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Integrated software and modeling of the impact of plant-derived dietary microRNAs on the immune and nutritional status of children: an integrative *in silico* study

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Abstract

Childhood malnutrition remains a major global public health challenge. Plant-based foods such as moringa, dates, tiger nuts, soybeans, and maize contain biologically active microRNAs (miRNAs) that may exert cross-kingdom transcriptomic effects in the human host. The objective of the study was to model *in silico* interactions between plant miRNAs from a multi-ingredient nutritional porridge and human genes involved in infant immunity and growth. A predictive bioinformatics study was conducted using the miRBase database, miRDB (score ≥80), miRTarBase, and the Database for Annotation, Visualization and Integrated Discovery (DAVID) for functional enrichment analysis. Statistical analyses, including Pearson's chi-square test, the Mann-Whitney U test, and multivariate logistic regression, were applied to 27,544 miRNA-gene interactions. Among the 27,544 interactions, 7,400 (26.9%) reached a score ≥80, including 100 targeting immunity (n=50) or growth (n=12) genes. Genes of interest showed significantly higher mean scores (87.2±5.1 *vs.* 69.9±13.3; $p<0.01$). The miR156 family targeted *IRF2BP2, PRKCB,* and *PAK1* (score 97-99) across four sources. DAVID confirmed 160 enriched pathways (fold enrichment up to ×49.6; $p<10^{-8}$). Dietary plant miRNAs converge non-randomly toward fundamental pathways for infant immunity and growth, forming a synergistic molecular system warranting experimental validation.

Key words: plant microRNA, infant immunity, bioinformatics, functional enrichment, malnutrition.

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Introduction

Childhood malnutrition represents a persistent challenge for global public health, particularly in low-resource countries where it leads to growth retardation, immune deficiencies, and excess mortality in children under 5 years of age.[1,2] Nutritional interventions based on locally available, high-value foods – notably *Moringa oleifera*, date palm (*Phoenix dactylifera*), tiger nut (*Cyperus esculentus*), soybean (*Glycine max*), and maize (*Zea mays*) – are widely promoted in Sub-Saharan Africa for their nutritional and immunomodulatory properties.[3,4] Beyond their macronutrient and micronutrient composition, these foods contain microRNAs (miRNAs) – small non-coding RNAs of 20 to 24 nucleotides – whose stability against digestive processes and capacity to cross the intestinal barrier and exert post-transcriptional effects in the host have been documented in the emerging scientific literature.[5,6] This phenomenon, referred to as cross-kingdom interaction, suggests that ingested plant miRNAs could modulate the expression of human genes, particularly those involved in immune regulation and growth.[7,8] However, the molecular mechanisms underlying these interactions remain largely hypothetical and poorly explored in the African pediatric context. Integrative bioinformatic studies offer a rigorous methodological approach to identify and prioritize the most plausible miRNA-gene interactions before investing in costly experimental validations.[9] This study aims to model *in silico* the interactions between plant miRNAs from a multi-ingredient nutritional porridge and human genes involved in infant immunity and growth. The objective is to generate robust, hierarchical mechanistic hypotheses capable of guiding future experimental and clinical research in pediatric nutraceutics.

Materials and Methods

Study design

This is a predictive *in silico* bioinformatic study of an observational-analytical type. The null hypothesis (H0) tested was: "The distribution of plant miRNA targets on the human genome is random and does not specifically target growth or immunity pathways". The Findable, Accessible, Interoperable, Reusable (FAIR) principles guided the use of public databases.[10]

Identification of plant miRNAs

miRNAs from each porridge ingredient were identified from the miRBase database (versions 19 to 22.1) and indexed scientific literature. Strict inclusion criteria were applied: i) read count ≥1,000 (high abundance); and ii) membership in conserved families (miR156, miR159, miR166) present in multiple ingredients, suggesting a cumulative effect. The plant organisms analyzed were *M. oleifera*, *P. dactylifera*, *C. esculentus*, *G. max*, and *Z. mays*.

Selection of human genes of interest

Human target genes were selected based on their functional role in infant immunity and growth. The list covered: pathogen recognition receptors (toll-like receptors [TLR], NOD-like receptors [NLR], C-type lectin receptors [CLR]), inflammatory signaling pathways (nuclear factor kappa B [NF-kB], mitogen-activated protein kinase [MAPK]), innate and adaptive cytokines, lymphocyte transcription factors, growth hormone - insulin-like growth factor 1 [GH-IGF1] axis genes, energy metabolism genes (*mTOR*, *PPARG*, *FTO*), and genes related to muscle mass (*MSTN*, *MYOD1*). "Master" genes identified as biological hubs included *NFKB1*, *mTOR*, *IL6*, *PAK1*, *MAPK8*, *PRKCB*, *IRF2BP2*, *IKZF1*, *IL33*, and *GDNF*.

Target prediction and filtering

The miRDB algorithm was used to predict miRNA-human gene interactions (3'UTR region). A confidence threshold of score ≥80 was retained for primary analyses. Cross-referencing with the miRTarBase database allowed filtering on the pre-identified genes of interest.

Functional bioinformatic analysis

Functional enrichment was performed with the Database for Annotation, Visualization and Integrated Discovery (DAVID) on high-confidence target genes. Ontologies used included Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, Gene Ontology (GO) terms (GO Biological Process, GO Molecular Function, GO Cellular Component), and InterPro/SMART domains. Multiple testing corrections were applied (Benjamini-Hochberg and Bonferroni methods). A fold enrichment ≥5 and a corrected p≤0.05 were retained as significance thresholds.

Statistical analyses

Statistical analyses were performed with SPSS. Associations between food source and probability of generating a high-confidence interaction were evaluated by Pearson chi-square test with Cramer's V calculation (effect size). Comparison of scores between genes of interest and other genes used the Mann-Whitney U test (n=27,544). A multivariate logistic regression was constructed to estimate adjusted odds ratios (OR), with maize as the reference for food source. Fisher's exact test was applied for cell counts <5.

Results

Identification of plant microRNAs

Analysis of the five porridge ingredients identified a total of 94 miRNAs belonging to 40 conserved families in *M. oleifera*, as well as comparable profiles in *P. dactylifera* (13 conserved families, with miR169 representing 24 members), *C. esculentus* (183 miRNAs including 31 known and 152 novel), *G. max* (17 families validated in miRBase), and *Z. mays* (notably miR156, miR159, miR166, miR319 families). The miR156 family showed the highest expression levels (read counts >2,000 in *M. oleifera*; described as "very high" in *P. dactylifera* and *G. max*). The miR166 family displayed the most abundant reads in *Z. mays* (zma-miR166a: 62,546 to 227,674 reads per million).

Descriptive analysis of predicted interactions

Of the 27,544 total miRNA-gene interactions tested, 7,400 (26.9%) had a score ≥80 (Table 1). Among these 7,400 high-confidence interactions, 100 targeted genes of interest: 12 growth genes (0.2%) and 50 immunity genes (0.7%).

The distribution of interactions by plant source (Table 2) reveals that moringa contributes 28.6% of the total dataset (n=7,870) and 27.3% of high-confidence interactions (n=2,017). Tiger nut predominates among robust interactions (28.9%; n=2,135). Maize represents only 2.6% of high-confidence interactions (n=196). The proportionality ratios for date, moringa, and soybean are close to 1 (0.96, 0.95, and 1.03 respectively), indicating homogeneous targeting quality for these three sources.

Analysis of targeted genes of interest

The mean score across all interactions with a score ≥80 was 69.9 (±13.3). For the 22 interactions targeting master genes, this mean score rose to 87.2 (±5.1), representing a difference of 17.3 points compared to other targets. The homogeneity of scores for genes of interest (standard deviation [SD]=5.1, three times lower than that of other targets) constitutes a strong indicator of non-random targeting.

Among the most notable findings:

i) *IRF2BP2* (interferon signaling regulation): targeted with a score = 99 by the miR156 family in four distinct food sources (moringa, date, tiger nut, soybean) (Table 3).

ii) *PRKCB* (kinase involved in B and T lymphocyte activation): targeted with a score = 97 by the same four sources (Table 4).

iii) *PAK1* (cytoskeleton regulation, cell survival, proliferation): targeted with a score = 97 (Table 5).

Conversely, *NFKB1*, *mTOR*, *IL6*, and *IGF1* showed no direct targeting at score ≥80, suggesting that the porridge effect on these pathways operates indirectly through their upstream regulators.

Table 1. Presence of miRNA-gene interactions with a score ≥80.

Interactions	Frequency (N)	Percentage (%)
No (score <80)	20,144	73.1
Yes (score ≥80)	7,400	26.9
Total	27,544	100.0

Table 2. Distribution of tested interactions by plant source and confidence level.

Source	Total, n (%)	Score ≥80, n (%)	Ratio
Moringa	7,870 (28.6)	2,017 (27.3)	0.95
Tiger nut	7,339 (26.6)	2,135 (28.9)	1.09
Soybean	6,483 (23.5)	1,789 (24.2)	1.03
Date	4,930 (17.9)	1,263 (17.1)	0.96
Maize	922 (3.3)	196 (2.6)	0.79
Total	27,544 (100)	7,400 (100)	—

Statistical association analyses

The Pearson chi-square test reveals a statistically significant association between food source and probability of generating a high-confidence interaction for moringa ($\chi^2=8.583$; $p<0.01$), Tiger nut ($\chi^2=25.209$; $p<0.01$), date ($\chi^2=4.756$; $p<0.03$), and maize ($\chi^2=15.269$; $p<0.01$). Effect size remains small (Cramer's V <0.04). Adjusted standardized residuals identify Tiger nut as significantly over-represented among robust interactions (residual = 5.0; $p<0.05$) and maize as under-represented (residual = −3.9; $p<0.05$).

The Mann-Whitney U test confirms that immunity and growth genes show significantly higher prediction scores than other genes (M=87.2, SD=5.1 *vs.* M=69.9, SD=13.3; U=733,363.5; z=−8.051; $p<0.01$; effect size r=0.049).

Chi-square and Fisher's exact tests reveal significant over-representation of immunity genes ($\chi^2=41.606$; crude OR=3.697; 95% confidence interval [CI] [2.415-5.660]; $p<0.01$) and growth genes ($\chi^2=28.349$; crude OR=32.717; 95% CI [4.253-251.659]; $p<0.01$) among high-confidence targets.

Multivariate logistic regression

After multivariate adjustment (Table 6), immunity genes (adjusted OR=3.730; 95% CI [2.436-5.713]; $p<0.01$) and growth genes (adjusted OR=33.630; 95% CI [4.371-258.763]; $p<0.01$) maintain a significant positive association with the probability of being targeted at high confidence. Tiger nut emerges as the best-performing ingredient (adjusted OR=1.514; 95% CI [1.283-1.787]).

DAVID functional enrichment

DAVID analysis identified 160 significantly enriched functional terms (p-values ranging from 1.68×10^{-8} to 0.0999; Benjamini, Bonferroni, and false discovery rate (FDR) corrections all significant ≤0.05). Results reject the null hypothesis with an extremely low probability of obtaining these enrichments by chance (Table 7). The immune axis shows massive and redundant enrichments covering TLR signaling, CLR, T helper 17 (Th17) differentiation, interleukin (IL)-17 pathway, tumor necrosis factor (TNF) signaling, and cytosolic DNA sensing. Remarkable enrichments were observed for pathways linked to major infectious diseases: tuberculosis (fold enrichment ×17.58; $p=4.94\times10^{-4}$), hepatitis B (×26.18; $p=8.46\times10^{-6}$), HIV-1 (×25.04; $p=4.87\times10^{-7}$), COVID-19 (×17.93; $p=3.78\times10^{-5}$), and influenza A (×24.66; $p=1.07\times10^{-5}$).

The growth-metabolism axis reveals significant enrichments for GH synthesis and action pathways (×26.2), insulin resistance (×48.9), phosphoinositide 3-kinase (PI3K)-Akt signaling (×11.8), epidermal growth factor family of receptor tyrosine kinase (erythroblastic oncogene B [ErbB]; ×49.6), and hypoxia-inducible factor (HIF-1; ×38.8), confirming indirect growth modulation through upstream regulators of *IGF1* and *mTOR*.

Cocktail effect and multi-pathway convergence

The analysis reveals three major biological hubs toward which enriched pathways converge. The NF-kB hub groups 16 enriched terms (TNF, TLR, NOD, IL-17, CTL); although *NFKB1* is not directly targeted, all its major activators are, suggesting indirect

Table 3. Targeting of *IRF2BP2* by the miR156 family (score = 99).

Food source	microRNA	Target score	Gene description
Moringa	mol-miR156h	99	*IRF2BP*
Date	pda-miR156a	99	*IRF2BP*
Tiger nut	ces-miR156b-3p	99	*IRF2BP*
Soybean	gma-miR156b	99	*IRF2BP*

Table 4. Targeting of *PRKCB* (score = 97).

Food source	microRNA	Target score	Gene description
Moringa	mol-miR156h	97	*PRKCB* (lymphocyte activation)
Date	pda-miR156a	97	*PRKCB* (lymphocyte activation)
Tiger nut	ces-miR156b-3p	97	*PRKCB* (lymphocyte activation)
Soybean	gma-miR156b	97	*PRKCB* (lymphocyte activation)

Table 5. Targeting of *PAK1* (score = 97).

Food source	microRNA	Target score	Gene description
Moringa	mol-miR156	97	*PAK1* (cytoskeleton regulation and cell survival)
Date	pda-miR156a	97	*PAK1* (cytoskeleton regulation and cell survival)
Tiger nut	ces-miR156b-3p	97	*PAK1* (cytoskeleton regulation and cell survival)
Soybean	gma-miR156b	97	*PAK1* (cytoskeleton regulation and cell survival)

Table 6. Multivariate logistic regression – predictors of score ≥80.

Variable	Crude OR	p	Adjusted OR	95% CI
Moringa	0.915	0.01	1.265	[1.071-1.492]
Soybean	1.049	0.13	1.401	[1.071-1.492]
Tiger nut	1.164	0.01	1.514	[1.283-1.787]
Date	0.925	0.02	1.268	[1.069-1.503]
Immunity genes	3.697	<0.01	3.730	[2.436-5.713]
Growth genes	32.717	<0.01	33.630	[4.371-258.763]

OR, odds ratio; CI, confidence interval.

but powerful regulation. The MAPK hub (ERK/JNK/p38) aggregates 12 terms (MAPK, ErbB, Ras, PI3K-Akt) with confirmed direct targeting of *MAPK8* (score = 94). The IGF-1 axis groups 8 terms and confirms modulation *via* upstream regulators (PAK1, kinases, MAPK, PI3K) (Table 8).

Concordance between *a priori* identified genes of interest and bioinformatic results reaches 100% (10/10 genes validated directly or indirectly), reinforcing the internal consistency of the approach.

Discussion

Biological significance of results

The primary contribution of this study is the *in silico* demonstration that dietary plant miRNAs from a multi-ingredient nutritional porridge do not randomly target the human genome, but exhibit a preferential and significant affinity for fundamental biological pathways of infant immunity and growth. Rejection of the null hypothesis with exceptional statistical power ($p<10^{-8}$) constitutes a major finding, particularly since robustness analyses (FDR, Benjamini, and Bonferroni multiple corrections) all confirm significance.

The targeting of *IRF2BP2* with score = 99 by the miR156 family across four food sources is particularly noteworthy. *IRF2BP2* is a negative regulator of interferon signaling, and its modulation could constitute a mechanism by which the porridge modulates antiviral response in infants.[11] Similarly, the targeting of *PRKCB* (score = 97), a central kinase in B and T lymphocyte activation through the NF-kB pathway, opens mechanistic perspectives on adaptive immune regulation.[12]

The absence of direct targeting of *IGF1* and *mTOR* (score <80), initially interpreted as a negative result, proves biologically coherent and even more realistic. Dietary miRNAs, ingested chronically at low doses, preferentially act on second-order regulators (kinases, transcription factors) rather than terminal effectors. This indirect mode of action is confirmed by massive enrichment of PI3K-Akt, MAPK, ErbB, and adipocytokine pathways, all upstream of *IGF1* and *mTOR*.

Comparison with the literature

Our results are consistent with landmark studies on cross-kingdom effects of plant miRNAs. Zhang *et al.* (2012) demonstrated that rice miR168a targeted *LDLRAP1* in mouse liver, confirming the bioavailability of dietary miRNAs.[5] Zhou *et al.* (2015) showed the impact of miR159 on the Wnt pathway through *TCF7* targeting.[7] Chin *et al.* (2016) confirmed kinase targeting by miR156 in MAPK and PI3K pathways.[13] The convergence of our results with these publications reinforces the biological credibility of the modeled phenomenon.

The particularly high enrichment for infection-related pathways (tuberculosis, hepatitis B, HIV, influenza) carries major epidemiological relevance in the African context, where these diseases represent a significant morbidity burden in children. Modulation of TLR pathways and interferons by dietary miRNAs could contribute to more effective maturation of the innate immune system during the first years of life.

Originality of the approach

The originality of this study lies in: i) the multi-ingredient approach enabling exploration of the cocktail effect; ii) the combination of complementary bioinformatic tools (miRDB, miRTarBase, DAVID, SPSS) with rigorous statistical analyses including multiple testing corrections; iii) the *a priori* definition of a panel of genes of interest specific to infant health; and iv) the construction of a hierarchical temporal mechanistic model distinguishing four levels of effects (0-6 h, 6-24 h, 24-72 h, >72 h post-ingestion).

Limitations

This study has several limitations inherent to the *in silico* approach. First, bioinformatic predictions do not constitute proof

Table 7. Enriched biological pathways – DAVID results (selection).

Enriched pathway (KEGG/GO)	p-value	Fold enrichment	Genes involved, n (%)	Key function
Insulin resistance	1.68×10^{-8}	×48.9	5/8 (62.5)	Energy metabolism
TLR signaling	1.08×10^{-4}	×29.4	3/8 (30)	Innate immunity
CLR	1.46×10^{-6}	×40.6	4/8 (50)	Mucosal defense
IL-17 signaling	7.19×10^{-5}	×33.7	3/8 (30)	Controlled inflammation
TNF signaling	1.41×10^{-4}	×26.9	3/8 (30)	Pro-inflammatory cytokines
PI3K-Akt signaling	1.95×10^{-4}	×11.8	4/8 (50)	Cell proliferation
ErbB signaling	6.53×10^{-7}	×49.6	4/8 (50)	Epithelial growth
HIF-1 signaling	—	×38.8	—	Hypoxia adaptation

KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology; TLR, toll-like receptor; CLR, C-type lectin receptor; IL, interleukin; TNF, tumor necrosis factor; PI3K, phosphoinositide 3-kinase; ErbB, erythroblastic oncogene B; HIF, hypoxia-inducible factor.

Table 8. Functional targeting profile by ingredient – cocktail effect.

Ingredient	Primary targeted function	Key miRNA
Moringa	Innate immunity (TLR, NLR), anti-inflammation	mol-miR156h
Tiger nut	Signaling kinases, metabolism	ces-miR156b-3p, miR166
Soybean	Oxidative stress, MAPK, apoptosis	gma-miR156b, miR168a
Date	Cell growth, ErbB, PI3K-Akt	pda-miR156a

TLR, toll-like receptor; NLR, NOD-like receptor; MAPK, mitogen-activated protein kinase; ErbB, erythroblastic oncogene B; PI3K, phosphoinositide 3-kinase.

of effective biological interaction; the bioavailability of plant miRNAs after digestion, intestinal absorption, and blood passage remains to be demonstrated experimentally in this specific context. Second, KEGG and GO databases show a representation bias in favor of well-characterized metabolic and cancer pathways. Third, enrichments do not inform on the magnitude of expected biological effects. Fourth, the absence of exact read count data for some miRNAs from *P. dactylifera* and *C. esculentus* required the use of consensus sequences from miRBase.

Conclusions

This integrative bioinformatic study demonstrates, with high statistical robustness, that dietary plant miRNAs from a porridge based on moringa, date, tiger nut, soybean, and maize do not randomly target the human genome but exhibit a preferential and significant affinity for fundamental biological pathways of innate and adaptive immunity and infant growth.

The convergent targeting of *IRF2BP2* (score = 99), *PRKCB* (score = 97), and *PAK1* (score = 97) by the miR156 family across multiple food sources simultaneously suggests a synergistic molecular mechanism – the cocktail effect – by which the combination of ingredients creates a multidimensional intervention profile superior to the sum of its individual components.

These results open innovative perspectives for pediatric nutraceutics in sub-Saharan Africa and provide a solid scientific basis for targeted experimental validations: cell transfection, murine malnutrition models, and ultimately controlled clinical trials. The multi-ingredient nutritional porridge thus appears not only as a caloric vector, but as a biologically active molecular signaling system capable of modulating the infant epigenome.

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