# ROCZNIKI PAŃSTWOWEGO ZAKŁADU HIGIENY

# ANNALS OF THE NATIONAL INSTITUTE OF HYGIENE



Quarterly 2024 Volume 75 Number 3 - SEPTEMBER

PUBLISHER: NATIONAL INSTITUTE OF PUBLIC HEALTH NIH – NATIONAL RESEARCH INSTITUTE Warsaw, Poland

# **ROCZNIKI PAŃSTWOWEGO ZAKŁADU HIGIENY** (ANNALS OF THE NATIONAL INSTITUTE OF HYGIENE)

Published since 1950

**Quarterly**, 4 issues in 1 volume per year (No 1 - March, No 2 - June, No 3 - September, No 4 - December) The journal is devoted to research studies on food and water safety, nutrition, dietetics, environmental hygiene, toxicology and health risk assessment, public health and other areas related to health sciences

Available at https://roczniki.pzh.gov.pl/

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ISSN 0035-7715 eISSN 2451-2311

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> Printing house: Agencja Reklamowa TOP Chocimska 4, 87-800 Włocławek tel.: + 48 54 427 09 70 http://www.agencjatop.pl

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X okazji świąt Bożego Narodzenia składamy naszym Czytelnikom życzenia zdrowia, spokoju i uśmiechu. Niech ten wyjątkowy czas przyniesie radość i odpoczynek od codziennych obowiązków.

WNowym Roku 2025 życzymy pomyślności i wielu sukcesów.

Redakcja Roczników Państwowego Zakładu Higieny

We wish a Merry Christmas to our Readers. May the spirit of Christmas bring you health, peace, and smile. We wish you happiness and much success in the New Year 2025.

> Editors of the journal 'Roczniki Państwowego Xakładu Higieny'



# **OD REDAKTORA NACZELNEGO**

Szanowni Państwo,

Oddajemy do Państwa rąk nr 3/2024 r. Roczników Państwowego Zakładu Higieny. Na wstępie wspominamy trzech wybitnych Profesorów, związanych z Narodowym Instytutem Zdrowia Publicznego PZH – Państwowym Instytutem Badawczym i Rocznikami Państwowego Zakładu Higieny, którzy odeszli w ostatnim miesiącu i pozostaną we wdzięcznej pamięci uczniów i współpracowników.

W bieżącym numerze znajdą Państwo publikacje z obszaru badań nad sposobem żywienia a ryzykiem rozwoju chorób i zaburzeń stanu zdrowia. R. Wierzejska przedstawia wyniki badań pilotażowych nad wpływem przyrostu masy ciała w ciąży na parametry urodzeniowe bliźniąt, H. Belaoufi i wsp. oceniają wpływ czynników społeczno-ekonomicznych i stylu życia na przestrzeganie diety śródziemnomorskiej wśród marokańskiej młodzieży a K. Kozik i B. Całyniuk wskazują na ryzyko wystąpienia ortoreksji psychicznej w grupie uczniów szkół średnich, związane z wybranymi zachowaniami żywieniowymi. Z kolei w obszarze badań nad obecnością składników odżywczych i substancji czynnych w produktach roślinnych, badacze z Maroka i Algierii wskazują na korzystne właściwości jadalnych roślin, dziko rosnących w tych krajach. A. Aboukhalaf i wsp. w swojej publikacji wykazali antybakteryjne i przeciwgrzybicze działanie metanolowych wyciągów z siedmiu rodzajów roślin, tradycyjnie spożywanych w Maroku a S. Essaih i wsp. zaprezentowali, z wykorzystaniem metody ICP-OES, że jadalne, dziko rosnace w tym kraju rośliny moga być znaczącym źródłem w diecie składników mineralnych, w tym potasu, magnezu, żelaza, sodu i manganu. H. Guenane i wsp. wskazali na obecność korzystnych dla zdrowia kwasów tłuszczowych w oleju z owoców Quercus ilex (dębu ostrolistnego), tradycyjnie spożywanych w Algierii oraz na antyoksydacyjne działanie związków fenolowych, w tym flawonoidów zawartych w odtłuszczonych i nieotłuszczonych wyciągach z tej rośliny. Ponadto w bieżącym numerze znalazła się publikacja N. Kuczki i wsp. oceniająca preferencje sensoryczne studentów dietetyki w odniesieniu do bogatobiałkowych jogurtów obecnych na polskim rynku oraz publikacja Ö.F. Kalkana i wsp. prezentująca wyniki badań, z wykorzystaniem modelu zwierzęcego, nad rolą soku z granatu, jako czynnika hamującego stres oksydacyjny indukowany przez czterochlorek wegla.

Jednocześnie w imieniu własnym i Redakcji Roczników Państwowego Zakładu Higieny pragnę wszystkim Czytelnikom i Współpracownikom życzyć radosnych Świąt Bożego Narodzenia i Szczęśliwego Nowego Roku.

Z poważaniem,

dr hab. Hanna Mojska, prof. NIZP PZH PIB Redaktor naczelna

Roczników Państwowego Zakładu Higieny



# **EDITORIAL INTRODUCTION**

Ladies and Gentlemen,

We would like to present to you No. 3/2024 of the journal Roczniki Państwowego Zakładu Higieny. At the beginning, we dedicate our memory to three outstanding Professors related to the National Institute of Public Health NIH – National Research Institute and the journal Roczniki Państwowego Zakładu Higieny, who passed away in the last month and will remain in the grateful memory of students and colleagues.

In the current issue you will find publications in the area of research on

nutrition and the risk of developing diseases and health disorders. R. Wierzejska presents the results of pilot studies on the impact of weight gain during pregnancy on the birth parameters of twins, H. Belaoufi et al. assess the impact of socio-economic factors and lifestyle on adherence to the Mediterranean diet among Moroccan adolescents, and K. Kozik and B. Calyniuk indicate on the risk of orthorexia nervosa in a group of high school students, related to selected eating behaviors. In turn, in the area of research on the presence of nutrients and active substances in plant products, researchers from Morocco and Algeria pointed out the beneficial properties of edible plants growing wild in these countries. In their publication, A. Aboukhalaf et al. demonstrated the antibacterial and antifungal effects of methanol extracts from seven types of plants traditionally consumed in Morocco, and S. Essaih et al. presented, using the ICP-OES method, that edible wild plants growing in this country can be a significant source of dietary minerals including potassium, magnesium, iron, sodium and manganese. H. Guenane et al. pointed out the presence of health-beneficial fatty acids in the oil from Quercus ilex (holm oak) fruit traditionally consumed in Algeria, and the antioxidant effect of phenolic compounds, including flavonoids contained in defatted and non-fat extracts from this plant. Additionally, the current issue includes a publication by N. Kuczka et al. assessed the sensory preferences of dietetics students in relation to high-protein yogurts available on the Polish market and the publication by **Ö.F. Kalkan et al.** presenting the results of research on an animal model on the role of pomegranate juice as a factor inhibiting oxidative stress induced by carbon tetrachloride.

At the same time, on behalf of myself and the Editorial office of the Roczniki Państwowego Zakładu Higieny, I would like to wish all Readers and Collaborators a very Merry Christmas and a Happy New Year.

Kind regards, H. Rei-

Assoc. Prof. Hanna Mojska, PhD Editor-in-Chief Roczniki Państwowego Zakładu Higieny





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# PAMIĘCI PROFESORA DR HAB. MARKA JAGIELSKIEGO (1943-2024)



Z głębokim żalem przyjęliśmy wiadomość o śmierci w dniu 28 listopada 2024 roku Pana prof. dr hab. Marka Jagielskiego, wieloletniego Kierownika Zakładu Bakteriologii Narodowego Instytutu Zdrowia Publicznego PZH – Państwowego Instytutu Badawczego oraz redaktora naczelnego kwartalnika Medycyna Doświadczalna i Mikrobiologia. Był autorem szeregu prac dotyczących biologicznych właściwości drobnoustrojów chorobotwórczych dla człowieka oraz opracowywania i wdrażania nowoczesnych metod biologii molekularnej do diagnostyki zakażeń bakteryjnych. Na szczególne podkreślenie zasługują zasługi Pana Profesora w kształceniu młodej kadry naukowej. Wypromował siedmiu doktorów, wykonując swoje obowiązki niezwykle sumiennie i z pełnym zaangażowaniem, zawsze gotowy wysłuchać, doradzić i wspierać. Prof. M. Jagielski był również wiceprezesem Polskiego Towarzystwa Mikrobiologii oraz Krajowej Izby Diagnostów Laboratoryjnych, której był jednym z założycieli. Był odznaczony wieloma odznaczeniami państwowymi i resortowymi.

Dla wszystkich pracowników Pan Profesor pozostanie mentorem i nauczycielem, osobą o wielkiej wiedzy, która chętnie dzieliła się swym doświadczeniem.

Pracownicy Zakładu Bakteriologii i Zwalczania Skażeń Biologicznych, Narodowy Instytut Zdrowia Publicznego PZH – Państwowy Instytut Badawczy, Warszawa Ŀ

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# IN MEMORIAM PROFESSOR MAREK JAGIELSKI (1943-2024)



It is with deep regret that we received the news of the death on November 28, 2024 of Prof. dr hab. Marek Jagielski, a long-time head of the Department of Bacteriology of the National Institute of Public Health – National Research Institute and the editor-in-chief of the quarterly Medycyna Doświadczalna i Mikrobiologia. He was the author of a many publications on the biological properties of pathogenic microorganisms for humans and the development and implementation of modern molecular biology methods for the diagnosis of bacterial infections. The Professor's merits in educating young scientific staff deserve special emphasis. He promoted seven doctors, performing his duties extremely conscientiously and with full commitment, always ready to listen, advise and support. Prof. M. Jagielski was also vice-president of the Polish Society of Microbiology and the National Chamber of Laboratory Diagnosticians, of which he was one of the founders. He was awarded many state distinctions.

For all employees, Professor will remain a mentor and teacher, a person of great knowledge who willingly shared his experience.

Employees Department of Bacteriology and Biocontamination Control, National Institute of Public Health NIH – National Research Institute, Warsaw, Poland

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# PAMIĘCI PROFESORA DR HAB. JANA DZIENISZEWSKIEGO (1934-2024)



Profesor dr hab. med. Jan Dzieniszewski zmarły w dniu 14 listopada 2024 roku, był wybitnym naukowcem i oddanym pacjentom lekarzem, pionierem i specjalistą w zakresie gastroenterologii i endoskopii. Przez wiele lat pracował w Instytucie Żywności i Żywienia (IŻŻ) w Warszawie, gdzie pełnił m.in. funkcję Kierownika Kliniki Chorób Metabolicznych i Gastroenterologii, a także przez kilka kadencji przewodniczył Radzie Naukowej IŻŻ. Przez długi czas pracował również w Mazowieckim Szpitalu Bródnowskim. Był jednym z pierwszych pracowników tego szpitala, w którym pełnił m.in. funkcję Ordynatora Oddziału Chorób Wewnętrznych i Gastroenterologii.

Profesor Jan Dzieniszewski urodził się 9 czerwca 1934 r. w Ostrowi Mazowieckiej. W roku 1952 rozpoczął studia na Akademii Medycznej w Warszawie na Wydziale Lekarskim, które ukończył w 1958 roku. W tym samym roku rozpoczął pracę na tej uczelni jako asystent w Klinice Chorób Wewnętrznych Studium Doskonalenia Lekarzy, gdzie zrobił specjalizację w zakresie chorób wewnętrznych. W 1964 roku uzyskał stopień doktora nauk medycznych.

W latach 1970-1980 pracował w Klinice Gastroenterologii Centrum Medycznego Kształcenia Podyplomowego w Warszawie na stanowisku adiunkta, a następnie docenta, gdzie organizował kształcenie podyplomowe lekarzy w dziedzinach niezabiegowych medycyny klinicznej. W 1974 roku uzyskał stopień naukowy doktora habilitowanego. W tym samym roku zrobił specjalizację w zakresie gastroenterologii. W 1984 roku Rada Państwa nadała mu tytuł profesora.

We wrześniu 1980 roku profesor Dzieniszewski objął stanowisko Kierownika Kliniki Chorób Metabolicznych i Gastroenterologii Instytutu Żywności i Żywienia z siedzibą w Szpitalu Bródnowskim w Warszawie. Kierował Kliniką do momentu przejścia na emeryturę w roku 2004. W tym okresie pod jego kierunkiem wielu lekarzy uzyskało specjalizację w zakresie chorób wewnętrznych i gastroenterologii, stopnie naukowe doktora i doktora habilitowanego. Po przejściu na emeryturę kontynuował aktywność zawodową i naukową, swoją radą i doświadczeniem służąc współpracownikom z Instytutu Żywności i Żywienia oraz Mazowieckiego Szpitala Bródnowskiego.

W roku 1988 został powołany na Specjalistę Krajowego w zakresie chorób wewnętrznych, sprawując tę funkcję do roku 1992.

Głównym obszarem działalności naukowej profesora Dzieniszewskiego była patofizjologia i klinika chorób trzustki, zakażenie *Helicobacter pylori* oraz leczenie żywieniowe. Był kierownikiem projektu "Opracowanie podstaw naukowych żywienia w szpitalach", realizowanego w latach 1999-2001, który wskazał na znaczenie prawidłowego żywienia w terapii pacjentów przebywających w szpitalach.

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Był autorem lub współautorem ponad 300 publikacji naukowych oraz redaktorem lub współredaktorem ponad 20 opracowań monograficznych dotyczących gastroenterologii i żywienia. W latach 1975-1985 pełnił funkcję redaktora naczelnego czasopisma Wiadomości Lekarskie. Był również członkiem rad naukowych wielu czasopism medycznych.

Profesor Dzieniszewski był członkiem licznych towarzystw i komitetów naukowych Polskiej Akademii Nauk (PAN). W latach 1970-1998 był wiceprezesem Zarządu Głównego Towarzystwa Internistów Polskich, a od 1980 r. członkiem i wielokrotnie wiceprezesem Zarządu Głównego Polskiego Towarzystwa Gastroenterologii. W latach 1985-2011 uczestniczył w pracach Komitetu Żywienia Człowieka i Komitetu Patofizjologii PAN.

Profesor Dzieniszewski za swoje osiągnięcia naukowe, zawodowe oraz zaangażowanie społeczne został odznaczony Srebrnym Krzyżem Zasługi i Krzyżem Oficerskim Orderu Odrodzenia Polski, Złotą Odznaką za Zasługi dla Warszawy, Złotą Odznaką za Zasługi dla Miasta Ostrowi Mazowieckiej, Medalem Akademii Medycznej we Wrocławiu. W roku 2000 otrzymał nagrodę Ministra Zdrowia za szczególne osiągnięcia w dziedzinie ochrony zdrowia.

Przede wszystkim jednak Profesor Jan Dzieniszewski był wspaniałym lekarzem – wzorem dla innych lekarzy. Nie tylko znakomicie diagnozował i leczył choroby, ale również był przyjacielem pacjentów. Pomagał dźwigać im cierpienie, dodawał otuchy oraz często poświęcał im swój osobisty czas. Tysiące pacjentów zawdzięcza Panu Profesorowi zdrowie i życie. W trudnych czasach dla naszego kraju był osobistym lekarzem i wspierał w chorobie Jego Świątobliwość Kardynała Stefana Wyszyńskiego oraz Jego Świątobliwość Kardynała Józefa Glempa.

Profesor Jan Dzieniszewski pozostanie w pamięci swoich współpracowników jako człowiek szerokich horyzontów, mentor i autorytet, osoba o nadzwyczajnej kulturze, a także wspaniały nauczyciel.

> Prof. dr hab. med. Mirosław Jarosz oraz Redakcja Roczników Państwowego Zakładu Higieny

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# IN MEMORIAM: PROFESSOR JAN DZIENISZEWSKI (1934-2024)



Professor Jan Dzieniszewski, MD, PhD, who died on November 14, 2024, was an outstanding scientist and physician dedicated to serving to his patients, a pioneer and specialist in the field of gastroenterology and endoscopy.

He worked for many years at the Institute of Food and Nutrition (IFN) in Warsaw, where he served, among others, as Head of the Department of Metabolic Diseases and Gastroenterology, and also chaired the Scientific Council of the IFN for several terms. For a long time he also worked at the Masovian Bródno Hospital. He was one of the first employees of this hospital, where he served, among others, as Head of the Department of Internal Medicine and Gastroenterology.

Professor Jan Dzieniszewski was born on 9 June 1934 in Ostrów Mazowiecka. In 1952 he began studies at the Medical Academy in Warsaw at the Faculty of Medicine, which he completed in 1958. In the same year, he began working at the academy as an assistant in the Department of Internal Medicine of the Doctors' Training College, where he specialised in internal medicine. In 1964, he was awarded the degree of Doctor of Medicine.

From 1970 to 1980, he worked at the Department of Gastroenterology of the Medical Centre for Postgraduate Education in Warsaw as an assistant professor and then as an associate professor, where he organised postgraduate training of doctors in non-surgical areas of clinical medicine. In 1974, he obtained his postdoctoral degree. In the same year he made a specialisation in gastroenterology. In 1984, the Council of State awarded him the title of professor.

In September 1980, Professor Dzieniszewski took up the post of Head of the Department of Metabolic Diseases and Gastroenterology at the Institute of Food and Nutrition, based in the Bródno Hospital in Warsaw. He held this position until his retirement in 2004. During this period, under his supervision, many doctors gained specialisation in internal diseases and gastroenterology, as well as doctoral and post-doctoral degrees. After retirement, he continued professional and scientific activity, offering his advice and experience to colleagues from the Institute of Food and Nutrition and the Mazovian Bródno Hospital.

In 1988 he was appointed National Specialist in Internal Medicine, holding this position until 1992.

Professor Dzieniszewski's main area of scientific activity was the pathophysiology and clinic of pancreatic diseases, *Helicobacter pylori* infection and nutritional treatment. He was the head of the project 'Preparation of scientific foundations of nutrition in hospitals', carried out in 1999-2001, which indicated the importance of proper nutrition in the treatment of patients staying in hospitals.

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Professor Dzieniszewski was a member of numerous societies and scientific committees of the Polish Academy of Sciences (PAN). In 1970-1998 he was Vice President of the Board of the Polish Society of Internal Medicine, and since 1980 a member and repeatedly Vice President of the Board of the Polish Society of Gastroenterology. From 1985 to 2011, he participated in the work of the Committee on Human Nutrition and the Committee on Pathophysiology of the Polish Academy of Sciences.

Professor Dzieniszewski has been awarded the Silver Cross of Merit and the Officer's Cross of the Order of Polonia Restituta for his scientific and professional achievements as well as his social commitment, the Golden Badge of Merit for Warsaw, the Golden Badge of Merit for the City of Ostrów Mazowiecka, and the Medal of the Academy of Medicine in Wrocław. In 2000 he received the award of the Minister of Health for special achievements in the field of health care.

Above all, however, Professor Jan Dzieniszewski was an excellent doctor – a role model for other doctors. He not only excelled in diagnosing and treating illnesses, but was also a friend to his patients. He helped them to bear their suffering, gave them encouragement and often devoted his personal time to them. Thousands of patients owe their health and lives to the Professor. In difficult times for our country, he was a personal doctor and supported His Holiness Cardinal Stefan Wyszyński and His Holiness Cardinal Józef Glemp in their illnesses.

Professor Jan Dzieniszewski will be remembered by his colleagues as a man of broad horizons, a mentor and authority, a person of extraordinary culture, and a great teacher.

Professor Mirosław Jarosz, MD, PhD and Editors Roczniki Państwowego Zakładu Higieny

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# PAMIĘCI PROFESORA DR HAB. STANISŁAWA BERGERA (1923-2024)



Z głębokim żalem przyjęliśmy wiadomość o śmierci Pana prof. dr hab. Stanisława Bergera w dniu 28 listopada 2024 roku.

Profesor Berger był wybitnym naukowcem i pionierem w dziedzinie nauki o żywieniu człowieka. W latach 1950-1957 zatrudniony był w Państwowym Zakładzie Higieny (obecnie Narodowy Instytut Zdrowia Publicznego PZH – Państwowy Instytut Badawczy) na stanowisku Kierownika Pracowni w Dziale Higieny Żywienia. Późniejsza kariera naukowa Pana Profesora związana była przede wszystkim ze Szkołą Główną Gospodarstwa Wiejskiego (SGGW) w Warszawie. Był współtwórcą i pierwszym dziekanem Wydziału Żywienia Człowieka i Gospodarstwa Domowego tej uczelni. Był doktorem honoris causa SGGW.

W trakcie wielu lat intensywnej działalności wypracował imponujący dorobek naukowy, obejmujący ponad 500 prac i książek, w tym podręczników i publikacji o zasięgu międzynarodowym. Prace prof. dr hab. Stanisława Bergera koncentrowały się m.in. na fizjologii i biochemii żywienia, ze szczególnym uwzględnieniem witaminy A i białek, a jego działalność znacząco wpłynęła na rozwój nauk o żywieniu zarówno w Polsce, jak i na świecie.

Profesor Berger współpracował także z redakcją Roczników Państwowego Zakładu Higieny. Pełnił funkcję sekretarza Redakcji czasopisma, a od 1984 r. wchodził w skład Komitetu redakcyjnego jako redaktor Działu Higieny Żywienia. Był również członkiem Komitetu Naukowego czasopisma, w ostatnich latach członkiem honorowym.

Dla wszystkich współpracowników i osób, które zetknęły się z Panem Profesorem w swojej pracy zawodowej, pozostanie on wybitnym autorytetem, a także mentorem i nauczycielem.

Pragnąc przypomnieć sylwetkę i dorobek Pana Profesora zamieszczamy tekst, który ukazał się w nr 3/2023 Roczników PZH z okazji 100. rocznicy Jego urodzin.

Redakcja Roczników Państwowego Zakładu Higieny Ŀ

# IN MEMORIAM PROFESSOR STANISŁAW BERGER (1923-2024)



It is with deep regret that we received the news of the death of Prof. Stanisław Berger on 28 November 2024.

Professor Berger was an outstanding scientist and pioneer in the field of human nutrition science. He was employed at the National Institute of Hygiene (now the National Institute of Public Health NIH – National Research Institute) in 1950-1957, as Head of the Laboratory in the Nutrition Hygiene Department. His later scientific career was mainly associated with the Warsaw University of Life Sciences (SGGW). He was a co-founder and the first Dean of the Faculty of Human Nutrition and Farming at this University. He was awarded an honorary doctorate by SGGW.

During his many years of intensive activity, he developed an impressive scientific output of more than 500 papers and books, including textbooks and international publications. Prof. Stanisław Berger's work has focused, among other things, on the physiology and biochemistry of nutrition, with particular emphasis on vitamin A and proteins. His work has significantly influenced the development of nutrition science both in Poland and worldwide.

Professor Stanisław Berger also collaborated with the editorial board of the Journal 'Roczniki Państwowego Zakładu Higieny'. He served as Secretary of the Editorial Board of the journal, and from 1984 he was a member of the Editorial Committee as Editor of the Nutrition Hygiene Section. He was also a member of the journal's Scientific Committee, in recent years an honorary member.

Professor Berger will remain an outstanding authority as well as a mentor and teacher for all colleagues and people who had contact with him in their professional work.

In order to remind you of the profile and contributions of Professor Berger to the development of nutritional sciences in Poland, we are publishing a text that was published in the issue 3/2023 of the journal 'Roczniki Państwowego Zakładu Higieny' on the occasion of his 100th birthday.

Editors Roczniki Państwowego Zakładu Higieny

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# **PROFESSOR STANISŁAW BERGER – THE JUBILEE OF 100TH BIRTHDAY**

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# ABSTRACT

Professor Stanisław Berger – Polish scientist, creator of human nutrition science, international authority, excellent lecturer, and consistent organizer of the nutritional scientific community - celebrates his 100th birthday. He was born on September 13, 1923. The history of Professor Stanisław Berger's long life is presented in this jubilee article.

**Key words:** Professor Stanisław Berger, scientist, creator of human nutrition, Warsaw University of Life Sciences (SGGW), National Institute of Public Health (PZH), Poland



Photo 1. Professor dr hab., dr h.c. Stanislaw Berger in the academic robes of the Warsaw University of Life Sciences (SGGW). (Photo from the collection of SGGW)

Polish scientist, creator of human nutrition science, international authority, excellent lecturer, and consistent organizer of the nutritional scientific community, as well as a science disseminator.

The history of the long life of Professor Stanisław Berger - celebrating his 100th birthday - is situated across two centuries, encompassing World War II, challenging post-war times, as well as difficult political and economic transitions culminating in Poland's accession to the European Union. It is also the story of the development of nutritional science - a field in which the Professor was a pioneer in Poland.

Professor Stanisław Berger was born on September 13, 1923, in Lublin, into an intelligentsia family with patriotic and independence traditions. His father was a lawyer and a professor at the Catholic University of Lublin, while his mother was a teacher. He completed his primary education in Lublin, but the outbreak of World War II plans changed for the young Stanisław Berger. Despite the difficulties caused by the occupation, he clandestinely passed the so-called "small matriculation" exam and subsequently, in 1943, obtained the title of an agricultural technician from a Polish agricultural school in Czernichów near Krakow.

After the outbreak of the Warsaw Uprising, he set out to aid the fighting in Warsaw, but he was intercepted by General Zygmunt Berling's units and drafted into an infantry regiment. With this regiment,

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Publisher: National Institute of Public Health NIH - National Research Institute

he headed westward alongside the Soviet offensive in 1945. In April 1945, he was severely wounded in battle near Bautzen, and, as he himself recalls, was miraculously saved in a local hospital. A few months later, not fully recovered from his injuries, he managed to reach Lublin and enroll in studies at the Faculty of Agriculture of the newly established Maria Curie-Skłodowska University (UMCS). He completed his agronomic studies, earning the title of a professional agricultural engineer and subsequently a master's degree in agronomic sciences in 1948.

# The academic and scientific career of Professor Stanisław Berger and scientific achievements

During his studies, Professor Berger engaged in academic and teaching activities as an assistant at the Department of Animal Physiology and Nutrition at the Faculty of Agriculture at Maria Curie-Skłodowska University in Lublin.

In 1949, he began a postgraduate specialized training in the Department of Nutrition Hygiene at the National Institute of Hygiene (today: National Institute of Public Health - National Institute of Hygiene), under the supervision of Professor Dr. Aleksander Szczygieł. In the years 1950-1957, he worked as a research assistant, assistant professor, and finally head of the Laboratory of Nutrition Physiology.

At the National Institute of Hygiene (PZH) in Warsaw he was engaged in research on the dietary habits and nutritional status of children and youths. He conducted studies on vitamin A and carotenoids, publishing a quantitative method for determining these compounds in plant products. He also conducted *in vivo* studies on the bioavailability and biological activity of provitamin A. As a talented young scientist specializing in the physicochemical and biological analysis of vitamin A and carotenoids, he received the Minister of Health's award in 1952 for his research.

The centenary jubilee of Professor Stanisław Berger's birth is an opportunity to recall his association with the National Institute of Hygiene, which remains very close due to his extended collaboration with the scientific journal "Roczniki Państwowego Zakładu Higieny" (Annals of the National Institute of Hygiene), which has been continuously published since 1950 until today. From 1954 to 1955, he served as the secretary of the editorial board, and in 1984, he became a member of the editorial committee of the journal as the editor of the Nutrition Hygiene Section. In addition to reviewing articles on nutritional topics, Professor Berger prepared summaries of scientific papers from "The British Journal of Nutrition". Due to limited and difficult access to foreign scientific literature, these abstracts, published from 1984 to 1988 in "Roczniki Państwowego Zakładu Higieny", constituted an invaluable source of knowledge about research and

scientific worldwide progress for scientists in Poland. After the journal was converted to an English language version in 2013, Professor Berger became a member of the International Scientific Committee. He currently holds an Honorary Membership in this Committee.

Regardless of work at the National Institute of Hygiene, Professor Stanisław Berger was also a senior assistant at the Department of Botany and Technical Microbiology in the Faculty of Chemistry at the Warsaw University of Technology from 1950 to 1952. In 1951, based on his doctoral thesis entitled "Seasonal Variations in the Content of Vitamin A and Carotene in Cow's Milk," he obtained his PhD at the Maria Curie-Skłodowska University (UMCS) in Lublin. In 1954, he was appointed an associate professor at the Department of Animal Nutrition at the Warsaw University of Life Sciences (SGGW). In 1957, as a young and promising scientist still working at the National Institute of Hygiene, he was awarded a one-year research fellowship from the Rockefeller Foundation to study at Cornell University in the United States. His work at the National Institute of Hygiene and the international collaboration significantly influenced his further academic career.

After returning from the fellowship, Stanisław Berger assumed the leadership of the newly established Department of Human Nutrition Technology and Hygiene at the Faculty of Agriculture at the Warsaw University of Life Sciences (SGGW). He was awarded the academic title of associate professor in 1961 based on his habilitation thesis entitled "Assessment of Carotene as Provitamin A based on its absorbability and conversion to cxerophthol under carious experimental conditions". In 1966, he attained the title of associate professor in agricultural sciences, and in 1976, he was granted the title of full professor.

A particularly significant phase of Stanisław Berger's professional career unfolded at SGGW during the years 1961-1962. During this time, along with Professor Eugeniusz Pijanowski, he played a key role in the establishment of the Faculty of Agricultural and Food Technology. He undertook the role of secretary of the newly formed Faculty Council and Vice-Dean from 1965 to 1966.

Many reviewers evaluating Professor Berger's scientific and educational contributions emphasize his impact on the field of human nutrition, including nutritional physiology, the role of protein, vitamin A and its precursors. They particularly commend Professor Berger's method for assessing vitamin A. Pioneering research on vitamin A precursors, its metabolism, and its utilization based on dietary protein content were predominantly conducted by the Professor in the United States. The modification of Professor Berger's micrometric method for spectrophotometric determination of carotene and vitamin A in blood is still ued in laboratories.

Professor Berger was also involved in evaluating the diet and nutritional status of various population groups, including youths being nourished collectively and individually. It's worth noting the comprehensive and collaborative research undertaken at the Department of Human Nutrition Technology and Hygiene under his leadership. This initiative, spearheaded by Professor Berger, encompassed multi-faceted research carried out in scientific cooperation between Poland and the United States. This research revolved around the accessibility and utilization mechanisms of carotene and vitamin A.

Furthermore, Professor Berger took part in other research endeavors of great significance, not only for the advancement of science but also for the economy. He authored a study for the Ministry of Agriculture on the possibilities and optimization of protein balance for the nutritional needs of the population in Poland.

During his work at Maria Curie-Skłodowska University in Lublin (1946-1949) and as well at the National Institute of Hygiene (PZH) in Warsaw (1950-1957), Warsaw University of Technology, Faculty of Chemistry (1950-1952), and Warsaw University of Life Sciences (SGGW) (1954-1999), he published over 500 original scientific papers, review articles, and popular science articles in the field of food science and human nutrition, with a particular focus on the role of vitamin A and protein metabolism. He also disseminated knowledge on human nutrition, economics, and food policy both domestically and internationally. A significant part of these works were published in foreign languages, which brought Professor Berger's name to prominence in international scientific field and resulted in numerous invitations to lecture at foreign universities and other research institutions.

Prof. DSc Włodzimierz Kamiński, while evaluating the scientific, educational, and organizational achievements of Professor Stanisław Berger, in connection with the initiation of proceedings to confer an honorary doctorate, emphasized that for nearly 50 years, Stanisław Berger's name has been recognized and highly esteemed in Polish and global food technology and nutrition science. His accomplishments are particularly impressive and deserving of the highest recognition for his role in promoting Polish science on the international stage.

# Pioneer of human nutrition science in Poland

The term "human nutrition," understood as a scientific discipline, was formulated many years ago by Professor Stanisław Berger, contributing to the institutionalization and conceptualization of this theoretical and research-oriented field. This led to the establishment in 1977 of the first scientific institution dedicated to human nutrition in Central Europe. Initiated by Professor Stanisław Berger, the Faculty of Human Nutrition and Rural Household Management was established at the Warsaw University of Life Sciences.

The conceptualization of human nutrition as a scientific discipline was formulated by Professor Berger in the form of the first definition of the science of human nutrition, along with its fundamental conceptual apparatus, including the terminology specific to this field. In the formulated definition of the science of human nutrition, its author emphasized that it is a scientific discipline dealing with the interrelationship between food and the human body at various levels: molecular, cellular, tissuebased, encompassing the entire organism, as well as populations.

The research work of the inventor of the definition of human nutrition science fully accounts for all these levels of interdependencies between food and the human body. They also find reflection at the population level. An example of such an approach is Professor Berger's viewpoint regarding food policy, which he stated, "is fundamentally based on nutritional and health categories". In this context, Professor Berger wrote that the role of science, education, and knowledge dissemination should increase to better understand the connection and impact of dietary habits on human development, health, and physical well-being. He argued that the cost often paid for nutritional deficiencies is higher than the expenses associated with preventing these adverse effects. Therefore, a consumption model should be created that serves the complete satisfaction of the needs of all citizens. Professor Berger advocated for increased investment in health education and the creating of lifestyles .

# An authority in the scientific community both nationally and internationally

In a speech dedicated to celebrating Professor Stanisław Berger's 80th birthday in 2003, Prof. Dr. habil. Jan Gawęcki asserted that the Jubilarian fully deserves the title of authority due to the following arguments:

- 1. Professor Stanisław Berger laid the foundation for the science of human nutrition, closely intertwined with the science of food. During the 1960s and 1970s, he published pioneering work on vitamin bioavailability, and in the subsequent decades, he charted paths for nutritional education and food policy.
- 2. He established the country's first Department of Human Nutrition Technology and Hygiene, as well as the first and still the only academic faculty educating in the field of human nutrition. Under

his guidance, nearly 500 individuals earned their Master of Engineering degrees, several attained doctorates, and a few achieved habilitation. Several outstanding professors emerged from his guidance. A substantial group of individuals from Poland and abroad owe their scientific careers, to a greater or lesser extent, to him. He reviewed dozens of doctoral and habilitation dissertations, as well as professorial applications, combining critical assessment of the work with kindness toward the creator.

3. Professor Berger's boundless energy and imperative to act ensured that over the course of 50 years, there hasn't been a significant nutritional matter, initiative, institution, or organization in which his name didn't appear – from campaigns like "Milk for Students" to his involvement in international organizations such as FAO, WHO, and UNICEF. His wisdom and captivating personality led to entrusting him with the most responsible roles, from being the head of the Department and Dean of the Faculty to Vice President of the International Union of Nutritional Sciences. His companionship and friendship were particularly valued.

It's worth noting that a characteristic distinguishing Professor Berger from many other scientific luminaries is his directness and lack of distance, particularly evident in personal interactions. Professor Stanisław Berger's authority is not only derived from his indisputable role in shaping the science of human nutrition, his research accomplishments, and publications, but also from his remarkable personality, his ability to forge connections with people, and his immense openness in interpersonal relationships.

# A lecturer, a students' friend and an indefatigable promoter of human nutrition science

Professor Stanisław Berger is not only an outstanding scientist but also a remarkable educator. He began his academic teaching career in 1946 at Maria Curie-Skłodowska University in Lublin and continued at the Warsaw University of Life Sciences (SGGW) from 1954. He conducted teaching activities in subjects such as animal nutrition, nutrition physiology, human nutrition biochemistry, selected aspects of nutrition technology, conducted seminars and specialized workshops for students. In his teaching, he always employed innovative forms of collaboration with students.

Professor Berger supervised the graduation of nearly 400 master's and engineering students, as well as 16 doctoral candidates, of which five have subsequently attained habilitation and become professors at SGGW or other Polish and foreign scientific institutions.

His unique approach towards students was characterized by treating them as individuals and with

a paternal attitude. He recognized them as the most essential and inherent value of the university. In return, students reciprocated this attitude with warmth, trust, and appreciation for Professor Berger as an authority figure and a true role model of an academic teacher and mentor.

Professor Berger based his teaching not only on Polish and international source materials but also on his own scripts and textbooks. His contribution to teaching population nutrition and training specialists in the field of human nutrition in Poland and worldwide is highly significant. His co-authored book "Polityka i organizacja żywienia ludności" (PWE, 1980) stands as the first academic textbook on this subject in Poland (and not only in Poland), for which the Minister of Science, Higher Education, and Technology awarded a scientific prize.



Photo 2. Professor Stanisław Berger at work in his office (Photo from the collection of SGGW)

Professor Stanisław Berger disseminated knowledge in the field of human nutrition by collaborating with economic and cultural entities, as well as through the press, radio, and television. He delivered numerous scientific and popular science presentations. The act of popularizing knowledge in human nutrition took on various forms, all guided by the principle of presenting scientific topics in a comprehensible manner for everyone, regardless of their academic background. This same approach is evident in Professor Berger's "Nutritional Rules," which is based on the widely recognized "7 U" principles of proper nutrition. The sequence of these principles is as follows: Variety; Moderation; Regularity; Skillful preparation of meals; Physical activity; Avoiding excess; Smiling. In Polish, '7U' reads: Urozmaicenie; Umiarkowanie; Uregulowanie; Umiejętne przygotowanie potraw; Uprawianie sportu; Unikanie nadmiaru; Uśmiechanie się.

Professor Stanisław Berger's "7 U" as the foundation for healthy eating is a recipe for a healthy lifestyle, incorporating physical activity and smiling, as well as a recipe for longevity. He himself is a living example of this, always wearing a smile and maintaining an optimistic outlook on life and people. These principles are widely propagated by Professor Berger's protégés in educational programs concerning human nutrition.

# Organizer of scientific life in Poland and abroad

Professor Stanisław Berger exceptional scientific achievements and organizational skills on a global scale were rooted in his prior experiences and specialized scientific practices in countries like the United Kingdom, the Netherlands, Czechoslovakia, Sweden, and the USSR. His work as a Rockefeller Foundation fellow at Cornell University in the USA was particularly influential. He also made the most of the unique opportunity afforded by his work at the FAO Secretariat in Rome (1967-1971), where he was involved in the World Agricultural Development Plan. Later, as the Head of the Nutritionists Training Section, he provided advice and training to nutritionists in developing countries.

His active involvement in prestigious international organizations, numerous presentations at international conferences, and lectures at foreign universities (in the USA, India, the UK, Italy, and Hungary) led Stanisław Berger to attain an indisputable global position in the field of human nutrition science. Notable international activities that brought him recognition include:

- Active membership in the International Union of Nutritional Sciences (IUNS) since 1957, where he served as Vice President from 1993 to 1997.
- Vigorous participation in the Federation of European Nutritional Societies (FENS), where he served as President from 1987 to 1991, and in the European Academy of Nutritional Sciences (EANS) as a member.
- Involvement in various United Nations agencies, such as FAO, UNICEF, WHO, ILO, and IAEA, and co-founding the United Nations University (UNU), in Tokyo.

Professor Berger has been, or is, a member or chairperson of several scientific organizations in Poland, including the Polish Biochemical Society, Polish Nutritional Science Society, Polish Food Technologists Society, as well as committees related to Food Technology, Food Chemistry, and Human Nutrition under the Polish Academy of Sciences, the Polish Committee for the International Union of Nutritional Sciences, the National Committee of the International Council for Science (ICSU) under the Polish Academy of Sciences, and the Food Economy Committee of the Polish Scientific Society for Food Production (NOT).

He has served as an editor in numerous national scientific journals (e.g., Roczniki Państwowego Zakładu Higieny - Annals of the National Institute of Hygiene, Żywienie Człowieka i Metabolizm, Polish Journal of Food and Nutrition Sciences, Przemysł Spożywczy) and foreign scientific journals (e.g., International Journal of Food Sciences and Nutrition, UK; Nutrition - the International Journal of Applied and Basic Nutritional Sciences, USA).

For many years, he has been disseminating knowledge about food and nutrition through radio and television broadcasts, which earned him the Radio and Television Award in 1991. As a co-founder of the Polish Nutritional Science Society in 1980, he became its first chairman (currently Honorary Chairman). He held the position of chairman of the Polish-Swiss Society from 1983 to 1996 and served as the president of the newly established Association of Polish Experts of the United Nations - POLSENZ from 1992 to 1996. From 1999 to 2002, Professor S. Berger was a member of the Board of the Council of Scientific Societies under the Presidium of the Polish Academy of Sciences.

He is also the patron of the Gastronomic Schools Complex in Warsaw at Majdańska street 30/36, and the ceremony for bestowing his name upon the institution took place on September 1, 2011. In recognition of his immense contributions to the field of human nutrition science in Poland and worldwide, Professor Stanisław Berger was awarded an honorary doctorate by SGGW in 1995. In 1996, he was elected a Fellow of the Royal Society of Medicine (UK) and served as Vice



Photo 3. Professor S. Berger awarded the Commander's Cross with Star of the Order of Polonia Restituta (Photo from the collection of SGGW)

Chairman of the Council of the Federation of Polish Medical Societies from 1996 to 2003.

Professor Berger's name and information about his scientific, professional, and organizational activities are included in various foreign bibliographic publications.

He has been honored with numerous state and ministerial decorations and awards, including the Commander's Cross with Star and the Knight's and Officer's Crosses of the Order of Polonia Restituta, the Medal of National Education, the B. Rumiński Medal, and the Gold Honorary Badge of the Chief Technical Organization (NOT). He was also honored three times with the Minister's Award.

# **A Living Legend**

Even though Professor Stanisław Berger has been retired for many years, he remains present in the life of his *Alma Mater*, especially through the group of academic teachers he established. They strive to creatively develop the ideas initiated by Professor Berger within the Institute of Human Nutrition Sciences and the Faculty of Human Nutrition at the Warsaw University of Life Sciences, following the current structure of the university.



Photo 4. Professor S. Berger celebrated his 99th birthday (2022) (Photo from the collection of SGGW)

Professor Stanisław Berger's undisputed authority ensures that he is not only active within the circles of his University but also on the international stage. An anecdote from one of his former students illustrates this: during the 22nd IUNS-ICN International Congress of Nutrition in Tokyo, Japan, in December 2022, the student randomly met an elderly man. Upon learning that she was from Poland, the man immediately smiled and said, "Stan Berger." Once he discovered that she was a protégé of Professor Berger, they engaged in a friendly conversation. This man turned out to be Prof. Mohd Ismail Noor, who personally knew Professor Berger and recalled their collaboration in the International Union of Nutritional Sciences. He particularly remembered Professor Berger's memorable speech during the 19th IUNS-ICN congress in Bangkok in 2009, during which Professor Berger was honored with the title of "Living Legend". This heartfelt encounter underlined how important Professor Stanisław Berger is to the community of nutritionists.

Professor Berger's professional life has earned him unquestionable fame, placing him on the highest pedestal in the field of human nutrition science. He serves as an unreachable model for us, a treasury of knowledge and wisdom. On the occasion of his 100th birthday jubilee, we wish Professor Berger many more years of life filled with health and smiles.

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https://roczniki.pzh.gov.pl/

Rocz Panstw Zakl Hig 2024;75(3):229-236

https://doi.org/10.32394/rpzh/192206

# ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF MOROCCAN WILD EDIBLE PLANTS SELECTED BASED ON ETHNOBOTANICAL EVIDENCE

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# ABSTRACT

**Background.** Despite the extensive literature focusing on identifying novel antimicrobials of plant origin, little work has been undertaken to examine the antimicrobial activity of wild edible plants.

**Objective.** The current research aimed to determine the *in vitro* antimicrobial activity of methanolic extract of some common wild edible plants.

**Material and Methods.** Disc diffusion and broth micro dilution methods were used to evaluate the antimicrobial activity of extracts of *Mercurialis annua*, *Ziziphys lotus*, *Rubia peregrina*, *Origanum vulgare*, *Papaver rhoeas*, *Foeniculum vulgare*, and *Dysphania ambrosioides* against known human microorganisms' pathogens.

**Results.** The result indicated that most of the extracts exhibited a range of *in vitro* growth inhibitory action against all bacterial strains and yeasts tested with inhibition zones ranging from 11 mm to 32 mm, MIC value ranging from 0.048 to 50 mg/ml and MBC and MFC values ranging from 0.048 to 100 mg/ml. Among the seven plant extracts tested, *O. vulgare* was the most effective showing high antimicrobial activity against all tested microbial strains. All plant extracts exhibited bactericidal activities against all the tested bacteria strains except for those of *R. peregrina, P. rhoeas* and *F. vulgare* which showed a bacteriostatic activity against *E. coli* and *Pseudomonas* sp. Antifungal activity was shown only by *O. vulgare, F. vulgare* and *D. ambrosioides* against both *C. albicans* and *C. neoformans*.

**Conclusion.** These findings highlight the potential of wild edible plants to control human pathogenic microbes and demonstrate that these plants could be used as starting points for the development of novel antimicrobial compounds.

Keywords: antibacterial activity, antifungal activity, wild edible plants, MIC, MBC, MFC, Morocco

# **INTRODUCTION**

The most relevant approach known to combat microbial diseases is the use of antibiotics. The latter are a class of chemicals with potent antimicrobial effects used to treat diseases related to pathogenic infections [1]. However, recent decades have seen indiscriminate use, overuse, and unnecessary prescription of antibiotics leading to antibiotic resistance and the spread of microorganisms [2]. As resistance to many synthetic antibiotics increases, a need to find effective, newer, less toxic antimicrobial drugs is of paramount importance. Plants have been recognized since ancient times, as valuable sources of natural chemicals with antimicrobial activities, which can be used as a cheaper alternative to expensive synthetic antibiotics [3, 4]. Morocco ranks among the countries with one of the richest plant diversities in the Mediterranean area [5]. Wild edible plants (WEPs) are a crucial element of this diversity. These plants are rich in protein, fiber, minerals and vitamins and their consumption helps fight malnutrition and food insecurity [6]. Additionally, WEPs are considered an excellent natural source of bioactive molecules associated with positive effects against various chronic diseases such as oxidative stress, cancer,

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Publisher: National Institute of Public Health NIH - National Research Institute

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cardiovascular diseases, immune dysfunctions and other diseases [7-9]. Nevertheless, most studies in Morocco and other parts of the world focused on medicinal and aromatic plants. On the other hand, studies on WEPs and their biological properties have been somewhat neglected by the scientific community. To this end, the aim of this study was to evaluate *in vitro* antibacterial and antifungal activities against common microbial pathogens of methanolic extracts of some selected wild edible plants (WEPs). The selection of plants was based on their traditional use in the treatment of several types of infectious diseases including diarrhea, respiratory tract infections, skin and wound infections, etc.

# **MATERIAL AND METHODS**

## **Collection of plant material**

The different parts of wild edible plants selected (Table 1) were collected in March 2022, from Sidi Bennour province (32° 39' 8.50" N, 8° 25' 39.68" W) in central Morocco. The plants were identified by a taxonomist in Department of Biology, Chouaïb Doukkali University, (El Jadida, Morocco) and voucher specimens of plants have been deposited in herbarium of the same department. Table 1 shows the botanical name, family, parts used, food use, and ethnomedicinal use of plants under this study.

#### **Preparation of crude extracts**

After collection, the samples were washed with distilled water and dried in an oven at 37°C for one week. The completely dried materials were ground

into fine powder, and then extracted by maceration method with methanol. The obtained extract was subsequently filtered through a Whatman No. 1 filter paper, and the filtrate was evaporated under reduced pressure using a Rota evaporator. Dried extract was stored in refrigerator at 4°C till further use.

# Antimicrobial activity

#### Microorganism strains

Extracts obtained from different plants were tested for antimicrobial activity against six strains of bacteria (three strains of Gram-positive bacteria) and three strains of Gram-negative bacteria) and two strains of fungi provided from the Institute Pasteur Paris Collection (CIP) and the American Type Culture Collection (ATCC): *Enterococcus faecalis* (ATCC19433), *Staphylococcus aureus* (ATCC25923), *Bacillus subtilis* (ATCC 66331), *Escherichia coli* (CIP54127), *Citrobacter freundii* (ATCC8090), *Pseudomonas* sp., *Candida albicans* (48.72), and *Cryptococcus neoformans* (CIP 960).

## **Disk Diffusion Assay**

Screenings of extract for antimicrobial activity was done by the disc diffusion method [18]. In order to do this, bacterial and fungal sterile physiological saline suspension was prepared to 0.5 of the McFarland standards (1.5×10<sup>8</sup> CFU/mL) from bacterial colonies grown on nutrient agar overnight at 37°C and yeast grown on Sabouraud at 37°C for 48 h. Then the bacterial suspensions were spread on Mueller Hinton Agar and the yeast suspensions were spread on Sabouraud Dextrose Agar. A paper discs (6 mm

Table 1. Wild edible plants tested for their antimicrobial activity in the study

Botanical name (family)	Common name	Parts used	Food uses	Ethnomedicinal uses
Origanum vulgare L. (Lamiaceae)	Zaatar	Leaves	Arome/Spices/ Drink [10]	Cold-respiratory problems, antiseptic, diarrhea, influenza, cough, intestinal parasites [10, 11]
Papaver rhoeas L. (Papaveraceae)	Belaaman	Aerial parts	Vegetables, Spices [12, 13]	Urogenital disorder-cough-rheumatism- dermatological problems, pulmonary infection [14]
Foeniculum vulgare Mill (Apiaceae)	Besbas Beldi	Aerial parts	Vegetables/Arome/ Snack [10, 12, 13]	Asthma, renal disease, eczema, cough, parasitic disease [15]
Dysphania ambrosioide L. (Amaranthaceae)	Mkhinza	Leaves	Vegetables/Arome [13]	Anthelmintic, antidiarrheic, colds, detersive, gynecological disorders, influenza [10, 11, 14]
Mercurialis annua L. (Euphorbiaceae)	Horriga lmelssa	Aerial parts	Vegetables/Spices [13]	Internal parasitosis, wound healing, rheumatism [12, 16]
Rubia peregrina L. (Rubiaceae)	ELfowa	Roots	Spices, Drinks [10, 12, 13]	Hepatitis, liver problems [17]
Ziziphus lotus L. Lam (Rhamnaceae)	Nbeg/Sedra	Fruits	Snack [10, 12]	Anthelmintic, wounds healing, urinary tract infections, pulmonary infection [10, 14]

diameter) that were impregnated with 60  $\mu$ L of extract at concentration of 100 mg/ml, were placed on the inoculated agar surface. Petri dishes were left for 2 h at 4°C to allow the diffusion of the extract before incubation at 37±2°C for 18-24 h for bacteria and at 28±2°C for 48 h for the yeast activity. After incubation, the diameters of the inhibition zones were measured in mm. Fluconazole and Ampicillin were used respectively as positive controls and methanol as negative control.

# Determination of the minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) of F. vulgare extract was determined by quantitative the micro-dilution method using resazurin as viability indicator [19]. Volume of 100 µl aliquots of Mueller-Hinton broth were placed in sterile 96-microwell plates and 50 µL of each extract, were adjusted to (50; 25; 12.5; 6.25; 3.12; 1.56; 0.8; 0.4; 0.2 and 0.1 mg/ml), and added to the wells. To each well containing the mixture, was added 50 µl of the Microbial suspension (1×107 CFU/ml) prepared in Mueller-Hinton broth for bacteria and prepared in Sabouraud broth for yeast. The plates were incubated for 24-48 h at 37±2°C and for 48-96 h at 27±2°C respectively. After incubation, 5 µl of resazurin (1 mg/ml) was added to each well and the incubation continued for 45 min. Finally, the MIC was recorded.

# Minimal bactericidal concentration (MBC) and minimal fungicidal concentration (MFC) determinations

All the well plate showing no growth after MIC tests were reinoculated for the determination of the MBC and MFC. The broths were incubated according to growth requirement of each microorganism. The absence of growth in the recovery medium was evidence of bactericidal and fungicidal activities. Moreover, the ratio MBC/MCI and MFC/MCI of each sample was calculated to assess the antimicrobial power. If the ratio  $\leq$ 4, the effect is bactericidal/fungicidal and when the ratio >4, it's bacteriostatic/fungistatic [20].

## RESULTS

# Antimicrobial activity

Screening of the antimicrobial activity of methanolic extracts of the studied plants was performed initially by the disc diffusion method against known human microorganisms' pathogens. These microorganisms were frequently encountered in infectious diseases. The results were summarized in Table 2. It was observed that except *Z. lotus* all plant extracts studied exhibited varying degrees of antimicrobial activity

against all bacterial strains and yeasts tested with inhibition zones ranging from 11 mm to 32 mm. According to Aldoweriej et al., 2016 [21], the crude extracts tested have high antimicrobial activity when the inhibition zone is >15 mm; moderate antimicrobial activity if it is of 10-14 mm; low antimicrobial activity if the zone is 7-9 mm.

As can be seen, *O. vulgare* was the most effective among the seven plant extracts tested. It showed high antibacterial activity against all Gram-negative bacteria, Gram-positive bacteria tested with zones ranging from 22 mm to 32 mm.

	Gra	m-positive bac	teria	Gran	n-negative bac	teria	Yea	sts
MICTODIAL CONTROL strains	S. aureus	E. feacalis	Bacillus sp.	C. freundii	E. coli	Pseudomonas sp.	C. albicans	C. neoformans
F. vulgare	19±1.72	$20 \pm 1.03$	13±1.00	17±0.56	16±1.65	17±1.03	12±1.23	14±0.63
P. rhoeas	15±0.5	$14{\pm}1.00$	13±1.45	15±0.23	13±0/33	11±1.17	IN	IN
O. vulgare	$31 \pm 0.00$	$30{\pm}1.03$	32±0.37	32±0.55	22±0.65	28±1.00	$16 \pm 0.17$	18±0.55
M. annua	17±1.13	$18 \pm 0.34$	17±0.19	17±1.52	$18\pm 1.03$	$16 \pm 0.39$	IN	IN
Z. lotus	IN	IN	IN	12	IN	IN	IN	IN
D. ambrosioides	15±0.3	$14\pm 0.52$	18±0.13	$11\pm 0.22$	12±0.52	12±0.12	$16 \pm 0.45$	$14 \pm 0.78$
R. peregrina	16±0.5	$14{\pm}0.7$	13±0.85	$14{\pm}0.1$	$16 \pm 0.38$	14±0.12	IN	IN
Amplicillin	$26 \pm 0.00$	27±0.0	27±0.00	25±0.00	28±0.00	$24{\pm}0.00$	ND	ND
Fluconazol	ND	ND	ND	ND	ND	ND	$26 {\pm} 0.00$	27±0.00
NI – no inhibition;	ND – not deter	mined						

The highest antibacterial activity of *F. vulgare* was against *E. faecalis* (20 mm) and the highest antibacterial activity of *P. rhoeas* and *R. peregrina* was against *S. aureus* (15 mm, 16 mm respectively). *M. annua* displayed also a high antibacterial activity against *E. faecalis* as well as *E. coli* (18 mm). *D. ambrosioides* exhibited high antibacterial activity against *Bacillus* sp. (18 mm). Antifungal activity was shown only by *O. vulgare*, *F. vulgare* and *D. ambrosioides* against both *C. albicans* and *C. neoformans*. None of the other plant extracts showed antifungal activity.

# Minimum inhibitory and bactericidal/Fungicidal concentrations of extracts

The tests of the studied extracts efficacy on the microbial strains used, was determined by measuring the minimum inhibitory concentration, MIC was determined for only microorganisms which showed a zone of inhibition and were sensitive to the plant extracts in the preliminary test using the disc diffusion method. MIC values varied from 0.048-50 mg/ml. Most active plants against test microorganisms are shown in Table 3. The most active plant against *E. coli, E. faecalis, Bacillus* sp., *Pseudomonas* sp. and *C. neoformans* was *O. vulgare* (0.048, 0.048, 0.097, 0.097 and 6.25 mg/ml respectively), against *C. freundii* were *M. annua* and *O. vulgare* (0.39 mg/ml), against *E. coli* was *F. vulgare* (0.78 mg/ml), against *C. albicans* was *D. ambrosioides* (6.25 mg/ml).

More precise data on the antimicrobial properties were obtained through the determination of bacteriostatic/fungistatic and bactericidal/fungicidal concentrations. As reported in Table 3, MBC and MFC values ranged from 0.048 to 100 mg/ml. As can be also seen all plant extracts exhibited bactericidal activity against bacteria strains except for those of *R. peregrina*, *P. rhoeas* and *F. vulgare* which showed a bacteriostatic activity against *E. coli* and *Pseudomonas* sp. Antifungal activity was shown by *O. vulgare*, *F. vulgare* and *D. ambrosioides* against both *C. albicans* and *C. neoformans*.

#### DISCUSSION

The current problem associated with antibiotic resistance represents a serious threat to public health, requiring surveillance, which continuously challenges the healthcare sector in a large part of the world [22, 23]. This problem is compounded by the decreasing effectiveness and increasing toxicity of antibiotics; which confront the scientific community with the obligation to seek new alternatives. Wild edible plants could be those alternatives. In the present study, the antibacterial and antifungal activities of seven

Moroccan wild edible plants were investigated against six bacterial and two fungal reference strains.

In accordance with previous findings, it appears that Gram-positive bacteria are more sensitive to the extracts examined in this study than Gram-negative bacteria [24]. This difference in sensitivity could be related to the different composition of the Gramnegative and Gram-positive bacteria cell walls [25].

Findings from the current study revealed that except Z. lotus, all plant extracts tested showed varying degrees of antimicrobial activity against any of the microorganisms tested. A maximum activity against all microorganism tested was also obtained with the extracts of the plant O. vulgare. In addition, high MIC, MBC and MFC values are obtained with these extracts as well as a bactericidal and fungicidal activities against all tested strains. In accordance with these results, other studies have reported an antimicrobial activity of methanolic extract of O. vulgare [26], while, contrary to the present study data, no antimicrobial activity against E. coli and C. albicans were found by these studies. These discrepancies could be related to strain specificity or to plant geographical variations. O. vulgare was also found to be significantly active against S. aureus, P. aeruginosa and E. coli. Likewise, Mehreen et al., 2016 [27]. Another investigation carried out in Peru, showed that ethanolic extract of O. vulgare has antibacterial activity against S. aureus, E. coli and P. aeruginosa. [28]. The antimicrobial potential of O. vulgare could be explained by its high content in monoterpenic hydrocarbons and in phenolic compounds such as carvacrol, thymol, p-cymene and 1-octacosanol [29, 30].

The extract from the Plant F. vulgare was found to have antimicrobial activity against all bacteria and fungi, with the highest activity obtained against E. faecalis. In agreement with these results, Aboukhalaf et al., 2020 [24] and Zellagui et al., 2011 [31] are have previously reported an inhibitory effect of extracts from F. vulgare aerial part against S. aureus, E. coli, E. faecalis and, C. albicans. Antagonistic activities of aqueous and organic extracts of F. vulgare against some human pathogenic bacteria such as E. faecalis, S. aureus, E. coli, P. aeruginosa, Salmonella typhi, and Shigella flexneri are also found by Kaur and Arora 2008 [32]. Another study showed the efficacy of F. vulgare extract against C. albicans [33]. Studies on F. vulgare identified the phenylpropanoid derivativedillapional as the compound responsible for the observed antimicrobial activity, scopoletin which is a coumarin derivative was also identified [34].

Another selected plant examined in this study is *D. ambrosioide*. It is considered as an important wild edibleplantwellknownandwidelyusedforthetreatment of respiratory, urogenital, gastrointestinal, vascular and nervous disorders, and for metabolic disturbances

Table 3. The antimid	crobial activity para	meters						
Plant s	pecies	L	<i>u</i>	012.200	M	D monocutin 2	D ambuoriation	7 104000
Microbi	ıl strains	r. vuigure	r. rnoeus	U. Vuigare	м. аппиа	n. peregrina	D. amorosioiaes	Z. 101US
	MIC	0.39	3.125	0.048	0.097	25	25	ND
ŭ	MBC	0.39	6.25	0.048	0.097	100	50	ND
D. aureus	MBC/MIC	1	2	1	1	4	2	ND
	Decision	Bc	Bc	Bc	Bc	Bc	Bc	ND
	MIC	0.097	6.25	0.048	0.097	12.5	12.5	ND
L frankis	MBC	0.097	12.5	0.048	0.097	25	12.5	ND
E. Jeucaus	MBC/MIC	1	2	1	1	2	1	ND
	Decision	Bc	Bc	Bc	Bc	Bc	Bc	ND
	MIC	6.25	12.5	0.097	0.39	25	12.5	ND
	MBC	6.25	25	0.097	0.78	50	12.5	ND
bacuus sp.	MBC/MIC	1	2	1	2	2	1	ND
	Decision	Bc	Bc	Bc	Bc	Bc	Bc	ND
	MIC	0.78	6.25	0.39	0.39	1.56	12.5	ND
	MBC	0.78	12.5	0.39	0.78	6.25	12.5	ND
C. Jreunau	<b>MBC/MIC</b>	1	2	1	2	4	1	ND
	Decision	Bc	Bc	Bc	$\operatorname{Bc}$	Bc	Bc	ND
	MIC	0.78	6.25	1.56	3.125	12.5	25	ND
L	MBC	3.125	50	3.125	6.25	100	50	ND
E. COII	MBC/MIC	2	8	2	2	8	2	ND
	Decision	Bc	Bs	Bc	Bc	Bs	Bc	ND
	MIC	0.39	12.5	0.097	0.39	12.5	12.5	ND
	MBC	6.25	100	0.197	0.78	100	12.5	ND
rseuuomonus sp.	MBC/MIC	16	8	2	2	8	1	ND
	Decision	Bs	Bs	Bc	$\operatorname{Bc}$	Bs	Bc	ND
	MIC	25	ND	25	ND	ND	12.5	ND
C albianc	MBC	50	ND	25	ND	ND	25	ND
C. albicans	MBC/MIC	2	ΠN	1	ND	ΠN	2	ND
	Decision	$\mathbf{Fs}$	ND	Fs	ND	ND	Fs	ND
	MIC	12.5	ND	9	ND	ND	25	ND
C unoformana	MBC	50	ND	12.5	ND	ND	50	ND
C. neojormans	MBC/MIC	4	ND	2	ND	ND	2	ND
	Decision	$\mathbf{Fs}$	ND	$\mathbf{F}_{\mathbf{S}}$	ND	ND	Fs	ND
Bc - bactericidal; B	s - bacteriostatic; Fs	s - fungistatic; ND	- not determined					

such as diabetes and hypercholesterolemia [35]. Its leaves are used in the preparation of traditional dishes as dietary condiment [35]. The results concerning the antimicrobial activity against Gram-positive, Gramnegative bacteria and yeasts observed in this study are contradictory to those of another study reporting no antimicrobial activity of *D. ambrosioides* against a wide range of microbial strains, namely *S. aureus*, *E. coli*, *P. aeruginosa* and *C. albicans* [35].

*P. rhoeas* was reported to produce diverse chemicals including fatty acids, hydroxyl-phenol groups, anthocyanin, flavonoid (flavon-3-ols, flavones and tannins), alkaloids typical rhoeadine groups that show antimicrobial activity [36, 37]. The extract of *P. rhoeas* was found to have moderate antimicrobial potential against both Gram-positive and Gramnegative bacteria, this result is in accordance with those found by Aboukhalaf et al., 2020 [24]. However, the lack of sensitivity of *C. albicans* and *C. neoformans* to *P. rhoeas* observed in the present study is in disagreement with other literature findings that showed an antifungal activity of *P. rhoeas* against *C. albicans* [24, 38, 39].

Concerning *M. annua*, bactericidal activities against all tested bacteria, are obtained with the extract of this plant. The present study results are different from those found in other researchers reports on antimicrobial activity of *M. annua* [24, 40]. This difference could be due to different factors related to the climate, the season, the geographical location, the time of the plant harvest, the part of the plant used for analysis, as well as the solvents chosen and the extraction procedure used.

For *R. peregrina*, there is only one report on antimicrobial activity by Ozgen et al., 2003 [41], indicating a potential inhibitory effect of this species against *Bacillus subtilis*, *S aureus* and *E. coli* and a limited inhibitory effect on *C. albicans*, this result is similar to our finding.

# CONCLUSIONS

In conclusion, there is a strong antimicrobial activity against bacteria and fungi of wild edible plants such as, *O. vulgare*, *F. vulgare*, *D. ambrosioides*, *M. annua*, *P. rhoeas* and *R. peregrina* that deserve to be considered as a prospective tool in the treatment of diseases related to pathogenic infections. These plants could, in addition, be used in food industry, as a new natural additive to maintain the quality and shelf life of food products. Further investigations are however required, to demonstrate the *in vivo* efficacy, stability, and ability of these studied extracts to control microbial strains.

# **Conflict of interest**

The authors declare no conflict of interest.

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Received: 21.07.2024 Revised: 08.08.2024 Accepted: 09.08.2024 Published online first: 10.09.2024



Rocz Panstw Zakl Hig 2024;75(3):237-245

https://doi.org/10.32394/rpzh/192523

ORIGINAL ARTICLE

# FATTY ACID COMPOSITION AND ANTIOXIDANT CAPACITY OF DEFATTED, NON-DEFATTED AND OILS EXTRACTS OF *QUERCUS ILEX* FRUIT FROM ALGERIA

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# ABSTRACT

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**Background.** The nutritional value and health-promoting properties cause the fruits (acorns) of *Quercus ilex* to have great potential for use in the food industry as functional ingredients and antioxidants source.

**Objective.** In this study, the amount of total phenolic compounds, flavonoids in different extracts (defatted, non-defatted) and composition of fatty acids in the fruits oils of *Quercus ilex* were investigated. Besides, antioxidant activity was determined.

**Material and Methods.** Fatty acids were extracted with n-hexane and determined by gas chromatography with mass spectrometry detection (GC-MS). Total phenolic and flavonoids contents in the extracts were measured spectrophotometrically and the antioxidant activities were tested by the DPPH (2,2-diphenyl-1-picrylhydrazyl), free radical scavenging assay, free radical-scavenging ABTS and total antioxidant capacity.

**Results.** The amount of total phenolic and flavonoid compounds in the defatted Q. *ilex* were 634.36±27.41 mg GAE/g DW and 96.85±2.13 mg RE/g DW, respectively. Unsaturated fatty acids were detected in higher amounts than saturated fatty acids. The primary unsaturated fatty acids of the *Quercus ilex* oil were oleic acid (65.38%), 9,12-octadecadienoic acid (16.64%) and palmitic acid (12.81%). Besides, defatted Q. *ilex* extract showed remarkable DPPH and ABTS radical scavenging activity with IC<sub>50</sub> values of 0.008±0.0008, 0.005±0.001 mg/ml respectively, while high total antioxidant capacity of the non-defatted extract with VCEAC value 0.13±0.006.

**Conclusions.** *Q. ilex* oil contained high amounts of polyphenols, high essential fatty acids and antioxidant potential for producing specific health promoting antioxidants in food and pharmaceutical industry.

Key words: Quercus ilex, total phenolics, total flavonoids, fatty acids, GC-MS, antioxidants

# **INTRODUCTION**

The Algerian ecosystem is rich in important medicinal plants and natural sources that are widely used in traditional medicine to treat various pathological phenomena. It is worth noting that Algerian medicinal plants have promising biological activities due to their unique secondary metabolites [1-9].

Oak acorns, one of the species of *Quercus* genus, are of vital importance for both humans and animals. They have been widely used as food for many thousands of years in many regions worldwide

[10]. According to Bainbridge et al. (2006) acorns were a staple food throughout Europe, the Mideast, North Africa, Asia, and North America [11]. Acorns are nutritional dense functional food with health properties. Some of the health benefits are attributed to the high level of phenolic compounds found in acorns. These phenolic compounds provide acorn fruit with high levels of antioxidants, which could have potential health benefits [10, 12]. The green oak (*Quercus ilex*) is a tree of *Fagaceae* family, it is mainly found in the western part of the Mediterranean basin. The fruits are achenes called acorns, they are a rich source of

Publisher: National Institute of Public Health NIH - National Research Institute

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carbohydrates, amino acids, proteins, lipids and sterols [13].

In Algeria the acorns of *Q. ilex* are widely used in traditional nutrition, but without knowing its biological properties attributed to their bioactive molecules such as polyphenol. There are reports on the antioxidative action of some acorn components like the skin and the endosperm that can contribute to the antioxidant ability [13]. The potential use of acorn oil appears to be promising, as indicated by a chemical composition that is rich in phytochemicals, especially sterols, tocopherols, and terpenic alcohols, suggesting possible applications in the pharmaceutical industry. α-tocopherol, a chainbreaking antioxidant that traps peroxyl free radicals, is the principal and most potent lipid-soluble antioxidant in plasma and low-density lipoprotein [14] and is frequently present in Quercus species [13]. The potential of acorn oil as antioxidant supplement could add value to an underutilized agricultural product. For long, different parts of the Quercus tree have been used as natural remedy for the treatment of gastrointestinal inflammations and disorders. Extracts from leaves, bark and wood have shown antioxidant, antimicrobial, anti-inflammatory, antitumor and gastroprotective properties, attributed to their high content of phenolic compounds [15]. Also, the intake of polyphenols is associated with their beneficial bioactivity related to cardiovascular protection, inhibiting the oxidation of low-density lipoprotein cholesterol, and to glucose metabolism, promoting absorption and helping to prevent hyperglycemia [16-18].

In recent times, acorns oil has attracted the attention of the scientific community [19, 20]. The three primary fatty acids found in acorn oil are oleic (C18:1), linoleic (C18:2), and palmitic (C16:0) acids [21]. Tocopherols and phytosterols are present in high amounts in acorns oils [19, 22, 23].

Acorns oil has been used in the dietary industry since the nineteen sixties [21]. The oil is also used in cosmetic preparations and combined with other ingredients like avocado oil and beeswax to treat skin irritation and eczema [19]. Studies have shown that acorns oil possess similar nutritional quality and physicochemical properties as olive oil [19, 24, 25, 26].

The object of this research was to measure the total polyphenol and flavonoid of (defatted, non-defatted) extracts, the fatty acid composition of the oil extract of *Quercus ilex* fruit from Algeria, and to examine the antioxidant activity using DPPH, ABTS and phosphomolybdenum tests.

# **MATERIAL AND METHODS**

# **Plant collection**

Q. *ilex* fruits were directly gathered from two or three individual trees from Laghouat of Algeria. The

mature acorns were taken and identified by the Process Engineering Laboratory, University of Laghouat, Algeria. Collected fruits (acorns) were sorted and cleared of all impurities, separated from the shell, the oak acorns were subsequently in the shade and at room temperature then powdered using electric grinder and passed through a 425  $\mu$ m sieve, then conserved for future use.

# **Oil extraction**

*Q. ilex* fruits oils were extracted using Soxhlet method. Acorn powder (10 g) was weighed into a cellulose extraction cartridge and the Soxhlet apparatus (JOAN Lab Glassware; China) containing the cartridge was fitted to a distillation flask containing 100 mL *n*-hexane. After 6 hours of extraction, the extract was filtered and dehydrated with anhydrous sodium sulphate and the solvent was evaporated under vacuum at 50°C [27]. The defatted of *Q. ilex* was left in a fume hood overnight to evaporate the remaining solvent and then stored in sealed containers at -18 to -16°C until use.

# **Preparation of phenolic extracts**

*Q. ilex* fruits were thoroughly rinsed and air dried. They were ground to fine powder and 50 g of fruits was defatted with *n*-hexane and then extracted with ethanol for 72 hours. Another 50 g of fruits (non-defatted) was soaked in ethanol for 72 hours. All the filtrates of each extract were concentrated using rotary evaporator at 40°C. The crude extracts were weighed and stored at 4°C till further use.

### **Total phenolic content**

Total phenolic content of the extract was determined by spectroscopic method using Folin--Ciocalteu's reagent [28]. A volume of 100 µL of (defatted, non-defatted) extracts were added to 200 µL of 1:10 diluted Folin-Ciocalteu's reagent and 2 mL of water. After 3 min, 1 mL of saturated sodium carbonate solution was added. After 2 hours of incubation at room temperature, the absorbance was measured at 765 nm using a UV-Vis-spectrometer (Specord 200 Plus, Analytik Jena, Jena, Germany). Simultaneously, a standard curve was prepared using various concentrations of gallic acid (ranging from 0.25 to 1 mg/mL) and subjected to the same reaction conditions. The results were expressed as milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g).

# Measurement of flavonoid content

Aluminum-chloride colorimetric assay was used to determine the total flavonoid contents in the extract as previously reported by Shengwei et al. (2019) [29]. Briefly, 1 mL of (defatted, non-defatted) extracts were mixed with the same volume of 2% aluminum trichloride (AlCl<sub>3</sub>) solution and allowed to stand for 15 min. The absorbance of the mixture was then determined at 430 nm with a UV-Vis spectrometer (Specord 200 Plus, Analytik Jena, Jena, Germany). Simultaneously, a standard curve was prepared using various concentrations of rutin (ranging from 0.01 to 0.1 mg/mL) and subjected to the same reaction conditions. The data were expressed as milligrams of rutin equivalents per gram of dry extract (mg RE/g).

# **Antioxidant Activity**

# Determination of antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical

The antioxidant activity was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. Experiments were carried out according to the method of Apriliyanti et al. (2020) [30]. The reduction of the radical is followed by a decrease in the absorbance at 517 nm. A volume of 0.2 mL of extract was put into test tubes and 1.8 mL of DPPH solution was added. The tubes were covered with parafilm and kept in the dark for 1 h. Absorbance at 517 nm was measured with a UV-Vis spectrophotometer (Jasco V-530). Each assay was carried out in triplicate.

The inhibition activity (I(%)) was calculated as follows:

$$I(\%) = (A_0 - A_1)/A_0 \times 100$$

Where  $A_0$  is the absorbance of the control,  $A_1$  is the absorbance of the extract/standard. The extract concentration providing 50% of radical scavenging activity (IC<sub>50</sub>) was calculated from the graph of inhibition percentage against extract concentration. Ascorbic acid, BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene) were used as standards.

# Determination of ABTS radical-scavenging

ABTS free radical activity was performed according to the method of Li et al. (2018) with slight modification [31]. ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6sulfonic acid)) cation was produced by reacting ABTS solution (7 mM) with potassium persulfate (2.45 mM) and then the mixture to stand in dark (12-16 hours, at the room temperature). The mixture was diluted with ethanol to give an absorbance of  $0.700\pm0.02$  at 734 nm for the study. The extract (15 µL) and ABTS solution (1.485 mL) were mixed. The mixture absorbance was read at 734 nm after the incubation period (15 min, at room temperature). The results were expressed as IC<sub>50</sub> values.

# Determination of total antioxidant capacity

Total antioxidant capacity (TAC) of extract was determined by the method reported by Kandasamy et al. (2021) [32]. The assay is based on the reduction of Mo (VI) to Mo (V) by samples and formation of green colored phosphate Mo (V) complex at acidic pH. 0.3 mL of extract was mixed with 3 mL of reaction mixture containing 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate into the test tubes. The test tubes were incubated at 95°C for 10 min to complete the reaction. After cooling at room temperature, extract absorbance was measured at 695 nm using a spectrophotometer against a blank solution.

## Fatty acids composition

The fatty acid profile was determined by derivatization, followed by gas chromatography, coupled with mass spectrometry detection (GC-MS). The methyl esters were prepared by the following procedure: the fatty acids in the oils were esterified into methyl esters by saponification with 0.5 N methanolic NaOH and transesterified with 14% BF3 (v/v) in methanol [33], then analysed by GC-MS.

#### **GC-MS** analysis

The analysis of the samples was carried out in the Technical Platform of Physico-Chemical (PTAPC-CRAPC)-Laghouat-Algeria, Analysis using a SHIMADZU GCMS-QP2020 Instruments, equipped with a fused Rxi®-5ms capillary column (Phase: Crossbond® 5% diphenyl/95% dimethyl polysiloxane). Its dimensions are: 30 m  $\times$  0.25 mm and 0.25  $\mu$ m film thickness. This column has similar phase to the following columns: HP-1ms, HP-1msUI, DB-1ms, DB-5ms, DB-1msUI, Ultra-1, VF-1ms, ZB-1, ZB-1ms and considered also equivalent to USP G1, G2, G38 phases. A volume of 0.5 µL of sample was injected in split mode (1:10). Injector and detector temperatures were maintained at 250°C and 300°C, respectively the column temperature was programmed at: 80°C fixed for 4 min then increased to 200°C with an increase increment of 3°C/min then fixed for 5 min, after that raised to 300°C with an increase increment of 10°C/min, and maintained at that temperature for 5 min. The carrier gas used was helium (99.995%) purity) with a flow rate of 1 mL/min. The mass spectrometer conditions were as follow: ionization voltage 70 eV, ion source temperature 200°C, and electron ionization mass spectra were acquired over the mass range of 45-600 m/z [34].

### Statistical analysis

All experiments were performed in triplicate. The values of different parameters were expressed as the mean  $\pm$  standard deviation (Mean  $\pm$  SD).

# RESULTS

#### Total phenolic and flavonoid contents

As shown in Table 1, total phenolic contents (TPC) are given as gallic acid equivalents by reference to standard curve. TPC of Q. *ilex* fruits showed highly significant differences ( $p \le 0.05$ ) depending on extracts. Defatted Q. *ilex* extract showed a high phenolic content (634.36±27.41 mg GAE/g of DW), which was higher than that found in non-defatted Q. *ilex* (469.92±26.00 mg GAE/g of DW).

The results of the determination of flavonoids by the aluminum chloride method are presented in Table 1. TFC were determined as rutin equivalents (RE). Flavonoids content of defatted *Q. ilex* (96.85±2.13 mg RE/g of DW) was higher (p<0.05) than that determined in non-defatted *Q. ilex* extract (84.98±9.15 µg RE/mg dry extract).

## Antioxidant capacity

# DPPH radical scavenging activity

Substances which are able to perform reduction by either hydrogen or electron-donation can be considered as radical scavengers and therefore antioxidants. The color change degree of DPPH radicals from violet to yellow upon reduction indicates the radical scavenging potential of the antioxidant. Results showed that both extracts exerted considerable dose-dependent scavenging activity on DPPH radical (Table 2).

In this research, *Q. ilex* extract was being assessed its antioxidant activity as a free-radical scavenger (DPPH test) and expressed in  $IC_{50}$  value. An  $IC_{50}$ value was defined as extract concentration to show radical scavenging activity (RSA) of 50%. The highest antioxidant activity indicated by the lowest  $IC_{50}$ , ascorbic acid, BHA (butylated hydroxyanisole) and (BHT) butylated hydroxytoluene were employed as positive controls.

However, defatted *Q. ilex* extract with  $IC_{50}=0.0080.0008 \text{ mg/mL}$ , was significantly more potent (p<0.05) than non-defatted *Q. ilex* that gave an  $IC_{50}$  value of  $0.13\pm0.04$  mg/mL, while the lowest ( $IC_{50}=0.70\pm1.07$  mg/mL) were observed in Q. *ilex* oil were found to be lower to ascorbic acid, BHA and BHT (0.0022\pm0.0003, 0.0033\pm0.0005 and 0.0042\pm0.0002 mg/mL).

#### *ABTS radical scavenging activity*

In this research, the conversions for the  $ABTS^+$ radical cation inhibition or hunting capacities of each *Q. ilex* samples in various extracts were investigated in comparison with the standard Trolox. The trial data for ABTS radical scavenging potential of each plant extract is shown in Table 2.

For the activity against ABTS radical the IC<sub>50</sub> values showed the defatted *Q. ilex* as the most active with an estimated value of IC<sub>50</sub>=0.005±0.001 mg/mL, followed by the non-defatted *Q. ilex* extract (0.006±0.001 mg/mL). The *Q. ilex* oil exhibit the lowest IC<sub>50</sub> with a value of 0.016±0.007 mg/mL. However, these values classified as slightly lower when compared with the reference substance (Trolox 0.004±0.0002 mg/mL).

Thus, the ABTS scavenging effect increased in the order of Q. *ilex* oil < non-defatted Q. *ilex* < defatted Q. *ilex* < Trolox.

# Total antioxidant capacity

PM assay is based on the reduction of phosphate Mo (VI) to phosphate Mo (V) by the sample and subsequent formation of a bluish green colored phosphate Mo (V) complex at acid pH. The phosphomolybdenum

Table 1. Total phenolic and flavonoids contents in *Q. ilex* extracts

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<i>Q. ilex</i> extracts	Total phenolic content (mg GAE/g DW)	Total flavonoids content (mg RE/g DW)
Non defatted Q. ilex	469.92±26.00	84.98±9.15
Defatted Q. ilex	634.36±27.41	96.85±2.13

Table 2. Antioxidant activity of extracts for *Q. ilex*, expressed in  $IC_{50}$  (mg/mL) for DPPH and ABTS assay, VCEAC (vitamin C equivalents mmol of vitamin C/g dry weight) for phosphomolybdate assay

<i>Q. ilex</i> extracts	IC <sub>50/DPPH</sub> (mg/mL)	IC <sub>50/ABTS</sub> (mg/mL)	Phosphomolybdate assay VCEAC
Non defatted Q. ilex	0.13±0.04	0.006±0.001	0.13±0.006
Defatted Q. ilex	$0.008{\pm}0.0008$	$0.005 {\pm} 0.001$	$0.017 {\pm} 0.004$
<i>Q. ilex</i> oil	$0.70{\pm}1.07$	$0.016{\pm}0.007$	$0.0007 \pm 0.0003$
Ascorbic acid	$0.0022 \pm 0.0003$	ND	ND
BHT	$0.0042 \pm 0.0002$	ND	ND
BHA	0.0033±0.0005	ND	ND
Trolox	ND	$0.004 \pm 0.0002$	ND
method is routinely applied in the laboratory to evaluate the total antioxidant capacity of plant extracts [35]. The total antioxidant capacity of the extracts was determined by the Phosphomolybdate method. The different extracts of *Q. ilex* (defatted, non-defatted and oil) showed different degrees of total antioxidant capacity (Table 2). Non defatted *Q. ilex* extract showed the highest total antioxidant capacity (VCEAC=0.13±0.006) and followed by defatted *Q. ilex* (VCEAC=0.017±0.004). While, the *Q. ilex* oil indicated the lowest total antioxidant capacity (VCEAC=0.0007±0.0003). This capacity might be associated to the existence of antioxidant compounds in *Q. ilex*.

#### Fatty acid compositions

The fatty acid composition of acorn oil was presented in Table 3. As shown, the fatty acids in acorn oil were saturated fatty acids (SFA) and unsaturated (UFA). The total contents of SFA, monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) were 16.28%, 66.80% and 16.64%, respectively. The most abundant fatty acids determined in the acorn oil samples were oleic acid (C18:1) (65.38%), 9,12-octadecadienoic acid (C18:2) (16.64%) and palmitic acid (C16:0) (12.81%). Furthermore, minor quantities of 11-eicosenoic acid (C20:1), methylnonadecanoic acid (C20:0), cis-13-octadecenoic acid, (C18:1) and 20-methyl-heneicosanoic acid (C22:0) were detected (below 0.5%).

### DISCUSSION

The results generally of phenolic confirmed that the ethanol solvent (non-defatted) extract provide satisfactory phenolic content and defatted extract

Table 3. The fatty acid compositions (%) of Q. ilex

Peak no.	Compound name	Area %
1	Palmitic acid (C16:0)	12.81
2	9,12-octadecadienoic acid (C18:2)	16.64
3	Oleic acid (C18:1)	65.38
4	Vaccenic acid (C18:1)	1.00
5	Stearic acid (C18:0)	3.05
6	11-eicosenoic acid (C20:1)	0.32
7	Methylnonadecanoic acid (C20:0)	0.32
8	Cis-13-octadecenoic acid (C18:1)	0.10
9	20-methyl-heneicosanoic acid (C22:0)	0.10
	$\sum$ SFA	16.28
	$\sum$ MUFA	66.80
	$\sum$ PUFA	16.64

SAFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids

were found to be more effective for isolation and determination of phenolic compounds from different plant materials; and this statement is a confirmation for our findings reported in the present study. The higher phenolic content of the defatted compared with the non-defatted extracts showed that defatting the plant before extraction could enhance the availability of phenols in the plant (Table 1). The higher content of phenols in the defatted extracts of all the plants compared to the non-defatted extracts could be as a result of the removal of fatty substances from the extracts making the phenolic compounds more polar.

Noteworthy, results observed in the present work were higher than those previously reported by Zarroug et al. (2021) [36]. TPC and TFC of Tunisian *Q. ilex* flour were 23.56 mg GAE/g DW and 7.08 mg CE/g DW. The study of Custódio et al. (2013) [37] on acorns collected from Portugal, revealed lower contents of TPC and TCT, but higher levels of TFC. However, the content of TPC was in accordance with that (33.11%) registered by Masmoudi et al. (2020) on *Q. suber* on acorns collected from Portugal, revealed lower contents of TPC and TCT, but higher levels of TFC. However, the content of TPC was in accordance with that (33.11%) registered by Masmoudi et al (2020) on *Q. suber* [38].

Taib et al. (2024) reported that the total polyphenol contents were found to be 98.3 and 212.5 mg GAE/g of dry extract for *Q. faginea* and *Q. rotundifolia*, respectively. While, the total flavonoid contents were 6.2 and 10.3 mg/g of dry extract for *Q. faginea* and *Q. rotundifolia*, respectively [39].

Recent research reported that the flavonoid contents were ranged between 212.26 and 279.82 mg CE/kg dry weight and 122.99 and 131.6 mg CE/kg of oil for *Q. flour* and *Quercus* oil, respectively. The significant higher amount was obtained in *Q. ilex* species for the two different extracts [39]. These results are close to that found by Sousa et al. (2021) who showed that the bark ethanol extracts *Q. rotundifolia* bark had a high proportion of phenolic compounds (572.8 mg GAE/g extract; 3.7 g GAE/g dry bark), in which flavonoids constituted the major classes (247.6 mg CE/g extract. While, the bark water extracts contained a much lower amount of phenolic compounds; total phenolics 219.5 mg GAE/g of extract, flavonoids 162.5 mg CE/g of extract) [40].

DPPH is routinely used to evaluate the free radical scavenging capacity of chemical compounds. This assay enlightens the effect of the antioxidant molecules toward a stable free radical through a known mechanism involving a hydrogen atom transfer coupled to a fast electron transfer. Phenolic compounds scavenge DPPH by their ability to form o-quinone intermediates upon free radical H-atom abstraction and its subsequent disproportionation [41]. Santos et al. (2010) tested the scavenging activity of different extracts prepared from cork of *Q. suber* using DPPH radicals and reported an IC<sub>50</sub> values ranging from 2.79 to 5.84 µg/mL [42]. According to studies conducted by Makhlouf et al. (2019) the flour extract of *Q. ilex* species exhibited the strongest scavenging capacity on DPPH (52.62 g TE/g dry weight) [27]. The obtained data on the antioxidant activity shows that the methanolic extract of acorn can be used to reduce the stable DPPH radical with IC<sub>50</sub> values of 318 µg/ml [36]. *Quercus* extract exhibited the strongest scavenging capacity against DPPH radicals. These findings were directly related to the higher content of phenolic and flavonoid compounds.

ABTS scavenging activity is also one of the most commonly used method to evaluate the antioxidant properties in fruits and plants. This activity varied from 1.27 to 3.23 mg TE/g for acorn oil and from 36.19 to 44.50 mg TE/g for acorn flour as reported by Makhlouf et al. (2019) [27].

The significant antioxidant activity of *Quercus* fruit is due to the inductive effect of the natural antioxidants present in the fruit such as phenolic compounds and flavonoids which reduce and discolor free radicals (ABTS<sup>++</sup>) because of their ability to yield hydrogen [43, 44].

Phosphomolybdate is another important in vitro antioxidant assay to access the total antioxidant capacity of the plant extract. The assay principal follows the conversion of Mo (VI) to Mo (V) by extract or the compound which possess antioxidant potential resulting in green phosphate Mo (V). The electron/hydrogen donating pattern of antioxidants depends upon its structure and series of redox reactions occurring in the activity [45]. Our findings showed that non defatted Q. ilex extract has good antioxidant potential due to presence of flavonoid and phenolic contents. Phosphomolybdenum assay showed significant correlation with total flavonoid contents as well as total phenolic contents. Jan et al., also reported the best phosphomolybdenum activity of aqueous extract and a significant correlation with TPC and TFC [46].

The saturated and unsaturated fatty acids are influenced by environmental conditions, such as temperature, rainfall, and genotypes [47]. The amount of fatty acids, on the genotypic, is one of the most influential factors [48, 49]. Some authors have observed, that fatty acid contents were significantly influenced by years, various physiological, geographical, ecological and cultural factors [48,50]. Indeed, the level, of this fatty acid, has been shown to be greatly influenced by the impact of abiotic conditions, such as water availability [51].

Since the acorn oil was rich in both oleic and linoleic acids, it might be considered healthier for the

human diet [52]. It has long been acknowledged that plant oils containing relatively low concentrations of omega-6, and higher levels of MUFA (mainly oleic acid) may contribute to the lower rate of CHD and a nutritional perspective [53].

The minor difference in fatty acid levels noticed within the same species could be explained by various factors, including oak acorn maturity, differences in oil processing, environmental conditions, or different harvest dates [52, 22]. As previously stated, the main MUFA found in acorn oils is oleic acid. Numerous studies have clearly demonstrated the health benefits of this fatty acid, particularly in reducing cardiovascular disease risk and preventing type 2 diabetes mellitus, besides contributing to improving hemostasis, glucose metabolism, and endothelial dysfunction [54]. Zarroug et al. (2021) also determined the MUFA and PUFA were 67.94% and 17.35%, respectively. The most abundant fatty acids were oleic acid (67.2%), linoleic acid (16.46%) and palmitic acid (11.51%) [36].

Karabas (2013) showed myristic, arachidonic, palmitoleic and gadoleic acids were minor fatty acids constituting 0.09%, 0.37%, 0.08% and 0.61% [55]. Furthermore, the main fatty acids in acorn oils of *Quercus* were oleic (54.77%, 49.88), linoleic (23.04%, 28.25%), and palmitic acid (18.23%, 17.57%) in *Q. ilex* and *Q. coccifera* species, respectively [56]. However, Cantos et al. (2003) reported the concentration of oleic acid was >63% of total fatty acids in all cases, followed by palmitic and linoleic acids at similar concentrations (12-20%) [13]. The most abundant fatty acids of *Q. aegilops* (QA), *Q. infectoria* (QI), and *Q. calliprinus* (QC) were oleic (53.3-56.1%), linoleic 21.3-23.4%, palmitic 17.8-18.7%, linolenic 1.5-1.6 %, and stearic acid 1.02-1.60% [19].

### CONCLUSION

According to the current study, mature fruits of Q. ilex are revealed to be an interesting source for the production of oil. Furthermore, acorns possessed rich sources of potentially functional components (such as flavonoids and phenolics) and showed higher antioxidant capacity. Fatty acid composition showed the richness of oil with more than 67% of unsaturated fatty acids playing the potential preventative role in reducing the coronary heart disease and cancer. Results showed that Q. ilex oils were generally characterized as high methyl oleate. All these good qualities make acorn oil a suitable ingredient for many industrial applications in food, pharmaceutical, nutraceutical and cosmetic domains. Highlighting the good chemical composition, oxidative stability and the richness in natural antioxidants of acorn oil.

#### **Conflict of interest**

The authors declare no conflict of interest.

#### Acknowledgments

The authors are grateful to the DGRSDT and the Ministry of Higher Education and Scientific Research of Algeria for PRFU Project (A16N01UN030120230001), and the team of Medicinal Chemistry Department, Theodor Bilharz Research Institute, Egypt.

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Received: 02.07.2024 Revised: 19.08.2024 Accepted: 21.08.2024 Published online first: 13.09.20243



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Rocz Panstw Zakl Hig 2024;75(3):247-254

https://doi.org/10.32394/rpzh/192744

ORIGINAL ARTICLE

## SELECTED EATING BEHAVIORS AND THE RISK OF ORTHOREXIA NERVOSA IN A GROUP OF HIGH SCHOOL STUDENTS

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## ABSTRACT

**Background.** Orthorexia nervosa (ON), or the obsessive desire to eat only healthy foods, is closely linked to eating behaviors. Among adolescents, the risk of developing ON and its consequences, including weight loss and malnutrition, can be particularly significant due to the crucial impact of eating behaviors on an individual's psychophysical development. **Objective.** The aim of the study was to examine the relationship between the eating behaviors of high school students and the risk of ON.

**Material and Methods.** Observational study was conducted using the PAPI method among 514 students aged 14-19, of which 59.3% (N=305) were female. The research tool was a proprietary, validated questionnaire that included the ORTO-15 test (cutoff score=35) and the BSQFVF. The questionnaire was used to assess the frequency of consumption of selected food products, the level of dietary fiber intake, the quantity and regularity of meals consumed, as well as other eating behaviors of the participants. The individual responses were then analyzed according to the risk of ON using correlation coefficients.

**Results.** The prevalence of ON risk was found to be 32.1% (N=165). The percentage of individuals at risk of ON was higher among vegetarians. The results regarding the frequency of consumption of animal protein sources, dietary fiber intake, the number of meals and their regularity were similar in both the group at risk of ON and the group not at risk. Over 80% of individuals who never consumed bars, gummies, and candies were at risk of developing ON.

**Conclusions.** The risk of ON was higher among vegetarians. Individuals who excluded highly processed foods from their diet were more susceptible to developing ON.

Key words: adolescents, orthorexia nervosa, eating behaviors, ORTO-15, ORTO-35

### **INTRODUCTION**

A proper diet is one of the factors that ensure the appropriate psychophysical development of a young organism. The selection of food products and dishes should be rational and diverse, and the diet should meet all individual nutritional needs. A deficiency in certain vitamins and macro- and micronutrients can lead to impaired nervous system function, including a reduced ability to learn, which is crucial at a young age [1]. The eating behaviors of adolescents partially do not meet the recommendations for proper nutrition, as revealed by nationwide study. The most common dietary mistakes include insufficient intake of vegetables and fruits, whole grain bread, fish, and milk and dairy products, as well as excessive consumption of sweets, fast food, and sweetened carbonated beverages [2]. One of the responses to these issues is nutritional education, which emphasizes promoting proper eating behaviors [3]. Unfortunately, this emphasis can sometimes be misinterpreted, and efforts to improve dietary habits may become obsessive, potentially leading to the development of ON [4].

The term orthorexia (Lat. orthorexia nervosa; ON) originates from the Greek words *ortho* (proper) and *orexia* (appetite/desire). In 1997, doctor Steven Bratman first used this term to describe the obsession with healthy eating caused by health concerns, which he observed in his patients and himself [5]. ON has not yet been included in the ICD-10/ICD-11 or DSM-5

Publisher: National Institute of Public Health NIH - National Research Institute

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classifications of diseases. It is unclear whether ON should be classified as an unspecified eating disorder due to its close connection with eating, or as an obsessive-compulsive disorder due to the presence of symptoms typical of these disorders, such as intrusive thoughts about food, meal preparation rituals, and social isolation [6, 7, 8, 9]. ON is characterized by an excessive focus on a subjectively defined "ideal" or "clean" way of eating. This leads to the elimination of many foods, such as dairy and gluten-containing products, due to a subjective belief that they are detrimental to health. Specific rituals and beliefs related to food may develop, such as consuming only raw products due to fear of thermal processing. Orthorexics are particularly concerned with the quality and safety of food (especially the potential for microbiological contamination or pesticide additives), the method of preparation, and the materials used for food packaging. Deviations from the established dietary rules result in guilt, anxiety, and reduced self-esteem for the individual suffering from ON. Weight loss and malnutrition are consequences of ON. It is also suspected that, in the long term, ON may lead to anaemia and osteoporosis. Due to the similarity of these effects to those of anorexia nervosa (AN), special attention must be paid to differentiating between the two disorders. A significant distinguishing factor of ON from AN is the lack of preoccupation with one's body weight and the obsessive pursuit of a certain physique. The motivation for dietary restrictions in orthorexics is the desire to avoid serious illnesses or to improve health, rather than achieving a specific body weight [10, 11, 12].

Eating behaviors are a major aspect of ON, yet there is a limited amount of research on their impact on the risk of developing ON among adolescents. On one hand, individuals whose eating behaviors need improvement may encounter difficulties in maintaining dietary balance during attempts to change, partly due to fear of reverting to unhealthy behaviors. On the other hand, individuals who already meet dietary recommendations may feel increased pressure to maintain or further improve their eating behaviors due to their nutritional knowledge. Considering that eating disorders (EDs) are more likely to develop during adolescence [13], the described situations, combined with additional risk factors such as perfectionism, body dissatisfaction, and frequent exposure to idealized body images portrayed on social media [14, 15, 16], may potentially contribute to the development of ON. The aim of this study was to investigate the relationship between the eating behaviors of high school students and the risk of developing ON.

## **MATERIAL AND METHODS**

Observational, retrospective study was conducted using the PAPI method in February 2024 at the First

High School in Tychy, following the approval from the school's principal. The research tool was an anonymous, validated survey questionnaire consisting of:

- Questions regarding the participants' gender, age, weight, and height. The data obtained were used to calculate the Body Mass Index (BMI kg/m<sup>2</sup>), which was interpreted according to the recommendations of the Global Nutrition Report (<-1 SD: underweight; >+1 SD: overweight; >+2 SD: obesity) [17], using field tables with z-scores developed by WHO [18]. This section also included a question about whether the participants followed a vegetarian diet.
- Questions regarding the frequency of consumption 2. of: products that are sources of animal protein (red meat, poultry, eggs, fish and seafood, milk and dairy products, cheese) and highly processed foods (bars/candies/gummies, cakes/cookies/ donuts, sweetened breakfast cereals, chips/ crackers/pretzels/popcorn, ice cream, processed meat products, fries, instant meals, pizza/ kebabs/burgers/hot dogs, sweetened carbonated beverages, energy drinks). Respondents indicated the frequency of consumption of these products by choosing one of the following options: daily/4-6 times a week/2-3 times a week/once a week/less than once a week/never.
- 3. ORTO-15 A questionnaire assessing the risk of ON. The study employed a Polish validation of the test with a cutoff score of 35 points [19]. Based on the test results, two groups were identified: individuals at risk of ON (<35 points) and those not at risk of ON (≥35 points). The questionnaire consists of 15 questions regarding an obsessive approach to healthy eating, with responses ranging from always/often/sometimes/never. Each response is assigned a score from 1 to 4, with responses indicating a risk of ON scoring 1 point, and those indicating healthy eating behaviors scoring 4 points. The total score ranges from 15 to 60, with lower scores indicating a higher risk of ON.
- 4. BSQFVF A questionnaire to assess the intake of fruits, vegetables, and fiber, modified by Czarnocińska et al. [20]. The frequency of consumption of fruit and vegetable juices, fruits, salads, potatoes, legumes, vegetables, bran, cereals, coarse grains, wholemeal and wheat bread was assessed. Each response was assigned a score: daily 4 points, 4-6 times a week 3 points, 2-3 times a week 2 points, once a week 1 point, less than once a week 0 points. Based on the total score, acceptable fiber intake (≥20 points) and unacceptable intake (<20 points) were distinguished.</p>

### Characteristics of the study group

All participants were students of the First High School in Tychy, and being a student at this school was the only inclusion criterion for the study. The students were asked to complete a survey questionnaire during a nutritional education session conducted during classes. The study included 514 participants, comprising 305 (59.3%) females and 209 (40.7%) males, aged between 14 and 19 years (15.88 $\pm$ 1.27). A vegetarian diet was followed by 34 (6.6%) individuals. The lowest BMI value was 14.34 kg/m<sup>2</sup>, and the highest 36.31 kg/m<sup>2</sup>.

#### Statistical analysis

The obtained results were processed in MS Excel and subjected to statistical analysis using Statistica 13.0. Based on the interpretation of the skewness coefficient, appropriate measures of central tendency (mean, median) and dispersion (standard deviation, interquartile range) were determined. Depending on the type of scale assigned to the respective qualitative variables, appropriate correlation coefficients were selected – Cramer's V ( $V_c$ ) and Phi coefficient ( $\phi$ ). The interpretation of the correlation strength was based on the following guidelines: 0 indicates no stochastic relationship between variables, 1 represents a complete correlation between variables (very weak correlation (x<0.1), weak  $(0.1\ge x<0.3)$ , moderate  $(0.3\ge x<0.5)$ , strong  $(0.5 \ge x < 0.7)$ , very strong  $(0.7 \ge x < 0.9)$ , and almost certain (0.9 $\ge$ x<1.0), where x represents V<sub>c</sub> or  $\phi$ ).

In order to validate the author's part of the questionnaire, 11 subjects (6 women, 5 men) were asked to complete the questionnaire twice with a one-week interval. Cohen's Kappa value was then calculated (0.14-1) and the level of response repeatability was determined based on it (very good response repeatability was obtained for 2 questions, good for 11, medium for 6, poor for 6 and minimal for 1).

## RESULTS

## Orthorexia nervosa risk considering gender, BMI and vegetarian diet of subjects

The risk of ON was noted in 32.1% (N=165) of the respondents. The average score obtained in the ORTO-15 was  $36\pm6.0$ , with the lowest score being 21, and the highest 46.

The gender of the participants was not a predisposing factor for a higher risk of ON, unlike following a vegetarian diet (Table 1). The risk of ON was almost the same among females and males. More than half of the vegetarians were at risk for ON, compared to 30.2% among those following a traditional diet. The average score on the ORTO-15 test among vegetarians was  $33.0\pm8.0$ , while for non-vegetarians, it was  $37\pm5.0$ .

The risk of developing ON was the same regardless of the participants' age or BMI (Table 2). However, among individuals at risk for ON, a higher percentage were overweight or obese compared to those not at risk.

	No risk of ON	Risk of ON	φ	
Women N (%)	208 (68.2)	97 (31.8)	0.009	
Men N (%)	141 (67.5)	68 (32.5)	0.008	
Traditional diet N (%)	335 (69.8)	145 (30.2)	0.15	
Vegetarian diet N (%)	14 (41.2)	20 (58.8)	0.15	

Table 1. Risk of orthorexia nervosa vs. gender of subjects and vegetarianism

 $\Phi$  – Phi coefficient

Table 2. Risk of orthorexia nervosa vs. BMI interpretation and age of subjects

	Total N (%)	No risk of ON N (%)	Risk of ON N (%)	V <sub>c</sub>
Underweight	68 (13.2)	47 (13.5)	21 (12.7)	
Normal weight	383 (74.5)	264 (75.6)	119 (72.1)	0.04
Overweight	54 (10.5)	33 (9.5)	21 (12.7)	0.04
Obesity	9 (1.8)	5 (1.4)	4 (2.4)	
14 years	77 (14.9)	55 (15.9)	22 (13.3)	
15 years	141 (27.4)	95 (27.2)	46 (27.9)	
16 years	134 (26.1)	89 (25.5)	45 (27.3)	0.04
17 years	97 (18.9)	66 (18.9)	31 (18.8)	0.04
18 years	58 (11.3)	39 (11.1)	19 (11.5)	
19 years	7 (1.4)	5 (1.4)	2 (1.2)	

V<sub>c</sub> - Cramer's V coefficient

## Orthorexia nervosa risk and selected eating behaviors of subjects

The highest percentage of respondents declared the same responses regarding selected eating behaviors, regardless of their risk of ON (Table 3). More than half of the subjects in both groups consumed 4-5 meals per day. Over 40% of respondents reported eating every 3-4 hours. Regular breakfast consumption was declared by only half of the respondents in both groups. The highest percentage of participants drank 1.5-2 liters of water daily. However, 61.2% of those at

risk of ON drank 1.5-3 liters of water, whereas 72.5% of those not at risk drank 1-2 liters of water, indicating a higher daily water intake among individuals at risk of ON.

In 67.1% of respondents, dietary fiber intake was noted to be at an unacceptable level (Figure 1). Among those at risk for ON, a higher percentage of subjects achieved acceptable fiber intake compared to those not at risk for ON. The mean score obtained on the BSQFVF for those at risk of ON was  $17.82\pm5.03$ , while among those not at risk, it was  $16.89\pm4.88$ .

Table 3. Risk of orthorexia nervosa vs. breakfast intake, amount of water drunk, number of meals per day and length of breaks between them

		Total N (%)	No risk of ON N (%)	Risk of ON N (%)	V <sub>c</sub>	
	1-2	51 (9.9)	33 (9.5)	18 (10.9)		
Number of meals	3	177 (34.4)	124 (35.5)	53 (32.1)		
per day	4-5	267 (51.9)	180 (51.6)	87 (52.7)	0.04	
	6 and more	19 (3.8)	12 (3.4)	7 (4.2)		
	Yes, always	259 (50.4)	172 (49.3)	87 (52.7)		
Breakfast intake	Yes, but not always	184 (35.8)	126 (36.1)	58 (35.2)	0.04	
	No	71 (13.8)	51 (14.6)	20 (12.1)		
	I don't drink water	4 (0.8)	3 (0.9)	1 (0.6)		
	0.5 l and less	34 (6.6)	24 (6.9)	10 (6.1)		
Amount of water	Approximately 1 l	126 (24.5)	91 (26.1)	35 (21.2)	0.14	
drunk per day	1.5-21	227 (44.2)	162 (46.4)	65 (39.4)	0.14	
	2.5-3 1	85 (16.5)	49 (14.0)	36 (21.8)		
	Above 31	38 (7.4)	20 (5.7)	18 (10.9)		
	1 h and less	8 (1.6)	6 (1.7)	2 (1.2)		
	2 h	50 (9.7)	33 (9.5)	17 (10.3)		
Length of breaks	3-4 h	233 (45.3)	154 (44.1)	79 (47.9)	0.05	
between meals	5 h	41 (8.0)	30 (8.6)	11 (6.7)	0.05	
	6 h and above	23 (4.5)	16 (4.6)	7 (4.2)		
	Are highly irregular	159 (30.9)	110 (31.5)	49 (29.7)		

V<sub>c</sub> - Cramer's V coefficient



 $\Phi$  – Phi coefficient

Figure 1. Orthorexia nervosa risk vs. level of dietary fiber intake

## consumption of selected food products

The highest percentage of respondents reported the same frequency of consumption of animal protein

Risk of orthorexia nervosa and frequency of sources, regardless of their risk of ON (Table 4). Over half of the individuals consuming red meat daily were at risk of ON. Similarly, 41.9% of individuals who never consumed red meat were also at risk of ON.

		Never N (%)	Less than 1 a week N (%)	1 a week N (%)	2-3 times a week N (%)	4-6 times a week N (%)	Daily N (%)	V <sub>c</sub>
Dedmost	n/risk	36 (58.1)	108 (73.0)	69 (69.0)	98 (72.6)	29 (59.2)	9 (45.0)	0.16
Ked meat	risk	26 (41.9)	40 (27.0)	31 (31.0)	37 (27.4)	20 (40.8)	11 (55.0)	0.10
Doultary	n/risk	13 (46.4)	15 (55.6)	48 (66.7)	178 (76.7)	81 (63.8)	14 (50.0)	0.20
Poultry	risk	15 (53.6)	12 (44.4)	24 (33.3)	54 (23.3)	46 (36.2)	14 (50.0)	0.20
Б	n/risk	11 (52.4)	50 (78.1)	89 (72.4)	132 (72.5)	50 (54.4)	17 (53.1)	0.10
Eggs	risk	10 (47.6)	14 (21.9)	34 (27.6)	50 (27.5)	42 (45.6)	15 (46.9)	0.19
Fish & seafood	n/risk	52 (68.4)	161 (70.6)	112(70.0)	22 (53.7)	1 (12.5)	1 (100)	0.19
	risk	24 (31.6)	67 (29.4)	48 (30.0)	19 (46.3)	7 (87.5)	0 (0)	0.18
Milk & dairy	n/risk	3 (30.0)	14 (70.0)	14 (60.9)	71 (71.0)	103 (70.5)	144 (67.0)	0.12
products	risk	7 (70.0)	6 (30.0)	9 (39.1)	29 (29.0)	43 (29.5)	71 (33.0)	0.13
Chasses	n/risk	7 (43.8)	12 (57.1)	24 (66.7)	80 (69.0)	131 (70.4)	95 (68.3)	0.11
Cheeses	risk	9 (56.2)	9 (42.9)	12 (33.3)	36 (31.0)	55 (29.6)	44 (31.7)	0.11

Table 4. Risk of orthorexia nervosa vs. frequency of consumption of products that are sources of animal protein

n/risk – no risk of ON; risk – risk of ON;  $V_{\rm C}$  – Cramer's V coefficient

Table 5. Risk of orthorexia nervosa vs. frequency of consumption of highly processed foods

		Never N (%)	Less than 1 a week N (%)	1 a week N (%)	2-3 times a week N (%)	4-6 times a week N (%)	Daily N (%)	V <sub>c</sub>
Bars, candies,	n/risk	4 (16.7)	48 (53.9)	54 (64.3)	126 (73.3)	82 (80.4)	35 (81.4)	0.21
jellies	risk	20 (83.3)	41 (46.1)	30 (35.7)	46 (26.7)	20 (19.6)	8 (18.6)	0.31
Cookies,	n/risk	7 (26.9)	111 (62.7)	110 (73.3)	87 (75.6)	24 (72.7)	10 (76.9)	0.22
cakes, donuts	risk	19 (73.1)	66 (37.3)	40 (26.7)	28 (24.4)	9 (27.3)	3 (23.1)	0.23
Salty snacks,	n/risk	15 (48.4)	153 (65.7)	101 (68.7)	65 (82.3)	13 (68.4)	2 (40.0)	
e.g. crisps, crackers	risk	16 (51.6)	80 (34.3)	46 (31.3)	14 (17.7)	6 (31.6)	3 (60.0)	0.17
Sweetened	n/risk	83 (57.6)	123 (71.1)	46 (69.7)	54 (68.3)	24 (77.4)	19 (90.5)	
breakfast cereals	risk	61 (42.4)	50 28.9)	20 (30.3)	25 (31.7)	7 (22.6)	2 (9.5)	0.17
T	n/risk	14 (40.0)	227 (69.9)	66 (68.0)	34 (77.3)	8 (80.0)	0 (0)	0.21
Ice cream	risk	21 (60.0)	98 (30.1)	31 (32.0)	10 (22.7)	2 (20.0)	3 (100)	0.21
Processed	n/risk	23 (42.6)	43 (66.2)	62 (74.7)	95 (68.8)	82 (79.6)	44 (62.0)	
meats, e.g. sausages	risk	31 (57.4)	22 (33.8)	21 (25.3)	43 (31.2)	21 (20.4)	27 (38.0)	0.22
Fast food	n/risk	11 (42.3)	229 (67.7)	79 (69.9)	25 (78.1)	4 (100)	1 (100)	0.15
Fast 1000	risk	15 (57.7)	109 (32.3)	34 (30.1)	7 (21.9)	0 (0)	0 (0)	0.15
Errica	n/risk	28 (59.6)	227 (66.4)	68 (74.7)	19 (76.0)	5 (71.4)	2 (100)	0.10
Files	risk	19 (40.4)	115 (33.6)	23 (25.3)	6 (24.0)	2 (28.6)	0 (0)	0.10
Instant mools	n/risk	146 (60.8)	161 (72.5)	23 (82.1)	17 (85.0)	1 (50.0)	1 (50.0)	0.16
mistant means	risk	94 (39.2)	61 (27.5)	5 (17.9)	3 (15.0)	1 (50.0)	1 (50.0)	0.10
Sweetened carbonated beverages	n/risk	60 (47.6)	141 (77.5)	59 (68.6)	59 (73.8)	18 (72.0)	12 (80.0)	
	risk	66 (52.4)	41 (22.5)	27 (31.4)	21 (26.2)	7 (28.0)	3 (20.0)	0.26
Energy drinks	n/risk	243 (67.9)	72 (72.0)	10 (50.0)	13 (61.9)	5 (83.3)	6 (66.7)	0.10
Energy driffks	risk	115 (32.1)	28 (28.0)	10 (50.0)	8 (38.1)	1 (16.7)	3 (33.3)	0.10

 $n/risk - no risk of ON; risk - risk of ON; V_{c} - Cramer's V coefficient$ 

A similar trend of higher ON risk for extreme consumption frequencies was observed for poultry and eggs. Increased ON risk was evident with relatively higher frequencies of fish and seafood consumption (2-3/4-6 times per week). For milk and dairy products, as well as cheese, higher ON risk was found among individuals who never consumed these products.

Among those excluding particular highly processed foods from their diet, a higher percentage were at risk of ON (Table 5). Among individuals who never consumed bars, candies, and jellies, 83.3% were at risk of ON. Conversely, among those who consumed them 2-3/4-6 times per week or daily, over 70% were not at risk. A similar trend was observed for the consumption of cakes, cookies, donuts, salty snacks, ice cream, processed meats, fast food, and sweetened carbonated beverages. The highest percentage of individuals at risk of ON most often never consumed instant meals, sweetened breakfast cereals, and carbonated beverages, whereas among those not at risk, the highest percentage consumed these products less than once a week.

#### DISCUSSION

In the conducted study, the prevalence of the risk of ON was found to be 32.1% (N=165). Other researchers adopting the same cutoff score (ORTO-35) obtained results at similar levels – 27.8%, 28.3%, 34.9% [21, 22, 23], or slightly lower – 13.7% [24].

The authors of ORTO-15, when introducing the tool in 2005, obtained an ON prevalence of 6.9% (N=36) in a group of 525 subjects [25]. If the present study, conducted on a similar sized group of subjects (N=514), had adopted the cutoff recommended for ORTO-40, it would have yielded an ON prevalence of 80.7% (N=415). This is an almost 12-fold increase over 20 years. On the one hand, this may indicate a tendency for the ORTO-15 test to overestimate the risk of ON, which is also confirmed by the results of other studies - an increase from 27.8% to 76.7%, following a change in the cutoff score in the study by Łucka et al. [21]. On the other hand, it reflects a growing interest in healthy eating and, unfortunately, an improper approach to it that has developed in society over the past two decades.

No influence of gender on the risk of ON was observed. A similar observation was noted by Australian, Hungarian and Polish researchers [21, 26, 27]. The lack of gender variation in the incidence of ON risk, may be a specific aspect of ON, stemming from its source – fear for one's health [10]. This concern, may affect both genders equally, unlike the pressure to have a slim figure, which affects women more often and is a risk factor for EDs such as AN or bulimia nervosa (BN) [28].

The transition from a traditional diet to a vegetarian diet at a young age involves certain dietary restrictions. This may also be a time when a young person starts paying attention to the nutritional value of the foods they consume. If an excessively strict dietary regime is applied, the above situation could lead to the development of EDs [29]. In the present study, a higher risk of ON was demonstrated among vegetarians, which is consistent with the results of other studies [30, 31, 32]. Dittfeld et al. [33] noted a relationship involving an inversely proportional increase in the risk of ON to age and length of adherence to a vegetarian diet, indicating a higher risk of ON among younger and novice vegetarians. This draws attention to the fact that switching to a plant-based diet at a young age should be done under supervision and with parental support.

In the study conducted, those at risk of ON consumed more dietary fiber, drank more water, and avoided breakfast less often compared to those not at risk, which can be considered manifestations of more positive eating behaviors. A similar relationship, involving a higher intensity of pro-healthy eating behaviors, has been noted in other studies [22, 34, 35]. Adopting proper eating behaviors, especially at a young age, is a desirable phenomenon. At the same time, however, the emphasis on maintaining a healthy dietary approach and balance should be as strong as the emphasis on improving eating behaviors, which could reduce the risk of ON or other EDs. Excessive restrictions - even on highly processed foods - are not beneficial for mental health [36, 37]. The risk of ON was higher among those who completely excluded products commonly (and rightly) considered unhealthy, e.g., candy bars, chips, fast food dishes. However, the risk was higher, even compared to those with lower consumption of these products (less than once a week). In this case, wouldn't occasional appetite-driven consumption of products considered unhealthy be more beneficial from a psychodietetic point of view?

#### **Strengths and limitations**

The study was conducted on a large sample using a contact method; however, all participants attended the same school. Expanding the study to include students from other schools would provide a valuable evaluation, giving it a regional perspective.

### CONCLUSIONS

The risk of developing orthorexia nervosa was higher among vegetarians. Individuals who excluded highly processed foods from their diet were more prone to developing ON. Apart from the aforementioned factors, no significant differences were observed in the eating behaviors of those at risk of ON compared to those not at risk.

## **Conflict of interest**

The authors declare no conflict of interest.

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Received: 23.07.2024 Revised: 28.08.2024 Accepted: 29.08.2024 Published online first: 12.09.2024



https://roczniki.pzh.gov.pl/

Rocz Panstw Zakl Hig 2024;75(3):255-260

https://doi.org/10.32394/rpzh/192790

ORIGINAL ARTICLE

## MINERAL ELEMENTS OF SOME WILD PLANTS OF TRADITIONAL USES IN THE MOROCCAN RIF MOUNTAINS

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### ABSTRACT

**Background.** Wild edible plants (WEPs) that are part of the Mediterranean diet and used in Moroccan traditional food and therapy are now less or not used. This requires their valorization to avoid the threat of their disappearance. The objective of this work was therefore to determine the mineral content in the parts of some WEPs species used for their health benefits in the Rif in Morocco.

**Material and Methods.** The parts of the WEPs species used, the leaves of *Tetraclinis articulata* (Vahl) masters, the aerial parts of *Lavandula stoechas* L., the stems of *Rubia peregrina* L., the seeds of *Ammodaucus leucotrichus* Coss. & Dur., known under the local names of Laaraar, Halhal, Foua, and Kamoun soufi respectively, are washed with distilled water, dried in an oven and crushed. The extracts of the powders obtained are analyzed for the quantification of mineral elements by ICP spectrophotometer.

**Results.** The analysis results of ICP-OES show that the aerial parts of *Lavandula stoechas* L. are rich in K, Mg, Fe, Na, and Zn. The stems of *Rubia peregrina* L. are rich in Fe, Na, Mg, Zn, K, Ca, and Mn. The leaves of *Tetraclinis articulata* (Vahl) masters are rich in Fe, Ca, K, Na, Mn and Mg and the seeds of *Ammodaucus leucotrichus* Coss. & Dur. are rich in K, Ca, Mg, Na.

**Conclusion.** The results reported in the samples of the WEPs analyzed present significant contents in minerals, in particular in K, Fe, Ca, Mg, Na, Zn and Mn and show that in addition to their medicinal values these species have a nutritional potential and could contribute to the dietary balance.

Key words: wild edible plants, Morocco, nutrients, minerals, analysis

## **INTRODUCTION**

The rural world of Mediterranean countries has always been a repository of culinary and medicinal knowledge of many plant species in ethnobotany. In Mediterranean regions, the *Asteraceae*, *Lamiaceae* and *Apiaceae* families are the most represented in many ethnobotanical studies for food or medicinal use. These are used as infusions, decoctions, poultices, etc. in traditional cooking and in the treatment of several diseases [1].

Today, nutritional problems still persist and dominate on a global scale despite the efforts and progress of health systems. Even when they are in moderate forms, consequences of malnutrition and micronutrient deficiencies, much more considerable than previously recognized, have been reported over the last two decades. At the same time, changes in dietary habits have been observed due to several

factors including demography, economic and technological progress, urbanization and the shift from a rural lifestyle to a more sedentary urban one. These factors have affected agricultural systems and policies, socio-cultural and economic characteristics and lifestyle in addition to globalization. The impact of these factors on the dietary balance in terms of energy and micronutrient intake linked to the change in dietary habits, also affects the nutritional and health statuses of populations. Deficiencies in these nutrients are indeed known for their adverse effects on the populations health and performance.

Revaluing forgotten or unused plant biodiversity as an additional source of carbohydrates, vitamins, fats, proteins and minerals through the use of wild plant species can help reduce these health problems [2]. These plants were, indeed, a dietary component of the Mediterranean diet and contribute to its sustainability. In addition, balanced diets have evolved in recent

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Publisher: National Institute of Public Health NIH - National Research Institute

years towards a decrease in red meat and an increase in vegetables and fruits [3]. Indeed, global dietary guidelines recommend increasing the consumption of fruits and vegetables to mitigate the threat of dietrelated diseases, including metabolic disorders, cancer and cardiovascular diseases [4]. Therefore, promoting these plants will ensure important nutritional sources while respecting biodiversity. It is in this perspective that this work aimed to determine the chemical composition of four wild plants used in cooking and traditional medicine as declared by populations of previous ethnobotanical surveys carried out in the Casablanca settat region [1] and in the Rif of Morocco. Therefore, the question in this work is of promoting these plants as essential sources of minerals that can contribute to nutrient intake and combat certain dietary deficiencies and various diseases. Specifically the objective is to determine the mineral composition of four species: Tetraclinis articulata (Vahl) masters, Lavandula stoechas L., Rubia peregrina L., Carum carvi, Ammodaucus leucotrichus Coss. & Dur. collected in the Tanounat region.

#### MATERIAL AND METHODS

#### Sample preparation

Four different wild plants used by the population of the Moroccan Rif Mountains were selected namely *Tetraclinis articulata* (Vahl) masters, *Lavandula stoechas* L., *Rubia peregrina* L. and *Ammodaucus leucotrichus* Coss. & Dur. were collected in 2023. The identification of the scientific nomenclature of these plants was determined using a digital herbarium, the help of the National Herbarium (Rabat, Morocco), the Agronomic and Veterinary Institute and with the help of a botanist from the Faculty of Sciences of El-Jadida (UCD, Morocco) as well as the literature documents [5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16]. The samples preparation and analysis are conducted according to the protocole used in the study team laboratory [17, 18].

The selected plants are species that are used in traditional medicine and in cooking dishes in the Taounat region of Morocco. These WEP were harvested at their phenological maturity stage, when the stems and leaves are well developed but still tender. After washing the used plants parts with distilled water, the samples were dried in an oven at 37°C for one week. The dried material was milled into powder (1 mm) using an electric blender (Moulinex type LM 207, France), and then stored for later analysis (Figure 1). To 1 g of plant powder, a volume of 100 mL of boiling distilled water was added, the mixture was filtered through Whatman filter paper No. 1 after standing for 5 minutes at room temperature and the water was

evaporated to dryness using a rotary evaporator to obtain the dried aqueous extract.

## Mineral content determination by ICP-OES spectrometer

The plant powder and aqueous extract was analyzed repeated 3 times for their mineral content namely P, K, Ca, Mg, trace elements and heavy metals determined using a complete ICP-OES spectrometer from Agilent Technologis (serial number 17390015). Na determination was performed by an atomic absorption spectrophotometer (Agilent Technologis, serial number 17360001). One gram of powder from each sample was weighed into crucibles and then placed in a muffle furnace at 525°C for 4 hours to complete calcination. The resulting ashes were then broken with nitric acid (65%) under a fume hood added as approximately 2 ml of concentrated acid per crucible and 100 ml of ultrapure water. The resulting liquid is poured into smoothed and sealed polypropylene bottles to allow suspended particles to settle for 24 hours [19, 20].

#### **RESULTS AND DISCUSSION**

Table 1 presents the results of the analysis of the proximal composition of the aerial parts of the species studied: Lavandula stoechas L. (local name: Halhal), stems of Rubia peregrina L. (Foua), seeds of Ammodaucus leucotrichus Coss. & Dur. (Kamoun soufi) and leaves of Tetraclinis articulata (Vahl) masters (Laaraar). The table shows that the mineral contents values that are determined after three repetitions and expressed per kg dry weight, are different in samples analyzed from a species to another. The aerial parts of Lavandula stoechas L. were characterized by high contents of several micronutrients and trace elements (Figure 1) with potassium as the main element present at a concentration of 16234.36 mg/kg, followed by magnesium 4223 mg/kg, iron 3158.52 mg/kg, sodium 1116.00 mg/kg and zinc 320.29 mg/kg. Table 1 also shows that these minerals were the main elements among a total of 23 minerals determined in this study and are in decreasing order K>Mg>Fe>Mn>Na>Zn. The contents of these plants in Ca, Al, Mn, Cr, B, Ba, N and Cu were lower while those of the other mineral elements analyzed (P, As, Cd, Co, Li, Mo, Sb) were presented at values of less than 3.

The same results have been previously obtained in the aerial parts of the plants analyzed for mineral content by ICP spectrometry in western Algeria [21] and in Izmir, Turkey [13]. Similarly, as revealed in the present study, the same trace elements were determined in Turkish and Algerian plants. These contents of K, Mg, Na and trace elements Fe, Zn, Cu and Mn were expressed per 100 g of dry weight.

No	3
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Mineral	Symbol		<i>Lavandula</i> stoechas Halhal	Ammodaucus leucotrichus Kamoun soufi	<i>Rubia peregrina</i> Foua	<i>Tetraclinis</i> <i>articulata</i> Laaraar
Nitrogen	N	mg/kg	10.34±0.13	1.71±0.09	1.10±13	0.96±12
Phosphorus	Р	mg/kg	0.09±0.36	0.18±0.01	0.15±18	0.07±4.39
Potassium	K	mg/kg	16234.36±7.65	17470.98±99	2890.20±49	1234.87±7.29
Sodium	Na	mg/kg	1116.00±13	1193.53±26	3207.19±18.43	1217.70±8.39
Calcium	Ca	mg/kg	$142.78 {\pm} 0.89$	8346±16	1302.66±9.79	1293.66±7.83
Magnesium	Mg	mg/kg	4223±0.09	2204±40	189.66±8.29	179.66±8.78
Aluminium	Al	mg/kg	130.55±9.49	114.30±4.4	104.30±7.92	214.30±5.2
Arsenic	As	mg/kg	<1	<1	<1	<1
Boron	В	mg/kg	47.24±0.13	27.91±10	25.31±9.49	28.70±7.39
Barium	Ba	mg/kg	29.41±0.09	3.22±0.04	17.13±7.87	18.61±4.56
Cadmium	Cd	mg kg	< 0.3	< 0.3	<0.3±10.05	< 0.3
Cobalt	Co	mg/kg	<1	<1	<1	<1
Chromium	Cr	mg/kg	$60.50 {\pm} 0.08$	3.70±0.06	6.83±3.02	4.51±12.28
Copper	Cu	mg/kg	$8.98{\pm}0.04$	6.46±4.44	5.95±9.49	7.01±6.47
Iron	Fe	mg/kg	3158.52±6.22	1524.59±5.24	3879.20±6.97	9076.32±7.29
Lithium	Li	mg/kg	<1	2.58±0.06	1.30±10	1.11±18.29
Manganese	Mn	mg/kg	2189.73±0.22	14.40±6	238.70±8.64	529.64±12
Molybdenum	Mo	mg/kg	<1	<1	<1	<1
Nickel	Ni	mg/kg	$1.63 \pm 0.56$	<1	<1	$1.74 \pm 8.28$
Lead	Pb	mg/kg	$1.94 \pm 3.23$	1.86±0.02	1.20±2.28	2.90±4.95
Antimony	Sb	mg/kg	<1	<1	<1	<1
Selenium	Se	mg/kg	1.36±10.45	1.15±0.07	<1	<1
Zinc	Zn	mg/kg	320.29±0.23	15.53±0.9	210.36±14.34	137.31±9.49

Table 1. Mineral content of 4 plants



Figure 1. Photos taken during chemical analysis

Furthermore, the content of 142.78 mg/kg of Ca revealed in the present study is similar to that obtained in the Algerian study. However, this result is different compared to that of the Turkish plant where this mineral was not detected. The geographical location of the studies conducted on the plant *Lavandula stoechas* L. (Morocco, Algeria and Turkey) may be the cause of the difference in the physicochemical characteristics that may influence the mineralogical composition of the plant. This may explain the similarity of the research results between Morocco and the neighboring country Algeria, which differ from those obtained for Turkey. The mineral content may also vary depending on the growing area, the vegetation period of the plant and the characteristics of the soil [22].

As for the plant Rubia peregrina, the results of the analyses per kg dry weight carried out show that the content of the stems of this species constitutes an important source of iron (3879.20 mg/kg), sodium (3207.19 mg/kg), potassium (2890.20 mg/kg) and calcium (1302.66 mg/kg). It also contains in smaller quantities contents of magnesium (189.66 mg/kg), manganese (238.70 mg/kg), zinc (210.36 mg/kg), aluminum (104.30 mg/kg), boron (25.31 mg/kg), barium (17.13 mg/kg), chromium (6.83 mg/kg) and copper (5.95 mg/kg) as well as contents evaluated at less than 3 mg/kg in other mineral elements analyzed such as N, P, As, Cd, Co, Li, Mo, Ni, Pb, Sb, Se were found in this species of plant. These results could not be compared to those in the literature as at the best of our knowledge, there are no similar quantitative studies on the mineralogical composition of this plant stems, which reveals the originality of the present research work.

On the other hand, maximum contents of mineral components are also reported in the leaves of Tetraclinis articulata for iron (9076.32 mg/kg), calcium (1293.66 mg/kg), potassium (1234.87 mg/kg), sodium (1217 mg/kg) in the following order Fe>Ca>K>Na as well as manganese (529.64 mg/kg), magnesium (189.66 mg/kg), aluminum (214.30 mg/kg), zinc (137.31 mg/kg), boron (28.70 mg/kg), barium (18.61 mg/kg), chromium (4.51 mg/kg) and copper (7.01 mg/kg) (Table 1). The other mineral elements analyzed, N, P, As, Cd, Co, Li, Mo, Ni, Pb, Sb, Se, were found to be at values <3 mg/kg. To our knowledge, the majority of studies conducted on this plant have focused on its biological activities, and there is few research focused on the dosage of these mineral elements in the used part of this plant. This observation can be explained by a lack of knowledge on the use of this plant by populations elsewhere.

The results of the analyses of the seeds of *Ammodaucus leucotrichus* show that the seeds of this plant were characterized by high contents of potassium 17470.98 mg/kg, calcium 8346 mg/kg, magnesium

2204 mg/kg, sodium 1193.53 mg/kg and aluminum 114.30 mg/kg and lower contents of boron 27.91 mg/kg, zinc 15.53 mg/kg and manganese 14.40 mg/kg. The analysis also revealed other mineral elements N, P, As, Ba, Co, Cr, Cu, Li, Mo, Ni, Pb, Sb, Se at contents lower than 3 mg/kg. Similar results o the composition of this plant in the Sahara of Algeria or in the seeds of Ammodaucus leucotrichus have been reported for their meningeal content [21]. Indeed, the same trace elements and macroelements were revealed with roughly similar contents. The high content of these minerals in these plants could contribute to reduce malnutrition problems related to the deficiency of these elements, such as growth retardation and anemia which are prevalent in several populations [21]. In addition, calcium, whose daily requirements in human are in the order of 1000 to 1500 mg, also plays a role in bone structure and contributes to the sensitivity of nerves and muscles as well as its role in the activation of enzymes involved in digestion and metabolism [22, 23, 24, 25].

The importance of phosphorus (P) is also known for the health of the skin, hair, nails and nervous system, in addition to ensuring the neutrality of body fluids. The recommended dietary intake of P is 800 to 1300 mg per day [23, 25]. The minerals Na and K also play an important role in muscle and nerve function and the daily requirements are 6 g/day for Na and 2 to 4 g per day for K. Na deficiency can lead to circulatory, respiratory and nervous system disorders. Insufficient levels of K can lead to unbalanced blood pressure, irregular heartbeat and disorders of the renal and urinary systems [23, 25]. Regarding Mg, it plays a role as a cofactor for many enzymes and is involved in energy metabolism. It is the vital mineral required for the absorption of minerals, Ca, P, Na and K, by the body. The recommended daily intake of Mg is 200 to 400 mg. Mg deficiency is implicated in depression, migraine, cardiovascular disease, and hypertension [23, 25]. For boron, human studies suggest its involvement in the metabolism of calcium, copper, magnesium, amino acids, blood sugar, triglycerides, and estrogens. It is involved in erythropoiesis, immune defenses, and brain function as well as in anti-inflammatory action. The best documented effects concern its positive impact on bones, particularly calcification and stabilization of bone mass. Iron is a mineral necessary for energy production that provides oxygen to cells. Daily iron requirements are 10 to 15 mg and iron deficiency leads to anemia. As for zinc, it has an essential role in cell growth and differentiation. Zinc also plays an important role in the treatment of diabetes mellitus, infectious diseases, depression, and fatty liver. The daily requirement of Zn is 10-12 mg. A deficiency of this mineral leads to sweating of the joints, enlargement of the liver and

spleen, poor appetite, and growth retardation [23, 25]. As for copper, it is essential for the brain, nerves, and connective tissue and helps iron to make hemoglobin. The daily requirement of copper is 2-3 mg.

The results obtained for the dried plants studied, it can be deduced that the contribution of 1 g of aerial parts of Lavandula stoechas L. (locally called Halhal) is 16.234 mg of potassium, 4.223 mg of magnesium, 3.158 mg of iron, 1.116 mg of sodium and 0.320 mg of zinc. Similarly, 1 g of stems of Rubia peregrina (Foua) provides 3.879 mg of iron, 2.890 mg of potassium, 3.207 mg of sodium, 1.302 mg of calcium, 0.189 mg of magnesium, 0.238 mg of manganese and 0.210 mg of zinc. Tetraclinis articulata (Laaraar) leaves can also provide iron (9.076 mg), calcium (1.302 mg), magnesium (0.189 mg), manganese (0.238 mg) and zinc (0.210 mg). In addition, 1 g of Ammodaucus leucotrichus (Kamoun soufi) seeds provides 17.470 mg of potassium, 8.346 mg of calcium, 2.204 mg of magnesium and 1.193 mg of sodium.

### Limitations of the study

The plant parts analyzed, belong to plants collected from a single site, which does not reflect the variability of mineral contents that may exist depending on the growing geographical area of these plants and soil characteristics.

## CONCLUSIONS

The results of this study reveal the richness of the plants examined in minerals whose contents are confirmed in other studies conducted in Turkey and Algeria for some species such as *Lavandula stoechas* L., *Ammodaucus leucotrichus*. However, the mineral contents of *Tetraclinis articulata* and *Rubia peregrina* determined quantitatively by ICP spectroscopy are reported for the first time in this study. These data highlight the prospect of using these plants and their extracts as an alternative to chemicalbased synthetic products in industrial sectors.

This work also suggests that thanks to their mineral constituents, the parts of the plants studied could be used as a real natural source to enrich certain nutraceutical recipes or drinks. In addition, these wild plant species can be used as fortifiers for bakery products including bread and its derivatives, for an effective dietary strategy aimed at enhancing the nutritional value of these foods.

### Acknowledgements

The investigation was supported by the Moroccan Ministry of Higher Education and Research.

### Funding

No funding was received for this article.

#### **Conflict of interest**

The authors declare no conflict of interest.

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Received: 21.07.2024 Revised: 25.08.2024 Accepted: 30.09.2024 Published online first: 01.10.2024





https://roczniki.pzh.gov.pl/

Rocz Panstw Zakl Hig 2024;75(3):261-273

https://doi.org/10.32394/rpzh/194469

ORIGINAL ARTICLE

## ADHERENCE TO MEDITERRANEAN DIET IN MOROCCAN SCHOOL-AGE ADOLESCENTS: SOCIODEMOGRAPHIC, SOCIOECONOMIC AND LIFESTYLE DETERMINANTS

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## ABSTRACT

**Background.** Despite its benefits, the Mediterranean diet (MD) is abandoned or not adopted by the younger generations in most Mediterranean countries.

**Objective.** The aim here was to examine some factors determining adherence to MD in Moroccan school-age adolescents. **Material and Methods.** A cross-sectional survey was conducted in a school setting among 386 students (148 boys and 238 girls), aged 14 to 18 years, randomly selected and from different socioeconomic strata. A structured questionnaire was used to collect sociodemographic, socioeconomic and lifestyle data. Physical activity was assessed using the short French version of the IPAQ questionnaire. The KIDMED test was used to assess adherence to the Mediterranean diet. Weight, height and waist circumference were measured according to WHO standards. Body mass index (BMI) and waist-to-height ratio (WHtR) were calculated. Statistical ANOVA test was used for to compare multiple means, Student's t-test for independent samples, *Chi*<sup>2</sup> test for categorical variables and multinomial logistic regression analysis to identify factors associated with adherence to the MD amongst adolescents sample.

**Results.** Adherence to the MD in this population was very low, with nearly 53% having poor adherence and only 7.7% reporting optimal adherence. Multinomial regression analyses showed that poor adherence is correlated with female gender (p=0.042), low paternal education (p=0.004), limited number of meals (p=0.006), advanced age (p=0.005) and inadequate sleep duration (p=0.027). In contrast, better adherence appears to be correlated with a high number of meals per day (p<0.001) and a better socio-professional class of the father (p<0.001).

**Conclusion.** This study revealed a low prevalence of good adherence to MD confirming a transition of the study adolescents towards a Westernized diet. Targeted nutritional intervention programs should be implemented to improve adherence to the MD among Moroccan adolescents, by promoting healthy eating habits.

**Key words:** *Mediterranean diet, sociodemographic, socioeconomic, lifestyle characteristics, school-age adolescents, Morocco* 

## **INTRODUCTION**

The Mediterranean diet, recognized by UNESCO in 2010 as an intangible cultural heritage of humanity, is the traditional dietary pattern that characterizes the eating habits of populations living along the Mediterranean basin including Morocco, Greece, Spain and southern Italy [1]. This quasi-vegetarian diet is characterized by abundant consumption of plantbased foods (fruits, vegetables, legumes, unrefined natural cereals, nuts), moderate consumption of eggs, fish, dairy products and alcohol, and reduced consumption in quantity and frequency of saturated fats, sweets and red and processed meat. The main source of unsaturated fat is olive oil [2, 3]. In addition, the MD involves the consumption of traditional, local, seasonal products, minimally processed food products that promote biodiversity [4], and are environmentally friendly [5]. These elements qualify this model as much as a sustainable diet [6]. The MD richness is also related to social, cultural and life habits qualitative criteria, such as conviviality, culinary activities, frugality and sobriety [7]. All combined with regular physical activity and adequate rest [3, 8]. The Mediterranean diet is associated with a lower prevalence of several diseases, such as type

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Publisher: National Institute of Public Health NIH - National Research Institute

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2 diabetes, hypertension, cardiovascular diseases and some cancers among others, that are all associated with overweight [9, 10]. Indeed, the Mediterranean diet is low in calories and rich in vitamins and minerals derived from vegetables and fruits, whole grains, nuts, virgin olive oil and fish, which guarantees sufficient intakes of micronutrients [11]. This explains also the absence of B type vitamin deficiency  $(B_1, B_2, niacin, B_2, niacin, B_2)$ folates or  $B_{12}$  in the Mediterranean basin and the high intakes of antioxidant vitamins (vitamins E and C) and carotenes [12, 13]. All of these characteristics made WHO considering MD as a healthy diet [14]. However, despite the health benefits of the Mediterranean diet, there has been a gradual abandonment of this dietary pattern in the recent decades by the inhabitants of the Mediterranean basin and the adoption of a more Westernized dietary pattern, particularly by the younger generations [15, 16]. Indeed, during this period, many factors have influenced their eating habits [17]. This has been partly attributed to the process of globalization through the widespread diffusion of Western-type dietary patterns [18]. Furthermore, it is suggested that sociodemographic, socioeconomic and lifestyle characteristics significantly affect adherence to the Mediterranean diet among adolescents [16]. Previous studies have found that optimal adherence to the Mediterranean diet is associated with younger age [16], regular physical activity [15], and high paternal education [19], while other researchers have correlated adherence to the MD with maternal education [16]. On the other hand, the HELENA study [20], has reported an inverse association between adherence to the Mediterranean diet and sedentary behaviors related to screen time, similar results were found in the literature. In addition, better adherence to Mediterranean diet is correlated with optimal sleep duration [16]. It was indeed, reported that the lack of sleep appears to contribute to eating disorders such as anorexia, bulimia, and binge eating disorder and that, inadequate sleep duration is in addition, associated with low intake of minimally processed foods and high intake of ultra-processed foods [21]. Morocco, among other Mediterranean countries, has been facing, in recent years, a change in lifestyle including a nutrition transition and a shift from an active to a sedentary lifestyle [22]. This dietary transition is characterized by a shift from a traditional diet based on cereals and legumes to a diet composed of more animal products and tending to be excessive in relation to the energy needs of a sedentary life [23]. This phenomenon has led to a gradual decrease in malnutrition in young children but is also one of the actors involved in the high prevalence of excess weight in countries that are supposed to adopt a traditional Mediterranean diet [23].

Overweight and obesity, generally considered as risk factors for noncommunicable diseases (NCDs), also represent a public health problem in Mediterranean countries. This is the case in Morocco where excess weight affects both children and adults nationwide [24]. Thus, according to data from the latest 2018 National Survey on Population and Family Health, 10.8% of children under 5 years of age are overweight, of whom 2.9% are obese. In comparison with these data, a proportion of 10.4% was recorded in 2003-2004 against 10.7% in 2011 of overweight children [25]. On the other hand, the World Health Organization (WHO) reported that one in ten Moroccan children and adolescents, or 10.3% of boys and 9.9% of girls aged 5 to 19 years, are obese [24].

According to WHO, adolescence is a period of rapid growth, beginning with puberty and ending in adulthood [26]. This critical transition phase in life is marked by a constellation of developmental and behavioral changes, including a decline in healthy eating habits [27]. Given the psychological and behavioral complexity and particularities that accompany this stage of life, an individualized approach is required in many areas, mainly nutrition and nutritional needs [28]. The search for autonomy among young people, the nutritional transition and the lack of nutritional education are factors that disrupt eating habits with effects on health [29]. In Morocco, few studies have been conducted on the adherence of school adolescents to the Mediterranean diet. This lack of data is among the motivations for undertaking this work.

This study aims to assess the level of MD adherence using the KIDMED test in a sample of Moroccan adolescents attending school and to determine the association between sociodemographic, socioeconomic and lifestyle factors and adherence to the Mediterranean diet among school-age adolescents in Morocco.

#### MATERIAL AND METHODS

#### **Study population**

After the exclusion of 23 respondents, 386 adolescents enrolled in a public secondary school in the city of Azemmour, in the province of El-Jadida in the Casablanca-Settat region (Morocco), were recruited to participate in this cross-sectional study during the 2022/2023 and 2023/2024 school years.

The study included the schoolers aged 14-18 years enrolled in a public secondary school in the city of Azemmour who agreed to participate, those suffering from physical disabilities or who were absent on the days of the data collection were excluded from the study. Among the total of 23 respondents excluded from the study, 12 did not complete their questionnaires, 6 participants were absent during the anthropometric measurement sessions and 5 adolescents voluntarily withdrew.

## **Data collection**

A questionnaire was used to collect the various information. It is inspired by published sources, was translated into Arabic and adapted to the particularities of the Moroccan population.

# Socioeconomic and sociodemographic characteristics

The sociodemographic variables collected in this study are gender, age, parents' level of education (illiterate, primary, secondary or university), place of residence (urban or rural), household size, family structure (nuclear or composite) and marital status of parents (married, divorced, father or mother deceased). The information concerning socioeconomic characteristics included the socio-professional class (SPC) of parents classified into four categories according to the definition of Orban-Seghebarth and collaborators [30]. The first category SPC1 corresponds to large traders and liberal professions; SPC2 to civil servants and executives; SPC3 to craftsmen, employees, workers, farmers, drivers and shop assistants; and finally, the SPC4 category was that of people without any paid profession.

The other information collected is the household income which is classified into three categories: 1 - low (<300  $\in$ ), 2 - medium ( $\geq$ 300 and <500  $\in$ ) and 3 - high ( $\geq$ 500  $\in$ ). Finally, the type of housing is categorized into owner or tenant.

### Assessment of adherence to the Mediterranean diet

The KIDMED index developed by Serra-Majem et al., in 2004 has been extensively used to assess adherence to the Mediterranean diet and general dietary habits including skipping breakfast and fastfood consumption in different areas of the world.

This tool has proven good validity and reliability for evaluating adherence to MD among young populations in the Mediterranean region [31, 32]. It has demonstrated suitable psychometric properties for assessing adherence to the MD in schoolchildren [33].

The index consists of sixteen questions answered yes or no with a total score ranging from -4 to +12 [34]. The questions with negative connotations are: skipping breakfast, eating in a fast-food restaurant more than once a week, eating industrial pastries for breakfast and, eating sweets or candies several times a day received a score of -1, while the others received a score of +1. The total score was classified into three categories indicating an optimal adherence when the score is  $\geq 8$ , a need for improvements to achieve optimal adherence if the score is between 4 and 7, and finally indicating very poor dietary quality if the score is  $\leq 3$  [34]. In addition to the KIDMED index, the participants were also requested to answer questions on the number of meals consumed daily.

## Anthropometric measurements

Anthropometric variables were measured on each adolescent according to the World Health Organization standards [35]. Weight was recorded on each participant with light clothing and without shoes, using a TANITA BC-313 digital scale ( $150\pm0.05$  kg).

Height was assessed to the nearest 0.1 cm, using a height rod. The respondent stood without shoes, legs straight, arms hanging and relaxed and heels together, with head and buttocks in contact with the wall. Adolescents' waist circumference (WC) was measured in a standing position with the feet 2.5 cm apart, using a tape measure placed without compression midway between the lower costal margin and the anterior superior iliac crest on the mid-axillary line, at the end of a normal expiration and without exerting pressure on the skin.

Waist-to-height ratio (WHtR) was calculated and a value of 0.5 was used to define the presence or absence of abdominal obesity in boys and girls [36].

Body mass index (BMI), or Quetelet index, was used as an indicator of general obesity and calculated by dividing weight in kg by the square of height in meters (kg/m<sup>2</sup>). The reference standards established by WHO in 2007 are used to calculate z-score values for BMI/age using the WHO software, AnthroPlus (version 1.0.4, 2010) used to assess growth in children and adolescents worldwide [37]. Four categories are defined to classify the adolescents according to these values. They are underweight when z-score  $\leq$ -2SD, normal weight if -2 SD< z-scores <+1 SD, overweight if z-score  $\geq$ +1 SD and obese when z-score  $\geq$ +2 SD.

## Physical activities and sedentary behavior

Physical activity (PA) was assessed using the validated French short version of the modified International Physical Activity Questionnaire (IPAQ) for adolescents [38], according to which the respondent indicated the level of (PA) performed during the 7 days preceding the administration of the questionnaire. The frequency (number of days per week) and duration (minutes/day) of practice of three types of physical activity: vigorous, moderate and walking were recorded for each participant.

A mean "Metabolic Equivalent of Task" (MET) score, expressed as metabolic equivalent of task relative to rest, was derived for each type of activity: 3.3 for walking, 4.0 for moderate activity and 8.0 for vigorous activity [38]. The MET-minute score was then calculated by multiplying the MET score by the minutes performed, giving the energy expenditure (in metabolic equivalent of task: MET-minutes per week) for each type of activity.

Sedentary behavior and specifically screen time was assessed by asking the respondents to estimate the average time in hours per day that they spent in front of a screen (watching TV, playing computer games and using a mobile phone), a screen time of 2 hours per day was considered sufficient [39].

Mean sleep duration in hours per night was calculated as the difference between the reported bedtime in the evening and the wake-up time in the morning, with sleep duration classified as inadequate if less than 8 hours per night and adequate if greater than or equal to 8 hours per night [40].

#### **Statistical analysis**

Data analysis was performed using zmienić na: IBM SPSS Statistics for Windows version 26.0 (IBM Corp., Armonk, NY, United States). Quantitative variables are presented as means  $\pm$  standard deviations (SD) or as means ± standard error (SE), while qualitative variables are presented as frequencies and percentages. Adherence to the Mediterranean diet is classified into three categories based on established reference standards. Analysis of variance (ANOVA) was used to compare multiple means, Student's t-test was used for independent samples and the Chi<sup>2</sup> test was used to compare categorical variables. In addition, multinomial logistic regression analysis was performed to identify factors associated with adherence to the Mediterranean diet among Moroccan school-going adolescents. Differences were considered statistically significant for p-values less than 0.05.

### **Ethical considerations**

The survey was conducted after obtaining authorizations (N° 0483/2022 and N° 2015/2023) from the provincial education directorate of the Casablanca-Settat region, Morocco. Subjects were informed of the objectives and methodology of the research, as well as the possibility of withdrawing from the study at any time if they wished. All participants and their parents gave their consent before the start of the survey.

## RESULTS

## Sociodemographic and socioeconomic characteristics of the study population

Table 1 shows the data on the sociodemographic and socioeconomic characteristics of the study population. A total of 386 school-going adolescents were recruited in this cross-sectional study, of which 61.6% were female. The mean age of the respondents was  $15.5\pm0.9$  years, with the majority of the adolescents (59%) falling

into the 14-15 age group. The data also show that the majority (76.68%) of the adolescents resided in urban areas, while 23.32% resided in rural areas, and most of the subjects (88.86%) belonged to nuclear families. Regarding the education level of the respondents' parents, a proportion of 44.56% of the fathers had a low education level and 24.61% a higher education level. On the other hand, more than half of the mothers (56.74%) were with low level of education and only

Table1.Sociodemographicandsocioeconomiccharacteristics of the study population

Characte	N (%) or M±SD				
Sociodemographic characteristics					
Mean	15.5±0.9				
	14-15	228 (59.07%)			
Age categories	16	99 (25.65%)			
(years)	17-18	59 (15.28%)			
Sar	Boys	148 (38.34%)			
Sex	Girls	238 (61.6%)			
Area of regidence	Urban	296 (76.68%)			
Alea of lesidelice	Rural	90 (23.32%)			
	None	67 (17.36%)			
Father education	Primary	105 (27.20%)			
level	Secondary	119 (30.83%)			
	University	95 (24.61%)			
	None	140 (36.27%)			
Mothers education	Primary	79 (20.47%)			
level	Secondary	110 (28.50%)			
	University	57 (14.77%)			
	Married	349 (90.41%)			
Parente civil status	Divorced	16 (4.15%)			
T arents ervir status	Widow or widower	21 (5.44%)			
True of formily	Nuclear	343 (88.86%)			
Type of family	Composite	43 (11.14%)			
Socioe	conomic character	istics			
	SPC 2	127 (32.90%)			
Fathers SPC	SPC 3	245 (63.47%)			
	SPC 4	14 (3.63%)			
	SPC 2	26 (6.74%)			
Mothers SPC	SPC 3	46 (11.92%)			
	SPC 4	314 (81.35%)			
	Low <300 €	152 (39.38%)			
Monthly household income	Middle ≥300 and <500 €	112 (29.02%)			
	High ≥500 €	122 (31.61%)			
Type of hebitat	Owner	319 (82.64%)			
Type of naonal	Tenant	67 (17.36%)			

N (%) – number (percentage); M $\pm$ SD – mean  $\pm$  standard deviation; SPC – socio-professional class;  $\in$  – Euro

14.77% had a high education level. The results on the professional situation of the adolescents' parents show that 63.47% belonged to the class of employees, workers, craftsmen, farmers and traders (SPC3) while the majority of the mothers of these adolescents (81.35%) were without paid profession. Also, nearly 39% of the participants came from families with low monthly income. In addition, the marital status of the parents shows that the majority (90.41%) were married.

# Anthropometric characteristics of the population studied by sex

Table 2 represents the anthropometric parameters of the study population by sex. The adolescents studied had on average  $1.65\pm0.08$  m and weighed approximately  $58.35\pm10.39$  kg, their BMI was on average of  $21.39\pm3.51$ , the majority of them (79%) had a normal weight and their WHtR was on average  $0.43\pm0.05$ . Abdominal adiposity was present in (11.92%) of the study population, with a very high prevalence in girls (p<0.001). Thus, overweight including obesity affects 18.4% of the adolescents' sample, 23.5% in girls against 10% in boys.

BMI was significantly higher (p<0.001) in girls compared to boys, while height was significantly higher in boys (p<0.001).

# Lifestyle characteristics by gender of the study population

Table 3 illustrates the lifestyle characteristics of school-aged adolescents by gender. The table data show that the studied adolescents devoted significantly more time to vigorous physical activity (1637.96±90.75 MET-min/week) and walking (1610.11±64.28 MET-min/week) compared to moderate physical activity (1019.59±54.28 MET-min/week). The comparison by gender shows that boys spent 591.55 MET-min/ week more than girls on vigorous physical activity

Variables		Total N (%) or M±SD	Girls N (%) or M±SD	Boys N (%) or M±SD	p-value
Weigl	nt (kg)	58.35±10.39	57.72±10.36	59.37±10.41	0.129
Heig	ht (m)	1.65±0.08	$1.61 \pm 0.06$	1.71±0.07	< 0.001
BMI (	kg/m²)	21.39±3.51	22.21±3.48	20.08±3.15	< 0.001
WC (cm)		71.97±8.11	72.12±8.86	71.74±6.75	0.640
WHtR		0.43±0.05	$0.44{\pm}0.05$	0.41±0.03	< 0.001
	Underweight	9 (2.33%)	3 (1.30%)	6 (4.10%)	
BMI z-score	Normal weight	306 (79.27%)	179 (75.20%)	127 (85.80%)	0.004
categories	Overweight	55 (14.25%)	44 (18.5%)	11 (7.40%)	0.004
	Obesity	16 (4.15%)	12 (5.00%)	4 (2.70%)	
WILLED astagonias	Without AO	340 (88.08%)	197 (8.80%)	143 (96.60%)	<0.001
write categories	With AO	46 (11.92%)	41 (17.20%)	5 (3.40%)	

 Table 2. Anthropometric characteristics of the study population by sex

N (%) – number (percentage); M $\pm$ SD – mean  $\pm$  standard deviation; WC – waist circumference; WHtR – waist-to-height ratio; AO – abdominal obesity; *Chi*<sup>2</sup> test, t-test for independent samples: p<0.05

Table 3. Lifestyle characteristics in the study population by sex

Variables		Total N (%) or M±SE	Girls N (%) or M±SE	Boys N (%) or M±SE	p-value	
	Intense PA	1637.96±90.75	1411.15±88.78	2002.7±185.37	< 0.001	
Physical activity	Moderate PA	1019.59±54.28	894.3±61.41	1221.08±99.5	0.001	
	Walking	1610.11±64.28	1453.3±63.37	1862.27±131.81	0.023	
	<8 h	195 (50.5%)	117 (49.2%)	78 (52.7%)	0.459	
Sleep duration / night	≥8 h	191 (49.5%)	121 (50.8%)	70 (47.3%)	0.458	
Time spent in front of screens/day	≤2 h	74 (19.2%)	54 (22.7%)	20 (13.5%)	0.026	
	>2 h	312 (80.8%)	184 (77.3%)	128 (86.5%)	0.026	
	1-2	27 (6.99%)	20 (8.40%)	7 (4.70%)		
Number of meals/	3	193 (50.00%)	131 (55.00%)	62 (41.90%)	0.004	
day	4 or more	166 (43.01%)	87 (36.60%)	79 (53.40%)		

N (%) – number (percentage); M $\pm$ SE – mean  $\pm$  standard error; PA – physical activity; h – hours; *Chi*<sup>2</sup> test, ANOVA one-way: p<0.05

(p<0.001), 302.43 MET-min/week more on walking (p=0.023) and 326.78 MET-min/week more on moderate physical activity (p<0.001). Regarding the duration of nighttime sleep, the majority of adolescents do not meet WHO recommendations on sleep, with no significant difference between the two sexes. In addition, 80.8% of the teenagers studied spent more than 2 hours a day in front of a screen, with a significantly higher proportion of boys than girls exceeding WHO sleep recommendations (p=0.026).

Regarding the number of meals per day, a significantly higher proportion of boys (53.40%)consumed 4 or more meals per day compared to girls (p=0.004) while girls are more likely to eat 3 meals per day (55.00%).

# Data on the adherence of the study population to the Mediterranean diet

The results regarding the proportion of positive responses of the adolescents in the study to the KIDMED test according to gender are presented in Table 4. These data show that fruits and fruit juices were consumed daily by less than half of the participants (47%) with only 11% of them taking a second portion

each day. In addition, a large proportion (94%) of these students ate vegetables every day with 37% consuming them more than once a day. An almost daily consumption of pasta or rice was also observed in 59% of the respondents. Regarding protein-based foods, more than a quarter of this group (27%) regularly ate fish and three out of four adolescents (74%) consumed legumes more than once a week. Furthermore, as expected in a Mediterranean country, over 84% of people consumed olive oil regularly and nearly 59% regularly consumed dried fruits. On the other hand, regular breakfast consumption was reported by more than half of the students (54%), of whom 85% consumed cereals, 54% milk or dairy products and 32% commercially available bakery or pastries for breakfast. Furthermore, a few adolescents said they ate two yogurts and/or 40 g of cheese per day. The data show that, on the other hand, 66% of the sample frequented fast food restaurants more than once a week and around one out of two ate sweets and/or candies several times a day. Data analyses also show significant differences between the individual components of the KIDMED score and gender. Furthermore, the mean KIDMED score was significantly different between

Table 4. Percentages of positive responses of adolescents to KIDMED test according to gender

Questions	Total N (%)	Girls N (%)	Boys N (%)	p-value
Eat a fruit or fruit juice every day	183 (47.41%)	114 (29.3%)	69 (17.87%)	0.807
Eat a second fruit every day	45 (11.66%)	19 (4.92%)	26 (6.73%)	0.004
Eat raw vegetables (salad) or cooked once a day	363 (94.04%)	224 (58.03%)	139 (36.01%)	0.936
Eat raw or cooked vegetables more than once a day	146 (37.82%)	82 (21.24%)	64 (16.58%)	0.083
Eat fish regularly (at least 2 to 3 times a week)	105 (27.20%)	63 (16.32%)	42 (10.88%)	0.682
Eat at least once a week in a fast-food restaurant	256 (66.32%)	155 (40.15%)	101 (26.16%)	0.529
Eat dried vegetables (legumes) more than once a week	288 (74.61%)	174 (45.07%)	114 (29.53%)	0.39
Eat pasta or rice at least 5 times a week	152 (39.38%)	95 (24.61%)	57 (14.76%)	0.784
Eat cereals and their derivatives (bread,) for breakfast	331 (85.75%)	191 (49.48%)	140 (36.26%)	< 0.001
Eat dried fruits regularly (dates, almonds, walnuts, peanuts	229 (59.33%)	139 (36.01%)	90 (23.31%)	0.64
Eat olive oil in your home regularly	328 (84.97%)	196 (50.77%)	132 (34.19%)	0.068
Skip breakfast	175 (45.34%)	120 (31.08%)	55 (14.24%)	0.011
Eat milk and its derivatives (butter, cheese, yogurt) for breakfast	210 (54.40%)	128 (33.16%)	82 (21.24%)	0.755
Eat industrial pastries for breakfast (croissant, biscuit,)	127 (32.90%)	87 (22.53%)	40 (10.36%)	0.053
Eat 2 yogurts or 40g of cheese (4 pieces) every day	37 (9.59%)	23 (5.95%)	14 (3.62%)	0.947
Eat sweets, chocolates, candies, several times a day	211 (54.66%)	134 (34.71%)	77 (19.94%)	0.412
KIDM	ED Index			
Low adherence	207 (53.63%)	141 (59.20%)	66 (44.60%)	
Moderate adherence	149 (38.60%)	81 (34.00%)	68 (45.90%)	0.019
Optimal adherence	30 (7.77%)	16 (6.70%)	14 (9.50%)	
KIDMED Mean Score	4.27±2.2	4±2.27	4.7±2.01	0.002

N (%) – number (percentage); M $\pm$ SD – mean  $\pm$  standard deviation; t-test, *Chi*<sup>2</sup> test: p<0.05

genders, namely 4.7 $\pm$ 2.01 in boys versus 4 $\pm$ 2.27 in girls (p=0.002). In addition, the proportion of boys who ate a second portion of fruit per day was significantly higher than that of girls (p=0.004). On the contrary, more girls consumed significantly cereals and their derivatives at breakfast than boys (49% versus 36.26% respectively; p<0.001) but also, they skipped breakfast more than boys (p=0.011). The results of the present study revealed a low percentage of adolescents who

have optimal adherence (7.77%) while nearly (38.60%) of these adolescents had moderate adherence and 53.63% had low adherence. In fact, the group of adolescents with low adherence included a higher percentage of girls (59.20%), while the groups with moderate and high adherence included more boys (45.90% and 9.50%, respectively) than girls (34% and 6.70%, respectively) (p=0.019).

Characteristics		Total N (%)	Low adherence	Moderate adherence	Optimal adherence	p-value	
		14 (70)	N (%)	N (%)	N (%)		
Age categories	14-15	228 (59.07%)	106 (51.2%)	100 (67.1%)	22 (73.3%)	-	
	16	99 (25.65%)	57 (27.5%)	35 (23.5%)	7 (23.3%)	0.002	
() •••••)	17-18	59 (15.28%)	44 (21.3%)	14 (9.4%)	1 (3.3%)		
Sex	Boys	238 (61.66%)	141 (68.1%)	81 (54.4%)	16 (53.3%)	0.019	
	Girls	148 (38.34%)	66 (31.9%)	68 (45.6%)	14 (46.7%)	0.019	
A	Urban	90 (23.32%)	50 (24.2%)	36 (24.2%)	4 (13.3%)	- 0.40	
Alea of residence	Rural	296 (76.68%)	157 (75.8%)	113 (75.8%)	26 (86.7%)		
	None	67 (17.36%)	40 (19.3%)	25 (16.8%)	2 (6.7%)	<0.001	
Father education	Primary	105 (27.2%)	72 (34.8%)	28 (18.8%)	5 (16.7%)		
level	Secondary	119 (30.83%)	62 (30%)	50 (33.6%)	7 (23.3%)		
	University	95 (24.61%)	33 (15.9%)	46 (30.9%)	16 (53.3%)		
	None	140 (36.27%)	83 (40.1%)	51 (34.2%)	6 (20%)	0.01	
Mother education	Primary	79 (20.47%)	46 (22.2%)	29 (19.5%)	4 (13.3%)		
level	Secondary	110 (28.5%)	52 (25.1%)	49 (32.9%)	9 (30%)		
	University	57 (14.77%)	26 (12.6%)	20 (13.4%)	11 (36.7%)	1	
	Married	16 (4.15%)	11 (5.3%)	4 (2.7%)	1 (3.3%)	1	
Parents civil status	Divorced	349 (90.41%)	179 (86.5%)	142 (95.3%)	28 (93.3%)	0.07	
	Widow or widower	21 (5.44%)	17 (8.2%)	3 (2%)	1 (3.3%)		
Type of family	Nuclear	43 (11.14%)	26 (12.6%)	15 (10.1%)	2 (6.7%)	0.548	
	Composite	343 (88.86%)	181 (87.4%)	134 (89.9%)	28 (93.3%)		
	SPC 2	117 (30.31%)	52 (25.1%)	50 (40.3%)	15 (50%)		
Fathers SPC	SPC 3	245 (63.47%)	143 (69.1%)	87 (58.4%)	15 (50%)	0.002	
	SPC 4	14 (3.63%)	12 (5.8%)	2 (1.3%)	0 (0%)		
	SPC 2	26 (6.74%)	12 (5.8%)	9 (6%)	5 (16.7%)		
Mothers SPC	SPC 3	46 (11.92%)	34 (16.4%)	11 (7.4%)	11 (7.4%) 1 (3.3%)		
	SPC 4 314 (81.35		161 (77.8%)	129 (86.6%)	24 (80%)		
Monthly household income	Low <300 €	152 (39.38%)	101 (48.8%)	44 (29.5%)	7 (23.3%)		
	Middle ≥300 and <500 €	112 (29.02%)	61 (29.5%)	44 (29.5%)	7 (23.3%)	<0.001	
	High ≥500 €	122 (31.61%)	45 (21.7%)	61 (40.9%)	16 (53.3%)		
	Owner	67 (17.36%)	42 (20.3%)	16 (10.7%)	9 (30%)	0.01	
Type of habitat	Tenant	319 (82.64%)	165 (79.7%)	133 (89.3%)	21 (70%)	0.01	
	<5	247 (63.99%)	124 (59.9%)	100 (67.1%)	23 (76.7%)		
Household size	5-7	126 (32.64%)	77 (37.2%)	42 (28.2%)	7 (23.3%)	0.166	
catgories	>8	13 (3.37%)	6 (2.9%)	7 (4.7%)	0 (0%)	1	

Table 5. Factors associated with adherence to the MD in the adolescents studied

N (%) – number (percentage); SPC – socio-professional class; € – Euro; Chi<sup>2</sup> test: p<0.05

Table 5 presents the results of the bivariate analysis of factors associated with adherence to the Mediterranean diet in the adolescents studied. Indeed, according to the data of the present study, nearly 51% and 62% of adolescents whose fathers and mothers had a low level of education, respectively, did not adhere to the Mediterranean diet (p < 0.001 and p = 0.01, respectively). On the other hand, optimal adherence to the Mediterranean diet seems to be associated with younger age, such that 73% of adolescents in the 14-15 age group have better adherence compared to their 17-18-year-old counterparts (3%) (p=0.002). Furthermore, a significantly higher proportion of adolescents whose father and mother belonged to SPC2 had optimal adherence, suggesting that a better socioeconomic situation seems to be associated with good adherence to the Mediterranean diet (p=0.002 and p=0.009, respectively). In addition, good adherence was marked in 53% of children from households with a high monthly income (p<0.001). Contrary to what was expected, 79% of participants whose parents' housing status was home ownership had low adherence. The results obtained here do not show any influence of other parameters studied such as the area of residence, the marital status of the parents, the type of family and the size of the household on adherence to the Mediterranean diet.

The results regarding the association between adherence to the Mediterranean diet and lifestyle characteristics are illustrated in Table 6. According to the results of the table, no association was found between adherence to the Mediterranean diet and the different components of physical or sedentary activity, namely, intense physical activity (p=0.281), moderate physical activity (p=0.123) or walking (p=0.689), nor with screen time (p=0.449). On the other hand, better adherence to the Mediterranean diet is positively correlated with optimal sleep duration (p=0.004) and the number of meals per day (p<0.001). Indeed, 70% of adolescents who ate four or more meals per day, and those with an efficient sleep duration had a good adherence to the Mediterranean diet compared to their counterparts.

Table 7 presents the results of the logistic regression analysis of factors associated with adherence to the Mediterranean diet. Indeed, to eliminate confounding factors, following the bivariate analysis, and to explore the relationship between adherence to the MD, with moderate adherence as reference modality, and all significant determinants, a multinomial regression analysis was conducted. As shown in Table 7, this analysis showed that the risk of having poor adherence is associated with female gender compared to male gender (OR=1.68; 95% CI: 1.02-2.76). In addition, adolescents whose mothers do not have a profession (OR=0.45; 95% CI: 0.20-0.98) are less likely to have low adherence compared to their counterparts, suggesting that housewives put a lot of time and care into meal preparation. Moreover, a father's secondary or university education is associated with a significantly reduced chance of poor adherence to the MD compared to primary education (p=0.017, OR=0.45; 95% CI: 0.23-0.87 and p=0.004, OR=0.28; 95% CI: 0.12-0.66) respectively. Furthermore, participants in the 17-18 age group were more likely to have low KIDMED scores (OR=2.9; 95% CI: 1.38-6.12). On the other hand, inadequate sleep duration was correlated with a higher likelihood of having low adherence to the diet (OR=1.72; 95% CI: 1.06-2.78). Furthermore, adolescents who ate 1-2 meals per day were 5.25 times more likely to have low adherence (OR=5.25; 95% CI: 1.62-17.04), compared to their peers. In contrast, good

Characteristics		Total N (%) or M±SE	Low adherence N (%) or M±SE	Moderate adherence N (%) or M±SE	Optimal adherence N (%) or M±SE	p-value
Physical activity	Intense PA	1637.96±90.75	1632.92±119.97	1702.55±152.08	1352±334.39	0.281
	Moderate PA	1019.59±54.28	934.6±76.93	966.84±68.42	1868±259.84	0.123
	Walking	1610.11±64.58	1728.81±91.58	1441.47±98.95	1628.55±211.69	0.689
Sleep duration/ night	<8 h	195 (50.5%)	119 (57.5%)	67 (45%)	9 (30%)	0.004
	≥8 h	191 (49.5%)	88 (42.5%)	82 (55%)	21 (70%)	0.004
Time spent in front of screen/day	≤2 h	74 (19.2%)	36 (17.4%)	30 (20.1%)	8 (26.7%)	0.440
	>2 h	312 (80.8%)	171 (82.6%)	119 (79.9%)	22 (73,3%)	0.449
Number of meals/ day	1-2	27 (6.99%)	23 (11.1%)	4 (2.7%)	0 (0%)	
	3	193 (50%)	111 (53.6%)	73 (49%)	9 (30%)	< 0.001
	4 or more	166 (43.01%)	73 (35.3%)	72 (48.3%)	21 (70%)	

Table 6. Adherence to the Mediterranean diet in the study population according to lifestyle characteristics

N (%) – number (percentage); M $\pm$ SE – mean  $\pm$  standard error; PA – physical activity; h – hours; *Chi*<sup>2</sup> test, ANOVA one-way: p<0.05

Characteristics		Poor adherence		Optimal adherence		
		OR (IC 95%)	p-value	OR (IC 95%)	p-value	
C	Girls	1.68 (1.02-2.76)	0.042	1.4549 (0.58116-3.642)	0.423	
Sex	Boys	Reference				
Father education level	None	0.63 (0.30-1.31)	0.213	0.46 (0.076-2.79)	0.397	
	Primary	Reference				
	Secondary	0.45 (0.23-0.87)	0.017	0.78 (0.20-3)	0.714	
	University	0.28 (0.12-0.66)	0.004	1.51 (0.32-7.05)	0.602	
Mother education level	None	0.67 (0.34-1.32)	0.251	1.14 (0.26-4.98)	0.858	
	Primary	Reference				
	Secondary	0.69 (0.34-1.42)	0.32	1.30 (0.32-5.3)	0.711	
	University	1.43 (0.57-3.600)	0.448	3.14 (0.68-14.43)	0.141	
	SPC 2	0.92 (0.48-1.76)	0.812	0.70 (0.21-2.33)	0.564	
Fathers SPC	SPC 3	Reference				
Tamers Sr C	SPC 4	2.12 (0.43-10.55)	0.358	1.14×10 <sup>-5</sup> (1.14×10 <sup>-5</sup> -1.14×10 <sup>-5</sup> )	< 0.001	
Mothers SPC	SPC 2	0.5971 (0.16-2.23)	0.443	3.62 (0.28-47.50)	0.326	
	SPC 3	Reference				
	SPC 4	0.45 (0.20-0.98)	0.045	2.22 (0.23-20.98)	0.486	
Age categories (years)	14-15	Reference				
	16	1.48 (0.82-2.66)	0.193	1.34 (0.45-4)	0.595	
	17-18	2.9 (1.38-6.12)	0.005	0.44 (0.05-3.79)	0.452	
Sleep duration/night	<8 h	1.72 (1.06-2.78)	0.027	0.54 (0.21-1.39)	0.201	
Number of meals/day	1-2	5.25 (1.62-17.04)	0.006	6.86×10 <sup>-7</sup> (6.86×10 <sup>-7</sup> -6.86×10 <sup>-7</sup> )	< 0.001	
	3	Reference				
	4 or more	0.86 (0.52-1.40)	0.451	2.52 (0.97-6.58)	0.058	

Table 7. Results of the multinomial logistic regression analysis of factors associated with adherence to the Mediterranean diet

OR - odds ratio; SPC - socio-professional class

adherence was correlated with a negligible chance to a limited number of meals per day (OR= $6.86 \times 10^{-7}$ ; 95% CI:  $6.86 \times 10^{-7}$ - $6.86 \times 10^{-7}$ ). Conversely, a higher number of meals per day seems to be a good predictor of optimal adherence (OR=2.52; 95% CI: 0.97-6.58), although this is not significant (p=0.058). Also, a strong adherence to the Mediterranean diet is correlated with an extremely low probability (almost zero) to the professional occupation of the father SPC4 (OR= $1.14 \times 10^{-5}$ ; 95% CI:  $1.14 \times 10^{-5}$ - $1.14 \times 10^{-5}$ ) which suggests an association of optimal adherence to the MD with a better socio-professional class.

## DISCUSSION

The data from the present cross-sectional study revealed the sociodemographic, socioeconomic and lifestyle factors determining adherence to the traditional Mediterranean diet among the youth population in Morocco verifying positively the formulated research hypothesis. Indeed, the results of this study, which focused on a sample of 386 Moroccan school-age adolescents, attests that adherence to the MD among this population was extremely poor, with a very low percentage of the adolescents having optimal adherence, while 38% of the participants were moderately adherent to this diet. Also, poor adherence is correlated with female gender, low level of education of the father, limited number of meals per day, advanced age and inadequate sleep duration. On the other hand, better adherence appeared to be correlated with high number of meals per day and a better socio-professional class of the father. It has been previously reported that the degree of adherence to the Mediterranean diet differs between Mediterranean countries and even within the same country [41, 42]. The low rate of optimal adherence revealed by the present study corroborates the results of a previous research conducted on a population of Greek adolescents aged 12-18 years [16]. The present data also show that the poor adherence reported here may be related to a reduced frequency of consumption

of the basic food groups characterizing the traditional MD. Indeed, the results show a majority of adolescents who did not take a second portion of fruit or fruit juice each day (89%), did not consume raw or cooked vegetables more than once a day (63%), did not regularly eat fish (73%) and who ate fast food more than once a week (66%). In addition, the consumption of sweets and/or candies several times a day is recorded in more than half of the participants. Furthermore, a small percentage of the participants of this young population ate two yogurts and/or 40 g of cheese per day.

However, our results contrasted with the study of Azekour et al. (2020) [43] and the DIMENU study [44] which reported that 58% and 61% of their respective participants, had moderate adherence to MD. In the present study sample, girls are more likely to have poor adherence compared to boys. Also, the reported results reveal that the proportion of boys who ate a second portion of fruit per day was significantly higher than that of girls and that more girls skipped breakfast than boys. These results are consistent with those found in the literature [16], while Depboylu and Kaner (2023) [19] found no correlation. These results could be explained by various factors, including social and personal aspects. Indeed, boys tend to participate more in sports and physical activity as judged by the observations reported in this study, and this tendency may be associated with healthier food choices. On the other hand, adolescent girls may face more societal pressures regarding body image than boys, such a situation could lead to a greater emphasis on restrictive diets or alternative eating habits, which could distance them from adopting the Mediterranean diet. This study also revealed that older adolescents presented lower adherence, this finding followed previous studies [16, 19], this result may be explained by the absence of parental control, the increased influence peers, the acquisition of autonomy and financial independence in this age group and could be associated with inadequate nutritional intake and poor nutritional status [45].

Another significant finding of the present study was the association between optimal adherence to the Mediterranean diet and the high level of education of the father, and this has also been suggested by other studies [19, 43]. A weak correlation between a level of parental education and a low level of nutritional knowledge and a limited awareness of nutrition-related issues has in fact already been demonstrated before [19], while other data, namely those of the PASOS study [46] (Physical Activity, Sedentarism, lifestyles and Obesity in Spanish youth) reported that optimal adherence to the Mediterranean diet was associated with the level of income and the highest degree of maternal education [47]. Similar results to the latter have been reported previously [16, 46]. Certainly, mothers with a high level of education can influence their children's food choices through the availability and accessibility of certain foods, as well as by being a role model for them. However, it is also possible that higher educational status is associated with higher income and, therefore, greater availability of healthy foods [48].

An association of inadequate sleep duration with low adherence to the Mediterranean diet was also revealed by the present results in accordance with other studies [16, 19]. Sleep deprivation is known to contribute to eating disorders such as anorexia, bulimia, and binge eating disorder. In addition, insufficient sleep duration is associated with low consumption of minimally processed foods and high consumption of ultra-processed foods [21].

The present study further reports that a limited number of meals per day is associated with poor adherence to MD. These data are in agreement with those of some authors [49], while others found no correlation [15], moreover, skipping meals was associated with poor compliance with MD [47]. Likewise, skipping meals is correlated with unnecessary snacking, especially unhealthy food choices and energy-dense foods rather than the nutrient-dense items that are part of the MD [15].

Previous research has mentioned an association between a high KIDMED score and regular physical activity and reduced screen time [15, 20, 43], in that sense, some studies have found an association between absence of distractions or leisure (such as television) during mealtimes and higher degree of adherence to a MD. The lack of the latter might enhance family supervision and support the development of healthy eating habits, potentially mitigating the negative impact of television food advertising on the dietary behaviors of children and adolescents [48]. Unexpectedly, and contrary to previous studies, our findings did not find any significant variations in this score according to the levels of this variable.

#### Strengths and limitations

This study reported interesting and original data on the determinants of adolescents' adherence to MD. However, these results should be considered in light of their strengths and limitations.

To the best of our knowledge, this is the first study to analyze the association between adherence to the Mediterranean diet and sociodemographic, socioeconomic, and lifestyle factors among schoolgoing adolescents in Morocco.

A major strength of this study is that physical activity and adherence to the Mediterranean diet were assessed by internationally validated adolescent questionnaires, such as the IPAQ and KIDMED. Another strength is that data collection was conducted through face-to-face interviews, which could reduce the bias of self-reported data.

On the other hand, among the limitations of this study is that the cross-sectional design does not allow conclusions on causality. In addition, the validity of the KIDMED questionnaire and its adaptation to the particularities of the Moroccan population have not yet been carried out in Morocco. Also, the number of girls and boys included in the study sample is not balanced.

## CONCLUSIONS

The data reported in this study indicate that adherence to the Mediterranean diet in the adolescent population surveyed was very low with more than half of adolescents having worse adherence and a low percentage with high adherence to this diet. These results confirm the progressive nutritional transition underway in this population, diverting its diet from a healthy traditional diet to a Westernized diet as previously described.

Further, the present study results reveal that poor adherence to MD was correlated with female gender, low level of education of the father, limited number of meals, advanced age and inadequate sleep duration. On the other hand, better adherence appears to be correlated with a high number of meals per day and a better father socio-professional class.

Given that adolescence is a critical phase for the adoption and maintenance of healthy habits and that adolescents are the adults of tomorrow and to avoid the onset of health problems early in young adults, interventions targeting Moroccan adolescents should be planned. Also research studies accompanying and evaluating these interventions must also be undertaken to establish adequate strategies. Such interventions should use practical advice to orient adolescents towards the benefits of the Mediterranean diet to improve their adherence to this diet. In addition, nutrition education should include families to ensure an improvement in the quality of their food choices. Finally, policy makers should also consider food cost policies, by lowering the prices of healthy foods or providing financial support to low-income households, especially in times of economic and societal disruptions, when inequalities make people in poverty more vulnerable.

### Abbreviations

UNESCO – United Nations Educational, Scientific and Cultural Organization; MD – Mediterranean diet; KIDMED – Mediterranean Diet Quality Index for Children and Adolescents; IPAQ – International Physical Activity Questionnaire; WHO – World Health Organization; BMI – body mass index; WHtR  waist-to-height ratio; PA – physical activity; MET – Metabolic Equivalent of Task

### Acknowledgments

The authors thanks all those who, through their various contributions, led to the implementation of this study.

#### **Conflicts of Interest**

The authors declare no conflicts of interest.

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Received: 28.08.2024 Revised: 17.09.2024 Accepted: 10.10.2024 Published online first: 23.10.2024



Rocz Panstw Zakl Hig 2024;75(3):275-282

https://doi.org/10.32394/rpzh/195562

ORIGINAL ARTICLE

## EVALUATION OF GESTATIONAL WEIGHT GAIN IN WOMEN WITH TWIN PREGNANCIES AND ITS RELATIONSHIP TO NEONATAL BIRTH WEIGHT. A PILOT STUDY

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## ABSTRACT

**Background.** To date, there have been no studies in Poland on weight gain in women with twin pregnancies in relation to recommendations.

**Objectives.** The aim of this study was to analyze the gestational weight gain of women with twin pregnancies depending on their body weight before pregnancy, and to assess the relationship between the observed weight gain and the neonatal birth weight.

**Material and Methods.** The study was conducted among 50 women in twin pregnancies and their 100 newborns delivered after 36 weeks of gestation. Gestational weight gain was assessed based on the American Institute of Medicine guidelines for women in twin pregnancies. Data on the pre-pregnancy body weight and gestational weight gain were collected by interviewing the patients. Neonatal data were obtained from the hospital medical records.

**Results.** Normal gestational weight gain was observed only in 38% of the women. In women with monochorionic pregnancy, too low body weight gain occurred almost 3 times more often than in women with dichorionic pregnancy (74% vs. 26%), (p<0.001). Women with monochorionic pregnancies also gave birth to statistically significantly more newborns with low birth weight (<2500 g), compared to women with dichorionic pregnancies (62.5% vs. 37.5%) (p=0.007). The mean birth weight of newborns born to mothers with excessive weight gain was 151 g higher than children born to mothers with normal weight gain (2727 g vs. 2576 g) (p=0.035).

**Conclusions.** In the majority of studied women in twin pregnancies, gestational weight gain was not compliant with the current recommendations. The risk of insufficient weight gain is higher in women with monochorionic pregnancies compared to women with dichorionic pregnancies.

Key words: twin pregnancy; gestational weight gain; neonatal birth weight

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## **INTRODUCTION**

Over the past 30 years, there has been a considerable increase in the number of multiple pregnancies worldwide. In the US, France, and Australia twin births now account for 3.0–3.5% of all births (one birth in about 30 births) [1, 2], and in Poland in 2022 they accounted for 1.3%, giving a number of 3787 births [3]. The main factor contributing to multiple pregnancies is the use of assisted reproductive technology, followed by older age of women giving birth and possibly maternal obesity prior to pregnancy [2, 4].

In both singleton and twin pregnancies, the gestational weight gain (GWG) is an important factor affecting the course of pregnancy. Too low maternal weight gain increases, among others, the risk of preterm birth, while an excessive weight gain – the risk

of gestational diabetes and hypertension [5, 6]. The weight gain of pregnant women also correlates with neonatal birth weight. Mothers with insufficient weight gain more frequently give birth to small-for-gestational-age children, while among the newborns of mothers who gained too much, macrosomia is more common [7, 8].

According to some data, the body weight gain of mothers with a twin pregnancy starts sooner than those with a singleton pregnancy [9], while according to others, weight gain is the same until the 18th week of pregnancy and only after that time does it increase significantly in the case of twin pregnancies [10]. The literature also suggests that in women with twin pregnancies, weight gain in individual trimesters of gestation is more important than the total gain. It is believed that adequate weight gain at the beginning

Publisher: National Institute of Public Health NIH - National Research Institute

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of pregnancy has a significant impact on the development and functioning of the placenta, and in multiple pregnancies the placenta probably matures and ages more rapidly, which shortens the time of optimal nutrient supply to the fetus [9, 11, 12]. Some experts claim that, the weight gain of women in twin pregnancies in the first half of gestation also affects the weight of both newborns to the greatest extent [13]. The results of other studies indicate that only in the second trimester weight gain has a statistically significant relationship with neonatal birth weight [12].

When it comes to the total maternal body weight gain, many countries, including Poland, adopted the guidelines of the American Institute of Medicine of 2009 [14, 15]. As in the case of a singleton pregnancy, the weight gain of women in twin pregnancies depends on the Body Mass Index (BMI) before pregnancy. In contrast to the guidelines for women with singleton pregnancies, no weight gain values have yet been developed for obese women depending on the degree of obesity [14, 16]. It is worth noting that a body weight gain in women pregnant with twins who had a normal pre-pregnancy body weight is 47–56% higher compared to women expecting one child, while in obese women – even 111–120% higher.

To the best of authors' knowledge, there have been no studies in Poland on the weight gain of women with twin pregnancies and its relationship with the birth weight of newborns. Therefore, the aim of this study was to analyze the gestational weight gain of women with twin pregnancies in relation to BMI before pregnancy, and to assess the relationship between the observed weight gain and the neonatal birth weight.

#### **MATERIAL AND METHODS**

#### Study design

This retrospective study was conducted among 50 women with twin pregnancies and their 100 newborns delivered after 36 weeks of pregnancy at the 1<sup>st</sup> Department of Obstetrics and Gynecology, Medical University of Warsaw (years 2021–2022) or at the Department of Obstetrics, Perinatology and Neonatology, Centre of Postgraduate Medical Education in Warsaw (year 2023). Although delivery before 37 completed weeks of gestation is considered preterm birth, in case of uncomplicated monochorionic

twin pregnancies it is recommended to terminate the pregnancy between 36 and 37 weeks due to the lowest rate of neonatal complications [17]. The exclusion criteria for patients were age below 18 years, non-Polish nationality, and pregnancies complicated by twin-to-twin transfusion syndrome or with congenital anomalies of either of the twins. The patients gave their written consent to participate in the study. The study was approved by the Bioethics Committee of the National Institute of Public Health NIH – National Research Institute in Warsaw under No. 6/2021.

Gestational weight gain was assessed based on the American Institute of Medicine guidelines for women in twin pregnancies [14] (Table 1).

As stated in the Table 1, the weight gain for women who were underweight before pregnancy is not established, therefore, as other authors [18], for such women (10% of the group) we assumed the gain as for women with normal body weight. Some studies also show that the weight gain in underweight women equal to that in women with normal body weight is optimal to reduce the risk of adverse perinatal outcomes [19, 20].

Given that our study included pregnant women whose gestation exceeded 36 weeks, it is worth emphasizing that the assessment of gestational weight gain is in line with the above-mentioned guidelines, which were developed on the basis of the weight gain of women who gave birth to twins weighing no less than 2500 g after the end of the 36th week of pregnancy. The minimum gestational length of 36 weeks for assessing the weight gain in women with twin pregnancies in the context of the guidelines is also used by other authors [5].

### **Data collection**

Data on the body weight of women before pregnancy, their height, gestational weight gain, diet, taking vitamin and mineral preparations, course of pregnancy, lifestyle during pregnancy and sociodemographic data were collected by the method of an interview conducted by a dietician during the perinatal period. Maternal weight gain was calculated as the difference between mothers' self-reported prenatal weight or weight measured at the last obstetric visit and the pre-pregnancy weight. As this part of the study was based on a survey, pre-pregnancy weight

Table 1. Weight gain recommendations for women with twin pregnancies [14]

Pre-pregnancy BMI (kg/m <sup>2</sup> )	Nutritional status	Recommended weight gain for women with twin pregnancies (kg)
<18.5	Underweight	Not established
18.5–24.9	Normal body weight	17–25
25.0–29.9	Overweight	14–23
≥30.0	Obesity	11–19
and gestational weight gain were expressed rounded to 1 kg. The latter was categorized into three groups: below, in line with or above the recommendations.

Neonatal data (sex and birth weight) were obtained from the hospital medical records. Birth weight of the newborns was measured using a physician beam

scale. The characteristics of women and newborns are presented in Table 2.

# Statistical analysis

The following descriptive statistics were determined for the analyzed variables: percentage

Table 2. Maternal and neonatal characteristics

Maternal characteristics						
Number of women, n	50					
including:						
monochorionic pregnancies, n (%)	23 (46)					
dichorionic pregnancies, n (%)	27 (54)					
Age (in years), mean $\pm$ SD	31.7±4.6					
Education, n (%)						
higher	36 (72)					
other	14 (28)					
Place of residence, n (%)						
city/town	46 (92)					
rural/village	4 (8)					
Number of pregnancies, n (%)						
first	26 (52)					
subsequent	24 (48)					
Gestational age (in weeks), median (min-max)	36 (36–38)					
Gestational age of monochorionic pregnancy (in weeks), median (min-max)	36 (36–37)					
Gestational age of dichorionic pregnancy (in weeks), median (min-max)	37 (36–38)					
Maternal Body Mass Index (BMI) prior to conception, median (min-max)	22.5 (16.6–38.9)					
Gestational diabetes, n (%)	10 (20)					
Hypertension, n (%)	3 (6)					
Anaemia, n (%)	17 (34)					
Smoking during pregnancy, n (%)	0 (0)					
Supplementation with vitamin-mineral preparations (multicomponent), n (%)	49 (98)					
Calcium intake from milk and dairy products (mg), median (min-max)	641.1 (0.0–2900.4)					
Daily vitamin D intake						
with food ( $\mu$ g), median (min-max)	2.4 (0.4–9.1)					
with food and dietary supplements (µg), median (min-max)	52.1 (1.2–154.6)					
Caffeine intake from coffee and tea (mg), mean±SD (mg)	91.7±73.2					
Fish consumption (at least once a week), n (%)	20 (40)					
Food consumption during pregnancy compared to before pregnancy, n (%):						
no change (same amount of food consumed)	23 (46)					
10–20% more food consumed	12 (24)					
approximately 30% more food consumed	10 (20)					
approximately 50% more food consumed	3 (6)					
more than 50% more food consumed	1(2)					
less food consumed	1 (2)					
Neonatal characteristics	100					
Number of newborns, n Sex of the newborn $n (0/)$	100					
sex of the newborn, n (%)	15 (15)					
fomela	45 (45)					
Noonotal weight (g) maan + SD	2557.8+205.6					
Number of neuhorne with low birth weight ( $(2500 \text{ c}) = 0.00$	<u> </u>					
Number of new off is with low birth weight (<2500 g), $\pi$ (%)						
Dirth meislet discondence, p. (0/)						
Difful weight discordance, fi (70)	51 (16, 56)					
Noonatal hoad aircumfaranaa (am) madiar (min max)	$\begin{array}{c} 31 (40-30) \\ 22 (20, 25) \end{array}$					
Neonatal about circumference (cm), median (min-max)	<u> </u>					
Ineonatal chest circumference (cm), median (min-max)	51(2/-55)					
Apgar score at 5 minutes (points), median (min-max)	10 (8–10)					

frequencies for qualitative variables, arithmetic mean with standard deviation for quantitative variables with a normal distribution and median and range of variability for other quantitative variables. The normality of distribution was tested using the Kolmogorov-Smirnov test.

Values of quantitative variables were compared between distinguished study subgroups using the Student's t-test or non-parametric Mann-Whitney test according to their distribution normality. The significance of differences in frequencies of qualitative characteristics were tested by the chi-square test or the exact Fisher's test depending on subgroup size. The comparison of statistical significance of differences in GWG expressed in kg, according to the nutritional status of women was performed using the Kruskal-Wallis test. To identify different pairs the Mann-Whitney test with Bonferroni correction for multiple comparisons was applied.

The significance level of 0.05 was adopted for all statistical analyses. They were carried out using SPSS software version 12.0 PL.

### RESULTS

The gestational weight gain in 62% of women in twin pregnancies was not compliant with the current American Institute of Medicine recommendations, with most of them gaining too little weight (Figure 1).

The prevalence of too low and normal weight gain in women was not statistically significantly related to women's pre-pregnancy BMI. In contrast, excessive weight gain occurred almost exclusively in women with normal BMI before pregnancy (difference on the border of statistical significance; p=0.071 - exact Fisher's test) (Table 3). The median weight gain for all women was 16 kg (min 4 kg – max 41 kg). The difference in GWG expressed in kg between 3 groups of women is statistically significant (p=0.015) (Table 3). This effect results from the difference between the weight gain of women with normal body weight in comparison to women with excessive weight (p=0.004).

Of the factors analyzed in the study that could be related to the gestational weight gain (maternal age, education, place of residence, number of pregnancies, pre-pregnancy BMI, use of multivitamin preparations, type of pregnancy, gestational diabetes, anemia, hypertension during pregnancy), only the type of pregnancy proved statistically significant. Women with monochorionic pregnancy were more likely to have too low body weight gain, compared to women with dichorionic pregnancy (74% vs. 26%), (p<0.001).

Maternal weight gain was statistically significantly associated with neonatal weight (p=0.035) and risk of low birth weight (<2500 g) (p=0.014). The mean birth weight of children born to mothers with excessive weight gain was 151 g higher compared to children born to mothers with normal weight gain (2727 g vs. 2576 g) (Table 4).



Figure 1. Gestational weight gain in women in relation to the recommendations

		Nutritional status		
GWG ranges	Underweight (BMI <18.5) Number of women, n=5 (100%)	Normal weight (BMI 18.5–24.9) Number of women, n=31 (100%)	Overweight/obesity (BMI ≥25.0) Number of women, n=14 (100%)	Significance (p-value)
CWC lower then			II II (10070)	
recommended	3 (60%)	13 (42%)	8 (57%)	NS*
GWG within recommendations	2 (40%)	12 (39%)	5 (36%)	NS*
GWG higher than recommended	0 (0%)	6 (19%)	1 (7%)	p=0.071
GWG (kg) median (min-max)	14 (9–25)	20 (10-41)	12 (4–36)	p=0.015

Table 3. Gestational weight gain in women depending on pre-pregnancy BMI

\* statistically insignificant

Nacratal kirth weight (a)	Gestational weight gain ranges						
Neonatai birtii weight (g)	too low GWG	normal GWG	excessive GWG				
mean±SD	2500±328.9	2576±261.1	2727±159.3				
min-max	1640–3445	1935–3270	2350-3030				
Significance vs. normal GWG (p-value; Student's t-test)	NS*	_	p=0.035				

Table 4. Gestational weight gain vs. birth weight of neonates

\* statistically insignificant

Regarding the risk of low birth weight, out of 40 such newborns (40% of the group), 25 of them (62.5%) were born to mothers with GWG below the recommendations, 14 (35.0%) were born to mothers with a normal GWG and only 1 newborn (2.5%) was born to mother with excessive GWG. Among the 60 children with normal birth weight, their distribution by maternal weight gain groups was as follows; 23 newborns (38.3%), 26 newborns (43.3%) and 11 newborns (18.3%), respectively. Statistically significant differences were also found in the total maternal weight gain of women giving birth to one or two babies weighing <2500 g vs.  $\geq 2500$  g (p=0.026). Newborns weighing less than 2500 g were born to mothers with lower weight gain (median 14 kg; min 4 kg - max 26 kg, whereas children weighing  $\geq 2500 \text{ g}$ were born to mothers with higher weight gain (median 18 kg; min 8 kg – max 41 kg).

As for the risk of having a low birth weight baby, the type of pregnancy and the woman's height were also statistically significant factors. Significantly more newborns with low birth weight were born to women with monochorionic pregnancies than to women with dichorionic pregnancies (62.5% vs. 37.5%) (p=0.007). In terms of women's height, all women <160 cm gave birth to both babies with low birth weight, while among taller mothers this was the case for only 19% (exact Fisher test =0.022).

### DISCUSSION

Nearly half of the women in twin pregnancies in our study did not meet the minimum weight gain according to the US guidelines. This is highly consistent with the results obtained by Amyx et al. in their study conducted in France [7]. Forty seven percent of women with twin pregnancies studied there had insufficient GWG, and the proportion of women with excessive GWG was also very similar in both studies (14% vs. 10%). In a study in Australia, 29% of women had too little weight gain and 24% had too much weight gain [21]. Similar result was found in Canada, with 27% and 30%, respectively [22]. In light of the recent meta-analysis of studies (2022), more than 35% of women in twin pregnancies gained weight below US recommendations, more than 21% gained above, and less than 44% gained weight correctly [23].

In twin pregnancies, as in singleton pregnancies gestational weight gain is associated with birth weight of the newborns [5, 8, 24, 25], which was also proven in our study. Children born to mothers with excessive weight gain were heavier than infants born to mothers with normal weight gain. The study by Lal and Kominiarek [18] showed that women with underweight or normal body weight before pregnancy, who did not achieve the minimum weight gain, more often gave birth to children weighing less than 2500 g or even less than 1500 g (57.2% and 10.6% of newborns), compared to women whose weight gain was higher than the recommendations (36.2% and 4.3% of newborns). A significantly higher percentage of children with low birth weight, born to mothers with a weight gain lower than in the recommendations, compared to the percentage of such children born to other mothers, was also shown in our study.

In our study, the risk of low birth weight was associated with the type of pregnancy, which is not surprising, since previous studies indicates that women with monochorionic pregnancies give birth to smaller babies than women with dichorionic pregnancies [26–29]. Moreover, the risk of low birth weight was related to the height of women. It is quite well known that maternal and paternal height reflects the genetic growth potential of the fetus, with taller mothers generally giving birth to larger children [30–32]. In the light of a meta-analysis of studies conducted in singleton pregnancies, short-statured women have a greater risk of giving birth to newborns with low birth weight [33].

The median GWG of studied women was 16 kg (min 4 kg – max 41 kg) and was exactly the same as in another study in Poland conducted among women with twin pregnancies (median 16.0 kg) [34]. Moreover, the same result was also found in a French study, although in this case the authors report a mean value – 16.1 kg [7]. The weight gain of women with normal BMI before pregnancy is also very similar in the literature. In our study, the median was 20 kg, in the American study 20.4 kg [35], and in the Australian study 19 kg [21]. As for the GWG of overweight or obese women, it was 21.3 kg and 13.6 kg in the American study

[35] and 17.5 kg and 15 kg in the Australian study [21], respectively. The smallest weight gain in obese women is a positive result, regardless, it is important to bear in mind that the recommended weight gain decreases with increasing BMI of the mothers-to-be, which is due to accumulated energy stores in the form of adipose tissue [14].

However, in the scientific publications there is a debate as to whether the US weight gain guidelines for twin pregnancies are optimal. Some experts argue that these recommendations should be used in everyday obstetric practice, as weight gain in accordance with guidelines reduces the risk of low birth weight, pregnancy-induced hypertension, and preterm birth [6]. Others believe that it is currently impossible to determine whether the American recommendations are adequate [21]. In particular, the guidelines do not seem optimal for women from Asian countries, due to the lower height of women. Among Japanese women with twin pregnancies, normal birth weight for both babies was found in more than 70% of women, whose weight gain in all BMI categories was significantly lower than American guidelines (by 36%-80%). It was 11.5-16.5 kg in underweight women, 10.3-16.0 kg in normal-weight women, 6.9-14.7 kg in overweight women and 2.2-11.7 kg in obese women in the preconception period [5].

There are several limitations to this study that should be considered when interpreting the results. The most important is the relatively small sample size, mostly due to the small overall population of women pregnant with twins. Another limitation is retrospective nature of the study, including self-reported pre-pregnancy weight. This might have affected the credibility of the BMI calculations and the later interpretation. However, as the study shows utilization of self-reported or measured pre-pregnancy weight for pre-pregnancy BMI classification results in identical categorization for the majority of women [36]. Still another limitation is that the study did not analyze the energy value of the women's diet, the level of their physical activity and socioeconomic status. Therefore, it is not known to what extent these factors were related to gestational weight gain, but in the case of diet, as many as 46% of women reported that they ate the same amount of food as before pregnancy, which could have influenced the results obtained. It is also worth noting that the study was conducted at tertiary care centers in Warsaw, which also run separate outpatient clinics for multiple pregnancies. For this reason the study group may not reflect the situation in patients from smaller centers.

# **CONCLUSIONS**

Almost half of the studied women with twin pregnancies had insufficient weight gain. This occurred significantly more often in women with monochorionic pregnancies than in women with dichorionic pregnancies. Women with monochorionic pregnancies also gave birth to statistically significantly more newborns with low birth weight.

### **Funding sources**

*This research received no external funding (Statutory Research No. FŻ-5/2023).* 

### **Conflicts of Interest**

Author declares no conflict of interest.

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Received: 02.10.2024 Revised: 30.10.2024 Accepted: 04.11.2024 Published online first: 13.11.2024



https://roczniki.pzh.gov.pl/

Rocz Panstw Zakl Hig 2024;75(3):283-292

https://doi.org/10.32394/rpzh/196309

# SENSORY EVALUATION AND CONSUMPTION PREFERENCES OF HIGH-PROTEIN NATURAL YOGHURTS AMONG STUDENTS OF DIETETICS

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# ABSTRACT

**Background.** High-protein yoghurts, are recommended by dieticians as a valuable source of wholesome protein. Consumers' expectations of yoghurts are intrinsically linked to the perceived quality and health benefits of these products. **Objective.** The aim of this research study was the sensory analysis and evaluation of consumer preferences towards commercially available high-protein natural yoghurts. The study was designed to identify which quality and nutritional characteristics are crucial for dietetics students when choosing such products. Moreover, the factors influencing the purchasing decisions of this group were analysed, which can provide valuable information for food producers and dieticians. **Materials and Methods.** The research study was conducted among 65 students of dietetics at the Medical University of Silesia in Katowice. The examination included the sensory evaluation of eight high-protein yoghurts by means of a five-point rating scale and the analysis of consumer preferences based on a proprietary questionnaire, which consisted of questions concerning the characteristics of the study group and questions assessing consumer preferences and dietary habits.

**Results.** Among the surveyed students of dietetics, 35.4% declared to consume high-protein yoghurts several times a week, mainly due to 'positive health benefits'. In the conducted sensory evaluation, yoghurts of brands 'A', 'C' and 'E' scored highest, while the plant-based alternative of brand 'D' scored lowest.

**Conclusions.** The high-protein yoghurts of brands 'A', 'C' and 'E' outstand in terms of taste and texture, which makes them the most popular products among consumers. Products from 'A', 'C' and 'B' brands are also widely available on the market, which favours their popularity. Taste proved to be a key factor in the decision to re-purchase. The largest number of respondents stated their willingness to purchase 'A' brand yoghurt again, while 'D' and 'F' brand products were the least popular, which may be due to differences in taste preferences and the availability of these products on the market.

Key words: sensory evaluation, consumption preferences, consumer preferences, high-protein yoghurts, natural yoghurts

# **INTRODUCTION**

There is a constant increase in the production of milk and dairy products among Polish producers nowadays [1]. As compared to traditional dairy yoghurts, the lower preference for plant-based alternatives may result from their different textural and taste properties [2].

Originally, yoghurts were only available in their natural form, but manufacturers are increasingly diversifying their range of products by adding a variety of fruit and cereal ingredients to create ever-new flavours [3, 4, 5]. Innovative products are constantly being introduced to cater for the various dietary preferences of potential consumers, including not only fermented milk drinks and cheese, but also buttermilk and kefir [3]. Moreover, the offered dairy products have different nutritional values and organoleptic properties that allow them to be differentiated according to their tenacity, compactness, density, hardness, as well as the texture they possess (liquid, thick, mixed) [6].

Over recent years, there has been an increase of nutritional knowledge and awareness among consumers in recent years, who more often expect food that fulfils certain alimentary, dietary and nutritional standards, such as reduced sugar content or the absence of artificial preservatives [7, 8]. The nutritional value of natural yoghurts is particularly related to their chemical composition and the form of the ingredients, which facilitates the absorption, digestion and assimilation of nutrients [9].

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Natural yoghurts are the healthiest choice recommended by nutritionists and the FFC (*Functional Food Centre*). These products have potentially more nutritious composition compared to milk and are identified as 'functional food', which contains biologically active compounds that provide clinically documented health benefits, in preventing and treating diseases, even chronic ones. The composition of such products should be based solely on live bacteria cultures and substances of dairy origin, and should eschew unnecessary additives such as flavourings, colourings, thickeners or flavour enhancers [3, 10].

Regular consumption of natural yoghurts, rich in probiotic bacteria such as *Lactobacillus and Bifidobacterium*, provides excellent cancer prevention, especially of the large intestine, improves intestinal peristalsis during the postpartum period, prevents the occurrence of diarrhoea, reduces the risk of prediabetes type II and improves lipid profile [3].

An increased amount of protein in a portion of yoghurt, will be beneficial in individuals who reduce body weight, as it will improve the feeling of satiety, thereby improving appetite control [11]. For physically active people whose muscles require adequate recovery after intense exercise, a rapid intake of protein following a training unit affects the phosphorylation of signalling proteins, hence increasing muscle protein synthesis [12]. However, among the elderly struggling with malnutrition or osteoporosis, a condensed form of protein yoghurt will provide the necessary amount of protein and calcium in a small portion [13].

Consumers' expectations of yoghurts are intrinsically linked to the perceived quality and health benefits of these products. Consumers look for freshness, proper texture and optimum level of acidity in yoghurt, all of which are identified with a healthy and natural product Sensory research, extensively discussed by Samotyja [14], indicates that the food sensory appeal, including taste, fragrance and texture, plays a key role in consumers' purchasing decisions These qualities highly influence the satisfaction with the product and, in the context of yoghurts, prohealth aspects, such as probiotic content and natural ingredients, which can enhance consumer loyalty, are also gaining importance.

In the context of research on yoghurts, analysing consumer preferences through sensory evaluations allows products to be better aligned with market expectations [14].

# The objectives and assumptions of the research study

The main objective of the conducted research study was a sensory and consumer preference analysis of high-protein natural yoghurts available on the market, in order to identify which quality and nutritional characteristics are crucial for dietetic students when choosing this type of product. The study also aimed at identifying which yoghurt brands are best suited to meet the dietary expectations of this group and to determine the factors that contribute to repetitive purchase.

The following specific objectives have achieved the main goal:

- To conduct a sensory evaluation of selected brands of high-protein yoghurts available on the Polish market, with particular emphasis on the quality of taste and texture.
- To analyse the high-protein yoghurt market in terms of its availability to consumers.
- To determine which high-protein yoghurts are most valued by students of dietetics, both in terms of nutritional value and taste preferences.
- To identify the yoghurts that are most frequently chosen by students of dietetics for their dietary requirements.
- To examine the quality and nutritional criteria that influence dietetic students' choice of specific brands of high-protein yoghurt and the factors that determine their purchase.

## MATERIAL AND METHODS

#### Course of the study and research group

The research study was conducted among 65 students of dietetics at the Medical University of Silesia in Katowice, at the Faculty of Public Health in Bytom, in 2023 (57 women and 8 men).

Inclusion criteria for the research study included: providing informed consent for participation in the study, confirming status as a first- or second-cycle dietetics student and completing a questionnaire. Exclusion criteria, on the other hand, included: the absence of student status or a different field of study than the one mentioned above. Participation in the study was voluntary and anonymous. Data were collected among students attending classes held between December 2023 and February 2024 at the Department of Dietetics at the Medical University of Silesia in Katowice, where product evaluation workstations were arranged. The study group was represented by people aged between 19 and 30 years.

The study material consisted of 8 natural highprotein yoghurts from different companies and stores (Table 1).

The research study was conducted in the sensory analysis laboratory of the Department of Dietetics at the Medical University of Silesia in Katowice, at the Faculty of Public Health in Bytom, which fulfils the requirements and assumptions of the PN-EN ISO 8589:2010 standard for sensory laboratories [15].

Food product	Sample designation	Qualitative composition
Natural skyr	А	pasteurized milk, live yoghurt bacteria cultures: <i>Str. thermophilus</i> , <i>L. bulgaricus</i>
Natural yoghurt, high protein	В	skimmed milk, milk proteins, yoghurt bacteria cultures (contain milk), lactase
Natural skyr, Icelandic yoghurt	С	pasteurized milk, live yoghurt bacteria cultures: <i>Str. thermophilus</i> , <i>L. bulgaricus</i>
Vegan alternative	D	soybean base (water, peeled soybeans (15.7%)), sugar, stabiliser (pectin), tricalcium citrate, acidity regulators (sodium citrates, citric acid), natural flavours, sea salt, antioxidants (tocopherol-rich extract, fatty acid esters and ascorbic acid), vitamins ( $B_{12}$ , $D_2$ ), yoghurt cultures ( <i>Str. thermophilus, L. bulgaricus</i> )
Natural yoghurt of the Icelandic type	Е	condensed skimmed milk, cream, milk proteins, yoghurt bacteria cultures
Natural skyr	F	skimmed milk, cultures of lactic acid bacteria, microbiological rennet, pasteurized milk, live yoghurt bacteria cultures: <i>Str. thermophilus</i> i <i>L. bulgaricus</i>
Natural yoghurt, high protein, 0% fat, lactose free	G	pasteurized milk, live yoghurt bacteria cultures
Natural protein yoghurt	Н	milk, yoghurt bacteria cultures

Table 1. Types of high-protein yoghurts applied in the research study\*

\*Own study based on food labels

The prepared yoghurt samples were designated with three-digit codes. Each person participating in the evaluation received 8 samples, which contained approximately 50 g of product. While conducting the research study, sensory evaluation (colour, fragrance, texture, appearance and taste) was performed. The order of the analysed characteristics was not accidental. The evaluation of the quality of high-protein yoghurts was conducted by means of a proprietary evaluation card containing a five-point scale (5 – very good product quality, 1 – disqualifying quality of the tested product), prepared on the basis of PN-ISO 22935-1 [16].

All participants taking part in the conducted tests were given a set of 8 coded samples of a certain weight for evaluation. In addition, the participants of the research study were given cards with enumerated quality indicators for all tested characteristics and a sheet used for sensory evaluation of the examined samples. For each tested characteristic, an importance coefficient was determined by multiplying the numerical scores awarded by the evaluators.

The chosen five-point method was applied because of its adequate level of difficulty, which was adapted to the skills and experience of the group of participants of the study.

In the study, an importance coefficient (IC) was used to assign appropriate significance to the different sensory evaluation criteria and product availability. This coefficient was designed to consider the different relevance of individual characteristics, such as taste, colour, texture, appearance and fragrance, when calculating the overall rating of the high-protein yoghurts. The value of the coefficient was based on the opinions of students of dietetics and the results of previous studies, which indicated which characteristics have the greatest influence on consumers' purchasing decisions [17]. Importance coefficients were used in the analysis of the questionnaire data to distinguish those characteristics that had the greatest influence on respondents' choice of products.

### **Proprietary questionnaire**

The second element of the research was aimed at assessing preferences and behaviours regarding the consumption of high-protein natural yoghurts. For that purpose, a proprietary questionnaire was developed. The first part of the questionnaire contained questions concerning the characteristics of the study group, while the second part included 13 closed, single-choice questions assessing consumer preferences and dietary habits. The self-designed questionnaire guaranteed the anonymity of respondents, who were informed about the purpose of the research study, its methodology and agreed to participate.

### **Statistical analysis**

All data obtained were catalogued and analysed through Microsoft 365 Excel 2024 and Statistica StatSoft Polska. The distribution of each parameter was checked with the Shapiro-Wilk test. A t-test for dependent samples was applied to analyse the parametric data. Cramér's V ( $V_c$ ) coefficient was used to determine the strength of the association. The level of statistical significance, p<0.05, was adopted in the calculations.

### RESULTS

Characteristics of the research group and consumer behaviour Sixty-five respondents participated in the research study. The vast majority of interviewees (87.7%) were women – 57 people while the male group was represented by 8 people (12.3%). he average age of the respondents was ( $\overline{x}$ =22.0 years). 44 individuals constituted the group of first-cycle studies students (67.3%), while 21 participants attended second-cycle studies (32.3%). The majority of respondents (60 persons) declared that they consume high-protein yoghurts, which accounts for 92% of the people involved. Five individuals do not consume such products at all (8%).

Twenty-three persons of all respondents declared that they consume natural high-protein yoghurts 'several times a week' (35.4%), while 19 persons indicated that they ingest them 'several times a month' (29.2%). 4,6% of respondents stated that they consume yoghurts 'less frequently than the above answers' (Figure 1).

Students who undertake employment declared consumption of high-protein yoghurts 'every day', more often than students who don't work: 23.3% and 5.7% respectively (Table 2). In both groups, students consumed high-protein yoghurts most frequently 'several times a week' 30.0% and 40.0% accordingly. There is a statistically significant correlation between the respondents' professional activity and the frequency of consumption of high-protein natural yoghurts (p<0.05). The strength of the correlation is moderate (Vc=0.367).

Yoghurt from the 'A' company was chosen by 17 respondents (48.6%) who declared themselves as professionally inactive students, while in the case of

employed students, yoghurt from the 'B' company was the most common choice with 10 individuals (33.3%).

There is a statistically significant correlation between the respondents' work activity and the preferred company for natural high-protein yoghurts (p<0.05). The strength of the correlation is moderate (Vc=0.377).

When asked about the reasons for purchasing natural high-protein yoghurts, 72% of respondents stated that they choose them for the positive health and nutritional benefits, while 17% of participants indicated that they buy them 'for the taste'.

Almost all respondents (92.3%) purchase the discussed products in supermarkets/ discount retailers. The remaining people surveyed, do not buy natural high-protein yoghurts (6.2%).

Respondents participating in the questionnaire are most likely to buy yoghurt cups of >150 g and  $\leq 200$  g (43.1%) and  $\geq 100$  g and  $\leq 150$  g (40.0%) (Table 3). For more than half of the students, the most preferred texture of the yoghurts they purchase is the traditional one, to be consumed with a spoon (53.8%), followed by the dense texture (29.2%) and the liquid form (13.8%). As far as respondents' taste preferences are concerned, they are diverse, but the leading choice is natural yoghurt (38.5%), flavoured (fruit) yoghurt accounting for 6.9% and non-fruit flavoured yoghurt accounting for 20.0%. Plant-based yoghurts are not among the preferred flavours of respondents. The prices of yoghurts that students most often buy are between >2.50 PLN and  $\leq$ 3.50 PLN (46.2%) and between >3.50 PLN and ≤5.50 PLN (40.0%).

When asked about the most common way of consuming natural high-protein yoghurt, 27 respondents (41.5%) indicated the answer as a 'standalone meal/snack', 24 people (36.9%) selected the answer as a 'meal/snack accompaniment' and the least frequent mode of yoghurt consumption was as a 'food and/or dessert ingredient' (16.9%).

The information on the yoghurt packaging has an impact on purchase decisions for more than half



Figure 1. Frequency of consumption of natural high-protein yoghurts

		Respondents acti	' professional vity			
		Student who don't work n=35	Professionally active student n=30	p-value	Cramér's V	Strength of correlation
		n (%)	n (%)			
on 1	Every day	2 (5.7%)	7 (23.3%)			
npti oteir	Several times a week	14 (40.0%)	9 (30.0%)			
nsur pro s	Once a week	2 (5.7%)	1 (3.3%)			
con nigh nurt	Several times a month	13 (37.1%)	6 (20.0%)	0.000	0.367	Moderate
y of ral h yogl	Once a month	1 (2.9%)	3 (10.0%)	0.000	0.507	Wioderate
enc atui	Less frequently than the above	2 (5.7%)	1 (3.3%)			
Frequ of n	I do not buy natural high-protein yoghurts	1 (2.9%)	3 (10.0%)			

Table 2. Respondents' professional activity as compared to frequency of consumption of high-protein natural yoghurts, n=65

Table 3. Characteristics of the most popular natural high-protein yoghurts, n=65

	n	%	
	<100 g	1	1.5
Yoghurt package	≥100 g and ≤150 g	26	40.0
	>150 g and ≤200 g	28	43.1
size	>200 g	4	6.2
	I do not pay attention to the size of the package	3	4.6
	I do not buy natural high-protein yoghurts	3	4.6
	traditional (to be consumed with a spoon)	35	53.8
Vo abunt toutuno	dense	19	29.2
Yoghurt texture	liquid (drinking yoghurt)	9	13.8
	foamy, mousse-like texture	2	3.1
	natural	25	38.5
	fruit flavoured (e.g. strawberry, blueberry)	24	36.9
Taste of yoghurt	flavoured – other than fruit (e.g. vanilla, chocolate)	13	20.0
	plant-based	0	0.0
	I do not buy such products	3	4.6
	>1.00 PLN and ≤2.50 PLN	6	9.2
	>2.50 PLN and ≤3.50 PLN	30	46.2
Price of yoghurt	>3.50 PLN and ≤5.50 PLN	26	40.0
	>5.50 PLN	0	0.0
	I do not buy natural high-protein yoghurts	3	4.6

of respondents (61.5%), while 18.5% claimed it was not influential and 20.0% of respondents were are indecisive about its importance.

When asked what determinants the respondents follow when choosing particular yoghurt, 31 people (47.7%) stated that the price of the products they purchase is a big determinant (Table 4). Within the group of respondents, 22 people (33.8%) declared that the brand was of average importance. On the other hand, 33 respondents (50.8%) indicated that the size of

the packaging is an influential factor in their purchases. The composition of the products (43.1%), taste (64.6%) and expiry date (49.2%) were of huge importance to those taking part in the survey. The product promotion was however of average importance, when it came to potential consumers' choice (35.4%).

To the question of whether the interviewees had previously tried the tested product, they gave the greatest number of affirmative answers for products such as 'A' (89.2%) and 'C' (81.5%). Conversely, they

	Importance								
Determinants of product quality	Huge		В	ig	Ave	rage	Little		
	n	%	n	%	n	%	n	%	
Price	16	24.6	31	47.7	16	24.6	2	3.1	
Brand	6	9.2	18	27.7	22	33.8	19	29.2	
Package size	4	6.2	33	50.8	22	33.8	6	9.2	
Product composition	28	43.1	27	41.5	10	15.4	0.0	0.0	
Taste	42	64.6	21	32.3	2	3.1	0.0	0.0	
Expiry date	32	49.2	19	29.2	10	15.4	4	6.2	
Promotion	13	20.0	23	35.4	15	23.1	14	21.5	

Table 4. Determinants of product quality that influence the choice of natural high-protein yoghurts, n=65

Table 5. Five-point assessment of high-protein yoghurts

Food product	Sample designation	Sample number	Quality differentiator	IC	n	Average scores of examined yoghurts	Average scores of assessed attributes	SD	Me	X <sub>min</sub> ÷X <sub>max</sub>
			Appearance	0.15	65	3.8	0.6	0.2	0.6	0.2÷0.8
			Colour	0.10	65	4.4	0.4	0.1	0.5	0.1÷0.5
Natural skyr	A	802	Fragrance	0.25	65	3.9	1.0	0.3	1.0	0.3÷1.3
			Texture	0.15	65	4.2	0.6	0.2	0.8	0.2÷0.8
			Taste	0.35	65	3.7	1.3	0.4	1.3	0.4÷1.8
			Total points	1	65	4.0	3.9	4	4.2	1.2÷5.2
			Appearance	0.15	65	4.1	0.6	0.1	0.6	0.3÷0.8
Natural			Colour	0.10	65	4.0	0.4	0.1	0.4	0.1÷0.5
yoghurt, high	В	112	Fragrance	0.25	65	3.4	0.8	0.3	1.0	0.3÷1.3
protein			Texture	0.15	65	4.1	0.6	0.1	0.6	0.2÷0.8
			Taste	0.35	65	3.1	1.1	0.4	1.1	0.4÷1.8
			Total points	1	65	3.7	3.5	3.7	3.7	1.3÷5.2
		100	Appearance	0.15	65	3.9	0.6	0.2	0.6	0.2÷0.8
Natural skyr,			Colour	0.10	65	4.4	0.4	0.1	0.5	0.2÷0.5
Icelandic	C		Fragrance	0.25	65	4.1	1.0	0.2	1.0	0.3÷1.3
yoghurt			Texture	0.15	65	4.2	0.6	0.2	0.8	0.3÷0.8
			Taste	0.35	65	3.7	1.3	0.4	1.4	0.4÷1.8
			Total points	1	65	4.1	3.9	4.1	4.3	1.4÷5.2
			Appearance	0.15	65	3.9	0.6	0.2	0.6	0.2÷0.8
			Colour	0.10	65	2.8	0.3	0.1	0.3	0.1÷0.5
Vegan	D	355	Fragrance	0.25	65	2.8	0.7	0.3	0.8	0.3÷1.3
ancinative			Texture	0.15	65	3.7	0.6	0.2	0.6	0.2÷0.8
			Taste	0.35	65	2.6	0.9	0.5	0.7	0.4÷1.8
			Total points	1	65	3.2	3.1	3.2	3.0	1.2÷5.2
			Appearance	0.15	65	4.2	0.6	0.2	0.8	0.2÷0.8
Natural			Colour	0.10	65	4.3	0.4	0.1	0.5	0.1÷0.5
yoghurt of the	Е	400	Fragrance	0.25	65	3.8	1.0	0.3	1.0	0.3÷1.3
Icelandic type			Texture	0.15	65	4.0	0.6	0.2	0.6	0.2÷0.8
			Taste	0.35	65	3.6	1.3	0.4	1.4	0.4÷1.8
			Total points	1	65	4.0	3.9	1.2	4.3	1.2÷5.2

			Appearance	0.15	65	3.4	0.5	0.2	0.6	0.2÷0.8
			Colour	0.10	65	4.3	0.4	0.1	0.4	0.1÷0.5
Natural skyr	F	526	Fragrance	0.25	65	3.7	0.9	0.2	1.0	0.3÷1.3
			Texture	0.15	65	3.8	0.6	0.2	0.6	0.2÷0.8
			Taste	0.35	65	3.8	1.3	0.4	1.4	0.4÷1.8
			Total points	1	65	3.8	3.7	3.8	4.0	1.2÷5.2
			Appearance	0.15	65	3.7	0.5	0.2	0.6	0.2÷0.8
Natural			Colour	0.10	65	4.4	0.4	0.1	0.5	0.1÷0.5
yoghurt, high	G	600	Fragrance	0.25	65	3.7	0.9	0.3	1.0	0.3÷1.3
lactose free			Texture	0.15	65	3.9	0.6	0.2	0.6	0.2÷0.8
			Taste	0.35	65	3.8	1.3	0.4	1.4	0.4÷1.8
			Total points	1	65	3.9	3.7	3.9	4.1	1.2÷5.2
			Appearance	0.15	65	3.8	0.6	0.2	0.6	0.2÷0.8
		222	Colour	0.10	65	4.5	0.4	0.1	0.5	0.2÷0.5
Natural protein yoghurt	Н		Fragrance	0.25	65	3.8	0.9	0.3	1.0	0.3÷1.3
			Texture	0.15	65	4.0	0.6	0.2	0.6	0.2÷0.8
			Taste	0.35	65	3.6	1.3	0.4	1.4	0.4÷1.8
I			Total points	1	65	3.9	3.8	3.9	4.1	1.3÷5.2

IC - Importance Coefficient; SD - standard deviation; n - the number of the sample population; Me - the median symbol; xmin÷xmax - minimum value÷maximum value

had tried products from the companies 'H' (87.7%), 'G' (84.6%), 'D' (72.3%) and 'F' (69.2%) least frequently. A comparable number of respondents had not tried or had tried 'E' yoghurt, 52.3% and 47.7% respectively, and 'B', 46.2% and 53.8% accordingly.

When asked about the availability of the analysed products in the store, respondents reported that 'A', 'C' and 'B' were the most accessible products, (98.5%), (92.3%) and (75.4%) respectively. The highest percentage of respondents (52.3%) advocated that 'G' is not a generally available product in stores.

# Sensory evaluation of natural high-protein yoghurts

The results of the sensory evaluation obtained due to the five-point method revealed that the people participating in the research study rated natural, Icelandic yoghurt 'A' with the highest score, followed by natural skyr 'C' and natural, Icelandic yoghurt 'E' (Me=4.3) (Table 5). Of all the yoghurts evaluated, vegetarian alternative 'D', received the lowest number of points awarded by respondents (Me=3.0).

# DISCUSSION

There has been a significant increase in consumer interest in protein-enhanced dairy products over recent years. This phenomenon represents a trend of sorts that responds to the growing public awareness and demand for products that support a healthy diet [18].

Natural high-protein yoghurts are increasingly becoming an integral part of the diets of physically active people, athletes, and those who care about a healthy and balanced diet. The nutrients and minerals included in the composition of high-protein products support recovery and rebuilding processes in the body, as well as contribute to better control of body weight [18, 19].

Consumption of high-protein natural yoghurts 'several times a week', was declared by 40.0% of nonworking students and 30.0% of students undertaking additional employment. In a research study conducted by Grębowiec and Korytkowska [20], 33.0% of respondents ingested dairy products 'several times a day', 29.0% of respondents declared that they consume yoghurts 'several times a week', while 27.0% of respondents chose the answer 'once a day'.

The most frequently chosen natural yoghurt among non-working students was 'A' (48.6%), which has no fat (0 g/100 g) and low sugar content (4.1 g/100 g). In contrast, employed students preferred natural yoghurt of brand 'B' (33.3%), characterised by a slightly higher level of fat (0.3 g/100 g) and sugar (6.6 g/100 g). The choice of the following protein yoghurts may be determined by students of dietetics who favour products with reduced fat content and those that are easily available in popular discount retailers. A study concerning the consumer evaluation of the attractiveness of yoghurts conducted by Dykiel et al. [21] revealed that the most preferred yoghurts are fruit yoghurts, with strawberry yoghurt being the most popular (52.7%). Natural yoghurts were favoured by only 25.3% of respondents.

Traditional fruit yoghurts can contain even as much as 12.5 g/100 g sugar and added glucose-fructose syrup. A study by Chollet et al. [22] proved that attempts to reduce the sugar content, which negatively affects the human body, are needed, but on the condition that the sweetness is acceptable to the consumer. Flavoured yoghurt with 10.0% added sugar was described as too sweet compared to yoghurt with 7.0% added sugar, moreover, the treatment of increasing coffee or strawberry flavours did not contribute to pushing the preference for reduced sugar levels in the product. The study provides a guideline for manufacturers to reduce the amount of added sugar in their products to an acceptable level for consumers, while utilising flavours prudently.

In a study conducted by Grębowiec and Korytkowska [20], the most common reasons for consuming dairy products included the responses 'suitable for direct consumption' (44.0%) and 'force of habit' (42.0%). In the self-reported research, 72.0% of respondents chose natural high-protein yoghurts due to 'positive health and nutritional benefits', while 17.0% of participants indicated the answer 'for taste'. With regard to the taste of the yoghurt, the students demonstrated diverse preferences, as natural taste was preferred by 38.5% of the respondents, fruit flavour by 36.9% and non-fruit flavour by 20.0% of the respondents.

Within the sample group, the most preferred texture of yoghurt is traditional, that is, to be consumed with a spoon -53.8% and dense -29.2%. A different preference was shown by a group of respondents in a study conducted by Kowalczuk and Szymanski [23], where the most frequently consumed types of yoghurt included flavoured drinking yoghurts (52.6%) and natural Greek-type yoghurts (43.6%).

According to the most recent *Retail* report (2023/2024, SpicyMobile Magazine) [24], for the Polish population, price (46.0%) and promotion (19.0%) are the most important factors when choosing a product, while product opinion (12.0%) and brand (9.0%) are not as relevant for consumers in their purchasing decisions. In the self-report research study, the largest number of people were guided by the taste of the product (64.6%), the expiry date (49.2%) and the price (24.6%). Similarly, as proven in the report, brand was not important to consumers (29.2%).

In Gutkowska's article [25], the authors emphasise that nowadays consumers pay the highest attention to the state of their health, so the decisions they make when making purchases largely determine physical and mental well-being. Moreover, convenience is also of utmost importance, allowing quick shopping and easy access to products. For some consumers, the concepts of ecology and local products also seem to be significant. According to the analysis of the responses, the author's survey, as many as 92.3% of respondents choose supermarkets/discount retailers as the place in which to buy natural high-protein yoghurt. It reinforces the belief regarding the convenience of shopping.

In the subsequent part of the conducted research study, the students of dietetics performed a consumer sensory evaluation of the high-protein yoghurts. The overall evaluation of the products included individual quality characteristics such as colour, fragrance, taste, texture and appearance, which were essential for the overall assessment of product quality. In a self- study, the results obtained using the five-point method revealed that high-protein yoghurts from the companies 'A', 'C' and 'E' received the highest scores, while the vegan alternative 'D' gained the lowest rating by the students of dietetics. Natural high-protein yoghurts of the brand 'A' and 'C' contained no fat in their composition (0.0 g/100 g of product), 'E' yoghurt had 2.0 g/100 g of fat, while plant-based alternative was characterised by a high fat content (3.3 g/100 g of product). In a study by Wichrowska and Wojdyła [26], yoghurts with a high fat content (above 3.0%) were characterised by the best organoleptic qualities, including taste, colour, fragrance, texture and appearance. Yoghurts with a low lactic acid content (less than 0.936%) and an excessively high content (more than 1.0%), however, were evaluated organoleptically worse compared to the others. Furthermore, it was demonstrated that refrigerated storage of yoghurt slightly deteriorated the colour, taste and fragrance of the yoghurts and had a greater effect on their texture and appearance, especially for yoghurts with lower fat content.

The research has shown that consumer acceptance of high-protein yoghurts depends on their diversified composition, which influences consumers' perceptions of specific product quality attributes. The palatability of high-protein yoghurts may significantly differ from the preferences and eating habits of people who do not consume such products. The results of the conducted research study predominantly depend on the individual preferences of the participants.

### Limitations of the study

The study has several important limitations that need to be considered when interpreting the results. It was conducted with a small sample of 65 dietetics students, which limits the generalizability of the results to a broader population. The specific profile of the study group, consisting solely of students in this field of study, may influence the results due to their expertise and interests in nutrition. In addition, the survey was local in nature, covering students from one university in Poland, which does not allow for regional differences in consumer preferences.

Although a five-point sensory evaluation scale was used, the results obtained may reflect the subjective opinions of the participants. The assessment of product availability was based on respondents' experiences, which could introduce error due to differences in local store offerings.

In addition, the survey was cross-sectional, which makes it impossible to analyze changes in consumer preferences over time or to assess their reactions to new products appearing on the market. Eliminating the above limitations, would produce more representative and comprehensive results in future surveys.

## The benefit to recipients of the manuscript

For an audience including students of dietetics and food manufacturers, the manuscript provides information on current trends and consumer expectations regarding protein products. The research study may act as a guide to the high-protein yoghurt market, assisting dietitians in selecting and recommending the highest quality products. For manufacturers, this report can be crucial in improving products, adjusting them to consumer preferences and increasing their market competitiveness.

## CONCLUSIONS

- Consumer evaluation of selected brands of highprotein yoghurts, conducted with a five-point method, revealed that yoghurts from companies 'A', 'C' and 'E' were rated highest in terms of taste and texture. Meanwhile, the plant-based alternative from company 'D' received the lowest marks awarded by the students of dietetics.
- 2. The majority of respondents identified taste as a key factor influencing their purchase decisions, suggesting that taste preference plays an important role in the re-purchase of high-protein natural yoghurts.
- 3. Natural high-protein yoghurts of the 'A' ,'C' and 'B' brands were rated as readily available on the market, which facilitates their purchase by consumers looking for this type of product.
- 4. The most popular yoghurts among non-working students are brand 'A', consumed several times a week, which may be due to their favourable nutritional values and low fat content. Conversely, students undertaking employment are most likely to reach for 'B' brand products, which are consumed daily. These preferences may reflect differences in lifestyle and perceptions of product availability as well as product quality depending on professional activity.
- 5. The most numerous group of respondents expressed their willingness to re-purchase natural highprotein yoghurt from company 'A'. In contrast, products from brands 'D' and 'F' were least

popular, which may be due to differences in taste preferences or availability of these products.

## Acknowledgements

The presented work was supported by the Student Scientific Association, operating at the Department of Food Technology and Quality Assessment, Department of Dietetics, Faculty of Public Health in Bytom, Medical University of Silesia in Katowice.

### **Conflict of interest**

The authors declare that they have no conflicts of interest concerning this article.

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Received: 17.09.2024 Revised: 20.11.2024 Accepted: 22.11.2024 Published online first: 10.12.2024



https://roczniki.pzh.gov.pl/

Rocz Panstw Zakl Hig 2024;75(3):293-302

https://doi.org/10.32394/rpzh/196986

ORIGINAL ARTICLE

# POMEGRANATE JUICE TREATMENT REVERSES CARBON TETRACHLORIDE (CCL4)-INDUCED INCREASED ACETYLCHOLINESTERASE ACTIVITY AND CELL DEATH VIA SUPPRESSION OF OXIDATIVE STRESS IN RATS

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# ABSTRACT

**Background.** Environmental pollution, including exposure to carbon tetrachloride (CCl4), poses serious health risks, particularly through oxidative stress, which may lead to neurodegenerative damage. Antioxidants, especially those found in natural products, show potential in mitigating these toxic effects. Pomegranate juice (PJ), rich in bioactive phytochemicals, has demonstrated antioxidant, anti-inflammatory, and neuroprotective properties.

**Objective.** This study aimed to investigate the protective effects of PJ on neurotoxicity induced by CCl4 in rats, assessing specific markers of oxidative stress, enzymatic activity, and apoptotic cell death.

**Material and Methods.** Twenty-eight male Wistar rats were divided into four groups: Control, CCl4, PJ, and CCl4+PJ. The CCl4 group received intraperitoneal injections of CCl4 (0.2 ml/100 g) twice weekly for six weeks, while the PJ group received PJ orally (4 ml/kg) daily for 30 days. The CCl4+PJ group received both treatments in sequence. Brain tissues were analysed for malondialdehyde (MDA), reduced glutathione (GSH), acetylcholinesterase (AChE), glutathione S-transferase (GST), glutathione reductase (GR), and carboxylesterase (CaE) activity. Apoptotic cell death was assessed using TUNEL staining.

**Results.** CCl4 exposure resulted in a marked increase in MDA levels and AChE activity in brain tissue (p<0.05), alongside a significant decrease in reduced GSH levels and GST activity (p<0.05). Treatment with PJ significantly lowered MDA levels and AChE activity in the CCl4+PJ group compared to the CCl4 group (p<0.05). However, GSH levels and GST activity showed no significant changes in the CCl4+PJ group. TUNEL staining indicated a reduction in apoptotic cells in the CCl4+PJ group versus the CCl4 group, suggesting reduced cellular damage with PJ treatment (p<0.05).

**Conclusions.** PJ demonstrates neuroprotective potential against CCl4-induced oxidative stress and neurotoxicity in rats by reducing oxidative markers and apoptosis. These findings suggest that PJ could serve as a natural protective agent against neurodegenerative risks associated with environmental pollutants like CCl4.

Keywords: carbon tetrachloride, neurotoxicity, pomegranate juice, acetylcholinesterase, oxidative stress

# **INTRODUCTION**

Environmental pollution is one of the most important global problems that threatens humankind and health among with global warming [1]. According to a recent study, more than 9 million people died due to environmental pollution originated effects per year [2]. Contaminants that introduced to water, soil and air can effect to human body and causes irreversible damage to the organs [3]. Carbon tetrachloride (CCl4)

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This article is available in Open Access model and licensed under a Creative Commons Attribution-Non Commercial 4.0 International License (CC BY-NC) (https://creativecommons.org/licenses/by-nc/4.0/) Publisher: National Institute of Public Health NIH - National Research Institute which may be found in both ambient outdoor and indoor air is a dangerous chemical [4]. In ambient air, concentrations around 0.1 to 1 parts per billion (ppb) are generally considered safe due to regulation [5]. Toxic effects are unlikely at these low levels for the general population. exposure limit (PEL) for CCl4 in workplaces is 10 parts per million (ppm) over an 8-hour time-weighted average [5]. This is intended to prevent chronic toxicity, though acute exposure to 200 ppm or more can cause immediate health risks, including symptoms like dizziness, nausea, and damage to organs. The IDLH (immediately dangerous to life or health) level is 200 ppm, where even short exposures can lead to significant toxicity [6]. In laboratory settings, acute toxicity studies show that doses of CCl4 at 50-100 mg/kg body weight can cause significant liver and kidney damage in animal models [6]. In humans, ingestion of as little as a few millilitres of liquid CCl4 can be fatal, and high vapour concentrations (over 1,000 ppm) are similarly lethal if inhaled [6]. The major effects of CCl4 on health are hepatic and kidney damage and central nervous system (CNS) depression [4]. Despite its usage has been highly restricted since it was identified as a very harmful toxic substance, it is still possible for humans to be exposed to this substance [7]. CCI4 induce brain damage via increasing oxidative stress by free radical toxicities, inflammation, and neurotoxicity [8]. CCl4 has been shown to induces oxidative damage by the generation of free radicals and increases lipid peroxidation which are the main signs of oxidative stress [9]. The formation of free radicals increases in tissues exposed to CCl4 [9]. As a result of the depletion of antioxidant mechanisms in the cells, the prooxidant-antioxidant balance is disrupted and tissue damage occurs [10]. This complex tissues damage can cause neurodegeneration in CNS [11]. Antioxidants used against CCl4 toxicity may have a protective effect in this neurodegenerative process [12]. Numerous studies have investigated the use of specific antioxidant molecules for preservative or therapeutic purposes and promising data are available [12-14]. Although there are medical treatments against acute poisoning that caused by environmental pollution, it is important to avoid chronic toxic effects of CCl4 like pollutions by reducing the risk factors with natural products.

The pomegranate juice (PJ) is a natural product that is rich in various phytochemicals, exerting a wide range of biological activities such as antioxidative, anti-inflammatory, and anti-apoptotic properties [15]. These rich compounds play an important role in the oxidation of unsaturated fatty acids and detoxification of free radicals and makes PJ an efficient beneficial natural product for heath [16]. According to antioxidant capacity PJ has been safely used for centuries in both traditional and modern medicine [17]. The neuroprotective effect of pomegranate phytochemicals has been demonstrated in a few studies [18-20]. Regarding neurodegenerative diseases, a lot of in vivo studies on beneficial effects of pomegranate have been devoted. However, there is limited data about its neuroprotective potential against CCl4 induced toxicity.

In current study investigated the potential protective efficacy of PJ on neurotoxicity induced by CCl4 in rats. To better understand the neuroprotective effects of pomegranate juice (PJ) on neurotoxicity induced by carbon tetrachloride (CCl4), several key markers were assessed. Malondialdehyde (MDA), a wellknown indicator of lipid peroxidation [21], and reduced glutathione (GSH), a vital antioxidant for neutralizing reactive oxygen species (ROS) and supporting cellular redox balance [22], were measured. Additionally, the activities of brain acetylcholinesterase (AChE), which is affected by CCl4 exposure [23], and brain glutathione S-transferase (GST), an enzyme crucial for detoxification by facilitating GSH conjugation to toxic compounds [22], were analysed. Glutathione reductase (GR), essential for recycling GSH from its oxidized form and maintaining cellular antioxidant capacity [24], as well as carboxylesterase (CaE), involved in ester metabolism and detoxification [25], were also evaluated. Together, these markers provide insight into the effects of CCl4 on oxidative stress, antioxidant defenses, and enzymatic activity impacted by neurotoxicity. Furthermore, we assessed apoptotic cell death to evaluate the potential protective effect of PJ.

# **MATERIAL AND METHODS**

#### **Preparation of pomegranate juice**

Pomegranates freshly sourced from Adıyaman, Turkey, were carefully prepared. After washing and draining, they were halved, and their seeds along with the white pulp were shredded together using an electric blender. The resulting juice was then preserved in 1 ml quantities at -20°C until used.

### Chemical composition of pomegranate juice

The chemical content of the PJ has been studied in the project 'My city is Adıyaman' as the following project number: TRC1/18/KBG/0.036. Project financial issues granted by Development of Urban Culture and Urban Awareness Financial Support Program. The PJ content determined as followed: phenolic acid 490.75 mg/kg, anthocyanin 137.1 mg/L, ellagic acid 175 mg/100 g, total flavonoids 63 mg/kg and total antioxidants 1530 mg/kg [26].

#### Animals

The study was conducted at the Adıyaman University Animal Experiment Centre. Ethical approval was obtained from the Adıyaman University Animal Experiments Centre Ethics Committee under the protocol number 2022/18. Twenty-eight adult male Wistar albino rats weighing 200-250 g were used in the study. The rats were kept at  $22\pm20^{\circ}$ C room temperature in a 12-hr light 12-hr dark cycle with ad libitum access to chow and water.

### **Experimental design**

The animals were randomly divided into four groups (n=7 for each group) as followed: Control group, CCl4 group, PJ group, CCl4+PJ group. Before all experimental procedures, the animals were housed in cages without any experimental applications for one week to allow for adaptation. Immediately after adaptation process experimental applications performed as followed:

Control group: The control group received the solvent of CCl4 twice a week for six weeks. Following this, they were administered saline (4 ml/kg) every other day for the next 30 days.

CCI4 group: CCI4 (Bayer,  $\geq$ 99.9% purity) was prepared in 50% olive oil and injected intraperitoneally (i.p.) at a dose of 0.2 ml/100 g twice a week for 6 weeks [27].

PJ group: In this group, pomegranate juice was administered to the animals via an orogastric tube at a dose of 4 ml/kg per day for 30 days [28].

CCI4+PJ group: In this group, the animals were injected i.p. with CCI4 at a dose of 0.2 ml/100 grams twice a week for 6 weeks. For the following 30 days, PJ was administered to the animals via an orogastric tube at a dose of 4 ml/kg per day.

After the 30-day application process, the animals were euthanized by decapitation and the brain tissues were removed. The brain tissues were kept at  $-20^{\circ}$ C for biochemical analysis.

### Homogenization of brain tissue

Brain samples from all groups were homogenized by using a polytron homogenizer (Heidolph RZ 2021, Germany) in a chilled homogenization buffer (0.1 M, pH 7.4 in potassium phosphate buffer; containing 0.15M KCl, 1mM EDTA, 1mM DTT) at four times the total tissue weight (w/v). Following homogenization, the homogenates were transferred to Eppendorf tubes and centrifuged at 16.000g for 20 minutes at 4°C (Sigma Centrifuge Model 2-16K, Sigma, St. Louis, MO). Upon centrifugation, the supernatant fraction was collected, and enzyme activities along with other parameters were assessed. Each sample underwent three replicate absorbance readings, with a repetition of the reading if there was a correlation difference exceeding 10% between the values obtained for the same samples.

# Determination of brain malondialdehyde (MDA) and reduced GSH levels

MDA and reduced GSH levels were assessed using a microplate reader spectrophotometer system (Thermo TM Varioskan Flash, Thermo Scientific). Brain MDA levels were determined by assessing the relative production of reactive substances of thiobarbituric acid [29]. Results presented as nmol/mg wet tissue weight. Reduced GSH activity was measured by its reaction with DTNB to form a compound absorbing at 412 nm [30]. Results expressed as nmol/mg wet weight tissue.

# Determination of brain acetylcholinesterase (AChE) activity

Brain AChE activity was assessed according to the method described by Ellman et al [31] (some modifications has been approached to method [32]) utilizing a Thermo TMV arioskan Flash microplate reader spectrophotometer system from Thermo Scientific. In this process, acetylcholine iodide (ACTI) served as the substrate, and product formation was determined based on color change. To begin, 10 µl of supernatant was pipetted into microplate wells. Subsequently, the final solution, consisting of 200 µl (0.701 mM ACTI and 0.136 mM 5.5-dithiobis-2nitro-benzoic acid (DTNB)) prepared in 0.1 M buffer (pH 8.0), was transferred into the microplate wells. Absorbance changes were recorded at 412 nm for 1 minute at 25°C. Specific AChE activity was calculated as nmol/min/mg protein. Total protein content in the tissue homogenates was determined using the Bradford method [33]. Following a 1:4 dilution, 5 µl of the diluted sample and 250 µl of Bradford solution were sequentially added to the microplate wells. This mixture was then incubated at room temperature in the dark for 15 minutes. Absorbance was measured at a wavelength of 595 nm, based on the resulting colour change.

# Determination of brain glutathione s-transferase (GST) activity

To measure GST activity, a solution of 20 mM 1-chloro-2.4-dinitrobenzene (CDNB) was prepared first in 96% ethanol, and this served as the substrate. Reductive glutathione (0.002 M) was utilized as the cofactor in the reaction [34]. Briefly, 10  $\mu$ l of supernatant, 100  $\mu$ l of phosphate buffer (0.1 M, pH 6.5), 100  $\mu$ l of the GSH mixture, and finally 10  $\mu$ l of CDNB were transferred into the microplate wells. These were then inserted into the microplate reader system, and the absorbance change was recorded at 344 nm for 2 minutes at 25°C. Specific GST activity was computed as nmol/min/mg protein.

### **Determination of brain glutathione reductase (GR)**

The analysis of GR activity was conducted using a modified method [35]. The assay solution comprised 50 mM Tris–HCl buffer (pH 8.0), 1 mM EDTA, 1 mM GSSG, and 0.1 mM NADPH. One enzyme unit was defined as the quantity that oxidizes 1  $\mu$ mol NADPH per minute under the specified assay conditions.

# Determination of brain carboxylesterase (CaE) activity

The previously described spectrophotometric methods were adapted for analysis of CaE activity using a microplate reader system [36, 37]. In the activity assay, p-nitrophenol acetate (PNPA) was dissolved in 26 mM 96% ethanol to serve as the substrate. A reaction solution comprising 5 ml of sample and 250 ml of 0.1 mM Trizma buffer (pH 7.4) was incubated for 3 minutes at 25°C. The reaction was initiated by adding 5 ml of the substrate to the reaction solution. Changes in absorbance were monitored at 405 nm for 2 minutes at 25°C. Specific CaE activity was calculated as nmol/min/mg protein.

### **TUNEL Analysis and apoptotic index**

After embedding brain samples from each group into paraffin blocks, sections 5-6 µm thick were placed on poly-L-lysine-coated slides. Apoptotic cells were identified using the ApopTag Plus Peroxidase In Situ Apoptosis Detection Kit (Chemicon, cat. no.: S7101, USA) according to the manufacturer's instructions. The tissues were deparaffinized with xylene, passed through graded alcohol series, and washed with phosphate-buffered saline (PBS). Following this, the tissues were incubated for 10 minutes with 0.05% proteinase K, and then incubated for 5 minutes with 3% hydrogen peroxide to block endogenous peroxidase activity. After washing the tissues with PBS, they were incubated for 6 minutes with Equilibration Buffer and then incubated for 60 minutes at 37°C in a humid chamber with the working solution (70% Reaction Buffer + 30% TdT Enzyme). After a 10-minute incubation in Stop/Wash Buffer, the tissues were treated with Anti-Digoxigenin-Peroxidase for 30 minutes, and apoptotic cells were visualized using the Diaminobenzidine (DAB) substrate. The sections were counterstained with Harris hematoxylin and mounted using an appropriate medium. The images were examined and photographed using a Novel N-800M microscope [38].

To evaluate the TUNEL staining, nuclei stained blue with Harris hematoxylin were considered normal, while cells with brown nuclear staining were evaluated as apoptotic. In randomly selected areas at 10x magnification, at least 500 normal and apoptotic cells were counted in the sections. The apoptotic index (AI) was calculated by determining the ratio of apoptotic cells to the total number of cells (normal + apoptotic), and statistical analyses were performed based on this ratio [38].

### Statistical analysis

The results are presented as mean  $\pm$  SEM. Group comparisons were conducted using one-way analysis of variance (ANOVA), followed by the Tukey–HSD test. Statistical significance was considered at p<0.05.

### RESULTS

#### **Brain AChE activity**

AChE activity significantly The increased only in CCl4 group that compared to the control (28.54±1.07 nmol/min/mg group protein and 21.03±0.92 nmol/min/mg protein, respectively, p<0.05). The AChE activities of the PJ (21.15±1.41 nmol/min/mg protein) and CCl4+PJ (22.47±1.01 nmol/min/mg protein) groups were significantly lower that compared to CCl4 group (p < 0.05). There was no significant difference neither in PJ nor in CCl4+PJ groups that compared to control group (p>0.05, see Figure 1).



The data represent as mean  $\pm$  SEM (n=7); \* – p<0.05 compared to the control group; \*\* – p<0.05 compared to the CCl4 group

Figure 1. Effects of CCl4 and PJ on the AChE activity in male rat brain

#### **Brain MDA levels**

The brain MDA concentration of the CCl4 group was significantly higher than the control group ( $0.98\pm0.03$  nmol/g and  $0.78\pm0.02$  nmol/g, respectively, p<0.05). There was no significant difference in the PJ ( $0.74\pm0.01$  nmol/g) and CCl4+PJ group ( $0.75\pm0.04$  nmol/g) that compared to the control group. In comparison to the CCl4 group, the brain MDA levels were lower in the PJ and CCl4+PJ groups (p<0.05, see Figure 2).

CCl4: carbon tetrachloride



The data represent as mean  $\pm$  SEM (n=7); \* – p<0.05 compared to the control group; \*\* – p<0.05 compared to the CCl4 group

Figure 2. Effects of CCl4 and PJ on the MDA level in male rat brain

### **Brain reduced GSH levels**

The brain reduced GSH levels were significantly lower in the CCl4, PJ and CCl4+PJ groups that compared to the control group  $(0.12\pm0.003 \text{ nmol/g}, 0.13\pm0.005 \text{ nmol/g} \text{ and } 0.13\pm0.004 \text{ nmol/g} \text{ respectively}, p<0.05)$ . In comparison to CCl4 group, there was no significant difference in both PJ and CCl4+PJ groups (p>0.05, see Figure 3).



The data represent as mean  $\pm$  SEM (n=7); \* – p<0.05 compared to the control group

Figure 3. Effects of CCl4 and PJ on the reduced GSH level in male rat brain

### **Brain CaE activity**

Brain CaE activity decreased in the CCl4, PJ and CCl4+PJ groups that compared to the control group (239.56 $\pm$ 6.16 nmol/g, 292.40 $\pm$ 9.64 nmol/g, 281.85 $\pm$ 5.24 nmol/g and 402.93 $\pm$ 21.53 nmol/g, respectively, p<0.05). However, the CaE activity was higher in both PJ and CCl4+PJ groups than in the CCl4 group (p<0.005, see Figure 4).



The data represent as mean  $\pm$  SEM (n=7); \* – p<0.05 compared to the control group; \*\* – p<0.05 compared to the CCl4 group

Figure 4. Effects of CCl4 and PJ on the CaE levels in male rat brain

### **Brain GST activity**

Brain GST activity were significantly lower in the CCl4, PJ and CCl4+PJ groups that compared to the control group ( $51.24\pm2.78$  nmol/g,  $56.94\pm1.98$  nmol/g,  $58.81\pm3.41$  nmol/g and  $69.81\pm2.28$  nmol/g respectively, p<0.05). In comparison to CCl4 group, there was no significant difference in both PJ and CCl4+PJ groups (p>0.05, see Figure 5).





Figure 5. Effects of CCl4 and PJ on the GST levels in male rat brain

### **Brain GR activity**

The brain GR activity was not significantly higher or lower in the CCl4, PJ and CCl4+PJ groups that compared to the control group ( $17.38\pm0.46$  nmol/g,  $17.01\pm1.02$  nmol/g,  $18.04\pm0.80$  nmol/g and  $20.52\pm1.67$  nmol/g respectively, p>0.05). In comparison to CCl4 group, there was no significant difference in both PJ and CCl4+PJ groups (p>0.05, see Figure 6).



The data represent as mean  $\pm$  SEM (n=7); p>0.05 compared to the CCl4 group

Figure 6. Effects of CCl4 and PJ on the GR activity in male rat brain

### **TUNEL findings**

Upon examining TUNEL staining under a light microscope to identify apoptotic cells, no statistically significant difference in TUNEL positivity was observed in the PJ group (Figure 8d) compared to the control group (Figure 8a) (p=0.637). However, a statistically significant increase in TUNEL

positivity was observed in the CCl4 group (Figure 8b) compared to the control group (p=0.0001). In contrast, a statistically significant decrease in TUNEL positivity was observed in the CCl4+PJ group (Figure 8c) compared to the CCl4 group (Figure 8b) (see Figure 7).



The data represent as mean  $\pm$  SEM (n=7); \* – p<0.05 compared to the control group; \*\* – p<0.05 compared to the CCl4 group

Figure 7. Effects of CCl4 and PJ on the apoptotic activity in



a - control, b - CCl4, c - PJ, d - CCl4+PJ; apoptotic cells are indicated with black arrows; DAB chromogen, Mayer's haematoxylin, scale bar: 50 µm

Figure 8. Photomicrographs of TUNEL staining in the cortical region of rat brains from all groups

### DISCUSSION

Although the brain is quite sheltered by many and physiological component, it anatomic is particularly susceptible to extensive oxidative damage due to its high lipid content, elevated energy demands, and limited antioxidant defence system [39]. ROS, generated under toxic conditions such as CCl4 exposure, increase neuronal vulnerability by causing oxidative alterations, ultimately contributing to the development of neurodegenerative diseases [40]. The maintenance of ROS levels and the regulation of metal overload are governed by cellular defence mechanisms, intracellular signalling pathways, and the physiological roles of antioxidants within the brain [39]. Numerous antioxidants, including polyphenols and vitamins, play crucial roles in ROS regulation, metal ion chelation, and cellular signalling to defend against oxidative stress in the brain [41]. AChE, a prominent enzyme in brain tissue, plays a significant role in the pathogenesis of neurodegenerative diseases. It can influence various processes including the inflammatory response, apoptosis, oxidative stress, and aggregation of pathological proteins [42]. Many studies have demonstrated that toxins and heavy metals typically lead to a reduction in AChE activity [43, 44]. In our study, CCl4 exposure led to a significant increase in AChE activity in the brain tissue, diverging from findings where toxins and heavy metals typically suppress AChE [44]. This unexpected increase may be attributed to CCl4's unique toxic mechanisms, which can induce an inflammatory response, apoptosis, and aggregation of pathological proteins through AChE modulation. Our findings align with studies on neurotoxic exposures like aluminium and cadmium, which similarly induce AChE activity in the brain by triggering stressresponse pathways [45]. PJ treatment, known for its polyphenol content, showed a reversing effect on this increase. Polyphenol-rich antioxidants like resveratrol, curcumin, and N-acetylcysteine have similarly been shown to stabilize or even reduce AChE activity after toxic exposure [46], suggesting PJ's potential to counteract neurotoxic effects mediated by AChE. This finding represents the novel aspect of our study. MDA, final products of polyunsaturated fatty acids peroxidation in the cells. An increase in free radicals causes overproduction of MDA and level is commonly indicator of oxidative stress and the antioxidant status [47]. It is known that pollutants and heavy metals causes increase of MDA levels. Lipid peroxidation, as measured by MDA levels, was significantly elevated in the CCl4 group, which aligns with the established increase in oxidative stress markers following exposure to toxins. Antioxidants such as vitamin E, melatonin, and curcumin have demonstrated efficacy

in reducing MDA levels in comparable models, reflecting their capacity to counteract oxidative lipid damage [48-50]. The reduction in MDA levels with PJ treatment supports its lipid-protective properties, yet PJ alone did not alter MDA levels, implying its antioxidative role is particularly relevant in mitigating CCl4-induced stress rather than as a standalone effect. Reduced GSH levels, a key marker of antioxidant defence, were significantly decreased in the CCl4-treated group, consistent with the depletion of cellular antioxidants under toxic stress. Interestingly, PJ treatment alone also reduced GSH levels, which might reflect the pro-oxidant tendencies of certain flavonoids when administered chronically. Similar findings have been observed with antioxidants like quercetin and epigallocatechin gallate (EGCG), which can exhibit pro-oxidant effects depending on their concentration and duration of exposure [51]. This dual role of PJ and other polyphenols suggests that, while protective in acute toxic stress, these compounds may modulate redox balance differently under extended treatment. GST, like reduced GSH, is also on the frontline of cellular defence against both acute and chronic oxidative stress toxicity [52]. A reduction was observed with CCl4 exposure, aligning with its known vulnerability to toxic insults. PJ treatment alone also reduced GST activity, again potentially due to the pro-oxidant properties of flavonoids in high doses or chronic exposure. Antioxidants like selenium and silymarin, which have been studied in CCl4 models, similarly demonstrate dose-dependent impacts on GST, either boosting or reducing its activity depending on treatment conditions [53, 54]. This suggests that the dose and duration of antioxidant treatment are critical in modulating GST activity, and further studies could explore optimal dosing regimens for PJ to balance its antioxidative and pro-oxidant effects.GR in an important component of cells that plays an essential role in the defence system against ROS by regenerating the glutathione [55]. Unlike GST, GR activity did not change significantly across groups, suggesting that the treatment period of our study may have been insufficient to alter this enzyme's activity, which plays a crucial role in glutathione regeneration. In some studies, low-dose CCl4 and moderate doses of antioxidants such as coenzyme Q10 show limited effects on GR [56], yet antioxidants in higher doses or different compounds, like alpha-lipoic acid, have demonstrated a significant impact on GR activity [57]. This highlights that PJ's effect on GR might also be dose-dependent, warranting further investigation to understand its role in modulating cellular redox balance at varying concentrations. CaE activity, involved in metabolizing endogenous and exogenous esters, was significantly reduced by CCl4, reflecting its susceptibility to oxidative damage [25].

PJ treatment in both CCl4 and standalone groups enhanced CaE activity, indicating a protective effect. This is consistent with antioxidants like melatonin and alpha-lipoic acid, which have been shown to maintain or even restore CaE activity following oxidative stress in CCl4 and similar models [58]. PJ's ability to enhance CaE activity suggests a protective role in maintaining esterase function, which is essential for the detoxification of environmental toxins and preservation of neural homeostasis. In TUNEL staining analysis, we observed a significant increase in apoptotic cells in the CCl4 group, while PJ treatment notably reduced apoptosis, indicating a neuroprotective effect. Studies with antioxidants like curcumin, EGCG, and selenium show similar anti-apoptotic effects in the context of CCl4-induced oxidative stress [59], supporting the potential of PJ to protect against cell death pathways. The reduction in TUNEL positivity in the PJ-treated group suggests that PJ's polyphenols may help stabilize cellular environments and mitigate the impact of ROS-induced apoptosis.

### CONCLUSIONS

In conclusion, our findings underscore the potential of PJ as a neuroprotective agent in CCl4-induced oxidative stress, with similarities to antioxidants. The dual effects observed, particularly the time dependent modulation of antioxidant enzymes and pro-oxidant properties of PJ, highlight the importance of optimizing treatment duration and dosing for effective neuroprotection. Further studies are needed to compare PJ's effects with these antioxidants across different dosages and durations to further elucidate its therapeutic potential in oxidative stress and neuroprotection.

### **Conflict of interest**

The authors declare that they have no conflicts of interest concerning this article.

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Received: 07.10.2024 Revised: 04.11.2024 Accepted: 06.12.2024 Published online first: 17.12.2024



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3. Kerner S, Chou C, Warmind M. Commensality: From Everyday Food to Feast. London: Bloomsbury Publishing PLC; 2015. ISBN 9780857857361.

# Book chapter:

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