JPMB | Journal of Plant Molecular Breeding
Published by Genetics and Agricultural Biotechnology Institute of Tabarestan

2024 | Volume 12 | Issue 1

Check for update

Open ACCESS

Edited by

Prof. Hematolah Pirdashti,
Department of Agronomy, Genetics and Agricultural Biotechnology Institute of Tabarestan, Sari Agriculture Sciences and Natural Resources University

Date

Received: 6 September 2024
Accepted: 29 October 2024
Published: 13 November 2024

Correspondence

Dr. Ibrahim Ajadi
ajadiibrahim669@gmail.com

Citation

Olahan ,G., Ajadi, I., and Sulaimon, W. (2024). Bioactive compounds and microbial evaluation of African walnuts (*Tetracarpidium conophorum* (Mull. Arg.) Hutch & Dalziel) retailed in Ilorin metropolis. *J Plant Mol Breed. 12* (1): 49-59.
Doi: 10.22058/jpmb.2024.2040416.1305.

Original paper

Bioactive compounds and microbial evaluation of African walnuts (*Tetracarpidium conophorum* (Mull. Arg.)) Hutch & Dalziel retailed in Ilorin Metropolis

Ganiyu Olahan, Ibrahim Ajadi*, Waliyat Sulaimon

Department of Plant Biology, Faculty of Life Sciences, University of Ilorin, Ilorin, Nigeria.

Abstract: The African walnut (*Tetracarpidium conophorum*) is a nutrient-dense tropical fruit that provides numerous health benefits and offers a wealth of nutritional value. This study investigated the bioactive compounds and microbes associated with African walnuts retailed in the Ilorin Metropolis. After preparing stock solutions from the obtained nuts, microbial isolations were carried out on Potatoes Dextrose Agar (fungi), Nutrient Agar, and Eosin Methylene Blue Agar (bacteria) using the pour plate method. Sixteen bioactive compounds of very significant therapeutic values were identified in the raw nuts using Gas Chromatography-Mass Spectrometry (GC-MS). Also, molecular identification of the fungal species and biochemical characterization of the bacterial species isolated from the nuts revealed the presence of four fungal species, namely *Aspergillus niger*, *Penicillium rolfsii, Rhizopus delemar* and *Rhizopus arrhizus*, as well as three bacterial species, namely *Bacillus cereus*, *Staphylococcus aureus*, and *Escherichia coli*. The GC-MS results revealed the nutritional and medicinal benefits of consuming cooked African walnuts; however, the microbial screening serves as a precaution for those consuming cooked African walnuts retailed in the Ilorin metropolis.

Keywords: GC-MS, microbial screening, molecular identification, African walnuts, antimicrobial.

Introduction

African walnut (*Tetracarpidium conophorum* (Mull. Arg.) Hutch & Dalziel) is a climbing plant belonging to the Euphorbiaceae family. It is native to the humid regions of Eastern and Western Nigeria, as well as, West Africa as a whole. In Eastern Nigeria, it is called Ukpa in Igbo, while in Western Nigeria, it is referred to as "Awusa" or "Asala" in Yoruba and "Okhue" or "Okwe" in Edo (Asuzu-Samuel and Nnamdi, 2023). They are characterized by their glossy, dark green leaves and small, round fruits, each containing two to four seeds or nuts. The nuts themselves are encased in a tough, woody black shell (Ayodeji and Aliyu, 2018).

Beyond their culinary appeal, African walnuts are revered for their nutritional richness, serving as a concentrated source of essential nutrients (Ayodeji and Aliyu, 2018). Each nut is packed with proteins, healthy fats, dietary fiber, vitamins (such as vitamin E, vitamin B6, and folate), and minerals (including calcium, magnesium, and potassium) (Salem et al., 2022). This nutrient profile not only makes African walnuts a valuable component of a nutritious diet but also confers numerous health benefits, ranging from cardiovascular support to cognitive enhancement on its consumption.

Moreover, African walnuts are a veritable treasure trove of bioactive compounds such as phenolic compounds, flavonoids, alkaloids, and terpenoids (Adetunji et al., 2021). These phytochemicals are endowed with potent antioxidant, antimicrobial, anti-inflammatory and anticancer properties, which contribute to the nut's therapeutic potential. The antioxidant activity of these compounds helps combat oxidative stress and free radical damage, thereby reducing the risk of chronic diseases such as heart disease, diabetes, and cancer (Mukarram et al., 2024).

The nuts of *Tetracarpidium conophorum* are used in Nigeria to increase sperm counts in men (Alenazy, 2023). It was proved that the extract of *Tetracarpidium conophorum* nut increases the viability and sperm output of male albino rats and this suggests that the nuts could be included in the formulation of male fertility drugs (Ikpeme et al., 2014). The nuts of *Tetracarpidium conophorum* have the potential to reduce hyperglycaemia; researchers also reported that the nuts increased the haemoglobin level and decreased urine output in the test group when compared with controls and could prevent diabetes associated with renal damage. In addition to its nutritional and medicinal attributes, African walnuts hold cultural and socioeconomic significance within West African communities (Amusa et al., 2016; Paulussen et al., 2017). Traditionally, the nuts are not only consumed as a snack but also incorporated into various dishes, desserts, and beverages. Furthermore, African walnut cultivation and retailing provide livelihood for many farmers and vendors across the region, contributing to the local economy and fostering social cohesion (Shigaeva and Darr, 2020). Despite its nutritional and medicinal significance, African walnuts are susceptible to microbial contamination during storage, leading to spoilage and potential health hazards (Edeh et al., 2024). In light of these factors, this study aimed to reveal the bioactive compounds present in African walnuts and identify the microorganisms responsible for their potential microbial spoilage

Materials and Methods

Sample collection

Fresh, uncooked walnuts (40 nuts) were obtained from Oja-Oba Market in Ilorin, where they were kept in open baskets placed in shaded areas to protect them from direct sunlight and moisture. After purchasing, the walnuts were transferred to Ziploc bags and brought to the laboratory for microbiological and phytochemical analyses. In the lab, they were stored at 4°C to ensure freshness and inhibit microbial growth until the analyses took place. The forty African walnut nuts were categorized into two groups of twenty nuts each; one for GC-MS analysis and the other groups for microbial assessment. The two groups of African walnut nuts were stored at room temperature prior to the analyses.

Sample preparation for GC-MS

The raw nuts in group one were shelled, sliced, dried, and milled, after which oil was extracted from them.

Extraction of oil from African walnuts

This was conducted using a Soxhlet apparatus. Approximately 40 grams of the pulp (from the milling exercise) were extracted individually with n-hexane in the Soxhlet apparatus. The solvent was then eliminated to obtain the oil. Any residual solvent in the oil was evaporated gently in a beaker at room temperature. The oil was subsequently refrigerated at 4°C until required, and when needed, it was brought back to room temperature before analysis by taking the oil out of the refrigerator and placed in a clean, dry environment at room temperature. The volatile compounds were then identified using Gas Chromatography-Mass Spectroscopy (GC-MS).

Phytochemical screening of the extracted oil

This was conducted using an Agilent Technologies 8890 Series GC System coupled with an Agilent 5977B Mass Selective Detector with Electron Impact Ionization (GC-MS). A HP-5 capillary column measuring 30 meters in length, with an internal diameter of 0.32 mm and a film thickness of 0.25 µm, was utilized, packed with 5% phenyl methyl siloxane. The following conditions were maintained: injection port temperature set at 250°C (split-less, pressure at 8.35 psi, purge flow of 30 ml/min, purge time of 1.0 min, total flow of 35.35 ml/min); the GC-MS operated in the chosen ion-monitoring mode.

The column oven initiated at 90°C for 3 minutes, followed by a 15-minute programming period at a rate of 30°C/minute (from 90 to 200°C), 5 minutes at a constant temperature (200 to 265°C), and a subsequent 15-minute period at a rate of 3°C/min (from 265 to 276°C). The separated components exited the column and entered the Mass Spectrometer (MS) for analysis. Compound identification was achieved by comparing the extract's retention indices and mass spectra fragmentation patterns with those in published literature. Additionally, the National Institute of Standards and Technology library sources were consulted to match the identified compounds from the walnut (Oladimeji and Adebo, 2023).

Microbiological analyses

Isolation and Identification of Bacterial Species -In the second group, nuts were carefully chosen, cracked open, and weighed using a precise electronic balance. These nuts were then transferred into a sterilized mechanical blender, where a sterile water solution was added in a specific ratio (1 part of walnut to 9 parts of sterile water) before blending. This blending process produced a concentrated solution known as the stock solution. From this stock solution, further dilutions were made up to the third level/category, each being ten times less concentrated than the previous one, with the most diluted solution being labeled as 10^{-3}. A milliliter of inoculum from the 10^{-3} dilution was carefully transferred into pre-labeled 3 sterile Petri dishes. Molten agar media made up of equal volumes of sterilized Nutrient Agar (NA) and Eosin Methylene Blue (EMB) were respectively poured into the Petri dishes. The agar plates were then left to solidify and were subsequently incubated at 37°C for 24 to 48 hours to allow bacterial growth. Following the incubation period, the bacterial isolates present on the agar plates were subjected to a series of biochemical tests to characterize their properties following the procedures of Afolabi et al. (2020). These tests included the catalase test, oxidase test, sugar fermentation test, citrate utilization test, nitrate reduction, casein hydrolysis, urea hydrolysis, and motility test.

Isolation and Identification of Fungal Species- From each of the prepared stock solutions, the pour plate method was utilized to isolate fungi on potato dextrose Agar (PDA), which were then incubated at a temperature of 28°C for 3 to 5 days for fungal growth. Thereafter, pure isolates of the fungal cultures were identified molecularly following a series of stepwise procedures. Firstly, total genomic DNA was extracted from each of the pure fungal isolates using a Zymo Research DNA kit, following a detailed protocol provided by the manufacturer. The Internal Transcribed Spacer (ITS) region of each fungal isolate's DNA was then amplified using a specific primer combination (ITS1 and ITS4), as described by Liu and Chen (2007). This amplification process was carried out using thermal cycler PCR equipment. Subsequently, the amplified DNA sequences were subjected to sequencing at Inqaba Biotech facility in Ibadan. Using the methods outlined by Stucky (2012), a consensus DNA sequence was generated from both the forward and reverse sequences obtained. This consensus sequence served as a unique genetic fingerprint for each fungal isolate.

Table 1. Bioactive compounds in African walnuts.

Peak no.	Name	R.T	Peak area (%)	MF	MW (g)	Compound Nature	Biological activity
1	Nonadecane	11.916	0.88	$C_{19}H_{40}$	268.5	Straight chain alkane	Antioxidant
2	Hexadecanoic acid, methyl ester	13.026	2.35	$C_{17}H_{34}O_2$	270.4	Fatty acids	Antimicrobial, Anti-inflammatory
3	n-Hexadecanoic acid	13.547	11.49	$C_{16}H_{32}O_2$	256.42	Saturated Fatty acids	Antioxidant, Antimicrobial
4	Eicosane	13.598	1.74	$C_{20}H_{42}$	282.5	Unsaturated fatty acid	Antioxidants, anti-inflammatory and cardiovascular protective properties
5	9,12-Octadecadienoic acids, methyl ester,(E,E)-	14.394	2.59	$C_{18}H_{32}O_2$	280.4	Fatty acid methyl ester	Anti-inflammatory
6	9-Octadecenoic acid, methyl ester	14.440	6.04	$C_{19}H_{34}O_2$	294.5	Fatty acids methyl ester	Antioxidant, anti-inflammatory nematicide and anticancer
7	Methyl stearate	14.623	1.23	$C_{19}H_{38}O_2$	298.5	Fatty acids methyl ester	Antimicrobial,antioxidant,Gastrin inhibitor
8	trans-13- Octadecenoic acid	14.937	18.87	$C_{18}H_{34}O_2$	282.5	Long chain fatty acid	Antioxidant
9	9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	14.966	5.39	$C_{18}H_{32}O$	264.4	Unsaturated fatty acid	Anti-inflammatory, antioxidant
10	Octadecanoic acid	15.069	4.68	C18H36O2	284.5	Saturated fatty acid	Anti-inflammatory ,anticancer, antimicrobial
11	1,15-Pentadecanedioic acid	15.944	1.68	C15H28O4	272.38	Saturated fatty acid	Antimicrobial ,antioxidant ,anti-inflammatory
12	9-Octadecenamide, (Z)-	16.694	2.24	C18H35NO	281.5g	Fatty acid	Anti-inflammatory , neuro protective potential
13	9-Octadecenal,(Z)-	17.747	6.01	C18H34O	266.5g	Fatty aldehyde	Antibacterial, antimicrobial
14	9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	17.816	4.15	C20H34O2	306.5	Fatty acid ester	Antibacterial , anti-inflammatory, neurological function
15	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	18.348	4.51	C19H38O4	330.5	Carboxylic acid	Antioxidants, nematicide and pesticide , anti-inflammatory
16	Bis(2-ethylhexyl) phthalate	18.714	4.67	C24H38O4	390.6	Phthalate esters	Oral toxicity during pregnancy

The generated ITS DNA sequences were compared with sequences available in the National Centre for Biotechnology Information's (NCBI) GenBank database. This comparison was facilitated using the Basic Local Alignment Search Tool for Nucleotides (BLASTN) Sequences, accessible through the GenBank repository web page. (https://www.ncbi.nlm.nih.gov/). By matching the nucleotide sequences of the fungal isolates with those in the database, their respective species were determined.

Results

GC-MS results

The gas chromatogram of the sample revealed the presence of 16 compounds with different retention times as presented in Figure 1. These compounds were identified through mass spectrometry attached with GC. Table 1 contains the retention times (R.T.s), molecular formulae, molecular weights, compounds nature, peak areas, biological activities and names of the 16 bioactive compounds identified in this study. Nonadecane, Hexadecanoic acid, methyl ester, 9,12-Octadecadienoic acids, methyl ester,(E,E)-, Eicosane, n-Hexadecanoic acid, trans-13-Octadecenoic acid, Methyl stearate, 9-Octadecenoic acid, methyl ester, 1,15-Pentadecanedioic acid, 9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-, Octadecanoic acid, 9-Octadecenal,(Z)-, 9-Octadecenamide, (Z)-, Bis(2-ethylhexyl) phthalate, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, 9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-, and 9,12,15-octadecatrienoic acid, (Z, Z,Z)-.

Microbiological analyses

The microbial counts on the unprocessed African walnuts were 7.4× 10^3 and 1.5×10^3, for fungi and bacteria respectively, providing insights into the microbial population present in the nuts (Table 2). Results of all the biochemical tests conducted revealed the identities of 3 bacterial species isolated from the nuts (Table 3), while Table 4 presents the unique NCBI accession numbers assigned to each of the fungal species isolated from the same nuts.

Table 2. Microbial load of raw African walnuts.

Isolates	Counts (×10^3) (cfu/g)
Bacteria	1.5
Fungi	7.4

Table 3. Results of biochemical tests conducted on the bacterial isolates.

Selective biochemical reaction	*Bacillus cereus*	*Staphylococcus aureus*	*Escherichia coli*
Gram reaction	+	+	-
Citrate test	+	+	-
Oxidase test	-	-	-
Motility	Non-Motile	Non-Motile	Motile
Catalase test	+	+	+
Sugar fermentation	+	+	+
Urea hydrolysis	+	+	+
Nitrate reduction	+	+	+
Casein hydrolysis	-	+	+

Table 3. NCBI accession numbers and matched fungal species.

NCBI No.	ID (%)	QC (%)	Matched organism
PP756477	99	99	Rhizopusdelemar
PP756479	99	98	Rhizopusarrhizus
PP756478	100	100	Aspergillusniger
PP756481	99	99	Penicilliumrolfsii

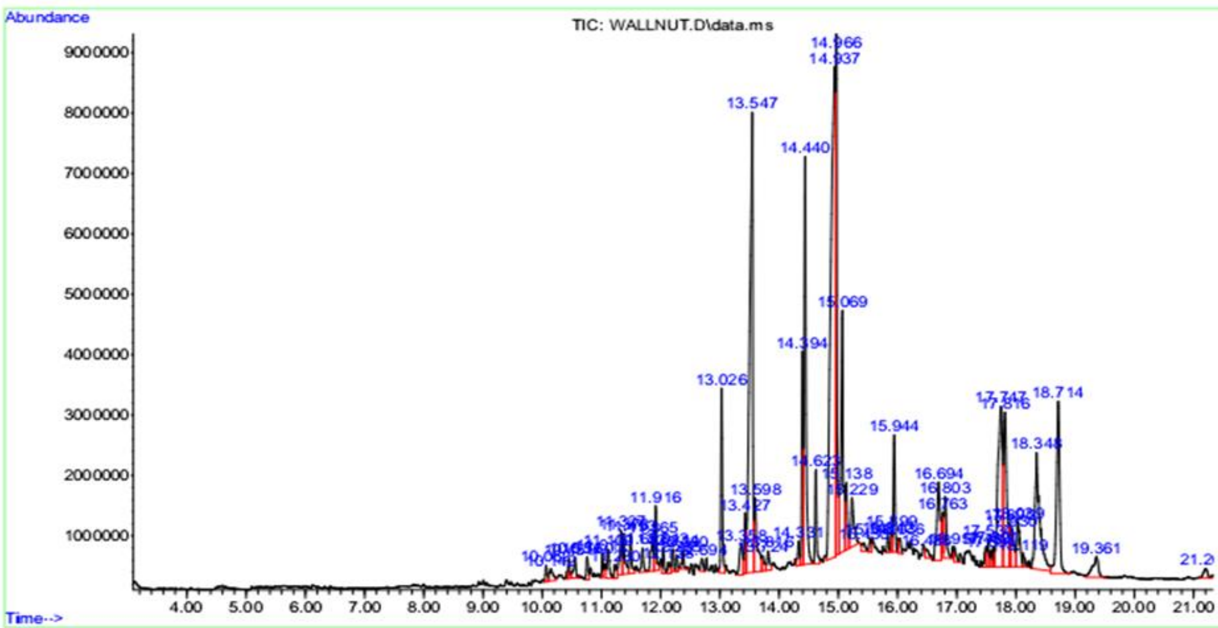

Figure 1. GC-MS chromatogram of African walnuts.

Discussion

In this study, the GC-MS analysis conducted on the oil extract of raw African walnuts revealed the presence of 16 bioactive compounds. Notably, the compound with the highest peak value was trans-13-Octadecenoic acid (68.87%). Krishnamoorthy and Subramaniam (2014) reported the anti-inflammatory and cancer-preventive properties of this compound which they got from *Solena amplexicaulis*. Nonadecane which has antioxidant, antibacterial, antimicrobial, antitoxic and antimalarial effects, according to Aziz et al. (2022) and El-Shahir et al. (2022) was also present in the oil extracted from African walnuts in this study. Hexadecanoic acid, methyl ester with hepatoprotective, antibacterial, and anti-inflammatory activities, according to Shaaban et al. (2021) and Gupta et al. (2021) were also reported in this study. 9,12-Octadecadienoic acid, methyl ester, present in the extracted oil has earlier been reported to have antimicrobial properties by Muzahid et al. (2023). Eicosane, another compound reported in this study has antifungal and antibacterial properties (Bhat et al., 2024). Semwal et al. (2018) had earlier reported that n-Hexadecanoic acid, found in the African walnuts in this study has antioxidant, anti-inflammatory, hypocholesterolemic, and cancer properties. Methyl stearate present in the African

walnuts in this study also has antibacterial properties (Nakaziba et al., 2022).
The CG-MS analysis of oil extracted from African walnuts in this study contained 1,15-Pentadecanedioic acid which Alenazy (2023) had earlier reported to have anti-inflammatory and antimicrobial activities. Similarly, 9-Octadecenoic acid, a methyl ester reported to have an antidiarrheal effect by Zahara et al. (2022) was found in the oil extracted from African walnuts. 9,12,15-octadecatrien-1-ol, (Z,Z,Z)-, an antioxidant and antibacterial according to Olivia et al. (2021) 9-Octadecenal, (Z)- and 9-Octadecenamide, (Z)- with have anti-inflammatory and flavoring roles in food respectively, according to Hatami et al. (2016) and Muzahid et al. (2023) were found in the oil extracted from African walnuts. Bis(2-ethylhexyl) phthalate with antibacterial and larvacidal properties (Javed et al., 2022) was found in the oil extracted from African walnuts; while 9,12,15-octadecatrienoic acid, (Z, Z,Z) and 2-hydroxy-1-(hydroxymethyl)ethyl ester having antimicrobial and anti-inflammatory properties. Salem et al. (2022) were also present in the oil extracted from African walnuts.
According to Matotoka et al. (2023), the effectiveness of any medicinal plant is assessed by linking its phytochemical compound constituents with significant biological functions in the human body. Given the significance of each of the compounds discovered from the oil extracted from African walnut nuts in this study, using GC–MS, walnut nuts can be classified as being medicinal.
This study identified three bacterial species: *Bacillus cereus*, *Staphylococcus aureus* and *Escherichia coli* based on outcomes of the biochemical tests conducted on each of them. These bacteria species have earlier been reported to be present in natural settings such as fruits, soil, and water (Kowalska et al., 2020; Ryu et al., 2021). *Bacillus cereus*, for instance, occurs naturally in fruit flora and it is linked to their spoilage (Garuba et al., 2024). The detection of *Escherichia coli* and *Staphylococcus aureus* suggests the usage of contaminated water in washing the walnuts or handling of the nuts with contaminated hands. *Staphylococcus aureus*, typically found in the nasal cavity and on human skin, can cause various illnesses in humans (Laux et al., 2019). In a prior investigation, Fakile et al. (2023) also identified *Bacillus subtilis* and *Staphylococcus aureus* as bacterial species associated with the spoilage of African walnut, underscoring the importance of adopting appropriate handling and storage procedures for walnut fruits.
The fungal species isolated from the examined walnuts in this study were molecularly identified as *Rhizopus delemar*, *R. arrhizus*, *Aspergillus niger*, and *Penicillium rolfsii*. Each species exhibited a query cover and percentage identity exceeding 98 % and 97 %, respectively, in the analysis, confirming their accurate identification. According to Raja et al. (2017), reliable molecular identification typically requires achieving a query cover of at least 80 % and a percentage identity of at least 97 % when comparing fungal species sequences to those in the NCBI database using BLASTN. The PCR technique was used in the current study to diagnose fungi isolated from nuts, as indicated by Freeman Weiss et al. (2021) that the traditional methods adopted in the diagnosis of fungi and based on the identification of phenotypic criteria are no longer sufficient due to the overlap of these criteria with other species in addition to the genetic difference between them. Their growth and phenotypic characteristics do not necessarily imply the difference in genotype, especially between isolates of the same genus and species.
The above fungal species align with those documented by Nooralden (2022) in their study on nut samples including walnut in the city of Kirkuk where fungi of both genus *Penicillium* and *Aspergillus* were isolated. *Aspergillus* is frequently found in various nuts because it produces a wide array of enzymes that allow it to thrive on diverse nutrients (Corbu et al., 2023). Additionally, it can adapt well to challenging environmental conditions and generate numerous spores that withstand unfavorable conditions (Paulussen et al., 2017). This aligns with Musangi et al. (2024), who found *Aspergillus* to be a common contaminant in nuts. The detection of *R. arrhizus* and *R. delemar* in this study corroborates earlier research identifying *Rhizopus* species as contaminants in nut samples. However, Eze et al. (2019) only isolated *Penicillium* sp. and *Fusarium* sp. in their investigation of raw African walnuts from the Eke Akpara market in Abia State.

Conclusion

Sixteen bioactive compounds were identified in the oil extracted from African walnuts, showing the nutritional and therapeutic significance of consuming these nuts. Also, three bacterial and four fungal species were isolated from the nuts, all of them are pathogenic.

Supplementary Materials

There is no supplementary material available for this article.

Author Contributions

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

Funding

This research received no external funding.

Acknowledgments

The authors wish to acknowledge the Inqaba Biotec facility in Ibadan, Nigeria, for providing assistance with the molecular identification of the recovered fungal isolates.

Conflict of Interest Statement

The authors declare no conflict of interest.

References

Adetunji, J.B., Adetunji, C.O., and Olaniyan, O.T. (2021). African walnuts: A natural depository of nutritional and bioactive compounds essential for food and nutritional security in Africa. *J. Food Secur.*: 331-354.

Afolabi, O.J., Oladele, O.O., and Olususi, F.C. (2020). Assessment of bacterial loads of *Clarias gariepinus* (Burchell, 1822) obtained from cultured and natural habitats. *J Basic Appl Zool* 81: 1-7.

Alenazy, R. (2023). Antimicrobial activities and biofilm inhibition properties of *Trigonella foenumgraecum* methanol extracts against multidrug-resistant *Staphylococcus aureus* and *Escherichia coli*. *Life* 13(3): 703.

Amusa, T., Adefalu, L., and Aderinoye-Abdulwahab, S. (2016). Socio-economic potentials and threats to the African walnut in tropical lowland rainforests of southwest Nigeria. *J Agric Res Dev* 15(2): 88-99.

Asuzu-Samuel, H.O., and Nnamdi, J.G. (2023). Isolation and characterization of the bioactive components in *Tetracarpidium conophorum* as dissolvent in methanol. *J. Adv. Res. Rev* 20(3): 1878-1883.

Ayodeji, A.E., and Aliyu, N. (2018). *Tetracarpidium conophorum* (African walnut) Hutch. & Dalziel: Ethnomedicinal uses and its therapeutic activities. *J. Med. Plant. Econ. Dev* 2(1): 1-10.

Aziz, M., Ahmad, S., Khurshid, U., Pervaiz, I., Lodhi, A.H., Jan, N., Khurshid, S., Arshad, M.A., Ibrahim, M.M., and Mersal, G.A. (2022). Comprehensive biological potential, phytochemical profiling using GC-MS and LC-ESI-MS, and in-Silico assessment of *Strobilanthes glutinosus* Nees: an important medicinal plant. *Molecules* 27(20): 6885.

Bhat, M.P., Kumar, R.S., Chakraborty, B., Nagaraja, S.K., Babu, K.G., and Nayaka, S. (2024). Eicosane: An antifungal compound derived from Streptomyces sp. KF15 exhibits inhibitory potential against major phytopathogenic fungi of crops. *Environ. Res.* 251: 118666.

Corbu, V.M., Gheorghe-Barbu, I., Dumbravă, A.Ș., Vrâncianu, C.O., and Șesan, T.E. (2023). Current insights in fungal importance—a comprehensive review. *Microorganisms* 11(6): 1384.

Edeh, J.A., Anjorin, T.S., Asala, S.W., Onyeiwu, S.C., and Akpan, G.E. (2024). Assessment of handling practices and microbial contamination of raw and cooked African walnut (*Tetracarpidium conophorum*) fruit snacks in Abuja Nigeria markets. *J Adv Res Rev* 22(1): 888-897.

El-Shahir, A.A., El-Wakil, D.A., Abdel Latef, A.A.H., and Youssef, N.H. (2022). Bioactive compounds and antifungal activity of leaves and fruits methanolic extracts of *Ziziphus spina-christi* L. *Plants* 11(6): 746.

Eze, V., Maduka, N., Ahaotu, I., and Odu, N. (2019). Microbiological quality and shelf life of pickled African Walnut (*Tetracarpidium conophorum*) preserved with lactic and citric acids. *Microbiol. Res. J. Int.* 26(1): 1-18.

Fakile, O., Solana, O., and Okolosi, J. (2023). Antimicrobial activity of african walnut (*Tetracarpidium conophorum*) oil against bacterial and fungal species causing food spoilage and food poisoning diseases. *J Food Agric.*

Freeman Weiss, Z., Leon, A., and Koo, S. (2021). The evolving landscape of fungal diagnostics, current and emerging microbiological approaches. *J. Fungus* 7(2): 127.

Garuba, T., Ajala, F.A., Olahan, G.S., and Lateef, A.A. (2024). Molecular identification of dominant microbes in Kola nut (*Cola nitida*). *Badeggi J. Agri Res Env* 6(1): 13-24.

Gupta, L., Vermani, M., Kaur Ahluwalia, S., and Vijayaraghavan, P. (2021). Molecular virulence determinants of *Magnaporthe oryzae*: disease pathogenesis and recent interventions for disease management in rice plant. *Mycology* 12(3): 174-187.

Hatami, S., Mohamadi Sani, A., and Yavarmanesh, M. (2016). Chemical composition and antibacterial activity of organic extra virgin olive oil from Iran. *Nutr Food Sci* 46(3): 388-395.

Ikpeme, E., Ekaluo, U., Udensi, O., Ekerette, E., Ekpo, P., and Asuquo, B. (2014). Sperm quality and hormone profile of male albino rats fed with seeds of African walnut (*Tetracarpidium conophorum,* Mull). *Annu. Res. Rev.* 4(9): 1379-1386.

Javed, M.R., Salman, M., Tariq, A., Tawab, A., Zahoor, M.K., Naheed, S., Shahid, M., Ijaz, A., and Ali, H. (2022). The antibacterial and larvicidal potential of bis-(2-ethylhexyl) phthalate from *Lactiplantibacillus plantarum*. *Molecules* 27(21): 7220.

Kowalska, J., Maćkiw, E., Stasiak, M., Kucharek, K., and Postupolski, J. (2020). Biofilm-forming ability of pathogenic bacteria isolated from retail food in Poland. *J. Food Prot.* 83(12): 2032-2040.

Krishnamoorthy, K., and Subramaniam, P. (2014). Phytochemical profiling of leaf, stem, and tuber parts of *Solena amplexicaulis* (Lam.) Gandhi using GC‐MS. *Int. Sch. Res. Not.* 2014(1): 567409.

Laux, C., Peschel, A., and Krismer, B. (2019). *Staphylococcus aureus* colonization of the human nose and interaction with other microbiome members. *Microbiol. Spectr.* 7(2): 10.1128/microbiolspec. gpp1123-0029-2018.

Liu, Y.-G., and Chen, Y. (2007). High-efficiency thermal asymmetric interlaced PCR for amplification of unknown flanking sequences. *Biotechniques* 43(5): 649-656.

Matotoka, M.M., Mashabela, G.T., and Masoko, P. (2023). Phytochemical content, antibacterial activity, and antioxidant, anti‐inflammatory, and cytotoxic effects of traditional medicinal plants against respiratory tract bacterial pathogens. *J Evid Based Complementary Altern Med* 2023(1): 1243438.

Mukarram, S.A., Wandhekar, S.S., Ahmed, A.E.M., Várallyay, S., Pandey, V.K., József, P., and Bela, K. (2024). Global perspectives on the medicinal implications of green walnut and its benefits: A comprehensive review. *Horticulturae* 10(5): 433.

Musangi, C.R., Juma, B.S., Mukhebi, D.W., Isoe, E.M., Kibiti, C.M., and Mbinda, W.M. (2024). Aspergillus population diversity and its role in aflatoxin contamination of cashew nuts from coastal Kenya. *PLoS One* 19(1): e0292519.

Muzahid, A.A., Sharmin, S., Hossain, M.S., Ahamed, K.U., Ahmed, N., Yeasmin, M.S., Ahmed, N.U., Saha, B.K., Rana, G.M., and Maitra, B. (2023). Analysis of bioactive compounds present in different crude extracts of *Benincasa hispida* and *Cucurbita moschata* seeds by gas chromatography-mass spectrometry. *Heliyon* 9(1).

Nakaziba, R., Amanya, S.B., Sesaazi, C.D., Byarugaba, F., Ogwal-Okeng, J., and Alele, P.E. (2022). Antimicrobial bioactivity and GC‐MS analysis of different extracts of *Corchorus olitorius* L leaves. *Sci. World J.* 2022(1): 3382302.

Nooralden, Z.N. (2022). Isolation and identification of fungi associated with walnuts, almonds, hazelnuts, cashews, pistachios and peanuts using molecular diagnostics technique (PCR) in the local markets of Kirkuk Governorate/Iraq. *J Pharm Negat Results*: 156-160.

Oladimeji, B.M., and Adebo, O.A. (2023). Dataset of metabolites extracted from African walnut (*Tetracarpidium conophorum*) using two different solvents. *Data in Brief* 47: 108930.

Olivia, N.U., Goodness, U.C., and Obinna, O.M. (2021). Phytochemical profiling and GC-MS analysis of aqueous methanol fraction of *Hibiscus asper* leaves. *Futur. J. Pharm. Sci.* 7: 1-5.

Paulussen, C., Hallsworth, J.E., Álvarez‐Pérez, S., Nierman, W.C., Hamill, P.G., Blain, D., Rediers, H., and Lievens, B. (2017). Ecology of aspergillosis: insights into the pathogenic potency of *Aspergillus fumigatus* and some other Aspergillus species. *Microb. Biotechnol.* 10(2): 296-322.

Raja, H.A., Miller, A.N., Pearce, C.J., and Oberlies, N.H. (2017). Fungal identification using molecular tools: a primer for the natural products research community. *J. Nat. Prod.* 80(3): 756-770.

Ryu, S., Shin, M., Yun, B., Lee, W., Choi, H., Kang, M., Oh, S., and Kim, Y. (2021). Bacterial quality, prevalence of pathogens, and molecular characterization of biofilm-producing Staphylococcus aureus from Korean dairy farm environments. *Animals* 11(5): 1306.

Salem, S.H., El-Maraghy, S.S., Abdel-Mallek, A.Y., Abdel-Rahman, M.A., Hassanein, E.H., Al-Bedak, O.A., and El-Aziz, F.E.-Z.A.A. (2022). The antimicrobial, antibiofilm, and wound healing properties of ethyl acetate crude extract of an endophytic fungus *Paecilomyces* sp.(AUMC 15510) in earthworm model. *Sci. Rep.* 12(1): 19239.

Semwal, P., Painuli, S., Badoni, H., and Bacheti, R.K. (2018). Screening of phytoconstituents and antibacterial activity of leaves and bark of *Quercus leucotrichophora* A. Camus from Uttarakhand Himalaya. *Sci. Rep.* 4: 1-6.

Shaaban, M.T., Ghaly, M.F., and Fahmi, S.M. (2021). Antibacterial activities of hexadecanoic acid methyl ester and green‐synthesized silver nanoparticles against multidrug‐resistant bacteria. *J. Basic Microbiol.* 61(6): 557-568.

Shigaeva, J., and Darr, D. (2020). On the socio-economic importance of natural and planted walnut (*Juglans regia* L.) forests in the Silk Road countries: A systematic review. *For. Policy Econ.* 118: 102233.

Stucky, B.J. (2012). SeqTrace: a graphical tool for rapidly processing DNA sequencing chromatograms. *J. Biomol. Tech.* 23(3): 90.

Zahara, K., Bibi, Y., Arshad, M., Kaukab, G., Al Ayoubi, S., and Qayyum, A. (2022). In-vitro examination and isolation of antidiarrheal compounds using five bacterial strains from invasive species *Bidens bipinnata* L. *Saudi J. Biol. Sci.* 29(1): 472-479.

JPMB | Journal of Plant Molecular Breeding
Published by Genetics and Agricultural Biotechnology Institute of Tabarestan

2024 | Volume 12 | Issue 1

ترکیبات زیست فعال و ارزیابی میکروبی گردوی آفریقایی (*Tetracarpidium conophorum*) (Mull. Arg.) Hutch & Dalziel در ایلورین

گانیو اولاهان، ابراهیم اجادی* و ولیات سلیمان

گروه زیست شناسی گیاهی، دانشکده علوم زیستی، دانشگاه ایلورین، ایلورین، نیجریه.

مقاله پژوهشی

چکیده: گردوی آفریقایی (*Tetracarpidium conophorum*) یک میوه استوایی مغذی است که ارزش غذایی بالایی داشته و برای سلامت انسان‌ها مفید می‌باشد. در این مطالعه ترکیبات زیست فعال و میکروب‌های مرتبط با گردوی آفریقایی موجود در کلان‌شهر ایلورین بررسی شد. پس از تهیه محلول‌های ذخیره از آجیل‌های نمونه‌گیری شده، جداسازی‌های میکروبی روی محیط PDA (قارچ)، نوترینت آگار و ائوزین متیلن بلو آگار (باکتری) به روش پورپلیت انجام شد. ۱۶ ترکیب فعال زیستی با ارزش های درمانی بسیار قابل توجه در آجیل خام با استفاده از کروماتوگرافی گازی - طیف سنجی جرمی (GC-MS) شناسایی شد. همچنین شناسایی مولکولی گونه‌های قارچی و شناسایی بیوشیمیایی گونه‌های باکتریایی جدا شده از آجیل، حضور چهار گونه قارچی به نام‌های *Aspergillus niger*، *Penicillium rolfsii*، *Rhizopus delemar* و *Rhizopus arrhizus* و همچنین سه گونه باکتری به نام‌های *Bacillus cereus*، *Staphylococcus aureus* و *Escherichia coli* را نشان داد. اگرچه نتایج GC-MS می‌تواند بیانگر برخی مزایای تغذیه ای و دارویی گردوی آفریقایی بوداده شده باشد، ولی مخاطرات آلودگی میکروبی گردوی آفریقایی برشته شده در بازارهای ایلورین را نیز باید مدنظر قرار داد.

کلمات کلیدی: GC-MS، غربالگری میکروبی، شناسایی مولکولی، گردوی آفریقایی، آنتی‌میکروبی.

ویراستار علمی

دکتر همت الله پیردشتی،
گروه زراعت، پژوهشکده ژنتیک و زیست‌فناوری کشاورزی طبرستان، دانشگاه علوم کشاورزی و منابع طبیعی ساری

تاریخ

دریافت: ۱۶ شهریور ۱۴۰۳
پذیرش: ۸ آبان ۱۴۰۳
چاپ: ۲۳ آبان ۱۴۰۳

نویسنده مسئول

دکتر ابراهیم اجادی

ajadiibrahim669@gmail.com

ارجاع به این مقاله

Olahan ,G., Ajadi , I., and Sulaimon , W. (2024). Bioactive compounds and microbial evaluation of African walnuts (*Tetracarpidium conophorum* (Mull. Arg.) Hutch & Dalziel) retailed in Ilorin metropolis. *J Plant Mol Breed. 12* (1): 49-59. doi: 10.22058/jpmb.2024.2040416.1305.