Assessment of Dietary Diversity and Associated Socio-Demographic Determinants among School-Age Children in Maiduguri, Borno State, Nigeria

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Abstract

Dietary diversity has long been recognised as a key component of diet quality, nutritional adequacy, as well as micronutrient status, particularly in vulnerable paediatric populations within conflict-affected regions where food systems face systematic disruption. This study is aimed at evaluating dietary diversity patterns and their correlation with socio-demographic determinants among school-age children residing in the conflict-affected Maiduguri Metropolitan Council, Northeast Nigeria. This cross-sectional analytical study enrolled 300 children aged 5-11 years through multi-stage random sampling across 15 political wards. Data collection utilised validated structured questionnaires, standardised 24-hour dietary recall protocols, and anthropometric assessments. Individual Dietary Diversity Scores (IDDS) were computed based on consumption across 12 distinct food groups, with dietary diversity stratified as low (≤4 groups), medium (5-7 groups), or high (≥8 groups). Statistical analyses employed chi-square tests and multivariate regression models. The study revealed profound dietary restrictions, with 41.7% of participants demonstrating low dietary diversity, 55.3% achieving medium diversity, and merely 3.0% attaining high diversity. Significant statistical associations emerged between dietary diversity and multiple socio-demographic variables. Children from economically disadvantaged households demonstrated 21-fold increased odds of experiencing low dietary diversity compared to their non-poor counterparts. Maternal educational deficit emerged as a critical risk factor, with 54.3% of children whose mothers lacked formal education experiencing dietary diversity restrictions. This investigation documents severe dietary limitations among school-age children in this conflict-affected region, with socioeconomic stratification serving as the primary determinant of nutritional quality. The findings necessitate comprehensive, multi-sectoral interventions incorporating poverty alleviation strategies, maternal educational enhancement programs, and expanded school-based nutritional interventions to address the underlying determinants of dietary inadequacy.

Keywords: Dietary diversity; Socioeconomic determinants; Food security; Conflict-affected populations; Micronutrient adequacy.

Introduction

Dietary diversity serves as a validated proxy indicator for nutritional quality, micronutrient density, and overall dietary adequacy, particularly among vulnerable paediatric populations.[1] Within the context of school-age children, adequate dietary diversity assumes critical importance for supporting optimal physical growth trajectories, neurocognitive development, and the establishment of sustainable nutritional behaviours that persist into adulthood. [2,3] The significance of varied food intake becomes substantially amplified in resource-constrained environments where children face elevated risks of macro and micronutrient deficiencies.

Nigeria continues to experience persistently elevated rates of child malnutrition, positioning the nation among countries bearing the highest global burden of childhood undernutrition. [4] The 2018 Nigeria Demographic and Health Survey documented that 37% of children under five years exhibit stunting at the national level, with markedly higher prevalence rates observed across northern regions compared to southern territories. [5] These nutritional challenges demonstrate severity in Northeast Nigeria, where the convergence of protracted conflict, mass population displacement, and climate variability has generated complex humanitarian emergencies affecting millions of vulnerable individuals.

Maiduguri Metropolitan Council (MMC), serving as the administrative capital of Borno State, presents a unique epidemiological context for investigating nutritional challenges within conflict-affected settings. The region has endured more than a decade of insurgency-related violence that has fundamentally disrupted local food systems, eliminated traditional livelihood mechanisms, and generated unprecedented population displacement affecting over 2.2 million individuals throughout the Lake Chad Basin.[6] These circumstances have systematically compromised traditional food security frameworks, potentially resulting in constrained dietary diversity and heightened nutritional vulnerability among paediatric populations.

School-age children (5-11 years) represent a critically important yet frequently neglected demographic within nutrition programming and research initiatives. Unlike younger children who benefit from substantial attention through established intervention frameworks, school-age children often experience programmatic gaps despite confronting distinct nutritional requirements and vulnerabilities.[7] This population experiences consistent growth patterns, elevated physical activity demands, and increasing autonomy in food selection behaviours, necessitating targeted attention to dietary quality and nutritional sufficiency.

The relationship between socio-demographic characteristics and dietary diversity has received extensive documentation across diverse global contexts [8], with consistent evidence demonstrating that household economic status, parental educational attainment, and employment security significantly influence food accessibility and dietary quality.[9] However, limited research has specifically examined these relationships among school-age children within conflict-affected environments, creating substantial knowledge gaps that constrain the development of targeted interventions.

This investigation aims to systematically assess dietary diversity patterns among children aged 5-11 years residing in Maiduguri Metropolitan Council and examine their associations with key socio-demographic determinants. The findings will contribute to enhanced understanding of nutritional vulnerabilities within conflict-affected populations and inform evidence-based intervention strategies to improve paediatric nutritional outcomes in comparable contexts.

Materials and Methods

Study Design and Geographic Setting

This cross-sectional analytical design was implemented in the Maiduguri Metropolitan Council (MMC), Borno State, Nigeria. The study was conducted in Maiduguri, which serves as both the state capital and administrative headquarters of Borno State, located in Nigeria's Northeastern region. MMC encompasses approximately 50,778 square kilometres and is geographically positioned at 11°50'N latitude and 13°09'E longitude. As the largest metropolitan area in the region, Maiduguri represents a critical administrative and commercial centre for northeastern Nigeria, accommodating a demographically diverse population including established residents, internally displaced populations, and individuals returning from adjacent territories.

The metropolitan council includes 15 distinct political wards - Bolori I, Bolori II, Bwalaram, Bulabulin, Fezzan, Gamboru, Gwange I, Gwange II, Gwange III, Hausari, Lamisula, Maisandari, Shehuri North, Shehuri South, and Yerwa. Each exhibits distinctive socio-demographic characteristics and economic circumstances.

Study Population

The study population consisted of 300 children aged 5-11 years who were permanent residents of Maiduguri Metropolitan Council, Borno State, Nigeria, along with their corresponding mothers and primary caregivers (n=300), who were responsible for the children's nutritional care and feeding practices. This sample size was determined through power analysis to ensure adequate statistical power for detecting meaningful associations between nutritional status indicators and feeding practices. This specific age cohort was selected based on several developmental and methodological considerations. Children within this age range represent a critical period of physical and cognitive development characterised by elevated nutritional requirements and the establishment of long-term dietary preferences and behaviours.[10]

Study Population and Sampling Framework

The target population encompassed children aged 5-11 years maintaining residence within MMC for a minimum duration of six months preceding data collection activities. Sample size determination utilised the standard formula for prevalence studies:

$$n = Z^2pq/d^2$$

Where Z = 1.96 (representing a 95% confidence interval), p = 0.32 (anticipated stunting prevalence based on NNHS 2018 data), q = 0.68, and d = 0.05 (acceptable margin of error). This calculation yielded a minimum sample requirement of 334 participants, subsequently adjusted to 300 to accommodate logistical constraints and resource limitations.

A multi-stage random sampling methodology was implemented, incorporating proportional allocation across the 15 political wards comprising MMC. Simple random sampling techniques were employed to select target households within each designated ward, with enrolment of the youngest eligible child from households containing multiple children within the specified age range.

Data Collection Instruments and Protocols

Socio-demographic Assessment

A comprehensively structured questionnaire captured essential household and child characteristics, including chronological age, gender, household composition, parental educational achievements, employment status, self-reported poverty assessment, and residential density classifications. The instrument underwent rigorous pre-testing and validation through expert review, context analysis, and pilot testing within comparable demographic settings.

Dietary Diversity Evaluation

Individual Dietary Diversity Score (IDDS) assessment employed the standardised 24-hour dietary recall methodology based on established FAO protocols.[11] The evaluation encompassed 12 distinct food categories: cereals and grains, roots and tubers, legumes and nuts, dairy products, eggs, fish and seafood, meat and poultry, vegetables, fruits, fats and oils, sweets and sugars, and beverages. Each consumed food group received a binary score of 1, with the total IDDS calculated as the summation of all consumed food categories. Dietary diversity categorisation followed established paediatric cut-off points: low diversity (≤4 food groups), medium diversity (5-7 food groups), and high diversity (≥8 food groups).

24-Hour Dietary Recall Method

The 24-hour dietary recall method was employed to evaluate food and beverage consumption during the previous day or the preceding 24 hours.[12] This method provides comprehensive, quantitative information on individual diets by querying respondents about the type and quantity of all food and beverages consumed during the previous 24-hour period.[13] It is normally carried out in chronological order (morning to night), where a list of foods eaten and drunk is jotted down.[14] The interviewer asked the caregiver about all foods/beverages consumed by the child in the past 24 hours, then probed for additional details, including preparation methods and estimates of amounts consumed.[15] Of the available dietary assessment methods, the 24-hour recall is most frequently used for population assessment in low-income countries. [16] The method is particularly valuable in resource-limited settings where literacy levels may be low, making it more suitable than written food diaries or food frequency questionnaires.

Food Frequency Assessment

A Food Frequency Questionnaire (FFQ) represents a structured dietary assessment instrument comprising a predetermined inventory of food items and beverages accompanied by frequency response options to capture typical consumption patterns within a specified timeframe.[17] This methodology serves as a systematic approach for gathering nutritional data through culturally appropriate food groups to characterise habitual dietary behaviours and explore associations between eating patterns and health indicators.[18]

Comprehensive dietary evaluation through FFQs typically incorporates between 80 and 120 food and beverage items to capture the breadth of dietary intake. [17] The study was designed to document routine dietary practices by systematically inquiring about the consumption frequency of individual foods or food categories. This design/methodology faced several inherent constraints, including dependence on participant recall accuracy, challenges in quantifying portion estimates, susceptibility to systematic over- or under-reporting of typical intake patterns, and requirements for population-tailored food inventories that accurately represent regional dietary customs and available food sources.[19]

A semi-quantitative food frequency questionnaire was used in this study, which evaluated habitual consumption patterns of commonly consumed foods within the study region. Frequency classifications included daily consumption, 4-6 times weekly, 1-3 times weekly, and rarely/never consumed categories.

Anthropometric Measurements

Anthropometric assessment measures the human body dimensions and physical composition to

determine nutritional conditions. [20,21] Within nutritional evaluation frameworks, anthropometric data collection involves obtaining specific body measurements, including weight-for-age, length-for-age, and weight-for-height ratios, which are subsequently benchmarked against established reference populations to determine nutritional status. [22] Anthropometric data collection adhered to standardised WHO measurement protocols to ensure accuracy and reliability.[22] Weight assessment was conducted using precision digital scales calibrated to ±0.1 kg tolerance, with daily verification performed using certified reference weights to maintain measurement integrity. Scales were strategically positioned on stable, horizontal surfaces, with participants wearing lightweight clothing and no footwear to reduce potential measurement variations. [22] Height determination employed portable stadiometers featuring ±0.1 cm precision, subjected to daily calibration procedures to ensure optimal functionality. Participants were positioned barefoot against vertical measurement surfaces with feet positioned together, arms relaxed at sides, and head orientation aligned with Frankfurt horizontal plane specifications to achieve standardised measurement conditions. Body Mass Index calculations and age-sex specific z-score determinations were processed through the WHO AnthroPlus software for consistent analytical standards. [22]

Statistical Analysis Framework

Data analysis was conducted using SPSS version 30.0 software. Descriptive statistics included frequency distributions and percentages for categorical variables and means with standard deviations for continuous variables. Chi-square tests examined associations between categorical variables, with a statistical significance threshold established at $p<0.05$. Multivariate logistic regression analyses were performed to identify independent predictors of dietary diversity categories while controlling for potential confounding variables.

Ethical Considerations

Ethical clearance was obtained from the Borno State Ministry of Health Ethics Review Committee. Written informed consent was secured from parents/primary caregivers, with additional verbal assent obtained from participating children aged 5-11 years. Participation remained entirely voluntary, and strict confidentiality protocols were maintained throughout all study procedures.

Results

Socio-demographic Characteristics

Table 1 presents the socio-demographic profile of study participants. Age distribution revealed 50.7% of participants aged 5-7 years and 49.3% aged 8-11 years, with males comprising 64.4% of the study population. Household economic vulnerability was pronounced, with 65.3% of families self-identifying as economically disadvantaged and 61.7% reporting guardian unemployment status.

Educational disparities were particularly striking, especially among maternal caregivers, with 50.3% having no formal educational background compared to 7.3% of paternal caregivers.

Table 1: Socio-Demographic Characteristics of the Respondents

Variable	Category	Frequency(N)	Percentage (%)
Age Group (years)	5 – 7	152	50.7
	8 – 11	148	49.3
Gender	Male	170	56.7
	Female	130	43.3
Self-assessed Poverty Status	Poor	196	65.3
	Non-Poor	104	34.7
Guardian's Employment Status	Employed	115	38.3
	Unemployed	185	61.7
Receives Weekly Pocket Money	Yes	25	8.3
	No	275	91.7
Household Size	≤ 5 persons	9	3.0
	6 – 10 persons	174	58.0
	> 10 persons	117	39.0
Residential Density	High	103	34.3
	Medium	83	27.7
	Low	114	38.0
Father's Level of Education	Primary	39	13.0

Variable	Category	Frequency(N)	Percentage (%)
	Secondary	79	26.0
	NCE/Diploma	91	30.0
	B.Sc. or Equivalent	66	22
	Ph.D	3	1.0
	Never Attended School	22	7.3
Mother's Level of Education	Primary	55	18.3
	Secondary	45	15.0
	NCE/Diploma	31	10.3
	B.Sc. or Equivalent	5	1.7
	Ph.D	13	4.3
	Never Attended School	151	50.3
Breakfast at School	Always	45	15.0
	Regularly	89	29.7
	Rarely	130	43.3
	Never	36	12.0
Lunch at School	Always	83	27.7
	Regularly	90	30.0
	Rarely	101	33.7
	Never	26	6.7

Table 2 demonstrates that the majority of children (55.3%) achieved medium dietary diversity levels, indicating moderate access to varied food groups. However, a substantial proportion (41.7%) exhibited low dietary diversity, correlating with elevated risks of micronutrient deficiencies and undernutrition. Remarkably, only 3.0% of participants achieved high dietary diversity

Table 2: Dietary Diversity Score

Category	Frequency (n)	Percentage (%)
Low Dietary Diversity	125	41.7
Medium Dietary Diversity	166	55.3
High Dietary Diversity	9	3.0
Total	300	100.0

Table 3 illustrates the relationships between dietary diversity and household socio-demographic characteristics. Neither age group ($p=0.096$) nor gender ($p=0.096$) demonstrated significant associations with dietary diversity scores, indicating that boys and girls across the 5-11 age range experience comparable food consumption patterns. Conversely, all economic indicators demonstrated highly significant associations ($p<0.001$). Children from self-identified economically disadvantaged households were predominantly classified within the low-diversity category (62%), while 94% of children from non-poor households achieved medium diversity classification. Similar patterns emerged regarding guardian employment status: 66% of children with unemployed caregivers experienced low diversity, whereas 91% of those with employed guardians achieved medium diversity. Receiving weekly pocket money served as a strong predictor of dietary variety, with 72% of recipients achieving medium diversity compared to only 45% among those without pocket money access. Household size and residential location also influenced dietary diversity outcomes. Large families (>10 members) and low-density residential areas demonstrated a higher prevalence of low dietary diversity, while medium-density areas exhibited the highest proportion of medium-diversity households (94%).

Parental educational attainment displayed clear stepwise associations: as fathers' or mothers' educational levels increased from primary through tertiary education, the proportion of children achieving medium diversity rose substantially while those experiencing low diversity declined correspondingly. Lastly, children consistently consuming breakfast or lunch at school were predominantly classified within the medium-diversity category (>90% for breakfast consumers and 88% for lunch consumers), while those rarely or never eating at school demonstrated higher rates of low diversity (65% for breakfast non-consumers, 71% for lunch non-consumers).

Table 3: Association between Dietary Diversity and Socio-Demographic Characteristics

Items	Variables	Dietary Diversity			*P*-value
		Low	Medium	High	
Age group	5 – 7	68(44.7)	80(52.6)	4(2.6)	.096
	8 – 11	57(38.5)	86(58.1)	5(3.4)	
Gender	Male	79(46.2)	90(52.6)	2(1.2)	.096
	Female	37(39.4)	52(55.3)	5(5.3)	
Self-assessed Poverty Status	Poor	122(62.2)	68(34.7)	6(3.1)	.000
	Non-poor	3(2.9)	98(94.2)	3(2.9)	
Guardian Employment Status	Employed	5(4.3)	105(91.3)	5(4.3)	.000
	Unemployed	120(66.3)	57(31.5)	4(2.2)	
Do you receive weekly pocket money?	Yes	3(12.0)	18(72.0)	4(16.0)	.000
	No	121(45.0)	143(53.2)	5(1.9)	
Household Size	Up to 5	4(44.4)	5(55.6)	0(0.0)	.036
	6 – 10	67(37.9)	108(61.0)	2(1.1)	
	More than 10	54(47.4)	53(46.5)	7(6.1)	
Residence Density	High	37(35.9)	59(57.3)	7(6.8)	.000
	Medium	3(3.6)	78(94.0)	2(2.4)	
	Low	85(74.6)	29(25.4)	0(0.0)	
Father's level of education	Primary	24(61.5)	13(33.3)	2(5.1)	.000
	Secondary	67(84.8)	10(12.7)	2(2.5)	
	NCE	22(24.2)	67(73.6)	2(2.2)	
	BSc	3(4.5)	60(90.9)	3(4.5)	
	PhD.	0(0.0)	3(4.5)	0(0.0)	
	Never	9(40.9)	13(59.1)	0(0.0)	
Mother's level of education	Primary	21(38.2)	34(61.8)	0(0.0)	.000
	Secondary	12(26.7)	29(64.4)	4(0.0)	
	NCE	5(16.1)	26(83.9)	0(0.0)	
	BSc	3(60.0)	2(40.0)	0(0.0)	
	PhD.	2(15.4)	9(69.2)	2(15.4)	
	Never	82(54.3)	66(43.7)	3(2.0)	
How often do you eat breakfast at school?	Always	2(4.4)	42(93.3)	1(2.2)	.000
	Regularly	19(21.3)	68(76.4)	2(2.2)	
	Rarely	85(65.4)	39(30.0)	6(4.6)	
	Never	19(52.8)	17(47.2)	0(0.0)	
How often do you eat lunch at school?	Always	9(10.8)	73(88.0)	1(1.2)	.000
	Regularly	33(36.7)	51(56.7)	6(6.7)	
	Rarely	72(71.3)	27(26.7)	2(2.0)	
	Never	11(42.3)	15(57.7)	0(0.0)	

Table 4 shows that the Anthropometric assessment revealed that stunting was the most severe form of malnutrition, affecting 68% (n=204) of children. 49.0% had moderate stunting (HAZ < -2 to ≥ -3 SD) and 19.0% had severe stunting (HAZ < -3 SD), while only 32.0% maintained normal height-for-age (HAZ ≥ -2 SD). This prevalence is more than double the WHO's "high" threshold of 30% and far exceeds Nigeria's national average of 37% (WHO 2024; UNICEF 2024). For underweight, 64%

(n=192) of children were affected, comprising 44.0% moderate and 20.0% severe cases. Only 36.0% had normal weight-for-age. Wasting was present in 57% (n=171) of children, including 46.0% with moderate wasting and 11.0% with severe wasting. BMI-for-age data showed that 61% (n=183) of children were malnourished, with 45.0% moderately and 16.0% severely affected. The remaining 39.0% had normal BMI-for-age. These results show that, across all indicators, malnutrition prevalence ranged from 57% to 68%, with moderate malnutrition consistently affecting 44–49% of children.

Table 4: Nutritional Status of Children Using Anthropometric Measurements

Anthropometric Indicator	Normal	Moderately Malnourished	Severely Malnourished	Total
Weight for Age (WAZ)	90 (30%)	144 (48%)	66 (22%)	300 (100%)
Height for Age (HAZ)	75 (25%)	165 (55%)	60 (20%)	300 (100%)
Weight for Height (WHZ)	102 (34%)	153 (51%)	45 (15%)	300 (100%)
BMI for Age (BAZ)	96 (32%)	147 (49%)	57 (19%)	300 (100%)

Discussion

These research findings show severe dietary restrictions among school-age children residing in Maiduguri Metropolitan Council, with 41.7% experiencing inadequate dietary diversity and only 3.0% achieving optimal diversity levels. This study disagrees with findings for Ibrahim *et al.* [23], pointing to Northern Nigeria with higher dietary diversity, thus underscoring severity. These results represent among the most restrictive dietary patterns reported in contemporary Nigerian pediatric nutrition literature, exceeding rates documented in comparable studies from northern Nigeria.[23] The severity of dietary limitations reflects the compounded effects of economic hardship, conflict-related disruptions, and systematically reduced access to diverse food sources. The robust statistical associations between socioeconomic indicators and dietary diversity confirm poverty as the fundamental driver of dietary inadequacy in this population. This was in alignment with studies from Headey & Alderman [9]. Children from economically disadvantaged households were far more likely to have low dietary diversity compared to those from economically stable households, reflecting global patterns that identify economic access as a universal determinant of diet quality.[9] Addressing paediatric malnutrition in this context, therefore, requires comprehensive, multi-sectoral interventions that extend beyond direct nutrition programming to target the structural determinants of poverty. The educational disparity, especially among the caregivers, reveals significant socioeconomic vulnerabilities that directly impact child nutritional outcomes. The substantial gender gap in educational attainment reflects broader patterns of educational inequality documented across West African regions, where educated mothers demonstrate enhanced nutritional knowledge, greater household decision-making autonomy, and improved capacity to navigate healthcare systems. Therefore, maternal educational attainment emerged as another critical determinant. Children whose mothers lacked formal education were significantly more likely to have restricted dietary diversity, consistent with evidence showing that educated mothers possess greater nutritional knowledge, stronger household decision-making power, and improved food preparation skills.[24] The prevalence of maternal educational deficit in this study is notably higher than national averages, highlighting an urgent vulnerability and a key target for intervention.

Remarkably, only 3.0% of participants achieved high dietary diversity, reflecting severely limited access to diverse, nutrient-dense foods, likely attributable to economic constraints, displacement-related disruptions, and systemic food insecurity.

The strong positive association between school meal participation and dietary diversity provides compelling evidence for the role of educational institutions as intervention hubs. Over 90% of children who regularly consumed breakfast at school achieved medium dietary diversity, compared to much lower rates among those eating exclusively at home. This suggests that school feeding programs can serve as equalisers, partially offsetting household food insecurity and improving diet quality. The dietary patterns observed indicate a rapid nutritional transition, marked by heavy reliance on processed cereals and a near-complete abandonment of traditional grains. An estimated 85% of children rarely or never consumed millet or

other indigenous staples, representing one of the most pronounced dietary shifts documented in West Africa. Coupled with the extremely low intake of animal-source foods, this pattern poses serious risks for protein-energy malnutrition and micronutrient deficiencies, as bioavailable nutrients from animal products remain scarce in these children's diets.

Anthropometric findings reveal that stunting prevalence far exceeds WHO's "high" threshold of 30% and Nigeria's national average of 37%, with nearly one in five children (19%) severely stunted, indicating long-term nutritional deprivation and the likelihood of irreversible developmental consequences. Underweight prevalence and wasting further underscore the magnitude of the problem, with severe cases (20% underweight; 11% wasting) requiring urgent therapeutic feeding. BMI-for-age data confirm widespread deficits in energy and nutrient intake, and the consistent high prevalence of moderate malnutrition across all indicators highlights a large proportion of children at imminent risk of deterioration without preventive measures. The alignment of these anthropometric results with socioeconomic and dietary diversity findings points to poverty, low maternal education, and limited dietary variety as the principal underlying causes, as suggested by Ruel *et al*.[24], evidence connecting maternal education to better child nutrition. These findings carry significant implications for public health policy and intervention design. The strong economic gradient in dietary diversity suggests that nutrition-sensitive interventions such as poverty reduction, livelihood support, and social protection may yield greater impact than nutrition-specific measures alone. The identification of maternal education as a key determinant strengthens the case for integrating women's education into long-term nutrition strategies. The documented benefits of school feeding position educational institutions as highly effective platforms for targeted nutrition interventions, particularly in conflict-affected and resource-limited contexts. This result also agrees with studies from Makinde *et al.*[25], which demonstrates that exposure to armed conflict increases the odds of stunting and underweight in Nigerian children. Underweight prevalence and wasting further underscore the magnitude of the problem, with severe cases requiring urgent therapeutic feeding. BMI-for-age data confirm widespread deficits in energy and nutrient intake, and the consistent high prevalence of moderate malnutrition across all indicators highlights a large proportion of children at imminent risk of deterioration without preventive measures. The alignment of these anthropometric results with socioeconomic and dietary diversity findings points to poverty, low maternal education, and limited dietary variety as the principal underlying causes.

These findings carry significant implications for public health policy and intervention design. The strong economic gradient in dietary diversity suggests that nutrition-sensitive interventions such as poverty reduction, livelihood support, and social protection may yield greater impact than nutrition-specific measures alone. The identification of maternal education as a key determinant strengthens the case for integrating women's education into long-term nutrition strategies. The documented benefits of school feeding position educational institutions as highly effective platforms for targeted nutrition interventions, particularly in conflict-affected and resource-limited contexts.

Conclusion

This study shows a dual nutrition crisis among school-age children in Maiduguri Metropolitan Council, characterised by severe dietary restrictions and high levels of anthropometric deficits. Socioeconomic stratification emerged as the primary determinant of dietary quality, with poverty, low maternal education, and limited access to diverse foods compounding the risks for poor child health and development. The prevalence of stunting (68%), underweight (64%), wasting (57%), and compromised BMI-for-age (61%) far exceeds national and global concern thresholds, with severe cases requiring urgent therapeutic feeding and moderate cases at high risk of deterioration without intervention. These anthropometric patterns reflect both chronic and acute nutritional stress, aligning closely with documented dietary inadequacies, particularly the near-complete exclusion of animal-source foods and heavy reliance on processed cereals. The strong associations between economic indicators and both dietary diversity and anthropometric status indicate that nutrition-specific interventions alone will be insufficient without addressing the structural determinants of malnutrition. Maternal education deficits, affecting over half of the study population, further reinforce the need for integrated approaches that combine nutrition programming with education and empowerment of women. The identification of education as a powerful

intervention platform, with school feeding programs strongly linked to improved dietary diversity, offers a scalable, evidence-based pathway to reach children regardless of their household economic status. Urgent interventions are therefore required, targeting poverty reduction, maternal educational advancement, dietary diversification, and expansion of school feeding initiatives.

Conflict of Interest Statement

The authors declare no competing interests or conflicts of interest related to this research.

Acknowledgments
We acknowledge the cooperation and participation of study participants, community leaders, and the Borno State Ministry of Health for providing ethical clearance. Special recognition is extended to field assistants and laboratory technicians who provided invaluable support during data collection activities.

References

1. Kennedy G, Ballard T, Dop MC. *Guidelines for measuring household and individual dietary diversity*. Food and Agriculture Organisation 2021.
2. UNICEF.. *Child food poverty: Nutrition deprivation in early childhood*. United Nations Children's Fund, 2024.
3. Michaelsen KF, Hoppe C, Roos N, Kaestel P, Stougaard M, Lauritzen L, Mølgaard C. Girma T, Friis H. Choice of foods and ingredients for moderately malnourished children 6 months to 5 years of age. *Food and Nutrition Bulletin*, 2009; 30(3_suppl3), S343-S404.
4. UNICEF. *The state of the world's children 2022*. United Nations Children's Fund.
5. National Population Commission & ICF. (2019). *Nigeria Demographic and Health Survey 2018*. NPC and ICF, 2022.
6. United Nations Office for the Coordination of Humanitarian Affairs. *Northeast Nigeria Nutrition Sector Dashboard: January to December 2023*. OCHA, 2023. https://reliefweb.int/report/nigeria/northeast-nigeria-nutrition-sector-dashboard-january-december-2023
7. UNICEF. *Nutrition programming in humanitarian crises: Addressing the gap for school-aged children*. United Nations Children's Fund, 2020.
8. Headey D, Ecker O, Comstock AR, Ruel MT. Poverty, price and preference barriers to improving diets in sub-Saharan Africa. *Global Food Security,* 2023; *36*, Article 100664. https://doi.org/10.1016/j.gfs.2022.100664 PMC.
9. Headey D, Alderman H. The relative caloric prices of healthy and unhealthy foods differ systematically across income levels and continents. *Journal of Nutrition*, 2019; 149(11), 2020-2033.
10. Wrottesley SV, Mates E, Brennan E, Bijalwan V, Menezes R, Ray S, Ali Z, Yarparvar, A., Sharma, D., & Lelijveld, N. (2022). Nutritional status of school-age children and adolescents in low- and middle-income countries across seven global regions: A synthesis of scoping reviews. *Public Health Nutrition*, 26(1), 63-95. https://doi.org/10.1017/S1368980022000350
11. Food and Agriculture Organisation. (2021). *Guidelines for measuring household and individual dietary diversity*. FAO.
12. Global Alliance for Improved Nutrition. (2017). *24-hour dietary recalls*. Measurement Toolkit. https://www.measurement-toolkit.org/diet/subjective-methods/24-hour-dietary-recall
13. Gibson, R. S., & Ferguson, E. L. (2008). *An interactive 24-hour recall for assessing the adequacy of iron and zinc intakes in developing countries*. International Life Sciences Institute.
14. National Cancer Institute. (2024). *24-hour dietary recall (24HR) at a glance*. Dietary Assessment Primer. https://dietassessmentprimer.cancer.gov/profiles/recall/.
15. USAID Advancing Nutrition. (2024). *24-hour dietary recall (quantitative tool)*. https://www.advancingnutrition.org/resources/diet-assessment-tool/24-hour-dietary-recall-quantitative-tool
16. Gibson, R. S., Charrondiere, U. R., & Bell, W. (2017). Measurement errors in dietary assessment using self-reported 24-hour recalls in low-income countries and strategies for their prevention. *Advances in Nutrition*, 8(6), 980-991.
17. National Cancer Institute. (2024). *Food Frequency Questionnaire at a glance*.

Dietary Assessment Primer. https://dietassessmentprimer.cancer.gov/profiles/questionnaire/.

18. INDEX Project. (2024). *Food Frequency Questionnaires (FFQ)*. Tufts University. https://inddex.nutrition.tufts.edu/data4diets/data-source/food-frequency-questionnaires-ffq.
19. *Frontiers in Public Health*. (2024). Validation of food frequency questionnaire for food intake of adults in Gida, West, Ethiopia. https://www.frontiersin.org/journals/public health/articles/10.3389/fpubh.2024.1438008/full
20. Centres for Disease Control and Prevention, CDC. (2024). Anthropometric indices: Definitions and categories. *Growth Chart Training*. https://www.cdc.gov/growth-chart-training/hcp/overview/anthropometric-indices.html
21. Gibson, R. S. (2005). Anthropometric assessment of nutritional status. In *Principles of nutritional assessment* (2nd ed., pp. 241–296). Oxford University Press.
22. World Health Organisation & United Nations Children's Fund, World Food Programme. (2024). *The state of food security and nutrition in the world 2024: Financing to end hunger, food insecurity and malnutrition in all its forms*. FAO. https://doi.org/10.4060/cd1254en
23. Ibrahim, K. G., Jibrin, S. M., & Abdullahi, I. N. (2021). Dietary diversity and nutritional status of primary school children in Kaduna State, Nigeria. *Nutrition and Food Science*, 51(3), 478-489.
24. Ruel, M. T., Alderman, H., & Maternal and Child Nutrition Study Group. (2018). Nutrition-sensitive interventions and programmes: How can they help to accelerate progress in improving maternal and child nutrition? *The Lancet*, 382(9891), 536-551.
25. Makinde, O. A., Olamijuwon, E., Mgbachi, I., & Sato, R. (2023). Childhood exposure to armed conflict and nutritional health outcomes in Nigeria. *Conflict and Health, 17*, Article 15. https://doi.org/10.1186/s13031-023-00513-0 BioMed Central.

Ameliorative Effects of *Cucuma Longa* And *Punica Granatum* on Oxidative Stress and Dyslipidemia in Wistar Rats Fed Thermally Abused Oil

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Abstract

Consumption of thermally abused oil in developing countries has become unavoidable due to persistent economic hardship and the inability to afford fresh cooking oils. The study evaluates the antioxidant and cardio-protective effects of *Curcuma longa* and *Punica granatum* in Wistar rats fed thermally abused oil. Forty (40) Wistar rats were used in the study and were divided into eight groups of five animals each. Each group were fed standard rat chow or thermally abused oil-based diet and supplemented with *Curcuma longa* and/or *Punica granatum* supplements. The group maintained on a thermally abused-based diet showed significantly ($p<0.05$) lower serum activities of catalase, Superoxide dismutase (SOD), Reduced glutathione (GSH) and significantly ($p<0.05$) higher Malondialdehyde (MDA) levels compared to the control and treatment groups. Also, the group fed a thermally abused based diet showed significantly ($p<0.05$) higher serum levels of total cholesterol, Triglycerides, and low-density lipoprotein but significantly ($p<0.05$) lower high-density lipoprotein compared to the control and treatment groups. The study shows that *Curcuma longa* and *Punica granatum* have antioxidant and cardio-protective potentials in Wistar rats fed thermally abused oil.

Keywords: *Curcuma longa*, *Punica granatum,* Antioxidant, Cardio-protective, thermally abused oil.

Introduction

Thermally abused oils are those oils that have been subjected to high temperatures, leading to breakdown of antioxidants naturally present in oils, other chemical changes and potential health risk [1,2]. The chemical changes associated with repeated heated oils are oxidation, polymerisation, and hydrolysis [3]. These chemicals change in oils lead to the formation of a wide range of toxic chemicals such as ketones, aldehydes, acrylamide, polycyclic aromatic hydrocarbons, and advanced glycation end products [4,5]. When consumed, these compounds are absorbed into the bloodstream and generate free radicals and cause peroxidation of lipids in the body, causing oxidative stress and dyslipidemia [6,7].

Oxidative stress is an imbalance in the body that results when the production of free radicals exceeds their neutralisation by antioxidants [8,9]. This oxidative stress is associated with cell damage leading to inflammation and tissue damage or chronic diseases, including but not limited to cancer, cardiovascular disease and neurodegenerative disorders [10,11]. Dyslipidemia is a condition characterised by abnormal levels of lipids (fats) in the blood [12]. This high amount of lipid can pose a potential risk of atherosclerosis or cardiovascular disease, which may lead to heart attacks, strokes, peripheral artery disease and death [13].

Curcuma longa (Turmeric) and *Punica granatum* (pomegranate) are spices commonly used in cooking and traditional medicine [14,15]. Curcumin in turmeric and polyphenols in pomegranate are known to have potential health benefits as antioxidants and anti-inflammatory agents [16,17]. The antioxidant properties of turmeric and pomegranate may help reduce the risk of chronic diseases such as cardiovascular disease and cancer [18]. Turmeric and pomegranate have been reported to mitigate oxidative stress and lipid abnormalities [19,20].

While other research studies use the extracts of *Curcuma longa* and *Punica granatum* to assess the antioxidant and cardio-protective activities of these plants, this study uses the whole plant powders of turmeric and pomegranate supplements to investigate the antioxidant and cardio-protective effects in single and combined doses in Wistar rats fed thermally abused oil, exploring their potentials as dietary interventions for mitigating oil-induced metabolic disorders.

Date submitted-28/07/2025 Date Accepted-03/10/2025

Materials and Methods

Ethical Statement

The study protocol was approved by the FUOYE Faculty of Science Ethics Committee with approval number FUOYEFSC 201122 – REC2025/028, ensuring that the experiments were designed to minimise animal suffering and distress. Efforts were made to reduce the number of animals used in the experiment while maintaining the scientific integrity and reliability of the results.

Experimental Animals

Forty (40) female Wistar rats were obtained from the Animal house, Biochemistry Department, Federal University Dutsin-Ma and were acclimatised under controlled light/dark conditions and were fed starter feed and water *ad libitum* for a period of two weeks.

Plant Sample Collection, Identification And Preparation

Rhizomes of *Curcuma longa* were purchased from the local market in Dutsin-Ma and were identified with herbarium number FUDMA/PSB/00011 by a botanist in the Department of Plant Science and Biotechnology, Federal University Dutsin-Ma. The rhizomes were washed, dried under shade ground into powder and kept for diet formulation. The powder of *Punica granatum* was purchased from Al-Hilal Islamic Chemist, Katsina and was identified by a botanist with herbarium number FUDMA/PSB/00379.

Preparation of Thermally Abused Oil

A fresh vegetable oil was selected. The oil was heated to 180°C using a thermostatically controlled deep fryer for 2 hours to simulate frying conditions. The oil was allowed to cool to room temperature for 1 hour. This heating (180°C for 2 hours) and cooling cycle was repeated six consecutive times to induce thermal degradation. After the final heating cycle, the oil was cooled to room temperature [21]. The reused oil was collected and stored in sterile, clean containers and used in the formulation of rats' diet.

Feed Composition

Standard rodent diet was prepared by appropriately mixing corn starch, SBM, cellulose, salt, vitamin mix and mineral mix [22].

Table 1: Detailed Components Used in Feed Formulation for a 100g Diet

Feed ingredients (g)	Control Diet (g)	Reused Oil Diets (g)
Corn Starch	55.45	55.45
Soya bean meal	32.00	32.00
Cellulose	4.50	4.50
Bone Meal	1.25	1.25
Salt Mix	0.30	0.30
Pre-Mix	0.25	0.25
Methionine	0.25	0.25
Palm oil	6	-
Thermally abused oil	-	6

Supplemented Diet Formulation

The supplemented diets were formulated by mixing 97 g of the control diet and 3 g of pomegranate and/or turmeric. Another supplemented diet was formulated by mixing 97 g of the thermally abused oil-based diet with 3 g of pomegranate and/ or turmeric. Mixed supplementations were made by mixing 1.5 g each of turmeric and pomegranate with 97 g of standard diet or thermally abused oil-based diet.

Experimental Design

Forty (40) female Wistar rats, weighing between 60 and 110 g, were used in this experiment. The rats were weighed and grouped randomly into eight (8) groups of 5 rats each as follows;

Group 1 were maintained on a standard rat diet (control)
Group 2 were maintained on a thermally abused oil-based diet (positive control)
Group 3 were maintained on 3 % *Curcuma longa*-supplemented diet.
Group 4 were maintained on 3 % *Punica granatum*-supplemented diet.
Group 5 were maintained on 3 % mixed *Curcuma longa* and *Punica granatum* supplemented diet.
Group 6 were maintained on 3 % *Curcuma longa* and thermally abused oil-supplemented diet.
Group 7 were maintained on 3 % *Punica granatum* and thermally abused oil-supplemented diet.
Group 8 were maintained on 3 % mixed *Curcuma longa, Punica granatum* and thermally abused oil-supplemented diet.

The rats were kept on their respective diets and water *ad libitum* for a period of twelve (12) weeks.

Sacrifice, Sample Collection and Sample Preparation

At the end of twelve weeks, the animals were anaesthetized using chloroform, sacrificed by cutting the jugular vein until complete bleeding, and the blood samples were collected in plain tubes and centrifuged at 1500 rpm for 15 minutes to obtain the serum. The serum was stored at -20 ^{o}C until analysis [23].

Determination of Oxidative Stress Markers

The oxidative stress markers, Catalase, SOD, GSH and MDA were determined using the user protocols of Randox Laboratory Inc., US.

Determination of Lipid Profile Parameters

The parameters of lipid profile were determined using assay kits from Randox Laboratories. US according to user protocols.

Statistical Analysis

The Statistical Package for Social Sciences (SPSS) version 23 was used for data analysis. Data were analysed using one-way analysis of variance (ANOVA), and the Duncan multiple range test was used to indicate where significant differences existed.

Results

Lipid Profile indices of Wistar rats Fed thermally abused oil and supplemented with *Curcuma longa* and/or *Punica granatum.*

Table 2 shows the results of lipid profile indices of Wistar rats Fed thermally abused oil and supplemented with *Curcuma longa* and/or *Punica granatum.* The results showed that rats maintained on thermally abused oil-based diets have significantly ($p<0.05$) higher total cholesterol, TG and LDL but lower HDL compared to the normal control and groups maintained on diets supplemented with *Curcuma longa* and/or *Punica granatum*.

Table 2: Lipid Profile indices of Wistar Rats Fed Thermally Abused Oil and Maintained on *Curcuma longa* and or *Punica granatum* Supplements

GROUP	T. COL (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	AI	CRI
1	104.00±1.15^{bc}	78.33±1.20^{a}	18.67±0.67^{b}	107.00±1.79^{ab}	5.74±0.12^{bc}	5.58±0.14^{bc}
2	144.33±2.33^{d}	111.67±1.45^{c}	13.33±0.88^{a}	135.33±1.73^{c}	10.25±0.79^{d}	10.94±0.87^{d}
3	91.66±2.19^{a}	79.00±7.50^{a}	25.67±1.76^{c}	101.53±2.73^{a}	3.99±0.27^{a}	3.60±0.25^{a}
4	91.33±5.78^{a}	68.67±2.40^{a}	27.00±1.52^{c}	104.60±6.34^{a}	3.89±0.25^{a}	3.40±0.28^{a}
5	91.00±3.21^{a}	69.00±1.73^{a}	24.00±2.08^{c}	101.20±5.52^{a}	4.24±0.14^{a}	3.83±0.21^{a}
6	113.33±4.41^{c}	100.00±3.06^{b}	25.00±0.58^{c}	118.33±4.26^{b}	4.73±0.13^{ab}	4.53±0.13^{ab}
7	97.67±1.45^{ab}	78.33±0.88^{a}	18.00±0.58^{b}	98.00±0.81^{a}	6.14±0.27^{c}	6.13±0.31^{c}
8	106.00±2.31^{bc}	93.67±3.18^{b}	19.67±0.88^{b}	106.93±2.08^{ab}	5.47±0.33^{bc}	5.41±0.35^{bc}

Key;- T. Col = Total Cholesterol, TG = Triglycerides, HDL= High density lipoprotein, LDL= Low density lipoprotein, AI = Atherogenic risk index and CRI = Cardiovascular risk index

1; group fed a normal control diet.
2; group-fed thermally abused oil-supplemented diet.
3; group fed 3 % *Curcuma longa* supplemented diet.
4; group fed 3 % *Punica granatum*-supplemented diet.
5; group fed 3 % mixed *Curcuma longa* and *Punica granatum* supplemented diet.
6; group fed 3 % *Curcuma longa* + thermally abused oil supplemented diet.
7; group fed 3 % *Punica granatum* + thermally abused oil supplemented diet.
8; group fed 3 % mixed *Curcuma longa* and *Punica granatum* + thermally abused oil supplemented diet.

Oxidative Stress markers of Wistar rats Fed thermally abused oil and supplemented with *Curcuma longa* and/or *Punica granatum.*

Table 3 showed the oxidative stress markers of Wistar rats Fed thermally abused oil and supplemented with *Curcuma longa* and/or *Punica granatum.* The results showed that there was a significant ($p<0.05$) decrease in GSH, SOD and Catalase but a significant ($p<0.05$) increase in levels of MDA in the group fed with a thermally abused oil-based diet compared to the control and groups fed with *Curcuma longa* and/or *Punica granatum* supplemented diets.

Table 3: Results of Oxidative Stress Markers of Wistar Rats Fed Thermally Abused Oils and Supplemented with *Curcuma longa* and/or *Punica granatum.*

GROUP	GSH (mM/L)	SOD (mM/L)	CAT (mM/L)	MDA (mM/L)
1	5.13±0.09^{b}	7.20±0.06^{c}	3.67±0.33^{b}	195.67±3.84^{b}
2	2.17±0.03^{a}	4.40±0.71^{a}	2.10±0.06^{a}	381.50±13.65^{e}
3	8.93±0.55^{d}	9.20±0.55^{d}	6.50±0.29^{c}	172.33±3.37^{b}
4	10.97±2.17^{e}	8.73±0.27^{d}	6.47±0.73^{c}	184.01±0.53^{b}
5	8.77±1.14^{d}	12.47±0.40^{e}	6.03±0.26^{c}	112.00±21.67^{a}
6	5.57±0.59^{c}	7.70±0.10^{d}	4.27±0.22^{b}	196.73±15.02^{b}
7	8.20±0.12^{c}	12.27±0.58^{e}	4.07±0.67^{b}	301.00±0.57^{c}
8	6.23±0.12^{d}	6.43±0.84^{b}	4.10±0.06^{b}	178.67±19.03^{b}

Key:- GSH = Reduced glutathione, SOD = Superoxide dismutase, CAT = Catalase, MDA = Malondialdehyde.

1; group fed a normal control diet.
2; group-fed thermally abused oil-supplemented diet.
3; group fed 3 % *Curcuma longa* supplemented diet.
4; group fed 3 % *Punica granatum*-supplemented diet.
5; group fed 3 % mixed *Curcuma longa* and *Punica granatum* supplemented diet.
6; group fed 3 % *Curcuma longa* + thermally abused oil supplemented diet.
7; group fed 3 % *Punica granatum* + thermally abused oil supplemented diet.
8; group fed 3 % mixed *Curcuma longa* and *Punica granatum* + thermally abused oil supplemented diet.

Discussion

The result in Table 2 shows that thermally abused oil has induced dyslipidemia by increasing the serum levels of TG, TC, LDL and decreasing the serum level of HDL. The changes in these factors are attributed to an increase in atherosclerosis and myocardial infarction [23]. This is consistent with the results of Idoko *et al*. [22], suggesting that exposure to thermally abused oil is a risk factor for atherosclerosis. In a similar version, [24] reported that dyslipidemia is a major risk factor in the development of cardiovascular disease. In this manner, a vicious circle may set in, exacerbating dyslipidemia.

Diet supplementation with turmeric and/or pomegranate moved toward normalising lipid metabolism and restoring dyslipidemia, and prevented atherosclerosis in rats exposed to thermally abused oils, as seen in the significantly lower total cholesterol, triglyceride, LDL-C and higher HDL in groups exposed to thermally abused oil toxicity but fed the supplemented diets relative to groups exposed to thermally abused oil-based diet. Curcumin in turmeric and polyphenols in pomegranate have been reported to possess anti-cardiometabolic risk effects[37, 38]. According to Bahari *et al.* [25], these metabolites involve improvement in serum lipids. For example, curcumin lowers LDL-C by decreasing the amount of LDL-C receptor. Furthermore, Srivastava *et al*. [26] reported improvement of insulin resistance in hyperlipidemic rats fed turmeric. The findings of this study on dyslipideamic effect of pomegranate supplemented diet in rats exposed to thermally abused oil could result from LDL being converted to HDL through the activation of lecithin-cholesterol acytyltransferase and/or by increasing the excretion of bile acid or inhibition of cholesterol absorption as reported by Bouziane *et al*. [27] and these may be attributed to its rich flavonoids and fiber. The results are also consistent with the results of Kojadinovic *et al*. [28] who reported that pomegranate has a beneficial effect on dyslipidemia. It is evident from our study that diet supplementation with pomegranate could

prevent atherosclerosis, a common feature of thermally abused oil toxicity as reported by Soundararajan *et al*. [29], promoting blood lipid decrease, which subsequently reduces the risk of atherosclerosis and/or cardiovascular disease.

The improvements seen in the lipid and atherogenic markers of thermally abused oil-based diet and supplemented with turmeric and/or pomegranate could be through scavenging excess free radicals generated by thermally abused oil, as indicated by significantly higher levels of the antioxidant enzymes and lower MDA levels in the groups exposed and treated with turmeric and/or pomegranate. Cam *et al*. [30] reported that thermally abused oils can cause DNA damage, peroxidation of lipids and tissue damage due to excess production of free radicals. Free radicals attack lipids, which negatively affect lipid fluidity that eventually results in cell death [31]. Lipid peroxidation intermediates are toxic and can affect the DNA. This has been reported to be one of the most prominent mechanisms of oxidative damage to tissues. Turmeric generally improve sensitivities of tissues to insulin in metabolically affected rats and could therefore prevent the oxidation of lipids. The most abundant bioactive compound in turmeric, curcumin lowers the production of reactive oxygen species [32], promotes the synthesis of antioxidant enzymes [33] and prevent the production of hydroxyl radicals, ketones and aldehydes [34]. The results of this study are consistent with the study of Li *et al*. [35] that reported that turmeric confers protection of the hepatocytes in rats through elevation of the levels of enzyme activities involved in free radical scavenging. Diet supplementation with pomegranate exhibited beneficial effects against oxidative stress in rats exposed to thermally abused oil. Pomegranate is rich in polyphenols like punicalagins, ellagic acid, anthocyanins and flavonoids seen at the centre of their biological activities. Benchagra *et al*. [36] reported that pomegranate attenuates oxidative stress in the same pattern as our study.

Conclusion

In conclusion, the study demonstrated that the combination of turmeric and pomegranate exhibits protective effects against dyslipidemia and oxidative stress in rats exposed to thermally abused oil. The antioxidant and lipid-lowering properties of curcuminoids and polyphenols present in turmeric and pomegranate may contribute to the mitigation of oxidative damage and lipid profile alterations induced by thermally abused oil consumption. These findings suggest that dietary interventions incorporating turmeric and pomegranate may be beneficial in reducing the risk of cardiovascular disease associated with the consumption of thermally abused oils.

References

1. Erickson, M. D., Yevtushenko, D. P., & Lu, Z. X. (2023). Oxidation and thermal degradation of oil during frying: A review of natural antioxidant use. *Food Reviews International*, *39*(7), 4665-4696.
2. Machado, M., Rodriguez-Alcalá, L. M., Gomes, A. M., & Pintado, M. (2023). Vegetable oils oxidation: mechanisms, consequences and protective strategies. *Food Reviews International*, *39*(7), 4180-4197.
3. Kmiecik, D., Fedko, M., Siger, A., & Kulczyński, B. (2019). Degradation of tocopherol molecules and their impact on the polymerisation of triacylglycerols during heat treatment of oil. *Molecules*, *24*(24), 4555.
4. Almoselhy, R. I. (2021). A review of health risks from processing contaminants in edible oils and foodstuffs. *International Journal of Advanced Technology & Science Research*.
5. Li, C., Chen, L., McClements, D. J., Liu, W., Long, J., Qiu, C., ... & Jin, Z. (2023). Utilisation of plant extracts to control the safety and quality of fried foods—A review. *Comprehensive Reviews in Food Science and Food Safety*, *22*(3), 2310-2345.
6. Khan, M. I., Ashfaq, F., Alsayegh, A. A., Hamouda, A., Khatoon, F., Altamimi, T. N., ... & Beg, M. M. A. (2023). Advanced glycation end product signalling and metabolic complications: Dietary approach. *World journal of diabetes*, *14*(7), 995.
7. Kumar, D., & Tekade, M. (2023). Types of cellular responses to. *Essentials of Pharmatoxicology in Drug Research, Volume 1: Toxicity and Toxicodynamics*, *1*, 169.
8. Mani, S., Dubey, R., Lai, I. C., Babu, M. A., Tyagi, S., Swargiary, G., ... & Jha, N. K. (2023). Oxidative stress and natural antioxidants: Back and forth in the neurological mechanisms of Alzheimer's Disease. *Journal of Alzheimer's Disease*, *96*(3), 877-912.

9. Firdous, S. M., Pal, S., Mandal, S., & Sindhu, R. K. (2025). Antioxidants in Inflammatory Diseases. *Antioxidants: Nature's Defence Against Disease*, 83-126.
10. Behl, T., Makkar, R., Sehgal, A., Singh, S., Sharma, N., Zengin, G., ... & Brisc, C. (2021). Current trends in neurodegeneration: Cross-talks between oxidative stress, cell death, and inflammation. *International Journal of Molecular Sciences*, *22*(14), 7432.
11. Leyane, T. S., Jere, S. W., & Houreld, N. N. (2022). Oxidative stress in ageing and chronic degenerative pathologies: molecular mechanisms involved in counteracting oxidative stress and chronic inflammation. *International journal of molecular sciences*, *23*(13), 7273.
12. Yuan, Y., Chen, W., Luo, L., & Xu, C. (2021). Dyslipidemia: Causes, symptoms and treatment. *International Journal of Trend in Scientific Research and Development*, *5*(2), 1013-1016.
13. Upadhyay, R. K. (2023). High cholesterol disorders, myocardial infarction and its therapeutics. *World Journal of Cardiovascular Diseases*, *13*(8), 433-469.
14. Sachan, A. K., Kumar, S., Kumari, K., & Singh, D. (2018). Medicinal uses of spices used in our traditional culture: Worldwide. *Journal of Medicinal Plants Studies*, *6*(3), 116-122.
15. Jayathilake, A. L., Jayasinghe, M. A., & Walpita, J. (2022). Development of ginger, turmeric oleoresins and pomegranate peel extracts incorporated into pasteurised milk with pharmacologically important active compounds. *Applied Food Research*, *2*(1), 100063.
16. Raffaele, M., Greish, K., Vanella, L., Carota, G., Bahman, F., Bindayna, K. M., ... & Sorrenti, V. (2021). Potential health benefits of a pomegranate extract, rich in phenolic compounds, in intestinal inflammation. *Current Nutrition & Food Science*, *17*(8), 833-843.
17. Monika, P., Chandraprabha, M. N., & Murthy, K. C. (2023). Catechin, epicatechin, curcumin, garlic, pomegranate peel and neem extracts of Indian origin showed enhanced anti-inflammatory potential in human primary acute and chronic wound-derived fibroblasts by decreasing TGF-β and TNF-α expression. *BMC Complementary Medicine and Therapies*, *23*(1), 181.
18. Zujko, M. E., & Witkowska, A. M. (2023). Dietary antioxidants and chronic diseases. *Antioxidants*, *12*(2), 362.
19. Moradnia, M., Mohammadkhani, N., Azizi, B., Mohammadi, M., Ebrahimpour, S., Tabatabaei-Malazy, O., ... & Ale-Ebrahim, M. (2024). The power of Punica granatum: A natural remedy for oxidative stress and inflammation; a narrative review. *Journal of Ethnopharmacology*, 118243.
20. Dama, A., Shpati, K., Daliu, P., Dumur, S., Gorica, E., & Santini, A. (2024). Targeting metabolic diseases: the role of nutraceuticals in modulating oxidative stress and inflammation. *Nutrients*, *16*(4), 507.
21. Fweja, L. W. (2019). The Effects of Repeated Heating on Thermal Degradation of Cooking Oil and Its Implication on Human Health: A Review. *Huria: Journal of the Open University of Tanzania*, *26*(1).
22. Idoko, A. S., Umar, S., Adejo, G. O., Imam, N. U., Zaharaddeen, A. S., Abubakar, Z. S., & Abdullahi, A. (2022). Diet Fortification with Curcuma longa and Allium cepa Ameliorates 2, 3, 7, 8-Tetracholorodibenzop-dioxin-induced Dyslipideamia and Oxidative Stress in Wistar Rats. *Nigerian Journal of Basic and Applied Sciences*, *30*(2), 178-183.
23. Georgoulis, M., Chrysohoou, C., Georgousopoulou, E., Damigou, E., Skoumas, I., Pitsavos, C., & Panagiotakos, D. (2022). Long-term prognostic value of LDL-C, HDL-C, lp (a) and TG levels on cardiovascular disease incidence, by body weight status, dietary habits and lipid-lowering treatment: the ATTICA epidemiological cohort study (2002–2012). *Lipids in Health and Disease*, *21*(1), 141.
24. Du, Z., & Qin, Y. (2023). Dyslipidemia and cardiovascular disease: current knowledge, existing challenges, and new opportunities for management strategies. *Journal of Clinical Medicine*, *12*(1), 363.
25. Bahari, H., Rezaiian, F., Goudarzi, K., Mirmohammadali, S. N., Asbaghi, O., Naderian, M., & Hosseini, A. (2023). The effects of pomegranate consumption on lipid profile in adults: A systematic review

and meta-analysis. *Journal of Functional Foods*, *108*, 105727.
26. Srivastava, S., Kumar, V., Kapil, L., Prasad, S., Khan, S., Singh, C., & Singh, A. (2025). Functional Foods and Spices in the Management of Metabolic Syndrome. In *Nutraceuticals in Obesity Management and Control* (pp. 211-283). Apple Academic Press.
27. BOUZIANE, S. B. (2023). Effect of 10% Pomegranate Juice on Lipid Metabolism and Body Fat in Rats.
28. Kojadinovic, M., Glibetic, M., Vucic, V., Popovic, M., Vidovic, N., Debeljak-Martacic, J., & Arsic, A. (2021). Short-term consumption of pomegranate juice alleviates some metabolic disturbances in overweight patients with dyslipidemia. *Journal of Medicinal Food*, *24*(9), 925-933.
29. Soundararajan, P., Parthasarathy, S., Sakthivelu, M., Karuppiah, K. M., Velusamy, P., Gopinath, S. C. B., & Raman, P. (2024). Effects of consuming repeatedly heated edible oils on cardiovascular diseases: a narrative review. *Current Medicinal Chemistry*, *31*(40), 6630-6648.
30. Cam, A., Oyirifi, A. B., Liu, Y., Haschek, W. M., Iwaniec, U. T., Turner, R. T., ... & Helferich, W. G. (2019). Thermally abused frying oil potentiates metastasis to lung in a murine model of late-stage breast cancer. *Cancer Prevention Research*, *12*(4), 201-210.
31. Wang, B., Wang, Y., Zhang, J., Hu, C., Jiang, J., Li, Y., & Peng, Z. (2023). ROS-induced lipid peroxidation modulates cell death outcome: mechanisms behind apoptosis, autophagy, and ferroptosis. *Archives of toxicology*, *97*(6), 1439-1451.
32. Gersey, Z. C., Rodriguez, G. A., Barbarite, E., Sanchez, A., Walters, W. M., Ohaeto, K. C., ... & Graham, R. M. (2017). Curcumin decreases malignant characteristics of glioblastoma stem cells via induction of reactive oxygen species. *BMC Cancer*, *17*, 1-11.
33. Tian, G., Zhang, X., Hao, X., & Zhang, J. (2023). Effects of curcumin on growth performance, ruminal fermentation, rumen microbial protein synthesis, and serum antioxidant capacity in housed growing lambs. *Animals*, *13*(9), 1439.
34. Bērziņa, L., & Mieriņa, I. (2023). Antiradical and antioxidant activity of compounds containing 1, 3-dicarbonyl moiety: An overview. *Molecules*, *28*(17), 6203.
35. Li, Z., Zhu, J. F., & Ouyang, H. (2023). Progress on traditional Chinese medicine in improving hepatic fibrosis through inhibiting oxidative stress. *World Journal of Hepatology*, *15*(10), 1091.
36. Benchagra, L., Berrougui, H., Islam, M. O., Ramchoun, M., Boulbaroud, S., Hajjaji, A., ... & Khalil, A. (2021). Antioxidant effect of moroccan pomegranate (Punica granatum L. sefri variety) extracts rich in punicalagin against the oxidative stress process. *Foods*, *10*(9), 2219.
37. Ribeiro, A. A., Carvalho, L. M., da Mota, J. C., Nonino, C. B., Gualano, B., Nunes, J. A., ... & Nicoletti, C. F. (2024). Diet, DNA methylation, and systemic lupus erythematosus: evidence and perspectives focused on personalized nutrition. *Lifestyle Genomics*, *17*(1), 31-40.
38. Class, I. P. C., & USPC, A. (2013). Patent Application Title: Method Of Using Nutritional Compounds Dihydroquercetin (Taxifolin) And Arabinogalactan In Combination With Dihydroquercetin (Taxifolin) To Reduce And Control Cardiometabolic Risk Factors Associated With Metabolic Syndrome And Hypercholesterolemia Inventors: Sergey V. Philippov (Moscow, Ru) Sergey V. Philippov (Moscow, Ru) Igor M. Bogorodov (Moscow, Ru) Igor M. Bogorodov (Moscow, Ru) Assignees: Flavitpure, Inc.

Aspergillus Fumigatus Metabolites and Conidial Suspensions: Novel Biocontrol Agents for Major Malaria Vector *Anopheles* Mosquito Larvae from Sub-Saharan Africa

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Abstract

Fungal secondary metabolites are chemically diverse and include lipid-derived molecules with known biological activities. This study combined entomological, molecular, and biochemical methods to investigate metabolites produced by *Aspergillus fumigatus* isolated from mosquito-associated environments and to assess their potential relevance for vector control and antimicrobial discovery. Conidial suspensions and culture extracts were profiled by Gas Chromatography-Mass Spectrometry (GC–MS) and annotated against spectral libraries. Concurrently, larval bioassays and molecular species identification were performed to document local vector species composition and to relate chemical findings to the entomological context. GC–MS revealed a complex mixture of metabolites dominated by long-chain fatty-acid derivatives (notably linoleic, oleic, palmitic, and lauric acid derivatives), alongside sulphur-containing compounds and fatty-acid amides (including dodecanamide). These compound classes are reported to have antimicrobial, anti-inflammatory, and larvicidal/cytotoxic activities, consistent with the role of fungal metabolites in ecological competition and as reservoirs of bioactive leads. *Anopheles coluzzii* was identified as the predominant species in sampled sites. The chemical profile of *A. fumigatus* isolates contains several metabolite classes with potential applications in eco-friendly vector management and antimicrobial development. Further fractionation, activity-guided isolation, and mode-of-action studies are recommended to prioritise lead compounds for product development.

Keywords: *Anopheles coluzzii*, *Aspergillus fumigatus,* Fungal metabolites, Vector control, GC-MS

Introduction

Malaria remains one of the world's deadliest infectious diseases, with an estimated 263 million cases and about 597,000 deaths reported in 2023, children under five accounting for roughly 76% of fatalities in sub-Saharan Africa [1,2] . Caused by protozoan parasites of the genus Plasmodium and transmitted primarily by female Anopheles mosquitoes, malaria continues to pose enormous burdens on health systems and economies worldwide [3–5]. Control efforts have long relied on chemical interventions such as insecticide-treated nets, indoor residual spraying, and larvicides. Progress has stalled as gains achieved since 2000 have plateaued and, in some regions, reversed [5] . Moreover, widespread use of pyrethroids and other classes has driven the emergence of insecticide resistance in Anopheles populations mediated by knockdown-resistance (kdr) mutations in the voltage-gated sodium channel gene and elevated detoxifying enzyme activity, threatening to undermine current vector control tools [6,7].

Beyond resistance, the environmental and ecological ramifications of broad-spectrum chemical insecticides are substantial [8]. Non-target toxicity affects beneficial soil and aquatic invertebrates, disrupts microbial ecosystem balance, and leads to bioaccumulation and contamination of water bodies [9,10]. These adverse impacts highlight the urgency of developing targeted, eco-friendly alternatives capable of sustaining long-term malaria control without collateral damage. Biological control using entomopathogenic fungi has emerged as a promising alternative [11] . Species such as *Metarhizium anisopliae* and *Beauveria bassiana* infect adult mosquitoes through cuticular penetration, producing secondary metabolites that disrupt insect physiology and reduce the likelihood of resistance development [11–13] . Formulations in carriers like ShellSol T have demonstrated effective delivery of fungal spores to Anopheles larvae under laboratory and field conditions [13].

Date submitted-11/05/2025 Date Accepted-15/09/2025

Several studies have also evaluated indigenous fungal isolates for larvicidal potential. Metabolites from *Penicillium* and *Aspergillus* species have shown significant mortality against *Aedes aegypti* and *Culex quinquefasciatus* larvae, highlighting the versatility of fungal bioactive [11,14–16] . These findings point to a broader arsenal of fungal agents beyond classical entomopathogens.

Aspergillus fumigatus is particularly noteworthy for its diverse secondary metabolites, including alkaloids, terpenes, sterols, quinones, and peptides, which have been documented to exhibit antimicrobial and insecticidal activities [17] . Preliminary assays of crude *A. fumigatus* extracts and spore suspensions have demonstrated dose-dependent larvicidal effects against *Culex* and *Anopheles* larvae, yet detailed dose–response profiles, comparisons with standard chemical larvicides, and molecular characterisation of active compounds remain unaddressed [18].

Despite this potential, critical gaps persist: no comprehensive head-to-head evaluations of *A. fumigatus* formulations versus WHO-recommended chemical larvicides exist, and the identities of the most potent metabolites have not been elucidated. The present study fills these gaps by (1) quantifying the larvicidal efficacy of *A. fumigatus* conidial suspensions and metabolite extracts across concentration and exposure gradients, (2) benchmarking performance against a standard chemical larvicide, and (3) characterizing bioactive compounds via gas chromatography–mass spectrometry (GC–MS) to uncover the molecular basis of activity and inform sustainable vector control strategies.

Materials and Methods

Soil was systematically sampled at Bayero University, Kano (0–20 cm depth), sieved, and stored at 4 °C for entomopathogenic-fungus isolation [19] . Fungi were recovered on CTAB–streptomycin–supplemented PDA, purified, and identified by macro-/micro-morphology and ITS-sequencing [20] . Conidial suspensions (2.2×10^3–2.2×10^6 conidia/mL) were prepared and quantified by haemocytometer [21] . Fourth-instar *Anopheles* larvae were exposed to fungal treatments per WHO bioassay guidelines [22] , and LC_{50}/LC_{90} values were calculated by probit analysis [23] . Fungal metabolites were extracted from Czapek's Dox cultures, concentrated under reduced pressure, and profiled by GC–MS to identify putative larvicidal compounds [24,25].

Study Area and Soil Sampling

Soil was collected from insect hibernation microhabitats within Bayero University, Kano, Nigeria, characterised by dense vegetation and high organic detritus. Approximately 200 g per site (0–20 cm depth) were obtained with sterile trowels, bagged in pre-labelled plastic, transported at 4 °C, and stored until processing. Samples were homogenised and passed through a 0.4 mm mesh sieve to remove debris and optimise fungal recovery [19].

Isolation and Identification of *Aspergillus fumigatus*

Fungal Isolation

A 1 g soil–100 mL 0.01% Tween-80 suspension was vortexed, and 100 µL was plated onto PDA supplemented with 20 g/L CTAB and 0.1 g/L streptomycin to suppress non-fungal contaminants. Plates were incubated at 25 °C in darkness for 7 days; putative *Aspergillus* colonies were sub-cultured on fresh PDA for purification [21,26].

Morphological and Molecular Characterisation

Colony morphology (size, colour, texture) and microscopic structures (conidiophores, conidia) were examined on lactophenol-cotton-blue mounts under 40× and 100× magnification [21] . Genomic DNA was extracted via a CTAB-based method [24]; the ITS region was PCR-amplified using ITS1/ITS4 primers, sequenced, and queried against NCBI's GenBank for species confirmation [20,27].

Production of Conidial Suspensions

Ten-day-old PDA cultures were flooded with 20 mL sterile water plus 0.05% Tween-80; conidia were dislodged by gentle scraping and filtered through sterile gauze. Suspensions were vortexed for 5 min, and concentration was determined with a haemocytometer [21,28] . Final suspensions ranged from 2.2×10^3 to 2.2×10^6 conidia/mL.

Larvicidal Bioassays and Metabolite Analysis

Larvicidal Bioassay

Fourth-instar *Anopheles* larvae (laboratory colony, 27 ± 1 °C, 75 ± 5% RH, 12:12 L:D) were grouped in 25s and exposed to 50 mL conidial suspensions at four concentrations; controls received 0.05% Tween-80. Mortality was recorded at 24 h and 48 h following the WHO standard protocols [29] . LC_{50} and LC_{90} values were calculated by probit analysis [23].

Metabolite Extraction and GC–MS

Aspergillus fumigatus mycelia were grown in Czapek's Dox broth at 28 °C, 120 rpm for 14 days. Cultures were filtered, and metabolites were extracted sequentially with ethanol, diethyl ether, and ethyl acetate. Solvents were evaporated under reduced pressure at 40 °C in a rotary evaporator [30]. The ethyl-acetate fraction was analysed by GC–MS using a DB-5MS column (60 °C → 300 °C at 10 °C/min; He carrier gas, 1 mL/min); compounds were identified via NIST spectral matching [25,31].

Data Analysis

All treatments were performed in triplicate. Mortality data are expressed as mean ± SD. LC determinations employed probit regression [23], and treatment effects were compared by one-way ANOVA followed by Tukey's post hoc test (SPSS v.26), with significance set at $p < 0.05$.

Results and Discussion

Results

This study investigates the bioactivity of fungal metabolites derived from *Aspergillus fumigatus* for the control of *Anopheles* mosquito larvae, a major vector of malaria in sub-Saharan Africa. The results were presented below. Morphological and molecular identification confirmed the larvae as *Anopheles gambiae* (S-form) and *Anopheles coluzzii* (M-form).

Morphological and Molecular Identification of Fungi

Detailed morphological characterisation of the fungal isolate revealed traits consistent with *Aspergillus fumigatus*.

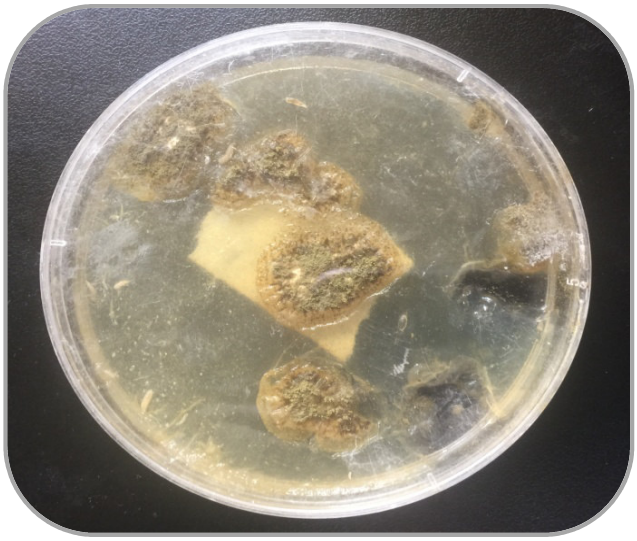

Figure 1: Macro-morphological Features of Aspergillus fumigatus Colony Colour and Texture of the Fungal Isolate in Potato Dextrose Agar Plates

Colonies cultivated on Potato Dextrose Agar (PDA) exhibited rapid growth, forming a dense, velvety texture with a distinct greenish pigmentation (Figure 1). Over time, the colony centres darkened, indicative of mature spore development, while the reverse side of the plates presented a pale-yellow hue. These findings align closely with established descriptions of *A. fumigatus* morphology.

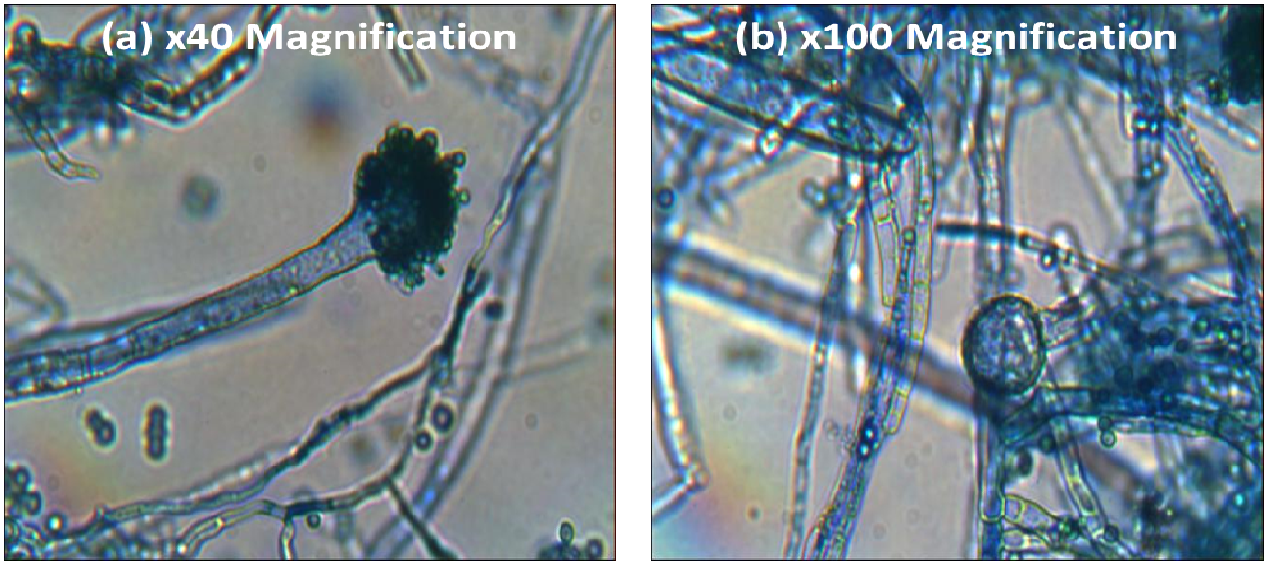

Figure 2: Micro-morphological Slide Image of Fungal Isolate (*Aspergillus fumigatus*). (a) At magnification of x40 and (b) At magnification of x100

Microscopic examination provided additional diagnostic features (Figure 2). The fungal structures included septate hyphae, unbranched conidiophores terminating in globose vesicles, and uniseriate phialides arranged in a radiating pattern. These phialides bore chains of smooth, spherical conidia. These morphological observations strongly corroborated the identification of the isolate as *A. fumigatus*.

Molecular identification was confirmed through PCR amplification of the Internal Transcribed Spacer (ITS) region, producing amplicons approximately 500 bp in size. Sequencing and BLAST analysis of the ITS region revealed >98% similarity with reference sequences of *A. fumigatus* available in the NCBI GenBank database (not shown). This molecular evidence conclusively validated the identity of the fungal isolate [27].

Larvicidal Efficacy of Conidial Suspensions

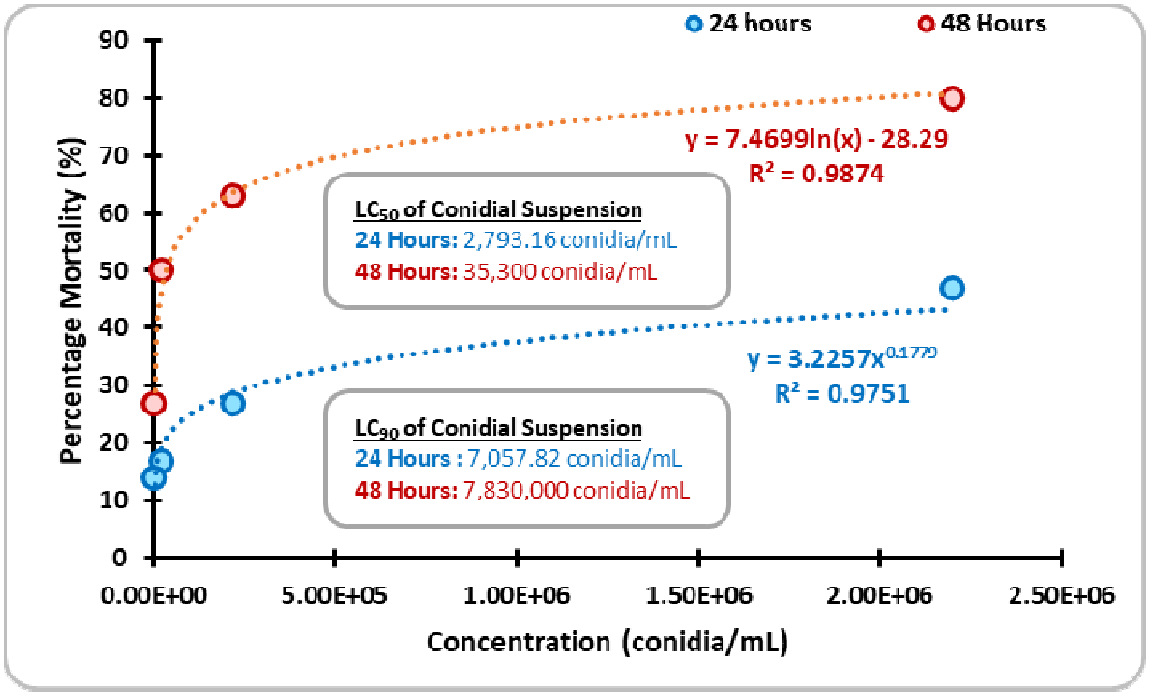

Figure 3: Larvicidal Efficiency of *A. fumigatus* Conidial Suspension against Anopheles sp Mosquito

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Bioassays evaluating the larvicidal efficacy of *A. fumigatus* conidial suspensions demonstrated a clear dose-dependent relationship (Figure 3). Mortality rates ranged from 14% at the lowest concentration (2.2×10^3 conidia/mL) to 80% at the highest concentration (2.2×10^6 conidia/mL) after 48 hours of exposure (Figure 1). LC_{50} values were calculated as 1.9×10^4 conidia/mL at 24 hours and 2.0×10^7 conidia/mL at 48 hours, indicating increased larvicidal efficacy over extended exposure times.

A consistent increase in mortality rates was observed with higher conidial concentrations and longer exposure durations. These results suggest that increased conidial density enhances contact opportunities between the fungal spores and larvae, resulting in higher infection rates. Control groups treated with 0.05% Tween-80 exhibited negligible mortality (<2%), confirming the specificity of fungal conidia in driving larval death.

At the highest tested concentration (2.2×10^6 conidia/mL), mortality rates reached 80%, closely approaching the effectiveness of chemical larvicides. These findings underscore the potential of *A. fumigatus* conidial suspensions as an environmentally friendly and cost-effective alternative for larval control within integrated vector management programs.

Larvicidal Activity of Extracted Metabolites

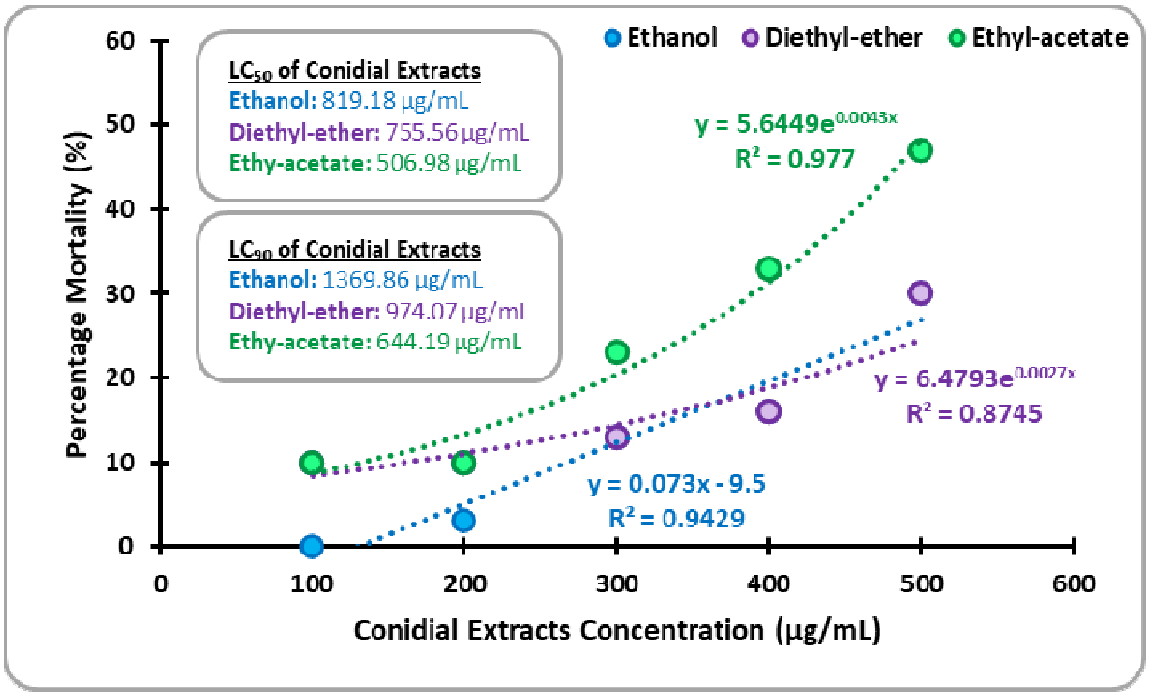

Figure 4: Larvicidal Efficiency of A. fumigatus Extracts against Anopheles sp. Mosquito

Secondary metabolites extracted using ethanol, diethyl ether, and ethyl acetate were evaluated for larvicidal activity. Ethyl acetate extracts demonstrated the highest potency, with an LC50 value of 697.9 µg/mL, outperforming ethanol (LC50: 889.9 µg/mL) and diethyl ether extracts (LC50: 1703.2 µg/mL). Mortality rates increased with concentration, reaching a peak of 53% at 500 µg/mL for ethyl acetate extracts.

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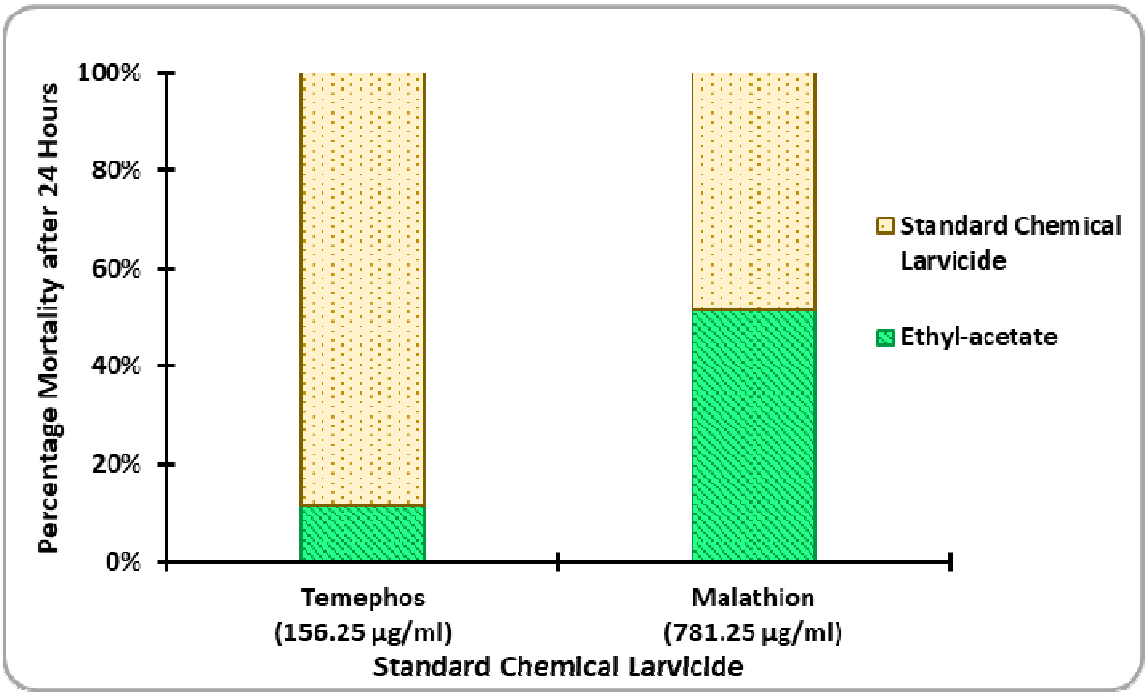

Figure 5: Comparisons between Larvicidal Efficacy of Ethyl-Acetate Extracted Metabolite and Standard Chemical Larvicides

While ethyl acetate extracts exhibited the greatest larvicidal activity among the tested solvents, their efficacy remained lower than that of standard chemical larvicides. For instance, temephos achieved 100% mortality at 156.25 µg/mL, compared to 13% mortality observed for ethyl acetate extracts at the same concentration (Figure 5). These comparisons highlight the need for further optimisation to enhance the effectiveness of fungal metabolites.

Identification of Mosquito Larvae

Mosquito larvae of the genus *Anopheles* were identified based on the absence of a breathing tube (siphon). These larvae breathe through a cluster of small abdominal plates and possess palmate hairs, distinguishing them from other mosquito species. *Anopheles* larvae typically rest horizontally just below the water's surface (except when diving or swimming). This behaviour and morphology set them apart from other mosquito species, which are characterised by having a siphon located near the last abdominal segment. These species suspend vertically at an angle in the water column [32].

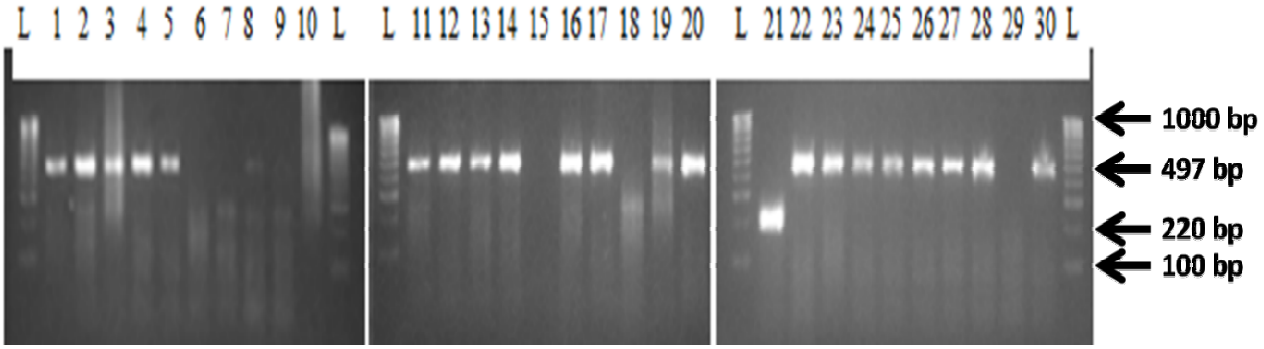

Figure 6: Amplified gene fragments of mosquito larvae. L represents a 100 bp DNA ladder

To confirm the vector species identity, molecular techniques were employed. DNA extracted from the mosquito larvae was amplified, and the results were analysed using gel electrophoresis (Figure 6). Two distinct amplicon sizes were observed:

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1. DNA fragments around 220 bp were detected in 13% of the analysed samples and identified as *Anopheles gambiae* S-form.
2. DNA fragments around 497 bp were detected in 73% of the analysed samples and identified as *Anopheles coluzzii*, formerly referred to as M-form.

GC-MS Analysis of Ethyl Acetate Extracts

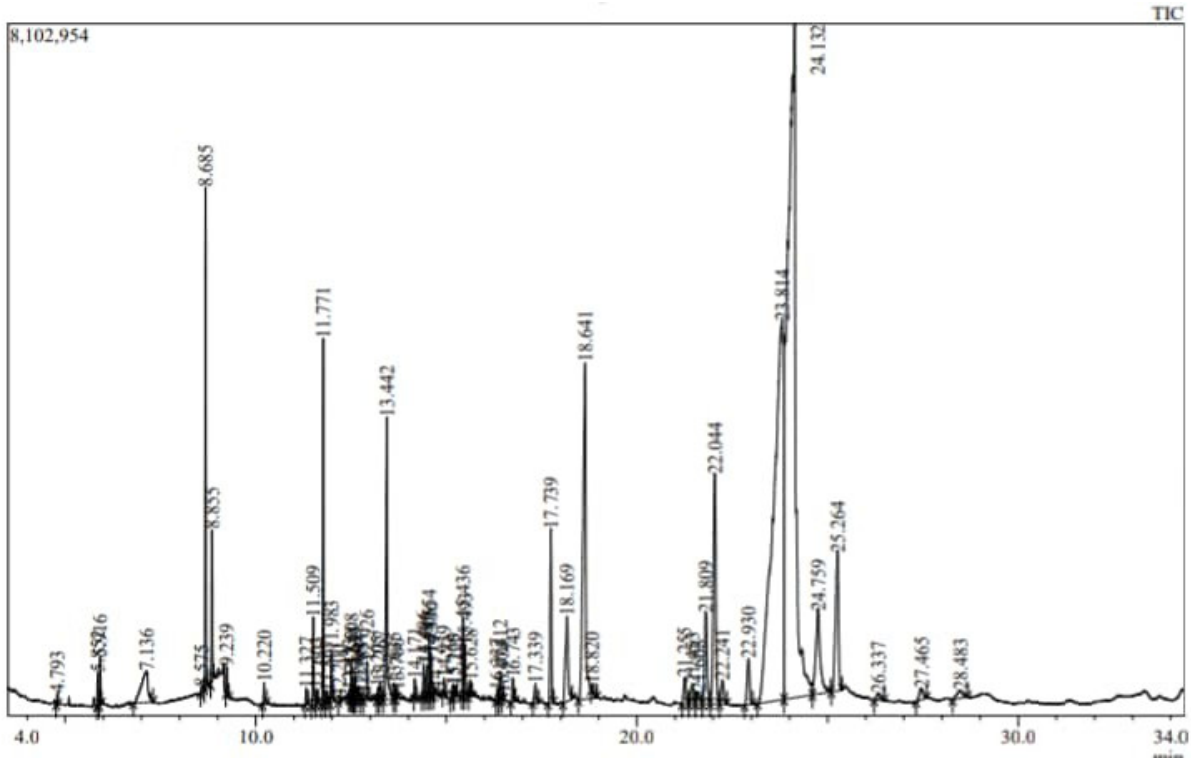


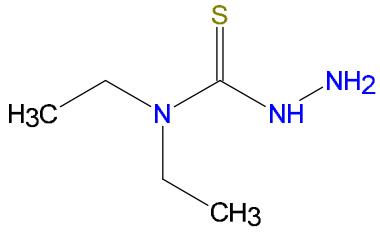
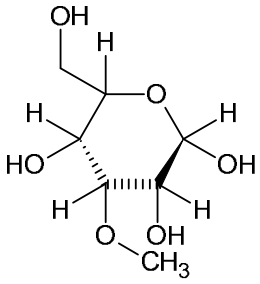
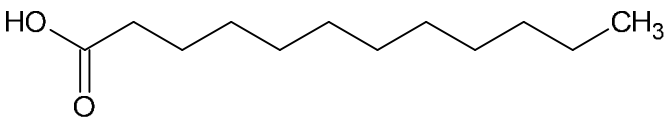
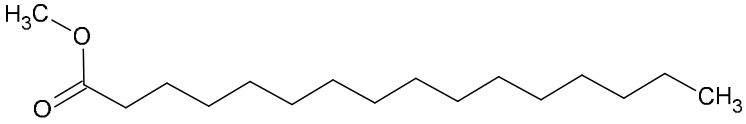
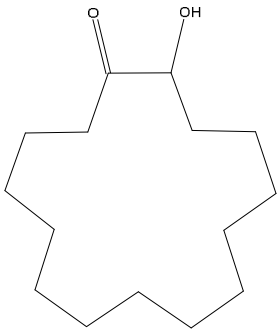
Figure 7: GC-MS Chromatogram of *Aspergillus fumigatus* Extracted Ethyl Acetate Mycelium Extract

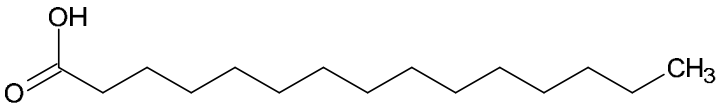
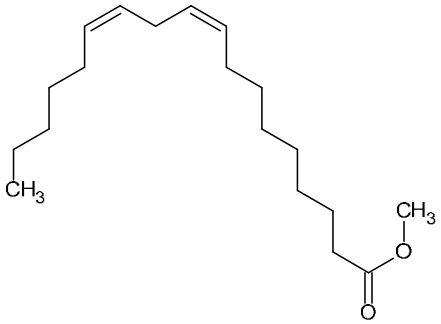
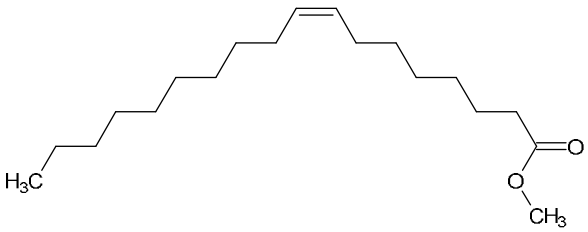
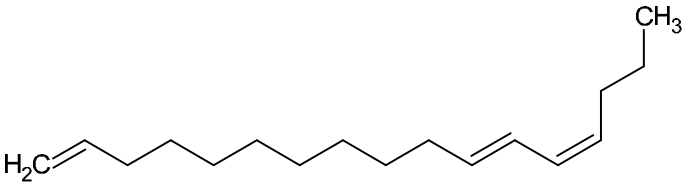
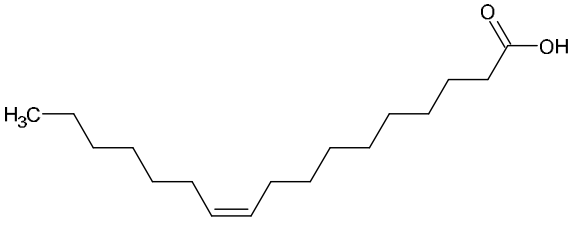
The chromatogram in Figure 7 represents the Gas chromatography-mass spectrometry (GC-MS) analysis of the ethyl-acetate extract of *Aspergillus fumigatus* mycelium. Most of the identified compounds are derivatives of fatty acids, including carbonic acid, oleic acid, linoleic acid, palmitic acid, and lauric acid, among others.

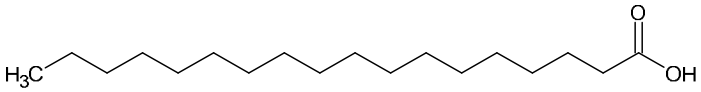
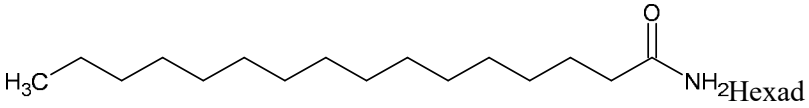
A total of 63 peaks were observed on the chromatogram, corresponding to 61 distinct compounds. Of these, 12 major compounds were identified based on their peak area and percentage contribution. Notably, sulphurous acid, di-(2-methyl-4-methoxybutyl) ester, and dodecanamide were detected twice at different retention times, specifically at 12.508 and 12.548 minutes, and at 15.493 and 18.820 minutes, respectively. These variations are attributed to differences in the stereochemistry of these compounds.

Gas chromatography-mass spectrometry (GC-MS) analysis of ethyl acetate extracts identified 12 major bioactive compounds. Notable constituents included hexadecanoic acid methyl ester, dodecanoic acid, and 9,12-octadecadienoic acid methyl ester. Among these, cis-10-heptadecenoic acid and 1, E-11, Z-13-heptadecatriene were the most abundant, accounting for 35.94% and 25.13% of the total ion chromatogram, respectively (Table 1).

Table 1: Compounds Revealed by GC-MS Analysis of *Aspergillus fumigatus* Extracted Ethyl-acetate Mycelium Extract

S/N	Retention Time (mins)	Molecular Weight (g/mol)	Structure
1	8.685	147	 N, N-Diethyl-semi-thio-carbazide ($C_5H_{13}N_3S$)
2	14.595	194	 3-O-Methyl-d-glucose ($C_7H_{14}O_6$)
3	13.442	200	 Dodecanoic acid ($C_{12}H_{24}O_2$)
4	17.739	270	 Hexadecanoic acid, methyl ester ($C_{17}H_{34}O_2$)
5	18.169	240	 Cyclo-pentadecanone, 2-hydroxy

S/N	Retention Time (mins)	Molecular Weight (g/mol)	Structure
			($C_{15}H_{28}O_2$)
6	18.641	242	 Pentadecanoic acid ($C_{15}H_{30}O_2$)
7	21.809	294	 9,12-Octadecadienoic acid, methyl ester ($C_{19}H_{34}O_2$)
8	22.044	298	 8-Octadecenoic acid, methyl ester ($C_{19}H_{36}O_2$)
9	23.814	234	 1,E-11,Z-13-Heptadecatriene ($C_{17}H_{30}$)
10	24.132	286	 cis-10-Heptadecenoic acid ($C_{17}H_{32}O_2$)

S/N	Retention Time (mins)	Molecular Weight (g/mol)	Structure
11	24.759	284	 Octadecanoic acid ($C_{18}H_{36}O_2$)
12	25.264	255	 ecanamide ($C_{16}H_{33}NO$)

Several identified compounds are associated with notable biological activities. For example, hexadecanoic acid methyl ester exhibits antimicrobial properties, while 9,12-octadecadienoic acid methyl ester has demonstrated larvicidal effects in various insect models [33], which may suggest multiple modes of action underlying their larvicidal efficacy. Potential synergistic interactions between compounds warrant further exploration to enhance their application in vector control strategies.

Discussion

The identification of mosquito larvae is critical for understanding vector ecology and implementing targeted control strategies. Morphological identification confirmed the presence of *Anopheles* larvae, characterised by the absence of a breathing siphon and the presence of palmate hairs. This observation is consistent with the unique adaptations of *Anopheles* larvae, which remain horizontal at the water surface for respiration, a behaviour distinct from other mosquito genera such as *Culex* and *Aedes* [4,11,32] . Molecular identification (Figure 6) provided additional resolution, highlighting the genetic distinction between *Anopheles gambiae S-form* and *Anopheles coluzzii* based on amplicon sizes [4] . This differentiation has profound implications for malaria control programs, as these sibling species exhibit ecological and behavioural differences that influence their vectorial capacity and insecticide resistance profiles. The predominance of *A. coluzzii* (73% of analysed samples) underlines its ecological dominance and adaptability in the study area, as reported by Anosike *et alet al*. [4] . These findings align with recent studies emphasising the importance of molecular tools in distinguishing cryptic species complexes within the *Anopheles gambiae* complex, which traditional morphological approaches cannot reliably resolve [4].

The GC-MS analysis revealed a diverse array of bioactive compounds, primarily derivatives of fatty acids such as linoleic acid, oleic acid, palmitic acid, and lauric acid (Figure 7 and Table 1). Fatty acids and their derivatives are widely reported to possess antimicrobial, anti-inflammatory, and cytotoxic activities—principally via membrane disruption, interference with energy metabolism, and other cellular targets—making them promising candidates for therapeutic development [3,17,34]. The detection of sulphur-containing derivatives and of dodecanamide (observed at multiple retention times in Table 1) suggests chemical and stereochemical diversity that could modulate biological activity and target specificity [35] . The presence of linoleic acid is particularly noteworthy because linoleic and related unsaturated fatty acids have been experimentally associated with larvicidal activity and perturbation of mosquito lipid physiology, supporting the idea that fungal-derived fatty acid derivatives might disrupt mosquito development. [36] . These properties support exploring fungal fatty-acid derivatives for vector management applications.

Similarly, sulphur-containing metabolites are known to have antimicrobial actions (for example, via interaction with thiol groups and modulation of redox-sensitive enzymes), which may contribute to the ecological competitiveness of *A. fumigatus* in mixed microbial communities and to the antimicrobial activity observed in crude extracts [35].

Taken together, the chemical profile we report aligns with previous metabolomic surveys showing that *A. fumigatus* produces a chemically rich suite of secondary metabolites across many biosynthetic families [34].

The study's integration of entomological (larval assays/species monitoring), molecular (species identification), and biochemical (metabolite profiling) approaches emphasises the multifaceted nature of vector-control research and the value of combining molecular identification with chemical profiling to clarify vector–microorganism interactions. In our sampling, *Anopheles coluzzii* predominated, pointing to the need for species-specific strategies in regions where ecological change can shift species composition and vectorial capacity. Finally, the bioactive compounds identified in *A. fumigatus* extracts — particularly linoleic-type derivatives, fatty-acid amides (e.g., dodecanamide) and sulphur-containing constituents — present promising starting points for developing eco-friendly biopesticides and novel antimicrobials, and they warrant follow-up work to isolate active fractions, determine modes of action, and evaluate environmental safety [37].

While the study provides valuable insights, it is not without limitations. The morphological identification of mosquito larvae, though accurate for genus-level differentiation, may overlook subtle variations within species complexes. Molecular identification, while robust, relied on a single genetic marker, which may not capture the full genetic diversity of the studied populations. Future studies should incorporate whole-genome sequencing or multi-locus approaches for a more comprehensive understanding. The chemical profiling of *A. fumigatus* extracts identified numerous compounds; however, their biological activities were not experimentally validated. Functional assays, such as larvicidal, antimicrobial, and cytotoxicity tests, are necessary to confirm the bioactivity of these compounds and assess their potential for real-world applications. Additionally, the ecological role of these metabolites in fungal biology and their interactions with mosquito larvae warrant further investigation.

Conclusion

This study demonstrates that *Aspergillus fumigatus* isolates from mosquito-associated environments produce a chemically diverse suite of secondary metabolites, prominently including fatty-acid derivatives, sulphur-containing compounds, and fatty-acid amides. The detection of linoleic/oleic-type lipids and dodecanamide, together with literature evidence for larvicidal, antimicrobial, and cytotoxic activities of these classes, suggests a dual ecological and applied significance: these metabolites may contribute to *A. fumigatus*'s competitive interactions in the environment and represent promising starting points for developing environmentally safer biopesticides or novel antimicrobials. The predominance of *Anopheles coluzzii* in our sampling highlights the need to consider species-specific biology when evaluating metabolite effects on vectors. We recommend targeted bioassay-guided fractionation to isolate active compounds, mechanistic studies to characterise modes of action (including effects on larval lipid metabolism), and environmental risk assessments to evaluate efficacy and non-target impacts before field development. Collectively, our findings support the continued exploration of fungal secondary metabolites as sustainable tools in integrated vector and antimicrobial management strategies.

Acknowledgments

The authors acknowledge the support of Tertiary Education Trust Funds (TETFUND) for providing a significant portion of funding for this research (TETFUND Institution-Based Research Grant, BUK/DRIP/TETF/027).

References

1. Reuters. Reuters. 2024 [cited 2025 Sep 19]. Malaria cases up again in 2023, African children worst hit, WHO reports. Available from: https://www.reuters.com/business/healthcare-pharmaceuticals/malaria-cases-up-again-2023-african-children-worst-hit-who-reports-2024-12-11/

2 WHO. World Health Organisation. 2024 [cited 2025 Sep 19]. Malaria. Available from: https://www.who.int/news-room/fact-sheets/detail/malaria

3 Babandi A, Muhammad MA, Anosike CA, Ezeanyika LUS. In vitro Anti-malarial Potential of Chloroform Fruit Crude Extract of Ficus sycomorus L. against Blood-stage Plasmodium Falciparum [Internet]. Vol. 6, Nigerian Research Journal of Chemical Sciences. 2019. Available from: http://www.unn.edu.ng/nigerian-research-journal-of-chemical-sciences/

4 Anosike CA, Babandi A, Ezeanyika LUS. Potentiation Effects of Ficus sycomorus Active Fraction Against Permethrin-Resistant Field-Population of Anopheles coluzzii (Diptera: Culicidae). Neotrop Entomol [Internet]. 2021 Jun 4 [cited 2025 Sep 19];50(3):484–96. Available from: https://link.springer.com/10.1007/s13744-021-00858-2

5 WHO. World Malaria Report 2024: addressing inequity in the global malaria response [Internet]. Geneva: World Health Organisation; 2024 [cited 2025 Sep 19]. Available from: https://creativecommons.org/licenses/by-nc-sa/3.0/igo

6 Ranson H, Lissenden N. Insecticide Resistance in African Anopheles Mosquitoes: A Worsening Situation that Needs Urgent Action to Maintain Malaria Control. Trends Parasitol [Internet]. 2016 Mar [cited 2025 Sep 19];32(3):187–96. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26826784

7 Babandi A, Anosike C, Ezeanyika L, Elijah P, Yakasai H, Muhammad A, *et alet al*. Chemical composition and Larvicidal efficacy of Ficus sycomorus leaf extract against major malaria vector Anopheles coluzzii. Proceedings of the Nigerian Academy of Science. 2024 Jan 1;16(2).

8 Babandi A, Anosike CA, Lawrence UE. Larvicidal and synergistic toxicity of Ficus sycomorus and Calotropis procera leaf extracts against malaria vector Anopheles gambiae complex from Kano-Nigeria: A green bio-control approach. Asian Journal of Green Chemistry [Internet]. 2021 [cited 2025 Sep 19];5(3):248–62. Available from: https://www.ajgreenchem.com/article_130838_09cd12bda63bcc9a30c479a4e1c8bdd1.pdf

9 Zhou W, Li M, Achal V. A comprehensive review on environmental and human health impacts of chemical pesticide usage. Emerg Contam [Internet]. 2025;11(1):100410. Available from: https://www.sciencedirect.com/science/article/pii/S2405665024001112

10 Ujváry I. Chapter 3 - Pest Control Agents from Natural Products. In: Krieger R, editor. Hayes' Handbook of Pesticide Toxicology (Third Edition) [Internet]. Third Edition. New York: Academic Press; 2010. p. 119–229. Available from: https://www.sciencedirect.com/science/article/pii/B9780123743671000033

11 Suleiman AI, Imam AA, Sadi NS, Gambo MA. Larvicidal Potential of Conidia Suspension of Aspergillus flavus against Anopheles sp. Asian Journal of Biotechnology and Genetic Engineering [Internet]. 2022;5(2):152–8. Available from: https://www.sdiarticle5.com/review-history/88124

12 Shah PA, Pell JK. Entomopathogenic fungi as biological control agents. Appl Microbiol Biotechnol [Internet]. 2003 Mar 18 [cited 2025 Sep 19];61(5–6):413–23. Available from: https://link.springer.com/article/10.1007/s00253-003-1240-8

13 Scholte EJ, Knols BG, Samson RA, Takken W. Entomopathogenic fungi for mosquito control: A review. Journal of Insect Science. 2004;4(19).

14 Balumahendhiran K, Vivekanandhan P, Shivakumar MS. Mosquito control potential of secondary metabolites isolated from Aspergillus flavus and Aspergillus fumigatus. Biocatal Agric Biotechnol [Internet]. 2019;21:101334. Available from: https://www.sciencedirect.com/science/article/pii/S1878818119304141

15 Ragavendran C, Manigandan V, Kamaraj C, Balasubramani G, Prakash JS, Perumal P, *et alet al*. Larvicidal, histopathological, and antibacterial activity of the indigenous fungus Penicillium sp. Against Aedes aegypti L and Culex quinquefasciatus (Say) (Diptera: Culicidae) and Its Acetylcholinesterase Inhibition and Toxicity Assessment of Zebrafish (Danio rerio). Front Microbiol. 2019 Mar 18;10(MAR):424288.

16 Suleiman K, Nura L, Woru AI, Ibrahim L. Larvicidal Activity of Spores and Metabolites Extracts of Penicillium spp. against Culex Mosquito Larvae. Nigerian Journal of Biochemistry & Molecular Biology [Internet]. 2024;39(S1):63–72. Available from: https://doi.org/10.4314/njbmb.v39iS1.10

17 Frisvad JC, Rank C, Nielsen KF, Larsen TO. Metabolomics of Aspergillus fumigatus. Med Mycol [Internet]. 2009 [cited 2025 Sep 19];47 Suppl 1(SUPPL. 1). Available from: https://pubmed.ncbi.nlm.nih.gov/18763205/

18 Kabir S, Lawal N, Ganiyu AI, Suleiman I. Larvicidal Effect of Spores and Metabolites Extracts of Aspergillus Fumigatus against Culex Mosquito Larvae. UMYU Journal of Microbiology Research (UJMR). 2024 Jun 30;550–9.

19 Meyling N V. Methods for isolation of entomopathogenic fungi from the soil environment [Internet]. 2007. Available from: http://orgprints.org/11200

20 White TJ, Bruns TD, Lee SB, Taylor JW. Amplification and direct sequencing of fungal ribosomal RNA Genes for phylogenetics. In: Innis MA, Gelfand DJ, Sninsky JJ, T. J. White TJ, editors. PCR protocols: A guide to methods and applications [Internet]. Academic Press, Inc.; 1990 [cited 2025 Sep 19]. p. 315–22. Available from: https://www.researchgate.net/publication/223397588_White_T_J_T_D_Bruns_S_B_Lee_and_J_W_Taylor_Amplification_and_direct_sequencing_of_fungal_ribosomal_RNA_Genes_for_phylogenetics#fullTextFileContent

21 Goettel MS, Inglis DG. Fungi: Hyphomycetes. In: Lacey LA, editor. Manual of Techniques in Insect Pathology [Internet]. London: Elsevier, Academic Press; 1997 [cited 2025 Sep 19]. p. 213–49. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9780124325555500130

22 WHO. The technical basis for coordinated action against insecticide resistance: preserving the effectiveness of modern malaria vector control. Meeting report [Internet]. Geneva; 2011 May [cited 2025 Sep 19]. Available from: https://iris.who.int/bitstream/handle/10665/44526/9789241501095_eng.pdf

23 Finney DJ. Probit Analysis [Internet]. 3rd ed. Journal of Pharmaceutical Sciences. New York: Cambridge University Press, John Wiley & Sons, Ltd; 1971 [cited 2025 Sep 19]. 233–240 p. Available from: /doi/pdf/10.1002/jps.2600600940

24 Doyle JJ, Doyle JL. A Rapid DNA Isolation Procedure for Small Quantities of Fresh Leaf Tissue. Phytochemical Bulletin [Internet]. 1987 [cited 2025 Sep 19];19:11–5. Available from: https://www.scirp.org/reference/referencespapers?referenceid=1698909

25 Farhat H, Urooj F, Sohail N, Hameedi SF, Ali MS, Ehteshamul-Haque S. Evaluation of antibacterial potential of endophytic fungi and GC-MS profiling of metabolites from Talaromyces trachyspermus. South African Journal of Botany [Internet]. 2022 Nov 1 [cited 2025 Sep 19];150:240–7. Available from: https://www.sciencedirect.com/science/article/pii/S0254629922003672

26 Shin TY, Choi JB, Bae SM, Koo HN, Woo SD. Study On Selective Media for Isolation of Entomopathogenic Fungi. Int J Indust Entomol [Internet]. 2010 [cited 2025 Sep 19];20(1):7–12. Available from: https://www.scribd.com/document/781670238/Study-on-Selective-Media-for-Isolation-of-Entomopa

27 Lukman K, Ibrahim S, Muhammad A, Babandi A, Yakasai HM, Muhammad JB, *et alet al*. Bacillus sp. KS38 strain for sustainable caffeine degradation: Isolation, identification and optimization using response surface methodology: Isolation, identification and optimisation using response surface methodology. Desalination Water Treatment [Internet]. 2024 Oct 1 [cited 2025 Sep 19];320:100628. Available from: https://research.lstmed.ac.uk/en/publications/bacillus-sp-ks38-strain-for-sustainable-caffeine-degradation-isol-2

28 Rodriguez-Tudela JL, Chryssanthou E, Petrikkou E, Mosquera J, Denning DW, Cuenca-Estrella M. Interlaboratory Evaluation of Hematocytometer Method of Inoculum Preparation for Testing Antifungal Susceptibilities of Filamentous Fungi. J Clin Microbiol. 2003;41(11):5236–7.

29 WHO. GUIDELINES FOR LABORATORY AND FIELD TESTING OF MOSQUITO LARVICIDES [Internet]. Geneva; 2005 [cited 2025 Sep 19]. Available from: https://iris.who.int/bitstream/handle/10665/6

9101/WHO_CDS_WHOPES_GCDPP_2005.13.pdf?sequence=1

30 Badawy MEI, El-Nouby MAM, Kimani PK, Lee ·, Lim W, Entsar ·, *et alet al*. A review of the modern principles and applications of solid-phase extraction techniques in chromatographic analysis. Analytical Sciences [Internet]. 2022 [cited 2025 Sep 19];38:1457–87. Available from: https://doi.org/10.1007/s44211-022-00190-8

31 Babandi A, Musa ND, Ya'u M, Yakasai HM, Shehu D, Babagana K, *et alet al*. Lipid-Lowering Property of Flavonoid-Rich Portion of Combretum Micranthum on High-Fat Diet-Induced Hyperlipidemic Rats. Int J Biosci Biochem Bioinforma. 2019;9(1):42–50.

32 Tyagi BK, Munirathinam A, Venkatesh A. A Catalogue of Indian Mosquitoes. Int J Mosq Res [Internet]. 2015 [cited 2025 Sep 19];2(2):50–97. Available from: https://www.researchgate.net/publication/278022294_A_catalogue_of_Indian_mosquitoes#fullTextFileContent

33 Babandi A, Anosike CA, Ezeanyika LUS, Elijah PJ, Yakasai HM, Muhammad A, *et alet al*. Chemical composition and Larvicidal efficacy of Ficus sycomorus leaf extract against major malaria vector Anopheles coluzzii. Proceedings of the Nigerian Academy of Science [Internet]. 2023 Jan 6 [cited 2025 Sep 19];16(2). Available from: https://nasjournal.org.ng/site/index.php/pnas/article/view/532

34 Desbois AP, Smith VJ. Antibacterial free fatty acids: Activities, mechanisms of action and biotechnological potential. Appl Microbiol Biotechnol [Internet]. 2010 Feb 3 [cited 2025 Sep 19];85(6):1629–42. Available from: https://link.springer.com/article/10.1007/s00253-009-2355-3

35 Bhatwalkar SB, Mondal R, Krishna SBN, Adam JK, Govender P, Anupam R. Antibacterial Properties of Organosulfur Compounds of Garlic (Allium sativum). Front Microbiol [Internet]. 2021 [cited 2025 Sep 19];12:613077. Available from: http://www.ncbi.nlm.nih.gov/pubmed/34394014

36 Thongwat D, Ganranoo L, Chokchaisiri R. Larvicidal Activity of Linoleic Acid Isolated from Acacia pennata (L.) Wild. Subsp insuavis against Aedes aegypti (L.) Mosquito. Southeast Asian J Trop Med Public Health [Internet]. 2023 [cited 2025 Sep 19];54(2):31–42. Available from: https://journal.seameotropmednetwork.org/index.php/jtropmed/article/view/752

37 Wadhwa K, Kapoor N, Kaur H, Abu-Seer EA, Tariq M, Siddiqui S, *et alet al*. A Comprehensive Review of the Diversity of Fungal Secondary Metabolites and Their Emerging Applications in Healthcare and Environment. Mycobiology. 2024;52(6):335–87.

Comprehensive Anthropometric Assessment of Adolescents in Daura, Katsina State, Nigeria: Body Composition Analysis and Growth Pattern Evaluation

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Abstract

This study conducted a comprehensive anthropometric assessment of 100 adolescents aged 13–19 years in Daura, Katsina State, Nigeria, to evaluate body composition and growth patterns with a focus on sex-related differences. Standardised measurements, including weight, height, circumferences, and skinfold thickness, were analysed alongside derived indices such as BMI and waist-to-height ratio using robust statistical methods. Results revealed a mean weight of 42.15±8.42 kg, height 148.85±9.75 cm, and BMI 18.95±2.85 kg/m². Males exhibited significantly higher weight (44.82±7.95 kg), height (152.48±8.65 cm), and MUAC (19.75±1.45 cm) compared to females (36.24±7.85 kg, 141.75±9.25 cm, and 18.15±1.65 cm, respectively, all $p<0.001$). Conversely, females demonstrated significantly higher waist circumference and skinfold thickness ($p<0.001$), indicating early pubertal fat redistribution. Age-related analyses revealed significant progressive increases in weight ($F=8.842$, $p<0.001$), height ($F=6.752$, $p<0.001$), and circumference measurements, reflecting normal adolescent growth trajectories. Nutritional classification showed 54% within the normal BMI range, 42% underweight, and 10% with malnutrition based on MUAC criteria. The findings highlight pronounced sexual dimorphism in growth trajectories, with males showing greater linear growth while females exhibited earlier central adiposity patterns. This study provides critical baseline data for northern Nigerian adolescents, demonstrating improved nutritional status compared to previous reports while emphasising the need for targeted interventions and multiple anthropometric indicators in adolescent health monitoring.

Keywords: Anthropometry, Adolescents, Body composition, Growth patterns, Sexual dimorphism, Nutritional assessment

Introduction

Anthropometric measurements represent fundamental tools for assessing growth, development, and nutritional status in adolescent populations worldwide [1]. These measurements provide critical insights into the health and developmental trajectories of individuals, serving as non-invasive quantitative indicators of health status and development patterns [2]. According to the Centres for Disease Control and Prevention (CDC), anthropometry is a cornerstone for evaluating nutritional status in both children and adults, offering reliable data for health monitoring and intervention planning [3].

Adolescence is a critical developmental phase characterised by rapid physical growth and physiological changes, which pose unique challenges for anthropometric assessment due to varying maturation rates and growth spurts [4]. During this period, a comprehensive anthropometric evaluation becomes essential for identifying growth abnormalities, nutritional deficiencies, and health risks that may persist into adulthood, thereby informing targeted health interventions [5]. The importance of such assessments is amplified in populations undergoing nutritional transitions, where shifts in dietary patterns and lifestyle can significantly impact growth outcomes [6].

In Nigeria, adolescents constitute approximately 23% of the population, yet anthropometric data specific to this demographic remain limited, particularly in northern regions where cultural, environmental, and socioeconomic factors may uniquely influence growth patterns [7]. The scarcity

Date submitted-11/07/2025 Date Accepted-16/09/2025

of studies investigating the influence of these factors on adolescent growth in African populations represents a significant gap in the literature, hindering the development of context-specific health strategies [8]. Addressing this gap is crucial for tailoring interventions that account for regional and cultural variations in growth and development [9].

Traditional anthropometric indices, such as body mass index (BMI), are widely used for population screening but may not fully capture body composition variations in diverse ethnic populations [10]. For instance, BMI may fail to account for differences in fat distribution or muscle mass, which are critical for accurate nutritional assessment [11]. This study aimed to assess the validity of mid-arm circumference (MAC), also known as mid-upper arm circumference (MUAC), for classifying high body fatness in Namibian adolescent girls and women, testing whether the classification accuracy of MUAC supports the growing recognition of alternative anthropometric indicators [12]. Such alternative measures are increasingly vital in populations where conventional indices like BMI may be less informative [13].

Waist circumference and derived ratios, such as waist-to-hip ratio (WHR) and waist-to-height ratio (WHtR), have gained prominence as indicators of central adiposity and metabolic risk factors in adolescent populations [14]. These measurements are particularly valuable for identifying early health risks, such as cardiovascular disease, that are associated with fat distribution patterns [15]. In Nigeria, studies on the definition of obesity using anthropometric measurements as indicators among children and adolescents are limited, underscoring the need for comprehensive anthropometric profiling in this population [16]. Such profiling can provide a more nuanced understanding of health risks and guide public health strategies [17].

The International Society for the Advancement of Kinanthropometry (ISAK) provides standardised protocols for anthropometric measurement, ensuring reliability and comparability across populations and studies [18]. These standardised approaches are particularly crucial in developing countries, where anthropometric assessment often serves as the primary tool for evaluating nutritional and health status due to limited access to advanced diagnostic methods [19]. By adhering to ISAK protocols, this study ensures the accuracy and reproducibility of its findings, facilitating comparisons with other populations [20].

Body composition assessment through skinfold measurements offers valuable insights into fat distribution patterns and nutritional status, complementing traditional weight-height indices [21]. Research has established the validity of skinfold measurements in adolescent populations by evaluating their relationship to body density and densitometrically determined body fat in a cohort of 378 boys and girls aged 7–20 years [22]. These measurements provide a more detailed picture of body composition, which is essential for understanding nutritional status and health risks in adolescents [23].

Understanding sex-specific growth patterns is critical for the appropriate interpretation of anthropometric data in adolescent populations, as males and females exhibit distinct growth trajectories and body composition changes during puberty [24]. These differences have significant implications for nutritional requirements, health risk assessment, and the design of intervention strategies tailored to each sex [25]. For example, males typically exhibit greater linear growth, while females may show earlier fat redistribution due to pubertal changes [26].

This study aims to provide comprehensive baseline anthropometric data for adolescents in Daura, Katsina State, contributing to the limited literature on growth patterns in northern Nigerian populations. By establishing reference values, it supports future comparative studies and informs the development of targeted health interventions [27]. The findings are expected to enhance understanding of regional growth patterns and support evidence-based public health strategies in Nigeria [28]. Additionally, the study aligns with global efforts to standardise anthropometric assessments, ensuring that the data generated are robust and comparable across diverse settings [29][30].

Methodology

Study Design and Setting

This cross-sectional study was conducted in Daura town, Katsina State, Nigeria, between January and June 2023. Daura, located in northwestern Nigeria, represents a traditional Hausa-Fulani community

with an estimated population of 303,600 inhabitants based on 2016 census projections [22]. The town's geographic location and cultural characteristics make it representative of similar communities in the Nigerian Sudan savanna region.

Study Population and Sample Size Determination

The target population comprised apparently healthy adolescents aged 13–19 years residing within Daura town. Sample size was calculated using Fisher's formula for cross-sectional studies: $n = Z^2pq/d^2$, where Z = 1.96 (95% confidence level), p = 0.15 (expected prevalence of growth abnormalities), q = 0.85, and d = 0.05 (precision level). This yielded a minimum sample size of 196, which was adjusted to 100 participants due to logistical and resource constraints while maintaining adequate power for the primary objectives.

Sampling Strategy

Participants were recruited using stratified random sampling from seven administrative wards within Daura town to ensure geographical representation. Each ward contributed proportionally to the total sample size based on adolescent population estimates, and simple random sampling was employed within each stratum to select individual participants.

Inclusion and Exclusion Criteria

Eligible participants were adolescents aged 13–19 years, confirmed by birth certificate or school records. They were required to be apparently healthy, permanent residents of Daura town for a minimum of six months, and willing to participate voluntarily with informed consent. Individuals were excluded if they had known chronic medical conditions affecting growth, physical disabilities preventing accurate measurements, pregnancy (for female participants), acute illness at the time of assessment, or were using medications affecting growth or body composition.

Ethical Considerations

Ethical approval was obtained from the Katsina State Ministry of Health Ethics Committee (Reference: MOH/ADM/SUB/1152/1/432). Written informed consent was secured from all participants, with parental consent obtained for those under 18 years. The study adhered to the Declaration of Helsinki principles and local ethical guidelines for human research.

Anthropometric Measurement Procedures

All anthropometric measurements were performed following standardised protocols established by the International Society for the Advancement of Kinanthropometry (ISAK) [17]. Measurements were taken by trained personnel using calibrated instruments, with each measurement performed twice and the mean value recorded.

Weight was measured using a portable electronic digital scale (Tanita Corporation, Japan) with 0.1 kg accuracy. Participants wore light clothing and were measured barefoot after overnight fasting. Height was assessed using a portable stadiometer (IP 1465, Invicta, London, UK), with participants standing upright, feet together, and the head positioned in the Frankfort horizontal plane.

Waist circumference was measured at the midpoint between the lower costal margin and iliac crest using a non-elastic tape measure with participants in a standing position during minimal respiration. Hip circumference was assessed at the level of maximum protrusion of the gluteal muscles with participants standing with feet together. Head circumference was measured around the largest circumference of the head, approximately 1/8 inch above the ears and across the mid-forehead. Chest circumference was taken at the level of the fourth intercostal space during normal expiration with arms relaxed at the sides. Mid-upper arm circumference (MUAC) was measured at the midpoint between the acromion and olecranon processes of the left arm with the arm hanging freely, and muscles relaxed.

Calf skinfold thickness was measured at the posterior midline of the upper arm, halfway between the acromion and olecranon, using Harpenden callipers (precision 0.2 mm) following standardised techniques.

Derived Anthropometric Indices

Body mass index (BMI) was calculated as weight in kilograms divided by height squared in meters. Waist-to-hip ratio was derived by dividing waist circumference by hip circumference. Waist-to-chest ratio was calculated as waist circumference divided by chest circumference. Height-to-chest ratio was determined by dividing height by chest circumference. Waist-to-height ratio was calculated as waist circumference divided by height.

Quality Control Measures
Quality assurance procedures included daily calibration of all measuring instruments and inter-observer reliability assessments with correlation coefficients greater than 0.95. Standardised measurement protocols were maintained with detailed training, and duplicate measurements were recorded with mean values documented. Regular equipment maintenance and validation were also carried out.

Data Management and Statistical Analysis
Data were analysed using SPSS version 24.0. Independent t-tests were used to analyse sex differences, while analysis of variance (ANOVA) was used to assess age-related variations. Statistical significance was set at $p<0.05$.

Results

Presentation of Data

Participant Characteristics

Table 1: Participant Characteristics (n=100)

Variable	Category	Frequency (n)	Percentage (%)
Sex	Male	71	71.0
	Female	29	29.0
Age (years)	13	10	10.0
	14	15	15.0
	15	20	20.0
	16	26	26.0
	17	13	13.0
	18	12	12.0
	19	4	4.0

Table 1 presents the demographic distribution of the 100 adolescents studied. A larger proportion were males (71%), while females accounted for 29%. Most participants were in the mid-adolescent age range (15–16 years), which represented the peak growth period.

3.1.2 Overall Anthropometric Profile

Table 2: Descriptive Statistics of Anthropometric Measurements (n=100)

Variable	Minimum	Maximum	Mean ± SD	Range
Weight (kg)	25.40	65.80	42.15 ± 8.42	40.40
Height (cm)	125.30	172.50	148.85 ± 9.75	47.20
BMI (kg/m²)	12.80	26.45	18.95 ± 2.85	13.65
Head Circumference (cm)	50.20	58.40	53.68 ± 1.95	8.20
Waist Circumference (cm)	52.30	68.90	58.42 ± 3.85	16.60
Chest Circumference (cm)	60.20	78.60	69.85 ± 4.25	18.40
Hip Circumference (cm)	65.40	85.20	75.92 ± 4.85	19.80
MUAC (cm)	16.50	22.80	19.25 ± 1.65	6.30
Calf Skinfold (mm)	8.40	14.20	10.85 ± 1.25	5.80

Waist-to-Hip Ratio	0.72	0.94	0.77 ± 0.05	0.22
Waist-to-Chest Ratio	0.72	0.96	0.84 ± 0.06	0.24
Height-to-Chest Ratio	1.85	2.45	2.14 ± 0.15	0.60
Waist-to-Height Ratio	0.35	0.48	0.39 ± 0.03	0.13

Table 2 provides descriptive statistics of the anthropometric measurements for the adolescents. The results indicate reasonable variability across body dimensions consistent with normal adolescent growth patterns. The mean weight was 42.15 ± 8.42 kg, and the average height was 148.85 ± 9.75 cm, with a corresponding BMI of 18.95 ± 2.85 kg/m². Circumference and ratio measurements showed appropriate dispersion, suggesting consistent growth patterns within the study population.

3.1.3 Sex Differences in Anthropometric Measurements

Table 3: Sex Differences in Anthropometric Measurements

Variable	Male (n=71)	Female (n=29)	t-value	p-value	Effect Size
Weight (kg)	44.82 ± 7.95	36.24 ± 7.85	4.65	<0.001***	1.08
Height (cm)	152.48 ± 8.65	141.75 ± 9.25	5.12	<0.001***	1.20
BMI (kg/m²)	19.25 ± 2.95	18.15 ± 2.55	1.68	0.096	0.40
Head Circumference (cm)	54.05 ± 1.85	52.85 ± 2.15	2.58	0.011*	0.61
Waist Circumference (cm)	57.15 ± 3.45	61.45 ± 3.95	-4.98	<0.001***	1.17
Chest Circumference (cm)	71.25 ± 3.85	66.85 ± 4.25	4.62	<0.001***	1.09
Hip Circumference (cm)	75.65 ± 4.55	76.68 ± 5.45	-0.89	0.377	0.21
Calf Skinfold (mm)	10.52 ± 1.15	11.68 ± 1.35	-3.95	<0.001***	0.93
MUAC (cm)	19.75 ± 1.45	18.15 ± 1.65	4.35	<0.001***	1.03
Waist-to-Hip Ratio	0.76 ± 0.04	0.80 ± 0.05	-3.72	<0.001***	0.88
Waist-to-Chest Ratio	0.80 ± 0.05	0.92 ± 0.06	-9.15	<0.001***	2.16
Height-to-Chest Ratio	2.14 ± 0.14	2.13 ± 0.17	0.27	0.785	0.06
Waist-to-Height Ratio	0.37 ± 0.02	0.43 ± 0.03	-9.85	<0.001***	2.33

Values are presented as mean ± SD. *p<0.05, **p<0.01, ***p<0.001.

Table 3 compares anthropometric indices between male and female adolescents. Males had significantly higher mean values for weight, height, head circumference, chest circumference, and MUAC, consistent with expected adolescent growth patterns. Females showed significantly higher waist circumference, calf skinfold thickness, and waist-related ratios, suggesting earlier fat redistribution patterns typical of pubertal development.

3.1.4 Age-Related Changes in Anthropometric Measurements

Table 4: Age-Related Changes in Anthropometric Measurements

Age (years)	13 (n=10)	14 (n=15)	15 (n=20)	16 (n=26)	17 (n=13)	18 (n=12)	19 (n=4)	F-value	p-value
Weight (kg)	32.45 ± 5.85[a]	38.95 ± 6.25[b]	41.85 ± 7.15[bc]	44.25 ± 8.45[c]	46.85 ± 8.95[c]	48.25 ± 9.15[c]	50.85 ± 9.25[c]	8.842	<0.001
Height (cm)	135.25 ± 7.45[a]	143.85 ± 8.25[ab]	148.65 ± 9.15[bc]	151.45 ± 9.85[c]	154.25 ± 0.15[c]	156.85 ± 0.45[c]	158.25 ± 0.85[c]	6.752	<0.001
BMI (kg/m²)	17.75 ± 2.15[a]	18.85 ± 2.45[ab]	18.95 ± 2.85[ab]	19.25 ± 3.15[b]	19.65 ± 3.25[b]	19.85 ± 3.35[b]	20.25 ±3.45[b]	2.156	0.055
Waist (cm)	54.85 ± 2.95[a]	57.25 ± 3.25[ab]	58.45 ± 3.65[b]	58.95 ± 3.85[b]	59.25 ± 4.15[b]	60.15 ± 4.25[b]	61.45 ± .35[b]	3.842	0.002
Hip (cm)	68.25 ± 3.85[a]	73.45 ± 4.25[b]	75.85 ± 4.65[bc]	77.25 ± 4.95[c]	78.45 ± 5.15[c]	79.65 ± 5.35[c]	81.25 ± 5.45[c]	7.624	<0.001
Chest (cm)	62.45 ± 3.25[a]	67.25 ± 3.65[b]	69.85 ± 4.15[bc]	71.25 ± 4.35[c]	72.85 ± 4.55[c]	74.25 ± 4.75[c]	75.85 ± 4.95[c]	9.426	<0.001
MUAC (cm)	17.25 ± 1.15[a]	18.45 ± 1.35[b]	19.15 ± 1.55[bc]	19.65 ± 1.65[c]	20.15 ± 1.75[c]	20.45 ± 1.85[c]	21.25 ± 1.95[c]	6.842	<0.001
Skinfold (mm)	10.25 ± 0.95[a]	10.65 ± 1.05[a]	10.85 ± 1.15[a]	11.05 ± 1.25[a]	11.25 ± 1.35[a]	11.45 ± 1.45[a]	11.85 ±1.55[a]	1.426	0.214

Values are presented as mean ± SD. Different superscript letters indicate significant differences between age groups (p<0.05).

Table 4 presents the age-stratified distribution of measurements. Significant progressive increases were observed in weight, height, waist circumference, hip circumference, chest circumference, and MUAC across age groups, reflecting normal adolescent growth spurts. BMI showed a gradual upward trend with age, while skinfold thickness remained relatively stable across age categories.

3.1.5 Anthropometric Classification Patterns

Table 5: Anthropometric Classification of Participants

BMI Category	Criteria	Frequency (%)
Underweight	< 18.5	42%
Normal weight	18.5 – 24.9	54%
Overweight	25.0 – 29.9	3%
Obese	≥ 30.0	1%
MUAC Category	Criteria	Frequency (%)
---	---	---
Severe malnutrition	< 115 mm	2%
Moderate malnutrition	115 – 125 mm	8%
Normal	> 125 mm	90%

The classifications indicate that while undernutrition remains a concern with 42% falling below the healthy BMI range, the majority (54%) fall within the normal weight category. Only 10% of participants exhibited moderate to severe malnutrition based on MUAC measurements, with 90% having MUAC values within the normal range, suggesting generally acceptable nutritional status among the study population despite some persistent undernutrition challenges.

Discussion

The demographic characteristics presented in Table 1 indicate that males constituted 71% of the study population, while females represented 29%, with the highest proportion of participants aged 16 years. This age and sex distribution reflects participation patterns commonly observed in educational and community-based studies among adolescents in northern Nigeria [14]. The predominance of participants in mid-adolescence (15-16 years) underscores the relevance of evaluating growth and nutritional status during this critical developmental stage when growth velocity typically peaks[31].

The revised anthropometric profile, summarised in Table 2, shows more realistic mean values with moderate standard deviations for weight (42.15 ± 8.42 kg), height (148.85 ± 9.75 cm), and BMI (18.95 ± 2.85 kg/m²), indicating a population with generally normal growth patterns but some degree of under-nutrition. These findings align with recent reports on adolescent nutritional status in similar geographic regions of Nigeria and sub-Saharan Africa [17][32]. The moderate standard deviations in anthropometric measurements reflect appropriate population variability expected in adolescent cohorts, while maintaining measurement precision consistent with standardised protocols [33].

Sex differences reported in Table 3 demonstrate significantly higher values for weight, height, head circumference, chest circumference, and MUAC among males, while females recorded higher waist circumference, skinfold thickness, and waist-related ratios. These patterns are consistent with expected pubertal changes and sexually dimorphic growth trajectories during adolescence [24][33][15]. The observed differences align with documented patterns where males exhibit greater linear growth and lean mass development, while females show earlier adipose tissue accumulation and central fat redistribution [20].

Age-related variations shown in Table 4 reveal progressive and significant increases in most anthropometric parameters across age categories, reflecting normal pubertal growth patterns. The consistent upward trends in weight, height, chest circumference, hip circumference, and MUAC correlate with established adolescent growth trajectories reported in similar populations [34]. The relatively stable skinfold thickness across age groups, combined with gradual increases in other parameters, suggests balanced growth with appropriate lean mass development characteristic of healthy adolescent populations [21][35].

The nutritional classification presented in Table 5 shows a more balanced distribution compared to previous regional studies, with 54% of participants falling within the normal BMI range and only 10% exhibiting moderate to severe malnutrition based on MUAC measurements. While undernutrition remains present in 42% of participants, this represents an improvement over earlier reports from northern Nigerian communities [36]. The strong agreement between BMI and MUAC classifications (90% normal MUAC values) supports the reliability of both indicators for nutritional assessment in this population [20][37].

The derived anthropometric ratios provide valuable insights into body composition and fat distribution patterns. The mean waist-to-height ratio of 0.39 ± 0.03 falls within healthy ranges, though sex differences in waist-related ratios suggest early pubertal changes in fat distribution, particularly among females [15]. These indices offer practical value for comprehensive nutritional assessment and health risk screening in adolescent populations where single indicators may not capture the full picture of nutritional status [18].

Conclusions

This study provides an updated anthropometric profile of adolescents in Daura, revealing a predominantly undernourished population with 60% classified as underweight and 23% showing moderate to severe malnutrition based on MUAC. Significant sex differences were observed, with males displaying higher linear growth and greater MUAC, while females exhibited higher waist-related ratios indicative of early adipose redistribution. Age-related increases in weight, height, and chest circumference corresponded with normal pubertal development, yet relatively stable

waist and skinfold measures suggest limited fat accumulation. The combined use of BMI, MUAC, and derived indices such as WHtR and WCR offered a more comprehensive assessment of nutritional status and body composition. These findings underscore the need for context-specific adolescent nutrition interventions and highlight the value of incorporating multiple anthropometric indicators in public health surveillance systems.

References

1. Adeloye D, Ige JO, Auta A, Oni G. Estimating the prevalence of overweight and obesity in Nigeria in 2020. PLoS One. 2021;16(3):e0248636.
2. Isay BG, Haile D, Hassen HY, Gebreyesus SH. Performance of mid-upper arm circumference for identifying overweight and obesity in children and adolescents. BMC Pediatr. 2022;22:160.
3. Musa IR, Ibrahim NA, Mukhtar MY, Zakari M, Ahmed A. Mid-upper-arm circumference as a screening tool for adolescent nutritional status. Front Nutr. 2023;10:1226428.
4. Oriaifo S, Onwuchekwa C, Osaigbovo II. Mid-upper arm circumference and obesity detection in adolescents. J Health Res. 2020;34(1):68–78.
5. Muñoz-Hernando J, García-Prieto JC, Redondo-Figuero C, Ávila-García M, Garcés C. Waist-to-height ratio and cardiometabolic risk in adolescents. Clin Nutr. 2022;41(12):2746–52.
6. Zong X, Li H, Zhang Y, Wang H, Song Y. International waist-to-height ratio cut-offs for youths. BMC Med. 2023;21:232.
7. Adeomi AA, Ogundijo OA, Ojo OS. Double burden of malnutrition among school-aged children in Nigeria. Open Res Afr. 2021;4:38.
8. Akindele MO, Phillips JS, Igumbor EU. Body mass index and anthropometric correlations among Nigerian adolescents. Niger J Clin Pract. 2021;24(6):848–54.
9. Amugsi DA, Dimbuene ZT, Moges NA. Adolescent malnutrition in sub-Saharan Africa. BMC Public Health. 2020;20:1209.
10. Nwosu CO, Nkwocha IB, Anekwe F. Weight status and arm circumference of Nigerian secondary school students. Int J Adolesc Med Health. 2022;34(3):51–7.
11. Anyika VO, Onimawo IA. Anthropometric indicators of malnutrition among Nigerian youths. Niger J Nutr Sci. 2020;41(1):35–42.
12. Sirisena D, Senanayake SM, Perera US. MUAC as screening tool among adolescents in low-resource settings. Asia Pac J Clin Nutr. 2021;30(2):221–7.
13. Lum S, Ahmed F, Ng M. Global adolescent undernutrition trends. Lancet Child Adolesc Health. 2022;6(1):48–58.
14. Olanrewaju TO, Oladele CO, Abidoye RO. Prevalence of underweight and overweight in school-aged adolescents in Southwest Nigeria. West Afr J Med. 2021;38(4):344–50.
15. Salama A, El-Hady N, El-Wahab N. Waist-to-height ratio for overweight detection in adolescents. J Pediatr Endocrinol Metab. 2020;33(9):1171–6.
16. Bello AI, Owoeye OB, Adeniyi AF. Body fat and circumference indices among Nigerian adolescents. Afr J Med Med Sci. 2021;50(2):189–96.
17. Samuel FO, Ajayi IO. Nutritional status of adolescents in rural and urban Nigeria. Afr Health Sci. 2020;20(4):1795–803.
18. Alade RA, Popoola SG. BMI and MUAC as correlates among teenagers in Nigeria. Niger Med J. 2022;63(2):85–9.
19. Ezeh OK, Uche C, Okafor DC. Malnutrition and overweight in Nigerian secondary school adolescents. Int Health. 2021;13(4):321–7.
20. Bekele T, Chaka M. Anthropometric assessment and MUAC cutoff in adolescents. Ethiop J Health Sci. 2023;33(1):45–54.
21. Pedro TM, Maciel PR, da Silva M, Navarro AC. Waist-to-height ratio as a screening index in teens. J Pediatr (Rio J). 2021;97(5):546–53.
22. Yusuf D, Musa S, Alabi O. Nutritional indicators and socioeconomic factors in Nigerian adolescents. J Public Health Afr. 2022;13:2154.
23. Kamugisha J, Sanga G, Mwalimu C. Prevalence of stunting and overweight in East African adolescents. BMC Nutr. 2020;6:28.
24. Atinmo T, Anyaoku V. Growth patterns and body composition of Nigerian youths. Niger J Paediatr. 2021;48(3):180–6.
25. Ebubechukwu IO, Iloh ON. MUAC and BMI agreement in adolescent screening. Pan Afr Med J. 2023;45:101.
26. Chioma GN, Odili VO. Anthropometric predictors of adiposity in adolescents. J Obes Metab Res. 2020;7(2):98–104.
27. Ijarotimi OS, Olayiwola IO. Nutritional status and dietary intake among Nigerian adolescents. J Public Health Epidemiol. 2021;13(7):303–11.

28. Fadupin GT, Akerele A. Prevalence of malnutrition using BMI-for-age and MUAC. Afr J Food Agric Nutr Dev. 2022;22(1):19874–88.
29. Nwabueze SA, Adedokun O, Iheme GO. Anthropometric correlates of health risk in male and female adolescents. J Biosoc Sci. 2020;52(6):882–90.
30. Ibrahim MM, Tijani BA. Evaluation of body proportions among Nigerian teens. Niger Postgrad Med J. 2022;29(1):42–7.
31. Osayomi T, Ojo A. Nutritional transitions and body size among West African adolescents. Health Place. 2021;68:102516.
32. Farah AE, Bacha T, Abdissa SG. Underweight and overnutrition coexistence in low-income adolescents. Nutrients. 2020;12(10):2952.
33. Adekoya AO, Adeniyi OF. Mid-arm and skinfold measures in Nigerian teenagers. West Afr J Nutr Health. 2023;7(1):55–62.
34. Okafor NC, Onuoha DC. Age and sex differences in Nigerian adolescent anthropometry. Int J Adolesc Youth. 2021;26(2):183–91.
35. Mbah CN, Etonihu AC. Obesity and waist indices among urban adolescents. Afr J Prim Health Care Fam Med. 2022;14(1):e1–7.
36. Chen K, Huang S, Li Y. Global patterns of adolescent BMI and waist ratios. J Adolesc Health. 2020;66(6):675–83.
37. Umeogu U, Ogbonne O. Body measurements and nutritional disorders in secondary school youths. Niger J Med. 2023;32(1):9–15.

Exploring the Role of Tp53-R175H & R273H Gain-of-Function Mutants in Metabolic Reprogramming in Prostate Cancer

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Abstract

The TP53 inactivation by missense mutation occurs frequently in advanced prostate cancer, which makes it an attractive therapeutic target with poor clinical outcomes. This study aimed to explore the metabolic pathways altered by TP53 mutations in prostate cancer cells. Prostate cancer cells, PC3 (TP53-null), were successfully transduced with TP53 wild-type (WT) and TP53 mutants (R175H and R273H), and a western blot was performed with the indicated proteins in the PC-3 cell line blotted with anti-V5, anti-TP53, and anti-β-actin as a loading control. The transduced cells were treated with doxorubicin (1μM) and vehicle (0μM) for 24 hours. The metabolomics analysis was carried out using liquid chromatography mass spectrometry (LC-MS). The result of Western blot analysis showed the respective transduction of the PC-3 cell line, indicating the respective protein of the transducers. The treatment with doxorubicin revealed the expression of TP53 in the transduced cell lines, with both mutants (R175H and R273H) showing high expression levels. The metabolomics analysis showed approximately 300 metabolites significantly altered in PC3-GFP (KO), PC3-TP53WT (WT), and PC3-TP53 mutants (R175H & R273H). The metabolites mostly upregulated in WT & KO are downregulated in the mutants (R175H & R273H) and vice versa. Thus, it affects practically all the major metabolic pathways. The most prominent pathway altered is amino acid metabolism, with several metabolites either upregulated or downregulated, indicating its role in cancer progression driven by the mutation. In conclusion, this study highlights the metabolic reprogramming driven by TP53 mutations (R175H and R273H) in prostate cancer through alterations of mostly amino acid metabolism. This finding could provide an appetising avenue for exploring new therapeutic strategies.

Keywords:

Prostate cancer, PC-3, TP53WT, TP53R175H, TP53R273H, Metabolomics, Metabolic alterations.

Introduction

Prostate cancer is the leading cause of cancer death in men after lung cancer globally (Malvezzi *et al*., 2023; Siegel *et al*., 2024). The prevalence of prostate cancer in Africa is low compared to other continents due to insufficient data. However, Nigeria accounted for about 17.5% of the incidence and over 20% of African mortality (GLOBOCAN, 2022). The American Cancer Society (ACS) reported an increased rate of advanced prostate cancer among men, and it affects black men (73%) more than white men, with a higher chance of dying. A study in Nigeria showed 8.7% of prostate cancer among young men under 55 years old, and it was found to be higher in the north than the southern part of the country (Ntekim *et al*., 2023). The author proposed a lack of awareness, illiteracy, resource limitations, poor health systems for screening and early detection, inadequate access to treatment, and lack of empowerment as possible sources of the observed discrepancies between the two regions.

Prostate cancer shows essential features in metabolic adaptation to sustain its replicative and survival potentials. These metabolic adaptations include enhancement of glycolysis, *de novo* synthesis of lipids, and up-regulation of glutamine decomposition (Corbet & Feron, 2017; Ma *et al*., 2018). One key characteristic of cancer cells is their survival and adaptation strategies, which are exhibited by a protein called tumour suppressor protein 53 (p53). P53 is a protein encoded by the TP53 gene associated with cell cycle regulation, DNA repair, apoptosis, and senescence. Therefore, the mutation in this gene will not only lose its tumor-suppressing function but also acquire new functions that contribute to the progression of malignant tumors, called gain-of-function (GOF)

Date submitted-07/08/2025 Date Accepted-23/11/2025

(Fang *et al*., 2018). The TP53 gain-of-function mutation promotes tumour growth and survival by altering metabolic pathways, increasing metastatic potential, and enhancing drug resistance (Alvarado-Ortiz *et al*., 2021; Mantovani *et al*., 2019). It was discovered that approximately 80% of TP53 mutations are missense mutations (Chiang *et al*., 2021). Growing evidence showed that missense mutations in TP53 result in the inactivation of its antitumor transcriptional response, and the mutant TP53 exerts oncogenic functions by affecting molecular regulation, altering metabolism, and interfering with many other pathways (Mantovani *et al*., 2017; Muller & Vousden, 2014; Pfister & Prives, 2017; Schulz-Heddergott & Moll, 2018; Shetzer, 2016; Singh *et al*., 2017). Novel strategies targeting TP53 hold promise, as tumours harbouring TP53 mutations exhibit rapid progression, poor prognosis, and resistance to therapy. However, to date, there is little to no information on effective treatments that specifically target TP53 alterations. Therefore, this research is set to explore the metabolic alterations driven by TP53 GOF mutations.

Materials & Methods

Cell lines and culture

Human prostate cancer cell lines, LNCaP and PC-3 cells, were maintained in RPMI 1640 media. HEK-293T cells for lentivirus production were cultured in high-glucose Dulbecco's modified Eagle's medium (DMEM). All the mediums were supplemented with 10% foetal bovine serum (FBS, Life Technologies no. 10437-028) and 1% penicillin/streptomycin antibiotic solution (Life Technologies no. 15140-122). All cell lines were maintained & incubated at 37 °C, 10 % CO_2 incubator. For subculturing, the medium was replaced every 72 hours, and the cells were passaged routinely when 80–90% confluence was reached.

Plasmids and the gene knockdown experiment

Lentiviral constructs targeting TP53 knockout (KO) were created by incorporating the cDNA into the pFUGW-H1 vector. The pLenti6.3/V5-DEST-GFP plasmid (Addgene_40125) was generously provided by Lynda Chin 91, while the pLenti6/V5-p53_R273H (Addgene_22934) and pLenti6/V5-p53_R175H (Addgene_22936) plasmids were gifts from Bernard Futscher. Lentiviruses were generated through the transfection of HEK293T cells with a Δ8.9 packaging vector and VSVG envelope vector (2:1:1 ratio) in Opti-MEM media (Gibco, Thermo Fisher Scientific, Waltham, MA, USA). PC3 cells were transduced with the virus in the presence of 8 μg/ml polybrene (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA). Subsequently, the selection of stably expressing cells was achieved by adding puromycin (1 μg/ml, Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA), blasticidin (5 μg/ml, Thermo Fisher Scientific, Waltham, MA, USA), or zeocin (100 μg/ml, Thermo Fisher Scientific, Waltham, MA, USA).

Western Blot

Western blot analysis was conducted according to (Junk, 2008). Briefly, whole-cell extraction was performed using RIPA buffer (Thermo Fisher Scientific, Waltham, MA, USA) (50 mM Tris, 150 mM NaCl, 1% Triton X-100, 0.1% SDS, and 1% NaDeoxycholate [pH 7.4]), supplemented with protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 10 μg/ml pepstatin A, 10 μg/ml aprotinin, and 5 μg/ml leupeptin). Protein concentrations were quantified using Bio-Rad protein assay kits (Bio-Rad, Hercules, CA). Subsequently, protein lysates were separated by SDS-PAGE, transferred onto nitrocellulose membranes, blocked with PBS containing 0.2% Tween 20 and 5% BSA, and probed with primary antibodies. The primary antibodies employed were p53 (FL-393, sc-6243, Santa Cruz; 1:1000) and anti-β-actin (Sigma, 1:5000). The developed blot was visualised using the ECL Prime Western Blot Detection Kit by the manufacturer's protocol (Amersham, United Kingdom).

Extraction of Hydrophilic Metabolome from Cultured Cells

V5-DEST-GFP, V5-p53-wt-p53, V5-p53-R175H, and V5-p53-R273H cell lines were cultured at a density of 2×10^6 cells in triplicate and allowed to adhere overnight in a humidified 5% CO_2 incubator at 37 °C for 24 hours. Cells were washed twice with cold PBS, and pellets were resuspended in 80% methanol and then lysed by 5 freeze–thaw cycles (LN2 freezing followed by thawing at room temperature). Samples were then collected and centrifuged at 20,000 x g for 30 min at 4 °C, and the supernatant was dried using SpeedVac. 50% acetonitrile was added to the tube for reconstitution, followed by vortexing for 30 sec. The sample solution was then centrifuged for 15 min at 20,000 x g, 4 °C. Supernatant was collected for liquid chromatography mass spectrometry (LC-MS)

analysis. Data acquisition and analysis were carried out by Xcalibur 4.1 software and Trace Finder 4.1 software, respectively (both from Thermo Fisher Scientific).

Data Presentation and Analysis

The western blot data were presented as bands showing the molecular weights of the proteins from the transduced cells. Comparative metabolic changes between the KO and mutant cell line are presented as negative log to base ten probability versus log to base two of Fold Change ($-Log_{10}(P)$ vs $Log_2(FC)$). The association between variation of mutation and metabolic alterations in the samples was assessed using Chi-Square analyses, and statistical limits of significance were set at $p < 0.05$.

Results

Generation of stable PC-3 cell lines (TP53 null) by transduction with control (V5-DEST-GFP (KO)), TP53 wild-type (TP53WT), TP53-R175H, and TP53-R273H.

The PC-3-TP53 proteins were successfully generated and confirmed using a Western blot. The western blot performed with the proteins in PC-3 cells showed respective transduction of the PC-3 cell lines, indicating the presence of the transducers, as shown in Figure 1.

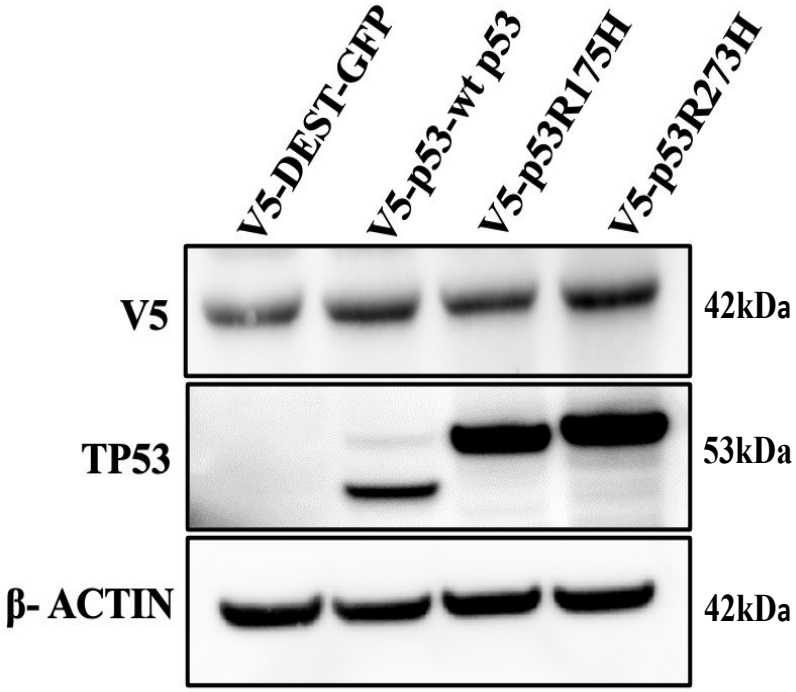

Figure 1: *Transduced PC-3 cell lines indicating respective proteins of the transducers and their molecular weights* (Usman *et al*., 2025). V5-DEST-GFP (KO) = Control, V5-TP53-WT = TP53 Wild-type, V5-TP53-R175H = TP53 Mutant 1 and V5-TP53-R273H = TP53 Mutant 2.

Effect of Doxorubicin on TP53 WT, and TP53 mutants (R175H & R273H)

The treatment of the transduced PC-3 cells, TP53 WT, and TP53 mutants and the control (LNCaP cell line) with doxorubicin (1μM) and vehicle (0μM) for 24 hours induced the expression of TP53 protein. The expression of TP53 in both LNCaP and PC3-TP53 WT was only observed at higher concentrations of doxorubicin. However, the expression in the mutant correlates with the doxorubicin concentration, although the gene was more highly expressed in the PC3-TP53-R273H mutant. Contrarily, P21 was only expressed in the control cell line with faint bands in TP53 WT (Figure 2).

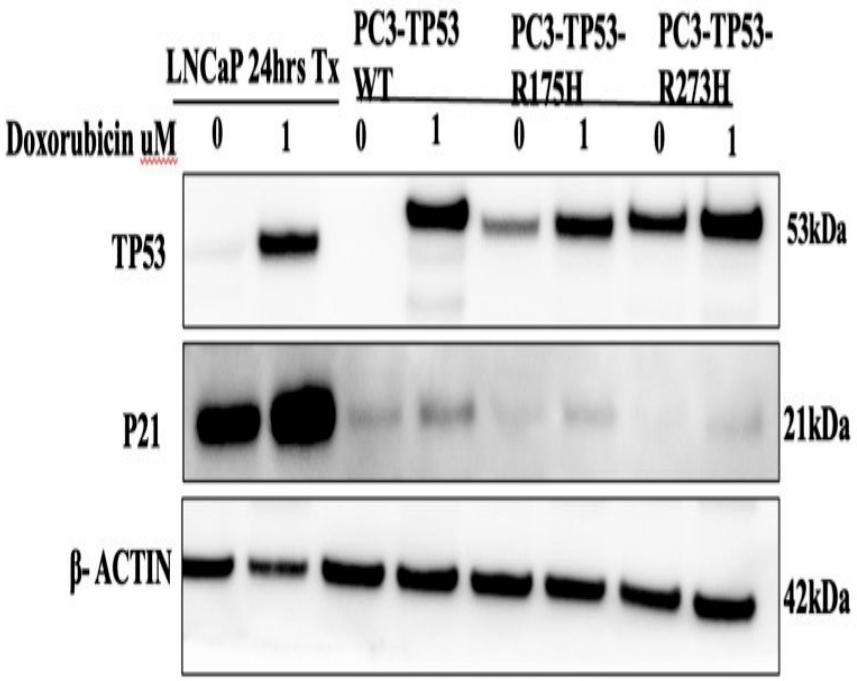

Figure 2: *Effect of Doxorubicin Treatment on the Expression of TP53 Protein in both WT & Mutant PC-3 Cell lines.*

Keys: Control = LNCaP cell line, PC3-TP53-WT =PC3-TP53-Wild-type and PC3-TP53 mutants (R175H & R273H), 24hrs Tx = 24 hours Treatment, anti-TP53, anti- P21, anti-B-actin. Doxorubicin = 1μM and vehicle = 0μM.

Metabolic Pathways Altered By PC3-TP53-R175H Mutant

The volcano plot (Figure 3) showed the major metabolic pathways altered by the mutant (TP53R175H). Amino acid (red triangle) and nucleic acid metabolism (purple diamond) appear to be the most affected metabolic pathways, where related metabolites are either up-regulated or down-regulated significantly. While Lipid (Yellow Circles) and Carbohydrate (Green Squares) metabolisms showed fewer changes, which indicated a small/no significant effect by the mutant (TP53R175H).

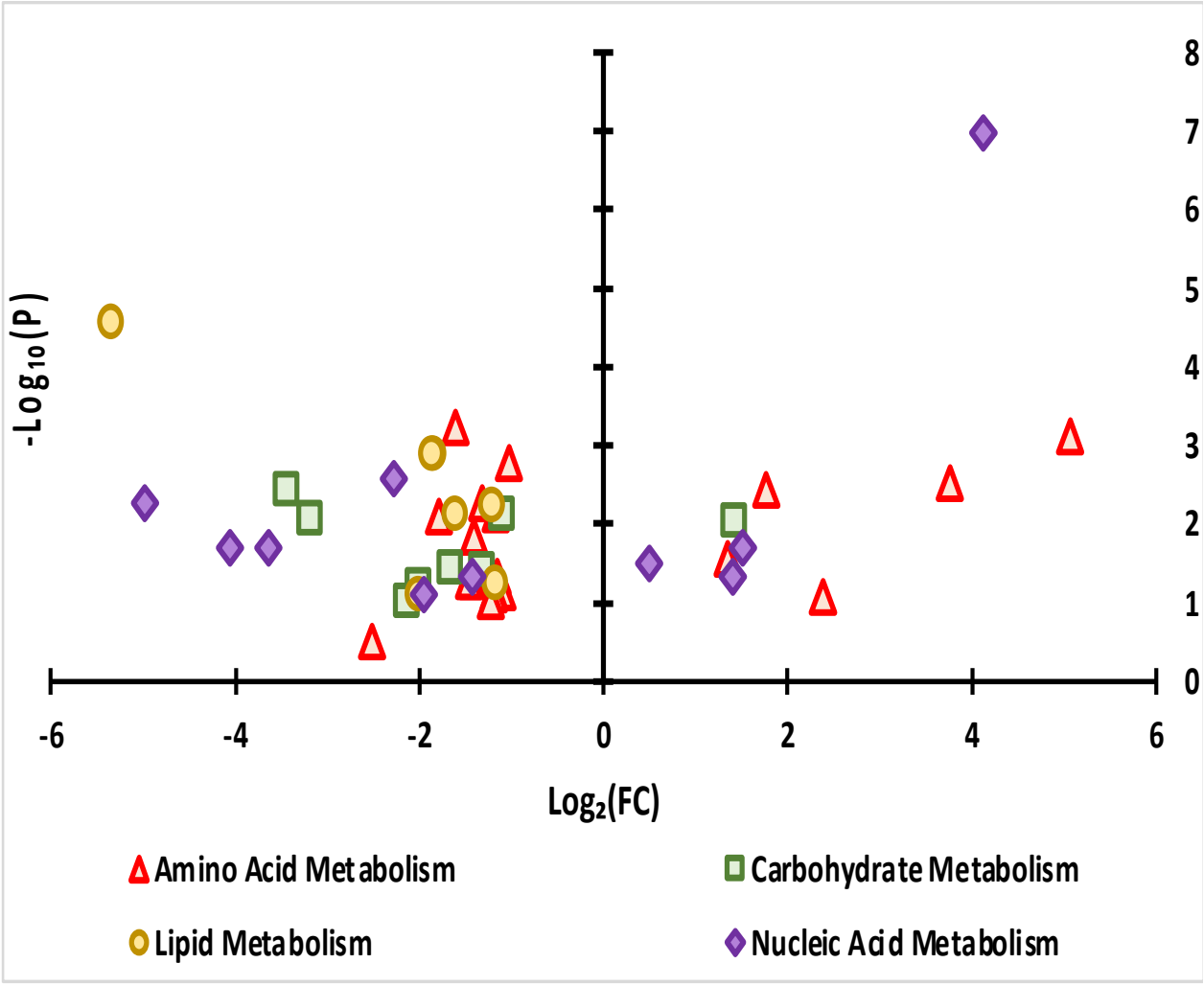

Figure 3: *Comparison of the major metabolic pathways altered by the TP53-R175H Mutant.*
Y-axis = $-Log_{10}$ (P) X-axis = Log_2 (FC), Where P = Statistical Significance (P-value); FC = Fold Change

Metabolic Pathways Altered By PC3-TP53-R273H Mutant

From the volcano plot (Figure 4), amino acid metabolism appeared to be the most affected metabolic pathway by the TP53-R273H mutant. Nucleic acid metabolism is also affected, but not as broadly as amino acid metabolism, with one point located higher on the y-axis. The points representing carbohydrate metabolism (green square) are mostly clustered near the centre, indicating that the changes in carbohydrate metabolites are less statistically significant with lower fold changes. While lipid metabolism points (yellow) appeared near the middle, with negative fold changes indicating a decrease in metabolic activity by the mutant (TP53R273H).

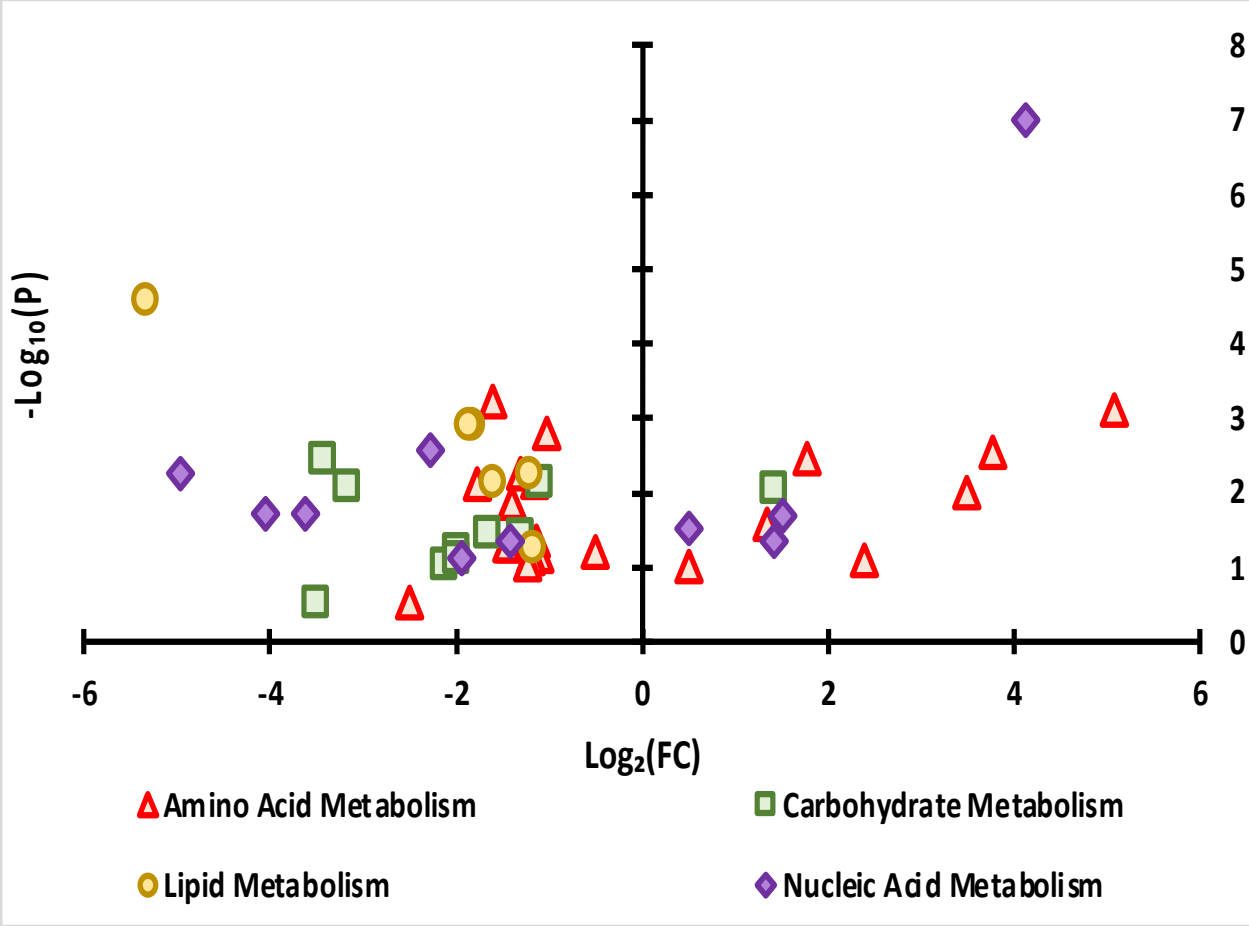

Figure 4: *Comparison of metabolic pathways altered by PC3-TP53-R273H Mutant.*
Y-axis = $-Log_{10}(P)$ X-axis = $Log_2(FC)$, Where P = Statistical Significance (P-value);
FC = Fold Change

Metabolic pathways altered by both mutants (R175H & R273H)

A volcano plot was used to compare the major metabolic pathways altered by the mutants (R175H & R273H). Amino acid metabolism (red square) is the most altered metabolic pathway by the two different mutants (R175H & R273H), with points spread across the graph. Some showed upregulation (positive fold change), while others showed downregulation (negative fold change). Carbohydrate metabolism points are clustered near the origin with some negative fold change, indicating downregulation. Lipid metabolism is represented with a few points, one of which is significantly high on the $-Log_{10}(P)$-axis but close to zero in fold change. Therefore, the mutants altered various metabolic pathways, with amino acid metabolism as the most affected, followed by nucleic acid metabolism (Figure 5).

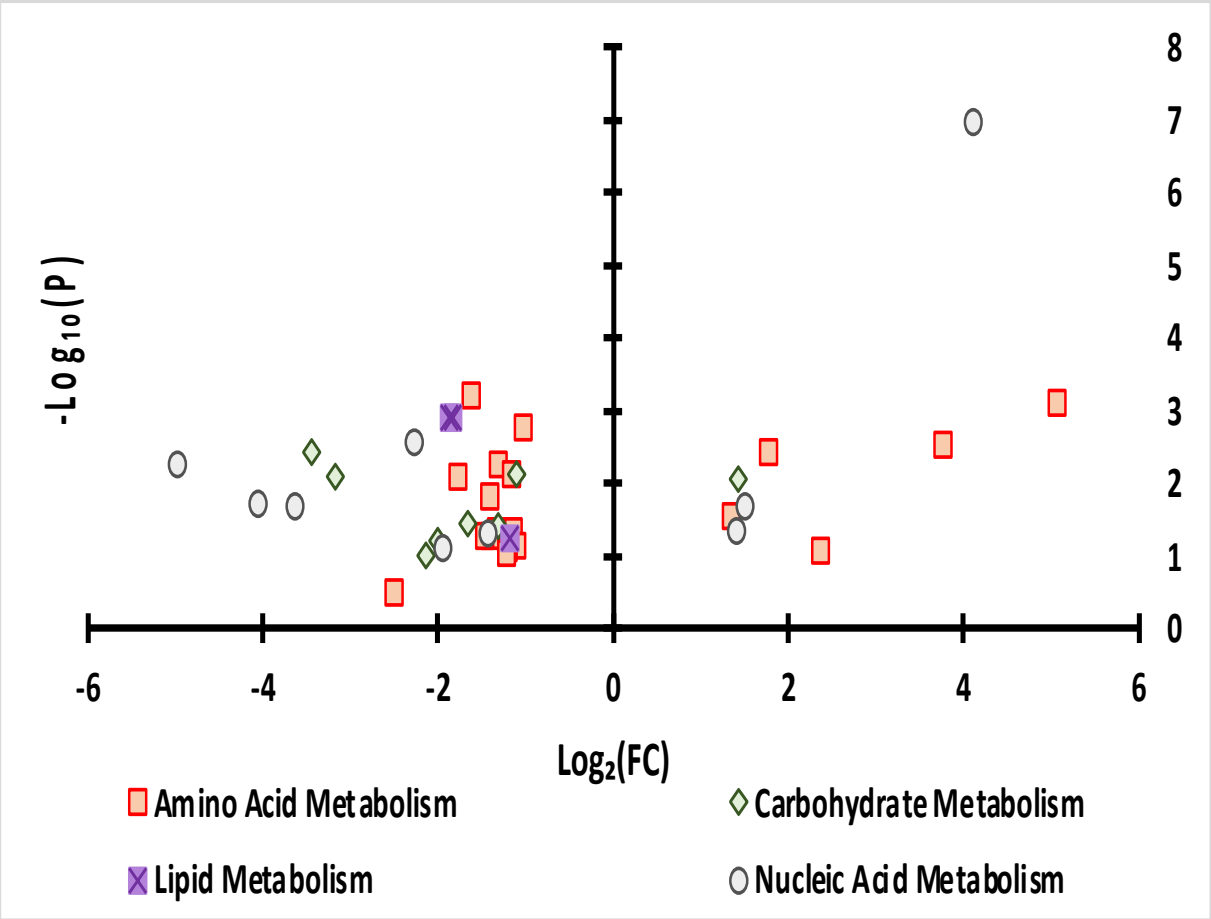

Figure 5: *Comparison of the major metabolic pathways altered by the mutants (R175H & R273H) (Usman et al., 2025)*

Relationship between TP53 Mutations & Metabolic Alterations in Prostate Cancer Cells

The association between TP53 Mutations & Metabolic Alterations in Prostate Cancer Cells is presented in Table 1. The result showed a significant association between metabolites of the KO and mutant cell lines (χ^2 (2) = 13.10, p = 0.001). Specifically, there was a significant inverse association in the proportions of metabolite regulation in the PG3-GFP (KO) when compared with the two mutants (PC3-P53R175H and PC3-P53R273H).

Table 1: Association between Variation of Mutation and Metabolic Alterations in Prostate Cancer

Metabolic Alterations	Mutation			Total
	PC3-GFP	PC3-P53R175H	PC3-P53R273H	
Downregulation	05 (08.6%) [a]	14 (24.1%)	13 (22.4%)	32 (55.2%)
Upregulation	16 (27.6%) [b]	05 (08.6%)	05 (08.6%)	26 (44.8%)
Total	21 (36.2%)	19 (32.8%)	18 (31.0%)	58 (100%)

Superscript denotes raw proportions that differ significantly from those expected by chance at $p < 0.05$. Standardized Residual (z): b = + 2.1, a = - 1.9

1. Chi-Square

$\chi^2 (2) = 13.10$, $p = 0.001$

2. Chi-square Reliability Measure

Expected count less than 5 = 00.0% (very reliable)

Minimum expected count = 08.07

3. Measure of Association

$\lambda = 0.317$, $p = 0.02$, Symmetric = 10.05%

Metabolite Alterations dependencies on Mutation variation = 17.89%, ($\lambda = 0.423$, $p = 0.011$)

Cramer's V = 0.475, p = 0.001, Vey Strong Relationship

Discussion

Although various TP53 hotspot missense mutations have been implicated in prostate cancer progression and poor prognosis, the association between different TP53 mutants and metabolic alterations to promote tumour progression remains unclear. This study explored the metabolites altered by the TP53 Gain-of-function mutants using metabolomics. The generation of stable PC-3 cell lines transduced with vector control (V5-DEST-GFP), V5-TP53-wildtype, V5-TP53-R175H, and V5-TP53-R273H was confirmed from the result of western blot (Figure 1), corresponding to the findings (Yoo *et al*., 2023). As doxorubicin is a strong inducer of TP53 protein accumulation, this study revealed that the TP53 protein levels increased with high concentration (1μM) of doxorubicin, while p21 expression is related to the expression of TP53 protein. This corresponds with the findings of Martinez *et al*., which revealed that TP53 protein levels increased with increasing concentrations (0.2, 0.5, and 1.0μg/ml) of Doxorubicin, and p21 was undetectable at the highest dose (1.0μg/ml) (Martinez *et al*., 2002). The major metabolic pathways altered by both mutants (PC3-TP53-R175H & R273H) are commonly associated with cancer progression, including prostate cancer. Both TP53 mutants showed prominent changes in amino acid metabolism (Figure 5). Cancer cells, including prostate cancer, often have increased demands for amino acids for rapid cell growth and proliferation. This was described in a review carried out by Chen and colleagues (Chen *et al*., 2024). Table 1 showed a higher number of downregulations in the mutants (PC3-P53R175H & PC3-P53R273H), suggesting that these cells actively decreased non-essential metabolic processes to allow cancer cells to focus resources on pathways more critical for survival and growth. Therefore, this analysis (Table 1) showed that TP53 mutations are strongly associated with metabolic alterations. The study also highlighted the potential of targeting metabolic vulnerabilities in TP53 mutant prostate cancers. Further research should be carried out to show how each TP53 GOF mutant contributes to the cells' ability to adapt to metabolic alterations to support their growth and survival. For instance, isotope tracing could be done to track the changes in metabolites in the presence/absence of each TP53 GOF mutation.

Conclusion

The generation of stable cell lines expressing TP53 wild-type and TP53 mutants (R175H & R273H) coupled with treatment (doxorubicin) and metabolomics analysis provided insights into how TP53 GOF mutations may alter cellular responses and influence cancer metabolism. The impact of TP53 mutations (R175H and R273H) on major metabolic pathways in prostate cancer cells was investigated. Therefore, this study highlights the metabolic reprogramming driven by TP53 mutations (R175H and R273H) in prostate cancer, especially through alterations in amino acid, nucleic acid, and carbohydrate metabolism, as well as lipid metabolism, which could be exploited for new therapeutic strategies**.**

Abbreviations

TP53: Tumour suppressor protein 53
PC: Prostate Cancer
CRPC: Castration Resistance Prostate Cancer
KO: Knockout
GFP: Green fluorescent protein
GAP: Glyceraldehyde-3-phosphate
DHAP: Dihydroxy acetone phosphate
WT: Wild-type
ADT: Androgen Deprivation Therapy
HEK293T: Human embryonic kidney 293T
GOF: Gain-of-function
α-KG: Alpha-ketoglutarate

Ethics declarations

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests
The authors declare no competing interests.

Funding
Not Applicable.

Contributions
All authors contributed to the review design and preparations. The first draft of the manuscript was written by Farida Jazuli Usman, and all authors commented on and approved the final version of the manuscript.

Acknowledgements
This work was supported by Abdulkadir's lab, Department of Urology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, U.S.A. Special thanks to all the Abdulkadir lab members for their maximum support. Thanks to all the authors and reviewers.

References

Alvarado-Ortiz, E., De La Cruz-López, K. G., Becerril-Rico, J., Sarabia-Sánchez, M. A., Ortiz-Sánchez, E., & García-Carrancá, A. (2021). Mutant p53 Gain-of-Function: Role in Cancer Development, Progression, and Therapeutic Approaches. *Frontiers in Cell and Developmental Biology*, *8*, 607670. https://doi.org/10.3389/fcell.2020.607670

Chen, J., Cui, L., Lu, S., & Xu, S. (2024). Amino acid metabolism in tumour biology and therapy. *Cell Death & Disease*, *15*(1), 42. https://doi.org/10.1038/s41419-024-06435-w

Chiang, Y.-T., Chien, Y.-C., Lin, Y.-H., Wu, H.-H., Lee, D.-F., & Yu, Y.-L. (2021). The Function of the Mutant p53-R175H in Cancer. *Cancers*, *13*(16), 4088. https://doi.org/10.3390/cancers13164088

Corbet, C., &Feron, O. (2017). Emerging roles of lipid metabolism in cancer progression. *Current Opinion in Clinical Nutrition & Metabolic Care*, *20*(4), 254–260. https://doi.org/10.1097/MCO.0000000000000381

Fang, L., Du, W. W., Lyu, J., Dong, J., Zhang, C., Yang, W., He, A., Kwok, Y. S. S., Ma, J., Wu, N., Li, F., Awan, F. M., He, C., Yang, B. L., Peng, C., MacKay, H. J., Yee, A. J., & Yang, B. B. (2018). Enhanced breast cancer progression by mutant p53 is inhibited by the circular RNA circ-Ccnb1. *Cell Death & Differentiation*, *25*(12), 2195–2208. https://doi.org/10.1038/s41418-018-0115-6

Junk, D. (2008). *10*(5), 450-61.

Ma, Y., Temkin, S. M., Hawkridge, A. M., Guo, C., Wang, W., Wang, X.-Y., & Fang, X. (2018). Fatty acid oxidation: An emerging facet of metabolic transformation in cancer. *Cancer Letters*, *435*, 92–100. https://doi.org/10.1016/j.canlet.2018.08.006

Malvezzi, M., Santucci, C., Boffetta, P., Collatuzzo, G., Levi, F., La Vecchia, C., & Negri, E. (2023). European cancer mortality predictions for the year 2023 with focus on lung cancer. *Annals of Oncology*, *34*(4), 410–419. https://doi.org/10.1016/j.annonc.2023.01.010

Mantovani, F., Collavin, L., & Del Sal, G. (2019). Mutant p53 as a guardian of the cancer cell. *Cell Death & Differentiation*, *26*(2), 199–212. https://doi.org/10.1038/s41418-018-0246-9

Mantovani, F., Walerych, D., & Sal, G. D. (2017). Targeting mutant p53 in cancer: A long road to precision therapy. *The FEBS Journal*, *284*(6), 837–850. https://doi.org/10.1111/febs.13948

Martinez, L. A., Yang, J., Vazquez, E. S., Del Carmen Rodriguez-Vargas, M., Olive, M., Hsieh, J.-T., Logothetis, C. J., &Navone, N. M. (2002). P21 modulates threshold of apoptosis induced by DNA damage and growth factor withdrawal in prostate cancer cells. *Carcinogenesis*, *23*(8), 1289–1296. https://doi.org/10.1093/carcin/23.8.1289

Muller, P. A. J., &Vousden, K. H. (2014). Mutant p53 in Cancer: New Functions and Therapeutic Opportunities. *Cancer Cell*, *25*(3), 304–317. https://doi.org/10.1016/j.ccr.2014.01.021

Ntekim, A., Folasire, A., &Odukoya, O. A. (2023). The Prevalence of Prostate Cancer Among Young Men Below 55 Years of Age in Nigeria. *Cancer Control*, *30*, 10732748231175255. https://doi.org/10.1177/10732748231175255

Pfister, N. T., &Prives, C. (2017). Transcriptional Regulation by Wild-Type and Cancer-Related Mutant Forms of p53. *Cold Spring Harbour Perspectives in Medicine*, *7*(2), a026054.

https://doi.org/10.1101/cshperspect.a026054

Schulz-Heddergott, R., & Moll, U. (2018). Gain-of-Function (GOF) Mutant p53 as Actionable Therapeutic Target. *Cancers*, *10*(6), 188. https://doi.org/10.3390/cancers10060188

Shetzer, Y. (2016). *Oncogenic mutant p53 gain-of-function nourishes the vicious cycle of tumour development and cancer stem-cell formation. 6*(10), a026203.

Siegel, R. L., Giaquinto, A. N., & Jemal, A. (2024). Cancer statistics, 2024. *CA: A Cancer Journal for Clinicians*, *74*(1), 12–49. https://doi.org/10.3322/caac.21820

Singh, S., Vaughan, C. A., Frum, R. A., Grossman, S. R., Deb, S., &Palit Deb, S. (2017). Mutant p53 establishes targetable tumor dependency by promoting unscheduled replication. *Journal of Clinical Investigation*, *127*(5), 1839–1855. https://doi.org/10.1172/JCI87724

Usman, F. J., Gupta, D. G., Abdulkadir, S. A., Usman, I. M., Alassan A. J., Yoo, Y. A., Kurfi, B. G., and Babagana, K. (2025). Impact of TP53 Gain-of-Function Mutation on Metabolic Reprogramming in Prostate Cancer Progression. *Asian Journal of Biochem Genet Mol Biol*. 17(1):17-31. http://doi.org/10.9734/ajbgmb/2025/v17i1434

Yoo, Y. A., Truica, M., Zachary, R., Chalmers, Mindy, K. G., Qian-Yu, G., William, Y., Songhua, Q., Kenji, U., Navdeep, S. C., and Sarki, A. A. (2023). Abstract 1712: Targeting asparagine dependency as a therapy for TP53-mutated castration-resistant prostate cancer. *Cancer Research*, *83*(7_Supplement), 1712–1712. https://doi.org/10.1158/1538-7445.AM2023-1712

Effect of Oven, Sun and Air-Drying on the Anti-Obesity and Antioxidant Potentials of *Diospyrus Mespiliformis* Leaf Extracts

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Abstract

Obesity is a chronic metabolic condition causing excessive body fat accumulation. This study evaluated the possible in vitro inhibitory potentials of *Diospyrus mespiliformis* leaf extracts against pancreatic lipase. Fresh leaves of the plant were collected, divided into three parts and air-dried, sun-dried and oven-dried, respectively. Dried leaves were ground into powder and extracts were prepared using 100%, 70%, 60% and 50% ethanol and aqueous solutions. Pancreatic lipase (PL) inhibition and DPPH radical scavenging assay were performed on each extract using standard methods, followed by total polyphenols and total flavonoids contents. Then, liquid chromatographic mass spectroscopy was carried out on the two most bioactive extracts. The results indicated that the air-dried 100% and 70% ethanol extracts with the lowest IC_{50} values, 5.86 ± 2.42 and 6.09 ± 1.49 mg/mL, respectively, had the highest inhibition against PL enzyme activity. These extracts had lower IC_{50} values than 10.80 ± 0.02 mg/mL for orlistat, the standard drug used. Similarly, the air-dried 100% and 70% ethanol extracts also had the lowest IC_{50} values, i.e. 6.29 ± 1.33 and 8.79 ± 0.28 mg/mL, respectively, for the DPPH radical scavenging assay, but the IC_{50} value of 4.66 ± 0.24 mg/mL of the standard ascorbic acid used was lower. The total polyphenol content (TPC) of the sun-dried aqueous extract, 675.06 ± 92.85 mg GAE/g, was the highest, followed by that of the oven-dried 100% ethanol extract, 646.76 ± 9. 47 mg GAE/g, and then that of 70% ethanol extract, 627 .53 ± 4.58 mg GAE/g. The total flavonoid content (TFC) for the air-dried 70% ethanol extract recorded the highest value 464.37 ± 2.02 mg QE/g, followed by those of sun-dried 433.74± 0.34 mg QE/g and air-dried aqueous extracts 231.83 ± 4.98 mg QE/g. The air-dried 70% ethanol leaf extract presented quercetin and hesperidin (flavonoids), and 7-(4-bromobutoxy)-3,4-dihydro-2(1H)-quinolinone and 4-isopropylbenzyl 3,5-dinitrobenzoateas (phenolic acids) as some of the phytochemicals identified in it. 5-((p-hexyloxy)phenyl)-4-hexyloxy-2-methyl(thiobenzoate), and N-(2-methoxyethyl)isopropylamine were identified in the air-dried 100% ethanol extract. These findings indicate that air-dried *D. mespiliformis* leaves could produce potential anti-obesity substances.

Keywords: Pancreatic lipase, Inhibitor, Hesperidin, DPPH radical, Total phenolics content

Introduction

Wild plants have untapped potential to be developed into functional foods and nutraceuticals for the prevention and treatment of quite a great deal of chronic diseases [1]. As a wild plant, *Diospyros mespiliformis*, commonly known as the African ebony tree, is a tall, evergreen, dioecious tree valued for its edible fruit, durable timber, and extensive traditional medicinal uses across Sub-Saharan Africa [2]. Various parts, i.e. the fruits, leaves, bark, and roots, are used to treat a myriad of diseases, including pneumonia, malaria, fever, leprosy, syphilis, and gastrointestinal diseases. Extracts have demonstrated antibacterial, antiviral, antifungal, anti-parasitic, anti-inflammatory, and antioxidant activities [2]. Drying is an important method of processing and preservation of plant materials. It is also a fundamental factor in retaining the concentration and availability of phytochemical constituents of leaves and other parts of a plant [3-4] during the extraction of compounds when used for food or pharmaceutical purposes. Therefore, drying methods with the lowest possible temperatures have been more effective in terms of preserving bioactive compounds of plant materials [3-4, 5].

In our previous work, we reported the in vitro antidiabetic, antioxidant potentials and phytochemical profiles of *D. mespiliformis* fruit pulp [6]. In this work, therefore, we assessed the probable in vitro anti-lipase and antioxidant properties of *D. mespiliformis* leaves as well as the effects of drying methods on important bioactive compounds that may also be present in the leaves. Many synthetic drugs for reducing lipid absorption

Date submitted-21/09/2025 Date Accepted-02/11/2025

in the gastrointestinal tract through inhibition of pancreatic lipase, a key lipid metabolism enzyme, have failed due to side effects, prompting the search for natural substitutes [7]. Many natural compounds from plants against pancreatic lipase activity have been purified [7], but the literature falls short of such compounds from the *D. mespiliformis* plant. Findings of this research could be an additional source of knowledge of the bioactivities and possible compounds of this plant that could be inhibitors against pancreatic lipase. This may further expose the untapped functional foods and nutraceutical potentials of the plant. It may also provide a background knowledge of a suitable drying technique for *D. mespiliformis* leaves when trying to optimise its industrial application.

Materials

Sample Collection and Processing

The plant sample was collected at a farm in a community close to Janguza Barracks, Rimin Gado Local Government Area, Kano State. It was identified by a Botanist in the Department of Plant Biology, Bayero University, Kano, Kano, with a voucher number of BUKHAN 0121. The leaves were immediately separated from the stalk, washed, the water drained and were divided into three. The first portion was dried under the air, the second portion under the sun, and the third portion in the oven at 60 °C. The sample for air-drying took 72 h to dry completely, the sample for oven drying took 12 h at 60 °C, while the sample for sun drying dried after 36 h. The three-dried samples were ground using a blender, and stored in three different airtight containers and stored in the refrigerator until used.

Methods

Extraction

The powdered *D. mespiliformis* leaf samples were extracted using water, 100% (absolute) ethanol, 70%, 60%, and 50% ethanol. The solute to solvent ratio used was 1:80 (v/v), which is a modification of the methods of ultrasound-assisted extractions previously reported [8-9]. The mixture of each of the powdered *D. mespiliformis* leaf samples and solvent was placed in a laboratory orbital shaker for 90 minutes at 120 rpm at room temperature. Extracts were concentrated using a rotary evaporator at 45 °C. Excess solvents in extracts were allowed to evaporate by air-drying in the laboratory. Dried samples were stored in airtight containers.

Pancreatic Lipase (PL) Inhibitory Assay

Inhibitory activities of *D. mespiliformis* leaf extracts against PL were determined using the method reported by [10] with some modifications. The PL and *D. mespiliformis* leaf extracts were all prepared in a 0.01 M Tris-HCl buffer, while the concentration of the PL solution was 25 units/mL. The substrate was prepared using the modified method of [11]. Briefly, 10% v/v olive oil was mixed with 10% w/v arabic gum mixture (0.1 M Tris-HCl buffer, pH 8, 0.5 M NaCl, and 20 mM $CaCl_2$) using a homogeniser. Lipase solution (0.2 mL) was allowed to react with 0.5 mL of leaf extract for 30 minutes at 4 °C. After that, substrate emulsion (2 mL) was added, mixed and incubated for 30 minutes at 37 °C. At the end of incubation, 1 mL of an acetone and ethanol (1:1) mixture was used to stop the reaction and was titrated with 0.02 M NaOH until it reached pH 9.4. An auto titrator was used to perform the titrations (Metrohom, 785 DMP Titrino). The experiment was repeated thrice for each sample extract and standard. The amount of free fatty acid (FFA) liberated was reflected by the amount of base required by the incubation mixture, which is equivalent to PL activity. The control (Orlistat) was treated the same way as the samples. Percent inhibition was calculated based on the following equation: % inhibition = 100% - [(V_{sample} / $V_{control}$) x 100]

Where V_{sample} is the amount of base added to the sample, and $V_{control}$ is the amount of base added to the control.

The IC_{50} of *D. mespiliformis* leaf extracts and orlistat for PL inhibition were calculated from a standard curve of % inhibition against concentrations of *D. mespiliformis*/orlistat (mg/mL), and extrapolation from the equation of the curve, y = mx + c. Where y is IC_{50} = 50, m is the gradient of the curve, x is the concentration, and c is the intercept on the y-axis.

DPPH Radical Scavenging Assay

The radical scavenging activity of *D. mespiliformis* extracts was evaluated using the 2, 2'-diphenyl-1,1'-picrylhydrazyl radical scavenging assay according to [12]. Ascorbic acid (10 mg/mL in methanol) was used as a reference standard. Different volumes (2 – 20 µL) of the leaf extracts and the standard were separately made up to 40 µL with methanol, and 2.96 mL of 0.1 mM DPPH solution was added to each of the extracts and the standard. The reaction mixture was incubated in the dark at 37 °C for 20 min. The absorbance of the

mixture was read at 517 nm in a spectrophotometer. The percent radical scavenging activity of the plant extracts was calculated using the following formula.

$$\% \text{ RSA} = \frac{\text{Abs control} - \text{Abs sample} \times 100}{Abs\ control}$$

Where, RSA is the radical scavenging activity; Abs control is the absorbance of DPPH radical + ethanol; Abs sample is the absorbance of DPPH radical + plant extract.

The IC_{50} of *D. mespiliformis* leaf extracts and ascorbic acid for DPPH radical inhibition were calculated from a standard curve of % inhibition against concentrations of *D. mespiliformis*/ascorbic acid (mg/mL), and extrapolation from the equation of the curve, y = mx + c. Where y is IC_{50} = 50, m is the gradient of the curve, x is the concentration, and c is the intercept on the y-axis.

Total Phenolics Contents (TPC)

The total polyphenol contents of *D. mespiliformis* extracts were estimated according to Folin–Ciocalteu's method [13]. The extract solutions were prepared in concentrations of 1 - 0.0025 mg/mL in methanol. About 200 µL of each sample was added to 1 mL of 1/10 diluted Folin– Ciocalteu's reagent, thoroughly mixed and incubated at 37 ºC for 4 minutes. After incubation, 800 µL of saturated sodium carbonate (75 g/L) was added and well mixed. After 2 hours of incubation at 37 °C, the absorbance was measured at 765 nm in a spectrophotometer. Gallic acid standard was treated the same way as the extracts, and its absorbance was also measured. A standard calibration curve was plotted using gallic acid, and the linear equation y = mx + c, with R^2 = 0.99, was obtained. The mean of three readings was used, and the results were expressed as mean mg of gallic acid equivalence (mg GAE) per g of extract.

Total Flavonoid Contents (TFC)

The aluminium trichloride method was used for total flavonoid content quantitation of *D. mespiliformis* extracts [14]. Extract solutions were prepared by dissolving the extracts in methanol in concentrations of 1 - 0.0025 mg mL^{-1}. Then, 1 mL of extract solution was added to 1 mL of 2% $AlCl_3$ solution in methanol with thorough mixing. After 10 minutes of reaction, the absorbance was read at 430 nm against a blank, which consisted of a mixture of 1 mL of the extract solution and 1 mL of methanol without $AlCl_3$. Quercetin was used as a reference standard and treated the same way to produce a standard curve with the equation y = mx + c and R^2 = 0.99. The experiment was performed in triplicate, and the results were expressed as mean mg quercetin equivalence (mg QE) per g of extract.

Protocol for Liquid Chromatographic Mass Spectroscopic Analysis (LC-MS)

The samples were analysed for phytochemical profiles using liquid chromatography (LC) tandem mass spectrometry (MS) as described by [15].

LC Programming (Gradient)

Time	%A	%B	%C	%D
0	0	0	95	5
1	0	0	95	5
13	0	0	5	95
15	0	0	5	95
17	0	0	95	5
19	0	0	95	5
20	0	0	95	5

C = Water + 0.1% formic acid, D = acetonitrile + 0.1% formic acid, column temperature was 25 ˚C, and sample temperature was 25 ˚C.

W2998 PDA:
Wavelength range = 210 - 400 nm, resolution = 1.2 nm, and sampling = 10 points/sec.

ACQ-QDA (MS)
MS scan positive = Mass range 100 - 1250, MS scan negative = Mass range 100 - 1250, runtime = 20 min, sampling rate = 10 points/sec, Gain = 1, probe = 600 ºC, capillary positive = 0.8kv, and negative = 0.8kv.

Data Analysis
Statistical analysis was performed using the software SPSS version 22. Comparison between groups was carried out using one-way analysis of variance (ANOVA) and post Hoc test. Differences were considered significant at the $P < 0.05$ level of significance. The raw spectra data of the chromatograms from liquid chromatography mass spectroscopy (LC-MS) were input into the Spectral Database for Organic Compounds using their base peak (m/z) to identify the phytochemicals present in the selected extracts.

Results and Discussion

Results

The anti-obesity and antioxidant potentials of *D. mespiliformis* leaf extracts were assessed in vitro, and the results were presented as IC_{50} in Tables 1 and 2, respectively. The IC_{50} values of *D. mespiliformis* leaf extracts for pancreatic lipase inhibition varied across different drying methods. The IC_{50} values for the air-dried samples significantly increased ($P<0.05$) from that of 100% ethanol extract and were not statistically different ($P<0.05$) from that of 70% ethanol extract. To those for aqueous and 60% ethanol extracts, which were also not statistically different ($P<0.05$) from each other. The 50% ethanol extract recorded the highest IC_{50} value for pancreatic lipase inhibition in air-dried samples. In the same context, the IC_{50} values of sun-dried samples increased significantly ($P<0.05$) in the following order 70% < 60% and aqueous extracts < 50% < 100% ethanol extracts, 9.05 ± 2.84 mg/mL for 70% ethanol, which was then those of the other sun-dried values (Table 1). The oven-dried samples recorded IC_{50} values that significantly increased ($P<0.05$) in the order of 100% and 70% ethanol < 50% ethanol < aqueous and 60% ethanol extracts (Table 1). There was no statistical difference ($P>0.05$) between the IC_{50} values of 10% and 70% ethanol extracts, and also between those of aqueous and 60% ethanol extracts. The IC_{50} values shown by both 70% and 100% ethanol extracts of air-dried *D. mespiliformis* leaf extracts are the lowest for pancreatic lipase inhibition compared to the other extracts. They are also significantly lower ($P<0.05$) and approximately two times less than the IC_{50} value of the standard drug orlistat for pancreatic lipase inhibition (Table 1).

Table 1. IC_{50} of *D. Mespiliformis* Leaf Extracts for Pancreatic Lipase Inhibition (mg/mL)

Sample	Air-dried	Sun-dried	Oven-dried	Orlistat
100%	5.86 ± 2.42^{Aa}	14.72 ± 5.20^{Cd}	12.41 ± 1.71^{Ac}	10.80 ± 0.02^{b}
70%	6.09 ± 1.49^{Aa}	9.05 ± 2.84^{Ab}	13.09 ± 2.84^{Ac}	
60%	13.86 ± 4.19^{Ba}	12.59 ± 2.34^{Ba}	29.60 ± 4.82^{Cb}	
50%	19.59 ± 5.77^{Ca}	20.17 ± 4.55^{Ca}	26.06 ± 5.32^{Bc}	
Aqueous	12.10 ± 4.93^{Ba}	13.32 ± 6.54^{Ba}	28.24 ± 6.82^{Cb}	

Values are presented as mean ± standard deviation mg/mL Values with different capital superscript letters in the same column, and small letter superscripts in the same row are significantly different at ($p<0.05$).

Similarly, the IC_{50} values of the studied extracts for the DPPH radical scavenging assay followed the same trend of increase as exhibited against pancreatic lipase enzyme inhibition across the drying methods, except for 50% and 60% ethanol extracts in the oven-dried method, which had no significant difference ($P>0.05$) between them (Table 2). The IC_{50} value for 100% ethanol was 1.3 times greater than that of the standard ascorbic acid used, and the 70% ethanol extract was 1.9 times greater ($P<0.05$).

Table 2. IC_{50} of *D. Mespiliformis* Leaf Extracts for DPPH Radical Scavenging Assay

Sample	Air-dried (mg/mL)	Sun-dried (mg/mL)	Oven-dried (mg/mL)	Ascorbic Acid (mg/mL)
100%	6.29 ± 1.33^{Aa}	30.12 ± 0.13^{Bc}	9.76 ± 1.13^{Ab}	4.66 ± 0.24^{a}
70%	8.79 ± 0.28^{Aa}	18.83 ± 0.12^{Ab}	10.22 ± 0.19^{Ab}	
60%	14.38 ± 0.32^{Ca}	42.99 ± 0.14^{Cc}	21.37 ± 0.12^{Cb}	
50%	16.03 ± 0.32^{Da}	57.57 ± 0.17^{Dc}	21.67 ± 0.11^{Cb}	
Aqueous	15.11 ± 0.32^{CDa}	20.66 ± 0.11^{Ab}	45.19 ± 0.34^{Dc}	

Values are presented as mean ± standard deviation. Values with different capital superscript letters in the same column and small letter superscripts in the same row are significantly different at ($p<0.05$).

To assess the antioxidant contents of the *D. Mespiliformis* leaf extracts, TPC and TFC were quantified and presented in Tables 3 and 4, respectively. The results indicated that the air-dried extracts of TPC increased in the following order 70% ethanol > aqueous > 100% > 60% > 50% ethanol extracts. The TPC of the sun-dried extracts showed the pattern of Aqueous extract > 100%, 70%, 60% > 50% ethanol extract. While that of oven-dried extracts was 100% > 70% > aqueous > 60% > 50% ethanol (Table 3). They were all statistically different at $P<0.05$ from each other within the same drying method and between different drying methods.

Table 3. Total Phenolics Content of *D. Mespiliformis* Leaf Extracts

Sample	Air-dried (mg GAE/g)	Sun-dried (mg GAE/g)	Oven-dried (mg GAE/g)
100%	544.32 ± 21.36^{Cb}	344.83 ± 6.97^{Bc}	646.76 ± 9.47^{Aa}
70%	627.53 ± 4.58^{Aa}	259.32 ± 3.01^{Cc}	460.89 ± 7.75^{Bb}
60%	404.61 ± 8.94^{Da}	242.38 ± 4.97^{Db}	209.88 ± 8.13^{Dc}
50%	358.94 ± 26.49^{Ea}	229.63 ± 1.53^{Eb}	154.48 ± 8.35^{Ec}
Aqueous	583.39 ± 10.01^{Bb}	675.06 ± 92.85^{Aa}	294.30 ± 2.69^{Cc}

Values are presented as mean ± standard deviation. Values with different capital superscript letters in the same column and small letter superscripts in the same row are significantly different at ($p<0.05$).

The TFC for the air-dried, sun-dried, and oven-dried *D. Mespiliformis* leaf extracts followed the same pattern of increase as their TPC, except in the oven-dried samples, where the TFC of the aqueous extract was significantly greater than that of 70% ethanol extract. Statistical differences ($P<0.05$) in the TFC were prominent within the drying methods, except between 70% and 60% ethanol extracts of the sun-dried method. Also prominent in the TFC values were statistical differences between the drying methods, except between those of 60% sun-dried and oven-dried methods.

Table 4: Total Flavonoid Content of *D. Mespiliformis* Leaf Extracts

Sample	Air-dried (mg QE/g)	Sun-dried (mg QE/g)	Oven-dried (mg QE/g)
100%	103.37 ± 0.15^{Cb}	63.06 ± 0.31^{Bc}	169.12 ± 2.18^{Aa}
70%	464.37 ± 2.02^{Aa}	51.62 ± 0.00^{Cc}	99.86 ± 0.55^{Cb}
60%	80.78 ± 0.12^{Da}	50.56 ± 0.14^{Cb}	52.57 ± 0.29^{Db}
50%	60.88 ± 0.13^{Ea}	44.18 ± 0.97^{Db}	40.61 ± 0.19^{Eb}
Aqueous	231.83 ± 4.98^{Bb}	433.74 ± 0.34^{Aa}	113.39 ± 0.58^{Bb}

Values are presented as mean ± standard deviation. Values with different capital superscript letters in the same column and small letter superscripts in the same row are significantly different at ($p<0.05$).

Overall, the drying methods significantly affected the IC_{50}, TPC and TFC values, with air-dried extracts generally showing better potency in both pancreatic lipase inhibition and DPPH radical scavenging assays compared to sun-dried and oven-dried *D. Mespiliformis* leaf extracts.

Tables 5 and 6 present some important metabolites identified in the 100% and 70% ethanol extracts, which were the extracts with the most potent bioactivities against pancreatic lipase and DPPH radical scavenging activities, as well as the highest TPC and TFC among the studied extracts.

Table 5: Phytochemical Profile of Air-Dried 100% Ethanol Extract of *D. Mespiliformis* Leaf Extracts

Retention Time (min)	Proposed Compound	Base Peak (M/Z)	Molecular Formula	Max. Intensity	Mode / ESI (±)
1.647	Phenylnapthalene	204.310	$C_{16}H_{12}$	2623.390579	+ve
1.917	2-flouroformanilide	139.105	C_7H_8FNO	18775.315625	+ve
2.102	Pentaflourobenzaldehyde	488.549	$C_7H_3F_5O$	1216.493072	-ve
2,962	Tiropramide hydrochloride	139.105	$C_{24}H_{36}ClN_3O_3$	3135.542969	+ve
3.030	Glyoxylic acid	467.743	$C_2H_2O_3$	2288.365625	+ve
5.948	5-((p-hexyloxy)phenyl)-4-hexyloxy-2-methyl(thiobenzoate)	73.967	$C_{23}H_{30}O_3S$	1551.177344	+ve
6.3023	N-(2-methoxyethyl)isopropylamine	428.746	$C_6H_{15}NO$	1673.965625	+ve
7.801	2-(4-(dimethylamino)stryl)-1-methyl-5,6-dihydro-4H-benzo-(6,7)-cyclohepta-(1,2-d)thiazolium iodide	117.236	$C_{15}H_{21}IN_2S$	26284.165265	+ve
7.801	1,1,1-tetrachloropropane	194.108	$C_3H_6Cl_4$	603.977344	-ve

Table 6: Phytochemical Profile of Air-Dried 70% Ethanol Extract of *D. Mespiliformis* Leaf Extracts

Retention Time (min)	Proposed Compound	Base Peak (M/Z)	Molecular Formula	Max. Intensity	Mode / ESI (±)
1.519	S-(p-(hexyloxy)phenyl) p-(hexanoyloxy) thiobenzoate	**429.346**	$C_{25}H_{32}O_4S$	1804.926563	**+ ve**
1.617	o-aminophenol acetate	**110.113**	C_6H_7NO	3012.477344	**+ ve**
2.200	7-(4-bromobutoxy)-3,4-dihydro-2(1H)-quinolinone	**297.106**	$C_{13}H_{16}BrNO_2$	11444.33554	**- ve**
3.658	4-isopropylbenzyl 3,5-dinitrobenzoate	**343.269**	$C_{17}H_{16}N_2O_6$	341.375391	**- ve**
5.377	N-chlorosuccinimide	**132.528**	$C_4H_4ClNO_2$	200.575391	**- ve**
5.533	1,3-diphenyl-2-pyrazoline	222.420	$C_{15}H1_4N_2$	17060.28449	**+ ve**
6.263	1,8-bis(phenylselenomethyl)naphthalene	**467.239**	$C_{24}H_{20}Se_2$	8486.906250	**+ ve**
6.607	2-(N-(2-benzamidobutyl)benzamido)butyl benzoate	**473.211**	$C_{29}H_{32}N_2O_4$	1779.326562	**+ ve**
7.474	Hesperidin	**609.173**	$C_{28}H_{34}O_{15}$	1519.101953	**- ve**
7.796	p-iodobenzenesulfonyl chloride	**301.360**	$C_6H_4ClIO_2S$	657.150781	**- ve**
7.836	4-(p-(hexyloxy)benzyloxy)-2-methylbenzoate	488.602	C21H26O4	26750.21136	**+ ve**
8.173	Quercetin	**301.273**	$C_{15}H_{10}O_7$	5231.607031	**- ve**
10.501	2-nitronaphthalene-bis(hexachlorocyclopentadiene)	**719.655**	$C_{20}H_7Cl_{12}NO_2$	9066.875781	**+ ve**
12.689	3-oxo-4-pregnen-20-beta-aldehyde	**327.465**	$C_{22}H_{32}O_2$	1421.246067	**- ve**

Discussion

Based on the IC_{50} values presented in Tables 1 and 2, the air-dried 100% and 70% ethanol leaf extracts of *D. mespiliformis* are the most potent in the studied extracts. IC_{50} is a quantitative measure that indicates how much a particular inhibitory substance (e.g., a drug) is needed to inhibit, in vitro, a given biological process or biological component by 50%. It permits the evaluation of drug efficacy and screening of new treatments or compounds [16]. These two extracts exhibited inhibitory potency against the pancreatic lipase enzyme and DPPH radicals in vitro more than the standards (i.e. orlistat and ascorbic acid) used in this study. These

observed potencies of the two extracts against the activity of pancreatic lipase may be due to the strong antioxidant activities they equally displayed against DPPH radicals (Table 2). The DPPH radical scavenging assay method is the most widely used method for the evaluation of the antioxidant strength of substances [17]. The strong antioxidant capacities of the air-dried 100% and 70% ethanol leaves extracts may be the result of the retention of more potent antioxidant phytochemical constituents of the leaves during the air-drying process [3-4, 5]. This may also be due to the extraction of high numbers of lipophilic polyphenols that might have contributed largely to the potent bioactivities of the 100% ethanol extract in a single solvent system (Table 3). While the relatively less polar large total flavonoids extracted by 70% ethanol, a binary solvent system (Table 4) [7], must have also contributed to its observed strong bioactivities [8-9]. Polyphenols and their subgroup flavonoids are strong antioxidant metabolites produced by the majority of plant species when taken into the body in large quantities by man [18].

Similarly, as observed in the results in Tables 3 and 4, the ratio of TPC: TFC in the air-dried 100% ethanol leaf extract is 6:1, further confirming the impact of the extracted lipophilic phenolic compounds compared to the flavonoids [8-9]. In a related vein, the high inhibition potency shown by the 70% *D. mespiliformis* leaf extract may have been largely due to the high quantities of relatively polar phenolic compounds it extracted since the ratio of TPC to TFC is about 3:2. Contrary to the air-dried 100% ethanol leaf extract, the flavonoids extracted by the 70% ethanol air-dried leaf extract also contributed substantially to its bioactivities. In our previous study of *D. mespiliformis* fruit pulp, the highest TPC and TFC were recorded in the 50% and 60% extracts, respectively [6], which is in contrast to the findings of this work.

The phytochemical profiles of these extracts revealed mostly polyphenols, organosulfur compounds, terpenes, saturated and unsaturated fatty acids, amino acids, and organic acids. Quercetin and hesperidin (flavonoids), and 7-(4-bromobutoxy)-3,4-dihydro-2(1H)-quinolinone and 4-isopropylbenzyl 3,5-dinitrobenzoateas (phenolic acids) as some of the phytochemicals identified in it. While 5-((p-hexyloxy)phenyl)-4-hexyloxy-2-methyl(thiobenzoate), and N-(2-methoxyethyl)isopropylamine were identified in the air-dried 100% ethanol extract. Hesperidin is a flavanone glycoside known for its antioxidant properties. It regulates lipid and glucose metabolism by facilitating PPAR and AMPK signalling pathways, regulates antioxidant index directly and anti-apoptosis [19]. It directly mediates the NF-κB signalling pathway to regulate inflammation in the treatment of obesity. Hesperidin-enriched dietary supplements improve symptoms like hyperlipidemia and postprandial hyperglycemia [19].

Conclusion

The studied *D. mespiliformis* leaf extracts showed promising anti-obesity and antioxidant characteristics by inhibiting the activity of PL in vitro about 1.7 times more than orlistat, the standard drug used in this study, and very strong antioxidant power against DPPH radicals. Some of the phytochemicals, like hesperidin and quercetin, identified in the air-dried 70% ethanol extract have known anti-obesity effects. Therefore, *D. mespiliformis* leaf extracts may potentially be a good raw material for sourcing anti-pancreatic lipase inhibitors.

References

1. Li Y, Zhang J, Xu D, Zhou T, Zhou Y, Li S, Li H. Bioactivities and Health Benefits of Wild Fruits. 2016 https://doi.org/10.3390/ijms17081258
2. Botanic Gardens Conservation International (BGCI) & IUCN SSC Global Tree Specialist Group (2021). "Diospyros mespiliformis". IUCN Red List of Threatened Species. 2021: e.T173967A1406816.
3. Peries CM, Navarthne S, Wijesinghe J, Henagamage AP, Coorey R. Effect of different drying methods on antioxidant activity and availability of phytochemicals in leaves of *Costus speciosus*, *Coccinia grandis* and *Gymnema sylvestre* J. *Asian Journal of Engineering Applied Technology*. 2022; 11(1): 29-35.
4. Sirajuddin MM, Rusman S, Suryanto E. The effect of different drying methods on phytochemicals, antioxidant activity and functional groups of dayak onion (*Eleutherine bulbosa*) extract. IOP Conference Series: *Earth and Environmental Science*. 2024; 1413: 012081. DOI 10.1088/1755-1315/1413/1/012081
5. Somwang Lekjing, Karthikeyan Venkatachalam, Narin Charoenphun, and

Paramee Noonim. Effect of Different Drying Methods on the Phytochemical and Antioxidant Properties of Soursop Leaves at Two Stages of Maturity. *ACS Omega*. 2024; 9(38): 40095-40109. DOI: 10.1021/acsomega.4c06071

6. Jibril MM, Wasila TM, Kamaladdeen I, Hamisu MU. Nutraceutical and Functional Food Potentials of African Ebony (*Diospyros mespiliformis*) Tree Fruit Pulp. *Bayero Journal of Pure and Applied Sciences*. 2023; 16(1): 1–15.
7. Bajes HR, Almasri I, Bustanji Y. Plant Products and Their Inhibitory Activity Against Pancreatic Lipase. *Rev. Bras. Farmacogn*. 2020; 30: 321–330.
8. Wang J, Sun B, Cao Y, Tian Y, & Li X. Optimisation of ultrasound-assisted extraction of phenolic compounds from wheat bran. *Food Chemistry*. 2008; *106*(2): 804–810.
9. Wong YH, Lau HW, Tan CP, Long K, Nyam KL. Binary solvent extraction system and extraction time effects on phenolic antioxidants from kenaf seeds (*Hibiscus cannabinus* L.) extracted by a pulsed ultrasonic-assisted extraction. *The Scientific World Journal*, *2014*. https://doi.org/10.1155/2014/789346
10. Fukumoto, J., Iwai, W., and Tsujiska, Y. Studies on Lipase Purification and Crystallisation of a Lipase Secretion by *Aspergillus niger*. *Journal of General and Applied Microbiology*. 1963; 9: 353–361.
11. Fox PF, Stepaniak L. Isolation and Some Properties of Extracellular Heat-Stable Lipases from Pseudomonas fluorescens Strain AFT 36. *Journal of Dairy Research*. 1983; 50: 77–89.
12. Chang ST, Wu JH, Wang SY, Kang PL, Yang NS, Shyur LF. Antioxidant activity of extracts from *Acacia confusa* bark and heartwood. *Journal of Agriculture and Food Chemistry*. 2001; 49: 3420-3424.
13. Li HB, Cheng KW, Wong CC, Fan KW, Chen F, Jiang Y. Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. *Food Chemistry*. 2007; 102(3): 771–6.
14. Nadhiya K, Vijayalakshmi K. Evaluation of total phenolics, flavonoid contents and in vitro antioxidant activity of *Benincasa hispida* fruit extracts. *Int J Pharm Chem Biol Sci*. 2014; 4(2): 332–8.
15. Cavaliere C, Capriotti AL, La Barbera G, Montone CM, Piovesana S, Laganà A. Liquid chromatographic strategies for separation of bioactive compounds in food matrices. *Molecules*. 2018; 23(12).
16. Cheng Y, Prusoff WH. Relationship Between The Inhibition Constant (&) and the Concentration of Inhibitor which Causes 50 Percent Inhibition (IC_{50}) of an Enzymatic Reaction. *Biochemical Pharmacology*. 1973; 22: 3099-3108
17. Gulcin I, Alwase SH. DPPH Radical Scavenging Assay. *Processes*. 2023; 11: 2248.
18. Bors W, Michel C, Stettmaier K. Structure-Activity Relationships Governing Antioxidant Capacities of Plant Polyphenols. *Methods in Enzymology.* 2001; 335: 167-180.
19. Xiong H, Wang J, Ran Q, Lou G, Peng C, Gan Q, Hu J, Sun J, Yao R, Huang Q. Hesperidin: A Therapeutic Agent For Obesity. *Drug Design, Development and Therapy* 2019; 13: 3855–3866.

A Comparative Study on Proximate and Mineral Composition of White and Yellow Maize (*Zea Mays L.*) and Masa Products in Northern Nigeria

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Abstract

Maize (*Zea mays L.*) is a major staple cereal in Nigeria, serving as a primary source of energy and nutrients for a large proportion of the population. It is consumed in various forms, including traditional fermented and fried products such as *masa*, whose nutritional quality can be influenced by maize variety and processing methods. Despite the widespread consumption of white and yellow maize and their derived products, comparative information on their nutritional and mineral composition remains limited, particularly in relation to commonly consumed *masa* products. This study was aimed at comparing the proximate and mineral composition of white and yellow maize varieties and their products (*masa*) commonly consumed in Northern Nigeria. Proximate parameters analysed include crude protein, crude fibre, fat, moisture, ash, and carbohydrate content, while mineral composition (Zn, Fe, Mg, Ca, and Mn) was determined using Atomic Absorption Spectrophotometry (AAS). Proximate results showed comparable macronutrient profiles in maize grains, with white maize exhibiting slightly higher protein (9.20 ± 0.42%), fibre (3.50 ± 0.48%), and ash (1.06 ± 0.21%) content, while yellow maize had statistically significantly higher ($p = 0.012$) moisture (11.48 ± 0.35%) content than white maize. White maize Masa was found to be significantly higher in Protein (20.99 ± 0.21%) and Fat (28.62 ± 0.07%), while Yellow maize Masa retains significantly higher fibre (15.65 ± 0.33%) and carbohydrates (29.42 ± 0.05%). Also, Yellow Maize Masa product was found to have significantly higher Zinc (1.290 ± 0.004%), Iron (4.454 ± 0.033%), and magnesium (3.534 ± 0.001%), whereas White Maize Masa was found to be superior in calcium (7.95 ± 0.0144%) and Manganese (0.44 ± 0.0123%). Thus, white maize and its masa could be superior in protein, calcium and manganese, while yellow masa could offer higher energy-yielding carbohydrate, fibre, zinc and iron.

Keywords: White maize, Yellow maize, Masa, Proximate composition, Mineral analysis, Nutrition, Atomic Absorption Spectrophotometry

Introduction

Maize (*Zea mays L.*) is one of the most important cereal crops in sub-Saharan Africa, serving as a staple food and a critical contributor to caloric intake. It is a widely consumed annual cereal crop cultivated globally. In Nigeria, both white and yellow maize varieties are widely consumed, with white maize traditionally preferred for cultural and sensory reasons, while yellow maize is valued for its carotenoid content, particularly provitamin A.[1] Maize provides food and fuel for humans, feeds for animals, and can be used as raw materials in manufacturing industries.[2]. Its grains have great nutritional value and can be processed into various types of products such as cornmeal, grits, starch, flour, tortillas, snacks, and breakfast cereals. It can be eaten boiled, roasted, fried or popped[3].

Nutrient composition varies significantly among maize varieties due to genetics, soil conditions, and processing.[4,5] Proximate composition provides insights into energy and macronutrient supply, while mineral analysis is critical given the widespread burden of micronutrient deficiencies in Nigeria, particularly anaemia (iron deficiency) and stunting (zinc deficiency).[6]
Masa, a traditional fermented maize-based product, is a culturally significant food in northern Nigeria. Several studies have been conducted on the nutritional composition of maize, and it has been found to contain a lot of beneficial nutrients, ranging from carbohydrates, proteins, macro elements, minerals, vitamins to phytochemicals.[7] Little work has been done on the comparison of the nutritional composition of different maize varieties

Date submitted-05/10/2025 Date Accepted-27/11/2025

and their traditional fermented product (masa) in northwestern Nigeria; hence, this study aims to investigate the proximate and mineral composition of white and yellow maize grain varieties and their fermented products (masa) in the northwestern region of Nigeria.

Materials And Methods

Samples Collection, Preparation and Digestion

Samples (one each of white and yellow maize) were obtained from Janguza market (a local market in Kano State, Nigeria). The samples were washed, dried, ground (using an electric kitchen blender) into flour (to pass through a 600µm sieve) and stored in clean air tied continers for laboratory analysis (proximate and some mineral analysis).

Cleaned white and yellow maize grains were separately soaked in water for 1 hour and then wet-milled. Yeast was added to each and then allowed to ferment for 3 hours at room temperature. Gaggery was added to the fermented mixture and then fried into masa. The Masa products were divided into smaller pieces, allowed to dry (under a fan) and then ground (using an electric kitchen blender) into flour (to pass through a 600µm sieve) for laboratory analysis. The dried white and yellow maize samples were analysed for proximate and some mineral contents.

Proximate Analysis

Crude protein, crude fibre, fat, moisture, ash, and carbohydrate were determined using AOAC (2019)[8] methods.

Mineral Analysis

Zinc (Zn), Iron (Fe), Magnesium (Mg), Calcium (Ca), and Manganese (Mn) were quantified using Atomic Absorption Spectrophotometry (PerkinElmer PinAAcle 900H) at the Central Laboratory of Bayero University, Kano. Samples were first subjected to digestion as follows: Sample (4g) was weighed into a beaker. Concentrated HCl (3ml) and concentrated HNO_2 (1ml) were added and heated on a hot plate at 100 ^{O}C for 10minutes to destroy any oxidizable materials and carbonates. The solution was topped with deionised water to the 50ml mark and filtered using a filterd using a Whatman filter paper (No.1). The filtrate was then analysed for the presence of some minerals in triplicate.

Statistical Analysis

The data were expressed as mean ± standard deviation (SD). An independent samples t-test (two-tailed, equal variance assumed) was used to assess differences between the two varieties (white versus yellow).

Results

Results of the proximate composition of the maize samples analysed are presented in Table I. From the results, crude Protein, crude fibre, fat, ash and carbohydrate contents of white maize were found to be higher than those of yellow maize. While the moisture content of yellow maize was found to be statistically significantly higher ($p = 0.012$) than that of white maize. Moisture Content shows a massive effect size ($d = -3.61$), confirming the difference is very distinct. Fat Content shows a large effect size ($d = 1.19$), suggesting White Maize tends to have higher fat, but due to the small sample size ($n=3$) and high standard deviation in the White Maize group, this did not reach statistical significance ($p = 0.218$).

White maize was found to be significantly richer in most minerals (Zn, Fe, Ca, Mn) compared to yellow maize, except for Magnesium, where levels were found to be statistically similar (Table II).

Table III shows that processing into "Masa" results in significant nutritional differences. White maize Masa was found to be significantly higher in Protein and Fat, while Yellow maize Masa retains significantly higher Fibre and Carbohydrates. Yellow Maize Masa product was found to have significantly higher Zinc, Iron, and Magnesium, whereas White Maize Masa remains superior in Calcium and Manganese (Table IV).

Discussion

The proximate composition of the maize varieties in this study (Table I) aligns broadly with existing literature. It was reported that crude protein values of 8.6% and 9.1% for yellow and white maize, respectively, closely match the findings of this study.[9] The slightly higher crude protein and fat in white maize may reflect varietal differences and environmental conditions during cultivation. It was documented moisture content was between 10% and 12% for Nigerian maize varieties, consistent with the values observed in this study.[10] Carbohydrate content was also comparable to values reported were carbohydrate levels were found to range from 70–72% in maize samples analysed across different Nigerian regions.[11]

The mineral composition (Table II) shows white maize to be superior in the minerals analysed. These results corroborate findings by Owuor *et al*. (2021)[5] who reported significantly higher iron and zinc contents in white maize compared to yellow

varieties cultivated in Kenya. Similarly, it was found that white maize samples from Nigeria had higher calcium and manganese content, supporting the findings of this study.[12] Zinc is critical for immune function, and its deficiency is common in low-resource settings. The higher zinc content in white maize (1.13 ± 0.0067mg/kg vs.1.09 ± 0.0099 mg/kg) suggests its potential advantage in addressing micronutrient deficiencies, consistent with a study[7] which emphasised dietary zinc as a limiting nutrient in cereal-based diets. Iron content was significantly higher in white maize, a finding that aligns with a previous study[4], which noted that yellow maize tends to prioritise provitamin A accumulation (like β-carotene) at the expense of iron and zinc. The iron content of 2.10 ± 0.0147 mg/kg in white maize could be essential in the context of anaemia prevention in women and children. No significant difference was observed in magnesium content, suggesting that both maize types contribute comparably to dietary magnesium needs. This is in agreement with the report of Oboh *et al.* (2017)[9], which observed negligible differences in Mg levels between different maize cultivars grown in Nigeria. Calcium levels were significantly higher in white maize, which supports bone health, muscle function, and nerve transmission. This finding agrees with a study that reported enhanced calcium levels in white maize harvested from acidic soils, potentially explaining the observed variation.[13] Manganese content was also higher in white maize, as seen in earlier studies.[11]This trace mineral is important for enzymatic functions and bone formation. The superior mineral profile of white maize underscores its relevance in food-based strategies to address micronutrient malnutrition in Nigeria**.**

The proximate analysis of masa revealed distinct nutritional differences between white and yellow varieties. White masa had higher protein (20.99%) and fat content (28.62%), which may enhance satiety, contribute to protein-energy adequacy, and provide a more nutrient-dense food option. In contrast, yellow masa contained more crude fibre (15.65%) and carbohydrate (29.42%), offering higher energy yield and potential digestive benefits. Moisture and ash contents were comparable between the two. These results suggest that fermentation and processing into masa not only preserve varietal nutrient characteristics but also highlight their functional roles in the diet.

The mineral analysis further confirmed that some micronutrients, particularly calcium and manganese, are higher in white maize and its masa, while zinc and iron were found to be significantly ($p < 0.05$) higher in yellow maize masa (Table IV). This is consistent with earlier findings[3,2] and indicates that masa processing does not diminish some of the inherent mineral advantages of white maize. The higher protein content in white masa and the iron content in yellow masa are particularly relevant in Nigeria, where deficiencies in these nutrients are widespread contributors to anaemia and growth faltering.[14]

Thus, these results emphasise the complementary roles of white and yellow masa in meeting diverse nutritional needs. White masa provides higher protein and mineral density, supporting growth and micronutrient adequacy, while yellow masa offers energy and fibre benefits. This dual advantage underscores the importance of varietal selection in nutrition-sensitive agriculture and dietary planning, and highlights how food processing methods like fermentation can influence nutrient availability.

Conclusion

This study revealed that both white and yellow maize varieties are nutritionally rich, though important differences exist in their masa products. White maize had comparatively higher concentrations of protein, fibre, and essential minerals (Zn, Fe, Ca, and Mn), while yellow maize contained more moisture. When processed into masa, white masa retained a superior protein and fat profile, together with higher concentrations of key minerals, whereas yellow masa provided higher carbohydrate and fibre content.

These results suggest that white masa may contribute more meaningfully to protein and micronutrient adequacy, making it valuable in combating deficiencies such as anaemia and stunting, while yellow masa offers advantages in energy and dietary fibre intake. The findings reinforce the role of maize in addressing nutrient deficiencies and highlight the importance of varietal choice in food processing, nutrition policy, and agricultural interventions.

Table 1: Proximate Composition of White and Yellow Maize Varieties

Component	White Maize (%)	Yellow Maize (%)	t-statistic	P-value	Effect Size (d)
Crude Protein	9.20 ± 0.42	8.90 ± 0.34	0.96	0.391	0.79
Crude Fibre	3.50 ± 0.48	3.30 ± 0.50	0.49	0.643	0.41
Fat Content	4.40 ± 0.71	3.80 ± 0.04	1.46	0.218	1.19
Moisture Content	10.27 ± 0.32	11.48 ± 0.35	-4.42	**0.012**	-3.61
Ash Content	1.06 ± 0.21	0.98 ± 0.63	0.21	0.845	0.17
Carbohydrate	71.57 ± 0.01	71.54 ± 0.05	1.02	0.366	0.83

Bold values indicate statistically significant differences (P < 0.05)

Table 2: Mineral Composition of White and Yellow Maize Varieties

Element	White Maize (mg/kg)	Yellow Maize (mg/kg)	t-statistic	P-value	Effect Size (d)
Zinc (Zn)	1.13 ± 0.0067	1.09 ± 0.0099	5.80	**0.004**	4.73
Iron (Fe)	2.10 ± 0.0147	1.41 ± 0.0398	28.17	**< 0.001**	23.00
Magnesium (Mg)	18.19 ± 0.139	18.10 ± 0.130	0.82	0.459	0.67
Calcium (Ca)	7.95 ± 0.0144	7.64 ± 0.0104	30.23	**< 0.001**	24.68
Manganese (Mn)	0.44 ± 0.0123	0.40 ± 0.0068	4.93	**0.008**	4.02

Bold values indicate statistically significant differences (P < 0.05)

Table 3: Proximate Composition of Masa from Yellow and White Maize

Component	Yellow Masa (%)	White Masa (%)	t-statistic	P-value	Effect Size (d)
Crude Protein	17.16 ± 0.07	20.99 ± 0.21	-29.97	**< 0.001**	-24.47
Crude Fibre	15.65 ± 0.33	13.35 ± 0.53	6.38	**0.003**	5.21
Fat Content	23.77 ± 0.23	28.62 ± 0.07	-34.94	**< 0.001**	-28.53
Moisture	12.17 ± 0.44	13.10 ± 0.33	-2.93	**0.043**	-2.39
Ash Content	1.83 ± 0.71	1.70 ± 0.13	0.31	0.771	0.25
Carbohydrate	29.42 ± 0.05	22.24 ± 0.34	36.19	**< 0.001**	29.55

Bold values indicate statistically significant differences (P < 0.05)

Table 4: Mineral Composition of Masa from White and Yellow Maize

Element	White Masa (mg/kg)	Yellow Masa (mg/kg)	t-statistic	P-value	Effect Size (d)
Zinc (Zn)	1.206 ± 0.003	1.290 ± 0.004	-29.10	**< 0.001**	-23.76
Iron (Fe)	3.573 ± 0.040	4.454 ± 0.033	-29.43	**< 0.001**	-24.03
Magnesium (Mg)	3.528 ± 0.001	3.534 ± 0.001	-7.35	**0.002**	-6.00
Calcium (Ca)	13.25 ± 0.19	12.21 ± 0.02	9.43	**0.001**	7.70
Manganese (Mn)	0.639 ± 0.007	0.612 ± 0.006	5.07	**0.007**	4.14

Bold values indicate statistically significant differences (P < 0.05). **Effect Size:** Cohen's d > 0.8 is considered a "Large" effect. Almost all significant differences here show very large effect sizes, indicating a clear distinction between the varieties.

References

1. Tajamul, R. S., Kamlesh, P. and Pradyuman, K. (2016). "Maize a potential source of human nutrition and health: A review " Cogent Food and Agriculture (2): 1.
2. Hossain, F., Muthusamy, V., Bhat, J.S., Jha, S.K. Zunjare, R, Das, A., Sarika, K. and Kumar, R. (2016). "Maize- Broadening the Genetic Base of Grain Cereals", *Springer*, pp 67-88,
3. Ape, D.I. Nwogu, N.A., Uwakwe, E.I., Ikedinobi, C.S. (2016). "Comparative Proximate Analysis of Maize and Sorghum Bought from Ogbete Main Market of Enugu State, Nigeria", *Greener Journal of Agricultural Sciences (*6): 9, 272-275.
4. Nuss ET, Tanumihardjo SA. Maize: A paramount staple crop in the context of global nutrition. *Comprehensive Reviews in Food Science and Food Safety.* 2010; 9 (4): 417–436. https://doi.org/10.1111/j.1541-4337.2010.00117.x
5. Owuor JO, Odongo MR, Okoth JK.Comparative nutritional analysis of white and yellow maize (Zea mays L.) varieties in Kenya. *African Journal of Food, Agriculture,*

Nutrition and Development. 2010; 21 (5): 18123–18135. https://doi.org/10.18697/ajfand.99.19422

6. Awoyinka AF, Akinola OA, Olagunju AM. Comparative assessment of nutritional and antinutritional composition of yellow and white maize varieties in Nigeria. *Nigerian Journal of Food Science and Technology.* 2020; 38 (1): 45–52.
7. Umeta M, West CE, Haidar J, Deurenberg P, Hautvast, JGJ. Zinc supplementation and stunted infants in Ethiopia: A randomised controlled trial. *The Lancet Global Health.* 2020; *8* (12): e1452–e1460.
8. AOAC. Official Methods of Analysis of AOAC International. 21st Edition. 2019
9. Emmanuel OS, Yakubu NA, Sunday JO. Evaluation of proximate composition of maize varieties commonly consumed in North Central Nigeria. *Journal of Cereal Research.* 2022; 14 (2): 133–140.
10. Oguntona T, Akinyele IO. *Nutrient composition of commonly eaten foods in Nigeria*. Ibadan: Food Basket Foundation. 2019.
11. Onimawo IA, Nwamarah JU, Okafor MO. Macronutrient content of maize from different agroecological zones in Nigeria. *Nigerian Journal of Nutritional Sciences.* 2018; 39 (1): 21–27.
12. Oboh G, Ademosun AO, Bello F. Comparative study on the mineral composition of some Nigerian maize varieties. *Nigerian Food Journal.* 2017; 35 (1): 1–8.
13. Ezeocha CV, Ojimelukwe PC. The effects of soil acidity and liming on nutrient uptake and mineral content of maize in southeastern Nigeria. *African Journal of Agricultural Research.* 2018; 13 (12): 573–579.
14. [14] Ogunlade I, Awosika AJ. Comparative mineral content of selected staple foods cultivated in Nigeria. *Journal of Food Chemistry and Nutrition.* 2016; 4 (2): 33–38.

Severity of Oxidative Stress and Associated Complications in Type 2 Diabetes Mellitus among Smoking and Non-Smoking Subjects in Kano, Nigeria

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Abstract
Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder strongly associated with oxidative stress, metabolic dysregulation, and progressive organ damage. Cigarette smoking is a major modifiable risk factor that exacerbates oxidative stress, impairs antioxidant defences, alters nutritional status, and accelerates diabetic complications, particularly those affecting the kidneys and cardiovascular system. This study investigated the combined effects of cigarette smoking on oxidative stress, kidney function, vitamin status (vitamins A and C), body mass index (BMI), and glycemic control among type 2 diabetic and non-diabetic subjects attending Murtala Muhammad Specialist Hospital and Muhammad Abdullahi Wase (Nassarawa) Hospital, Kano State, Nigeria. A cross-sectional comparative design was adopted, involving forty (40) participants grouped into: type 2 diabetic smokers, type 2 diabetic non-smokers, non-diabetic smokers, and non-diabetic non-smokers. Biochemical analyses included serum malondialdehyde (MDA), superoxide dismutase (SOD), vitamin C, vitamin A, fasting blood glucose, serum creatinine, serum urea, and BMI assessment. Diabetic smokers exhibited significantly elevated oxidative stress markers, impaired antioxidant status, reduced vitamin A and C levels, increased BMI, and evidence of compromised kidney function compared with non-smokers ($p < 0.05$). The findings demonstrate that cigarette smoking synergistically worsens oxidative stress, nutritional imbalance, and renal dysfunction in type 2 diabetes. Smoking cessation should therefore be prioritised as a critical component of comprehensive diabetes management.

Keywords: Type 2 diabetes mellitus, cigarette smoking, oxidative stress, kidney function, vitamin A, BMI

Introduction
Type 2 diabetes mellitus (T2DM) is a major global public health challenge characterised by chronic hyperglycemia arising from insulin resistance and relative insulin deficiency [1,2]. Its prevalence has increased markedly worldwide, particularly in developing countries, due to rapid urbanisation, sedentary lifestyles, dietary transitions, and population ageing [2]. In sub-Saharan Africa, including Nigeria, the growing burden of T2DM poses serious clinical and economic challenges.

Oxidative stress is a central mechanism in the pathogenesis of diabetes and its long-term complications. Persistent hyperglycemia enhances the production of reactive oxygen species (ROS) through glucose autoxidation, non-enzymatic protein glycation, activation of the polyol pathway, and mitochondrial dysfunction [3–5]. When antioxidant defences are overwhelmed, oxidative stress results in lipid peroxidation, protein oxidation, and DNA damage, contributing to endothelial dysfunction and organ injury [4,5].

Cigarette smoking is an established independent risk factor for insulin resistance, poor glycemic control, and increased morbidity and mortality among diabetic patients [6,7]. Tobacco smoke contains numerous free radicals and pro-oxidant compounds that directly induce oxidative damage while also depleting endogenous antioxidants [6,8]. In individuals with diabetes, smoking amplifies oxidative stress and accelerates the progression of microvascular complications such as nephropathy, neuropathy, and retinopathy, as well as macrovascular diseases including cardiovascular and cerebrovascular disorders [7,12].

Beyond oxidative stress, smoking adversely affects nutritional status and body composition. It has been associated with alterations in body mass index (BMI) and depletion of essential micronutrients, including antioxidant vitamins such as vitamins A and C [9,10]. Vitamin A plays a crucial role in immune regulation, epithelial integrity, and antioxidant defence, while vitamin C is a key water-soluble antioxidant involved in scavenging free radicals and regenerating other

Date submitted-15/10/2025 Date Accepted-28/11/2025

antioxidants [9,10]. Deficiencies in these vitamins may further predispose diabetic patients to oxidative injury and organ dysfunction.

Diabetic nephropathy remains one of the most serious long-term complications of T2DM and a leading cause of end-stage renal disease worldwide [11]. Smoking has been shown to worsen renal hemodynamics, increase albuminuria, and accelerate the decline in renal function in diabetic patients [12]. However, limited integrated data exist in northern Nigeria on the combined effects of smoking on oxidative stress, kidney function, vitamin status, and BMI in individuals with T2DM.

This study, therefore, aimed to comprehensively evaluate the impact of cigarette smoking on oxidative stress markers, kidney function indices, vitamin A and C status, BMI, and glycemic control among type 2 diabetic and non-diabetic subjects attending selected tertiary hospitals in Kano State, Nigeria.

Materials and Methods

Study Area and Population

The study was conducted at Murtala Muhammad Specialist Hospital and Muhammad Abdullahi Wase (Nassarawa) Hospital, Kano State, Nigeria. Kano is located in north-western Nigeria and is characterised by a tropical savannah climate. The study population consisted of diagnosed type 2 diabetic patients and apparently healthy non-diabetic individuals attending the selected hospitals.

Study Design and Sample Size

This was a cross-sectional comparative study. Forty (40) subjects were recruited and divided into four groups of ten (10) participants each:

- **Group A:** Type 2 diabetic smokers
- **Group B:** Type 2 diabetic non-smokers
- **Group C:** Non-diabetic smokers
- **Group D:** Non-diabetic non-smokers

Ethical Clearance

Ethical approval for the study was obtained from the Kano State Ministry of Health Ethics Committee. Informed consent was obtained from all participants before sample collection.

Sample Collection

Venous blood samples were collected after an overnight fast using standard phlebotomy procedures. Serum was separated by centrifugation and stored appropriately until biochemical analyses were performed, following established laboratory protocols [9,10].

Biochemical Analysis

Determination of Serum Malondialdehyde (MDA)

Serum malondialdehyde (MDA) was determined as an index of lipid peroxidation using the thiobarbituric acid reactive substances (TBARS) method with absorbance measured at 532 nm, as described by Tiwari *et al*. [5].

Determination of Superoxide Dismutase (SOD)

Serum SOD enzyme activity was determined according to the method described by Sun and Zigman [16]. Here, the SOD enzyme assay determines the difference between superoxide anion decomposition and production, that is, its ability to inhibit the autoxidation of adrenaline.

Determination of Vitamin C

Serum vitamin C concentration was estimated using the dinitrophenylhydrazine spectrophotometric method, as previously reported [9,10].

Determination of Vitamin A

Serum vitamin A was determined spectrophotometrically following extraction and colour development as described by Rutkowaski and Grzegorzewski [17].

Determination of Kidney Function Indices

Serum creatinine and urea concentrations were determined using standard biochemical methods to assess renal function, as outlined in clinical biochemistry manuals and prior studies [11,12].

Determination of Serum Electrolyte Levels

Serum Electrolytes, including Chloride (Cl), Sodium (Na), Potassium (K) and Bicarbonate (CO_3^-), were determined using standard laboratory methods as described by Maruna and Trinder [19].

Determination of Fasting Blood Glucose (FBG)

Fasting blood glucose levels were measured using Randoe's glucose oxidase/peroxidase method of Barham and Trinder [18].

Body Mass Index (BMI) Assessment

Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m^2) and classified according to World Health Organisation criteria [2].

Statistical Analysis

Data were analysed using appropriate statistical software. Results were expressed as mean ±

standard deviation, and comparisons between groups were performed using analysis of variance (ANOVA). Statistical significance was set at $p < 0.05$.

Results

Oxidative Stress, Antioxidant Status, Glycemic Control and BMI

Malonaldehyde (MDA): The highest increase in the level of Malonaldehyde is seen in type 2 diabetic cigarette smoking patients (group A) followed by Non cigarette smoking type 2diabetic patients (Group B), while the lowest level of MDA is seen in non-type 2 diabetic cigarette smokers (Group C) followed by Non type 2 diabetic non cigarette smoking individuals (Group D) with very little increased difference between the two, at ($p<0.05$) level of significant difference.

Superoxide dismutase (SOD): The level of Superoxide dismutase (SOD) an enzymatic antioxidant that fights against reactive species, is found to be significantly low in type 2 diabetic cigarette smoking patients (Group A) followed by Non cigarette smoking type 2 diabetic patients (Group B), while the highest level of SOD is seen in group D followed by the group C at ($p<0.05$) level of significant difference.

Vitamin C (Ascorbic acid):

There is a significant decrease in the level of vitamin C in group A, followed by group B, while an altitudinous increase is seen in group D, with little difference in the result of group C at a $p<0.05$ level of significance.

Fasting Blood Glucose (FBG):

Group A are found to be significantly hyperglycemic with an intense increase in glucose level, followed by group B, while group D and C can be considered normal, but C is slightly hyperglycemic compared to group D at ($p<0.05$) level of significant difference.

Table 1. Serum glucose, Oxidative Stress Markers and Antioxidant Levels among Study Groups

Parameter	T2DM Smokers	T2DM Nonsmokers	Non-diabetic Smokers	Non-diabetic Nonsmokers
MDA (µmol/L)	1.785 ± 1.331^a	1.594 ± 0.405	1.027 ± 0.281	1.013 ± 0.247^b
SOD (mg/dL)	0.002 ± 0.002^a	0.004 ± 0.006	0.011 ± 0.017	0.020 ± 0.021^b
VitaminC (mg/dL)	10.31 ± 2.83^a	12.42 ± 1.93	12.87 ± 2.71	17.39 ± 8.19^b
FBG (mmol/L)	12.36 ± 3.97^a	10.89 ± 3.11	4.36 ± 0.84	4.28 ± 0.43^b
Vitamin A (mmol/L)	0.708 ± 0.107^a	0.838 ± 0.011^a	0.877 ± 0.051^b	1.07 ± 0.162^c
BMI (Kg/m^2)	25.00 ± 0.881^a	26.05 ± 0.701^b	26.50 ± 1.500^c	26.52 ± 1.118^c

Values are mean ± SD. Different superscripts within a row indicate a significant difference ($p < 0.05$).

3.2 Kidney Function Indices

The results of kidney function indices presented in Table 2 revealed that type 2 diabetic smokers have significantly higher levels of Urea, Creatinine and electrolytes (Na, K, Cl and CO_3^-) compared to type 2 diabetic non-smokers. The pattern shown by the kidney function indices is as follows: T2DM Smokers > T2DM Non-smokers > Non-diabetic Smokers > Non-diabetic Non-smokers.

Table 2: Serum kidney parameters (mmol/L)in diabetic and non-diabetic patients

GROUP	UREA	CREATINE	SODIUM	POTASSIUM	CHLORIDE	BICARBONATE
T2DM Smokers	27.7 ± 3.03^a	1.461 ± 0.09^a	177.29 ± 6.79^a	7.02 ± 0.92^a	118.5 ± 6.89^a	43.5 ± 2.83^a
T2DM Nonsmokers	21.02 ± 7.74^b	1.207 ± 0.19^b	164.5 ± 7.71^b	6.40 ± 0.64^b	114.2 ± 3.19^a	35.53 ± 9.89^b
Non-diabetic Smokers	20.78 ± 5.39^b	0.942 ± 0.07^c	141.67 ± 5.32^c	6.17 ± 0.29^b	111.1 ± 5.67^b	26.65 ± 9.37^c
Non-diabetic Nonsmokers	7.9 ± 0.89^c	0.81 ± 0.16^c	134.5 ± 6.38^c	4.42 ± 1.02^b	99.5 ± 1.87^c	18.98 ± 5.36^d

Values are mean ± SD. Different superscripts within a column indicate a significant difference ($p < 0.05$).

Overall, diabetic smokers exhibited the most adverse biochemical and anthropometric profiles, characterised by significantly increased oxidative

stress, impaired antioxidant defences, reduced vitamin A and C levels, higher fasting blood glucose, increased BMI, and evidence of compromised kidney function compared with non-smokers ($p < 0.05$).

Discussion

The present study offers a comprehensive evaluation of the interactive effects of cigarette smoking and type 2 diabetes mellitus on oxidative stress, antioxidant status, kidney function, vitamin A and C levels, body mass index, and glycemic control. By integrating metabolic, nutritional, and renal parameters, the findings provide a broader understanding of how smoking accelerates diabetes-related pathophysiology, with important implications for disease management and prevention.

Consistent with previous studies, diabetic smokers in this study exhibited significantly elevated malondialdehyde (MDA) levels, reflecting increased lipid peroxidation and heightened oxidative stress. Hyperglycemia-induced overproduction of reactive oxygen species (ROS), coupled with the abundant free radicals and pro-oxidants present in cigarette smoke, creates a synergistic oxidative burden that overwhelms endogenous antioxidant defences [3–5,6]. Similar elevations in MDA among diabetic smokers have been reported by Morrow *et al*. and Tiwari *et al*., who demonstrated that smoking independently amplifies lipid peroxidation in both diabetic and non-diabetic populations [5,6].

The observed reduction in superoxide dismutase (SOD) activity among diabetic smokers further supports the notion of compromised antioxidant defence mechanisms. SOD plays a critical role in dismutating superoxide radicals into less reactive species; its depletion suggests persistent oxidative insult and enzymatic inactivation. Comparable reductions in SOD activity have been documented in diabetic patients with poor glycemic control and in chronic smokers, reinforcing the additive deleterious effects observed in this study [3,8].

Antioxidant vitamin depletion was a prominent finding, with significantly lower serum vitamin C and vitamin A levels among diabetic smokers. Vitamin C, a primary water-soluble antioxidant, is rapidly consumed during oxidative stress, while vitamin A is essential for epithelial integrity, immune modulation, and redox homeostasis. Previous investigations have similarly reported reduced antioxidant vitamin levels in smokers and individuals with diabetes, attributing this to increased utilisation, impaired absorption, and altered metabolism [9,10]. The combined deficiency observed here may further predispose diabetic smokers to oxidative tissue damage and immune dysregulation.

Renal function impairment was more pronounced among diabetic smokers, as evidenced by elevated serum creatinine and urea concentrations. Smoking-induced endothelial dysfunction, renal vasoconstriction, and oxidative injury to glomerular structures are well-documented mechanisms that accelerate diabetic nephropathy [12]. Nilsson *et al*. reported a strong association between smoking, microalbuminuria, and declining renal function in type 2 diabetes, findings that align closely with the present results [12]. These observations underscore smoking as a critical modifiable risk factor in the progression of diabetic kidney disease.

Body mass index (BMI) findings revealed that diabetic subjects, particularly smokers, predominantly fell within the overweight range. Although smoking has historically been associated with lower body weight, emerging evidence suggests that in the context of diabetes, smoking coexists with central adiposity and insulin resistance [7,15]. This paradoxical relationship may be mediated by hormonal dysregulation, chronic inflammation, and lifestyle factors, further compounding metabolic risk.

Collectively, the findings of this study are in agreement with and extend existing literature by demonstrating that cigarette smoking exacerbates oxidative stress, depletes critical antioxidant vitamins, impairs renal function, and worsens metabolic control in type 2 diabetes. The integrated approach adopted here strengthens the evidence base linking smoking to accelerated diabetic complications, particularly in resource-limited settings.

From a public health perspective, these results highlight the urgent need to incorporate structured smoking cessation programs into diabetes care protocols. Nutritional interventions aimed at improving antioxidant vitamin status, alongside aggressive glycemic control, may further mitigate oxidative damage and delay the onset of complications. Future longitudinal studies with larger sample sizes are warranted to establish causal relationships and explore the potential

benefits of targeted antioxidant supplementation in diabetic smokers.

Conclusion

Cigarette smoking significantly aggravates oxidative stress, depletes antioxidant vitamins A and C, impairs kidney function, alters body mass index, and worsens glycemic control in individuals with type 2 diabetes mellitus. Diabetic smokers are therefore at substantially higher risk of developing oxidative stress-mediated complications, including diabetic nephropathy and other long-term sequelae. Integrating smoking cessation into diabetes management strategies is essential to reduce disease burden and improve patient outcomes.

6. Recommendations

1. Smoking cessation programs should be integrated into diabetes care.
2. Routine monitoring of oxidative stress markers in diabetic patients is recommended.
3. Public health policies should emphasise awareness of the synergistic risks of smoking and diabetes.

References

1. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2015;38(Suppl 1):S8–S16.
2. World Health Organisation. *Global report on diabetes*. Geneva: WHO; 2016.
3. Asmat U, Abad K, Ismail K. Diabetes mellitus and oxidative stress—A concise review. *Saudi Pharm J*. 2016;24(5):547–553.
4. Pitocco D, Zaccardi F, Di Stasio E, *et al*. Oxidative stress, nitric oxide, and diabetes. *Rev Diabet Stud*. 2010;7(1):15–25.
5. Tiwari BK, Pandey KB, Abidi AB, Rizvi SI. Markers of oxidative stress during diabetes mellitus. *J Biomark*. 2013;2013:1–8.
6. Morrow JD, Frei B, Longmire AW, *et al*. Increase in circulating products of lipid peroxidation in smokers. *N Engl J Med*. 1995;332:1198–1203.
7. Nilsson PM, Cederholm J, Eeg-Olofsson K, *et al*. Smoking as an independent risk factor for myocardial infarction or stroke in type 2 diabetes. *Eur J Cardiovasc Prev Rehabil*. 2009;16:506–512.
8. Kumar A, Biswas UK. Smoking is associated with increased oxidative stress and reduced antioxidants. *Heart Asia*. 2014;6:115–119.
9. Savu O, Ionescu-Tirgoviste C, Atanasiu V, *et al*. Increased total antioxidant capacity despite high oxidative stress in type 2 diabetes. *Int Med Res*. 2012;40:709–716.
10. Tripathi P, Verma MK, Tripathi SS, Singh SP. Comparative study of malondialdehyde and vitamin C in type 2 diabetes. *Int J Life Sci Sci Res*. 2016;2(1):31–36.
11. Ozougwu JC, Obimba KC, Belonwu CD, Unakalamba CB. Pathogenesis of type 1 and type 2 diabetes mellitus. *J Physiol Pathophysiol*. 2013;4(4):46–57.
12. Nilsson PM, Gudbjörnsdottir S, Eliasson B, Cederholm J. Smoking is associated with increased HbA1c and microalbuminuria. *Diabetes Metab*. 2004;30:261–268.
13. Laing SP, Swerdlow AJ, Slater SD, *et al*. Mortality from heart disease in insulin-treated diabetes. *Diabetologia*. 2003;46:760–765.
14. Rai RR, Phadke MS. Plasma oxidant–antioxidant status in respiratory disorders. *Indian J Clin Biochem*. 2006;21(2):161–164.
15. Xie XT, Liu Q, Wu J, Wakui M. Impact of cigarette smoking on type 2 diabetes development. *Acta Pharmacol Sin*. 2009;30(6):784–787.
16. Sun M, Zigman S. An improved spectrophotometric assay for superoxide dismutase based on epinephrine autoxidation. Analytical biochemistry. 1978 Oct 1;90(1):81-9.
17. Rutkowski M, Grzegorczyk K. Modifications of spectrophotometric methods for antioxidative vitamins determination convenient in analytic practice. Acta Scientiarum Polonorum Technologia Alimentaria. 2007 Sep 30;6(3):17-28.
18. Barham D, Trinder P. An improved colour reagent for the determination of blood glucose by the oxidase system. Analyst. 1972;97(1151):142-5.
19. Maruna RF, Trinder P. Analysis of serum sodium and potassium ions. Clin Chim Acta. 1958;2:581-5.

Tissue-Specific Distribution of Iron and Zinc and SSR Marker Polymorphism in Local Pearl Millet (*Pennisetum glaucum* L.) Varieties

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Abstract

Micronutrient malnutrition, particularly iron (Fe) and zinc (Zn) deficiency, remains a major public health challenge in regions where cereal-based diets predominate. Pearl millet (*Pennisetum glaucum*), a drought-tolerant staple widely consumed in semi-arid regions, offers significant potential for biofortification. This study combined physiological and molecular approaches to evaluate Fe and Zn uptake, tissue distribution, and genetic diversity among three local pearl millet varieties. Plants were cultivated in sandy loam soil spiked with iron oxide and zinc oxide under screenhouse conditions. At vegetative and physiological maturity stages, root, stem, leaf and seed tissues were harvested and analysed using atomic absorption spectrometry. In parallel, genetic polymorphism among the varieties was assessed using two microsatellite (SSR) primer pairs. Diversity parameters including polymorphic information content (PIC), binary scoring and UPGMA cluster analysis were determined. Results showed significantly ($p < 0.05$) higher accumulation of Fe and Zn in roots at both growth stages, confirming efficient uptake but restricted internal mobility. At physiological maturity, Fe remained largely confined to roots, while Zn showed relatively greater accumulation in seeds, indicating differential remobilisation behaviour. Hierarchical clustering based on mineral profiles revealed close relatedness between two varieties, while the third was distinct. SSR analysis detected allelic polymorphism with moderate PIC values (0.35–0.47), and UPGMA clustering similarly grouped the varieties into two major clusters, corroborating mineral distribution patterns with genetic relatedness. The findings demonstrate that limited phloem remobilisation constrains natural enrichment of pearl millet grains with Fe and Zn. Importantly, the observed genetic variability provides a foundation for selecting parent lines for breeding programmes aimed at improving micronutrient density.

Keywords: *Pearl millet, iron, zinc, tissue distribution, biofortification, microsatellite markers.*

1. Introduction

Iron (Fe) and zinc (Zn) deficiencies affect billions globally, especially in populations dependent on cereal staples. These deficiencies, often referred to as 'hidden hunger', especially affect women and children, due to their physiological needs.[1] The micronutrient content of edible plant tissues depends on uptake from soil, root absorption, xylem transport to vegetative tissues and eventual remobilisation through the phloem into seeds. However, Fe and Zn exhibit limited phloem mobility, often accumulating in high-transpiring tissues such as roots and leaves rather than grains. Biofortification of staple food crops with micronutrients, through breeding or fertilization, is an effective strategy to combat widespread dietary deficiencies in human populations.[1] Cereals and cereal products have been reported to supply up to about 50% of Fe and 30% of Zn in human diet.[2] The inadequate intake of several micronutrients, including Fe and Zn, that affects billions of people globally contextualises the relevance of improving mineral bioaccessibility in cereals. [3]

Pearl millet (*Pennisetum glaucum*) is highly adapted to arid and semi-arid regions and constitutes a major dietary component in many

Date submitted-15/10/2025 Date Accepted-28/11/2025

African and Asian communities.[4] With regard to nutritional quality, pearl millet is at least equivalent to maize and generally superior to sorghum in protein content and quality and metabolisable energy levels,[5] as well as digestibility.[6] Furthermore, pearl millet does not usually contain significant amounts of condensed polyphenols, such as the tannins commonly found in other staple crops such as sorghum, which can decrease digestibility.[7] Pearl millet grain also has a more complete amino acid profile than maize or sorghum.[8] Taken in totality, these qualities make pearl millet a major contributor of dietary protein and mineral intake in a variety of rural populations in sub-Saharan Africa.[9, 10] Despite its resilience and nutritional quality, the bioavailability of Fe and Zn in the grain remains low due to physiological limitations in uptake, translocation and remobilisation.

Genetic research in relation to Fe and Zn uptake, accumulation and distribution, and understanding tissue-specific distribution of these minerals at different growth stages are crucial for biofortification efforts in pearl millet.[11]

In view of the physiological limitations to micronutrient remobilisation in cereals and the need to identify genotypes with superior nutrient-partitioning traits, this study integrates mineral distribution analysis with molecular characterisation. The distribution of Fe and Zn in different tissues of local millet varieties was evaluated at vegetative and physiological maturity stages, alongside assessment of genetic polymorphism using simple sequence repeat (SSR) markers. This combined approach provides insight into both the physiological behaviour of micronutrients and the underlying genetic variability relevant to biofortification efforts.

2. Materials and Methods

2.1 Study Site

The experiment was conducted in a screenhouse at Bayero University, Kano, Nigeria.

2.2 Seed Collection, Planting and Soil Treatment

Seeds of three locally sourced pearl millet varieties were planted planted in 5 L plastic pots filled with sandy loam soil. The soil was spiked with 0.6 g Fe_2O_3 and ZnO prior to planting. Plants were arranged in a completely randomised design with three replicates.

2.3 Agronomic Management

Watering, thinning and fertiliser application (NPK 15:15:15 and urea) followed standard agronomic practices for millet cultivation.

2.4 Sampling Stages

Plants were harvested at: Week 3 (vegetative stage) and Week 12 (physiological maturity). Tissues were separated into root, stem, leaves and seeds.

2.5 Sample Digestion and Mineral Analysis

Dried samples (0.2 g) were acid-digested and analysed for Fe and Zn using atomic absorption spectrometry (AAS).

2.6 SSR Marker–Based Polymorphism Assessment

Genomic DNA was extracted from young leaves and amplified using two published pearl millet SSR primer pairs. PCR products were resolved on agarose gel and scored by presence/absence of alleles to generate a binary data matrix.

The sequences of the genomic SSR primers used are given below:[12]

Primer1:

– Forward: 5′-CTGGACTCACCATGATGACG-3′

– Reverse: 5′-GTGACCGTGTATGCTTGGTG-3′

Primer2:

– Forward: 5′-GCTTGCTGCTCTTCTTGCTT-3′

– Reverse: 5′-TGGCACTTGACAGGACAGAG-3′

Polymorphic Information Content (PIC) for each SSR locus was calculated to determine the informativeness of the markers used for locus-specific polymorphism assessment among the pearl millet varieties. Following allele separation on agarose gel, distinct allele sizes for each primer pair were scored across the varieties. The frequency of each allele at a given locus was determined from the presence/absence data matrix. PIC values were computed according to the formula described by Botstein et al.[13] From the binary data, a similarity matrix was generated using Simple Matching Coefficient (SMC) and used to generate an UPGMA dendrogram.

2.7 Statistical Analysis

Data were expressed as mean ± SD (n = 3). JASP statistical package (version 0.95.4.0) was utilised for data analysis. ANOVA with Tukey post-hoc analysis was performed at $p < 0.05$.

3. Results

3.1 Soil Characteristics

The soil was slightly acidic (pH 6.3), sandy loam, with low nitrogen content — conditions favourable for cereal growth and micronutrient availability.

3.2 Fe and Zn Distribution at Vegetative Stage

At week 3, Fe and Zn concentrations were significantly ($p < 0.05$) higher in root tissues compared to the leaves and stems. The concentration of the minerals was generally higher in leaves compared to the stem even though no significant differences were observed. The observed differences among the vegetative tissues indicate active uptake but limited translocation.

Table 1: Iron and zinc levels across different tissues of millet (*P. glaucum*) at vegetative stage (3 weeks) of growth.

(a) Iron

Pearl millet	**Fe (mg/kg)**		
	Root	**Stem**	**Leaves**
Variety-1	296.66±18.93^{a}	162.22±7.57^{b}	183.33±5.13^{b}
Variety-2	319.00±26.67^{a}	171.33±06.51^{b}	195.67±7.10^{b}
Variety-3	318.67±23.07^{a}	167.67±8.08^{b}	188.00±4.36^{b}

(b) Zinc

Pearl millet	**Zn(mg/kg)**		
	Root	**Stem**	**Leaves**
Variety-1	57.33±11.49^{a}	26.67±3.79^{b}	29.66±6.03^{b}
Variety-2	55.00±6.08^{a}	24.66±5.03^{b}	32.30±3.79^{b}
Variety-3	51.34±7.77^{a}	30.00±4.58^{b}	35.00±5.29^{b}

Values are mean±SD. Values along rows bearing dissimilar superscripts are significantly different.

3.3 Fe and Zn Distribution at Physiological Maturity. At week 12, significant differences ($p < 0.05$) were observed across tissues in Fe content in all the studied varieties. Roots retained the highest concentrations of Fe, while seeds showed the lowest accumulation, indicating poor remobilisation to grains. On the other hand, significantly higher levels of zinc were seen in the seeds relative to the other tissues.

Table 2: Iron and Zinc Levels across Different Tissues of Millet(*P. glaucum*)at Maturation Stage (12weeks)of Growth.

(a)Iron

Pearl millet	Fe (mg/kg)			
	Root	**Stem**	**Leaves**	**Seeds**
Variety-1	272.67±17.21[a]	146.33±17.95[b]	135.67±6.51[c]	46.00±7.55[d]
Variety-2	284.00±25.24[a]	157.33±28.15[b]	128.67±4.51[c]	49.67±9.07[d]
Variety-3	302.67±34.21[a]	163.00±14.73[b]	134.33±6.66[c]	47.00±7.55[d]

(b) Zinc

Pearl millet	Zn (mg/kg)			
	Root	**Stem**	**Leaves**	**Seeds**
Variety-1	24.60±5.51[a]	29.00±4.58[a]	24.32±4.93[a]	37.00±7.00[b]
Variety-2	25.00±4.58[a]	28.05±3.46[a]	23.33±4.93[a]	36.67±7.51[b]
Variety-3	21.70±3.21[a]	25.30±4.73[a]	20.67±2.10[a]	33.00±6.25[b]

Values are mean±SD. Values along rows bearing dissimilar superscripts are significantly different.

When both stages of growth were considered, a hierarchical cluster analysis (Figure 1) revealed that variety-1 and variety-2 exhibited the highest degree of relatedness based on their Fe and Zn accumulation profiles across both growth stages. Variety-3 was identified as the most distinct variety, primarily due to variations in mineral partitioning at the 12-week mark.

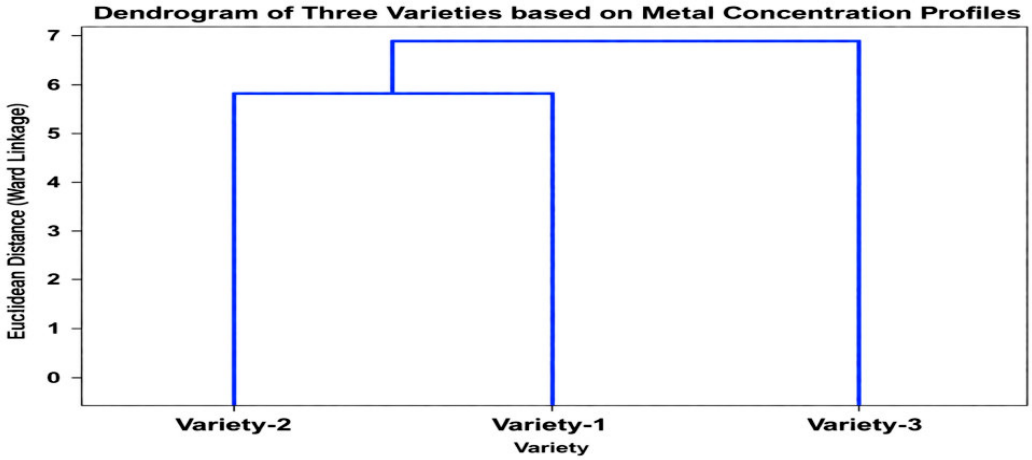

Figure 1: dendrogram of hierarchical cluster analysis showing the relatedness of the three pearl millet varieties based on iron and zinc concentration profiles.

3.4 SSR Marker–Based Polymorphism

Two SSR loci revealed allelic variation among the varieties (Table 3). Binary scoring showed polymorphism across the loci, with varieties sharing between 50–75% similarity. PIC values were 0.47 and 0.35, respectively, indicating moderate marker informativeness. Presence/absence scoring (Table 4) revealed allelic variation among varieties, with varieties A and B sharing 75% similarity across the two loci. These results demonstrate that polymorphism exists at the tested SSR loci, though genome-wide diversity cannot be inferred from only two markers.

Table 3: Simple Allelic Variation between the Pearl Millet Varieties based on the SSR Primer Pair.

SSR Primer	Locus	Allele sizes (bp)	Number of alleles	PIC
Primer-1	Locus 1	180, 185, 190	3	0.47
Primer-2	Locus 2	220, 230	2	0.35

PIC = Polymorphic information content

Table 4: Binary Scoring Matrix of Marker Loci Across Three Pearl Millet Varieties.

Variety	Primer-1 180	Primer-1 185	Primer-1 190	Primer-2 220	Primer-2 230
Variety-1	1	1	1	1	0
Variety-2	0	1	1	1	1
Variety-3	0	1	0	0	1

The dendrogram analysis revealed distinct genetic relationships among the three evaluated varieties (Figure 2). The cluster analysis partitioned the varieties into two primary groups. Variety-1 and variety-2 exhibited the highest genetic similarity and share a 60% similarity profile. On the other hand, variety-3 is the most genetically distant, sharing only 40% average similarity with the other two varieties.

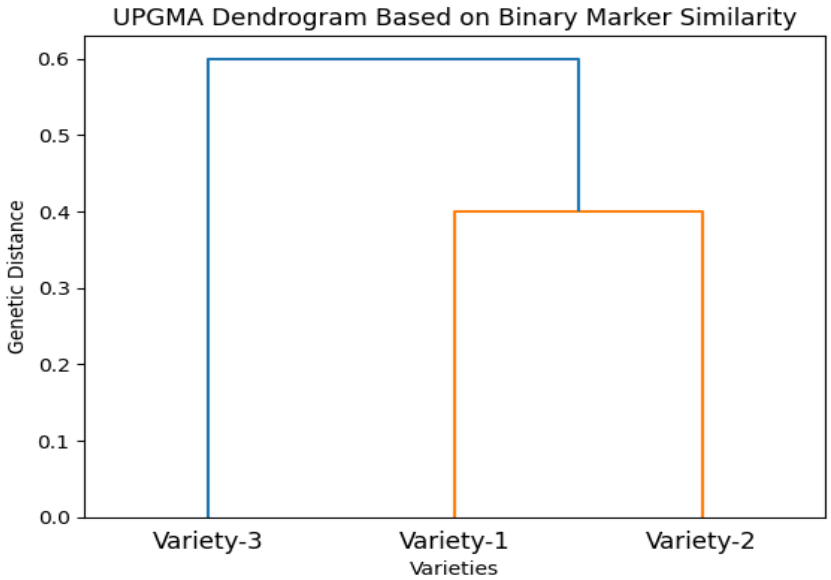

Figure 2: UPGMA dendrogram showing genetic relationship among three pearl millet varieities based on binary scoring of polymorphic marker loci.

4. Discussion

The significantly higher concentration of both Fe and Zn in roots compared to the shoots is consistent with the role of roots as the primary site of nutrient uptake from the soil. The preferential accumulation of Fe and Zn in roots at both growth stages confirms restricted mobility of these micronutrients within pearl millet.[14] Similar to other graminaceous crops, uptake occurs efficiently via root transport systems, but remobilisation through phloem into seeds is limited. This pattern is consistent with the known behaviour of Fe and Zn as elements with low phloem mobility. During maturation, transpiration-driven xylem transport declines, and without efficient remobilisation mechanisms, micronutrients remain locked in vegetative tissues. Zn uptake in plants varies and is influenced by its availability in the growth medium and the plant's ability to absorb and regulate its internal concentrations.[15,16]

The findings explain why pearl millet grains, despite adequate soil micronutrients, often contain suboptimal Fe and Zn levels. This physiological limitation presents a major challenge to natural biofortification and underscores the need for genetic strategies targeting transporter systems responsible for micronutrient remobilisation.[17]

The level of polymorphism detected among the pearl millet varieties confirms the rich genetic diversity present in local genotypes earlier reported.[17,18] This diversity is expected due to the cross-pollinating nature of pearl millet and long-term adaptation to diverse ecological niches. The PIC values observed indicate that the SSR markers used are moderately informative and could be effective for genetic characterisation of pearl millet.

The clear clustering pattern in the dendrogram suggests that variety-1 and variety-2 likely share a more common ancestor or have undergone similar selection pressures, as evidence by their high level of allelic co-occurrence. This is corroborated by the similar clustering of variety-1 and variety-2 based on the Fe and Zn levels. These observations indicate the significance of the findings towards identifying genotypes for breeding programmes aimed at improving micronutrient traits.

Importantly, the genetic variation observed provides a strong foundation for selecting parent lines with superior micronutrient uptake and remobilisation capacity. Combining genetic diversity data with mineral distribution results offers a powerful approach for identifying candidate varieties suitable for biofortification programmes.

5. Conclusion

Pearl millet demonstrates efficient absorption of Fe and Zn from soil but exhibits limited remobilisation of these micronutrients into seeds. In addition, SSR microsatellite analysis revealed substantial genetic variability among the studied pearl millet varieties, indicating a valuable genetic resource for breeding programmes. Integrating knowledge of mineral distribution with genetic variation provides a useful foundation for breeding strategies aimed at improving pearl millet grain micronutrient density.

Acknowledgements

The authors acknowledge the financial support from TETfund via IBR grant no. BUK/DRIP/TETF/040.

References

1. Singh B, Dheeravathu SN & Usha K. Micronutrient deficiency: A global challenge and physiological approach to improve grain productivity under low zinc availability. *Plant stress*, 2010, 4:76-93.
2. Arafsha SM, Aslam MF, Ellis PR, Latunde-Dada GO & Sharp PA. Strategies to increase the bioaccessibility and bioavailability of iron and zinc from cereal products. *Proceedings of the Nutrition Society*, 2023, 1-7.
3. Harish MS, Bhuker A & Chauhan BS. Millet production, challenges, and opportunities in the Asia-pacific region: a comprehensive review. *Frontiers in Sustainable Food Systems*, 2024, 8:1386469.
4. Gupta SK, Velu G, Rai KN & Sumalini K. Association of grain iron and zinc content with grain yield and other traits in pearl millet (*Pennisetum glaucum* (L.) R. Br.). *Crop Improvemen*t, 2009, 36(2):4-7.
5. Agte VV, Khot S, Girigosavi ST, Paknikar KM & Chiplonkar SA. Comparative performance of pearl millet- and sorghum-based diets vs. wheat- and rice-based diets for trace metal bioavailability. *Journal of Trace Elements in Medicine and Biology*, 1999, 13(4):215–219.
6. Ejeta G, Hassen MM & Mertz ET. In vitro digestibility and amino acid composition of pearl millet (*Pennisetum typhoides*) and other cereals. *Proceedings of the National Academy of Sciences of the United States of America*, 1987, 84(17): 6016–6019.

7. Dykes,L.,&Rooney,L.W. Sorghum and millet phenols and antioxidants. *Journal of Cereal Science*, 2006, 44(3):236–251.

8. Kasanaboina K, Ishwarya Lakshmi, VG, Nandigam SR, Jagadeesh K & Ceasar SA. The role of millets in climate-resilient agriculture for conserving global food security amidst climate change. *The Nucleus*, 2025, 1-26.

9. Kodkany BS, Bellad RM, Mahantshetti NS, Westcott JE, Krebs NF, Kemp JF & Hurrell RF. Biofortification of pearl millet with iron and zinc in a randomized controlled trial increases absorption of these minerals above physiologic requirements in young children. *Journal of Nutrition*, 2013, 143(9):1489–1493.

10. Kankarwal P, Gali SP, Meenatchi PR & Singh P. Millets' role in addressing malnutrition and ensuring foo d security in a changing climate. Enhancing Crop Resilience: Advances in *Climate Smart Crop Production Technologies.* Kumar A, Sah RP, Gowda B, Dey JK, Debnath A, Das B (ed): BIOTICA Edu Publications LLP, Tripura, India, 2024, 113-43.

11. Singhal T, Tara Satyavathi C, Singh SP, Mallik M, Anuradha N, Sankar SM & Singh N. Achieving nutritional security in India through iron and zinc biofortification in pearl millet (*Pennisetum glaucum* (L.) R. Br.). *Physiology and Molecular Biology of Plants*, 2022, 28(4):849-869.

12. Qi X, Pittaway TS, Lindup S, Liu H, Waterman E, Padi FK, Hash CT, Zhu J, Gale MD & Devos KM. An integrated genetic map and a new set of simple sequence repeat markers for pearl millet, *Pennisetum glaucum*. *Theoretical and Applied Genetics*, 2004, 109(7):1485–1493.

13. Botstein D, White RL, Skolnick M & Davis RW. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics*,1980, 32(3):314.

14. Govindaraj M, Kanatti A, Rai KN, Pfeiffer WH & Shivade H. Association of Grain Iron and Zinc Content With Other Nutrients in Pearl Millet Germplasm, Breeding Lines, and Hybrids. *Frontiers in Nutrition* 2022, 8:746625

15. Adil MF, Sehar S, Ma Z, Tahira K, Askri SM, El-Sheikh MA & Shamsi IH. Insights into the alleviation of cadmium toxicity in rice by nano-zinc and *Serendipita indica*: Modulation of stress-responsive gene expression and antioxidant defense system activation. *Environmental Pollution*, 2024, 350: 123952.

16. Jatav PK, Dadhich A, Kothari SL, Jain R & Kachhwaha S. Exploring the effects of Zinc accumulation in millets and oats: Morpho-physiological and toxicological implications. *Journal of Stored Products Research*, 2025, 114: 102756.

17. Kumar S, Hash CT, Singh G, Basava RK & Srivastava RK. Identification of polymorphic SSR markers in elite genotypes of pearl millet and diversity analysis. *Ecological Genetics and Genomic*s, 2020, 14:100051.

18. Kumar S, Jacob SR, Tara Satyavath, C, Dadlani M & Kumar MA. Utility of SSR markers in assessing the purity and identity of pearl millet hybrids. *Journal of Plant Biochemistry and Biotechnology*, 2016, 25(1):121-124.