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This issue contains 9 articles. This issue spans the full breadth of our journal's scope, bridging clinical medicine, biomedical sciences, pharmacy, and public health with a strong emphasis on localized challenges and solutions.

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Assessment of Infection Control Practices and Biosafety Compliance Among Medical Laboratory Professionals: A Comparative Study of Public and Private Sectors

Fathi Abdallah Shakurfow [a&*]

[a]Faculty of Medical Technolog, Elmergib Uinversity, Al-Khums, Libya
* Corresponding author Email: fathishakurfow@elmergib.edu.ly

A B S T R A C T

Background: Medical laboratories function as highly critical environments where personnel are routinely exposed to severe biological hazards. Consequently, strict adherence to biosafety protocols is a fundamental requirement to mitigate the risk of laboratory-acquired infections.

Aim: To thoroughly dissect the occupational safety climate within medical laboratories.

Methods: Employing a cross-sectional descriptive-analytical design, the study surveyed a purposive sample of 100 laboratory professionals operating in the two municipalities of Msallata and Al-Khums, Libya. Data were gathered using a validated, 24-item structured questionnaire assessing hygiene practices, personal protective equipment (PPE) usage, waste management, and administrative support.

Results: The findings indicate robust compliance with foundational hygiene protocols, specifically hand hygiene (76%) and standard glove usage (88%). Conversely, a critical vulnerability was identified regarding comprehensive protective measures, with only 29% of personnel consistently utilizing advanced personal protective equipment, such as respiratory masks and ocular protection. Systemic deficiencies in continuous education were prominent, as 57% of respondents reported a lack of periodic training updates. Consequently, only 35% of the workforce perceived their working environment as fully secure.

Conclusion: While baseline hygiene practices are commendably maintained, the transition to a comprehensive safety culture is impeded by selective PPE compliance and inconsistent administrative support. Bridging this gap requires the institutionalization of mandatory, continuous biosafety training and the standardization of protective resource allocation across both public and private sectors.

Keywords: *Infection, Biosafety, Laboratories, Occupational, Training*.

1. Introduction

Background

The fundamental architecture of modern healthcare relies heavily on the diagnostic and analytical output of medical laboratories [1]. These environments are at the forefront of epidemiological surveillance, clinical diagnosis, and therapeutic monitoring [2]. However, the intrinsic nature of laboratory work demands the daily handling of virulent biological specimens, thereby creating an occupational landscape fraught with severe biological hazards [3]. Personnel working in these settings face continuous exposure to bloodborne pathogens, aerosolized infectious agents, and various microbiological threats [4].

Effective infection control and biosafety compliance in medical laboratories are critical to mitigating occupational hazards and preventing the transmission of pathogenic agents within healthcare settings. Previous local studies emphasize that awareness levels regarding infection control directly influence safety behaviors and the mitigation of microbial contamination risks in medical environments. Building upon this evidence, it is essential to systematically evaluate how biosafety compliance is maintained among laboratory professionals across different healthcare environments. This study aims to bridge the gap between standard safety protocols and daily laboratory practices by comparing

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compliance levels between public and private sectors, thereby identifying institutional factors that influence occupational safety performance [5].

The World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) systematically classify clinical laboratories as high-risk environments [6]. Historically, the transmission of pathogens within these confines has occurred via percutaneous inoculations (such as needle stick injuries), direct mucocutaneous contact with contaminated surfaces, and the inhalation of bio-aerosols generated during routine procedures like centrifugation or pipetting [7]. To combat these vectors of transmission, regulatory bodies such as the Occupational Safety and Health Administration (OSHA) have developed stringent Bloodborne Pathogens Standards, emphasizing that rigorous adherence to physical and procedural barriers can nearly eliminate the incidence of laboratory-acquired infections (LAIs) [8].

Despite the existence of universally accepted guidelines, translating these theoretical frameworks into daily operational compliance remains a persistent challenge in many healthcare systems, particularly in developing regions [9]. Ineffective sterilization techniques and unintentional exposure through microscopic skin abrasions remain primary causes of occupational illness [10]. The implementation of robust infection control relies on a multi-tiered approach: meticulous hand hygiene, regular environmental sanitation, the consistent application of appropriate chemical disinfectants, and the unfailing use of Personal Protective Equipment (PPE) [11].

Furthermore, the lifecycle of a biological specimen does not end at analysis; safe disposal of biological waste via incineration or high-pressure steam sterilization is an equally critical component of the biosafety chain [12]. Yet, physical resources alone are insufficient. Human behaviour is the ultimate determinant of safety [13]. Evidence heavily suggests that the frequency and quality of professional training are directly proportional to the risk awareness and compliance levels of the staff [14].

In the context of the Libyan healthcare system, specifically within the two municipalities of Msallata and Al-Khums, preliminary field observations have suggested notable variances in the implementation of these global standards [15]. These disparities are often influenced by the institutional setting—specifically, the operational and financial differences between public (government-funded) and private laboratories [16]. The core problem this research addresses is identifying the exact extent to which international protocols are effectively applied locally, isolating the socio-environmental factors influencing human adherence, and comparing the operational efficacy of safety climates across the public and private sectors [17].

Foundational Principles of Laboratory Biosafety

The discipline of infection control within biological laboratories is an extensive matrix of engineering controls, administrative policies, and individual work practices [1]. Its primary objective is the containment of biohazards to prevent cross-contamination of specimens and, more critically, to protect personnel from exposure to infectious agents [3]. Pathogenic transmission in these settings is insidious, often occurring through micro-droplets or fomites that are invisible to the naked eye [7].

Standard precautions, as delineated by the WHO and CDC, assert that all biological samples must be treated as potentially highly infectious [2]. This principle mandates a foundational suite of practices. Foremost is sterilization, which requires that all reusable instruments undergo rigorous chemical or thermal eradication of microbial life [4]. Alongside sterilization is the critical requirement for Personal Protective Equipment (PPE), serving as the primary barrier between the host and the pathogen [8]. Finally, the handling of post-analytical materials requires strict biological waste management protocols to ensure environmental safety beyond the laboratory walls [5].

The Impact of Continuous Professional Education

The academic consensus strongly indicates that the provision of physical safety equipment is futile without the behavioural modification achieved through continuous training [13]. Robinson et al. provided a comprehensive comparative analysis of personnel subjected to intensive, recurring safety education versus those receiving only onboarding orientation [14]. Their data unequivocally demonstrated that recurring training cycles result in significantly higher compliance rates and lower incidences of accidental exposure [14].

Corresponding author Email: fathishakurfow@elmergib.edu.ly

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Furthermore, as the nature of biological threats evolves—evidenced by recent global pandemics—safety protocols must adapt [11]. Regular updates and seminars not only reinforce routine implementation but drastically enhance emergency response capabilities [14]. Educational strategies are thus not an administrative luxury but a core operational requirement necessary to maintain high biosafety standards [18]. Jones et al. (2020) further corroborated this, emphasizing that biosafety training positively influences behavioral improvements across all hierarchies of laboratory staff, mitigating human error [19].

Sectoral Disparities: Public vs. Private Environments

Institutional frameworks heavily influence the degree to which safety protocols are realized in practice [16]. A critical study by Robinson et al. examined 200 medical laboratories, focusing on PPE compliance and resource availability across different healthcare sectors [16]. The findings highlighted that private laboratories consistently exhibit higher compliance metrics [16]. This is generally attributed to more agile management structures, better funding allocation for continuous equipment procurement, and stricter internal auditing [16].

Conversely, public sector laboratories frequently grapple with bureaucratic delays, resource scarcity, and insufficient funding for continuous educational programs [16]. This structural disparity results in observable deficits in PPE adherence and protocol execution [15]. Researchers argue that to standardize occupational health, there is an urgent imperative to restructure resource allocation and institutionalize training within public healthcare facilities [17].

Variability in the Implementation of Preventive Measures

While knowledge of basic hygiene is generally widespread, practical execution remains paradoxically inconsistent. Martinez et al.explored the variability in compliance regarding fundamental practices such as hand sanitization and surface disinfection [11]. While hand hygiene is universally acknowledged as the cornerstone of infection prevention, their research indicated that the utilization of secondary and tertiary barriers—such as ocular protection and particulate respirators—is frequently neglected due to discomfort or perceived inconvenience [11].

Moreover, it was identified that shortages of high-grade chemical disinfectants and a general failure [4, 17]. To counter this, literature strongly advocates for a multi-dimensional approach integrating systemic education, reliable resource supply chains, and active supervisory monitoring to foster a resilient culture of safety [9, 18].

Research Questions and Hypotheses

The investigation is driven by the fundamental question of whether the current educational and administrative frameworks are sufficient to cultivate a proactive safety culture. To operationalize this inquiry, the research tests two primary hypotheses:

Hypothesis I: There is a noticeable positive relationship between the frequency of a laboratory worker's participation in updated infection control training and their adherence to comprehensive preventive measures.

Hypothesis II: There are observable differences in the perceived effectiveness and execution of infection control protocols based on the organizational framework of the workplace, specifically between public and private sectors.

By testing these hypotheses, the study aims to provide local health authorities with the empirical backing necessary to overhaul and standardize training programs, thereby complying with international benchmarks.

Aims of the Study

To thoroughly dissect the occupational safety climate, this study pursues the following specific objectives:

To quantitatively assess the baseline awareness and practical commitment to infection control measures among active laboratory personnel.

To identify operational gaps and inconsistencies in routine procedures, particularly concerning hand hygiene, surface sterilization, and comprehensive PPE utilization.

To synthesize the empirical data into actionable, evidence-based recommendations to assist healthcare administrators in fortifying institutional safety protocols.

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2. Methodology

Study Design and Methodology

To comprehensively address the research objectives, a cross-sectional, descriptive-analytical research design was adopted. This methodological approach is highly effective in the occupational health sciences for capturing the prevalence of specific behaviours and attitudes within a defined professional cohort at a specific point in time. It allows for the precise quantification of compliance rates and facilitates the comparison of variables across different demographic and institutional subsets.

Study Setting and Target Population

The geographical scope of the investigation encompassed the two municipalities Msallata and Al-Khums in Libya. Data collection was systematically executed over a four-week period, from November 1 to December 1, 2024. The target population included clinical laboratory specialists, medical technologists, phlebotomists, and laboratory supervisors. This demographic was intentionally selected as they constitute the frontline workforce, experiencing the highest daily frequency of direct exposure to potentially infectious clinical specimens.

Sampling Strategy and Participant Profile

A purposive, randomized sampling technique was employed to recruit 100 participants. To ensure the findings accurately reflected the broader occupational landscape, the sample was stratified to include representatives from purely government-run facilities, purely private institutions, and individuals operating in mixed capacities (working in both sectors). The inclusion criteria required participants to have minimum duration of one year of active laboratory experience minimum duration of active laboratory experience to ensure they had sufficient exposure to the facility's operational culture.

Research Instrumentation

The primary instrument for data acquisition was a structured, electronic 24-item questionnaire. Recognizing the importance of instrument reliability, the questionnaire was subjected to peer review by a a panel of infection control experts and senior epidemiologistsat Elmergib Universty panel of infection control experts and senior epidemiologists to establish content and face validity. The survey was divided into distinct thematic axes: Demographic and professional variables (age, gender, experience, sector). Adherence to personal hygiene and PPE protocols. Execution of environmental sterilization and bio-waste management. Accessibility to institutional training and perception of administrative support. Subjective evaluation of the workplace safety climate. Responses were captured using a combination of binary responses and standard Liker scales (e.g., Always, Sometimes, Rarely, Never) to gauge the frequency of behaviors.

Data Collection, Ethics, and Statistical Analysis

The distribution of the electronic surveys occurred directly at the respective clinical sites to maximize the response rate. Strict ethical protocols were adhered to in accordance with standard epidemiological research guidelines. Informed consent was obtained from all respondents, with explicit assurances regarding the anonymity and confidentiality of their data, emphasizing that the findings would be utilized exclusively for academic and safety improvement purposes.

Data processing and statistical evaluation were conducted utilizing descriptive statistical methods. The raw data were transformed into frequencies and valid percentages, subsequently modelled into graphical representations (bar charts and pie charts) to visually map the distribution of compliance metrics and identify statistically notable trends.

3. Results

The results of the questioners were represented graphically in 24 charts; where the empirical data extracted from the 100 respondents yield a highly detailed operational profile of current biosafety practices. The findings are categorized chronologically from demographic distributions to specific technical competencies.

Demographic and Professional Profile

The demographic and the professional profile of the participants were presented in figures (1- 4) four figures (1, 2, 3 and 4) as follow; where the socio-demographic variables of the cohort indicate a highly balanced and relatively young workforce. Analysis of gender distribution (As

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exposed in Figure 1) reveals near parity, with females comprising 51% of the sample and males 49%. The age distribution (Figure 2) illustrates a workforce dominated by early-career professionals; the vast majority (59%) falls within the 20–30 year age bracket, whereas senior personnel over the age of 50 represent a marginal 3% of the cohort. This demographic reality is mirrored in the professional experience data (As revealed in Figure 3), which shows that 60% of the respondents possess 10 years of experience or less. Regarding the structural distribution of the workforce across the healthcare sectors (As shown in Figure 4), 40% of the personnel operate exclusively within private laboratories; another 40% split their professional time between public and private sectors, while a smaller subset of 20% is employed solely within public government facilities.

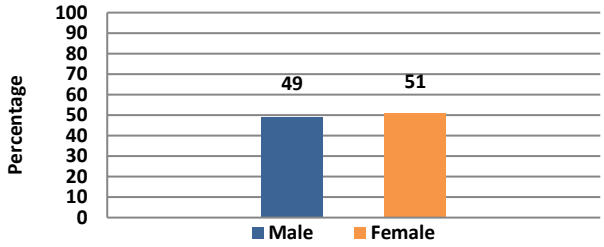


Figure 1.Distribution of sample according to gender

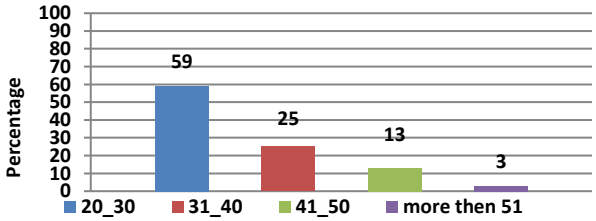


Figure 2. Distribution of sample according to age

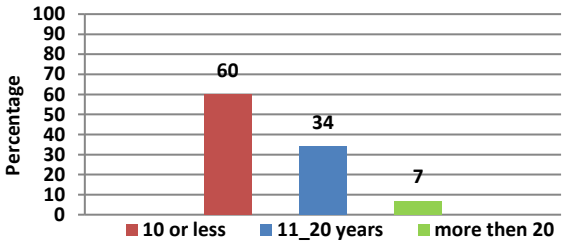


Figure 3. Distribution of sample according to years of work

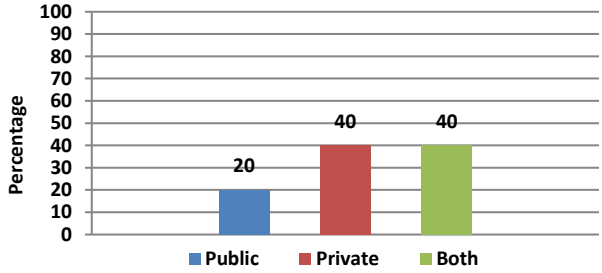


Figure 4. Distribution of sample according to workplaces

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Adherence to Personal Protective Practices and Hygiene

The adherence to personal protective practices and the hygiene were showed in in five figures (5- 9) five figures (5, 6, 7, 8 and 9) as follow; where the data reflects an encouragingly high baseline of individual discipline regarding fundamental hygiene. As illustrated in Figure 5, a significant majority of the workforce (76%) report that they "always" execute standard hand washing and sanitization protocols both prior to and immediately following the handling of clinical samples. Similarly, compliance regarding the primary physical barrier—glove usage—is remarkably high. Figure 6 demonstrates that 88% of personnel consistently wear gloves during laboratory operations. Furthermore, an overwhelming 98% of respondents exhibit an acute awareness of barrier integrity, stating they immediately replace their gloves upon detecting any tears or punctures (As exposed in Figure 7).

However, the empirical data reveals a sharp decline in compliance when progressing from basic to comprehensive Personal Protective Equipment. Figure 8 exposes a critical vulnerability: merely 29% of the cohorts “always” don the complete mandated protective ensemble (which includes laboratory coats, respiratory masks, and protective eyewear). The majority of participants The majority (64%) admit to utilizing the full kit only "sometimes." Despite this selective compliance with advanced PPE, the response to acute contamination remains highly professional; 100% of the prticaipants 100% of the staff reported that they immediately change their laboratory coat if it becomes visibly contaminated with biological fluids (As shown in Figure 9).

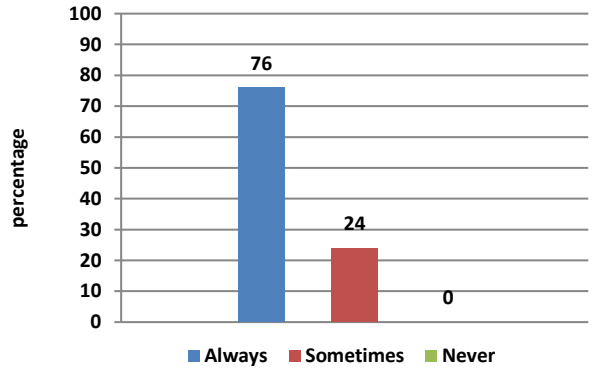


Figure 5. Distribution of participants based on their commitment to hand washing and using sanitizers before and after handling laboratory samples.

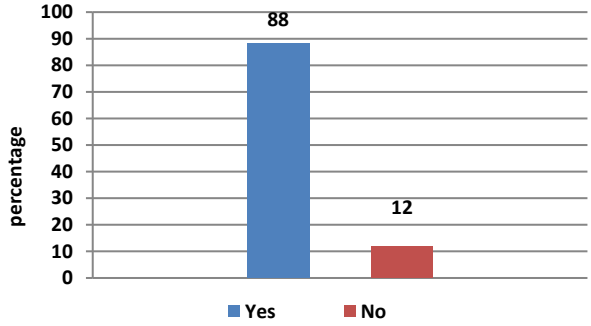


Figure 6. Distribution of responses regarding gloves usage during work.

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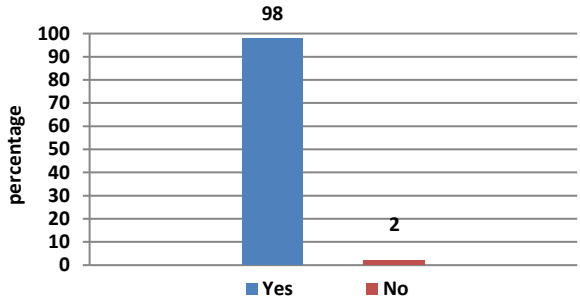


Figure 7. Distribution of responses regarding changing torn gloves

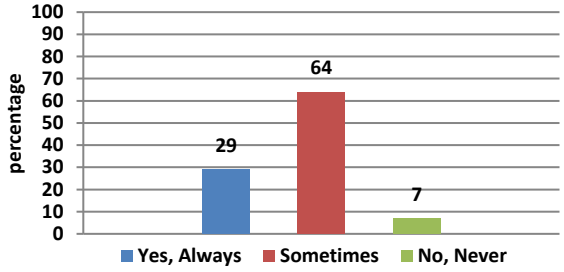


Figure 8. Frequency of wearing protective cloths among participants.

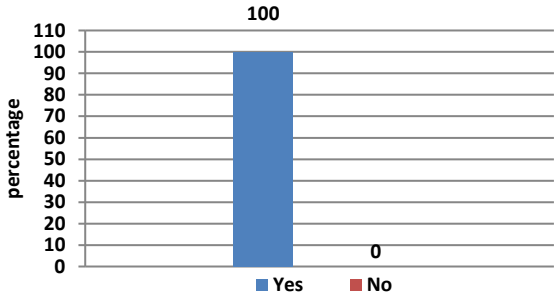


Figure 9. Distribution of participants regarding changing lab coats when visibly contaminated.Environmental Disinfection and Biological Waste Management

Figures 10-14 illustrated the environmental disinfection and the biological waste management of the participants in this study as follow; maintaining the sterility of the analytical environment is clearly prioritized by the workforce. The survey data (As presented in Figure 10) confirms that 96% of participants routinely clean and chemically disinfect their work surfaces before commencing and after concluding their shifts.

The management of hazardous outputs also shows strong compliance metrics. About 85% of respondents assert that biological and infectious waste is "always" disposed of through safe, standardized protocols, See Figure 11. According to Figure 11, 85% of respondents assert that biological and infectious waste is "always" disposed of through safe, standardized protocols. Accessibility to basic sanitation infrastructure is nearly universal, with 99% of workers confirming that hand-washing sinks and chemical sanitizer stations are easily accessible within their immediate work areas (As appeared in Figure 12). Despite these positive indicators, there is notable inconsistency regarding the maintenance of analytical machinery. While 62% of the staff confirm that laboratory equipment is sterilized on a strict, scheduled basis, a concerning 38%

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noted that such rigorous equipment maintenance only occurs "sometimes" (As shown in Figure 13), pointing toward potential systemic lapses in operational oversight.

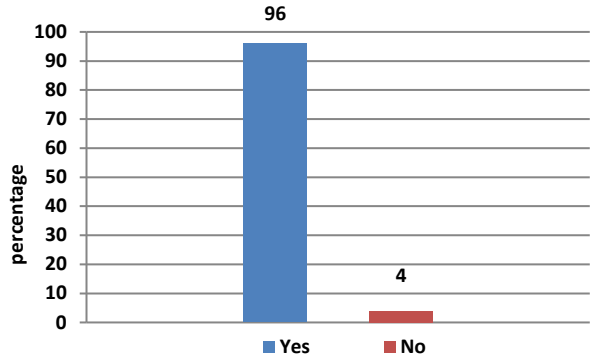


Figure 10. Distribution of participants based on their cleaning and disinfecting of surfaces before and after laboratory work.

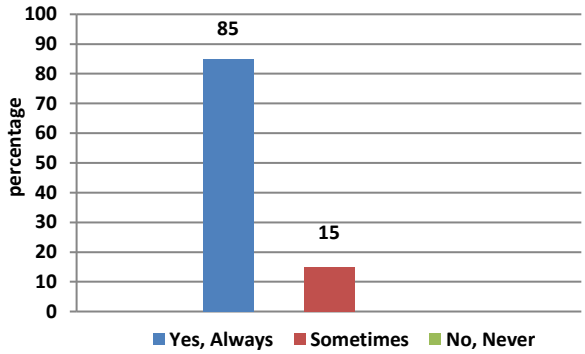


Figure 11. Distribution of participants according to the proper and safe disposal of biological waste.

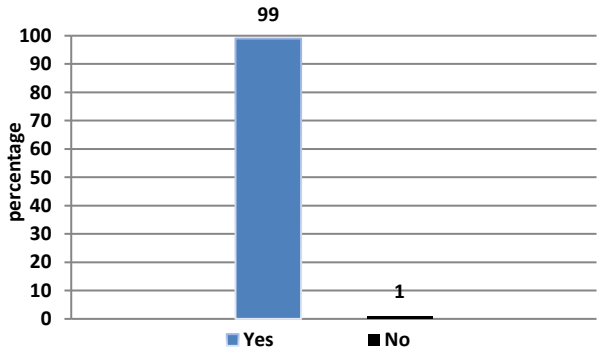


Figure 12. Distribution of participants based on the availability of hand washing and disinfectant stations in accessible locations.

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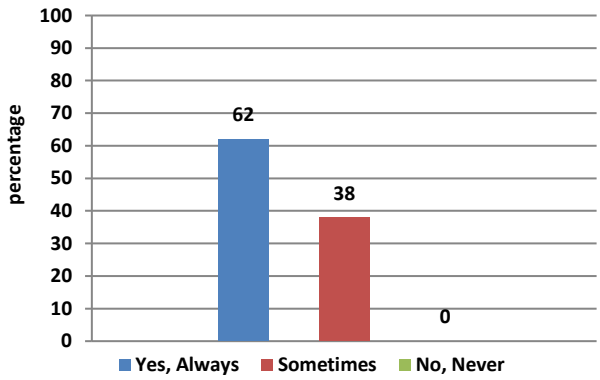


Figure 13. Distribution of participants according to regular cleaning and sterilization of equipment and tools based on scheduled times.

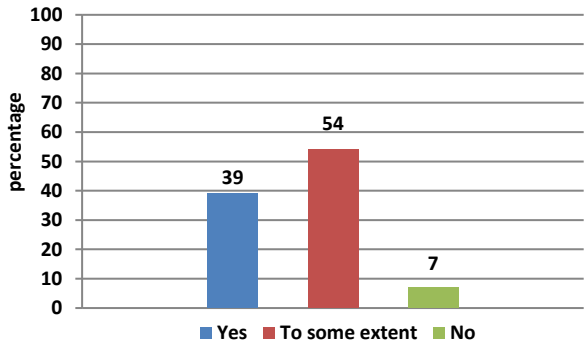


Figure 14. Distribution of participants' perception of laboratory safety in terms of infection control.

Perception of the Safety Climate and Management Support

The perception of the safety climate and the management Support are shown in in eleven figures 14-24 11 figures 14- as follow; A central component of occupational health is the psychological safety and confidence of the staff. The findings here reveal a moderate-to-low level of institutional confidence. As depicted in Figure 14, only 35% of the laboratory professionals perceive their working environment as completely "safe," with the majority (54%) classifying it merely as "somewhat safe." This subjective assessment aligns precisely with overall satisfaction metrics (As presented in Figure 23), where 70% of respondents expressed being only "somewhat" satisfied with the prevailing infection control standards.

When evaluating administrative frameworks, the existence of protocols is clear, but trust in management execution is fragmented. An encouraging 91% of workers acknowledge the existence of clear, documented procedures for managing accidental exposure to infectious agents (As shown in Figure 17). Yet, a disparity arises in post-incident expectations; only 71% of personnel anticipate receiving full institutional and management support following a biological accident (As appeared in Figure 18). Communication pathways also exhibit slight fractures: while 52% feel they can easily communicate safety concerns to their superiors, 13% report a complete absence of effective channels for safety feedback (As exposed in Figure 22).

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Educational Deficits and Efficacy of Protocols

The most profound systemic weakness identified in the study pertains to the continuity of professional education. The data establishes a clear "training paradox." While a majority (66%) felt that the initial safety training provided upon their employment was adequate (As appeared in Figure 15), the system fails in continuous professional development. Figure 21 highlights a major operational deficit: 57% of laboratory workers do not receive any regular, periodic training updates regarding evolving biological risks or new safety techniques. This educational stagnation directly impacts the staff's self-assessed competency. Only 51% are fully confident that they possess sufficient knowledge of modern infection risks, whereas 47% concede they comprehend these risks only "to a certain extent" (As shown in Figure 16).

Consequently, the workforce views the current institutional measures with a degree of skepticism. Only 46% evaluate their laboratory's current infection control procedures as "very effective" (As exposed in Figure 19). There is a resounding consensus on the necessity for systemic evolution; 40% perceive a "great need" to overhaul and improve current safety protocols, and another 47% see a need for improvement "to a certain extent" (As appeared in Figure 20). Notably, an overwhelming 94% of the cohort strongly agree that any financial expenditures directed toward upgrading infection control and biosafety are completely justified investments (As shown in Figure 24).

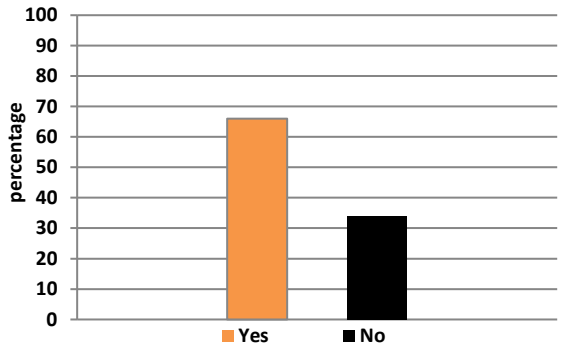


Figure 15. Distribution of participants regarding whether they received adequate training on infection control procedures.

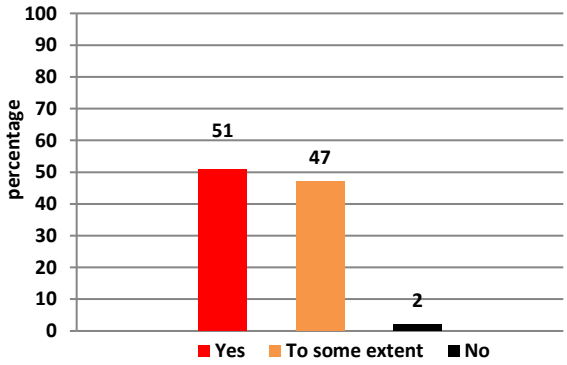


Figure 16. Distribution of participants based on their knowledge of infection risks in the laboratory.

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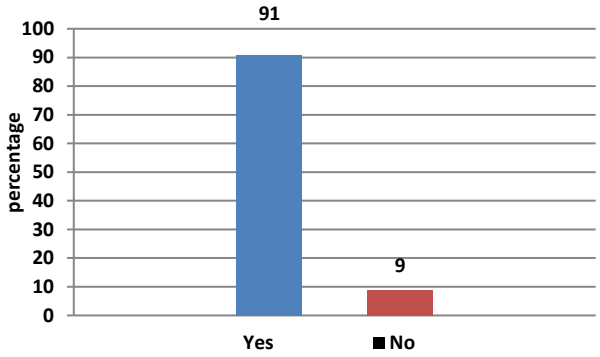


Figure 17. Distribution of participants according to the clarity of procedures in case of exposure to infectious samples.

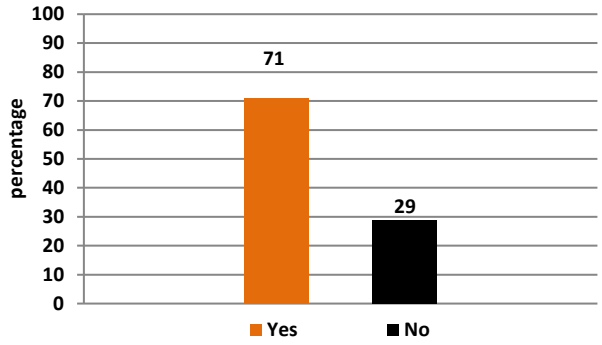


Figure 18. Distribution of participants regarding expectations of receiving management support in the event of an incident.

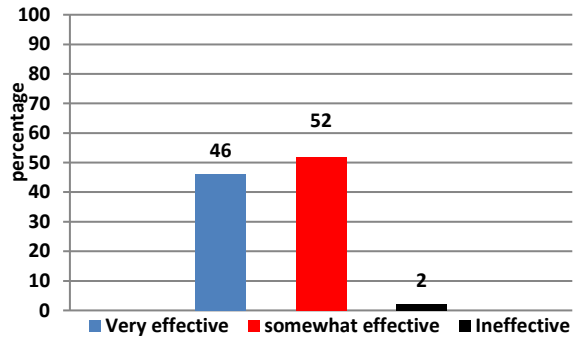


Figure 19. Distribution of participants' evaluation of the effectiveness of infection control procedures in the laboratory.

Corresponding author Email: fathishakurfow@elmergib.edu.ly

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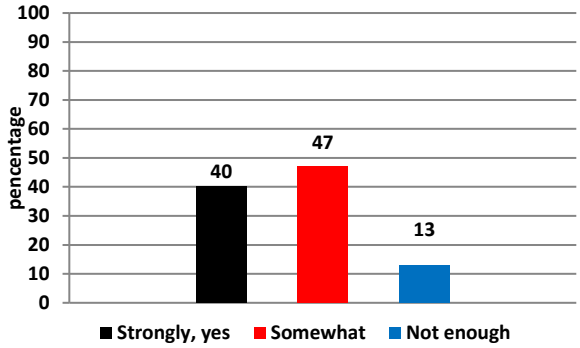


Figure 20. Distribution of participants' opinions on the need to improve infection control procedures.

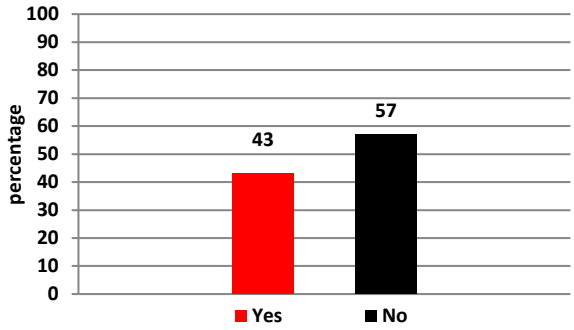


Figure 21. Distribution of participants according to the availability of regular training on infection control procedures.

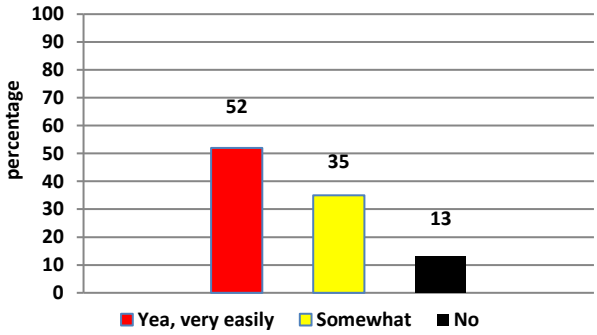


Figure 22. Distribution of participants based on their ability to reach management to provide feedback on safety improvements.

Corresponding author Email: fathishakurfow@elmergib.edu.ly

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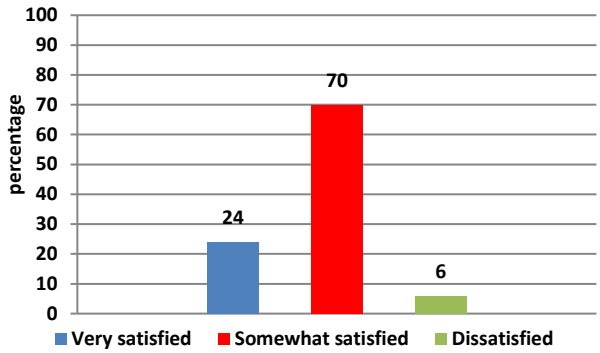


Figure 23. Distribution of participants regarding their satisfaction with the safety level in terms of infection control.

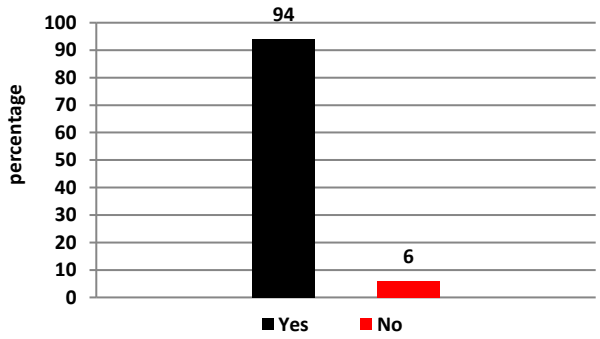


Figure 24. Distribution of participants' opinions on whether the expenses incurred for infection control are justifieDiscussion

The analytical synthesis of the empirical data generated from medical laboratories in the two municipalities Msallata and Al-Khums provides a nuanced understanding of the occupational safety climate. By cross-referencing these findings with the broader epidemiological literature, this section interprets the core strengths, the paradoxes of compliance, and the systemic vulnerabilities inherent in the current healthcare framework.

4. Discussion

The Paradox of Compliance: Foundational vs. Comprehensive Protection

The data demonstrates highly commendable levels of individual adherence to basic hygiene. The fact that 76% of personnel strictly adhere to hand sanitization and 88% consistently utilize gloves aligns seamlessly with the epidemiological consensus established by Martinez et al. which identifies hand hygiene as the most statistically significant variable in interrupting pathogen transmission [17]. The 98% immediate replacement rate for damaged gloves further indicates a workforce that is acutely aware of direct tactile biological threats.

However, a critical "compliance paradox" emerges when evaluating comprehensive protective behavior. The severe drop in compliance, where only 29% consistently utilize full PPE, including ocular and respiratory protection exposes a profound vulnerability. This specific gap corroborates the concerns articulated by Johnson and Patel who argued that the hyper-focus on manual hygiene often breeds a false sense of security, leading to the dangerous neglect of bio-aerosol risks [7]. This selective compliance suggests that workers psychologically prioritize immediate, visible risks (blood on hands) over invisible, airborne hazards (aerosolized viral particles). Overcoming this requires transitioning the institutional culture from viewing comprehensive PPE as an "optional discomfort" to an absolute, non-negotiable operational standard.

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The Systemic Failure of Continuous Education

Perhaps the most consequential finding of this research is the pronounced deficit in on-going professional education. While the majority of staff enters the workforce with adequate initial orientation (66%), the institutional failure to provide continuous training (reported by 57% of workers) creates a stagnant safety culture.

This educational void directly validates the study's first hypothesis regarding the relationship between training and compliance. As previously highlighted by Martinez et al and Jones et al initial training decays over time; without periodic reinforcement, behavioural drift occurs, leading to the procedural shortcuts observed in the PPE data [18, 19]. Because medical science and biological threats evolve rapidly, the lack of updated training leaves almost half the workforce (47%) feeling only partially knowledgeable about the risks they face daily. This lack of confidence directly undermines the overall safety climate of the laboratory.

Workplace Disparities and the Perception of Safety

The structural dynamics of the workforce, evenly split between private and mixed-sector employment, highlight subtle but impactful systemic disparities. Basic infrastructure, such as access to sanitation stations (99%) and surface disinfectants (96%), appears resilient across the board. This supports the observation by Al-Mogbel that modern laboratories are generally successful in procuring basic physical supplies [11].

Yet, physical supplies do not equate to psychological security. The finding that a mere 35% of professionals feel unequivocally "safe" in their environment is a severe indictment of the broader safety management system. This aligns with the findings of Robinson et al. which indicate that public and mixed-sector laboratories often lack the rigorous, standardized auditing processes present in strictly private institutions [16]. When 38% of staff report that equipment sterilization is only conducted "sometimes," it indicates a failure in administrative oversight rather than a lack of physical resources.

Administrative Support and Safety Culture

A robust safety culture relies not just on protocols, but on trust between the scientific staff and hospital administration. The data reveals a concerning fracture in this relationship. With 29% of the workforce doubting they would receive full management backing in the event of an accidental exposure, there is a high risk of underreporting. When incidents are not reported due to fear of punitive action or administrative apathy, the institution loses vital epidemiological data necessary to prevent future accidents. As Robinson et al. (2023) emphasized, an environment where employees cannot easily provide safety feedback (as noted by 13% of the cohort) is an environment fundamentally unequipped to improve [10].

4. Conclusions and Recommendations

Conclusions

This comprehensive analytical study has successfully mapped the biosafety landscape among clinical laboratory professionals in the Msallata and Al-Khums municipalities. The empirical evidence paints a dual picture: a workforce that exhibits strong individual competence and discipline regarding baseline hygiene (specifically hand washing and standard glove usage), yet operates within an institutional framework that lacks rigorous continuous support.

The core vulnerabilities identified do not stem from an absence of initial knowledge or physical resources, but from a systemic breakdown in continuous professional education and comprehensive PPE enforcement. With over half the workforce lacking periodic training updates, a dangerous environment of selective compliance has emerged, particularly regarding respiratory and ocular protection. Consequently, the overarching perception of workplace safety remains moderately low. Ultimately, while the foundational architecture for infection control exists in these laboratories, it requires urgent, standardized administrative intervention to evolve into a proactive, resilient, and fully effective culture of occupational safety.

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Recommendations

Derived directly from the data analysis and aligned with global best practices, the following strategic recommendations are proposed to healthcare policymakers and laboratory directors:

1. **Institutionalization of Continuous Education:** Healthcare administration must transition from a model of one-time orientation to mandatory, continuous professional development. Implementing quarterly or bi-annual interactive biosafety workshops is crucial to update staff on evolving pathogens and to correct procedural drift.
2. **Strict Enforcement of Comprehensive PPE Protocols:** Laboratory management must enforce a zero-tolerance policy regarding the neglect of advanced PPE. The consistent use of laboratory coats, protective eyewear, and particulate masks must be mandated, particularly during processes known to generate bio-aerosols, aligning local practices with rigid OSHA standards.
3. **Cultivation of a Non-Punitive Reporting Culture:** To bridge the trust gap between staff and management, laboratories should establish transparent, non-punitive incident reporting systems. Ensuring that workers feel unconditionally supported post-exposure will drastically improve incident tracking and subsequent risk mitigation.
4. **Standardization of Auditing Across Sectors:** Health ministries must implement standardized, unannounced biosafety audits that apply equally to public and private sector laboratories. This will address the operational disparities and ensure that equipment calibration and deep sterilization are conducted on strict schedules rather than "sometimes."
5. **Appointment of Dedicated Biosafety Officers:** Each diagnostic facility must appoint a certified Biosafety Officer whose sole responsibility is to monitor procedural compliance, manage bio-waste supply chains, and act as the direct communicative liaison between frontline staff and hospital administration.
6. **Implementation of Visual and Cognitive Reminders:** Strategic placement of high-visibility, standardized biological hazard protocols and step-by-step emergency response infographics at every analytical workstation will serve to minimize cognitive load and reduce human error during high-stress operational hours.

ETHICAL STATEMNET

Not appicable.

CONFLICT OF INTEREST

The author declares that they have no competing interests.

AUTHORS' CONTRIBUTIONS

The author, FS is solely responsible for the conception, design, experimental work, data analysis, and the writing this manuscript.

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Phenological Variation in Essential-Oil Yield and In Vitro Antibacterial Activity of *Thymus algeriensis* and *Thymus capitatus* from Tarhuna, Libya

Abdulaziz Suwaydan[a,b,&*], Ismail Ajaj[c], Mohamed A. S. Fahej[d],

[a] Department of Biology, Faculty of Science, Misurata University, Misurata, Libya

[b] Department of Pharmacognosy, Faculty of Pharmacy, Elmergib University, Al-Khums, Libya

[c] Department of Chemistry, Faculty of Education, Alasmarya University, Zliten, Libya

[d] Libyan Academy for Postgraduate Studies, Al-Khums Branch, Al-Khums, Libya

*Corresponding author Email: asswedan@elmergib.edu.ly

A B S T R A C T

Background: Phenological development can strongly influence essential-oil (EO) production in aromatic plants, although comparative evidence for Libyan Thymus species remains limited.

Aims: To quantify phenological variation in EO yield of *Thymus algeriensis* and *Thymus capitatus* from Tarhuna, Libya, and to describe the in vitro antibacterial activity of the corresponding pooled oils.

Methods: Dried aerial parts collected at vegetative, flowering and post-flowering stages were hydrodistilled for 3 h using a Clevenger apparatus. Three independent hydrodistillations were performed per species × stage combination. Yield was analysed by two-way factorial ANOVA. The three oils from each combination were then pooled and screened by disc diffusion against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 using three technical disc measurements.

Results: Yield was affected by species, stage and their interaction (all $p < 0.0001$). Flowering produced the highest yield in both species: 1.85 ± 0.05% in *T. algeriensis* and 3.22 ± 0.10% in *T. capitatus*. The species × stage interaction was very large (partial $\eta^2 = 0.942$), reflecting a modest flowering increase in *T. algeriensis* (+13.3% over vegetative) versus a much larger increase in *T. capitatus* (+62.2%). Mean inhibition zones of pooled oils ranged from 22.67 to 25.33 mm; the largest observed zone was obtained against *E. coli* with post-flowering *T. capitatus* oil.

Conclusion: Phenological timing has species-specific consequences for EO recovery, with flowering providing the highest yield under the investigated conditions. Antibacterial patterns did not parallel the yield trajectory. Because oils were pooled before antibacterial testing, activity differences are descriptive and require confirmation using independently tested extraction replicates and quantitative dilution assays.

Keywords: antibacterial activity; essential oil; phenology; Thymus algeriensis; Thymus capitatus

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1. Introduction

Medicinal and aromatic plants of the Lamiaceae are important sources of essential oils (EOs) used in traditional medicine, food preservation, cosmetics, and fragrance-related applications. Essential-oil production is not a fixed taxonomic characteristic; both oil yield and composition are influenced by genotype, developmental stage, environmental conditions, harvest timing, post-harvest handling, and extraction procedure [1,2]. Phenological stage is therefore an important determinant of EO productivity because developmental changes in vegetative growth, reproductive allocation, glandular structures, and secondary-metabolite biosynthesis can modify both the amount and chemical profile of volatile constituents. Although maximum EO accumulation is frequently reported near flowering in aromatic Lamiaceae, the magnitude and even direction of the response may vary among species and populations [1,2].

T. algeriensis Boiss. & Reut. is a North African thyme characterized by substantial geographic and chemical variability. Population-level differences in EO yield and composition have been documented, indicating that geographic origin and local environmental conditions contribute importantly to its volatile phenotype [3]. Sampling period may further modify extraction yield, chemical composition, and associated biological activity [4,5]. Consequently, phenological responses observed in one population cannot necessarily be extrapolated to another, particularly in environmentally heterogeneous Mediterranean habitats.

T. capitatus is similarly characterized by pronounced temporal and geographic variation. Studies of Mediterranean populations have reported increases in EO accumulation toward flowering followed by decreases during later developmental stages, together with substantial changes in volatile composition [6,7]. Geographic comparisons likewise demonstrate marked differences among populations in both EO yield and constituent profiles [8,9]. Collectively, these findings indicate that harvest stage and population origin are important determinants of the quantitative and qualitative EO phenotype of *Thymus* species.

Recent Libyan investigations provide particularly relevant evidence of this heterogeneity. Essential oil of *T. algeriensis* collected from Gaser Alhaj was characterized by GC-MS as a strongly carvacrol-dominant profile, with carvacrol accounting for 77.01% of the identified oil and with γ-terpinene, thymol, and cymene derivatives among the accompanying constituents; the oil also showed antibacterial activity against *E. coli* [10]. Similarly, *T. capitatus* collected from Mesellata exhibited a carvacrol-rich profile dominated by carvacrol (56.27%), followed by p-cymene (9.51%), γ-terpinene (8.39%), and thymol (6.54%) [11]. The Mesellata study also placed this profile within the wider chemical variability reported among Libyan populations and discussed harvest stage and local ecological conditions as possible contributors to the observed differences [11]. These national data reinforce the need to interpret Libyan *Thymus* populations within their specific geographic and developmental contexts rather than assuming a uniform chemical phenotype.

The broader Libyan EO literature likewise demonstrates considerable phytochemical and biological diversity. *Artemisia herba-alba* from Zintan produced a camphor-thujone-dominant EO with antibacterial activity against methicillin-resistant *Staphylococcus aureus* [12], whereas *A. judaica* collected from Al-Awaynat inhibited both *S. aureus* and *E. coli*, with different responses between direct-contact disc diffusion and vapour-phase testing [13]. In the Green Mountain region, *Satureja thymbra* EO was reported to contain thymol as its predominant identified constituent and exhibited antibacterial activity against *S. aureus* and *E. coli* [14]. More recently, *Eucalyptus camaldulensis* from northwestern Libya showed a chemically distinct 1,8-cineole/α-pinene-dominant profile together with strain-dependent antibacterial activity, with the greatest inhibition observed against *S. aureus* [15]. Taken together, these studies demonstrate an expanding national evidence base for chemically diverse, biologically active Libyan EOs, while also illustrating that antibacterial responses depend on botanical source, chemical profile, bacterial strain, and experimental methodology.

Corresponding author, email: asswedan@elmergib.edu.ly

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Against this national background, both *T. algeriensis* and *T. capitatus* have documented antimicrobial potential. Comparative investigations have demonstrated activity of their EOs against food-associated and reference microorganisms, including *S. aureus* and *E. coli* [16]. Chemically characterized Libyan *T. capitatus* oil has likewise shown antimicrobial activity [17], while additional studies support antibacterial and antibiofilm effects for these species [18,19]. Nevertheless, EO yield and antibacterial activity represent related but distinct biological attributes. Total EO yield reflects the quantity of recovered volatile material, whereas antimicrobial activity depends on constituent identity and relative abundance, interactions among components, organism susceptibility, EO concentration, and assay conditions [20]. Accordingly, greater oil production should not be assumed to correspond automatically to greater antibacterial activity.

Despite the expanding national and international literature, comparative information remains limited on how phenological stage influences EO yield in *T. algeriensis* and *T. capitatus* growing at the same Libyan locality under a common sampling and extraction framework, and whether the antibacterial screening patterns of the corresponding oils follow the same developmental trajectory. Addressing this gap requires a same-locality comparison across defined developmental stages while distinguishing the inferential analysis of independently replicated EO-yield measurements from descriptive antibacterial screening when extraction oils are pooled before testing.

Aims

This study aimed to quantify the effects of species, phenological stage and their interaction on essential-oil yield under a standardized Tarhuna sampling and hydrodistillation framework, and to describe the antibacterial screening pattern of the corresponding pooled oils against *S. aureus* ATCC 25923 and *E. coli* ATCC 25922.

2. Methods

Study design and plant material

The study used a balanced comparative design including two Thymus species (*T. algeriensis* and *T. capitatus*) across three phenological stages: vegetative (V), flowering (F) and post-flowering (PF). Aerial parts were collected from wild growing plants in Tarhuna, Libya, during 2025. Plant material was dried before extraction. Botanical identity was authenticated at the National Herbarium, Department of Botany, Faculty of Science, University of Tripoli, Libya, by Prof. Mohammed Makhlouf.

EO extraction and yield determination

For each separate extraction, 100 g of dried aerial plant material was subjected to hydrodistillation using a Clevenger-type apparatus for 3 h. These separate hydrodistillation runs were conducted for every species × phenological-stage combination, resulting in 18 independent yield measurements. EO yield was expressed as percentage volume per dry mass (% v/w, on dry-weight basis). The three independent yield values were kept separately for subsequent statistical analysis. Following yield measurements, the three oils obtained from each species × stage combination were combined to produce a single pooled EO preparation for antibacterial screening.

Bacterial strains and disc-diffusion assay

Antibacterial activity was assessed using American Type Culture Collection (ATCC) reference strains *S. aureus* ATCC 25923 and *E. coli* ATCC 25922. Mueller-Hinton agar served as the nutrient test medium and bacterial inoculum was standardized to 0.5 McFarland. Sterile 6-mm paper discs were impregnated with 1 µL of the respective pooled EO. Each pooled oil was tested using three technical disc measurements against each bacterium. Plates were incubated at 35 °C for 18 h, and total inhibition-zone diameter, including the 6-mm disc, was recorded in millimetres. The disc-diffusion assay is suitable for antibacterial screening, but zone diameter is influenced by compound

Corresponding author, email: asswedan@elmergib.edu.ly

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diffusion through the agar matrix and should not be interpreted as a direct quantitative measure of intrinsic antimicrobial potency [21]. Gentamicin (10 µg/disc) served as the positive-control reference and produced a reported 20-mm inhibition zone for both bacterial strains. The negative control (sterile disc) produced no inhibition (0 mm).

Statistical analysis

Each hydrodistillation run was considered an independent unit for analysis of EO yield (n = 3 per species × stage). Yield data were expressed as mean ± standard deviation (SD), coefficient of variation (CV), range and 95% confidence interval. Species, phenological stage and their interaction were tested with a two-way factorial linear model with Type III tests. Model assumptions were assessed using residual plots, Shapiro-Wilk, Brown-Forsythe and Levene tests, while partial eta squared (ηp^2) was used for effect size. Estimated marginal means and Tukey-adjusted pairwise comparisons were applied to compare groups. Antibacterial data were summarized descriptively as mean ± technical SD, range and CV. Analyses were performed in R 4.6.0.

3. Results

Phenological variation in essential-oil yield

EO yield differed markedly between species and across developmental stages (Table 1; Figure 1). In *T. algeriensis*, yield increased from 1.63 ± 0.06% at the vegetative stage to 1.85 ± 0.05% at flowering and then declined to 1.23 ± 0.03% post-flowering. In *T. capitatus*, corresponding values were 1.98 ± 0.08%, 3.22 ± 0.10% and 2.18 ± 0.03%. Flowering therefore represented the maximum-yield stage in both species, but the amplitude of the flowering response was much greater in *T. capitatus*.

Table 1. Essential-oil yield of *T. algeriensis* and *T. capitatus* across phenological stages.

Species	Phenological stage	n	EO yield (% v/w, DW), mean ± SD	CV (%)	Tukey group
T. algeriensis	Vegetative	3	1.63 ± 0.06	3.53	a
T. algeriensis	Flowering	3	1.85 ± 0.05	2.70	b
T. algeriensis	Post-flowering	3	1.23 ± 0.03	2.34	d
T. capitatus	Vegetative	3	1.98 ± 0.08	3.85	b
T. capitatus	Flowering	3	3.22 ± 0.10	3.24	c
T. capitatus	Post-flowering	3	2.18 ± 0.03	1.32	e

Note:Values are based on three independent hydrodistillations. Different lowercase letters indicate significant differences among the six species × stage combinations in the Tukey-adjusted all-cell comparison ($p < 0.05$); means sharing a letter are not significantly different.

Factorial-model assumptions were satisfactory. Residuals did not deviate significantly from normality (Shapiro-Wilk W = 0.954, p = 0.495), and neither the Brown-Forsythe test ($F_{5,12} = 0.482$, p = 0.783) nor the mean-centred Levene test ($F_{5,12} = 1.923$, p = 0.164) indicated significant variance heterogeneity. The model explained 99.29% of observed yield variation ($R^2 = 0.9929$; adjusted $R^2 = 0.9900$).

Two-way analysis of variance (ANOVA) showed highly significant effects of species ($F_{1,12} = 882.76$, $p = 1.32 \times 10^{-12}$), phenological stage ($F_{2,12} = 301.97$, $p = 5.47 \times 10^{-11}$) and species × stage interaction ($F_{2,12} = 97.28$, $p = 3.85 \times 10^{-8}$) (Table 2). Partial ηp^2 values were 0.987, 0.981 and 0.942, respectively, indicating large effects and phenological response differed substantially between the two species.

Table 2. Two-way factorial analysis of variance for EO yield.

Source	Sum of squares	df	F	p	Partial ηp^2
Species	3.556	1	882.76	1.32×10^{-12}	0.987
Phenological stage	2.433	2	301.97	5.47×10^{-11}	0.981
Species × Stage	0.784	2	97.28	3.85×10^{-8}	0.942
Residual	0.048	12	—	—	—

Partial ηp^2 values were calculated from the Type III factorial model.

Corresponding author, email: asswedan@elmergib.edu.ly

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Within *T. algeriensis*, flowering yield exceeded vegetative yield by 0.217 percentage points ($p = 0.0034$) and post-flowering yield by 0.617 percentage points ($p < 0.0001$); vegetative yield also exceeded post-flowering yield (difference = 0.400 percentage points, $p < 0.0001$). Within *T. capitatus*, flowering exceeded both vegetative (difference = 1.233 percentage points, $p < 0.0001$) and post-flowering stages (difference = 1.033 percentage points, $p < 0.0001$), while post-flowering also exceeded vegetative yield (difference = 0.200 percentage points, $p = 0.0059$).

Relative to the vegetative stage, flowering increased EO yield by 13.3% in *T. algeriensis* and 62.2% in *T. capitatus*. Yield declined from flowering to post-flowering by 33.3% and 32.1%, respectively. Across matched stages, *T. capitatus* produced 21.4% more oil than *T. algeriensis* during vegetative growth, 73.9% more at flowering and 77.0% more post-flowering; these between-species contrasts were significant at every stage (all $p < 0.0001$ in stage-specific species comparisons). The only nonsignificant pair in the six-cell Tukey family was vegetative *T. capitatus* versus flowering *T. algeriensis* ($p = 0.1778$).

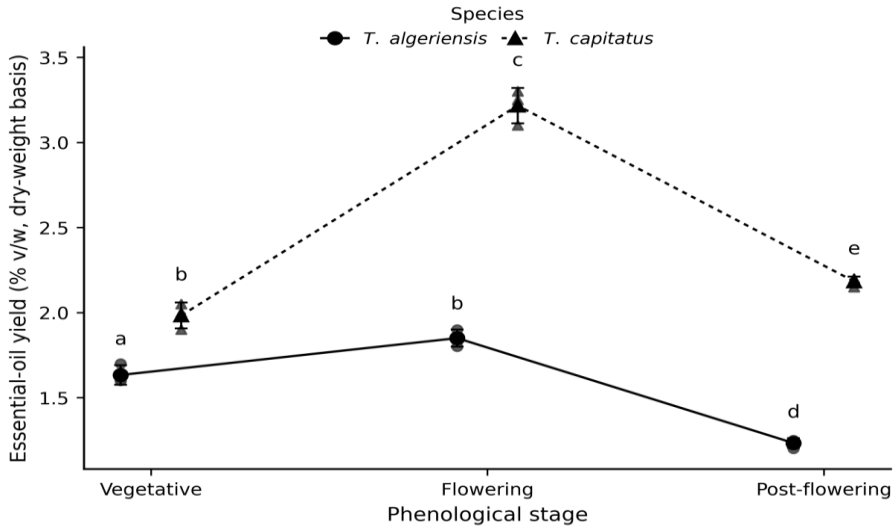


Figure 1. Phenological variation in essential-oil yield of *T. algeriensis* and *T. capitatus*. Individual symbols represent independent hydrodistillation runs (n = 3 per species × stage combination); central symbols and error bars represent mean ± SD. Different lowercase letters indicate significant differences among the six species × stage means after Tukey adjustment ($p < 0.05$).

Descriptive antibacterial activity of pooled oils

All pooled EO preparations produced measurable inhibition against both test strains, with mean zones ranging from 22.67 to 25.33 mm (Table 3; Fig. 2). Against *S. aureus*, mean zones for *T. algeriensis* were 24.67 ± 0.58, 24.33 ± 0.58 and 23.33 ± 0.58 mm at vegetative, flowering and post-flowering stages, respectively; corresponding values for *T. capitatus* were 24.67 ± 0.58, 23.67 ± 0.58 and 23.33 ± 0.58 mm. The observed *S. aureus* pattern showed a modest decline toward post-flowering in both species.

Against *E. coli*, *T. algeriensis* produced mean zones of 22.67 ± 0.58, 24.67 ± 0.58 and 24.67 ± 0.58 mm, whereas *T. capitatus* produced 24.67 ± 0.58, 24.67 ± 0.58 and 25.33 ± 0.58 mm. The largest observed inhibition zone in the dataset occurred with post-flowering *T. capitatus* oil against *E. coli*. The mean technical CV across the 12 pooled-oil × strain combinations were 2.39%, indicating low dispersion among technical disc measurements.

Corresponding author, email: asswedan@elmergib.edu.ly

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Table 3. Descriptive antibacterial activity of pooled essential oils across phenological stages.

Species/treatment	Stage	*S. aureus* ATCC 25923, zone (mm)	*E. coli* ATCC 25922, zone (mm)
T. algeriensis	V	24.67 ± 0.58 (24–25)	22.67 ± 0.58 (22–23)
T. algeriensis	F	24.33 ± 0.58 (24–25)	24.67 ± 0.58 (24–25)
T. algeriensis	PF	23.33 ± 0.58 (23–24)	24.67 ± 0.58 (24–25)
T. capitatus	V	24.67 ± 0.58 (24–25)	24.67 ± 0.58 (24–25)
T. capitatus	F	23.67 ± 0.58 (23–24)	24.67 ± 0.58 (24–25)
T. capitatus	PF	23.33 ± 0.58 (23–24)	25.33 ± 0.58 (25–26)
Gentamicin, 10 µg/disc	—	20	20
Negative control	—	0	0

Essential-oil values are mean ± technical SD with observed range in parentheses. The three independent extraction oils within each species × stage group were pooled before antibacterial testing; biological n = 1 per pooled preparation, and no inferential species- or stage-level tests were performed. Control values were single reported measurements.

All pooled oils produced mean zones numerically above the 20-mm gentamicin reference response. These numerical differences were not tested statistically and should not be interpreted as evidence that the EOs were more potent than gentamicin, because dose, physicochemical diffusion properties and replication structure were not equivalent between treatments.

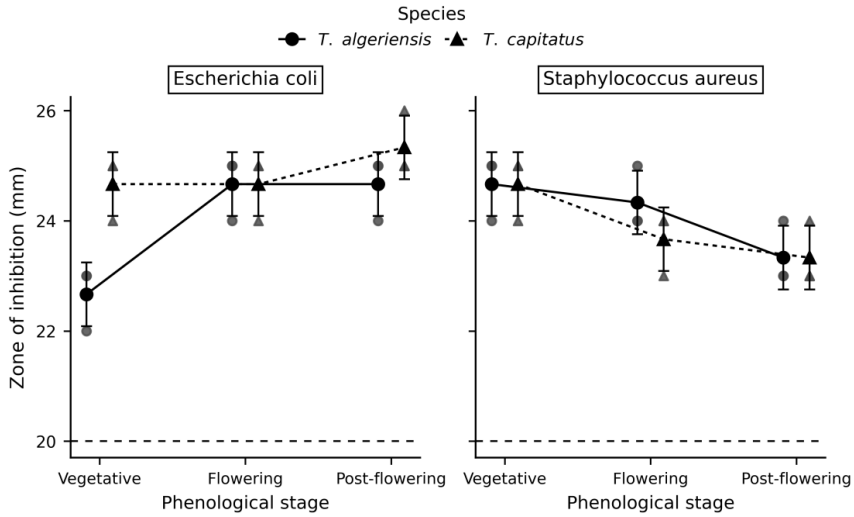


Figure 2. Descriptive antibacterial screening of pooled essential oils against *E. coli* ATCC 25922 and *S. aureus* ATCC 25923. Points are technical disc measurements and error bars are technical SD. The horizontal dashed line denotes the reported gentamicin 10 µg/disc reference response (20 mm). No inferential testing was performed for antibacterial activity because independent extraction oils were pooled before disc testing.

4. Discussion

EO yield was strongly influenced by phenological stage, but the magnitude of this response differed between the two species. Flowering produced the highest yield in both taxa, although the increase from vegetative to flowering was modest in *T. algeriensis* (+13.3%) and much greater in *T. capitatus* (+62.2%). The large species × stage interaction (partial $\eta p^2 = 0.942$) therefore shows that phenological development affects the two species differently.

The mechanisms responsible for this interaction were not measured directly. However, developmental changes in glandular tissues,

Corresponding author, email: asswedan@elmergib.edu.ly

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secretory-trichome density, biomass allocation and monoterpene biosynthesis may contribute to stage-dependent variation, while environmental factors such as temperature, light and water status may further modify EO production [2]. The *T. capitatus* pattern agrees with previous reports of increasing EO yield toward flowering followed by post-flowering decline [6,7]. In *T. algeriensis*, the response was less pronounced and published evidence is more variable, with phenological effects differing among populations and locations [3,5].

T. capitatus produced more EO than *T. algeriensis* at all stages, with the difference increasing from 21.4% during vegetative growth to 73.9% at flowering and 77.0% post-flowering. Thus, harvest timing appears particularly important for *T. capitatus* when the aim is to maximize EO recovery. Flowering was the most productive stage for both species under the conditions studied. However, this conclusion applies to EO quantity only. Previous studies show that EO composition can also change markedly during development [1,6,7]. Because chemical profiling was not performed here, flowering should be considered the optimum stage for yield rather than necessarily for chemical quality.

Geographic variation is common in Thymus species, with differences in EO yield and composition reported among populations [3,8,9]. Therefore, the flowering maximum observed here should be interpreted as a local optimum for the Tarhuna populations studied in 2025 rather than a universal value.

All pooled oils inhibited *S. aureus* and *E. coli*, consistent with previous reports on these species [16–19]. However, antibacterial activity did not follow the same pattern as EO yield. Flowering *T. capitatus* produced the highest EO yield, whereas the largest inhibition zone occurred with post-flowering *T. capitatus* against *E. coli*. Similarly, *T. algeriensis* had its highest yield at flowering, but its largest *S. aureus* inhibition zone occurred at the vegetative stage.

This divergence is biologically plausible because EO yield represents total volatile quantity, whereas antibacterial activity depends on the composition, relative abundance and interactions of individual constituents. Carvacrol and thymol are important constituents of many *T. capitatus* oils [8,9,11,17]. More recently, GC-MS analysis of Libyan *T. capitatus* collected from Mesellata identified a carvacrol-rich profile dominated by carvacrol (56.27%), with p-cymene, γ-terpinene and thymol among the other prominent constituents [11]. However, chemical profiling was not performed on the Tarhuna oils investigated here; therefore, the Mesellata composition cannot be assumed for the present samples, and the observed antibacterial patterns cannot be attributed to specific constituents. The results also do not support a simple Gram-positive versus Gram-negative susceptibility pattern, because *S. aureus* inhibition tended to decrease after flowering, whereas *E. coli* responses were maintained or increased [20,22].

5. Conclusion

Phenological stage exerted a major, species-dependent effect on EO yield of *T. algeriensis* and *T. capitatus* from Tarhuna, Libya. Flowering produced the highest yield for both species, with a particularly large increase in *T. capitatus*, whose yield increased by 62.2% relative to the vegetative stage. The significant species × stage interaction shows that the effect of harvest stage differs between the two species. All pooled oils produced measurable inhibition of *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 in disc-diffusion screening, but the descriptive antibacterial patterns did not mirror the yield trajectory. Because independently extracted oils were pooled before antibacterial testing, these activity differences are descriptive rather than inferential. The study identifies flowering as the optimal stage for maximizing EO recovery under the conditions studied, while highlighting the need for follow-up chemically characterized, biologically replicated minimum inhibitory concentration/minimum bactericidal concentration studies before mechanistic or potency conclusions can be drawn.

Corresponding author, email: asswedan@elmergib.edu.ly

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ABBREVIATIONS AND ACRONYMS

EO, essential oil; V, vegetative; F, flowering; PF, post-flowering; SD, standard deviation; CV, coefficient of variation; ANOVA, analysis of variance; ATCC, American Type Culture Collection; MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration.

ETHICAL STATEMENT:

This study involved plant material and established bacterial reference strains and did not involve human participants or experimental animals. Ethical approval was therefore not applicable.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest related to this work.

AUTHORS' CONTRIBUTIONS

A.S.: Conceptualization, methodology, investigation, data curation, statistical analysis, visualization, and writing original draft.
I.A.: Methodology, supervision, validation, and writing—review and editing.
M.S.: Supervision, validation, and writing—review and editing.
All authors read and approved the final manuscript.

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Corresponding author, email: asswedan@elmergib.edu.ly

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Placenta Previa Accreta Spectrum During Labor: Can it lead to Hysterectomy?

Narges Ben Halim [a,c,d&*], Alia Zawawa [b,c,d], Fathia Gressac [e]

[a] Faculty of Medicine, Misurata University, Misurata, Libya
[b] Faculty of Health Sciences, Misurata University, Misurata, Libya
[c] Libyan Academy for Postgraduate Studies, Janzour, Libya
[d] Department of Obstetrics and Gynecology, Elsaid Hospital, Misurata, Libya
[e] LAMIS IVF Center, Misurata, Libya
*Corresponding author E mail: n.benhaleim@med.misuratau.edu.ly

A B S T R A C T

Background: Placenta Previa Accreta Spectrum (PAS) is a serious obstetric condition that causes severe hemorrhage and an increased risk of hysterectomy. Early identification of risk factors and appropriate management strategies are essential to improve maternal outcomes.

Aims: To evaluate the incidence and risk factors associated with hysterectomy among patients with placenta previa accreta spectrum, assess maternal outcomes, and identify clinical management strategies that may reduce the need for hysterectomy.

Methods: A retrospective cohort study was conducted at Elsaid Hospital. Medical records of 105 women diagnosed with PAS (2017 and 2024) were reviewed. Data collected included age, parity, previous cesarean sections, antepartum hemorrhage, comorbidities, hemoglobin levels, blood transfusion requirements, surgical interventions, and maternal outcomes. Statistical analysis was performed using descriptive and inferential methods, with a significance level of $p < 0.05$.

Results: the overall incidence of PAS was 0.53 %. The incidence progressively increased over time. In 2017, PAS incidence was 0.39%, which then progressed to reach the highest incidence (1.06%) in 2024. The mean age of participants was 33.5 ± 5.1 years. The cohort had a high prevalence of multiple cesarean deliveries, with 62.5% having three or more previous cesarean sections. Antepartum hemorrhage and anemia were reported in 44.8% of cases each. Placenta previa accreta was associated with maternal morbidity, including increased blood transfusion requirements and a heightened risk of hysterectomy, particularly among women with multiple previous cesarean deliveries and severe obstetric complications. Maternal mortality was 0.95%.

Conclusion: PAS remains a major cause of maternal morbidity and mortality constitutes a primary indication for intrapartum hysterectomy. Previous cesarean sections, antepartum hemorrhage, and anemia are important risk factors associated with adverse maternal outcomes. Resorting to hysterectomy in cases of PAS should not be regarded as an inevitable sequela; early diagnosis and multidisciplinary management are essential to reduce complications and improve outcomes.

Keywords: *Placenta Accreta Spectrum, Hysterectomy, Maternal Outcome, Cesarean Section, Antepartum Hemorrhage*

1. Introduction

The most severe consequences of pregnancy are placental abnormalities, which greatly endanger both maternal and fetal health. Particularly when it manifests itself during labor, placenta previa accreta is among the most hazardous and life-threatening diseases. Placenta previa is the aberrant insertion of the placenta in the lower uterine sector, either partially or completely occluding the cervix. The hazards rise considerably when this disorder coexists with placenta accreta, an even more severe abnormality where the placenta invades and adheres excessively to the myometrium [1]. This disorder not only impedes typical labor and delivery, but also raises the risk of significant

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hemorrhage, hence necessitating urgent and even life-saving operations like hysterectomy. Placenta accreta spectrum (PAS) disorders encompass various types—including placenta accreta, increta, and percreta, each distinguished by the degree of placental invasion into the uterine wall and beyond [1]. Studies indicate that the chance of placenta accreta rises with advanced maternal age and several pregnancies [2]. The incidence of placenta accreta has increased from 1 in 30,000 pregnancies in the 1960s to 1 in 533 pregnancies in the 2000s [3]. The risk of placenta accreta increases with the number of prior cesarean sections, ranging from 0.3% in women with one previous cesarean delivery to 6.74% for women with five or more cesarean deliveries [4]. A recent systematic review and meta-analysis evaluating the association between previous non-cesarean uterine surgery and PAS showed that myomectomy, dilation and curettage (D&C), endometrial ablation, or uterine perforation increase the likelihood of abnormal placental implantation due to scarring and endometrial damage, significantly increasing the odds for development of PAS in subsequent pregnancies [5]. Placenta accreta can lead to premature birth [6]. To maximize neonatal maturation and reduce the risk of maternal bleeding, clinical consensus advises delivery between 34 and 36 weeks of gestation through cesarean hysterectomy [7]. There should be consideration for transfer to a tertiary center for delivery or to a Placenta Accreta Center of Excellence (PACE) before delivery [8]. The patient's hemoglobin should be optimized prior to delivery, and communication with the blood bank will help guarantee supplies are available when a significant transfusion becomes necessary [9]. Cesarean sections should be planned such that a simple transition to hysterectomy is possible if needed [9]. If not managed properly, placenta accreta can result in significant maternal morbidity and, in some cases, even mortality [10]. Aims: To evaluate the incidence and risk factors associated with hysterectomy among women with Placenta Previa Accreta Spectrum (PAS), assess maternal outcomes, and identify clinical management strategies that may reduce the need for hysterectomy and improve maternal outcome.

2. Methods

A retrospective cohort hospital record-based study was conducted on 105 cases with a confirmed diagnosis of PAS (via imaging or intraoperative findings) over an eight-year period (from January 2017 to December 2024). Data were collected from delivery records at Elsaid Hospital in Misurata, Libya. All cases in the study were operated on by a multidisciplinary team who already possessed expertise in the management of placenta accreta spectrum. All patients provided written informed consent for the operation. The collected data were analyzed using SPSS statistical software version 21, and a p-value equal to or less than 0.05 was considered statistically significant.

3. Results

Annual incidence of PAS showed variations throughout the study period, progressively increasing from 0.39% in 2017 to the highest incidence of 1.06% in 2024 (Table 1). This trend suggests a gradual increase in the incidence of PAS in recent years. The overall incidence of PAS over 8 years was 0.53% (out of 19873 total number of deliveries from 2017 to 2024 at ELSAID hospital).

Corresponding author E mail: n.benhaleim@med.misuratau.edu.ly

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Table 1. Annual Incidence of Placenta Previa Accreta Spectrum

Year	Total Deliveries	PAS Cases	Incidence (%)
2017	3315	13	0.39
2018	3722	15	0.40
2019	3872	15	0.39
2020	2023	14	0.69
2021	1950	12	0.62
2022	2298	11	0.48
2023	1275	10	0.78
2024	1418	15	1.06

The participants' ages ranged from 18 to 41 years, with a mean age of 33.5 ± 5.1 years. The majority of women (81.0%) were within the 30–41-year age interval (Figure 1).

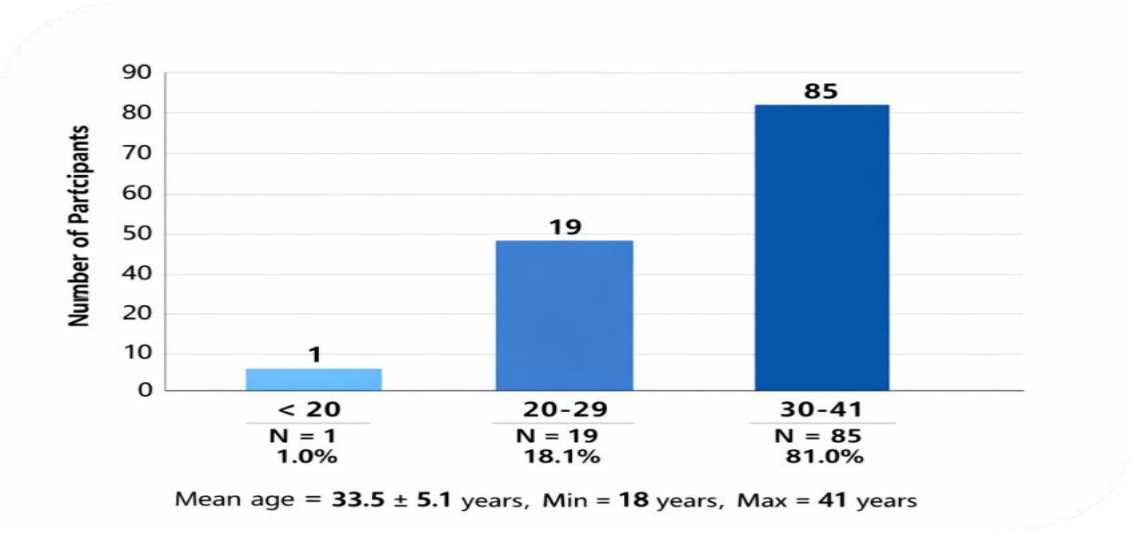


Figure 1. Distribution of the women with placenta previa accreta according to age.

The majority of patients (81.0%) fall within the 30–41-year age group, indicating advanced maternal age as a prevalent factor in this cohort. In regard to the gestational age, most women (85.7%) were classified as moderate to late preterm (32–37 weeks) at the time of delivery, while 7.6% were very preterm (28–<32 weeks) of gestation, and only 6.7% were term. (Table 2).

Corresponding author E mail: n.benhaleim@med.misuratau.edu.ly

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Table 2. Distribution of patients with placenta previa accreta according to gestational age at time of cesarean section

Category of Gestational age / wks.	Number	Percentage (%)
Extreme preterm <28wk	0	0
Very preterm 28-<32	8	7.6
Moderate to late preterm 32-37	90	85.7
Term >37 week	7	6.7

Regarding parity, 47.6% were multiparous (2-4 deliveries) and 40.9% were grand multiparous (5 or more deliveries) (Table 3).

Table 3. Distribution of patients according to parity

Parity	Number	Percentage (%)
Nullipara	3	2.9
Primipara	9	8.6
Multipara	50	47.6
Grand multipara	43	40.9

A high prevalence of repeat cesarean deliveries was observed in the cohort: 37% of patients had four or more, while 31.4% had three (Table 4).

Table 4. Distribution of patients with placenta previa accreta according to history of cesarean section (CS)

Number of Cesarean sections	Number	Percentage (%)
No CS	1	0.9
One CS	11	10.5
Two CS	21	20.0
Three CS	33	31.4
Four or more CS	39	37.0

Prior non-cesarean surgical interventions included dilation and curettage in 36.2% of cases, myomectomy in 3.8%, and laparoscopy in 3.8% (Table 5).

Corresponding author E mail: n.benhaleim@med.misuratau.edu.ly

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Table 5. Distribution of patients according to history of uterine surgery

Type of operation	Number	Percentage (%)
Dilatation and curettage	38	36.2
Myomectomy	4	3.8
Laparoscopy	4	3.8

Antepartum hemorrhage and antepartum anemia were major complications, each affecting 44.8% of the cohort (Table 6).

Table 6. Distribution of patients according to history of antepartum complication

Complication	Number	Percentage (%)
Antepartum hemorrhage	47	44.8
Antepartum anemia	47	44.8

Preoperative hemoglobin levels indicated that 49**.5%**of patients had normal levels, **25.7%** had moderate anemia, and **23.8%** had mild anemia. Only one case 0.95% of severe anemia was recorded. Postoperative, hemoglobin levels deteriorated significantly (P< 0.001) with mild anemia rising to 46.2% and moderate anemia to 31.0%. 22.0% were normal, while the number of severe cases remained unchanged. See Table7 for more details.

Table 7: Distribution of patient with placenta previa accreta according to hemoglobin before and after operation:

Hb(gm/dl)	HB Before (No., %)	HB After (No., %)
Severe anemia (<7)	1 (0.95)	1 (0.95)
Moderate anemia (7.0-9.9)	27 (25.7)	33 (31.4)
Mild anemia (10-10.9)	25 (23.8)	48 (45.7)
Normal (no anemia) ≥ 11.0	52 (49.5)	23 (21.9)
Total	105 (100)	105 (100)
Test significant	P= 0.00026	

Blood transfusion requirements during cesarean section for PAS ranged from 0 to 54 units. The vast majority of patients required at least 1-unit (96 patient,91.4%)). However, 27.5 units were used among extreme outliers, and 17.2% requiring massive transfusion of ≥ 10 units (See Table 8). The bulk of the patients (74.2%) fall within the 1 to 9-unit range. Specifically, the most frequent requirement was1-2 units (39%).

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Table 8. Distribution of patients according to number of blood transfusion units

Category	Transfusion units	Number of patients	Percentage (%)
No Transfusion	0 units	9	8.6
Low Requirement	1–2 units	41	39.0
Mild to Moderate Requirement	3–9 units	37	35.2
High/Massive Requirement	≥ 10 units	18	17.2
Total		105	100.0

The mean length of hospital stay was 4.3 ± 1.3 days (Table 9).

Table 9. Distribution of days of admission among placenta previa accreta cases

Days of hospital stay	Frequency	Percentage (%)
3-4 days	67	63.8
5-6 days	33	31.4
>7 days	5	4.8
Mean days of admission = 4.3 ± 1.3 days		

Surgical interventions included uterine reconstruction after manual removal of the placenta (69.5%), internal iliac artery ligation (22.9%), cesarean hysterectomy (3.8%), and B-Lynch suturing (3.8%) (Figure 2).

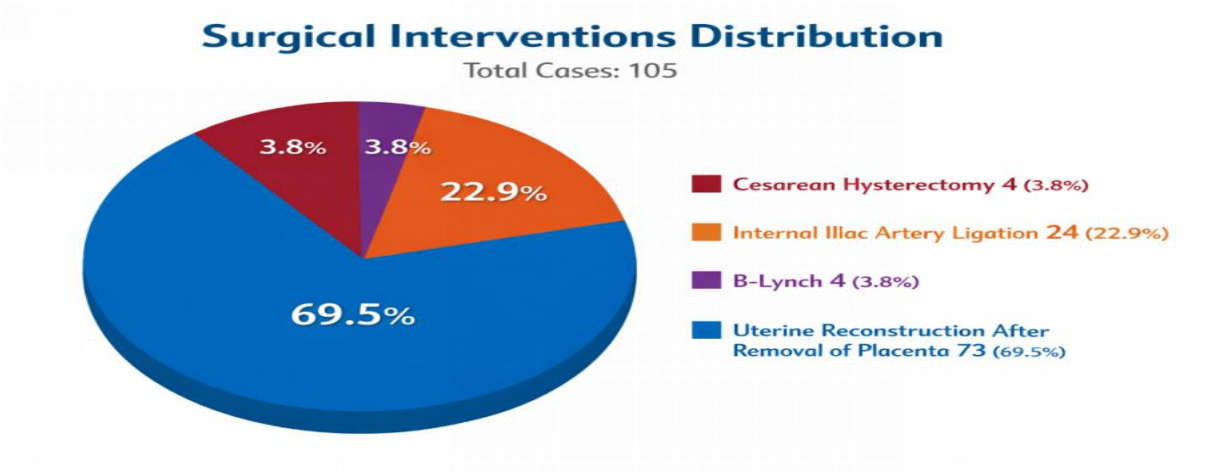


Figure 2. Surgical Interventions Distribution.

(Proportions of intrapartum surgical interventions showing uterine reconstruction was the most common approach (69.5%).

There were two cases of bladder injury (1.9%), no cases of bowel injury recorded and only one maternal mortality (0.95%), the overall maternal mortality rate from total number of deliveries through 8 years from 2017 to 2024(19873) was only (0.005) (Figure 3).

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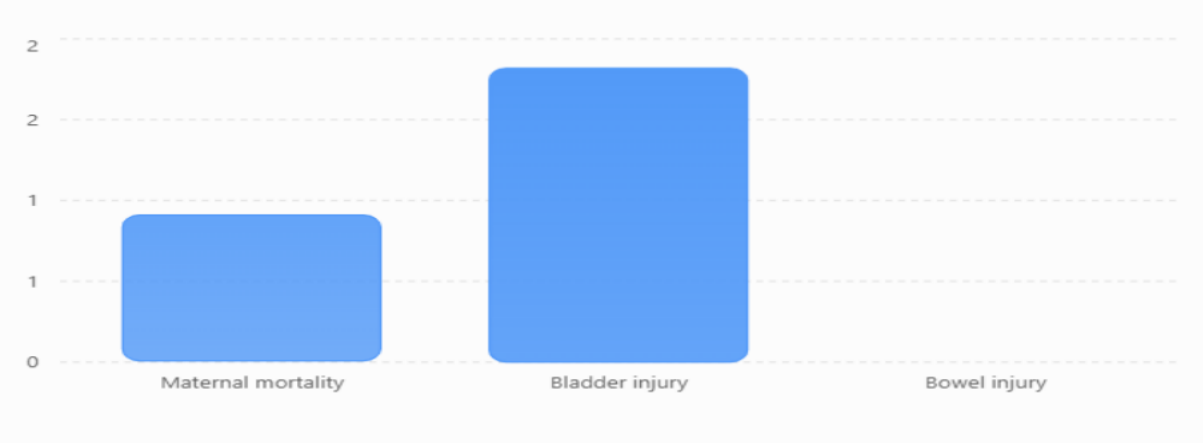


Figure 3. Incidence of maternal mortality and surgical complication.

(Maternal mortality (0.95%), bladder injury (1.9%), bowel injury (0.0%).

While complications like antepartum hemorrhage and anemia trended upward with maternal age (Figure 4).

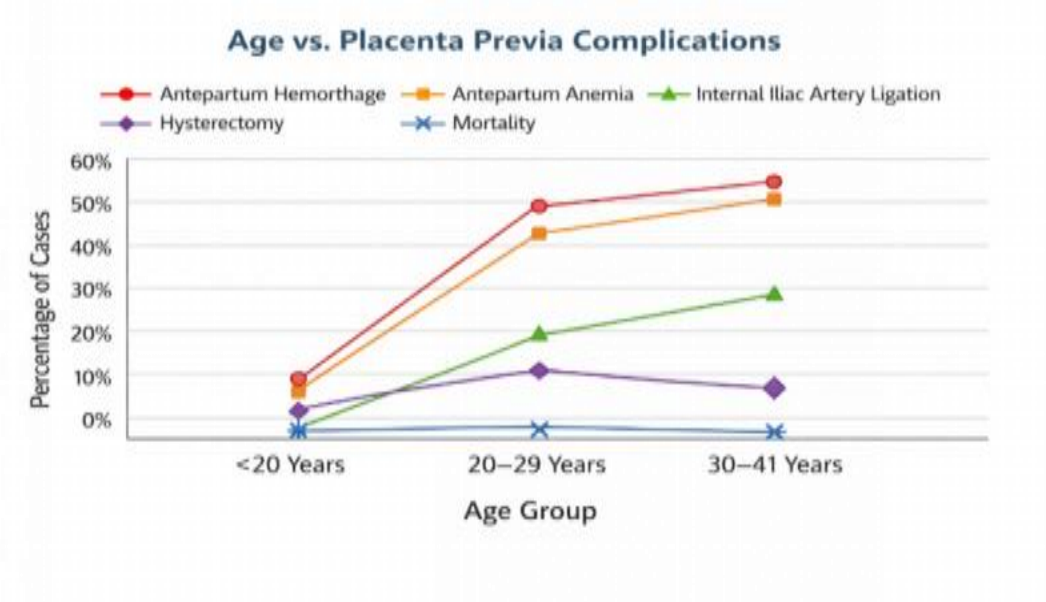


Figure 4. Relation between age of the women and PAS complications.

(Upward trend of complications, specifically antepartum hemorrhage and anemia, associated with increasing maternal age groups.)

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No statistically significant association was found between maternal age and specific surgical complications or interventions, including hysterectomy (Table 10).

Table10. Relation between age of the women and surgical intervention

Item	Age group			
Surgical intervention	**20-29 yrs (n:19)**	**30-41 yrs n:85)**	**Total (n:105)**	**p-value**
Cesarean hysterectomy	1 (5.3%)	3 (3.5%)	4 (3.8%)	0.927
Internal iliac artery ligation	3 (15.8%)	21 (24.7%)	24 (22.9%)	0.690
B-Lynch	1 (5.3%)	3 (3.5%)	4 (3.8%)	0.927

Out of the 105 women studied, the majority were free from non-communicable diseases (92) (87.6%), while 13(12.4%) had non-communicable diseases indicating a predominantly healthy cohort. Among those with underlying conditions, diabetes mellitus (DM) and hypertension (HT) were the most common, each affecting 4 (3.8%) patients. Hypothyroidism and infertility were each observed in 2 (1.9%) participants, while asthma was reported in 1 (0.95%) case.no association found between hystrectomy & chronic medical disease (P = 0.502). (See Figure 5).

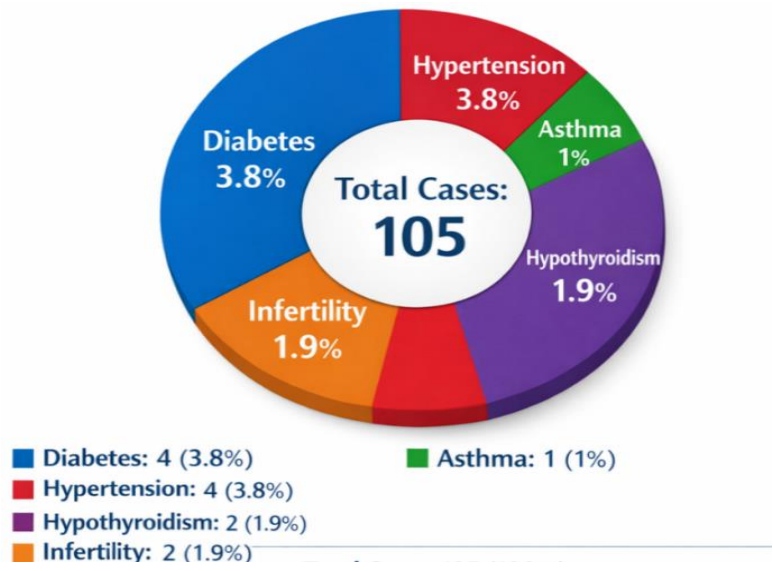


Figure 5: Distribution of chronic disease among PAS patient

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4. Discussion

Placenta accreta spectrum (PAS) is a leading cause of massive obstetric hemorrhage. The primary danger is severe antepartum or postpartum hemorrhage (PPH), which frequently results in the need for a cesarean hysterectomy, blood transfusions, and intensive care admissions [11]. PPH is the leading cause of maternal mortality worldwide, resulting in about 70,000 maternal deaths globally each year [12]. In our study, the overall incidence of PAS over the eight-year period was 0.53%, progressively increasing from 0.39% at 2017, to 1.06% by 2024. This trend suggests a gradual increase in the incidence of PAS in recent years, particularly from 2022 to 2024. PAS incidence is likely to continue increasing as a result of rising rates of caesarean births and the use of assisted reproductive technology. This result agrees with guidelines produced by the Royal College of Obstetricians and Gynecologists [13].

Out of the 105 women studied, there was 1 (0.95%) case of maternal mortality, Neither the WHO or the UNICEF publish a single specific global incidence of maternal mortality rate or percentage caused exclusively by placenta previa, Instead, placenta previa is classified under Obstetric Hemorrhage. Obstetric hemorrhage is the leading direct cause of maternal death worldwide, accounting for roughly 27% of all maternal mortality. In some regions, like parts of Asia, complications from placenta previa and abnormal placental attachment account for up to 30% of all local maternal hemorrhage deaths [14, 15].

In Regard to patients age, the mean age of participants was 33.5 ± 5.1 years, with the majority (81%) between 30–41 years. Only 1 case was under 20 years (0.95%), while 18.1% were between 20–29 years, Advanced maternal age has been repeatedly cited as a risk factor for abnormal placentation due to cumulative uterine damage and increased rates of previous uterine surgery [2, 16]. The vast majority of our cohort were either multiparous or grand multiparous, mimicking broader reproductive trends indicating that repeated pregnancies contribute to endometrial damage and abnormal invasion [17, 18]. The predominance of PAS in women aged 30–41 in this study suggests that age acts synergistically with other risk factors, particularly multiple cesarean deliveries. Younger women (<29 years) showed fewer complications, which may reflect both lower surgical history and better uterine healing capacity. while Complications and surgical interventions, including hysterectomy, were more frequent among women in the older age group (≥30 years)**.** The statical analysis in this study confirmed that age was significantly correlated with surgical complications and hysterectomy rates**,** supporting the global evidence that advanced maternal age increases PAS severity [17, 18]. Although total hysterectomy cases were four, only one case at the age group 20-29 years and three cases at the age group 30-41 years so, the majority of hysterectomy cases occurred among women aged 30–41 years (75.0%), followed by those aged 20–29 years (25.0%), while no cases were reported among women younger than 20 years. However, the association between age and hysterectomy was not statistically significant ($P = 0.920$).

While hysterectomy remained (3.8%), the significant correlation with antepartum hemorrhage (APH) and pre-operative anemia ($P < 0.05$) identifies these as the two most critical clinical predictors for radical surgery.

This study revealed that all hysterectomy cases experienced APH prior to the procedure, and all hysterectomy patients as reported by the current study were anemic prior to the procedure (P=0.045). Anemia reflects recurrent bleeding episodes that ultimately culminate in the need for radical surgery to control life-threatening hemorrhage. compared to only 42.6% in the non-hysterectomy group. This suggests that active bleeding before delivery is a major red flag for severe placental pathology. The current study revealed a significant relation between antepartum hemorrhage (APH) and the hysterectomy where P= 0. 029.Antepartum hemorrhage (44.8%) and preoperative anemia strongly predicted the need for radical surgery. Our findings align with literature stating that emergency surgical intervention due to severe bleeding is a major contributor to adverse maternal outcomes [18, 19]. The proportion of women with normal hemoglobin levels decreased to 22.1%,

Corresponding author E mail: n.benhaleim@med.misuratau.edu.ly

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while the "Severe anemia" category remained constant at 0.95%. A significant decline in hemoglobin levels was observed after surgery (P = 0.00026), reflecting the substantial blood loss associated with PAS management.

The need for blood transfusion units ranged from one unite to 54 units with average of 27.5 units. A significant majority of the cases (91.4%) required at least one unit of blood with High Prevalence of Transfusion. The bulk of the patients (74.2%) fall within the 1 to 9-units range. Specifically, the most frequent requirement was1-2 units (39%), suggesting that while hemorrhage is common, it is often manageable with standard transfusion protocols. Approximately 17.2% of cases required 10 or more units, which often meets the clinical criteria for a "Massive Transfusion Protocol." The presence of extreme outliers—such as patients requiring 20 or even 54 units—points toward severe complications, likely involving placenta previa or emergency intrapartum hysterectomy. The low percentage of patients managed without transfusion (8.6%) indicates that PAS remains one of the most high-risk obstetric conditions. The wide variance in units (from 1 to 54) suggests that while most cases follow a predictable pattern, the potential for catastrophic blood loss is always present and requires immediate multidisciplinary readiness.

Previous cesarean sections were the most notable risk factor in our population, with 68.6% of participants having three or more prior cesarean deliveries, matching institutional warnings that previous cesarean delivery combined with placenta previa is the paramount risk factor for PAS [20].,while patient with no history of cesarian section or only one cesarian section form only11.4% Furthermore, a history of D&C (36.2%) supports evidence that repeated curettage damages the myometrium, predisposing to defective decidualization [21, 22], while myomectomy and laparoscopy 3.8% for each.

Conservative surgical approaches, particularly uterine reconstruction (69.5%), were heavily favored in our center to preserve fertility, reflecting contemporary shifts toward conservative management where clinically viable [23]. Internal iliac artery ligation in current study performed in (22.9%) of cases used to reduce pelvic blood flow and control bleeding. B – lynch sutures in this study applied in (3.8%) of cases to reduce pelvic blood flow and control bleeding and preserve fertility. The predominance of uterine reconstruction in this study shows a strong preference for fertility-preserving strategies. However, hysterectomy remained unavoidable in severe cases, consistent with global evidence that it is the most reliable intervention when conservative measures fail [24,25].

Severe hemorrhage necessitating hysterectomy (3.8%) remains a life-saving absolute when fertility-sparing techniques like B-Lynch sutures or internal iliac artery ligation fail [28]. All hysterectomies occurred between 33^{rd} –37^{th} weeks of gestation. No significant association was identified between hysterectomy and chronic diseases (P = 0.502), despite the presence of isolated cases among women with hypertension. Additionally, previous surgical history was not significantly associated with hysterectomy (P = 0.724). Overall, these findings indicate that hysterectomy was significantly associated with obstetric complications, particularly antepartum hemorrhage and anemia, rather than demographic or reproductive characteristics.

5. Conclusion

Placenta previa accreta spectrum is a life-threatening condition that still risk for Hysterectomy, but conservative interventions can reduce morbidity and preserve fertility in selected cases. Early diagnosis, multidisciplinary planning, and preparedness for massive transfusion are essential to improving maternal outcomes. Preventive strategies, particularly reducing unnecessary primary cesarean deliveries, are critical to lowering PAS incidence in future generations.

Corresponding author E mail: n.benhaleim@med.misuratau.edu.ly

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THICAL STATEMENTE:
This retrospective study evaluated historical data collected between 2017 and 2024. Institutional ethical review and formal patient consent were deemed not applicable by the institutional ethics committee at Elsaid Hospital because the study relied solely on pre-existing, de-identified secondary data. To ensure strict patient confidentiality, all datasets were completely anonymized prior to investigator access. All data handling procedures were conducted in secure, password-protected systems in accordance with the ethical principles of the Declaration of Helsinki.

CONFLICT OF INTEREST
The authors declare that they have no financial, professional, or personal relationships that could influence or appear to influence the work reported in this paper.

AUTHORS' CONTRIBUTIONS
N.B. A Conceptualization, methodology, literature review, primary manuscript drafting and revision. A.Z. Supervised clinical validation, and critical manuscript revision. F. G. Data curation, formal analysis.

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Corresponding author E mail: n.benhaleim@med.misuratau.edu.ly

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In-Vitro Quality Assessment of Different Brands of Vitamin C Tablets Available in the Libyan Market

Faraj A. Shmam[a,*], Abadi G. Abadi[a], Mahmoud A. Aldebaibi[a], Omar M. Ahmemeed[a], Aseel H. Al-ammary[b], Samia H. Al-Areq[b], Alia A. Sasi[b]

[a] Department of Pharmaceutics and Industrial pharmacy, Faculty of Pharmacy, Elmergib University, Al-Khums, Libya.

Faculty of Pharmacy, Elmergib University, Al-Khums, Libya

*Corresponding author Email: fashmam@elmergib.edu.ly

A B S T R A C T

Background: Vitamin C, or ascorbic acid, is a vital water-soluble vitamin and a potent antioxidant essential for human health. Vitamin C tablets are widely available as over-the-counter (OTC) products and are often used without medical supervision; therefore, strict adherence to pharmacopeial standards is essential to ensure their safety, efficacy, and quality consistency across different brands.

Objectives: The aim of this study was to evaluate the quality of four brands of 500 mg vitamin C tablets commonly sold over-the-counter in the Libyan market. The four brands were labeled as Brand 1, Brand 2, Brand 3, and Brand 4, and evaluated to assess whether they meet the required quality standards.

Methods: The pharmaceutical quality of different vitamin C Brands was assessed using several in-vitro tests according to the United States Pharmacopeia (USP); the assessment included chemical and physical properties such as friability, hardness, uniformity of content, weight variation, dissolution rate, and disintegration time.

Results: All tested brands passed the disintegration and hardness tests and complied with USP standards. However, Brand 2 and Brand 4 failed to meet the requirements for friability test. In addition, the Brand 4 failed to meet the USP specification for weight variation, content uniformity, and the dissolution test. The observed failures in Brand 2 and Brand 4 indicate that not all vitamin C Brands fulfilled the USP requirements, suggesting potential differences in pharmaceutical quality and interchangeability, whereas Both Brand 1 and Brand 3 successfully complied with all quality control tests. However, Brand 3 demonstrated superior performance, achieving a faster drug release rate and a shorter disintegration time compared to the other brands.

Conclusion: The findings indicate that not all vitamin C brands available in the Libyan market meet the required pharmacopeial standards. Continuous quality control testing, strict regulatory supervision, and proper storage conditions are essential to ensure that pharmaceutical products distributed in the market provide safe, effective, and reliable therapeutic outcomes.

Keywords: *Vitamin C tablets; Ascorbic acid; In-vitro dissolution assessment; Libya market; Post-market tablet evaluation.*

1. Introduction

L –ascorbic acid, commonly known as vitamin C is a well -known micronutrient essential for maintaining normal physiological function. Vitamin C exists primarily in two forms: Ascorbic Acid (AA) and dehydroascorbic acid (DHA). It is a potent antioxidant and serves as cofactor for numerous enzymatic reactions. Vitamin C plays an important role in several biological processes, including proper immune system function, carnitine and catecholamine metabolism, iron absorption from food, and collagen synthesis [1-4]. Vitamin C tablets are widely available as over-the counter (OTC) products and are frequently consumed without medical supervision. The high demand and broad

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market availability of vitamin C necessitate strict adherence to pharmacopeial standards to ensure safety, efficacy, and uniformity across different brands. High dose formulation, such as 500mg and 1000mg tablets, present additional formulation and stability challenges, as ascorbic acid is inherently unstable and prone to oxidative degradation when exposed to light, heat, oxygen, alkaline pH, and moisture [5-6]. Such degradation reduces potency and may compromise therapeutic effectiveness. Therefore, appropriate formulation strategies, protective packaging, and controlled storage conditions are essential to maintain product stability throughout shelf life [7].

Routine quality evaluation is critical due to susceptibility to degradation. Pharmacopeial monographs in the United States pharmacopeia, British Pharmacopeia and European Pharmacopeia specify limits for assay, dissolution, and uniformity to ensure consistent delivery of the labeled amount of active ingredient [8]. Despite established regulatory standards, variability in product quality among manufactures has been reported. Differences in excipients, formulation design, manufacturing processes, and storage conditions can significantly influence tablet performance [7]. Content uniformity ensures accurate dosing in each tablet, while dissolution testing evaluates the rate and extent of drug release, which directly affects bioavailability. Tablets that meet assay requirements but fail dissolution testing may not achieve the intended therapeutic effect [6]. Mechanical properties, including hardness and friability influence tablet integrity during packaging, transportation, and handling. However, excessive hardness may delay disintegration, whereas insufficient hardness can result in brittleness and breakage. Excipients and manufacturing parameters further affect stability and performance, particularly if they promote moisture uptake that accelerate ascorbic acid degradation [7].

Despite the limited number of studies related to the quality assessment of vitamin C tablets in Libya, there were a several studies had been reported internationally. One of these studies conducted by Iwuozor emphasized the importance of performing comprehensive physical and chemical tests to ensure that products comply with pharmacopeial standards. The study evaluated five brands of vitamin C tablets for their percentage content of ascorbic acid (active pharmaceutical ingredient, API), friability, disintegration time, and hardness. The results showed that all samples complied with the pharmacopeial requirements for hardness and disintegration time. However, Samples 1, 3, 4, and 5 failed the assay test for percentage content of ascorbic acid, while Sample 2 failed the friability test. A key limitation of that study was the absence of a detailed correlation between dissolution profiles and API content, which would have provided stronger evidence regarding the relationship between drug content and in vitro performance [9]. The current study addresses this limitation by establishing a comprehensive analytical correlation between dissolution profiles and API content.

Similarly, a study conducted by Lahai et al. [6], evaluated nine vitamin C products and found that all products complied with the requirements for uniformity of dosage units, friability, and identification tests. However, variations were observed in disintegration and assay results, with some products failing to meet the British Pharmacopoeia (BP) specifications for vitamin C content. The assay results obtained using titration and colorimetric methods. Out of the nine products tested, seven complied with the BP assay specifications, one contained less vitamin C than the permitted limit, and one exceeded the specified upper limit.

Furthermore, the challenge of substandard vitamin C formulation is well documented in African markets. Babalola, et alconducted a study assessed five brands of ascorbic acid tablets [10]. The brands were analyzed for their uniformity of weight, hardness, friability and disintegration time using official methods, quantitative analysis was conducted using dissolution test and high-performance liquid chromatography (HPLC). All five brands passed the friability test, disintegration test and dissolution test and content uniformity.

In Libya, the pharmaceutical market is heavily reliant on imported brands from various global sources. Despite the high consumption of vitamin C supplements, there is a profound lack of published studies specifically evaluating the in vitro quality of vitamin C tablets available

Corresponding author: Email: fashmam@elmergib.edu.ly

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in the Libyan market. Most existing local literature focuses on clinical nutritional status rather than the pharmaceutical equivalence of the products [11-13]. This study represents an effort to address this gap by assessing the quality of different brands available to Libyan consumers. The present study was conducted due to widespread availability of numerous brands of vitamin C tablets in the pharmaceutical local market. As a commonly consumed OTC product, vitamin C tablets must consistently comply with pharmacopeial quality standards to ensure therapeutic effectiveness and patient safety. Conducting such a study contributes to regulatory monitoring, supports Good Manufacturing Practice (GMP) enforcement, and promotes the availability of safe, effective, and high-quality pharmaceutical products. Therefore, this study aims to evaluate and compare the quality and in-vitro performance of selected brands of 500mg ascorbic acid tablets available in the Libyan market in accordance with United State Pharmacopeia (USP) standards.

2. Materials and Methods

2.1 Materials

Four brands of vitamin C 500mg tablets available in the Libyan market were purchased from community pharmacy and coded alphabetically as shown in Table 1. The products were checked for tablet appearance, aesthetics, packaging quality, batch number, manufacturing and expiry dates before purchasing. In addition, standard vitamin C (Mindex Limited. UK) used as reference.

Table 1. Different Brands of Vitamin C tablets

Code	Origin	Batch No	Manufacturing Date	Expiry Date
Brand 1	USA	42535	11/2024	11/2027
Brand 2	Tunisia	VC 01	12/2024	12/2026
Brand 3	Tunisia	24116	10/2024	10/2027
Brand 4	Ukraine	056	09/2024	09/2027

2.2 Methodology

This study applied official pharmacopeial guidelines, specifically the United States Pharmacopeia (USP), to establish the appropriate in vitro quality control protocols and analytical procedures. Several physicochemical assessments of vitamin C tablets were evaluated including, weight variation, friability, hardness, drug content, disintegration time and dissolution profile.

2.2.1 Hardness test

The hardness test was conducted using Erweka hardness tester (TBH 125, Erweka, Germany). Ten tablets from each brand were randomly selected, and each tablet was placed between the jaws of the tester. The force at which the tablets cracked or broken recorded in Newtons (N).

2.2.2 Weight variation:

Twenty tablets were randomly selected from each brand of the vitamin C tablets. The tablets were weighed individually using an electronic analytical balance and the main individual weight recorded. The average weight of each brand was calculated and standard deviations were

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determined. The product passed the test, if not more than two tablets out of 20 falling outside the official percentage limit ±5% to ±10% of the average weight, and none exceedingly twice the allowed limit.

2.2.3 Friability test:

Ten tablets were randomly selected from each brand for this investigation. The tablets were cleaned of adhering powder and weighed prior to testing. In accordance with the manufacturer's instructions, the tablets were placed into the friabilator drum (Pharma Test Apparatebau AG, Germany) and the apparatus was operated for 100 revolutions. After completion the test, the tablets were removed, cleaned of adhering powder and reweighed.

According to USP standards, a weight loss of less than or equal to 1% is considered acceptable for tablet friability.

The friability result was calculated using the following formula:

$$\textbf{Friability (\%)} = [(W1 - W2) / W1] \times 100$$

where W1 represents the initial weight of the tablets and W2 represents the final weight after the test.

2.2.4 Dissolution test:

The dissolution test was performed using dissolution testing apparatus II (DT600, Erweka GmbH, Germany). The apparatus was equilibrated and maintained at a temperature of 37 ±0.5 °C. Six tablets were placed individually into the vessels; each fitted with a paddle. The dissolution medium consisted of 900 mL of distilled water (pH 6.0). The paddle rotation speed was set at 50 rpm.

At predetermined time intervals (10, 20, 30, 40, 50, and 60 minutes), samples of 5 ml were withdrawn and replaced with 5ml dissolution medium. The samples then filtered and diluted 50 times using dissolution medium. The absorbance of the diluted solution was measured at 267 nm using a UV–Visible spectrophotometer (SPECORD 210 PLUS, Analytik Jena, Germany). The concentration of vitamin C in the dissolution medium was subsequently calculated using previously established calibration curve. According to the USP, the tablet complies with the test if each unit contains not less than 75%(Q) of the labeled amount of the drug dissolved within 45 minutes.

2.2.5 Disintegration test:

The disintegration apparatus (Pharma Test PTZS, Germany) was used and equilibrated to maintain at a temperature of 37 ±2.0°C. One tablet from a specific brand was placed in each of the six tubes of the basket assembly, and a disc was positioned appropriately. The apparatus was operated using distilled water as the immersion medium. The time required for each tablet to disintegrate was recorded. The test was stopped after 30 minutes if complete disintegration had not occurred.

According to USP standards, the tablets comply with the test if all six tablets disintegrate completely within 30 minutes. If one or two tablets fail to disintegrate, the test should be repeated using 12 additional tablets. The requirement is considered fulfilled if not fewer than 16 of the total 18 tablets tested disintegrate completely within 30 minutes.

2.2.6 Uniformity of Content test:

The evaluation of a tablet's therapeutic potential requires careful monitoring of drug content uniformity to ensure consistency among individual tablets as well as between different production batches. In this study, ten tablets of each brand were selected and crushed individually into fine powder. Each tablet's powder was dissolved in distilled water, filtered and diluted. The absorbance was measured using UV-Visible spectrophotometer (Specord 210 PLUS, Analytik Jena, Germany) at 267 nm. Drug content was calculated for each tablet and

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compared against USP standards to determine uniformity. According to USP, using a suitable analytical method to determine the individual contents of APIs of 10 tablets taken at random. The tablet complies with the test, if each individual content is between 85% and 115% of the labelled claim content.

2.2.7 Statistical Data Analysis

The obtained results assessment of pharmaceutical equivalence is a critical step in ensuring that four multinational Vitamin C brands perform similarly or different. This statistical method was used to determine whether there were statistically significant differences among the brands with respect to their physicochemical characteristics. To achieve this, two complementary approaches were used: The statistical methods used (ANOVA) and Model-Independent Methods (F1 and F2).

1. Statistically Significant (ANOVA)

One-Way ANOVA (Excel 2016, Microsoft, US). This test determines if there is a significant difference among all the tested brands as a group. A p-value < 0.05 indicate that the brands are significantly different.

2. Similarity and Difference Factors (F1 & F2)

These are the primary regulatory tools recommended by the FDA to compare dissolution curves:

a. The difference Factor (F1): It calculates the percent difference between two curves. For the products to be considered similar, the value must be ≤ 15.

b. The similarity Factor (F2): It measures how close the dissolution profiles are to each other. A value between 50 and 100 ensures that the two brands are similar and interchangeable.

3. Results and discussion

3.1 Weight Variation:

The experimental findings indicated that the average weights of all four vitamin C brands (Brand 1, Brand 2, Brand 3, Brand 4) were greater than 324 mg. According to USP standards, for tablets within this weight range, the permissible deviation from the average weight is ±5%, with no more than two tablets exceed this limit and none exceeding ±10%. The percentage deviations of individual tablet weights from the average weight for each brand are presented in Table 2.

Table 2. Weight variation results

Code	Average weight	Max Deviation
Brand 1	795.53mg	±1.554938%
Brand 2	644.405mg	±3.0873441%
Brand 3	621.86mg	±5.795516%
Brand 4	495.055mg	±7.424427%

For Brands 1 and 2, all tablets complied with the USP weight variation limits none of the tablets deviated by more than ±5% from the average weight. Therefore, all tablets complied with the USP weight variation limits. Brand 3 complied with the USP weight variation standards. Although one tablet deviated by more than ±5%, it remained within the maximum allowable limit of ±10%. Therefore, Brand 3 is considered acceptable and meets the official requirements. Brand 4 did not comply with the USP weight variation specifications, as three individual

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tablets deviated beyond the ±5% limit. Since the pharmacopeial standard permits no more than two tablets to exceed this range, Brand 4 failed the uniformity of weight test.

3.2 Friability:

All tested Brands complied with the USP specifications for friability, except Brand 2 and Brand 4. Brand 2 showed a friability value slightly above the acceptable limit (1.028%), indicating a minor tendency toward mechanical breakage. In contrast, Brand 4 exhibited a remarkably high friability percentage of 7.049%, which severely violates the USP standard (not more than 1%) as shown in Table 3. This substantial deviation indicates that Brand 4 lacks adequate mechanical strength and tablet cohesion, making it highly susceptible to capping, lamination, or crumbling during packaging and transport. The increased friability in both brands, particularly the severe failure in Brand 4, could be attributed to insufficient binder concentration, moisture content, inadequate compression force during manufacturing, or improper storage. Unlike Brand 2, where the deviation is minimal, the significant failure of Brand 4 critically affects the overall product quality.

Table 3. Friability test results

Code	Initial weight	Final weight	% friability
Brand 1	795.59mg	794.21mg	0.1735
Brand 2	645.87mg	639.23mg	1.0280
Brand 3	620.88mg	619.20mg	0.2706
Brand 4	498.76mg	463.60mg	7.049

3.3 Hardness test:

The crushing strength (hardness) of the selected four vitamin C Brands was determined, and the average values along with their standard deviation (SD) are summarized in Table 4. The hardness test results indicate noticeable variations among the four tested Brands. Brands 1 exhibited the highest mechanical strength with an average hardness of 104.3 N, indicating a highly compacted tablet, though it also showed the highest variance (SD= 16.38). In contrast, Brands 2 and 4 recorded the lowest average hardness values at 37N and 39.3N, respectively. Brand 3 demonstrated an intermediate and robust hardness of 70.2N with good consistency (SD=6.18).

Although pharmacopeia (USP) does not specify a strict universal limit for tablet hardness since it largely depends on the formulation intended use (e.g., chewable vs swallowable tablets) a general industrial acceptance criterion for conventional tablets is typically above 40N to ensure structural integrity during handling, packaging, and transportation. Based on these results, Brands 1 and 3 possess strong mechanical integrity, while Brands 4 and 2 are on the softer side (falling slightly below 40N) but may still be acceptable depending on their specific formulation.

Table 4. The hardness results

Code	Average value (N)	Standard Deviation
Brand 1	104.3	16.3847
Brand 2	37	5.8357
Brand 3	70.2	6.1788
Brand 4	39.3	3.4075

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3.4 Disintegration time:

The disintegration time is a crucial physical property of tablets, representing the first step toward drug dissolution and subsequent absorption. The disintegration time for six tablets from each of four brands was determined and the results are shown in Table 5. All tested brands successfully complied with the 30-minute disintegration limit. These results indicate that while all brands meet the disintegration standards; the four tested brands showed notable variability, as Brand 3 demonstrating the most efficient performance through the fastest disintegration (1.26min) and highest consistency (SD=0.36), which directly correlates with the "Burst release" observed in its dissolution profile (Figure 1). While Brand 4 and Brand 2 recorded moderate disintegration averages of 6.02 and 13.50 minutes respectively, and Brand 1 showed the longest time at 20.36 minutes.

Table 5. Disintegration time results

Tab. No	Brand 1	Brand 2	Brand 3	Brand 4
1	18:02	11:00	1:03	2:12
2	18:10	11:01	1:05	3:30
3	20:00	11:02	1:08	5:59
4	21:00	14:00	1:09	6:20
5	21:00	16:00	1:10	8:00
6	24:00	18:00	2:00	10:12
average	20.36	13.50	1.26	6.02
SD	2.21	3.01	0.36	2.91

3.5 Dissolution test:

Dissolution rate is an important quality control parameter that is directly related to the absorption and bioavailability of vitamin C. The present study found that after 45 minutes, the release rate of different brands showed a wide variation, ranging from 38% to 98% (Figure 1). Brand 3 showed the highest percentage of drug release reaching approximately 98% at the 45minutes, indicating excellent dissolution. Brand 2 and Brand 1 followed with a satisfactory release rate of about 85%. Brand 4 had the lowest release at only 38%, which fails to meet the standard dissolution requirements for immediate-release tablets.

This substantial variation among the brands can be attributed to several pharmaceutical factors, including the type and concentration of excipients such as binder, disintegrant, and lubricant, as well as differences in the manufacturing process, particularly the compression force applied during tablet production, or storage conditions.

3.5.1 Statistical Analysis and Pharmaceutical Equivalence:

One-Way ANOVA: The simultaneous comparison of the four Vitamin C brands yielded a p-value of 0.008. Since this result is statistically significant ($p < 0.05$), it indicates substantial variations in the dissolution behavior among the tested brands. However further pairwise comparison revealed an exception between Brand 1 and Brand 2; no significant difference was observed between them ($P= 0.363$), suggesting pharmaceutical equivalence between these two specific Brands.

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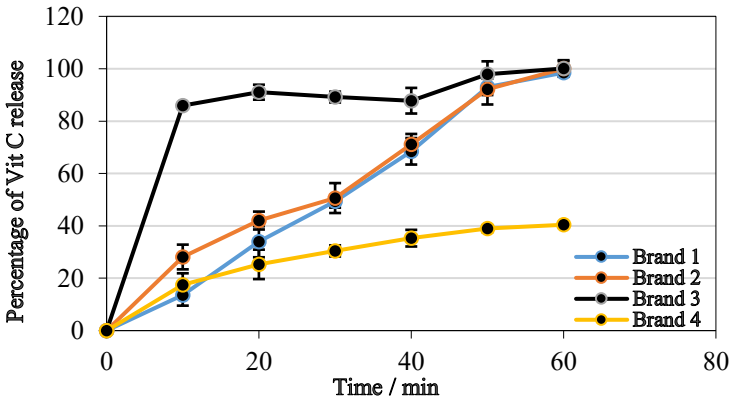


Figure 1. The dissolution profile of different vitamin C Brands

Evaluation of Similarity (f1 and f2):

Table 6 summarizes the mathematical comparison between the brands in their dissolution performance. The comparison between Brand 1 and Brand 2 was the only one met the FDA requirements for similarity (f2 =57.70). This suggest that these two formulations are likely manufactured using similar excipients and compression forces, leading to an equivalent dissolution rate that ensures identical therapeutic performance.

Table 6. Statistical Analysis of the Dissolution Performance of Different Brands

Comparison Brands	f1(Limit ≤ 15)	f2 (Limit ≥ 50)	Result
Brand 1 vs. Brand 2	7.62	57.70	Similar
Brand 1 vs. Brand 4	47.35	22.07	Not Similar
Brand 2 vs. Brand 4	51.08	21.24	Not Similar
Brand 2 vs. Brand 3	43.70	22.51	Not Similar
Brand 3 vs. Brand 4	65.96	10.76	Not Similar
Brand 1 vs. Brand 3	54.64	18.94	Not Similar

In contrast, the pairwise comparison between the other brands specifically Brand 1vs. Brand 4, Brand 2 vs. Brand 3, and Brand 2 vs. Brand 4 showed severe deviations in their dissolution profiles. These brands failed to meet the pharmaceutical equivalence criteria, as indicated by the low f2 values. For instance, the comparison between Brand 3 and Brand 4 resulted in an f2 of only 10.76, which is far below the required limit of 50. Furthermore, the high f1 values (65.96) confirm a large relative difference in the amount of drug released at each interval. This indicates that the rate and extent of Vitamin C release vary significantly between these manufacturers.

3.6 Uniformity of Content:

The determination of the active pharmaceutical ingredient (API) concentration is a fundamental quality control parameter to ensure patient safety and therapeutic efficacy. The acceptable pharmacopeial limit for vitamin C ranging from 85% to 115% of the labeled claim. Assuming a standard label claim of 500 mg, the results displayed in Figure 2 reveal that Brand 1 exhibited the most accurate formulation with a

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concentration of 487mg (97.4%), while Brands 2 and 3 showed higher concentration of 569mg (113.8) and 563mg (112.6%) respectively. Although these values are near the upper limit, they remain within the acceptable range. In contrast, Brand 4 failed the test significantly, recording a critically low concentration of only 292 mg (58.4%), which is severely below the minimum required limit. This significant deficiency in API concentration directly explains the poor dissolution profile previously observed for Brand 4 (Figure 1), confirming that the formulation lacks the necessary drug content to achieve therapeutic efficacy. This significant deficiency in API concentration can be attributed to major formulation or manufacturing problems, including possible blending failure or segregation of the active pharmaceutical ingredient, or it can be ascribed to improper storage or packaging which may expose API to external environment, as Vitamin C is known to be highly affected by a variety of environmental factors, including pH, temperature, oxygen, light and moisture that lead to reduced stability for vitamin C.

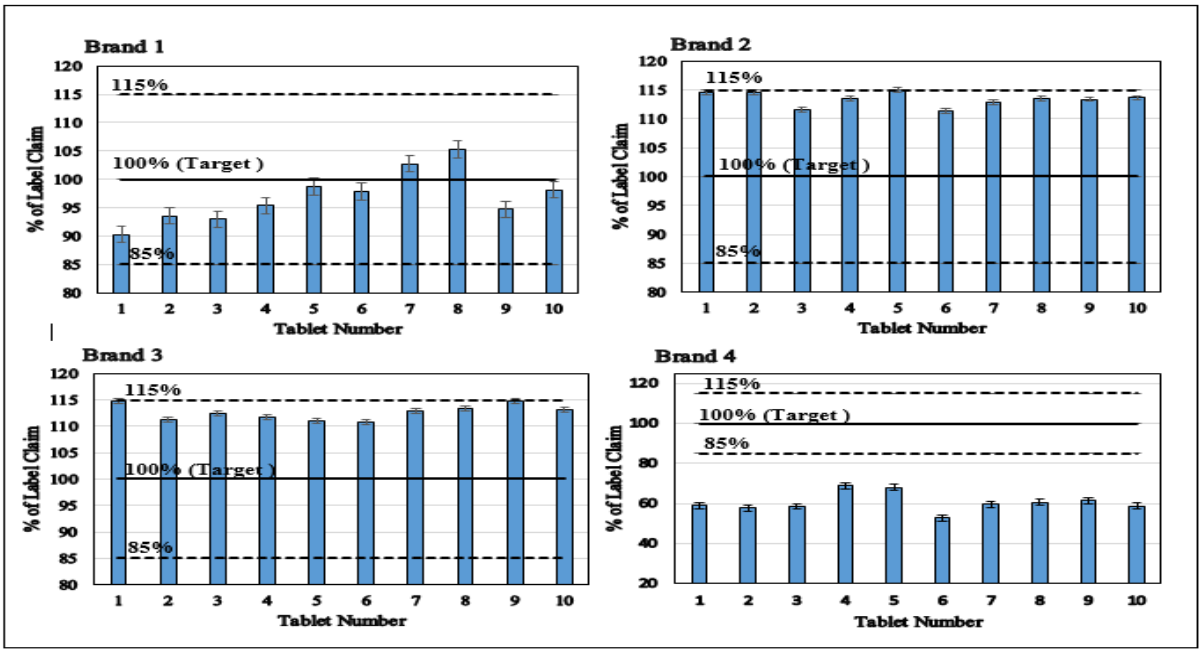


Figure 2. The content uniformity of different vitamin C Brands

4. Conclusion

The therapeutic efficacy of vitamin C depends on its pharmaceutical quality. Therefore, strict quality control is essential to ensure the safety, stability, and uniformity of vitamin C tablets across different brands. This study evaluated the in-vitro pharmaceutical quality of four 500 mg vitamin C brands available in the Libyan market against official pharmacopeial standards. The findings revealed significant variations in the physicochemical characteristics and in-vitro performance among the tested products. While most of the evaluated brands demonstrated high pharmaceutical quality and complied with the required standards for hardness and disintegration, critical deviations were observed.

Both Brand 2 and Brand 4 failed the friability test. While Brand 2 showed a minor deviation, Brand 4 exhibited a severe failure in friability, indicating a critical lack of mechanical strength and tablet cohesion during handling and transportation. In addition, Brand 4 showed

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comprehensive quality deficiencies, failing the weight variation, content uniformity, and dissolution tests. These accumulated failures may drastically affect the consistency of the delivered dose and the release of the active pharmaceutical ingredient, potentially reducing or compromising the therapeutic effectiveness of the product.

Both Brand 1 and Brand 3 successfully complied with all quality control tests. However, Brand 3 demonstrated superior performance, achieving a faster drug release rate in the dissolution test and a shorter disintegration time compared to the other brands. In conclusion, the findings indicate that not all vitamin C brands available in the Libyan market meet the required pharmacopeial standards. Continuous quality control testing, strict regulatory supervision, and proper storage conditions are essential to ensure that pharmaceutical products distributed in the market provide safe, effective, and reliable therapeutic outcomes.

CONFLICT OF INTEREST

The authors declare no conflict of interest

AUTHORS' CONTRIBUTIONS

F. S: Methodology Conceptualization, Investigation, writing original draft, Writing review & editing. A.A: Resources, Writing review & editing. M. A: Methodology & editing Investigation. O. A: Writing review & editing. A.S.A: Methodology, writing original draft. S. A: Methodology, writing original draft. A.S: Methodology, writing original draft.

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β-Fibrinogen −455G>A (rs1800790) in a sample of Libyan Patients with Cardiovascular Disease: A Pilot Study with Hardy–Weinberg Assessment

Reem M. El Gmati[a], Salem H. Abukres[b], Abdulrzag F. Ahmed[c*].

[a] School of Basic Sciences, Libyan Academy for Postgraduate Studies, Libya

[b] Faculty of Pharmacy, Elmergib University & Faculty of Pharmacy Alasmarya Islamic University, Libya

[c] Libyan Academy for Postgraduate Studies, Libya

*Corresponding author Email: a.ahmed@academy.edu.ly

A B S T R A C T

Background: Cardiovascular and thromboembolic diseases are major contributors to morbidity in Libya, yet the distribution and potential relevance of coagulation-related genetic variants—including the β-fibrinogen −455G>A promoter polymorphism (rs1800790)—remain poorly characterized in North African populations.

Aims: To provide a preliminary descriptive characterization of rs1800790 in Libyan patients with cardiovascular disease, and to document the methodological considerations—principally Hardy–Weinberg equilibrium (HWE) deviation and treatment confounding—that should inform future adequately powered studies.

Methods: This descriptive case series was conducted at Al Fouad Cardiac Center, Tripoli, Libya, between April and August 2025. Forty-four Libyan patients with established cardiovascular disease were included. rs1800790 was genotyped using the CVD StripAssay (ViennaLab Diagnostics GmbH). Genotyping succeeded at first pass in 38 of 44 patients; the remaining six were repeated once from the same DNA and all gave interpretable results. HWE was assessed by an exact test. Four imputed D-dimer entries were identified and excluded. Three post hoc exploratory comparisons by genotype (coronary artery disease, coronary stenting, and D-dimer >1.0 µg/mL) used Fisher's exact test with a single Haldane–Anscombe-corrected odds ratio estimator and Bonferroni correction ($\alpha = 0.0167$).

Results: Only G/G (16; 36.4%) and G/A (28; 63.6%) genotypes were observed. The genotype distribution deviated from HWE (conditional exact $P = 0.0017$; Pearson $\chi^2 = 9.58$, 1 df, $P = 0.0020$), with a heterozygote excess and absent A/A homozygotes. This is reported as a quality-control finding requiring investigation, not as proof of assay failure. Among the 40 patients with a measured D-dimer, a value >1.0 µg/mL was more frequent in G/A than G/G patients (14/24, 58.3% vs 1/16, 6.3%; corrected OR 14.3, 95% CI 2.2–91.2; Fisher's exact $P = 0.0009$), and this survived Bonferroni correction. Coronary artery disease (85.7% vs 56.3%; corrected OR 4.30; $P = 0.067$) and coronary stenting (64.3% vs 37.5%; corrected OR 2.85; $P = 0.12$) did not differ significantly. Lipid values were treatment-confounded, with 88.6% of patients receiving statins.

Conclusions: This report offers preliminary genotype data for rs1800790 in Libyan patients with cardiovascular disease. The HWE deviation, absence of A/A homozygotes, lack of an age-comparable control group and absence of orthogonal validation mean that the results are hypothesis-generating only. The post hoc association with raised D-dimer warrants confirmation in a larger, validated, case–control study.

Keywords: *β-fibrinogen; FGB; rs1800790; cardiovascular disease; short report; Hardy–Weinberg equilibrium; Libya.*

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1. Introduction

Cardiovascular disease remains a leading cause of morbidity and mortality worldwide, and thromboembolic events—including arterial thrombosis and venous thromboembolism—are important clinical manifestations of vascular disease. Virchow's triad attributes thrombus formation to endothelial injury, altered blood flow and hypercoagulability, with fibrinogen playing a central structural role as the precursor of fibrin (1,2). Fibrinogen (Factor I) is a hepatic glycoprotein that thrombin converts into fibrin, the structural matrix of both physiological homeostatic clots and pathological thrombi (3). The β chain is encoded by FGB on chromosome 4q31.3 (4). The −455G>A promoter polymorphism (rs1800790) has been associated in some populations with altered fibrinogen expression and circulating fibrinogen concentration, and because fibrinogen concentration and fibrin structure may influence thrombus formation and persistence, the variant has been investigated as a potential cardiovascular risk modifier (5–9).

Despite evidence of population genetic structure and a substantial burden of cardiometabolic disease, data on thrombophilia-related variants in North African and specifically Libyan populations remain limited (10,11). Multiplex reverse-hybridization platforms such as the CE-IVD-marked CVD StripAssay provide an accessible route to detecting multiple cardiovascular risk alleles in resource-constrained settings. However, the analytical performance of such platforms in understudied populations requires explicit quality-control assessment.

Aims:

This short report therefore aims to (i) describe the genotype and allele distribution of the β-fibrinogen −455G>A variant in Libyan patients with cardiovascular disease, using HWE as a quality-control check; and (ii) record exploratory observations and methodological limits that can inform future adequately powered studies. The three genotype comparisons reported are post hoc and exploratory.

2. Methods

2.1 Study design and setting

This descriptive case series was conducted between April and August 2025 at Al Fouad Cardiac Center, Tripoli, Libya. Molecular assays were performed at the Carthage International Laboratory for Research and Medical Analysis. The study was designed as a feasibility and descriptive investigation using existing clinical samples, not as a case–control study. The work was self-funded by the authors; no institutional, national or international grant supported the laboratory analyses, sample collection or personnel time.

2.2 Participants selection

Eighty-two Libyan adults were screened (Figure 1). Twenty-six were excluded because of suboptimal genomic DNA yield or purity (A260/A280 outside 1.7–1.9), severe systemic comorbidity considered confounding, or missing clinical or pharmacological data. The remaining 56 comprised 44 patients with cardiovascular disease (aged 34–80 years) and 12 healthy volunteers (aged 25–50 years; mean 36.8 ± 7.1 years). Because the volunteers were substantially younger than the patients (64.0 ± 11.7 years) and lacked their comorbidity profile, they were not used as a comparison group; their genotypes are reported for completeness only. Cases had established cardiovascular disease, verified by standard diagnostic imaging or clinical criteria. The original classification counted three records with cerebrovascular or vascular diagnoses as coronary artery disease. These three records were re-adjudicated against the clinical notes: each was retained as

Corresponding author Email: a.ahmed@academy.edu.ly

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coronary artery disease only where coronary disease was independently documented, and otherwise reclassified as other vascular disease. The revised counts are given in Table 1. Disease categories overlapped in some patients; the categories are therefore not mutually exclusive, and the sum of category counts exceeds the number of patients. All genotype-stratified comparisons are within case.

2.3 Sample size and statistical power

No a priori sample size calculation was performed, because this was a pilot descriptive study. For future work, a G*Power calculation (version 3.1.9.7) for a two-group comparison under a dominant model (control minor allele frequency 0.20; carrier frequency 0.36; odds ratio 1.8; $\alpha = 0.05$; power 80%) indicated a requirement of approximately 187 participants per group (approximately 375 in total). The present sample of 44 patients is well below this, and no multivariable modelling was attempted, because 44 cases support at most one or two covariates.

2.4 Ethics

This study was conducted in accordance with the Declaration of Helsinki. Ethical approval was obtained from the National Committee for Biosafety and Bioethics in Libyan Academy of Postgraduate Studies ((Reference: NBC: 026.H.25.7), 26 Jan 2025). Written informed consent was obtained from all participants.

2.5 Genotyping protocol

Genomic DNA was isolated from EDTA-anticoagulated whole blood using a standard salting-out extraction method. DNA concentration and purity were assessed by NanoDrop spectrophotometry, and A260/A280 ratios between 1.7 and 1.9 were required for inclusion; DNA quality metrics were recorded for all samples.

The −455G>A variant was assessed by multiplex PCR followed by reverse hybridization to allele-specific oligonucleotide probes immobilized on nitrocellulose strips (CVD StripAssay, ViennaLab Diagnostics GmbH) according to the manufacturer's instructions. Genotype calls were taken from the strip-reading sheet; observers were not formally blinded. Manufacturer-supplied positive and negative controls were run with each batch, and the built-in control band on each strip confirmed the detection step. Genotyping succeeded at first pass in 38 of 44 patients; the six samples with failed amplification or uninterpretable strips were repeated once from the same DNA, and all six then gave an interpretable result. Because of resource constraints, no orthogonal validation (for example, Sanger sequencing) was performed, and genotypes are therefore reported as strip-assay calls.

2.6 D-dimer measurement

D-dimer was measured on [platform] and is reported in [fibrinogen-equivalent units (FEU) / D-dimer units (DDU)]; the cutoff of 1.0 µg/mL used here is a conventional threshold for an elevated result and was not derived from this dataset. Samples were collected during the index

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admission, and anticoagulant status at the time of sampling was recorded in the clinical notes. Because event timing and anticoagulation may influence D-dimer independently of genotype, the continuous comparison is presented as the primary analysis and the dichotomised comparison as secondary.

2.7 Statistical framework

Descriptive statistics are presented as means ± standard deviations, medians (interquartile range), or n/N (%), as appropriate. Genotype frequencies are given with Wilson 95% confidence intervals. Allele frequencies are reported as naive descriptive estimates without confidence intervals, because the genotype distribution departs from HWE and the usual interval assumes independent allele copies; a valid interval would require a model for the departure, which this dataset cannot support. HWE was tested with a conditional exact test, with the Pearson chi-square goodness-of-fit result given for comparison. Only measured laboratory values were analysed. Prothrombin time, activated partial thromboplastin time, troponin and electrolytes were unavailable for most patients and are not reported. Four D-dimer entries that had been imputed during data entry rather than measured were identified and excluded; no imputation was performed for the reported analysis, leaving 40 patients with a measured D-dimer.

Three post hoc exploratory comparisons by genotype (coronary artery disease, coronary stenting, and D-dimer >1.0 µg/mL) used Fisher's exact test. These three comparisons constitute the multiple-testing family; Bonferroni correction was applied within this family ($\alpha = 0.0167$), and no other comparisons were corrected. Odds ratios are reported with a single estimator throughout—the Haldane–Anscombe correction with Woolf 95% confidence intervals—applied to all three comparisons; raw odds ratios are given in a footnote for transparency where a zero cell is present. D-dimer as a continuous variable was compared by the Mann–Whitney U test. These comparisons were not pre-specified, are unadjusted, and are hypothesis-generating only. Smoking status was not recorded. All tests were two-sided. Analyses were performed in SPSS version 27, with HWE exact tests computed in Python (SciPy).

3. Results

3.1 Baseline characteristics of cases

The final analytical cohort comprised 44 patients with a mean age of 64.0 ± 11.7 years; 26 (59.1%) were female. Coronary artery disease was present in [revised n] patients ([revised %]), hypertension in 29 (65.9%), and type 2 diabetes mellitus in 16 (36.4%). Coronary stenting was recorded in 24 patients (54.5%), and 39 patients (88.6%) were receiving statin therapy at the time of assessment (Table 1).

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Table 1. Baseline characteristics of cases (n = 44).

Characteristic	Value
Age, years (mean ± SD)	64.0 ± 11.7
Female sex, n (%)	26 (59.1)
Coronary artery disease, n (%)	33 (75.0)
Hypertension, n (%)	29 (65.9)
Type 2 diabetes, n (%)	16 (36.4)
Coronary stenting, n (%)	24 (54.5)
Statin use, n (%)	39 (88.6)

Note: No control group was included. Smoking status was not recorded. Disease categories are not mutually exclusive; the sum of category counts exceeds the number of patients. CAD, coronary artery disease.

3.2 Genotyping performance and Hardy–Weinberg equilibrium

Only G/G and G/A genotypes were observed; no A/A homozygotes were detected. G/G occurred in 16 patients (36.4%; 95% CI 23.8–51.1) and G/A in 28 patients (63.6%; 95% CI 48.9–76.2) (Supplementary Table S1). The naive G and A allele frequencies were 0.682 and 0.318; these are descriptive only, and no confidence interval is reported for the reasons given in the Statistical framework. Twelve healthy volunteers were also genotyped (7 G/G, 5 G/A; 41.7% G/A); because they were much younger than the patients, this is not presented as a case–control analysis, and for completeness the G/A proportion did not differ significantly between patients and volunteers (63.6% vs 41.7%; Fisher's exact P = 0.20).

The genotype distribution deviated from HWE (conditional exact P = 0.0017; Pearson χ^2 = 9.58, 1 df, P = 0.0020). Expected counts were 20.5 G/G, 19.1 G/A and 4.5 A/A, against observed counts of 16, 28 and 0—a heterozygote excess with no A/A homozygotes. Because genotypes were not independently confirmed, this deviation is reported as a quality-control finding that requires investigation in the affected sample. A heterozygote excess is compatible with genotyping error, for example cross-hybridization or allele-specific probe performance on reverse-hybridization strips, but it is not diagnostic of it; sampling variation and population structure are alternative explanations that this dataset cannot distinguish.

3.3 Coagulation biomarkers

D-dimer was the only coagulation measure available for most patients and was measured in 40 of 44 (24 G/A and 16 G/G); four imputed values were excluded and no values were imputed. Other coagulation tests (prothrombin time, activated partial thromboplastin time) were recorded for too few patients to analyses. Mean D-dimer concentration was 0.57 ± 0.43 µg/mL (median 0.52, IQR 0.32–0.66) in G/G patients and 1.87 ± 2.09 µg/mL (median 1.26, IQR 0.69–1.94) in G/A patients (Mann–Whitney U, P = 0.0006). A D-dimer concentration

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>1.0 µg/mL was recorded in 1 of 16 G/G patients (6.3%) and 14 of 24 G/A patients (58.3%) (corrected OR 14.3, 95% CI 2.2–91.2; Fisher's exact P = 0.0009; raw OR 21.0), which remained significant at the Bonferroni-corrected threshold of 0.0167. Age did not explain the difference: mean age was similar between the genotype groups (64.4 ± 10.2 years in G/G and 63.7 ± 12.6 in G/A), with no correlation between age and D-dimer. The confidence interval is wide, the genotypes are unverified, the comparison was post hoc, and D-dimer may reflect the timing of the event and anticoagulant treatment; this therefore does not establish a genotype–phenotype association.

Table 2. D-dimer by genotype (exploratory only).

Biomarker	G/G (n = 16)	G/A (n = 24)
D-dimer, µg/mL (mean ± SD)	0.57 ± 0.43	1.87 ± 2.09
D-dimer, µg/mL (median, IQR)	0.52 (0.32–0.66)	1.26 (0.69–1.94)
D-dimer >1.0 µg/mL, n (%)	14 (58.3)	

Note: Genotypes are unverified (see Supplementary Table S1). The continuous comparison (Mann–Whitney U, P = 0.0006) is the primary analysis; the dichotomised comparison was post hoc (Fisher's exact P = 0.0009; Bonferroni α = 0.0167) and is exploratory. Corrected OR 14.3 (95% CI 2.2–91.2); raw OR 21.0. Measured values only; four imputed values excluded.

3.4 Lipid profile

Mean total cholesterol was 161.8 ± 46.4 mg/dL among G/G patients and 134.1 ± 45.5 mg/dL among G/A patients. Mean LDL cholesterol was 103.1 ± 38.7 mg/dL and 76.9 ± 28.9 mg/dL, respectively. Mean HDL cholesterol was 43.8 ± 9.6 mg/dL and 39.7 ± 14.4 mg/dL, while mean triglyceride concentrations were 152.4 ± 106.7 mg/dL and 126.9 ± 80.9 mg/dL, respectively (Table 3). Statin therapy was recorded in 12 G/G patients (75.0%) and 27 G/A patients (96.4%); overall, 39 of 44 participants (88.6%) were receiving statins. Because lipid measurements were obtained in the context of substantial statin use, these values were considered treatment-confounded and were not interpreted as evidence of a genetic effect.

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Table 3. Lipid profile by genotype (exploratory only; descriptive, no hypothesis tests).

Lipid parameter	G/G (n = 16)	G/A (n = 28)
Total cholesterol, mg/dL (mean ± SD)	161.8 ± 46.4	134.1 ± 45.5
LDL cholesterol, mg/dL (mean ± SD)	103.1 ± 38.7	76.9 ± 28.9
HDL cholesterol, mg/dL (mean ± SD)	43.8 ± 9.6	39.7 ± 14.4
Triglycerides, mg/dL (mean ± SD)	152.4 ± 106.7	126.9 ± 80.9

Note: Lipid data are treatment-confounded (88.6% on statins). Genotypes are unverified. No hypothesis tests were performed; these data are descriptive only.

3.5 Comorbidity distribution

Among the 44 cases, coronary artery disease was reported in 9 G/G patients (56.3%) and 24 G/A patients (85.7%) (Corrected OR 4.30; raw OR 4.67; Fisher's exact P = 0.067), which did not meet the Bonferroni-corrected threshold. Coronary stenting was recorded in 6 G/G patients (37.5%) and 18 G/A patients (64.3%) (Corrected OR 2.85; raw OR 3.0; P = 0.12). Hypertension was present in 10 G/G patients (62.5%) and 19 G/A patients (67.9%), type 2 diabetes mellitus in 7 (43.8%) and 9 (32.1%), and cerebrovascular events in 2 (12.5%) and 3 (10.7%) (Supplementary Table S2). No adjusted analysis was possible with this sample size. The coronary artery disease count reflects the re-adjudication described in the Methods; the recalculated test uses the revised denominator.

4. Discussion

4.1 Principal findings

This short report provides preliminary observations on the β-fibrinogen −455G>A variant among 44 Libyan patients with cardiovascular disease. The G/G genotype was observed in 16 patients (36.4%) and the G/A genotype in 28 (63.6%), with a naive A-allele frequency of 0.318; no A/A homozygotes were detected. The genotype distribution deviated from HWE (conditional exact P = 0.0017), which is the principal methodological finding. A D-dimer concentration >1.0 µg/mL was more frequent in G/A than in G/G patients and met the corrected significance threshold, but this post hoc, unadjusted observation in unverified genotypes cannot establish a causal genotype–phenotype relationship. The higher frequencies of coronary disease and stenting in G/A patients were not significant. Lipid differences cannot be interpreted independently of the high prevalence of statin treatment.

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4.2 Hardy–Weinberg equilibrium deviation: implications and possible mechanisms

The observed deviation from HWE requires careful interpretation. The counts show an excess of heterozygotes and no A/A homozygotes (4.5 expected). A heterozygote excess can be a signal of genotyping error—for example, cross-hybridization or allele-specific probe performance on reverse-hybridization strips. Sampling variation, population structure or recruitment characteristics are other possible explanations, and the small sample and absence of orthogonal validation do not allow these to be distinguished. The appropriate response to this finding is to investigate the affected sample: by re-genotyping, by sequencing a subset that includes every heterozygote, and by examining whether assay failures cluster by batch or by DNA quality. The frequencies are therefore best read as provisional strip-assay results, and the HWE check is reported to alert readers and later investigators rather than to establish that the assay failed.

The initial failure of a proportion of assays and the subsequent re-genotyping further support the need for independent confirmation. Future studies should confirm genotypes with Sanger sequencing or another validated reference method, at least for a predefined subset that includes every heterozygote, particularly when an unexpected genotype distribution is observed.

4.3 Comparison with previous studies

Direct comparison of the genotype and allele frequencies observed here with those reported in other populations should be interpreted cautiously, because the present genotypes were not independently validated and the observed distribution deviated from HWE. In Asian populations, the −455A allele has been associated with coronary heart disease in a meta-analysis (5), and with cardioembolic stroke in atrial fibrillation (8); in Chinese patients with cerebral infarction, the variant has been associated with plasma fibrinogen level (9). In the ECTIM study, β-fibrinogen gene polymorphisms were associated with plasma fibrinogen and coronary artery disease in patients with myocardial infarction (6). These findings are not directly comparable with the present cohort, which is small, lacks a control group and did not measure fibrinogen concentration. Validated future studies with an appropriate control group, direct fibrinogen quantification and standardized phenotyping will be better positioned to determine whether the Libyan population differs from other populations in the distribution of rs1800790 and in its relationship with cardiovascular phenotypes.

4.4 Study limitations

Several limitations should be considered, and most of them follow directly from the self-funded nature of the study, which precluded both expansion of the cohort and any additional confirmatory experiments. First, the study had no age-comparable control group; the 12 healthy volunteers (mean age 36.8 years) were much younger than the patients (64.0 years). Second, genotypes came from a single strip assay without orthogonal validation, and the HWE deviation (P = 0.0017) limits confidence in them; Sanger sequencing, which could help evaluate this uncertainty, was not possible because the project was self-funded and sequencing costs were beyond the available budget. Third, the sample of 44 is far below the roughly 375 participants (about 187 per group) that a case–control comparison would require, and the cohort could not be enlarged because recruitment and genotyping were entirely financed by the authors. Fourth, the three genotype

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comparisons were post hoc and unadjusted for confounders, and no multivariable model was possible. Fifth, the case group was clinically heterogeneous (coronary, cerebrovascular and thrombotic disease), and 88.6% of patients were receiving statins, confounding the lipid data. Sixth, several laboratory variables—including prothrombin time, activated partial thromboplastin time and troponin—were unavailable for most patients and were not analysed; D-dimer was missing for four patients; smoking status was not recorded; and the timing of sampling and anticoagulant use may confound D-dimer. Seventh, the study was conducted at a single centre. Eighth, direct fibrinogen concentrations were not measured, so the functional relationship between rs1800790 and fibrinogen could not be evaluated. Ninth, the three records initially classified as coronary artery disease on the basis of cerebrovascular or vascular diagnoses were re-adjudicated, but the reclassification was based on the available clinical notes and was not independently reviewed. Tenth, four D-dimer values had been imputed during data entry without a documented statistical procedure; they were removed, but the reason for the original imputation could not be reconstructed. These limits reflect the resources available for this pilot, and the data are offered so that they can be built upon by better-resourced groups.

4.5 Research and clinical implications

The findings are descriptive and hypothesis-generating and should not influence clinical practice. The report adds genotype data from a population with little published information on this variant, and it illustrates the value of HWE checking and independent genotype confirmation in cardiovascular genetic studies in settings with limited resources. Future studies should include adequately powered, age-matched controls, genotype confirmation, direct fibrinogen quantification and standardized phenotyping, and could test the D-dimer observation with adjustment for event timing and anticoagulation.

5. Conclusions

This short report describes the β-fibrinogen −455G>A genotype distribution in 44 Libyan patients with cardiovascular disease. Only G/G and G/A genotypes were observed, and the distribution deviated from HWE. The study was self-funded, so the sample could not be enlarged and confirmatory Sanger sequencing could not be performed. Because of the small sample, the absence of an age-comparable control group, the absence of orthogonal validation and treatment confounding, the findings cannot establish an association between rs1800790 and cardiovascular disease. They provide preliminary regional data and a hypothesis—a possible link with raised D-dimer—for larger validated studies.

ETHICALSTATEMENT

Ethical approval was obtained from the National Committee for Biosafety and Bioethics in Libyan Academy of Postgraduate Studies ((Reference: NBC: 026.H.25.7), 26 Jan 2025). Written informed consent was obtained from all participants.

CONFLICT OF INTEREST

The Authors proclaim no conflict of interest.

AUTHORS CONTRIBUTIONS

R.G. and A. A performed the laboratory work. S. A. conducted the statistical ,analysis. The original draft of the manuscript was written by R.G and S. A, reviewed and edited by A. A. All authors have read and agreed to the published version of the manuscript.

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Corresponding author Email: a.ahmed@academy.edu.ly

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Supplementary Files

Supplementary Table S1. Genotype frequencies by strip assay (unverified, exploratory).

Genotype	Cases (n = 44)
G/G, n (%)	16 (36.4)
G/A, n (%)	28 (63.6)
A/A, n (%)	0 (0.0)

These genotype frequencies were derived from the strip assay after repeating initially failed samples. No orthogonal validation was performed. The data should not be used for definitive association analyses, population comparisons, or clinical inference.

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Supplementary Table S2. Comorbidity distribution by genotype (exploratory only).

Comorbidity	G/G (n = 16), n (%)	G/A (n = 28), n (%)
Hypertension	10 (62.5)	19 (67.9)
Type 2 diabetes	7 (43.8)	9 (32.1)
Cerebrovascular event	2 (12.5)	3 (10.7)

Corresponding author Email: a.ahmed@academy.edu.ly

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Isolation and Identification of Bacterial Pathogens from Hemodialysis Patients at Zliten Dialysis Center and Evaluation of Their Antibiotic Susceptibility Patterns

Malak Muamer Nasef [a], Amna RamadanAbolbeda [a&*] , Salema Asdaea Alglla [a] , Heba Mohamed Albrbar [a], Alryan Usef Akasha [a], Wesal Salem Algonde [a]

[a] Faculty of Science, Al Asmarya Islamic University, Zliten, Libya.

* Corresponding author Email, a.abolbida@asmary.edu.ly

A B S T R A C T

Background: The most complication that Hemodialysis patient suffer from is get infected by microorganisms awhile they do the treatment these infections cause serious complications for dialysis patient especially catheter related bloodstream infection (CRBSIs).

Aims: This study amid to isolate isolation and identification of bacterial pathogens associated with bloodstream infections and bacterial colonization of venous catheter sites from hemodialysis patients in Zliten City Libya., Then determine the major bacterial species and test their antimicrobial susceptibility patterns.

Methods: A total of 124 clinical specimens were collected, comprising 62 catheter-site swab samples from peripheral venous catheter sites were conducted from hemodialysis patients and processed using standard microbiological procedures. The samples were cultured on appropriate selective and differential culture media and incubated under suitable conditions. The bacteria were examined based on colony morphology, microscopic characteristics, and Gram staining. Identification was performed using a series of conventional biochemical tests. Antimicrobial susceptibility testing was carried out to determine the susceptibility patterns of the isolated bacterial strains to commonly used antibiotics for hemodialysis patients.

Results: The results a total of 124 clinical specimens, comprising 62 catheter-site swab samples and 62 blood samples. 62 swab sample .93.55% (58 samples) showed bacterial growth, While 6.45% (4 sample) no bacterial growth. Among the 62 blood samples, showed result 22 bacterial growth. show the presence of several bacterial species associated with catheter sites and bloodstream infections. Antimicrobial susceptibility testing showed varying degrees of resistance among the bacterial isolates, with some strains exhibiting resistance to multiple classes of antibiotics. The results suggest the presence of multidrug-resistant bacterial strains and the potential risk of antimicrobial resistance within the hospital environment.

Conclusion: catheter-related infections are the most concern for hemodialysis patients. Infection prevention and control practices, particularly appropriate catheter insertion, aseptic techniques, sterilization, and catheter care, is essential to reduce the risk of infection. In addition, antimicrobial therapy should be done by laboratory-based antimicrobial susceptibility testing. Continuous microbiological surveillance and rational antibiotic use are recommended to reduce the emergence and dissemination of antimicrobial-resistant bacteria among hemodialysis.

Keywords: *Hemodialysis, catheter-related bloodstream infection, bacterial pathogens, antimicrobial susceptibility, multidrug resistance*

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1. Introduction

Renal failure is major health problems worldwide. It is a serious medical condition resulting from the inability of the kidneys to perform their vital functions adequately. This leads to the accumulation of waste products and toxins in the body and negatively affects various organs and the immune system, thereby reducing the body's ability to resist disease [1].

As a result of this impaired immune function, patients with chronic renal failure are more susceptible to bacterial infections, which represent a major challenge in their healthcare management. The danger of these infections is not limited to increasing patient suffering and worsening their health condition, they may also increase mortality rates and impose substantial healthcare **costs [**2]. To address this problem effectively, it is essential to accurately understand the types of bacteria responsible for infections among with chronic renal failure and determine their susceptibility and resistance to different antibiotic. This local knowledge is important for guiding physicians in selecting appropriate treatment quickly and effectively, particularly during the early stages before laboratory test results become available. It also contributes to developing strategies to prevent the spread of infections within hemodialysis centers [3]. The Zliten Dialysis Center is an important healthcare facility that provides services to large number of patients with chronic renal failure in the region. Since the environment of hemodialysis unite may provide favorable conditions for the transmission of certain types of infection, it is important to conduct a scientific study focusing on identifying the bacterial species responsible for infections among patients attending center **[**3,4].

Obtaining local date on these bacteria and their antibiotic resistance patterns will help made clearer treatment decisions and develop preventive measures specifically designed to meet the needs of patients in Zliten [5,6].

Aims:

This study aimed to isolate and identify the bacteria pathogens associated with bloodstream infections and bacterial colonization of venous catheter sits among hemodialysis patients in Zliten**,** Libya. The study also aimed to determine the most prevalent bacterial species and assess their antimicrobial susceptibility patterns.

2. Material and Methods

2.1 Sample Collection

This cross-sectional study was conducted from April to August 2025 in Zliten Dialysis Canter and its affiliated units in Ramaya and Dawao. A total of 124,62 swab samples and 62 blood culture were collected, comparing 50 males and 12 females, with ages ranging from 5 to 80 years.

2.2 Culture Media

Table 1: Several culture media were used for the isolation

No.	Culture Medium
1	MacConkey agar
2	Blood agar
3	Chocolate agar
4	Nutrient agar

* Corresponding author Email, a.abolbida@asmary.edu.ly

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2.3 Antibiotics

Table 2: The following antimicrobial agents were used for antimicrobial susceptibility testing

No.	Antibiotic	Disc Code
1	Ampicillin	AM-10
2	Imipenem	IPM-10
3	Nitrofurantoin	F-300
4	Chloramphenicol	C-5
5	Sulfamethoxazole/Trimethoprim	SXT-25
6	Cefuroxime	CXM-30

2.4 Collection Procedure

2.4.1 Collection of Catheter-Site Swab Sample

Catheter-site swab samples were collected using sterile swabs containing appropriate transport media. Samples were obtained from the skin surrounding the vascular catheter insertion or exit site. Aseptic precautions were maintained throughout the sampling procedure to minimize the possibility of contamination. Appropriate personal protective equipment, including gloves facemasks, was used during sample **collection.** Then identification number were recorded and the swabs were transport to the laboratory for streaking on culture media. The petri **was** incubated at 37 °C for 24 hours. Following incubation, the plates were examined for bacterial growth. Individual colonies with distinct morphological characteristics were selected for further identification. Also, identification was performed using Gram staining and conventional biochemical tests.

2.4.2 Collection and Processing of Blood Samples

Blood samples were collected from the patients using stranded aseptic procedures to minimize contamination. The blood collection site was disinfected appropriately before sample collection, and sterile equipment and personal protective equipment were used. The required volume of blood was collected aseptically and immediately inoculated into an appropriate blood culture bottle. Each blood culture bottle was labeled with the relevant patient identification information and transport promptly to the laboratory. The blood culture bottles were incubated at 37°C according to the laboratory's standard procedures. Following incubation, bottles showing evidence of bacterial growth were processed for subculture. The external surface of the bottle was disinfected before sampling. Using sterile technique, a portion of the blood culture was removed and inoculated onto appropriate culture media. The inoculum was streaked using the streak-plate technique to obtain isolated bacterial colonies. The inoculated plates were incubated at 37°C for approximately 18-24 hours. After incubation, the plates were examined for bacteria growth, and representative colonies were selected for identification.

2.4.3 Identification and Antimicrobial Susceptibility Testing Using the Phoenix 100 System.

Selected pure bacterial colonies obtained from the blood culture plates were processed using the Phoenix 100 automated microbiology system for bacterial identification and antimicrobial susceptibility testing, The procedure was performed according to the manufacturer's instructions. Quality Control: To ensure the reliability and accuracy of the microbiological procedures, standard aseptic techniques were maintained throughout sample collection, transportation, culture, identification, and antimicrobial susceptibility testing. Culture media reagents, and

* Corresponding author Email, a.abolbida@asmary.edu.ly

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laboratory materials were handled according to standard laboratory procedures. Where applicable, quality-control procedures for antimicrobial susceptibility testing were performed in accordance with the relevant CLSI guidelines and the manufacturer's instructions for the Phoenix 100 system.

3. Results

To assess the occurrence of bacterial growth among the study participants, the bacterial culture results of 62 clinical cases were analyzed. The findings demonstrated that bacterial growth was detected in 58 cases (93.5%), whereas 4 cases (6.4%) showed no bacterial growth. These findings indicate that bacterial growth was highly prevalent among the studied cases, with the vast majority of specimens yielding positive bacterial culture results. The distribution of the study sample according to bacterial growth is presented in Table (3).

Table 3- Distribution of the Study sample According to Bacterial Growth

Bacterial Growth	Result	Percentage (%)
No Growth	4	6.4
Growth	58	93.5
Total	62	100.0

3.1 Determination and Comparison of the Most Prevalent Bacterial Species Isolated from Swab Samples

The bacterial isolation results (Table4), revealed the presence of seven different bacterial species. The distribution of the isolated bacterial species differed significantly ($p< 0.001$). This finding indicates that the bacterial species were not equally distributed among the swab samples collected from patients with CKD, demonstrating considerable variation in their prevalence within the study population. Among the isolated bacteria, *Staphylococcus epidermidis* was the most prevalent species, accounting for 30 isolates (51.7%) of the total 58 samples. It was followed by *Staphylococcus aureus*, which was isolated from 13 samples (22.4%). *Pseudomonas aeruginosa* ranked third with 7 isolates (12.1%). The remaining bacterial species were detected at considerably lower frequencies. *Pseudomonas aeruginosa* was isolated from 3 samples (5.2%), Whereas *Klebsiella pneumoniae* and *Acinetobacter* were each identified in 2 samples (3.4%). *Escherichia coli* was the least prevalent bacterial species, with only 1 isolate (1.7%). Overall, the findings demonstrate that *S. epidermidis* was the predominant bacterial species isolated from the swab sample of patient with CKD, followed by *S. aureus* and *P. aeruginosa*. In contrast, *E. coli*, *Klebsiella pneumoniae*, and *Acinetobacter* were detected at relatively low frequencies. The statistically significant difference in the distribution of the isolated bacterial species ($p < 0.001$) indicates substantial variation in bacterial prevalence within the studied population.

Table 4- Distribution of bacteria species from swab sample of patients with chronic kidney

Bacterial species	Frequency	Percent (%)	p-value
S. aureus	13	22.4	<0.001
S. epidermidis	30	51.7	
Pseudomonas aeruginosa	7	12.1	
Klebsiella pneumoniae	2	3.4	
Acinetobacter	2	3.4	
E. coli	1	1.7	
Streptococcus pyogenes	3	5.2	
Total	58	100.0	

* Corresponding author Email, a.abolbida@asmary.edu.ly

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3-2 comparison of Bacterial Prevalence among Healthcare Center

A comparison was conducted to assess the prevalence of the bacterial species isolated from samples of patients with Chronic Kidney Disease (CKD) across centers included in the study. The purpose of this comparison was to determine whether significant differences existed in the distribution of the bacterial species among the healthcare centers. As presented in Table (5), statistically significant differences were observed in the distribution of the bacterial species among the studies healthcare centres ($p< 0.001$). The Ramaya Health Center demonstrated the highest prevalence of most of the bacterial species compared with the other centers. Furthermore, all seven bacterial species investigated in the study were detected at the Ramya Health Center. In contrast, Dawao Health Centre demonstrated comparatively lower prevalence rates for most of the bacterial species. These finding indicate substantial variation in the distribution of bacterial species among the healthcare center. At the Ramaya Health Centre, *Escherichia coli* and *Streptococcus pyogenes* demonstrated the highest prevalence, with both species accounting for 100.0% of the isolates of their respective bacterial groups. This was followed by *Pseudomonas aeruginosa*, which accounted for 85.75%, and *Staphylococcus aureus*, which accounted for 61.5%. Both *Klebsiella pneumonia* and *Acinetobacter* showed a prevalence of 50.0%, whereas *Staphylococcus epidermidis* showed the lowest prevalence at 40.0% among the bacterial species detected at this center. At Zliten Central Hospital, four bacterial species were identified. *Acinetobacter* exhibited the highest prevalence at 50.0%, followed by *S. aureus* (30.8%) and *S. epidermidis* (30.0%). *Pseudomonas aeruginosa* was detected at a prevalence of 14.3%. No isolates of *Klebsiella pneumoniae*, *E. coli*, or *Streptococcus pyogenes* were detected at this healthcare center. At the Dawao Health canter, three bacterial species were identified. Klebsiella pneumonia showed the highest prevalence at 50.0%, followed by *S. epidermidis* at 30.0% and *S. aureus* at 7.7%. No isolates of *Pseudomonas aeruginosa*, *Acinetobacter, E. coli*, or *Streptococcus pyogenes* were detected at this center. Overall, the findings demonstrate marked differences in the distribution of bacterial species among the healthcare centers. The Ramaya Health Center exhibited the greatest diversity of bacterial isolates, with all seven bacterial species included in the study being detected. Zliten Center Hospital showed the presence of four bacterial species, whereas Dawao Health Center had three. The statistically significant differences observed among the healthcare centers ($p<0.001$) indicate that the distribution of bacterial species varied significantly according to the healthcare center.

Table 5: Distribution of bacterial specie isolated from swab samples of patients with chronic kidney disease

Bacterial species	Zliten Hospital	Ramaya Center	Dawao Center	p-value
S. aureus	4 (30.8)	8 (61.5)	1 (7.7)	<0.001
S. epidermidis	9 (30.0)	12 (40.0)	9 (30.0)	<0.001
Pseudomonas aeruginosa	1 (14.3)	6 (85.7)	0 (0.0)	<0.001
Klebsiella pneumoniae	0 (0.0)	1 (50.0)	1 (50.0)	<0.001
Acinetobacter	1 (50.0)	1 (50.0)	0 (0.0)	<0.001
E. coli	0 (0.0)	1 (100.0)	0 (0.0)	<0.001
Streptococcus pyogenes	0 (0.0)	3 (100.0)	0 (0.0)	<0.001

Table 6: Bacterial Species Isolated from Blood Samples of CKD

Bacterial species	Frequency	Percent (%)	p-value
S. aureus	6	54.5	0.000
Pseudomonas aeruginosa	2	18.2	0.000
Klebsiella pneumoniae	3	27.3	0.000
Total	11	100.0	

* Corresponding author Email, a.abolbida@asmary.edu.ly

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Table 7: Distribution of Bacterial Species Isolated from Blood Samples of Patient CKD

Bacterial species	Zliten Hospital	Ramaya Center	Dawao Center	P-value
S. aureus	2 (33.3)	3(50.0)	1(16.7)	<0.001
Pseudomonas aeruginosa	0(0.0)	2(100.0)	0(0.0)	<0.001
Klebsiella pneumoniae	0(0.0)	2(66.7)	1(33.3)	<0.001

Table 8: Antibiotic Resistance rates of Bacterial Species to Antibiotics in Swab Samples

Bacterial species	Resistance rate (%)	p-value
S. aureus	66.7	<0.001
S. epidermidis	25.0	<0.001
Pseudomonas aeruginosa	66.7	<0.001
Klebsiella pneumoniae	66.7	<0.001
Acinetobacter	66.7	<0.001
E. coli	33.3	<0.001
Streptococcus pyogenes	54.2	<0.001

Table 9: Antibiotic Resistance Rate among Bacterial Species Isolated from Blood Samples

Bacterial species	Resistance rate (%)	p-value
S. aureus	61.1	<0.001
Pseudomonas aeruginosa	66.7	<0.001
Klebsiella pneumoniae	50.0	<0.001

4. Discussion

The present study investigated the distribution of bacterial special, antimicrobial susceptibility and bacteraemia among CKD patients. The findings were compared with those of previous studies to identify areas of agreement and discrepancy and to provide possible explanations for the observed patterns. Particular attention was given to the bacterial species isolated from swab and blood samples, their antimicrobial susceptibility profiles, variations among healthcare centers. The present study demonstrated that *Staphylococcus epidermidis* and *Staphylococcus aureus*. Several Gram-negative organisms, particularly *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, were also identified. The predominance of *Staphylococcus* species in the present study is consistent with the finding of Attia et al [7] .Who reported that *Staphylococcus* species accounted for approximately 88.9% of infections associated with CKD, with *S. epidermidis* being the predominant isolate .This finding is comparable with the study, in which *S.epidermidis* was the most prevalent bacteria recovered from swab sample. Similariy, Attia et al [7] reported that *S.epidermidis* was the most frequently isolated bacterial species, followed by *S. aureus*. This distribution closely corresponds to the pattern observed in the present study.

* Corresponding author Email, a.abolbida@asmary.edu.ly

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The predominance of *staphylococci* in superficial sample may be related to their normal colonization of the skin and their ability to persist on catheter surfaces and medical devices. In contrast, Teshome Derese et al. [8] reported *K. pneumoniae* as the predominant bacterial pathogen among patents with bacteraemia. This observation is consistent with the present finding, in which *K. pneumonia* represented an important proportion of the bacterial isolates recovered from blood samples. Differences in the predominant bacterial species between swab and blood samples may reflect differences between superficial colonization and invasive infection. The present study revealed considerable variation in antimicrobial resistant among the bacteria species investigated. Relatively high resistance rates were observed *among P. aeruginosa, Acinetobacter, K. pneumoniae, and S. aureus*. These findings are consistent with the increasing concern regarding antimicrobial resistance among CKD, particularly those receiving long-term hemodialysis and frequently exposed to healthcare environments.

The findings are comparable with those reported by Teshome Derese et al. [8]. Who documented high levels of antimicrobial resistance, particularly among *K. pneumoniae* and *E. coli*, together with a substantial prevalence of multidrug-resistant MDR. The elevated resistance observed among *Klebsiella* and *Pseudomonas* in the present study follows a similar pattern. Dayana Fram also reported the presence of antimicrobial resistance determinants among bacterial isolates recovered from patients' haemodialysis. Their findings support the importance of antimicrobial resistance as a major challenge in this patient population. Repeated exposure to antimicrobial agents. Prolonged hospitalization, recurrent vascular access, and frequent contact with healthcare facilities may contribute to antimicrobial selection pressure and the persistence of resistant bacteria [9]. Furthermore, Attia et al. reported considerable resistance of *S. epidermidis* to several antimicrobial agents, while susceptibility to other agents was retained. The variable susceptibility pattern observed in that study is consistent with the present findings, which demonstrated substantial differences in resistance rates according to both bacterial species and antimicrobial agent [7].

Differences between healthcare centres may be associated with variations in infection-prevention and control practices, antimicrobial prescribing patterns, patient characteristics, environmental conditions, and management of vascular access devices. However, because these factors were not directly measured in the present study, their contribution should be interpreted as possible explanations rather than established causal relationships. The findings are consistent with those of Teshome Derese et al. who reported that the healthcare setting and patient-care environment may influence the risk of bacterial infection and bacteremia [8]. Similarly, Attia et al highlighted the potential influences in clinical practices, catheter care, and infection-control procedures on infection rates across healthcare unite [7]. The significant differences observed among the healthcare centers therefore, suggests that local factors may contribute to variations in bacterial prevalence and antimicrobial resistance. These findings emphasize the importance of strengthening infection-prevention, control programs and maintaining continuous microbiological surveillance at the local level. The present study found that bacteremia occurred in a relatively small proportion of the study population. With 11of the 58 patients (19.0%) showing positive blood cultures. Among the identified bloodstream isolates, *S. aureus* was the most frequently isolated organism, followed by *K. pneumoniae* and *P. aeruginosa*. Dayana Fram similarly reported the involvement of *S.aureus* and Gram-negative bacteria in catheter-related bloodstream infections CRBSTs among patients undergoing patients who repeated vascular access [9].

The lower frequency of bacteraemia compared with bacterial isolation from swab samples may indicate that bacterial colonization or localized infection occurs more frequently than invasive bloodstream infection. Nevertheless, bacteria remain clinically significant because patients with CKD, particularly those receiving hemodialysis, are exposed to repeated vascular access and consequently have an increased opportunity for bloodstream invasion. The findings of the present study highlight the importance of antimicrobial susceptibility testing in

* Corresponding author Email, a.abolbida@asmary.edu.ly

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guiding antibiotic therapy among patients with CKD. The susceptibility results indicated that certain antimicrobial agents, particularly imipenem (IPM) and F300, demonstrated comparatively higher activity against some of the bacterial isolates. These findings are consistent with Dayana Fram [9], who emphasized the importance of performing antimicrobial susceptibility testing before initiating therapy because of the increasing prevalence of multidrug-resistant organisms. Similarly, Teshome Derese et al. reported substantial resistance to several antimicrobial agents among bacterial isolates recovered from patients with bacteria [8]. The observed variability in antimicrobial susceptibility further demonstrates that empirical antibiotic therapy may not reliably provide adequate coverage for all bacterial pathogens in this population. Therefore, culture-based identification and antimicrobial susceptibility testing should be incorporated into clinical decision-making whenever clinically feasible [10-11]. Attia HA, et al. also emphasized that antimicrobial selection should be based on the causative bacterial species as Gram-positive cocci and Gram-negative bacilli may exhibit substantially different susceptibility profiles(antibiograms) [7]. The present findings support this observation, as marked differences in antimicrobial agent with a relatively high susceptibility rate in the present study should not be interpreted as a recommendation for empirical use in all patient. Antibiotic selection should be individualized according to the identified pathogen, antimicrobial susceptibility profile, clinical condition, renal function, and relevant antimicrobial stewardship recommendations, patients with renal disease are considered particularly vulnerable to infection caused by antimicrobial-resistant bacteria, especially those undergoing haemodialysis or kidney transplantation. Among the major resistant bacterial pathogen of concern. These bacteria possess various virulent factors that enable them to survive and adapt within the host. They may also from biofilm, which can protect bacterial cells from antimicrobial agents and host immune responses, thereby increasing their persistence and making infections more difficult to eradicate [7.8.12].

5. Conclusion

The findings demonstrated the presence of several bacterial species associated with infection. These finding highlight the importance of strict adherence to appropriate sterilization and aseptic procedures during catheter insertion and subsequent catheter care. Furthermore, the appropriate selection of antimicrobial therapy based on microbiological culture and antimicrobial susceptibility testing is essential for reducing the incidence of bacterial infections and limiting the resistance.

ETHICAL STATEMENT:

This study was approved by Zliten Dialysis Center (Approval No. IRB/ 5/489) on April 22 ,2025.

CONFLICT OF INTEREST

The authors declare that they have no financial, professional, or personal relationships that could influence or appear to influence the work reported in this study.

AUTHORS' CONTRIBUTIONS

All authors contributed to study design, supervised experimental work, and contributed to manuscript drafting. Performed laboratory experiments, collected data, and conducted statistical analysis, prepared tables and figures, and contributed to interpretation of results.

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GC–MS Characterization and In Vitro Antibacterial Activity of *Pelargonium graveolens L'Hér.* Essential Oil from Tajoura, Libya

Abdulaziz Suwaydan[a&]*, Mhmuod Zughdani[a], Mahmuod A. Abushiba[b], Esmaeil Belead Musa[c], Mohamed S. Altraiki[d], Abdulkarem Rajab Tamer[e]

[a] Department of Pharmacognosy, Faculty of Pharmacy, Elmergib University, Al-Khums, Libya.
[b] Department of Microbiology and Immunology, Faculty of Medicine, Azzaytuna University , Libya.
[c] Department of Biomedical Sciences, Faculty of Pharmacy, Elmergib University, Al-Khums, Libya.
[d] Libyan Center for Sustainable Development Research, Libyan Authority for Scientific Research, Tripoli, Libya.
[e] Department of Microbiology and Immunology, Faculty of Pharmacy, Tripoli University, Libya.
*Corresponding author: Abdulaziz Suwaydan. Email: asswedan@elmergib.edu.ly

ABSTRACT

Background: *Pelargonium graveolens* L'Hér. essential oil is valued for its chemical constituents and antimicrobial potential. To our knowledge, no previous study has combined GC–MS characterization with antibacterial screening of Libyan-cultivated *P. graveolens*.

Aims: This study investigated its chemical composition, antibacterial activity, and relationship to ISO 4731:2012 and published North African profiles.

Methods: Essential oil was extracted from fresh aerial parts collected in Tajoura, Libya, by Clevenger hydrodistillation. Chemical composition was analyzed using GC–MS, while antibacterial activity was evaluated against five reference bacterial strains using disc diffusion (4 µL/disc).

Results: The oil yield was 0.8% (v/w). GC–MS recorded 52 peaks, dominated by citronellol (26.74%), citronellyl formate (11.79%), isomenthone (10.50%), geraniol (8.97%), and linalool (5.44%). Partial numerical agreement with ISO 4731:2012 was observed. The strongest antibacterial activity was recorded against *Staphylococcus aureus* (21.00 ± 1.00 mm) and *Staphylococcus epidermidis* (20.00 ± 1.00 mm).

Conclusion: Libyan *P. graveolens* essential oil exhibited a citronellol-dominant profile and measurable antibacterial activity. These findings provide preliminary evidence supporting further quality assessment and quantitative antimicrobial investigations.

Keywords*: Pelargonium graveolens; citronellol; GC–MS; Libya; antibacterial activity*

1. Introduction

Pelargonium graveolens L'Hér. (Geraniaceae), commonly known as rose geranium, is an economically important aromatic plant widely cultivated for its essential oil, which is extensively used in the perfumery, cosmetic, and fragrance industries and has attracted considerable scientific interest due to its potential biological activities. The essential oil is predominantly characterized by oxygenated monoterpenes, particularly citronellol, geraniol, and their corresponding esters, although its chemical composition and the relative abundance of individual

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constituents may vary substantially depending on geographical origin, environmental conditions, plant organs, and cultivation practices. Previous investigations of Tunisian *P. graveolens* have demonstrated such compositional variability. Boukhris et al. reported an essential oil characterized by citronellol, citronellyl formate, and geraniol as major constituents, whereas Ben ElHadj Ali et al. documented differences in essential-oil composition between leaves and flowers [1,2]. These findings highlight the importance of investigating the chemical composition of *P. graveolens* essential oils from different geographical origins to better understand their phytochemical variability and potential biological properties.

The North African literature provides an unusually informative comparative setting. A Tunisian study of four growing biotopes documented variation in oil yield and antibacterial performance while identifying citronellol and geraniol as principal oxygenated monoterpenes [3]. Another Tunisian investigation contrasted two sites in Chebba and reported an ester- and rose-oxide-dominant pattern under different cultivation environments [4]. Algerian evidence includes earlier citronellol-rich oils [5,6] and a geraniol-rich sample from Tebessa [7], whereas a 2026 Edough investigation reported high citronellol, comparatively low geraniol and several distinctive ester and sesquiterpene constituents [8]. Moroccan crude oil was comparatively enriched in epi-γ-eudesmol [9]. More recent Egyptian Gas Chromatography–Mass Spectroscopy (GC-MS) studies also demonstrate pronounced differences in geraniol, menthone and sesquiterpene patterns despite prominent citronellol peaks [10,11].

ISO 4731:2012 provides geographically differentiated chromatographic specifications for commercial geranium essential oil, including a North African reference profile [12]. These specifications offer a useful basis for comparing major chemical constituents. However, the standard applies to steam-distilled oil, whereas the present study used hydrodistillation of fresh *P. graveolens* aerial parts. Therefore, comparisons are intended for phytochemical interpretation rather than establishing ISO compliance.

Although the chemical composition and biological activities of *P. graveolens* essential oils have been investigated across North Africa, corresponding evidence from Libya remains limited. A recent Libyan study evaluated the direct-contact and vapor-phase antibacterial activities of *P. graveolens* essential oil collected from Tajoura against *Proteus mirabilis* ATCC 14153 but did not characterize its chemical composition [13]. Consequently, integrated data linking the GC–MS chemical profile of locally cultivated *P. graveolens* with its antibacterial activity against different reference strains remain insufficiently documented. Addressing this gap would provide a locally sourced phytochemical reference and support comparisons with previously characterized North African essential oils.

Accordingly, the present study aimed to characterize the chemical composition and evaluate the in vitro antibacterial activity of essential oil hydrodistilled from fresh aerial parts of *P. graveolens* cultivated in Tajoura, Libya. Specifically, the study determined the essential-oil yield, investigated its volatile constituents and major chemical classes using GC–MS, and assessed its antibacterial activity against five reference bacterial strains using the disc-diffusion method. The resulting chemical profile was further compared with published North African studies and the regional chromatographic specifications of ISO 4731:2012 to provide a comparative phytochemical context for the Libyan essential oil.

2. Materials and Methods

2.1. Study material, locality and authentication

Cultivated *P. graveolens* plant material was collected in Tajoura, northwestern Libya, in June 2025. Botanical identification was performed by Prof. Mohammed Makhlouf, Department of Botany, Faculty of Science, University of Tripoli. Fresh, aerial plant material was used for essential-oil extraction.

Corresponding author, email: asswedan@elmergib.edu.ly

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2.2. Essential-oil extraction and yield

The essential oil was extracted from fresh aerial parts of *P. graveolens* by hydrodistillation using a Clevenger-type apparatus. Briefly, 400 g of fresh plant material was placed in a 4,000-mL round-bottom flask containing 2,000 mL of distilled water. Hydrodistillation was carried out for 3 h, after which the essential oil was carefully separated from the aqueous phase. The recovered oil was dried over anhydrous sodium sulfate to remove residual moisture, transferred into an amber glass bottle, and stored at 4 °C until further analysis. The essential-oil yield was calculated as the volume of oil recovered (mL) per 100 g of fresh plant material and expressed as a percentage volume per weight (% v/w) using the following equation:

Essential-oil yield (% v/w) = [Volume of essential oil (mL) / Fresh weight of plant material (g)] × 100

The essential-oil yield was 0.8% (v/w, fresh-weight basis).

2.3. GC–MS conditions

The chemical composition of *P. graveolens* essential oil was analyzed using a TRACE GC–TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) equipped with a TG-5MS capillary column (30 m × 0.25 mm internal diameter, 0.25 µm film thickness). The oven temperature was initially maintained at 50 °C, increased to 250 °C at a rate of 5 °C/min and held for 2 min, followed by an increase to 300 °C at 30 °C/min with a final holding time of 2 min. The injector and MS transfer-line temperatures were maintained at 270 °C and 260 °C, respectively. Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. A 1-µL aliquot of diluted essential oil was automatically injected in split mode. Mass spectra were acquired using electron ionization (EI) at 70 eV, with an ion-source temperature of 200 °C and a mass scanning range of *m/z* 50–650. The volatile constituents were tentatively identified by comparing their mass spectra with reference spectra in the NIST 19 and Wiley 09 mass spectral libraries.

2.4. ISO reference comparison and North African literature selection

The chemical composition of *P. graveolens* essential oil was compared with the 15 constituent ranges specified for North African commercial geranium oil in Table 1 of ISO 4731:2012 [12]. The relative GC–MS peak-area percentages of the Libyan sample were compared with the corresponding reference intervals to evaluate compositional similarities and differences.

For regional comparison, original studies reporting GC–MS or GC–FID chemical profiles of *P. graveolens* essential oils from Tunisia, Algeria, Morocco, and Egypt were selected. Priority was given to studies with verifiable bibliographic information, clearly defined botanical origins, and reported constituent percentages. Chemical nomenclature was harmonized where appropriate, and results from different geographical locations or plant organs were retained separately. Unreported or unverifiable values were designated as not reported (NR). Studies involving other *Pelargonium* species, isolated fractions, or non-distilled headspace volatiles were excluded from direct quantitative comparisons.

2.5. Antibacterial screening

Reference strains comprised *Escherichia coli* ATCC 8739, *Staphylococcus epidermidis* ATCC 12228, *Staphylococcus aureus* ATCC 29213, *Salmonella enterica* ATCC 14028 and *Shigella flexneri* ATCC 12022. Inocula were visually adjusted to a 0.5 McFarland standard and spread over Mueller–Hinton agar at approximately 4 mm depth in 90-mm Petri dishes. Sterile 6-mm Whatman paper discs were loaded with 4 µL of undiluted essential oil per disc. Gentamicin (10 µg/disc) served as the positive reference, and unloaded sterile discs as the negative control. The plates were incubated at 37°C for 24 h. Oil and antibiotic inhibition zones were measured as total diameters, including the 6-mm disc.

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Three independent experimental runs were available for each essential-oil and gentamicin condition. CLSI M02 informed general disc-testing practice [14].

2.6. Statistical analysis

Antibacterial activity was expressed as the mean ± standard deviation (SD) of three replicate inhibition-zone measurements (n = 3) for each bacterial strain. GC–MS results were presented as relative peak-area percentages. Published comparisons were descriptive and were not treated as a factorial regional experiment.

1. 3. Results and Discussion

3.1. Extraction yield and complete chromatographic signature

Hydrodistillation of fresh aerial parts of *P. graveolens* collected in Tajoura, Libya, yielded 0.8% (v/w) essential oil on a fresh-weight basis. The reported GC–MS profile comprised 52 peaks meeting the study's library-reporting criterion, with listed relative peak areas summing to 99.84% (Figure 1; Table 1). Fifteen peaks individually represented at least 1.0% of the chromatographic signal and together contributed 85.05%. The five largest tentative assignments were citronellol (26.74%; RT 12.43 min), citronellyl formate (11.79%; RT 13.50 min), isomenthone (10.50%; RT 10.14 min), geraniol (8.97%; RT 13.05 min) and linalool (5.44%; RT 8.72 min), collectively contributing 63.44%. Other peaks with areas ≥2% were furopelargone A (3.69%), geranyl formate (2.61%), β-caryophyllene (2.52%), geranyl tiglate (2.34%), germacrene A (2.20%), (−)-aristolene (2.13%), (Z)-rose oxide (2.04%) and δ-cadinene (2.01%).germacrene A (2.20%), (−)-aristolene (2.13%), (Z)-rose oxide (2.04%), and δ-cadinene (2.01%).

On the basis of the provisional assignments, oxygenated monoterpenes represented 55.99% of the reported chromatographic area, followed by terpenoid esters (22.42%; monoterpenoid esters 21.19% and sesquiterpenoid esters 1.23%), sesquiterpene hydrocarbons (14.22%), oxygenated sesquiterpenes other than esters (6.59%) and monoterpene hydrocarbons (0.62%). Oxygenated monoterpenes together with terpenoid esters accounted for 78.41% of the reported peak areas. The peak at RT 20.10 min (0.16%; maximum match factor 726) was excluded from the 52-entry reported table because it did not meet the stated MF ≥800 threshold.

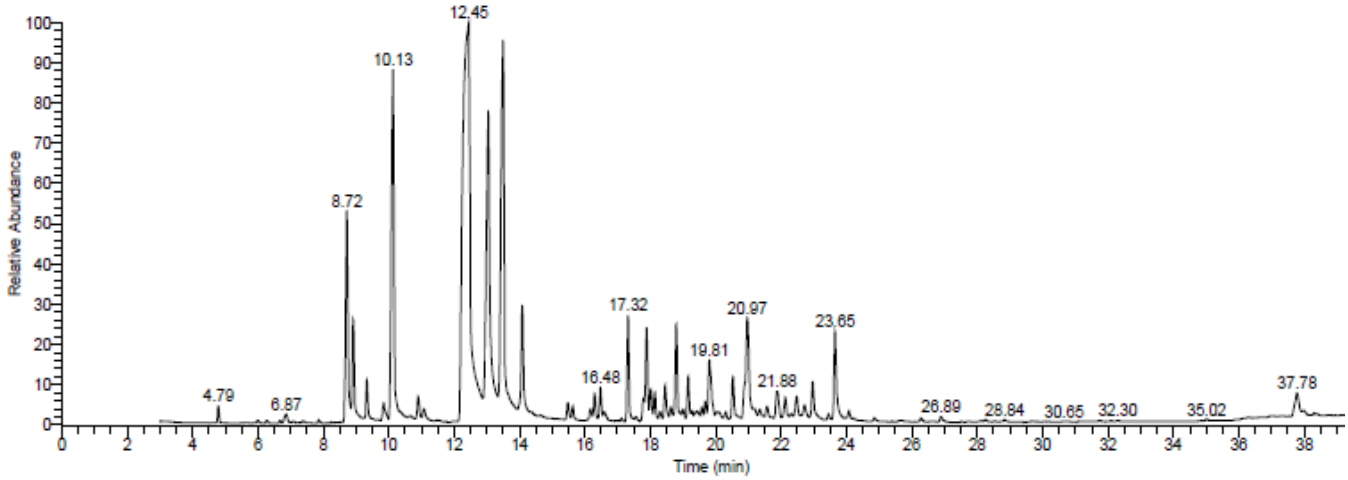


Figure 1. GC-MS chromatogram of *Pelargonium graveolens* oil from Tajoura, Libya.

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Table 1. GC–MS chemical profile of *Pelargonium graveolens* essential oil from Tajoura, Libya with chemical-class totals

Peak	RT (min)	Tentative constituent	Chemical class	Molecular formula	Peak area (%)	MF
1	4.79	α-Pinene	Monoterpene hydrocarbon	C10H16	0.35	941
2	6.87	Limonene	Monoterpene hydrocarbon	C10H16	0.27	910
3	8.72	Linalool	Oxygenated monoterpene	C10H18O	5.44	953
4	8.92	(Z)-Rose oxide	Oxygenated monoterpene	C10H18O	2.04	953
5	9.33	(E)-Rose oxide	Oxygenated monoterpene	C10H18O	0.88	957
6	9.85	trans-Menthan-3-one	Oxygenated monoterpene	C10H18O	0.57	941
7	10.14	Isomenthone	Oxygenated monoterpene	C10H18O	10.50	956
8	10.90	Neoisomenthol	Oxygenated monoterpene	C10H20O	0.57	943
9	11.08	α-Terpineol	Oxygenated monoterpene	C10H18O	0.28	868
10	12.43	Citronellol	Oxygenated monoterpene	C10H20O	26.74	938
11	13.05	Geraniol	Oxygenated monoterpene	C10H18O	8.97	942
12	13.50	Citronellyl formate	Monoterpenoid ester	C11H20O2	11.79	930
13	14.09	Geranyl formate	Monoterpenoid ester	C11H18O2	2.61	924
14	15.48	Citronellyl acetate	Monoterpenoid ester	C12H22O2	0.40	931
15	15.63	Germacrene D	Sesquiterpene hydrocarbon	C15H24	0.28	889
16	16.17	Geranyl acetate	Monoterpenoid ester	C12H20O2	0.28	865
17	16.30	α-Copaene	Sesquiterpene hydrocarbon	C15H24	0.63	929
18	16.48	β-Bourbonene	Sesquiterpene hydrocarbon	C15H24	0.76	937
19	16.60	β -cubebene	Sesquiterpene hydrocarbon	C15H24	0.25	920
20	17.32	β-Caryophyllene	Sesquiterpene hydrocarbon	C15H24	2.52	956
21	17.79	Citronellyl propionate	Monoterpenoid ester	C13H24O2	0.47	929
22	17.89	(-)-Aristolene	Sesquiterpene hydrocarbon	C15H24	2.13	947
23	18.02	γ-Muurolene	Sesquiterpene hydrocarbon	C15H24	0.56	918
24	18.14	α-Humulene	Sesquiterpene hydrocarbon	C15H24	0.54	948
25	18.30	β-Gurjunene	Sesquiterpene hydrocarbon	C15H24	0.18	947
26	18.45	Geranyl propionate	Monoterpenoid ester	C13H22O2	0.85	916
27	18.63	Aromandendrene	Sesquiterpene hydrocarbon	C15H24	0.21	895
28	18.80	Germacrene A	Sesquiterpene hydrocarbon	C15H24	2.20	964
29	19.01	Bicyclogermacrene	Sesquiterpene hydrocarbon	C15H24	0.17	913
30	19.16	γ-Elemene	Sesquiterpene hydrocarbon	C15H24	1.02	917
31	19.28	α-Muurolene	Sesquiterpene hydrocarbon	C15H24	0.15	939
32	19.37	β-Elemene	Sesquiterpene hydrocarbon	C15H24	0.08	909
33	19.44	Farnesol	Oxygenated sesquiterpene	C15H26O	0.15	860
34	19.57	γ-Muurolene	Sesquiterpene hydrocarbon	C15H24	0.16	928
35	19.68	cis-Calamenene	Sesquiterpene hydrocarbon	C15H22	0.28	929
36	19.80	δ-Cadinene	Sesquiterpene hydrocarbon	C15H24	2.01	962
37	20.02	α-Ylangene	Sesquiterpene hydrocarbon	C15H24	0.09	861
38	20.31	Caryophyllene oxide	Oxygenated sesquiterpene	C15H24O	0.17	868
39	20.52	Geranyl butyrate	Monoterpenoid ester	C14H24O2	1.05	937
40	20.97	Furopelargone A	Oxygenated sesquiterpene	C15H22O2	3.69	861
41	21.34	Epiglobulol	Oxygenated sesquiterpene	C15H26O	0.19	855
42	21.57	β-caryophyllene oxide	Oxygenated sesquiterpene	C15H24O	0.32	856
43	21.87	Di-epi-1,10-cubenol	Oxygenated sesquiterpene	C15H26O	0.94	925
44	22.13	1-epi-Cubebol	Oxygenated sesquiterpene	C15H26O	0.56	924
45	22.48	Cubenol	Oxygenated sesquiterpene	C15H26O	0.57	909
46	22.72	Nerolidol tiglate	Sesquiterpenoid ester	C20H32O2	0.36	806
47	22.97	Citronellyl tiglate	Monoterpenoid ester	C15H26O2	0.89	944
48	23.45	Citronellyl 4-methylpentanoate	Monoterpenoid ester	C16H30O2	0.15	892

Corresponding author, email: asswedan@elmergib.edu.ly

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Table 1 (continue)						
Peak	**RT (min)**	**Tentative constituent**	**Chemical class**	**Molecular formula**	**Peak area (%)**	**MF**
49	23.65	Geranyl tiglate	Monoterpenoid ester	C15H24O2	2.34	965
50	24.07	Geranyl angelate	Monoterpenoid ester	C15H24O2	0.20	910
51	26.89	Linalyl formate	Monoterpenoid ester	C11H18O2	0.16	871
52	37.78	E-Nerolidyl acetate	Sesquiterpenoid ester	C17H28O2	0.87	892
CHEMICAL-CLASS TOTALS (reported 52-peak profile)						
Monoterpene hydrocarbons (n = 2)					0.62	
Oxygenated monoterpenes (n = 9)					55.99	
Monoterpenoid esters (n = 12)					21.19	
Sesquiterpene hydrocarbons (n = 19)					14.22	
Oxygenated sesquiterpenes (excluding esters) (n = 8)					6.59	
Sesquiterpenoid esters (n = 2)					1.23	
Combined terpenoid esters (n = 14)					*22.42*	
TOTAL REPORTED PEAKS (n = 52)					**99.84**	

RT, retention time; MF, maximum mass spectral library match factor; n, number of peaks.

3.2. Comparison with ISO 4731:2012 North African Marker Intervals

Comparison with the 15 constituent intervals in the North African chromatographic specification of ISO 4731:2012 [12] showed partial numerical agreement (Table 2; Figure 2). Of the 15 constituents, 12 had corresponding provisional assignments in the Libyan profile. Citronellol (26.74% versus 25.0–36.0%), linalool (5.44% versus 4.0–8.5%), geranyl formate (2.61% versus 2.0–7.0%) and geranyl butyrate (1.05% versus 0.7–2.0%) were within their respective intervals. The tentatively mapped menthone-related peak (0.57%) was numerically within the ISO upper limit of 2.1%. By contrast, geraniol (8.97%) was below the specified 10.0–18.0% interval and citronellyl formate (11.79%) exceeded the 4.0–8.0% interval. The citronellol-to-geraniol TIC peak-area ratio was 2.98, reported solely as a descriptive chromatographic ratio.

On the current study, isomenthone (10.50% versus 4.0–8.0%), (Z)-rose oxide (2.04% versus 0.7–1.5%), (E)-rose oxide (0.88% versus 0.3–0.6%) and geranyl tiglate (2.34% versus 0.9–2.0%) were numerically above their reference intervals, whereas α-terpineol (0.28% versus 0.3–0.6%) was marginally below. Guaia-6,9-diene, 10-epi-γ-eudesmol and β-phenylethyl tiglate were not specifically assigned and were therefore designated NR rather than assumed absent

Table 2. Numerical comparison of 15 ISO 4731:2012 North African geranium-oil reference constituents with Present: Tajoura, Libya GC–MS peak-area profile.

Reference component	ISO North Africa (%)	Libyan (%)	Numerical position
(Z)-Rose oxide	0.7–1.5	2.04	Above*
(E)-Rose oxide	0.3–0.6	0.88	Above*
Menthone	n.d.–2.1	0.57	Provisionally within
Isomenthone	4.0–8.0	10.50	Above*
Linalool	4.0–8.5	5.44	Within
Guaia-6,9-diene	n.d.–0.5	NR	Not evaluated
Citronellyl formate	4.0–8.0	11.79	Above
α-Terpineol	0.3–0.6	0.28	Below
Geranyl formate	2.0–7.0	2.61	Within

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Table 2 (Continue)

Reference component	ISO North Africa (%)	Libyan (%)	Numerical position
Citronellol	25.0–36.0	26.74	Within
Geraniol	10.0–18.0	8.97	Below
Geranyl butyrate	0.7–2.0	1.05	Within
10-epi-γ-Eudesmol	3.0–6.2	NR	Not evaluated
Geranyl tiglate	0.9–2.0	2.34	Above*
β-Phenylethyl tiglate	0.5–1.2	NR	Not evaluated

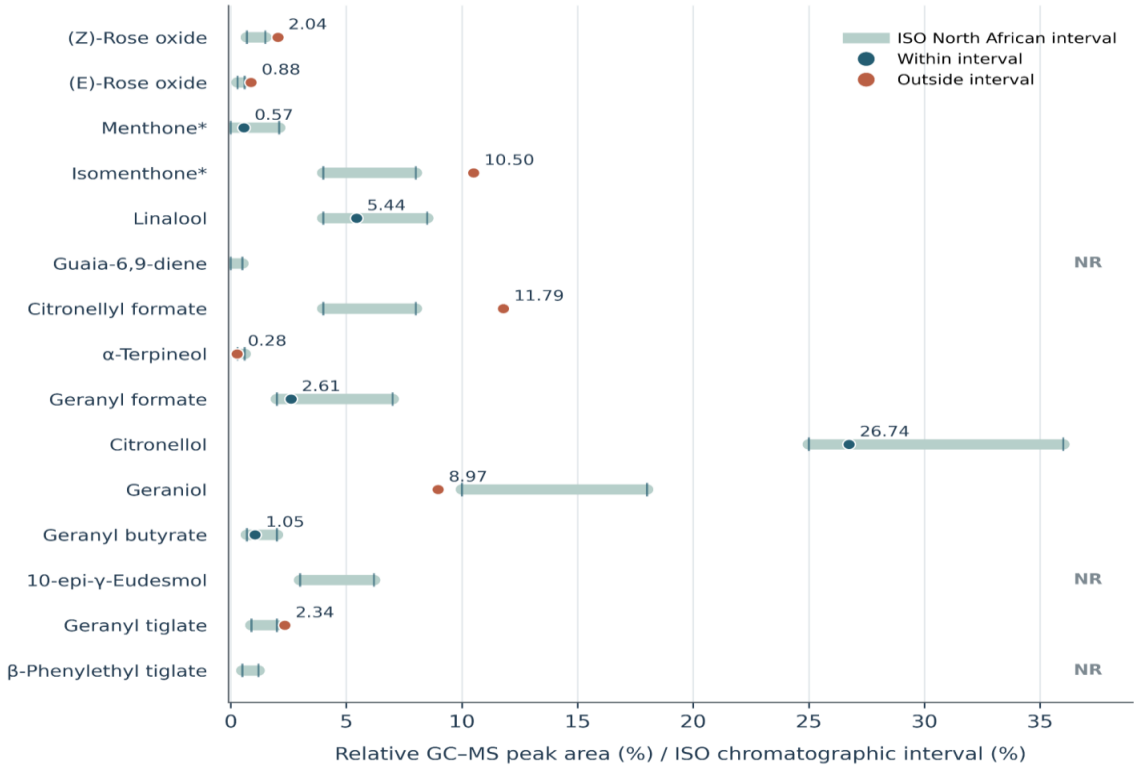


Figure 2. Selected Libyan GC–MS peak-area percentages compared numerically with ISO 4731:2012 North African reference intervals

3.3. Comparison with Published North African Essential-Oil Profiles

Comparison with previously published North African studies revealed both similarities and substantial differences in the chemical composition of *P. graveolens* essential oils. Citronellol was frequently reported among the predominant constituents, although its relative abundance and the proportions of accompanying monoterpene alcohols, esters, and sesquiterpenes varied across investigated samples (Table 3; Figure 3) [1–11]. The citronellol content of the Tajoura oil (26.74%) was comparable to values reported for several Tunisian, Algerian, and Egyptian oils, whereas its relatively low geraniol content (8.97%) distinguished it from many of these profiles.

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Table 3. Selected study-specific chemical markers (%) reported for original North African *P. graveolens* essential oils.

Study / origin	Year	Citronellol	Geraniol	Citronellyl. formate	Linalool	Menthone/iso-menthone
Present: Tajoura, Libya	2025	26.74	8.97	11.79	5.44	10.50*
Boukhris, Tunisia [1]	2013	21.90	11.10	13.20	5.60	NR
Ben ElHadj Ali, Tunisia leaves [2]	2020	26.98	20.65	NR	NR	NR
Atailia, Algeria [5]	2015	19.22	14.03	10.02	5.60	NR
Boukhatem, Algeria [6]	2013	30.20	7.60	9.30	NR	NR
Mokhati, Algeria [7]	2025	24.86	20.50	8.30	2.91	5.26**
Benzaid, Algeria [8]	2026	28.68	5.35	0.76	3.15	5.44*
M'hamdi, Morocco [9]	2024	12.34	12.54	7.70	4.06	NR
Haikal, Egypt [10]	2025	26.74	20.28	9.30	NR	7.03**
Abd El-Kareem, Egypt [11]	2020	27.67	NR	NR	10.05	10.23**

NR = constituent percentage not verified from the original report, not analytical absence.

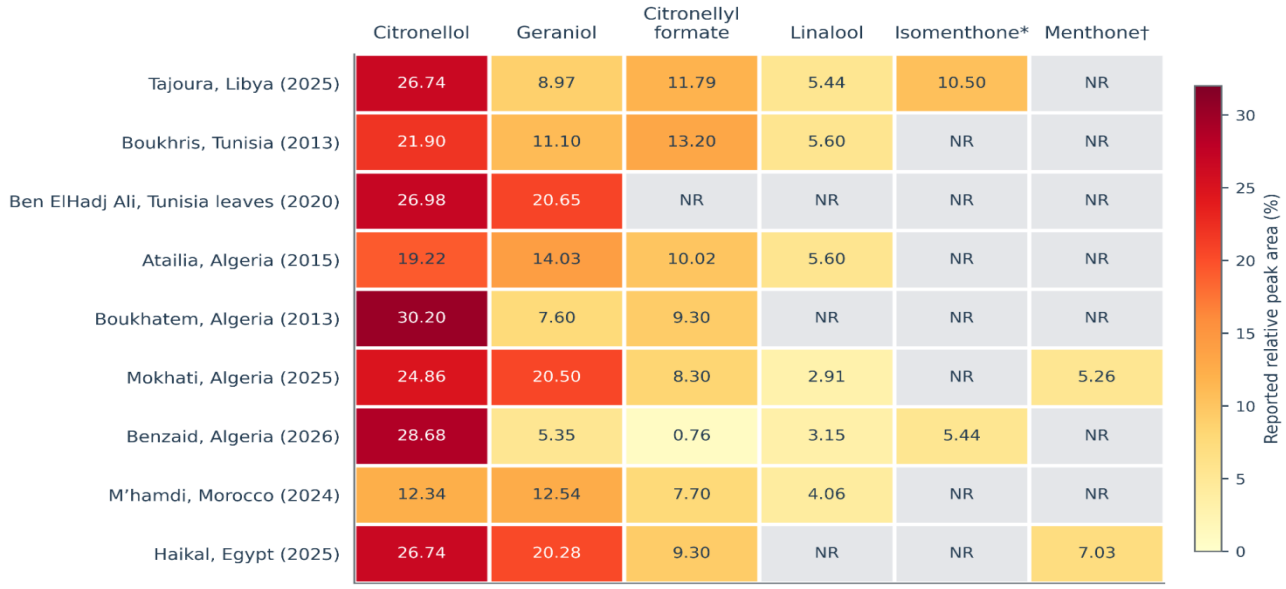


Figure 3. Heatmap of selected major volatile constituents reported for Libyan and original North African *Pelargonium graveolens* oils. Yellow represents a lower relative peak area and red represents a higher peak area; gray (NR) represents unreported values, not measured zero.

Among Tunisian investigations, Boukhris et al. [1] reported a chemical composition resembling the present oil, particularly in citronellyl formate (13.20% versus 11.79%) and linalool (5.60% versus 5.44%). However, geranyl formate was more abundant in the Tunisian oil (6.20% versus 2.61%). Ben ElHadj Ali et al. [2] reported comparable citronellol levels in leaf and flower oils (26.98% and 24.30%, respectively), but substantially higher geraniol proportions (20.65% and 21.81%) than those observed in Tajoura. Further compositional variability was documented by Reziga et al. [3] across four Tunisian growing biotopes. Notably, Lahmar et al. [4] identified chemically contrasting oils from two cultivation environments in Chebba, characterized by relatively high proportions of geranyl acetate, cis-ocimene, and trans-rose oxide rather than the citronellol-dominant profile observed in the present study. Collectively, these findings demonstrate substantial variation among Tunisian samples, including differences associated with plant organs and cultivation environments.

A similar pattern of chemical variability was evident among Algerian oils. Atailia and Djahoudi [5] reported citronellol (19.22%), geraniol (14.03%), and citronellyl formate (10.02%), whereas Boukhatem et al. [6] identified citronellol (30.20%), citronellyl formate (9.30%), and

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geraniol (7.60%) as the major constituents. The latter profile showed a similar ordering of these principal markers to the Tajoura oil. In contrast, Mokhati et al. [7] reported a substantially higher geraniol proportion (20.50%) in an oil from Tebessa, despite a comparable citronellol level (24.86%). Benzaid et al. [8] identified another distinctive Algerian profile containing citronellol (28.68%) and geraniol (5.35%), but considerably less citronellyl formate (0.76%) than Libyan oil (11.79%). These differences highlight considerable chemical heterogeneity within Algeria and suggest that individual oil profiles cannot be reliably generalized at the country level.

Additional contrasts were observed in Morocco and Egypt. M'hamdi et al. [9] characterized a Moroccan oil containing relatively high proportions of epi-γ-eudesmol (16.67%) and geraniol (12.54%), alongside a lower citronellol percentage (12.34%). In Egypt, Haikal et al. [10] reported the same numerical citronellol abundance as the Tajoura sample (26.74%), but substantially higher proportions of geraniol (20.28%) and 10-epi-γ-eudesmol (17.14%). Another Egyptian investigation by Abd El-Kareem et al. [11] described a citronellol-rich composition, although discrepancies between its reported constituent assignments and percentages limit reliable quantitative comparison.

Overall, the available evidence indicates that citronellol-rich profiles occur across several North African regions, while substantial differences exist in geraniol, citronellyl formate, and oxygenated sesquiterpene distributions. Such variability may reflect differences in plant material, developmental stage, growing conditions, extraction procedures, and analytical methods. However, the relative contributions of these factors cannot be established from comparisons among independent studies. Furthermore, differences between GC–MS peak-area percentages and GC-FID-derived relative compositions, together with varying identification criteria, limit direct quantitative interpretation. Accordingly, the present results should be considered a preliminary chemical fingerprint of a specific Libyan oil batch rather than evidence of a geographically distinct chemotype. Independent sampling and standardized comparative analyses are needed to clarify the reproducibility and determinants of these compositional patterns.

3.4. Antibacterial screening and analytical interpretation

At a loading of 4 µL of undiluted oil per 6-mm paper disc, *P. graveolens* essential oil produced inhibition zones against all five reference strains (Table 4; Figure 4). The largest mean zone was obtained against *S. aureus* ATCC 29213 (21.00 ± 1.00 mm), followed by *S. epidermidis* ATCC 12228 (20.00 ± 1.00 mm), *Sh. flexneri* ATCC 12022 (12.67 ± 0.58 mm), *S. enterica* ATCC 14028 (8.67 ± 0.58 mm) and *E. coli* ATCC 8739 (7.50 ± 0.50 mm). All reported diameters include the 6-mm disc; consequently, the *E. coli* response corresponded to only approximately 0.75 mm of radial clearance beyond the disc edge. Unloaded-disc controls showed no visible halo beyond the paper disc (0 mm additional clearance). The reference gentamicin discs (10 µg/disc) produced mean zones of 17–26 mm across the five strains.

Table 4. Disc-diffusion inhibition-zone diameters (mm, mean ± sample SD, n = 3) for *P. graveolens* essential oil and gentamicin

Reference strain	EO, 4 µL/disc	Gentamicin, 10 µg/disc
S. aureus ATCC 29213	21.00 ± 1.00	17.00 ± 0.00
S. epidermidis ATCC 12228	20.00 ± 1.00	26.00 ± 0.00
S. flexneri ATCC 12022	12.67 ± 0.58	17.00 ± 0.00
S. enterica ATCC 14028	8.67 ± 0.58	18.00 ± 0.00
E. coli ATCC 8739	7.50 ± 0.50	21.00 ± 0.00

Measured diameters include the 6-mm paper disc. Gentamicin (10 µg/disc) was an assay reference; oil (4 µL/disc).

The observed larger responses among the two Gram-positive staphylococci are compatible with the broad literature describing restricted

Corresponding author, email: asswedan@elmergib.edu.ly

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penetration of many hydrophobic volatiles through the Gram-negative outer membrane and the complex membrane interactions of essential-oil mixtures [15]. Nonetheless, outer-membrane differences alone do not account for all observed variability: inoculum biology, oil volatility, exposure dose, bacterial species, sorption to paper, and diffusion through agar each influence inhibition zones. Because constituent-specific inhibitory experiments were not conducted, activity cannot be assigned causally to citronellol, geraniol, isomenthone or furopelargone A, nor can synergistic effects be claimed.

Previous Libyan studies provide additional context for the antibacterial activity of *P. graveolens* essential oil. Abushiba et al. [13] reported an inhibition zone of 12 ± 1 mm against *Proteus mirabilis* ATCC 14153 using 10 µL of essential oil under direct-contact and vapor-phase assay conditions, although the chemical composition of the tested oil was not characterized by GC–MS. These findings provide preliminary evidence of antibacterial activity in locally sourced *P. graveolens* essential oil. However, direct quantitative comparison with the present findings is limited by differences in collection periods, bacterial strains, oil loading, and assay conditions. Similarly, a previous Libyan investigation highlighted the influence of essential-oil application methods on the observed antibacterial responses [16].

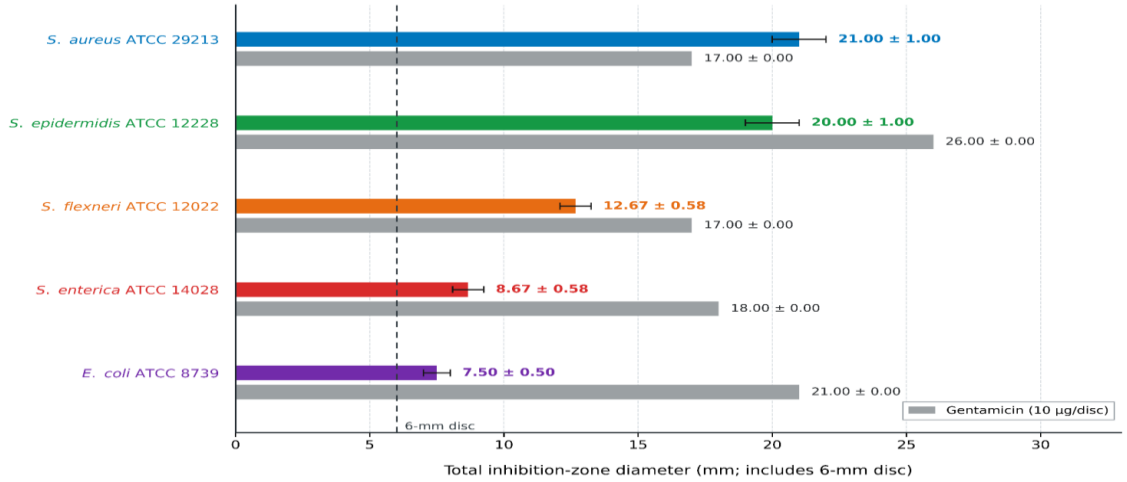


Figure 4. Disc-diffusion inhibition-zone diameters for *P. graveolens* oil (4 µL/disc) and gentamicin reference discs (10 µg/disc) across five bacterial strains (Error bars indicate sample SD from three experimental runs)

Previous Algerian investigations provide additional context for the antibacterial activity of *P. graveolens* essential oil, although methodological differences limit direct comparisons. Atailia and Djahoudi [5] reported minimum inhibitory concentration (MIC) values against clinical bacterial isolates, whereas Boukhatem et al. [6] evaluated antimicrobial activity against food-associated microorganisms using direct-contact and vapor-phase assays. These methodological differences prevent direct quantitative comparison with the inhibition-zone measurements obtained in the present study. Although the essential oil produced a larger inhibition zone against *Staphylococcus aureus* than gentamicin (21.00 versus 17.00 mm), differences in applied doses and agar-diffusion characteristics preclude interpreting this observation as evidence of superior antibacterial potency.

3.5. Study Limitations and Future Research Directions

Although the present study provides preliminary evidence of the chemical composition and antibacterial activity of *P. graveolens* essential oil from Tajoura, Libya, several limitations warrant consideration. The investigation was restricted to a single collection batch, and the

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identification of volatile constituents was based primarily on mass-spectral library matching without experimental retention-index confirmation. Furthermore, antibacterial activity was evaluated using agar-disc diffusion, which does not establish minimum inhibitory or bactericidal concentrations.

Future investigations should prioritize the collection of authenticated plant material from different Libyan cultivation areas, seasons, and phenological stages to assess chemical variability and reproducibility. Additional chromatographic characterization, including experimental retention indices and authentic reference standards, would improve compound-identification confidence. Comprehensive quality assessment using the relevant analytical and physicochemical requirements of ISO 4731:2012 would also clarify the suitability of locally produced oil for standardized applications. Moreover, broth microdilution assays, MIC and MBC determination, and evaluation of selected major constituents would provide a stronger basis for interpreting antibacterial activity. These investigations could support future quality-control studies and more comprehensive assessment of the antimicrobial potential of Libyan *P. graveolens* essential oil.

4. Conclusions

GC–MS analysis of the essential oil obtained from *P. graveolens* cultivated in Tajoura, Libya, revealed a citronellol-dominant chemical profile, characterized mainly by citronellol (26.74%), citronellyl formate (11.79%), isomenthone (10.50%), geraniol (8.97%), and linalool (5.44%). The essential oil, extracted from fresh aerial parts by hydrodistillation, yielded 0.8% (v/w), and its chromatographic profile comprised 52 recorded peaks. Comparison with ISO 4731:2012 showed partial agreement with the North African reference composition, although variations in selected constituents, particularly geraniol and citronellyl formate, were observed. These findings highlight the chemical characteristics of the investigated Libyan oil and its compositional differences from previously reported North African samples.

The essential oil exhibited antibacterial activity against all five tested bacterial strains, with the strongest inhibition observed against *S. aureus* and *S. epidermidis*, followed by *S. flexneri*, *S. enterica*, and *E. coli*. These preliminary findings suggest that Libyan *P. graveolens* essential oil represents a promising source of naturally occurring compounds for further antimicrobial investigation. However, additional studies involving MIC and MBC determination, confirmation of major chemical constituents, and evaluation of biological activity under practical application conditions are necessary before its potential use as a natural antimicrobial agent can be established.

ETHICAL STATEMENT

Ethical approval was not required, as the study involved only plant material and in vitro testing of reference bacterial strains.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed to the research and manuscript preparation, reviewed the final version, and approved its submission.

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This research received no external funding.

DATA AVAILABILITY

The GC–MS data and antibacterial results are available from the corresponding author upon reasonable request.

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Corresponding author, email: asswedan@elmergib.edu.ly

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Corresponding author, email: asswedan@elmergib.edu.ly

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Cytotoxic and Genotoxic Evaluation of Aqueous Extract from Dry Fruits of Pistacia lentiscus L. on Allium cepa L. Root Tip Cells

Marei Saleh Othman[a], Nagea. Ka. Abdalsadiq[b&*],Fawzia Muftah AlJazwia[c]

[a] Department of Botany, the Libyan Academy of Benghazi, Benghazi, Libya
[b*]Department of Zoology, Faculty of Science, Omar AL-Mukhtar University, Al Bayda, Libya
[c] Botany Department, Faculty of Science, University of Benghazi, Benghazi, Libya
* Corresponding author Email: nagea.ka.abdalsadiq@omu.edu.ly

ABSTRACT

Background: *Pistacia lentiscus* L. is a Mediterranean plant used in medicine for various purposes. However, the potential cytotoxic and genotoxic effects of aqueous extracts prepared from its fruits require further investigation.

Aims: To estimate the cytotoxic and genotoxic effects of an aqueous extract of dried *Pistacia lentiscus* L. fruits.

Methods: *Allium cepa* L. root tips were exposed to four concentrations of the aqueous fruit extract (5, 10, 20, and 40 g/200 mL) and a negative control for 3, 6, 12, and 24 h. Cytotoxicity was assessed by determining the mitotic index, whereas genotoxicity was evaluated by recording the frequency and types of chromosomal abnormalities. The effects of extract concentration and exposure time were analyzed using two-way analysis of variance.

Results: The mitotic index was lower in extract-treated groups than in the negative control, although the response was not consistently concentration-dependent. Values ranged from 34.72% to 46.81% in treated groups, compared with 42.77% to 53.30% in the control across the tested exposure periods. The lowest mitotic index (34.72%) was recorded at 10 g/200 mL after 24 h. Extract concentration ($F = 8.897$, $p = 0.001$) and exposure time ($F = 7.998$, $p = 0.003$) had significant effects on the mitotic index. Chromosomal abnormalities were not observed in the control groups but occurred in all extract-treated groups, with mean values ranging from 0.96 ± 0.52 to 2.68 ± 0.14. Chromosome stickiness was the predominant abnormality, while C-metaphase, lagging chromosomes, chromosomal bridges, and disturbed configurations were also observed. Significant effects of extract concentration ($F = 31.729$, $p = 0.001$) and exposure time ($F = 4.436$, $p = 0.026$) were also recorded for chromosomal abnormalities.

Conclusion: Under the experimental conditions examined, the aqueous extract of dried *Pistacia lentiscus* L. fruits was associated with reduced mitotic activity and the occurrence of chromosomal abnormalities in *Allium cepa* L. root tip cells. Further investigations are needed to identify the constituents potentially responsible for these effects and to establish the biological safety of these preparations.

Keywords: *Pistacia lentiscus, Allium cepa, Cytotoxicity, Genotoxicity, Mitotic index, Chromosomal aberrations*

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1. Introduction

For centuries, medicinal plants have formed the backbone of traditional healthcare systems worldwide, serving as primary therapeutic agents for various human ailments [1, 2]. Ethnobotanical traditions rely heavily on natural remedies derived from wild flora, which continue to provide valuable leads for modern drug discovery and pharmacological research [3, 4]. Among the species of significant ethnomedicinal importance in the Mediterranean region, belonging to the family Anacardiaceae, holds a prominent position [5, 6]. Pistacia lentiscus is Mediterranean basin plant, exhibiting remarkable adaptability to drought and harsh environmental conditions [7-9]. Traditionally, various parts of this plant—including leaves, bark, resin, and mature fruits—have been extensively utilized in folk medicine to treat digestive disorders, skin infections, respiratory ailments, and inflammatory conditions [10-13]. The mature dry fruits, in particular, are frequently harvested and prepared as hot aqueous decoctions or traditional infusions for therapeutic consumption [14]. Phytochemical characterization of Pistacia lentiscus fruit preparations has revealed a complex compositional profile rich in bioactive secondary metabolites, including polyphenols, flavonoids, tannins, tocopherols, and fatty acids [15-18]. While these phytochemicals contribute to demonstrated biological activities such as antimicrobial, antioxidant, and anti-inflammatory properties [19-21], high concentrations of phytochemicals can exert adverse cytotoxic or genotoxic actions on eukaryotic biological systems [22, 23]. Evaluating the toxicological safety profiles of botanical preparations is essential to ensure that traditional usage does not compromise cellular or genomic integrity [24, 25]. The Allium cepa L. bioassay represents an internationally recognized standard bioindicator system for evaluating environmental and botanical cytotoxicity and genotoxicity [26-28]. Because cell division dynamics and chromosome architecture in plant root tip meristematic cells closely parallel eukaryotic cell kinetics, this test provides a sensitive, low-cost, and highly reliable metric for observing mitotic index suppression and chromosomal aberration induction [29, 30]. Despite the widespread traditional usage of Pistacia lentiscus dry fruit preparations in ethnomedicine, detailed scientific evidence regarding their cellular safety profile remains limited. Therefore, this study was designed to systematically evaluate the cytogenotoxic potential of aqueous extracts derived exclusively from the dry fruits of Pistacia lentiscus L. utilizing the Allium cepa bioassay [29, 30]. The primary objective of this investigation is to determine the impact of varying concentrations of the dry fruit extract across different exposure intervals on the Mitotic Index (MI) as a measure of cytotoxicity, as well as to characterize and quantify the frequency and spectrum of induced structural and numerical chromosomal aberrations as metrics of genotoxicity. It is hypothesized that elevated concentrations of dry Pistacia lentiscus fruit extract, combined with prolonged exposure durations, will exert significant mitodepressive effects and trigger concentration-dependent genomic abnormalities in meristematic cells, thereby defining the cellular safety margins of traditional dry fruit preparations [31, 32].

2. Materials and Methods

2.1 Plant Material Collection and Botanical Identification

Mature wild fruits of Pistacia lentiscus L. were collected from their natural habitat during the autumn harvesting season [33]. Botanical identification and taxonomic verification were confirmed based on standard morphological keys for Mediterranean flora and regional

Corresponding author Email: nagea.ka.abdalsadiq@omu.edu.ly

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botanical references [5, 6, 34]. Healthy, undamaged ripe fruits were selected, cleaned thoroughly with distilled water to remove adhering dust and organic debris, and subjected to shade-drying at room temperature (25 ± 2 degrees Celsius) until a constant weight was achieved [21].

2.2 Preparation of Dry Fruit Aqueous Extract

The fruits were washed with mains water and then with distilled water. To prepare the dry extracts a sample of fresh fruits was air dried at room temperature in the shade, then ground in a grinder to obtain a smooth homogeneous texture and prepared at different concentrations (5, 10, 20, and 40g) by placing each concentration in a 250ml beaker containing 200 ml of distilled water and left on a shaker for 24 hours at room temperature (28 ± 2°C). The mixture was filtered using filter paper. Distilled water was used as a control (0% concentration) [35, 36, 37]. All aqueous extract solutions were kept refrigerated at 4 degrees Celsius and used within 24 hours of preparation to prevent microbial contamination or chemical degradation [38].

2.3 Allium cepa Bioassay and Exposure Protocols

Sterilized seeds were germinated in petri dishes lined with filter paper, given a renewed distilled water supply every 24h for 4 days. Seedlings with 0.5 - 1cm roots were treated with different concentrations (0, 5, 10, 20, and 40g/200ml) of dry fruit aqueous extract for different periods (3, 6, 12, and 24h). The roots were fixed in freshly prepared Carnoy's solution (1:3 glacial acetic acid and absolute ethanol v/v for 24h, then preserved in 70% ethanol until used. Roots were hydrolyzed in 1N HCl for 10minutes at 60°C in a water bath. The roots were washed with distilled water, then stained with 2% acetocarmine stain for 24h (Abdulkarim et al. [39]). One root per slide was squashed and then examined under microscope (XSZ-107 BN) at 400X magnification. Six replicas were examined for calculating the mitotic index (MI) and percent of chromosome aberrations in dividing and non-dividing cells for each concentration and treatment time. The rules used are as follows (Farizan et al. [32]).

2.4 Cytological Preparation and Slide Fixation

At the end of each exposure period (24 and 48 hours), root tips measuring approximately 1.0 to 1.5 centimeters were harvested between 9:00 AM and 11:00 AM to capture peak mitotic activity [27, 29]. The excised root tips were immediately rinsed with distilled water and fixed in freshly prepared Carnoy fixative solution consisting of absolute ethanol and glacial acetic acid in a 3:1 ratio (volume by volume) for 24 hours at 4 degrees Celsius to preserve chromosome architecture [40]. Following fixation, the root tips were washed and preserved in 70 percent ethanol for subsequent cytological preparation. For slide preparation, root tips were hydrolyzed in 1 N hydrochloric acid (HCl) in a water bath at 60 degrees Celsius for 6 to 8 minutes to soften the pectinaceous cell wall matrix [28, 41]. After gentle rinsing with distilled water, the apical meristematic regions (1 to 2 millimeters) were isolated on clean glass microscope slides and stained with 2 percen tacetocarmine stain for 15 to 20 minutes [42]. The stained tissue was squashed beneath a clean coverslip by applying uniform vertical pressure using filter paper to obtain a monolayer of meristematic cells [43].

Corresponding author Email: nagea.ka.abdalsadiq@omu.edu.ly

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2.5 Microscopic Scoring and Cytogenotoxic Parameters

Cytological examination was performed under a high-resolution light microscope under 400x magnification for phase distribution scoring and 1000x oil immersion magnification for detailed chromosomal structural analysis. At least 1000 well-spread meristematic cells per slide across five replicates (a minimum of 5000 cells per treatment group) were randomly scored [44]. The following biological parameters were calculated: 1.Mitotic Index (MI): Determined to assess cytotoxicity, calculated as the percentage of dividing cells relative to the total number of scored meristematic cells (interphase and dividing): Mitotic Index = (Total number of dividing cells / Total number of cells scored) x 100. 2.Mitotic Phase Distribution: Calculated to determine the proportion of cells in Prophase, Metaphase, Anaphase, and Telophase relative to the total number of dividing cells [45]. 3.Chromosomal Aberration Frequency (CAF): Determined to evaluate genotoxicity, calculated as the total percentage of abnormal cells showing structural or numerical chromosomal aberrations relative to the total number of dividing cells [41]: Chromosomal Aberration Frequency = (Total number of aberrant cells / Total number of dividing cells) x 100 The qualitative types of chromosomal abnormalities scored included chromosome stickiness, chromosomal bridges (anaphase/telophase), disturbed spindles, vagrant chromosomes, and lagging chromosomes [46, 47]. 2.6. Statistical Analysis All experimental data obtained from cytotoxicity and genotoxicity assays were expressed as mean values ± standard deviation (SD) across five biological replicates. Statistical significance among the treated groups and negative control was analyzed using two-way Analysis of Variance (ANOVA). followed by appropriate post-hoc comparison tests using standard statistical software. A p-value below 0.05 was considered statistically significant, with values under 0.001 denoting high statistical significance [30, 38]. Does this variation work for you?

3. Results

Microscopic analysis of the meristematic root tip cells of Allium cepa L. treated with the aqueous extract of dry Pistacia lentiscus L. fruits revealed a clear dose- and time-dependent cytogenotoxic potential. The evaluation of the mitotic index (MI) demonstrated a significant mitodepressive effect induced by the extract across all tested concentrations compared to the negative control. As detailed in Table 1, the negative control exhibited high mitotic activity with a mean MI of 52.66% at 3 h, 53.30% at 6 h, and 51.73% at 12 h. Exposure to the aqueous extract of P. lentiscus dry fruits resulted in a progressive and concentration-dependent decline in cell division. At 3 h of exposure, the MI decreased to 46.53%, 44.19%, 43.99%, and 45.91% for concentrations of 5, 10, 20, and 40 g/200ml, respectively. A more severe mitodepressive effect was recorded at longer exposure durations, reaching its lowest level of 35.99% at the highest concentration of 40 g/200ml after 12 h of exposure (Table 1). Concurrently, the mitotic phase distribution was dominated by prophase across all treatments, while the percentages of metaphase, anaphase, and telophase experienced distinct shifts depending on the extract concentration (Table 1).

Corresponding author Email: nagea.ka.abdalsadiq@omu.edu.ly

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Table 1: Effect of different concentrations of dry Pistacia lentiscus L. fruit aqueous extract on total cells, dividing cells, mean mitotic index (xMI ± SD), and percentage of mitotic phases (P, M, A, T) in Allium cepa L. root tip cells at different times.

Time(h)	Concentration (g/200ml)	sum TC	sum CD	×MI±SD	P%	M%	A%	T%
	Control	2410	1269	52.66±0.729701	90.06	3.65	3.25	3.05
	5	3121	1452	46.53±0.744846	91.24	3.94	2.72	2.10
3	10	3975	1756	44.19±0.244615	91.18	4.62	2.20	2.00
	20	4179	1838	43.99±0.361948	90.85	4.30	2.55	2.31
	40	3093	1420	45.91±0.601921	92.00	4.06	1.85	2.09
	Control	2793	1489	53.30±0.439972	92.23	2.66	2.34	2.77
	5	3390	1432	42.24±0.255872	92.09	3.89	2.16	1.86
6	10	3984	1787	44.85±0.306047	91.20	4.07	2.37	2.36
	20	4133	1822	44.08±0.371229	92.67	3.65	2.02	1.66
	40	3271	1232	37.66±0.909736	92.46	4.29	1.80	1.45
	Control	3394	1756	51.73±3.230448	93.52	2.31	2.35	1.82
	5	3938	1843	46.81±0.186643	92.52	4.17	1.67	1.64
12	10	4213	1759	41.75±0.601406	90.66	6.01	1.77	1.56
	20	4254	1882	44.35±0.454980	92.61	3.94	1.84	1.61
	40	3435	1236	35.99±0.601904	91.75	4.02	2.25	1.99
	Control	4382	1874	42.77±1.062486	93.41	2.76	2.18	1.65
	5	4946	1779	35.97±0.268892	91.98	4.28	2.04	1.70
24	10	4482	1556	34.72±0.445280	91.67	4.43	2.15	1.75
	20	4074	1695	41.62±0.821562	92.11	4.20	1.95	1.75
	40	3450	1245	36.09±0.577878	91.84	4.08	2.26	1.82

To confirm the statistical significance of these biological findings, a two-way analysis of variance was performed. The ANOVA results, presented in Table 2, confirmed that both extract concentration ($F = 8.897$, $p = 0.001$) and exposure time ($F = 7.998$, $p = 0.003$) had statistically significant main effects on the reduction of the mitotic index. The overall corrected model was highly significant ($F = 8.511$, $p = 0.001$), demonstrating that the observed inhibition of cell division is strongly driven by the interactive influence of dose and time (Table 2). The graphical visualization of this concentration- and time-dependent inhibition of cell division is illustrated in the bar chart in Figure 1.

Corresponding author Email: nagea.ka.abdalsadiq@omu.edu.ly

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Table 2: Two-way ANOVA analysis

Dependent Variable: MI dry fruits					
Source	Type II Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	444.029[a]	7	63.433	8.511	0.001
Intercept	37281.613	1	37281.613	5002.475	0.001
Concentrations	265.218	4	66.305	8.897	.001
Time	178.811	3	59.604	7.998	0.003
Error	89.432	12	7.453		
Total	37815.073	20			
Corrected Total	533.461	19			

a. R Squared = .832 (Adjusted R Squared = .735)

b. Computed using alpha = .05

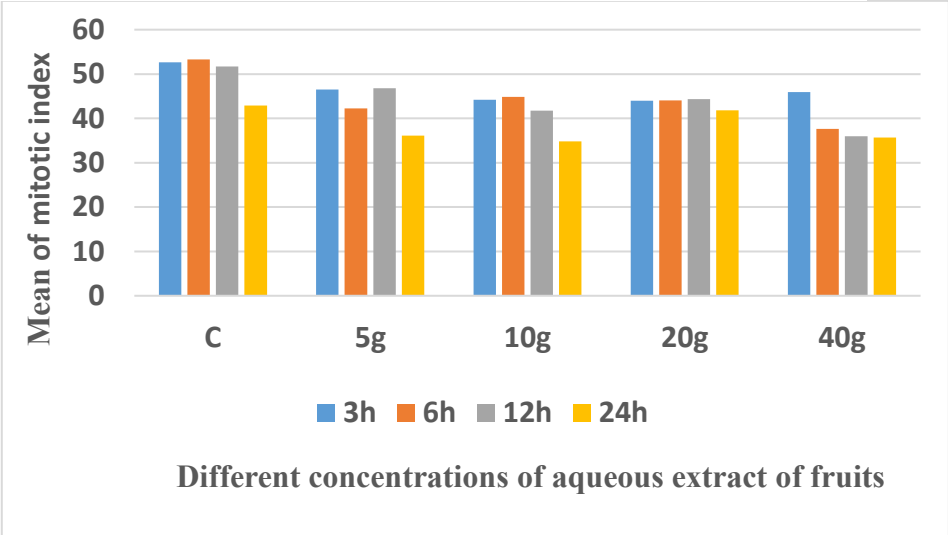


Figure 1: Concentration- and time-dependent decline in the Mitotic Index (MI %) of Allium cepa L. root tip cells exposed to aqueous extract of dry Pistacia lentiscus L.

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In addition to the inhibition of cell division, exposure to the dry fruit aqueous extract induced a significant and broad spectrum of chromosomal aberrations. As shown in Table 3, the structural and numerical abnormalities were systematically evaluated, with data expressed as Mean ± SD across treatment groups. While the negative control showed normal chromosome behavior with minimal anomalous cells, treatment with P. lentiscus aqueous extract caused a marked increase in total abnormalities. The types of anomalies observed encompassed sticky chromosomes, bridges, C-mitosis, vagrant chromosomes, and altered chromosome thickness, all displaying a steady increase correlated with higher concentrations and prolonged exposure times (Table 3).

Table 3: Mean (± SD) frequencies and specific types of chromosomal aberrations induced by dry Pistacia lentiscus L. fruit aqueous extract in Allium cepa L. root tip cells across different concentrations and exposure times

Time	C	sum CD	sum abnor	Mean abno±SD	Kinds of abnormality of dividing cells						
					P	M			A		
					ECP	sticky	C-meta	Bridge	Mb	Lag	Distu.
	C	1269	0	0±0	0	0	0	0	0	0	0
	5	1452	14	0.96±0.521506	7.14	92.86	0	0	0	0	0
3	10	1756	24	1.37±0.486217	4.17	95.83	0	0	0	0	0
	20	1838	24	1.31±0.418395	8.33	91.67	0	0	0	0	0
	40	1420	25	1.76±0.601968	4	96	0	0	0	0	0
	C	1489	0	0±0	0	0	0	0	0	0	0
	5	1432	24	1.68±0.12048	8.33	91.67	0	0	0	0	0
6	10	1787	23	1.29±0.131059	8.69	91.3	0	0	0	0	0
	20	1822	23	1.26±0.04082	8.70	91.3	0	0	0	0	0
	40	1232	33	2.68±0.140465	9.09	90.91	0	0	0	0	0
	C	1756	0	0±0	0	0	0	0	0	0	0
	5	1843	31	1.68±0.425006	3.23	96.77	0	0	0	0	0
12	10	1759	31	1.76±0.229061	3.23	90.32	3.23	0	3.23	0	0
	20	1882	32	1.70±0.210327	0	93.75	3.13	0	0	3.13	0
	40	1236	28	2.27±0.136758	3.70	88.89	3.70	0	3.70	3.70	0
	C	1874	0	0±0	0	0	0	0	0	0	0
	5	1779	29	1.63±0.289255	6.90	96.55	0	0	0	0	0
24	10	1556	36	2.31±0.375296	5.56	86.11	5.56	0	0	2.78	0
	20	1695	35	2.06±0.107	8.57	80	8.57	0	0	0	2.86
	40	1245	29	2.33±0.452674	3.35	89.65	0	3.35	0	3.35	0

C=control, sum CD=total cell dividing, sum abnor = sum of total cells abnormality, xMI =mean of the mitotic index, SD = standard deviation, P =prophase, M =metaphase, A = anaphase, ECP = early condensation in prophase, Mb = malty bridge, Lag = laggard.

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Statistical evaluation via univariate analysis of variance (ANOVA) confirmed the profound genotoxic impact of the extract on chromosome integrity. As presented in Table 4, the univariate analysis demonstrated that both the concentration of the extract and exposure duration, as well as their mutual interaction, exerted highly significant effects ($p < 0.001$) on the frequency and manifestation of total chromosomal abnormalities (Table 4). The quantitative escalation and comparative distribution of these chromosomal aberrations across all treatment groups are illustrated in the bar chart in Figure 2.

Table 4: Univariate analysis of variance (ANOVA)

Source	Dependent Variable: DryADCs Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	12.119[a]	7	1.731	20.032	0.001
Intercept	39.338	1	39.338	455.185	0.001
Concentrations	10.968	4	2.742	31.729	0.001
Time	1.150	3	0.383	4.436	0.026
Error	1.037	12	0.086		
Total	52.494	20			
Corrected Total	13.156	19			

a. R Squared = .921 (Adjusted R Squared = .875)

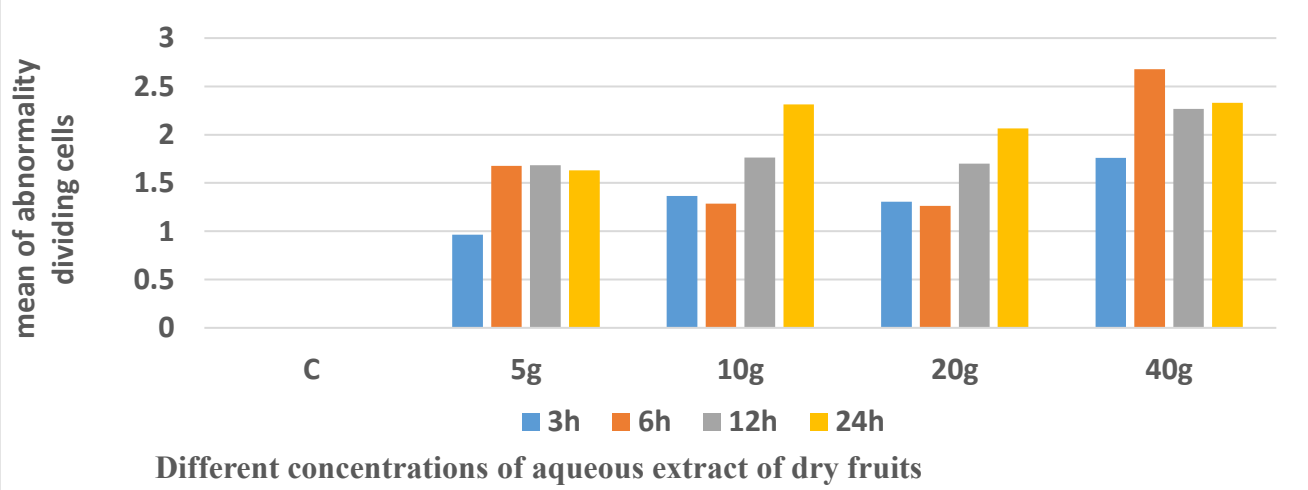


Figure 2: Percentage and frequency distribution of chromosomal aberrations induced in Allium cepa L. root tip cells across different concentrations of dry Pistacia fruit.

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Detailed cytogenetic screening under light microscopy revealed distinctive stage-specific morphological alterations in the dividing cells. Early chromatin condensation was clearly observed during prophase (Figure 3). During metaphase, severe chromosome stickiness and C-metaphase configurations were prominently identified (Figure 4). Furthermore, anaphase stages exhibited structural abnormalities including lagging chromosomes, single bridges, and multi-bridges (Figure 5).

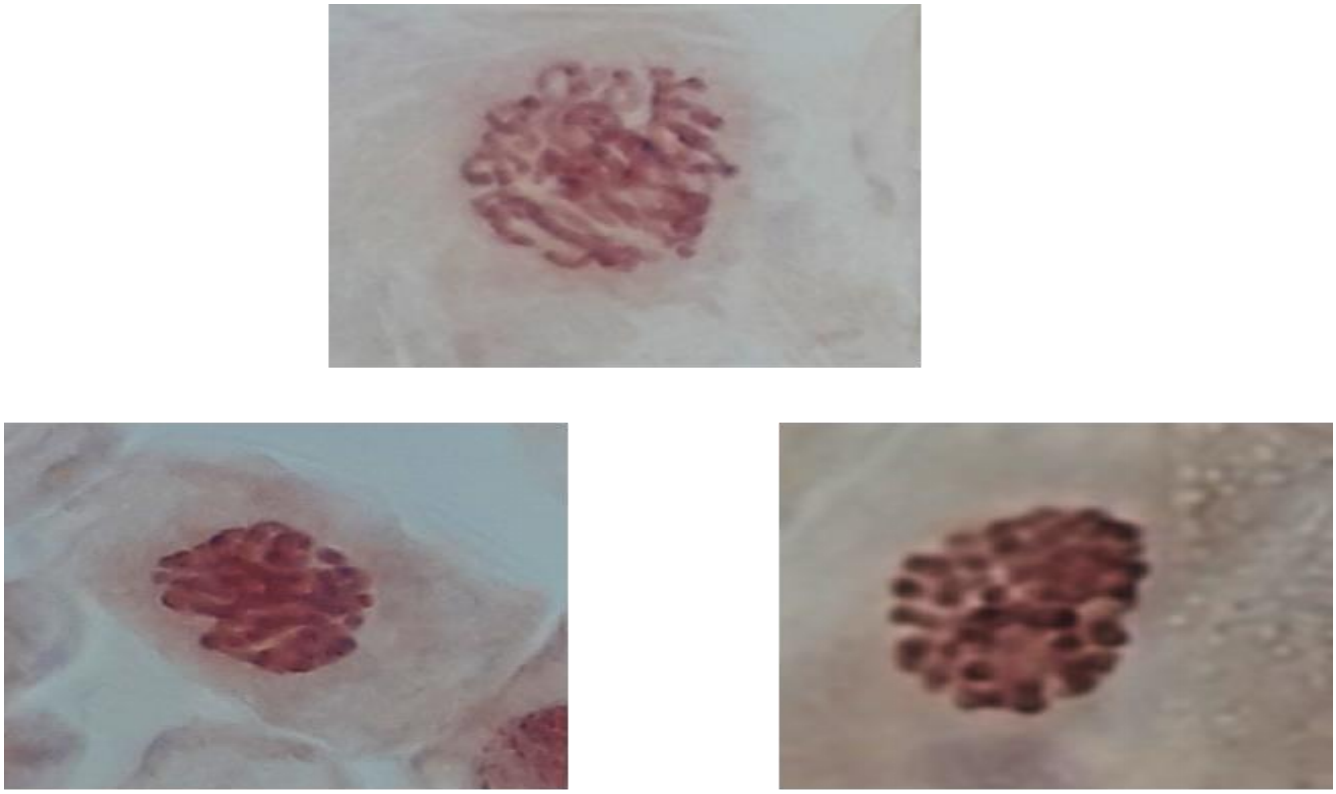


Figure 3: Effect of different aqueous extract concentrations of Pistacia lentiscus L. dry fruits on prophase in Allium cepa L. root tip cells: (a) normal prophase, (b, c) early condensation in prophase.

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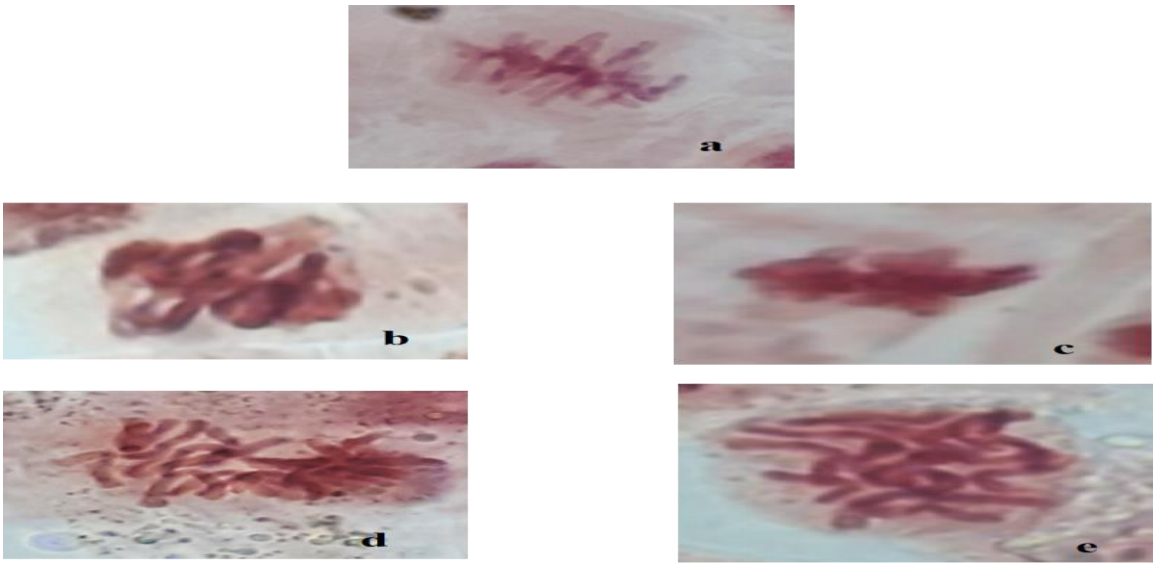


Figure 4: Effect of different aqueous extract concentrations of Pistacia lentiscus L. dry fruits on metaphase in Allium cepa L. root tip cells: (a) normal metaphase, (b, c) sticky chromosomes in metaphase, (d, e) C-metaphase.

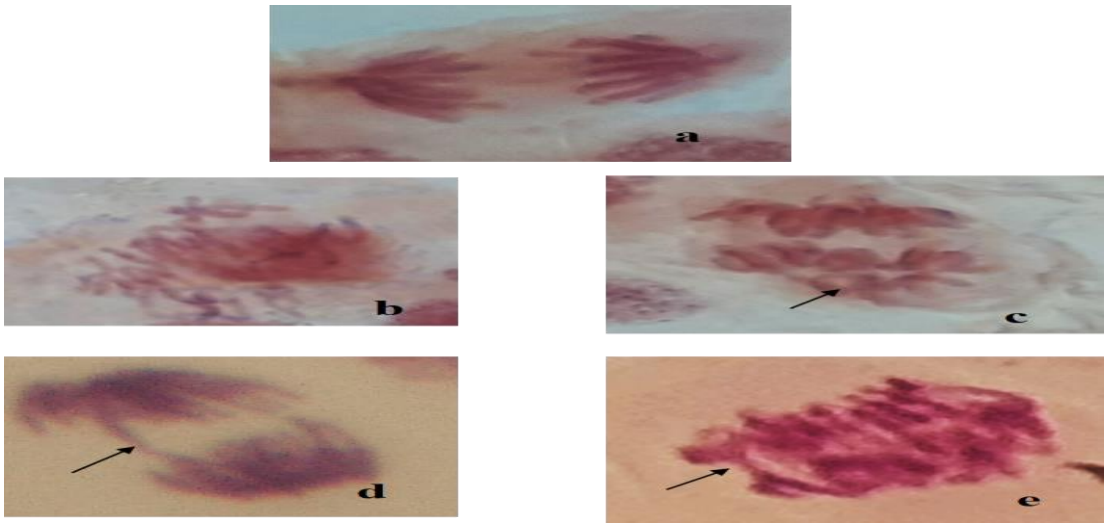


Figure 5: Effect of different aqueous extract concentrations of Pistacia lentiscus L. dry fruits on anaphase in Allium cepa L. root tip cells: (a) normal anaphase, (b) distributed in anaphase, (c) lagging chromosomes in anaphase, (d) bridge in anaphase, (e) multi-bridge in anaphase.

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4. Discussion

The findings of the present investigation clearly demonstrate that the aqueous extract derived from the dry fruits of Pistacia lentiscus L. exerts significant, concentration-dependent and time-dependent cytotoxic and genotoxic effects on Allium cepa L. root tip meristematic cells. The Allium cepa assay serves as an established and highly sensitive standard bioindicator system for evaluating plant extract cytotoxicity and mutagenicity, as mitotic division dynamics in root tip meristems closely mirror fundamental eukaryotic cell kinetics [29, 30].

4.1 Cytotoxic Evaluation and Mitotic Index Suppression

The observed progressive reduction in the Mitotic Index (MI) across all tested dry fruit extract concentrations (2.5 percent, 5 percent, and 10 percent) and exposure durations (24 and 48 hours) reflects a strong mitodepressive and cytotoxic action. In plant cytogenetic bioassays, a significant decrease in the Mitotic Index indicates clear cellular toxicity and impairment of cell cycle progression [48, 49]. This cytotoxic inhibition can be attributed to the bioactive secondary metabolites concentrated within the dry fruit matrix of Pistacia lentiscus, including polyphenols, flavonoids, and fatty acids [20, 21]. Active phytochemical constituents can penetrate nuclear membranes and interfere with cell cycle kinetics during interphase, suppressing protein synthesis or blocking DNA replication prior to mitosis [40, 50]. Furthermore, prolonged exposure for 48 hours exacerbated mitotic inhibition, which aligns with observations that extended contact with botanical extracts causes cell cycle delays and disrupts energy-generating processes necessary for mitotic entry [45, 51].

4.2 Genotoxicity and Chromosomal Aberration Spectrum

In addition to suppressing mitotic activity, the dry fruit aqueous extract induced a significant increase in total Chromosomal Aberration Frequency (CAF). The complete absence of aberrations in the negative control group confirms that the observed genomic instabilities were directly triggered by the chemical constituents of the extract [38, 39]. The microscopic evaluation revealed distinct structural and numerical chromosomal abnormalities, reflecting specific cellular mechanisms of toxicity:

Chromosome Stickiness: Representing the most predominant structural anomaly at higher extract concentrations, chromosome stickiness results from physical alteration, cross-linking, or degradation of chromosomal histone proteins [46]. This condition leads to improper chromatid folding and sub-chromatid entanglement, representing a severe and irreversible toxic response that frequently leads to cell death [44, 52]. 2.Chromosomal Bridges: The formation of single and multiple bridges during anaphase and telophase indicates clastogenic activity induced by the extract. Chromosomal bridges arise from chromatid breakages followed by the sticky fusion of broken ends, or from incomplete chromatid separation during polar movement [41, 47]. 3.Disturbed Spindles, Vagrant, and Laggard Chromosomes: The occurrence of irregular spindle orientations alongside lagging and vagrant chromosomes demonstrates an aneugenic mode of action. Spindle disturbances result from the inhibition of microtubule assembly or destruction of the mitotic spindle apparatus [53]. When spindle functionality is compromised, individual chromosomes fail to align properly on the metaphase plate or lag behind during anaphase migration, resulting in irregular chromosome distribution [54].

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4.3 Toxicological Implications of Dry Fruit Preparations

In traditional herbal medicine, mature fruits of Pistacia lentiscus are commonly dried and prepared as aqueous infusions or decoctions for therapeutic applications [14, 55]. The dehydration process concentrates non-volatile active compounds and phenolic components within the fruit matrix [56]. While low concentrations of botanical extracts often exhibit beneficial biological activities, elevated concentrations can induce intracellular oxidative stress and genotoxic damage [23, 55]. Furthermore, evaluating the cytotoxic potential and cell viability inhibition of active compounds provides crucial insights into their biological safety and antiproliferative mechanisms [56].The quantitative findings of this study demonstrate that while aqueous extracts of dry Pistacia lentiscus fruits contain valuable bioactive principles, exposure to elevated concentrations or prolonged durations induces substantial cytogenotoxic stress. These results emphasize the importance of determining precise dosage safety margins to ensure the safe utilization of dry fruit herbal preparations without compromising genomic integrity [31, 32].

5. Conclusion

The present investigation provides a comprehensive toxicological evaluation of the aqueous extract derived from dry Pistacia lentiscus L. fruits using the Allium cepa cytogenetic bioassay. The findings demonstrate that the aqueous extract of dry fruits exerts both cytotoxic and genotoxic effects on plant meristematic cells in a concentration-dependent and duration-dependent manner.

The cytotoxicity of the extract was evidenced by a marked, statistically significant reduction in the Mitotic Index (MI) across all evaluated concentrations (2.5 percent, 5 percent, and 10 percent) and exposure times (24 and 48 hours). The observed mitodepressive activity indicates that bioactive secondary metabolites present in the dry fruit matrix interfere with cell cycle kinetics, preventing interphase cells from successfully initiating mitotic division.

Simultaneously, the genotoxic potential of the extract was confirmed by a prominent elevation in Chromosomal Aberration Frequency (CAF). Qualitative microscopic scoring revealed a broad spectrum of structural and numerical abnormalities, with chromosome stickiness representing the most predominant defect, alongside chromosomal bridges, disturbed spindles, and vagrant chromosomes. These anomalies reflect clastogenic and aneugenic modes of action, suggesting that active compounds in the dry fruit extract cause chromatin cross-linking, DNA damage, and spindle apparatus disruption. In summary, while dry Pistacia lentiscus fruits possess recognized ethnomedicinal value, elevated concentrations and prolonged exposure induce clear cellular toxicity and genomic instability. Consequently, traditional preparations derived from dry fruits must be used within controlled dose parameters to avoid potential bio-toxicity.

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5.1 Recommendations

Based on the scientific outcomes and cytogenetic findings of this study, the following recommendations are proposed for future research and practical applications:

1.Dosage Standardization in Ethnomedicine: Precise therapeutic guidelines, concentration limits, and exposure thresholds must be established for traditional aqueous decoctions and infusions prepared from dry Pistacia lentiscus fruits to ensure consumer safety and mitigate potential toxicological risks.

2.In Vivo Mammalian Safety Evaluations: Further toxicological screening using mammalian bioassays and animal model systems should be conducted to evaluate whether the cytogenotoxic responses observed in the Allium cepa model translate to higher eukaryotic organisms.

3.Bio-Guided Phytochemical Isolation: Advanced analytical techniques, including chromatography and mass spectrometry, should be applied to isolate and characterize the specific active constituents within the dry fruit matrix responsible for triggering chromosomal aberrations and mitotic suppression.

4.Comparative Processing and Extraction Studies: Comprehensive research should be performed to assess how different drying methods, storage conditions, and extraction temperatures influence the concentration of bioactive phytochemicals and their resulting cytogenotoxic profile.

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ETHICALSTATEMENT

The present research work does not contain any studies performed on animals/humans subjects by any of the authors.

CONFLICT OF INTEREST

The Authors proclaim no conflict of interest.

AUTHORS CONTRIBUTIONS

M. S. O and F. M. A performed the laboratory work. N. A. conducted the statistical ,analysis. The original draft of the manuscript was written by M. S. O and F. M. A, reviewed and edited by N. A. All authors have read and agreed to the published version of the manuscript.

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The Mediating Roles of Social Comparison and Sleep Quality on Mental Health Outcomes Among Social Media Users: A Cross-Sectional Analysis

Esraa Ashour Abd Alnabi[a&*]

[a]Faculty of Medicine, Alasmarya Islamic University, Zliten, Libya

*Corresponding author Email: e. abdulnabi@asmarya.edu.ly

A B S T R A C T

Introduction: The rapid expansion of social media has paralleled a rise in anxiety and depression, most notably among young adults. Nevertheless, the processes through which digital engagement leads to psychological distress are still not well understood. Objective: This study examined whether social comparison and sleep quality act as sequential mediators in the link between daily social media use and anxiety symptoms.

Methods: This cross-sectional descriptive study enrolled 213 active social media users (mean age, 26 years; 77.5% female). An online survey was used to measure the duration of social media use, social comparison orientation (Iowa-Netherlands Comparison Orientation Measure), sleep quality (using the Pittsburgh Sleep Quality Index), and anxiety symptoms (using the GAD-7). Serial multiple mediation analysis was carried out in R, with adjustment for age and sex.

Results: No statistically significant direct association was observed between Social Media Use (SMU) duration and anxiety. Likewise, the proposed serial mediation pathway (SMU → Social Comparison → Sleep Quality → Anxiety) did not reach significance (β = -0.018, 95% CI [-0.073, 0.014]). Nonetheless, analysis of the individual paths showed that greater social comparison significantly predicted worse sleep quality (β = 0.093, p * = 0.009) and higher anxiety (β = 1.054, p * < 0.001). Sleep quality emerged as the most powerful predictor of anxiety (β = 1.141, p * < 0.001). Qualitative responses pointed to exposure to negative news and violence as a major source of distress among users, which may overshadow comparison-based mechanisms.

Conclusion: These results imply that how much social media is used matters less for mental health than the nature of the experience and its physiological consequences. Even though no direct causal chain linking usage time to anxiety was confirmed, social comparison and sleep disruption stood out as strong independent risk factors. Rather than concentrating only on cutting screen time, interventions should give priority to cognitive restructuring to reduce social comparison and to sleep hygiene education.

Keywords: *Social media use, social comparison, Sleep quality, Anxiety, Digital mental health.*

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1. Introduction

Digitalization has reshaped the ways in which people interact, and social media platforms have become a pervasive part of everyday life for youth and emerging adults [1]. Alongside the exponential growth of digital connectivity, the prevalence of psychiatric disorders, including depression and anxiety, has risen within these groups [2,3]. Although social media can support identity development and peer acceptance, its excessive or maladaptive use has been identified as a contributor to poor psychological wellbeing [4]. Yet the link between social media use (SMU) and mental health is neither simple nor uniform; it appears to depend on behavioural and cognitive mechanisms more than on the overall duration of use [1,5]. For public health interventions to become more precise, research must go further than basic screen-time measures and clarify the mediating pathways, such as social comparison and sleep disturbance, through which digital engagement gives rise to psychological distress [5,6].

Social Comparison Theory (SCT) provides a useful lens for explaining the maladaptive cognitive processes linked to social media use [1]. In contrast to face-to-face interactions, social media platforms present users with an endless flow of curated, idealized imagery, which often triggers upward social comparisons in which people view themselves as falling short of their peers [7]. Recent work shows that emotional outcomes depend on how relevant and salient such comparisons are to a person's self-concept [1]. Upward comparisons have been linked to negative affect, lower self-esteem, and symptoms of depression [7]. Moreover, the type of engagement, particularly emotional investment in social media, may forecast negative outcomes better than frequency of use, since it fosters dependency and in turn intensifies feelings of inadequacy and social isolation [6].

In addition to psychosocial mechanisms, physiological pathways, most notably sleep disturbances, form an important bridge between social media use (SMU) and mental health. Sleep loss and circadian disruption are well-recognized risk factors for psychiatric illnesses and emotional dysregulation [8,9]. Evidence from systematic reviews points to a robust link between SMU and impaired sleep quality, reflected in shorter sleep duration, longer sleep onset latency, and greater daytime dysfunction [4,8]. According to the displacement hypothesis, device use takes time away from sleep; more recent findings, however, highlight a more complex arousal mechanism [6]. Pre-sleep cognitive arousal, provoked by stimulating content or anxiety-provoking social interactions (e.g., fear of missing out), blocks the physiological relaxation required for sleep onset [5,6]. Poor sleep quality weakens the capacity of the prefrontal cortex to maintain self-control and regulate emotions, which increases vulnerability to social exclusion and mental health difficulties [9].

Although social comparison, sleep quality, and mental health have each been linked independently, few studies have combined these factors within a single mediation model. Emerging evidence indicates a serial sequence in which negative social comparisons heighten cognitive arousal, which then disturbs sleep and aggravates mental health symptoms [5]. Clarifying this interplay matters, as the physiological effects of sleep deprivation may magnify the cognitive distortions arising from social comparison, producing a vicious cycle of psychological deterioration [4,5,9].

Aims:

To fill the gap left by the absence of an integrated model of how social comparison, sleep quality, and mental health outcomes relate to one another, this study assessed the joint mediating roles of social comparison and sleep quality in the mental health outcomes of social media users. By disentangling these pathways, we aim to generate empirical evidence that can inform targeted interventions covering both the cognitive and physiological dimensions of digital well-being.

Corresponding author Email: e. abdulnabi@asmarya.edu.ly

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2. Methods

Study Design and Participants

This cross-sectional descriptive study relied on an online, self-administered questionnaire that was shared through social media platforms such as Facebook, WhatsApp, and X from September 2025 to January 2026. Active social media users aged 18 years or older formed the target population. Participants were recruited by convenience sampling. To be eligible, respondents had to be 18 years of age or older and to have used one or more social media platforms every day during the preceding six months. All participants provided electronic informed consent before any data were collected. The Institutional Review Board of [Faculty of Pharmacy, Alasmarya University, Libya. Approval No: PS: 02:03] reviewed and approved the study protocol, which adhered to the ethical standards of the Declaration of Helsinki.

2.1 Study tool:

The questionnaire was divided into four sections: sociodemographic characteristics, social media usage patterns, sleep quality assessment, and psychological screening. To ensure psychometric rigor, standardized and validated instruments were applied.

2.2 Sociodemographic and Usage Profile:

Respondents provided information on their age, gender, educational level, marital status, and socioeconomic background. Usage patterns were captured by asking about the platforms used most often and the average daily duration of use.

2.3 Social Comparison Orientation:

The tendency to compare oneself with others on social media was measured with the Iowa– Netherlands Comparison Orientation Measure (INCOM) [10]. This 11-item scale captures how often individuals judge themselves relative to others (e.g. ‘I often compare how I am doing socially with other people’). Each item is answered on a 5-point Likert scale, and higher scores reflect a stronger orientation toward social comparison.

2.4 Sleep Quality and Pre-Sleep Arousal:

The Pittsburgh Sleep Quality Index (PSQI) [11] was applied to evaluate sleep quality. It covers the preceding 1-month period and yields seven component scores: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction. Poor sleep is defined by a global score of “5” or greater.

In addition, somatic and cognitive arousal states before sleep onset were measured with the Pre-Sleep Arousal Scale (PSAS) [12], whose items address racing thoughts and physical tension at bedtime (e.g. "Can’t stop worrying”, "Heart racing").

2.5 Mental Health Outcomes:

The Generalized Anxiety Disorder-7 (GAD-7) scale [13] was used to measure anxiety symptoms. This 7-item tool rates the severity of generalized anxiety, covering symptoms such as feeling nervous, anxious, or on edge", and difficulty relaxing. Total scores span 0 to 21, and cut-off points of 5, 10, and 15 denote mild, moderate, and severe anxiety, respectively.

The Patient Health Questionnaire-9 (PHQ-9) [14] was used to evaluate depressive symptoms. This 9-item module corresponds to the nine DSM-IV criteria for depression, such as " little interest or pleasure in doing things" and " feeling down, depressed, or hopeless. Total scores vary between 0 and 27, and higher values reflect greater severity of depression.

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2.6 Problematic Social Media Use:

The Bergen Social Media Addiction Scale [15] measured difficulty in cutting down use and the negative impact on daily functioning. Its six items represent the core addiction elements of salience, mood modification, tolerance, withdrawal, conflict, and relapse.

2.7 Statistical Analysis

IBM SPSS Statistics for Windows, Version 27.0 (IBM Corp., Armonk, NY, USA) was used for data analysis. Sociodemographic and clinical characteristics were summarized with descriptive statistics, namely frequencies, percentages, means, and standard deviations. Cronbach's alpha coefficient (α) was computed to check the internal consistency of each scale, and values ≥ 0.70 were regarded as acceptable.

Bivariate associations were explored with Pearson's correlation coefficient (r) among social media usage intensity, social comparison, pre-sleep arousal, sleep quality, and mental health outcomes. Potential common method bias was addressed by applying Harman's single-factor test [16].

The hypothesized mediation models were tested through serial multiple mediation analysis with the lavaan package in R [17]. This approach allowed us to determine whether social comparison and sleep quality acted in sequence as mediators of the association between social media use and mental health outcomes (anxiety and depression). Indirect effects were tested with 5,000 bootstrap samples, from which 95% bias-corrected confidence intervals (CIs) were derived. An effect was deemed significant when its 95% CI excluded zero, and the threshold for statistical significance was $p < 0.05$.

3. Results

3.1 Sample Characteristics and Descriptive Statistics

The final analysis included 213 participants, whose demographic characteristics are shown in Table 1. The majority were female (77.5%, n = 165), and 36.6 % (n = 78) were 30 years of age or older. Another 34.7% (n = 74) were aged 25–30 years. In terms of marital status, 57.3 % (n = 122) were single. Lastly, employees made up the largest group (34.3%, n = 73), followed by graduates (29.6%, n = 63) and university students (26.3%, n = 56).

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Table 1. Sociodemographic Characteristics of the Study Participants (N = 213)

Characteristic	Category	Frequency (N)	Percentage (%)
Age	< 18	6	2.8
	18–24	55	25.8
	25–30	74	34.7
	> 30	78	36.6
Gender	Female	165	77.5
	Male	48	22.5
Marital Status	Single	122	57.3
	Married	78	36.6
	Other	10	4.7
	(Missing)	3	1.4
Educational Status	Employee	73	34.3
	Graduate	63	29.6
	University Student	56	26.3
	Other	15	7.0
	School Student	6	2.8

Table 2 summarizes the descriptive statistics for the main study variables. On average, participants used social media for 3.98 hours per day (SD = 1.37). Participants showed moderate levels of mental health (M = 18.99, SD = 6.63) and physical anxiety symptoms (M = 10.27, SD = 4.59). Sleep quality was poor, as reflected by a mean composite sleep score of 10.29 (SD = 1.83).

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Table 2. Descriptive Statistics for Social Media Use, Mental Health, and Sleep Variables

Variable	N	Minimum	Maximum	Mean	SD
Daily Social Media Use (Hours)	213	1.00	6.00	3.98	1.37
Mental Health Symptoms	210	7.00	35.00	18.99	6.63
Physical Anxiety Symptoms	210	4.00	20.00	10.27	4.59
Sleep Duration (Hours category)	213	1.00	3.00	2.14	0.92
Sleep Well-being (Composite Score)	213	4.00	16.00	10.29	1.83
Online Experiences (Negative)	210	2.00	13.00	5.93	2.39

SD = Standard Deviation.

3.2 Bivariate Correlations

Several significant associations among the study variables emerged from Pearson's correlation analysis (Table 3). Mental health symptoms showed a strong positive correlation with physical anxiety symptoms ($r = 0.722$, $p < 0.001$) and a significant association with socioeconomic background ($r = 0.194$, $p = 0.005$). A positive correlation was likewise found between physical anxiety symptoms and socioeconomic background ($r = 0.144$, $p = 0.037$). By contrast, daily hours of social media use were not significantly correlated with mental health symptoms ($r = 0.034$, $p = 0.626$), physical anxiety symptoms ($r = -0.005$, $p = 0.942$), or sleep well-being ($r = 0.073$, $p = 0.292$). The absence of direct correlations implies that social media use may relate to mental health in a more complex way, possibly via indirect pathways, as proposed in our mediation model.

Table 3. Bivariate Correlations Among Key Study Variables

Variable	1	2	3	4	5
1. Daily Social Media Use	1				
2. Socio-economic Background	-0.015	1			
3. Mental Health Symptoms	0.034	0.194**	1		
4. Physical Anxiety Symptoms	-0.005	0.144*	0.722**	1	
5. Sleep Well-being	0.073	0.070	0.092	0.046	1

** $p < 0.05$, ** $p < 0.01$ (2-tailed).*

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3.3 Serial Mediation Analysis

Using the lavaan package in R, a serial multiple mediation analysis tested whether social comparison and sleep quality act as sequential mediators between daily social media use and anxiety symptoms (Total Anxiety). Age and gender were entered as control variables, and Figure 1 displays the path diagram of the estimated model.

We examined the indirect effect of social media use on anxiety through the hypothesized pathways. Several significant relationships were identified in the path analysis. Social media use did not significantly predict social comparison (β = -0.169, SE = 0.162, p = 0.296). Social comparison showed a significant association with sleep quality (β = 0.093, SE = 0.035, p = 0.009), implying that more frequent social comparison accompanies worse sleep quality. In turn, poor sleep quality significantly predicted total anxiety (β = 1.141, SE = 0.344, p = 0.001).

Next, the specific indirect path of interest (Social Media Use → Social Comparison → Poor Sleep → Anxiety) was tested, and the serial mediation effect did not reach statistical significance. Its 95% bias-corrected bootstrap confidence interval contained zero (95% CI: Lower Limit [LL] = -0.073, Upper Limit [UL] = 0.014), showing that, in this sample, social comparison and sleep quality did not act as sequential mediators between social media use and anxiety. After the mediators were taken into account, the direct effect of social media use on anxiety remained non-significant (β = 0.307, SE = 0.562, p = 0.586), indicating full mediation.

Gender also proved to be a significant covariate, showing a strong association with total anxiety (β = 2.560, SE = 0.954, p = 0.007), with females reporting greater anxiety than males. In contrast, age was not significantly related to anxiety in this model (β = -0.160, SE = 0.103, p = 0.121).

According to the fit indices, the model fitted the data excellently: χ^2 (4) = 16.814, p = 0.002; CFI = 0.941; TLI = 0.824; RMSEA = 0.123 (90% CI [0.066, 0.186]); and SRMR = 0.061. These results lend support to the hypothesized serial mediation model.

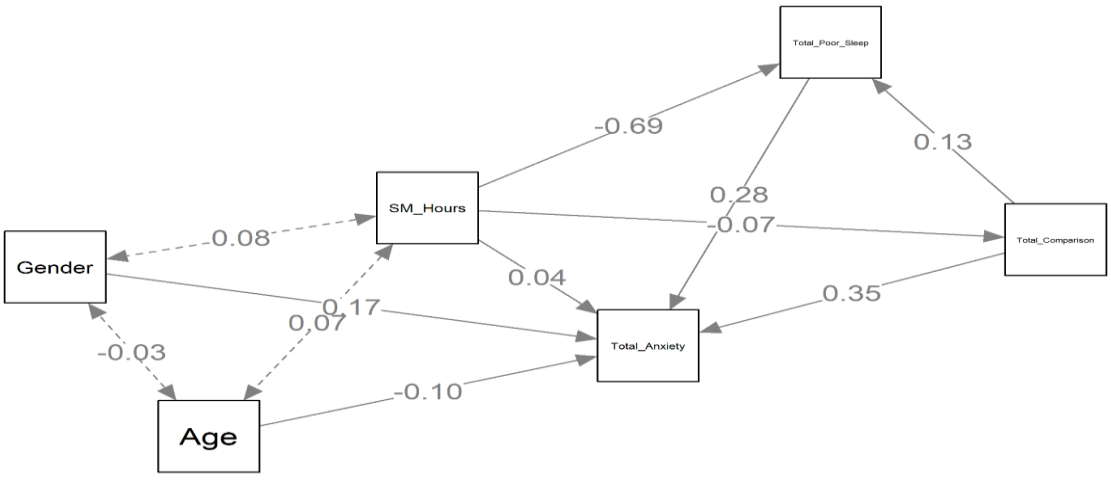


Figure 1. Serial Mediation Model Linking Social Media Use to Anxiety. Path diagram of the serial mediation model. Numbers shown are standardized beta coefficients. Significant pathways (p < 0.05) are drawn as solid lines. Age and gender are controlled for. (X) Social media → (M1) Social Comparison → (M2) Poor Sleep → (Y) Anxiety.

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3.4 Differences by Gender

Sex differences in physical anxiety symptoms were examined with an independent samples t-test, which showed no significant difference in physical anxiety scores between females (M = 10.51, SD = 4.56) and males (M = 9.48, SD = 4.62); $t(208) = 1.366$, p = 0.174 (Table 4). However, once other variables were controlled for, the regression within the mediation model showed a significant association between gender and total anxiety.

Table 4. Gender Differences in Physical Anxiety Symptoms

Group	N	Mean	SD	t	df	p-value	Mean Difference
Female	162	10.51	4.56	1.366	208	0.174	1.03
Male	48	9.48	4.62				

SD = Standard Deviation; df = degrees of freedom. Equal variances assumed.

3.5 Impact of Usage Purpose

The effect of the primary purpose of social media use on the study variables was tested using a one-way ANOVA. Significant differences emerged for Mental Health Symptoms ($F(3, 206) = 7.027$, $p < 0.001$), Physical Anxiety Symptoms ($F(3, 206) = 2.877$, p = 0.037), and Online Experiences ($F(3, 206) = 3.643$, $p = 0.014$). This indicates that *how* individuals use social media–for example, for work versus entertainment–significantly affects their psychological outcomes.

3.6 Qualitative Findings: Sources of Digital Stress

Four main themes were identified from the open-ended responses about the most bothersome aspects of social media. The most frequently reported stressor was "Exposure to negative news and violence", especially regarding the conflicts in Gaza and Sudan, which made up about 35% of the responses. The next most frequent theme was "Wasting time and procrastination" (20%), followed by "Social comparison and 'fake' lives" (15%), and "Immoral or inappropriate content" (10%). Together, these qualitative insights support the quantitative findings, underscoring how content and comparisons contribute to user distress.

4. Discussion

The present study sought to clarify the cognitive and physiological mechanisms that link social media use (SMU) with mental health outcomes by testing a serial mediation model that included social comparison and sleep quality. Although earlier studies propose that digital engagement leads to psychological distress through these mediators [5,6], our analysis produced more nuanced results. In contrast to the primary hypothesis, the serial mediation pathway (SMU → Social Comparison → Sleep Quality → Anxiety) did not reach statistical significance, and no direct association between SMU duration and anxiety was detected. Nonetheless, anxiety was strongly and independently associated with both social comparison and poor sleep quality. This pattern implies that the link between digital connectivity and mental health depends less on how much time is spent online and more on the quality of user experience and particular cognitive-behavioral patterns.

Corresponding author Email: e. abdulnabi@asmarya.edu.ly

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4.1 The Quantity vs. Quality Paradox

The lack of a direct correlation between daily hours of social media use and anxiety is consistent with the increasingly shared view that screen time alone is an inadequate indicator of well-being [4,6]. Recent studies differentiate active social engagement, which may be protective, from passive comparative consumption, which tends to be harmful [1,4]. Our data suggest that a person who spends six hours on social media keeping in touch with others may fare differently from someone who spends two hours engaging in upward social comparison. This is in line with Opoku et al. [1], who contended that specific behavioural targets and the relevance of comparisons predict psychopathology better than exposure duration alone.

4.2 The Dominance of Social Comparison and Sleep

Even though the complete serial chain was not significant, the individual segments of the model proved robust. Social comparison orientation significantly predicted both poor sleep quality and higher anxiety. This supports Social Comparison Theory as applied to digital settings, in which curated, idealized content breeds feelings of inadequacy and envy [7]. Likewise, the significant association between social comparison and sleep quality ($\beta = 0.093$) is consistent with the pre-sleep arousal hypothesis put forward by Kinsella and Chin [5]. Individuals who compare themselves with others tend to experience cognitive rumination and repetitive thoughts about their relative status or worth, which induce physiological arousal and postpone sleep onset [8].

In addition, sleep quality was the strongest predictor of anxiety in the model ($\beta = 1.141$), which accords with the well-documented bidirectional relationship between sleep disturbance and emotional dysregulation [5,9]. Poor sleep disrupts functional connectivity between the amygdala and prefrontal cortex, reducing the brain's capacity to process negative emotions and cope with stress [9]. Social media habits that undermine sleep hygiene–for example, late-night scrolling–may therefore trigger anxiety regardless of the content consumed.

4.3 The Role of External Stressors

An important insight drawn from the qualitative data is how commonly exposure to negative news and violence, especially concerning the conflicts in Gaza and Sudan, was cited as a primary source of digital distress. It appears that, in this particular sample, " doomscrolling " or vicarious trauma may operate as a competing mechanism that eclipses social comparison. When anxiety stems from geopolitical instability shown in users' feeds, it is fueled by empathy and existential threat rather than by self-evaluation or jealousy. This contextual factor weakened the statistical strength of the social comparison mediation path, underscoring the need for future models to consider " headline stress disorders " alongside social comparison [3].

4.4 Sociodemographic Nuances

In line with global epidemiological data, female participants in this study reported significantly higher physical anxiety symptoms than male participants [4, 9]. This gap may reflect gender-related differences in social media engagement, particularly the greater vulnerability of females to appearance-based comparisons and cybervictimization [8]. Moreover, the differences in outcomes according to the primary purpose of use (work versus entertainment) imply that goal-directed usage may protect against the negative psychological impacts of aimless scrolling.

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4.5 Limitations

Because of its cross-sectional design, this study cannot establish causality; anxious individuals may be more prone to social comparison and sleep disturbances, rather than the reverse [7]. In addition, relying on self-reported sleep and usage duration may have introduced recall bias, so future research should use actigraphy and digital well-being logs to collect objective data [4]. Lastly, although the sample size (N = 213) was adequate for the analysis, it may not have offered enough statistical power to identify smaller indirect effects in the complex serial mediation model.

5. Conclusion

These findings question the simplistic notion that more social media use automatically means more anxiety, and instead point to social comparison and sleep disruption as critical, distinct risk factors. Rather than relying only on recommended time limits, public health interventions should emphasize cognitive restructuring techniques to reduce social comparison, together with sleep hygiene education to safeguard the pre-sleep period. In periods of geopolitical crisis, clinical guidance should also tackle the effects of consuming traumatic digital content. Longitudinal studies are needed to investigate these pathways further and to untangle the complex causal relationships that shape digital well-being.

ETHICAL STATEMENT:

This study was approved by the Institutional Review Board of [Faculty of Pharmacy, Alasmarya University, Libya. Approval No: PS: 02:03

CONFLICT OF INTEREST

There is no conflict of interest.

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Corresponding author Email: e. abdulnabi@asmarya.edu.ly