., 2019). Type 2 diabetes, hypertension, and cardiovascular illnesses are only a few of the many cardiometabolic problems (Figure 4) closely associated with obesity, a global health concern. Traditional risk evaluations frequently use body mass index (BMI) and waist circumference. However, they do not adequately account for the complexity of metabolic disorders linked to obesity. The adiponectin-to-leptin ratio (Figure 5) provides a more sophisticated approach that shows the equilibrium between two important adipokines that are essential for metabolic regulation (Landi et al., 2018). Obese people tend to have lower adiponectin and higher leptin levels. Consequently, a lower adiponectin-leptin ratio is associated with higher cardiovascular risk and adipose tissue malfunction. This ratio can potentially be a more precise and predictive marker for obesity-related health problems than adiponectin or leptin levels measured separately (Manna and Jain, 2015).

A concise compendium of the main characteristics of leptin and adiponectin is presented in Table 1, including

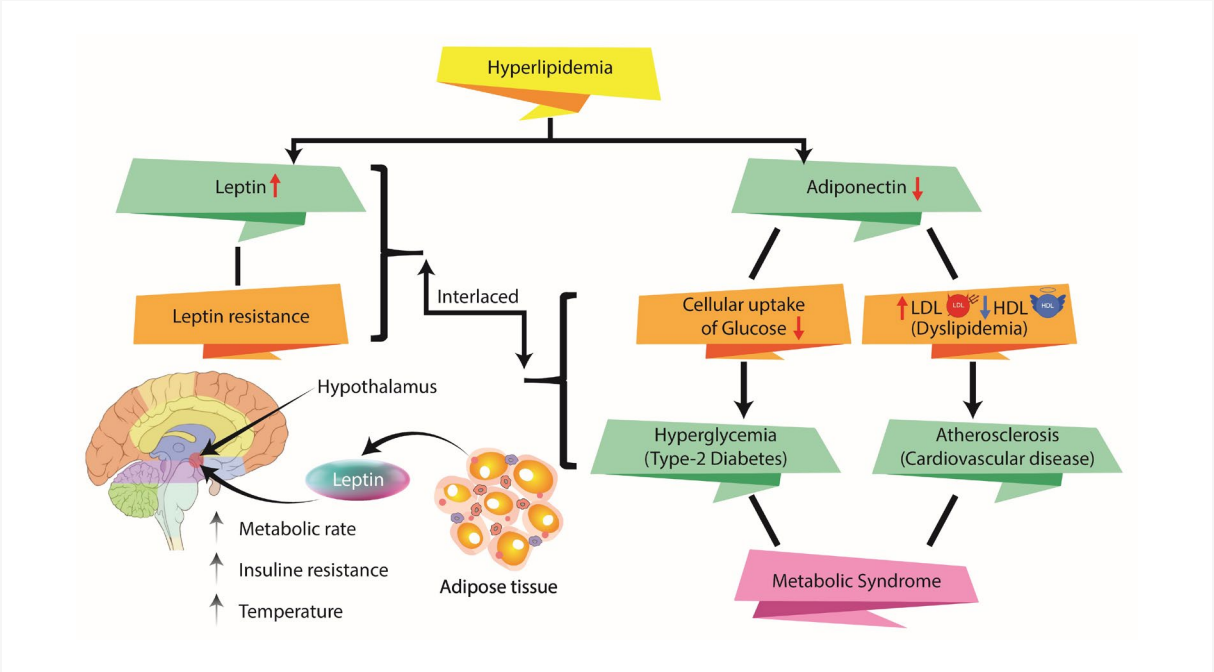

Figure 3. Involvement of the leptin-adiponectin axis in health and disease.

Slika 3. Vključenost leptin adiponektinske osi v zdravje in bolezen.

information on their sources, roles, clinical consequences, and assessment methods. This study highlights the divergent functions of these hormones in metabolism and their connections with obesity and related illnesses. It also lists the locations of the corresponding genes and primary receptors of these hormones.

In addition to leptin and adiponectin, adipokine encompasses resistin, angiotensinogen, visfatin, omentin, and various cytokines such as TNF-α, IL-6, etc. (Al-Suhaimi and Shehzad, 2013). Adipose tissue secretes a variety of bioactive adipokines. Resistin has been linked to inflammation and insulin resistance and may be a factor in metabolic diseases (Liu et al., 2020). Adipose tissue is the source of angiotensinogen, which is also known to regulate blood pressure and may affect cardiovascular health. Visfatin, a nicotinamide phosphoribosyltransferase, is involved in inflammation and metabolism (Saddi-Rosa et al., 2010). On the other hand, Omentin is linked to insulin sensitivity and possesses anti-inflammatory properties (Sperling et al., 2023). Moreover, pro-inflammatory chemicals released by adipose tissue include complement factors and cytokines like TNF-α and IL-6, which contribute to the low-grade inflammation linked to obesity and its health riss. Investigating these less well-known adipokines helps us comprehend the complex connections among inflammation, metabolic disorders, and obesity (Zorena et al., 2020).

Resistin

The primary source of the protein hormone resistin in the human body is adipose tissue (fat cells). The physiological function has been the focus of continuous investigation and discussion. It was first believed to be a significant factor in type 2 diabetes. Insulin resistance is characterized by reduced glucose uptake and high blood sugar levels caused by resistin interfering with insulin signalling in target tissues such as the liver and muscles. This implies that metabolic problems occur because of their pathogenic role (Berger, 2001).

Nevertheless, recent research has reported contradictory findings. Resistin-specific physiological and pathological functions are still being studied. Because resistin can affect inflammation and is expressed in immune cells, some studies suggested that it may have immunregulatory activities. It may play a role in the chronic low-grade inflammation observed in obesity-related disorders and is

Table 1. Primordial characteristics of adiponectin and leptin.

Tabela 1. Prvotne značilnosti adiponektina in leptina.

Parameter	Adiponectin	Leptin
Chemical nature	Protein hormone	Protein hormone
Source	Produced by adipose tissue	Produced by adipose tissue
Gene location	Chromosome 3	Chromosome 7
Association with adipocytes	Inversely related to adiposity	Positively association with adiposity
Primordial function	Promotes fatty acid oxidation	Crucial to maintaining an equilibrium between food consumption and energy use
	Insulin sensitivity enhancement	Body weight and adiposity
	Inhibits inflammation	Influences of thermogenesis and energy balancing
	Cardioprotective	Regulates appetite and energy expenditure
Circulating Levels	Generally higher in lean body weight and lower in obesity	Usually higher in obese
Clinical Implications	Lower levels associated with insulin resistance, metabolic syndrome, and cardiovascular risks	High levels associated with increased appetite, obesity, and metabolic syndrome
Typically measured in	Blood plasma (µg/mL)	Blood plasma (ng/mL)
Methods of Measurement	ELISA, immunoassays, Western blotting, and polymerase chain reaction (PCR)	ELISA, immunoassays, Western blotting, and PCR techniques
Primary Receptors	AdipoR1 and AdipoR2	Ob-R (Leptin receptor)

associated with controlling genes relevant to inflammation (Acquarone et al., 2019).

Overall, resistin plays a complicated and multidimensional role in numerous physiological and pathological processes, although its precise role in metabolism and illness is not fully understood. More research is required to clarify its mechanism and therapeutic implications (Li et al., 2021).

Angiotensinogen

Angiotensinogen has recently drawn attention as an adipokine. It has been recognized for its function in the renin-angiotensin system (RAS) as a precursor to angiotensin peptides implicated in blood pressure regulation. Angiotensinogen is one of the bioactive substances called "adipokines" secreted by adipose tissue and produced by adipocytes. It has a fascinating physiological function as an adipokine that links the regulation of systemic blood pressure with adipose tissue (Jin et al., 2023).

Angiotensinogen is released into the bloodstream by adipocytes and can be cleaved by the enzyme renin to yield angiotensin I. Eventually, angiotensin II, a potent vasoconstrictor that enhances blood pressure. Thus, angiotensinogen produced from adipose tissue may be involved in blood pressure regulation in response to alterations in body fat and metabolic disorders associated with obesity (Ramalingam et al., 2017).

Pathologically, dysregulation of angiotensinogen secretion can lead to cardiovascular issues. Increased adipose tissue mass in obesity frequently results in increased angiotensinogen production. This can encourage RAS overactivation, leading to blood pressure elevation, i.e., hypertension, salt retention, and vasoconstriction. Moreover, hypertension can be exacerbated by adipose tissue inflammation, which is a frequent characteristic of obesity and can trigger the release of angiotensinogen. By linking adipose tissue to the renin-angiotensin system, angiotensinogen contributes to the physiological regulation of blood pressure. It is crucial to comprehend the function of adipose tissue and its potential as a therapeutic target in managing obesity-related cardiovascular diseases. However, its pathological overproduction or dysregulation in obesity can lead to hypertension and cardiovascular complications (Yvan-Charvet et al., 2011).

Visfatin

Nicotinamide phosphoribosyltransferase, or visfatin, is an adipokine primarily released by visceral fat tissue, i.e., stored around internal organs, including the heart,

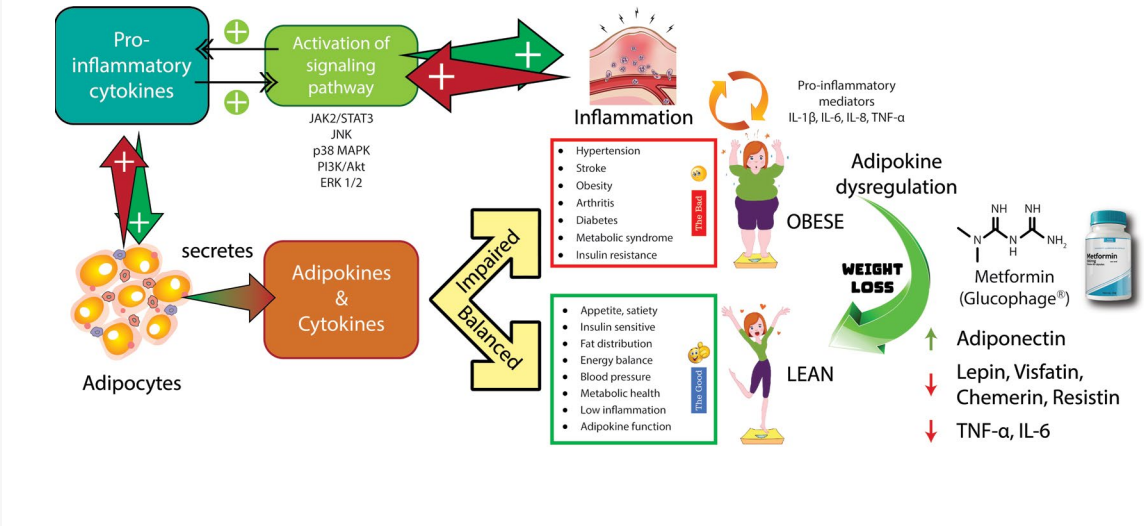

Figure 4. The overall effects of various adipokine on bodily functions via multiple signalling pathways.

Slika 4. Splošni učinek različnih adipokinov na telesne funkcije prek več signalnih poti.

liver, and intestines. Visfatin is required for several metabolic activities in the body. It contributes to the formation of nicotinamide adenine dinucleotide (NAD^+, NADH, NADPH), which is necessary for cellular signalling, energy metabolism, and DNA repair. Visfatin also exerts insulin-mimetic effects, reducing blood glucose levels by boosting peripheral glucose absorption and insulin sensitivity. Maintaining glucose homeostasis is vital to its function (Xie et al., 2020).

Pathologically, several illnesses, including obesity, type 2 diabetes, and cardiovascular disorders, have been linked to increased visfatin levels. Pro-inflammatory conditions caused by obesity-linked visfatin release from expanded visceral fat may aggravate insulin resistance and result in metabolic syndrome. Visfatin initially functions in the context of type 2 diabetes to offset insulin resistance, but persistently elevated levels may also be linked to pancreatic beta-cell malfunction. Furthermore, visfatin has been linked to atherosclerosis because of its involvement in vascular inflammation and endothelial cell dysfunction (Abdalla, 2022.).

Furthermore, visfatin is involved in various other clinical situations, such as in certain types of cancer, where it may facilitate tumor growth and metastasis (Figure 2b). This is explained by its function in NAD^+ production, which is necessary for the rapid division of cancer cells. In conclusion, visfatin has a dual role in physiological processes. Although essential for normal metabolic activities, dysregulation of this hormone has been associated with many diseases (Lin, 2019).

Omentin

Adipose tissue also releases a protein called omentin adipokine, which is involved in many human processes. Omentin is physiologically recognized for its advantageous effects on cardiovascular health and metabolism. It protects against insulin resistance and type 2 diabetes by improving insulin sensitivity, which in turn helps control blood sugar levels. In addition to its cardiovascular preventive benefits, omentin has anti-inflammatory properties. It reduces arterial stiffness, enhances endothelial function, and may minimize the risk of atherosclerosis (Chait and Den Hartigh, 2020).

Pathologically, changes in omentin levels are linked to several metabolic diseases. Omentin levels are frequently shown to be lower in people with obesity, type 2 diabetes, and cardiovascular conditions. This decline may have an impact on how these illnesses start and worsen. The deficiency of omentin in cardiovascular diseases may enhance vascular inflammation and endothelial dysfunction, whereas the diminished secretion of omentin in obesity exacerbates insulin resistance. Furthermore, rheumatoid arthritis and other inflammatory conditions are associated with reduced omentin levels, indicating a broader role for omentin in inflammatory disorders (Zhou et al., 2014).

Cytokines

Cytokines such as TNF-α and IL-6 are important adipokines. These cytokines control normal physiological/immunological responses, insulin sensitivity, and energy consumption. For instance, adipocytes secrete TNF-α and IL-6, which support healthy immune response and control of inflammation. They assist the body in responding to wounds or infections by taking part in the acute phase response (Makki et al., 2013).

However, their function may become harmful under pathological circumstances, mainly when obesity is present. These cytokines are overproduced in obese people because of excess adipose tissue, resulting in a chronic low-grade inflammatory state. One of the leading causes of insulin resistance, a feature of type 2 diabetes, is this ongoing inflammation. Elevated levels of TNF-α and IL-6 disrupt insulin signalling pathways, worsening insulin resistance. Furthermore, they facilitate endothelial dysfunction and the build-up of atherogenic lipids in blood vessels, which contribute to the pathophysiology of atherosclerosis and cardiovascular disorders (Saxena et al., 2020; Zatterale et al., 2020; Kumar et al., 2020). Complement factors are components of the innate immune system and have two functions. They are crucial to the immune complex, clearance of dead cells, and pathogen defense. However, the secretion of these hormones can be altered in obesity, leading to a pro-inflammatory state and increasing the risk of metabolic problems. Overall, excessive cytokines production in pathological conditions, such as obesity, results in chronic inflammation, which in turn promotes the development of insulin resistance, type 2 diabetes, and cardiovascular diseases. However, cytokines such as TNF-α, IL-6, and complement factors are necessary for normal bodily functions.

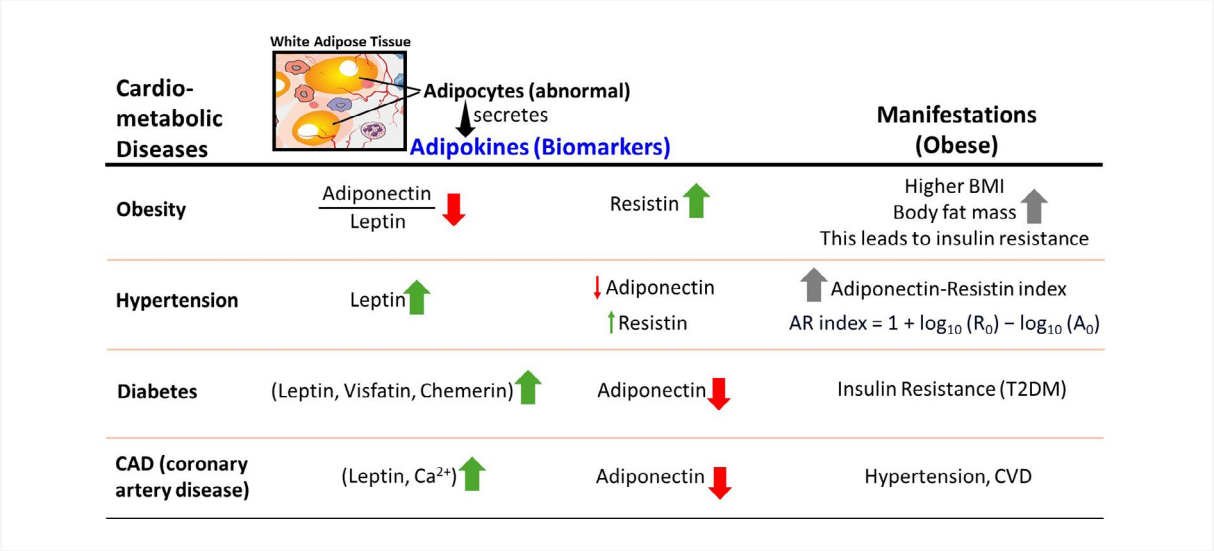

Figure 5. Cardiometabolic problems: Obesity, hypertension, Type 2 diabetes (T2DM), cardiovascular diseases (CVD), and CAD are closely associated with adipokine levels and serve as biomarkers for the identification of metabolic disorders (Green and red arrows show their increasing and decreasing concentrations, respectively).

Slika 5. Kardio-metabolne težave: Debelost, sladkorna bolezen tipa 2, hipertenzija in CAD so tesno povezani s koncentracijo adipokinov (zelena in rdeča puščica kažeta naraščajočo oziroma padajočo koncentracijo adipokinov).

Conclusion

The distinct features of adipocytes are critical to metabolic health and provide important information and consequences for comprehending the complex processes underlying obesity, insulin resistance, and metabolic diseases. Adipocytes are dynamic endocrine organs that release various adipokine, such as adiponectin, leptin, visfatin, and omentin, which significantly affect metabolic homeostasis. They are not only passive cells that store fat. By improving lipid metabolism and glucose utilization, the adipokine adiponectin, which has anti-inflammatory and insulin-sensitizing properties, can protect metabolic health. On the other hand, leptin controls hunger and energy expenditure, thereby helping to maintain an equilibrium between energy and body weight. Insulin resistance and obesity can result from dysregulation of these adipokines. Visfatin is linked to inflammation and glucose metabolism. Omentin is a newly identified adipokine that exhibits potential for enhancing insulin sensitivity and guard against cardiovascular issues. Understanding the distinct characteristics of these adipokines can help develop novel therapeutic approaches by offering vital insights into the pathophysiology of metabolic disorders. New strategies for treating obesity, type 2 diabetes, and associated metabolic disorders may be possible by focusing on adipocyte-derived factors like adiponectin and leptin or utilizing the therapeutic potential of newly discovered adipokine such as visfatin and omentin. In conclusion, critical components of the intricate network of metabolic health are the unique properties of adipocytes and the adipokine they secrete. Novel treatments and a better comprehension of the complex mechanisms underlying metabolic health and illness are potential outcomes of further investigation into these molecules and their mechanisms of interaction.

Author Contributions

Conceptualization, J.K.G., K.K., and N.W.; methodology, Y.S.; software, N.W.; validation, K.K., N.W.; formal analysis, J.K.G.; investigation, Y.S.; resources, J.K.G.; data curation, Y.S.; writing—original draft preparation, J.K.G and N.W., Y.S.; writing—review, editing, and paper communication, K.K.; visualization, K.K.; supervision, K.K., N.W.; project administration, J.K.G.; funding acquisition, N.W. All authors have read and agreed to the final version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest to disclose.

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