ISOLAMENTO, CARACTERIZAÇÕES, ANTICÂNCER, CITOTOXICIDADE, TOXICIDADE *IN SILICO* E AVALIAÇÕES ANTIMICROBIANAS DA PROTEÍNA DE SEMENTES DE *MORINGA OLEIFERA*

ISOLATION, CHARACTERIZATIONS, ANTI-CANCER, CYTOTOXICITY, *IN SILICO* TOXICITY, AND ANTIMICROBIAL EVALUATIONS OF *MORINGA OLEIFERA* SEED PROTEIN

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RESUMO

Introdução: A semente de *Moringa oleifera*, que é amplamente utilizada como uma fonte acessível de tratamento doméstico de água, também é conhecida por suas várias aplicações farmacológicas em todo o mundo. **Objetivo:** Este estudo avalia a toxicidade e o potencial farmacológico da proteína da semente de *Moringa oleifera* ao estabelecer o perfil químico. **Método:** Espectrometria de massa de tempo de voo de dessorção/ionização a laser assistida por matriz (MALDI-TOF-MS) e cromatografia líquida acoplada com espectrometria de massa de ionização por eletrospray (LC-ESI-MS/MS) foram usados para analisar o conteúdo de proteína/peptídeo da proteína purificada. A proteína também foi investigada quanto ao potencial anticâncer *in vitro* nas linhas de células Hela e MDA-MB-231, citotóxica na linha de células 3T3 e toxicidade para camarões de salmoura. Além disso, foi avaliada a ação antimicrobiana da proteína (polipeptídeo). **Resultados e discussão:** O MALDI-MS revelou três frações de proteína com valores de 3,4, 4,6 e 6,9 kDa. Em contraste, a análise por LC-ESI-MS/MS revelou dipéptidos e tripéptidos, que incluem serinilarginina, leucilprolina, leucilmethionilglicina, isoleucilglicilmetinina, ácido glicilarginilaspártico, isoleucylglutamicacidmethionine, difenilalanina, asparaginyllaspartylhistidine, seril-tirosil tirosina, fenilalanilasparaginiltirosina, e proliltrofanilglicina. A proteína da semente exibiu toxicidade extremamente baixa em ambas as linhas de células de câncer de mama 3T3 e MDA-MB-231, enquanto os padrões, doxorubicina e cicloheximida tiveram $IC_{50} = 0,5 \pm 0,07$ e $0,8 \pm 0,10$ $\mu\text{g/mL}$, respectivamente. Além disso, a proteína não mostrou atividade antibacteriana ou antifúngica contra todos os organismos testados. A proteína também não exibiu letalidade contra o camarão de água salgada. Os resultados de toxicidade *in-silico* indicaram que os peptídeos não são imunotóxicos, carcinogênicos ou mutagênicos, pois a maioria pertencia à classe V de toxicidade, exceto serilarginina prevista para classe de toxicidade IV (prejudicial se ingerido). Portanto, a ingestão do remanescente de proteína na água pode estar dentro do limite tolerável. **Conclusão:** Os resultados obtidos sugerem que o conteúdo protéico da semente de *Moringa oleifera* é não citotóxico. O resultado valida ainda mais o potencial de segurança do material de sementes desengorduradas e desengorduradas usadas como fontes potenciais de alimento para humanos e animais.

Palavras-chave: Peptídeo; MALDI; ESI-MS/MS; Doxorubicina; *in silico*.

ABSTRACT

Background: *Moringa oleifera* seed, which is widely utilized as an affordable source of domestic water treatment, is also reputable for its various pharmacological applications globally. **Aim:** This study evaluates the toxicity and pharmacological potentials of *Moringa oleifera* seed protein while establishing the chemical profile. **Method:** Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and liquid chromatography coupled with electrospray ionization mass spectrometry (LC-ESI-MS/MS) were used to analyze the protein/peptide content of the purified protein. The protein was also investigated for *in vitro* anti-cancer potential on Hela and MDA-MB-231 cell lines, cytotoxic on 3T3 cell line, and toxicity to brine shrimps. In addition, the antimicrobial action of the protein (polypeptide) was evaluated. **Results and Discussion:** The MALDI-MS revealed three protein moieties with values of 3.4, 4.6, and 6.9 kDa. On the other hand, LC-ESI-MS/MS analysis revealed dipeptides and tripeptides, which include serinylarginine, leucylproline, leucyl-methionyl-glycine, isoleucyl-glycyl-methionine, glycyl-arginyl-aspartic acid, isoleucyl-glutamyl-methionine, diphenylalanine, asparaginyl-aspartyl-histidine, seryl-tyrosyl-tyrosine, phenylalanyl-asparaginyl-tyrosine, and propanolyl-tryptophanyl-glycine. The seed protein exhibited extremely low toxicity on both 3T3 and MDA-MB-231 breast cancer cell lines, while the standards, doxorubicin, and cycloheximide had $IC_{50} = 0.5 \pm 0.07$ and 0.8 ± 0.10 $\mu\text{g/mL}$, respectively. Further, the protein showed no antibacterial or antifungal activity against all tested organisms. The protein also exhibited no lethality against brine shrimp. The *in-silico* toxicity results indicated that the peptides are not immunotoxic, carcinogenic, or mutagenic as most belonged to the tox class V except serylarginine predicted for tox class IV (harmful if swallowed). Hence, the intake of the protein remnant in water could be within the tolerable limit. **Conclusion:** The results obtained suggested that the protein content of the *Moringa oleifera* seed is non-cytotoxic. The result further validates the safety potential of the defatted and debittered seed material used as potential food sources for both humans and animals.

Keywords: Peptide; MALDI; ESI-MS/MS; Doxorubicin; In-silico

1. INTRODUCTION:

The seed of *Moringa oleifera* Lam. belonging to the family *Moringaceae*, is widely consumed globally, especially in African and Asian rural settings, for its nutritional and medicinal values (Falowo *et al.*, 2018). However, some measures of toxicity such as mutagenicity and genotoxicity are reportedly associated with the consumption of the seed (Araújo *et al.*, 2013; Villasenor *et al.*, 1989). The toxicity associated with seed consumption has been a challenge for the extensive utilization of the seed for various food and ethnomedicinal purposes (Falowo *et al.*, 2018; Liu *et al.*, 2018). A previous study on the *Moringa oleifera* seed has indicated the various phytochemical constituents, including niazimicin, niazidin, glucomoringin isothiocyanate, niazinin acetate, niazinin triacetate, niazirin, glucotropaeolin, triolein, and trivaccenin (Atolani *et al.*, 2020).

Previous work indicated that the seed extracts with high lipid content possess good antioxidant and low proteinase inhibition potentials, among others (Atolani *et al.*, 2018), other studies (Njan *et al.*, 2018) further revealed that the alcoholic extract of the *M. oleifera* seed could induce infertility in the male animal by attenuating semen pH, whilst also exhibiting

hepatotoxic effects due to persistent increase in ALP and AST even after withdrawal of the extract being administered. We further reported that the seed extractives exhibited high renal toxicity in another (Olorundare *et al.*, 2015). Having extensively examined the chemical composition and pharmacological activities and the toxicity associated with the various extracts of the *M. oleifera* seed, we here investigated the composition of the seed protein and the corresponding toxicity potential, which hitherto has not been well explored. The study, among other things, indicates a contribution of the seed protein to the overall inherent toxicity of the *M. oleifera* seed.

2. MATERIALS AND METHODS:

2.1. Plant Material

The seeds of *Moringa oleifera* were obtained as previously indicated (Atolani *et al.*, 2020). The seed was authenticated and documented with the voucher number UILH/002/1008 at the herbarium of the Department of Plant Biology, University of Ilorin, Nigeria. The air-dried seeds were de-shielded and pulverized. The powdered material was kept in a cool place for further work.

2.2. Extraction

Powdered seed material (2,000 g) were subjected to exhaustive extraction using ethanol for 9 days to remove all lipid-soluble extractives. This was followed by extraction with water for 5 days. The aqueous extract was thereafter filtered and partitioned with dichloromethane (DCM) to get rid of the non-polar content of the aqueous extract. The resultant aqueous solution was allowed to settle down for the precipitation of the protein. After about five days the precipitate collected via decantation was washed repeatedly with solvents such as hexane, DCM, and ethanol sequentially. The purified protein fraction was dried, and the solid mass obtained was further washed with DCM to remove traces of lipid, followed by methanol to remove other organic impurities, and cold water was finally used to wash it. The purified *M. oleifera* seed protein-tagged (MOSP) was kept in a cool dark place until further analyses (Mann *et al.*, 2001).

2.3. Peptide Profiling: HPLC-TOF/Q-TOF-ESI-MS/MS (TANDEM-MS)

The peptide profile of the MOSP was determined by subjecting the digested sample to Agilent Technologies High-Pressure Liquid Chromatography (HPLC) 6200 series-coupled to a Time-of-Flight Mass Spectrometry (TOF/Q-TOF-ESI-MS/MS). The HPLC was equipped with a binary pump, column component, and HiP Sampler Model G4226A, and mass MS Q-TOF component G6550A. Gradient elution mode was applied for the analysis as mobile phase A composed of 100% water and mobile phase B was 100% acetonitrile. The gradient program was set at 95.00 % A: 5.00 % Band increased to A: 0.00 % B:100.00 %. The flow rate was set to 0.3 mL/min as the maximum pressure limit was 1200.00 bars at a stop-time of 30 min. 3.00 μ L of the analyte was injected via needle wash injection mode as the right temperature was set at 45°C. The switch time of 0.01 min was used. The MS Q-TOF has a dual AJS ESI ion source and an MS/MS (TANDEM) absorption threshold of 5 with a mass acquisition range of 163 to 1000 at a scan rate of 1.00 spectra per second. The MOSP was dissolved in DMSO and warmed at 50°C before analysis. The mass determination in selective ion monitoring (SIM) mode was done with both positive and negative ion electrospray ionization methods for effective quantification of the analyte following standard procedure (Grimalt *et al.*, 2005).

2.4. Protein Analysis

The MOSP was characterized using Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) Bruker Daltonic, model Ultraflex III, equipped with smart beam laser, Germany. A prepared sinapinic acid matrix was used for a scan range of 5 – 100 KDa. An aliquot (1 μ L) of the sample was injected into the machine following standard dried droplet preparation (Gauss *et al.*, 1999).

2.5. Cytotoxicity evaluation – cell viability assay (HeLa, MDA-MB-231, and 3T3 cell lines)

The MOSP was assessed for cytotoxicity via MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyl-tetrazolium bromide) colorimetric assay (Mosmann, 1983). In this assay, HeLa (Cervical Cancer Cell), MDA-MB-231 (Breast Cancer Cell line ATCC- HTB-26), and 3T3 (Mouse fibroblast, ATCC-1658) were maintained in a standard culture medium consisting of a mixture of streptomycin, penicillin, Dulbecco's Modified Eagle's Medium and 5% fetal bovine serum, in an atmosphere of 5% CO₂ and 37°C. At 80% confluence, cells were sub-cultured and seeded (1x10⁵ cells/mL) into 96-well plate. After 24 h incubation, test compounds (1-100 μ M) prepared in the fresh medium were added, and incubation was carried out for 72 h. Cell viability was assessed by MTT colorimetric measurement at 570 nm on a microplate reader (Molecular Devices, CA, USA). Doxorubicin and cycloheximide were used in this assay as positive drug controls for breast, cervical, and HeLa cancer cell lines. All experimental preparations were made in triplicate. The cytotoxicity was estimated using Equation 1.

$$\% \text{Inhibition} = 100 - \left[\left(\frac{A_{\text{Sample}} - A_{\text{negative control}}}{A_{\text{positive control}} - A_{\text{negative control}}} \right) * 100 \right] \text{ (Eq. 1)}$$

where A is the Absorbance of either sample or control

2.6. Assay for antifungal activity

The antifungal activity evaluation of MOSP, standard agar dilution protocol as described previously elsewhere (Osho *et al.*, 2015), was adopted. The test organisms used include *Candida albicans*, *Aspergillus niger*, *Microsporium canis*, *Trichophyton rubrum*, *Fusarium lini*, *Candida glabrata*. Briefly, 12 mg/mL MOSP stock solution in

dimethylsulphoxide solution was prepared for further serial dilution. Inoculum of each fungus was introduced to each tube on Sabouraud dextrose agar growth media. Meanwhile, an agar surface streak method was used to evaluate non-mycelial growth. The solvent and miconazole were respectively used as negative and positive controls as the tubes were incubated for 72-168 h at 27-29°C. Linear growth (mm) in media was measured, and growth inhibition was determined with reference to the control. The minimum inhibitory concentration (MIC) for test compounds and reference drugs was estimated dose-response plot obtained by repeating the assay process at different dose levels of test compounds or reference drug (10, 25, 50, 100 mg.mL⁻¹).

2.7. Antibacterial assay

The antibacterial potential of MOSP was evaluated against both gram-negative and gram-positive bacterial which include *Salmonella typhi* (ATCC 14028), *Bacillus subtilis* (ATCC 23857), *Staphylococcus aureus* (NCTC 6571), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 10145), and in Mueller Hinton medium in a 96 well plate following a previously reported protocol (Sarkar *et al.*, 2007). The test compounds (300 µg/mL) were added to the wells except for the control well. Imipenem was used as drug control. After 20 h incubation, Alamar Blue Dye was added, and this was followed by a 3h-controlled shaking at 80 rpm. The color change of the dye from blue to pink was taken as the growth of the bacterial strains. Absorbance reading was taken at 570 and 600 nm on a microplate reader. All experiments were carried out in triplicate.

2.8. Toxicity assay using brine shrimp

Brine shrimp toxicity assay, a rapid, inexpensive, general toxicity bioassay was used to examine the toxicity of MOSP following a procedure described previously (Kivack *et al.*, 2001). The sample (20 mg) dissolved in DMSO was diluted to concentrations in the range 10 to 1000 µg/mL. Etoposide prepared in a similar solution served as positive control while distilled water served as the negative control. Shrimp survival was estimated periodically for 24 h, and the data was collected appropriately.

2.9. Computational toxicity studies

The *in silico* evaluations of the absorption, distribution, metabolism, excretion, and toxicity,

otherwise known as ADMET properties, were performed using the ProTox-II platform and SuperCYPsPred webserver (Banerjee *et al.*, 2018; Atolani *et al.*, 2020). While ProTox-II is a virtual platform for the evaluation of chemical-based toxicities, the SuperCYPsPred web server is a prediction model for five major CYPs isoenzymes, namely; CYP1A2, CYP2C19, CYP2D6, CYP2C9, and CYP3A4. Precisely, over 80% of clinical drug metabolism are accounted for using the indicated CYPs isoenzymes.

2.10. Data presentation and statistical analysis:

Data analysis was achieved using a Finney computer software to estimate IC₅₀ values at 95% confidence interval. The results represent the mean ± standard error of the mean values of three different experiments except where indicated otherwise.

3. RESULTS AND DISCUSSION:

3.1. Results

3.1. MALDI-TOF-MS Analysis

The characterization of complex protein mixtures using MALDI and TOF-MS are well established and reported in the literature. The MALDI analysis of MOSP (Figure 1) revealed the presence of three proteins with size values of 5712 (3.4 kDa), 7801 (4.6 kDa), and 11462 (6.9 kDa).

3.2. HPLC-MS/MS Analysis

Electrospray ionization - MS/MS is a viable option for identifying peptides (Ghebremichael *et al.*, 2005). Here, an MS/MS with a relative threshold of 0.010% coupled to HPLC operated at both positive and negative modes was used to analyze the MOSP to identify the peptide and amino acid constituents of the digested protein. A total of 8 and 6 peptides/amino acids were obtained in positive and negative modes, respectively (Tables 1 and 2).

3.3. Result of Cytotoxicity Assay

The cytotoxicity of MOSP was examined on HeLa, MDA, and 3T3 cell lines to evaluate its anti-cancer properties or otherwise. A low degree of inhibition (14.3%) was recorded on HeLa cell lines while there was no detectable activity on both MDA-MB-231 and the 3T3 cell lines (Table 3).

3.4 Antibacterial and Antifungal Activities

The result of the antimicrobial activity of the MOSP is as indicated in Table 4. The MOSP did not show significant antibacterial activity against all the organisms (both gram-negative and gram-positive) tested compared to the standard imipenem and miconazole.

3.5. The Brine Shrimp Toxicity Evaluation

The brine shrimp test is a viable means of evaluating the toxicity potential of natural products. The result of the brine shrimp lethality test is as indicated (Table 5).

3.6. *In silico* Evaluation

Computational toxicity prediction studies using ProTox-II platform (Banerjee *et al.*, 2018; Atolani *et al.*, 2020) suggest that all the peptides at the positive mode ESI are safe concerning their acute toxicity profiles, mostly belonging to the tox class V (may be harmful if swallowed); except serylarginine predicted for tox class IV (harmful if swallowed). Leucylproline, Glycine, N-(N-L-methionyl-L-leucyl)- shows probable binding to corticotropin-releasing factor Receptor 1 (off-targets/toxicity targets) with an average of 71.57 and 74.7. Tanimoto similarity to known ligands of the targets, respectively. N-Formyl-methionyl-leucyl-phenylalanine shows probable binding with the Opioid Receptor Mu with an average similarity of 80.97 (Tanimoto) with its known ligands (Table 6).

3.2. Discussion

The MALDI-TOF-MS analysis (Figure 1) result was corroborated by a previous report where three protein peaks were also obtained (Ghebremichael *et al.*, 2005). It was also reported that more than one protein family is present in MOSP (Gassenschmidt *et al.*, 1995). Various analyses of plant protein also indicated that closely related proteins are formed during different developmental Stages in plants. Interestingly, a non-protein and non-polysaccharide compound has been isolated from *M. oleifera* seed (Okuda *et al.*, 2001).

The DB difference of not more than ± 15 ppm was observed in both modes from the marching (Tables 1 and 2). Peptides are partly hydrolyzed or degraded products of protein which can be further hydrolyzed to the basic amino acid units. Peptides have amino acid units ranging

from 2 to 50 linked by peptide bonds (Nelson and Cox, 2005). Naturally occurring peptides have varying complexities, compositions, and functions. Two dipeptides, Serinyl-Arginine (Ser-Arg) and leucyl-proline (Leu-Pro), and four tripeptides obtained include leucyl-methionyl-glycine (Leu-Met-Gly), isoleucyl-glycyl-methionine (Ile-Gly-Met), glycyl-arginyl-aspartic acid (Gly-Arg-Asp), isoleucyl-glutamyl-methionine (Ile-Glu-Met) were obtained in the positive mode. Other peptides detected included N-formyl-methionyl-alanine and N-formyl-methionyl-phenyl alanine; both peptides are amino acids of methionine. Two dipeptides, namely di-phenylalanine (Phe-Phe) and tyrosyl-phenylalanine (Tyr-Phe), were obtained in the negative mode. In contrast, four tripeptides obtained are asparaginyl-aspartyl-histidine (Asn-Asp-His), seryl-tyrosyl-tyrosine (Ser-Tyr-Tyr), Phenylalanyl-asparaginyl-tyrosine (Phe-Asn-Tyr), and propanolyl-tryptophanyl-glycine (Pro-Trp-Gly). The peptides are well reported in seeds of plants (Gassenschmidt *et al.*, 1995; Ghebremichael *et al.*, 2005).

From the *In silico* Evaluation result, Table 6. None of the peptides showed inhibitive action against CYP3A4, CYP2D6, CYP2C9, CYP2C19, and CYP2A as predicted using SuperCYPs modeling.

The protein of *M. oleifera* (Table 3) was not active against the cervical, HeLa cancer cell line, MDA-MB-231, and the fibroblast cell line (3T3). The aqueous extract of the seed, which is known to contain glucosinolate and isothiocyanates, has been reported to be cytotoxic to breast adenocarcinoma (MCF-7) cells (Rahaman *et al.*, 2017). The chemical compounds include 4(α -L-rhamnosyloxy) phenylacetone nitrile, 4-hydroxyphenylacetone nitrile, and 4-hydroxyphenylacetamide, isolated from the seed exhibited mutagenic effects in a mouse model (Villasenor *et al.*, 1989). The seed extracts are well reported to be cytotoxic to various cancer cell lines while also possessing anti-inflammatory activities (Al-Asmari *et al.*, 2015). In light of these previous reports, our results may indicate that the cytotoxic constituent of the seed is not the protein.

The polar and non-polar extracts of *M. oleifera* seed were reported to induce sensitivity in fungi, bacterial and viruses which included: *Scenedesmus obliquus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus sterothophilus*, and *Herpes Simplex* virus type 1 (HSV 1) and *Polio* virus type 1 (Ali *et al.*, 2004). The seed extractives have also shown potency against some water-borne bacterial (Ferreira *et al.*, 2011; Kumar and Gopal,

1999).

The MOSP showed no lethality (Table 5) compared to the control as the same number of survivors was obtained for all tested concentrations. This implies that the change in concentration does not affect shrimp viability, thereby strengthening the prediction that the protein of the seed of *M. oleifera* is non-toxic at the tested concentrations. This suggests that the toxic characteristic of the seed of *M. oleifera* may not be due to the protein content.

The bioactivities abounding in seeds of plants cannot be over-emphasized. The seeds of plants have been reported to possess significant cytotoxic activity, antimicrobial potential, and antioxidant activities among others (Adeyemi *et al.*, 2020). In this study, the protein of the seed of *M. oleifera* has been observed to possess no significant cytotoxic activities on normal (non-transformed) and cancer cell lines. The extremely low activity recorded against the test organisms is an indication that the defatted and debittered seed powder would have no adverse effect in the fortification of flour for edible purposes (Ogunsina *et al.*, 2011). Furthermore, it showed no lethality against brine shrimp, and this was also in agreement with existing reports.

4. CONCLUSIONS:

The protein of *M. oleifera* seed from a native source has been purified via a simple technique characterized by ESI-MS and MALDI-MS. The protein showed no lethality on shrimp and no significant toxicity *in silico*. In addition, no cytotoxic action was observed on breast cancer, cervical cancer, and normal cell lines. Thus, the results suggest that MOSP is not responsible for the toxicity reported in the consumption of *M. oleifera* seed. It is presumed that the toxicity of the seed resides in the non-protein extractives of the seed.

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Figure 1: MALDI profile of MOSP

Table 1: Qualitative peptide composition at the positive mode ESI

SN	Name	RT	Mass	Formula	Hits (DB)	DB Diff (ppm)
1	Ser Arg	0.94	261.1415	C ₉ H ₁₉ N ₅ O ₄	10	8.28
2	Leu Pro	1.00	228.1442	C ₁₁ H ₂₀ N ₂ O ₃	5	14.19
3	N-formylmethionylalanine	1.26	248.0842	C ₉ H ₁₆ N ₂ O ₄ S	10	-4.46
4	Leu Met Gly	6.19	319.1602	C ₁₃ H ₂₅ N ₃ O ₄ S	10	-11.5
5	Ile Gly Met	8.77	319.1597	C ₁₃ H ₂₅ N ₃ O ₄ S	10	-9.77
6	N-formylmethionylphenyl alanine	8.78	324.1148	C ₁₅ H ₂₀ N ₂ O ₄ S	6	-1.17
7	Gly Arg Asp	8.91	346.1583	C ₁₂ H ₂₂ N ₆ O ₆	10	5.03
8	Ile Glu Met	9.51	391.1799	C ₁₆ H ₂₉ N ₃ O ₆ S	10	-5.61

Table 2: Qualitative peptide composition at the negative mode ESI

SN	Name	RT	Mass	Formula	Hits (DB)	DB Diff (ppm)
1	Asn Asp His	5.98	384.1451	C ₁₄ H ₂₀ N ₆ O ₇	6	-15.09
2	Ser Tyr Tyr	8.89	431.1705	C ₂₁ H ₂₅ N ₃ O ₇	10	-2.83
3	Phe Asn Tyr	15.78	442.1875	C ₂₂ H ₂₆ N ₄ O ₆	10	-5.18
4	Phe Phe	16.72	312.148	C ₁₈ H ₂₀ N ₂ O ₃	1	-1.79
5	Tyr Phe	16.94	328.1418	C ₁₈ H ₂₀ N ₂ O ₄	3	1.55
6	Pro Trp Gly	18.77	358.1652	C ₁₈ H ₂₂ N ₄ O ₄	10	-3.1

Table 3: Cytotoxicity (IC₅₀) of *Moringa oleifera* seed peptides

Compounds	HeLa %Inhibition/ [IC ₅₀ ± SD (µg/mL)]	MDA %Inhibition/ [IC ₅₀ ± SD (µg/mL)]	3T3 (µg/mL)
MOSP	14.3 [ND]	0.00 [ND]	0.00 [ND]
Cycloheximide	NA [NA]	NA [NA]	89.19 (0.8 ± 0.10)
Doxorubicin	100 (0.90 ± 0.10)	NA [NA]	NA [NA]

ND: Not determined; NA: Not applicable; IC₅₀: 50% inhibition concentration. Results represent mean ± standard error of mean of triplicate determinations

Table 4: The antibacterial and antifungal activities of the MOSP

Organisms	%Inhibition of MOSP	%Inhibition of Imipenem	MIC of Miconazole (µg/mL)
Bacterial (Gram Negative)			
<i>Escherichia coli</i> ATCC 25922	< 30	90	-
<i>Pseudomonas aeruginosa</i> ATCC 10145	< 30	92	-
<i>Salmonella typhi</i> ATCC 14028	< 30	93	-
Bacterial (Gram Positive)			
<i>Bacillus subtilis</i> ATCC 23857	< 30	95	-
<i>Staphylococcus aureus</i> NCTC 6571	< 30	93	-
Fungi			
<i>Trichophyton rubrum</i>	< 10	-	70.00
<i>Candida albicans</i>	< 10	-	110.00
<i>Aspergillus niger</i>	< 10	-	20.00*
<i>Microsporum canis</i>	< 10	-	98.40
<i>Fusarium lini</i>	< 10	-	73.80
<i>Candida glabrata</i>	< 10	-	110.80

* Amphotericin B was used.

Table 5: Effect of MOSP on Brine shrimp viability

Conc ($\mu\text{g/mL}$)	Number of Shrimps	Number of Survivors	Mortality via MOSP (%)	Mortality via Etoposide (%)
10	30	27 ± 0.0	10 ± 0.0	-
100	30	27 ± 0.0	10 ± 0.0	-
1000	30	27 ± 0.0	10 ± 0.0	46.6 ± 0.0

Results represent mean $\pm$ standard deviation of triplicate determinations

Table 6: Computational toxicity prediction using the ProTox-II platform for peptide obtained at the positive mode ESI.

Compounds	Molecular formula	Predicted Acute Toxicity (LD_{50})	Toxicity Target	Average Similarity (%)
Serylarginine	$\text{C}_9\text{H}_{19}\text{N}_5\text{O}_4$	2000 mg/kg Tox Class IV	-	-
leucylproline	$\text{C}_{11}\text{H}_{20}\text{N}_2\text{O}_3$	3000 mg/kg Tox Class V	Corticotropin releasing factor Receptor 1	71.57
Glycine, N-(N-L-methionyl-L-	$\text{C}_{13}\text{H}_{25}\text{N}_3\text{O}_4\text{S}$	3000 mg/kg Tox Class V	Corticotropin releasing factor Receptor 1	74-76
Arginyl-glycyl-aspartic acid	$\text{C}_{12}\text{H}_{22}\text{N}_6\text{O}_6$	3000 mg/kg Tox Class V	-	-
N-Formylmethionylalanine	$\text{C}_9\text{H}_{16}\text{N}_2\text{O}_4\text{S}$	5000 mg/kg Tox Class V	-	-
N-Formyl-methionyl-leucyl-phenylalanine	$\text{C}_{15}\text{H}_{20}\text{N}_2\text{O}_4\text{S}$	5000 mg/kg Tox Class V	Opioid Receptor Mu	80.97