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SHORT COMMUNICATION

Comparing Heavy Metals, Nutritional Value, and Toxicity in Organic *vs* Conventional *Moringa oleifera* Leaves from Chennai District

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Abstract

Background: The analysis of organic versus conventional *Moringa* powder reveals significant differences in heavy metal content and underscores their implications for safety and nutritional value. **Aim:** To compare the concentrations of heavy metals, proximate composition, and brine shrimp lethality between organic and conventional *Moringa oleifera* leaves sourced from Chennai District. **Methods:** Organic and conventional *M. oleifera* leaves were collected from Chennai District. The samples were dried at 40°C and ground into powder. The concentrations of heavy metals—lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg)—were determined using Atomic Absorption Spectrophotometry. Proximate analysis was conducted to measure moisture content, crude fat, crude protein, and ash value. Additionally, a brine shrimp lethality assay was performed to evaluate the toxicity of the samples, with IC50 values calculated for each. **Results:** Heavy Metal Concentrations: Organic *M. oleifera* (Sample "I") contained significantly lower levels of heavy metals compared to conventional *M. oleifera* (Sample "II"). Chromium: 1.76 mg/kg (organic) vs. 12.10 mg/kg (conventional); Cadmium: 0.02 mg/kg (organic) vs. 0.20 mg/kg (conventional); Lead: 0.48 mg/kg (organic) vs. 2.05 mg/kg (conventional); Arsenic: 0.06 mg/kg (organic) vs. 0.21 mg/kg (conventional). These findings underscore the greater safety of organic *M. oleifera*, as excessive heavy metal exposure is linked to respiratory issues, kidney damage, developmental problems, and increased cancer risk. Proximate Composition: The nutritional profiles of the samples were similar: Crude Protein: 23.88 g/100g (organic) vs. 23.81 g/100g (conventional) and Crude Fat: 6.36 g/100g (organic) vs. 6.28 g/100g (conventional). This suggests that both samples offer comparable nutritional benefits, although the organic variant is safer due to reduced heavy metal content. The organic sample exhibited a higher IC50 value, indicating lower toxicity compared to the conventional sample in brine shrimp lethality assay. **Conclusion:** The study highlights significant differences in heavy metal content, toxicity, and safety between organic and conventional *M. oleifera* leaves collected from Chennai District. While both samples displayed similar nutritional profiles, the organic sample proved to be the safer choice due to substantially lower heavy metal contamination, likely attributable to organic farming practices that avoid synthetic chemicals.

Major Findings: The study found that organic *Moringa oleifera* leaves have significantly lower heavy metal content (Cr, Cd, Pb, As) than conventional ones, making them safer. Both types had similar nutritional values, but organic samples showed lower toxicity in the brine shrimp lethality test, likely due to the absence of synthetic chemicals. Organic *Moringa* is the safer and preferable option.

Keywords: Atomic Absorption Spectrophotometry, Brine Shrimp Lethality, Heavy Metal Analysis, *Moringa oleifera*, Proximate Analysis

1. Introduction

Moringa oleifera, native to the Indian subcontinent, commonly known as the drumstick tree, is a multipurpose tree with a quick growth rate. The "miracle tree" gets its name from its remarkable nutritional, medical, and commercial benefits. Since it has been grown for millennia, practically every

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part of the *M. oleifera* tree—its leaves, pods, seeds, blossoms, and roots—is used for a variety of uses[1]. Because of its extensive array of health advantages and rich nutritional profile, the leaves in particular are widely prized. Because *moringa* can grow well in marginal and drought prone environments of semi-arid and arid regions it is a very important crop for these zones. Its rich nutritional value and the ability to be used in so many ways make it to be the focus of researchers, farmers and health expert. *Moringa* leaves are nutritionally rich packed with calcium, potassium, iron, magnesium and essential amino acids in addition to vitamins A, C, and E; this vegetable has antioxidants such as quercetin and chlorogenic acid that reduce severe chronic diseases caused by inflammation and oxidative stress[2]. Organic *Moringa* may have higher nutritional value due to natural farming methods. Additionally, organic *Moringa* tends to have lower microbial contamination and a lower risk of heavy metal contamination, unlike conventional *Moringa* which uses synthetic fertilizers and pesticides. This comparison provides valuable insights into the environmental, health, economic, and social implications of different farming practices. This study compares the nutritional and medicinal benefits of organic and conventional *Moringa* leaves, highlighting which option is better suited for addressing the needs of the modern world. *Moringa* has long been used in traditional medicine to treat injuries, arthritis, diabetes, and various other ailments due to its antibiotic and anti-inflammatory properties. These traditional uses are increasingly supported by scientific evidence, highlighting *Moringa*'s exceptional nutritional value. This makes it a vital resource for improving nutrition standards and combating malnutrition[3].

2. Materials and methods

2.1 Materials Required

Organic *Moringa oleifera* leaves and Conventional *M. oleifera* leaves from organic India store - Tnagar, Chennai and Kazhani native farms Chennai , Nitric acid (HNO_3), Sulfuric acid (H_2SO_4), Hydrogen peroxide (H_2O_2), Standard solutions of heavy metals lead (Pb), Cadmium (Cd), Arsenic (As), Mercury (Hg), Reagents for proximate analysis (sulfuric acid, sodium hydroxide, solvents for fat extraction), Artificial seawater, Brine shrimp eggs (Artemia salina).

Storage - Heavy metal solutions should be stored in a sealed container made of high-density polythene or Teflon

2.2 Equipments Used

Drying oven, Grinder or mill, Analytical balance, Soxhlet extractor Kjeldahl apparatus, Muffle furnace, Incubator- at Sri Ramachandra institution of higher education and research center, Porur. Computer with statistical analysis software (SPSS, Excel), Software for data visualization (GraphPad Prism version 10).

2.3 Identification of the Plant

The samples of *M. oleifera* was collected from different places in the Chennai District so as to ensure that the selected samples include both conventional agriculture and organic agriculture. Consequently, the identification process proved vital for samples reliability to be ensured. The species identification of plants was ascertained using standard plant identification key and herbarium records. This entailed comparing the obtained morphological features with the descriptions of various parts of *M. oleifera*; including leaves shape, flowers and seed pods. To ensure that all subsequent analysis was performed on actual samples of *M. oleifera* and to confirm the credibility of the study's results, accurate identification was essential.

2.4 Plant Extraction

Upon identification, the collected leaves of *M. oleifera* plant were prepared for analysis I. The leaves were first rinsed in distilled water to get rid of surface pollutants namely dust and pesticides. They were then weighed and placed in a drying oven at 40°C until they attained a stable weight equivalent to the elimination of the moisture content. This was necessary in order to keep any microbial activities and enzymatic actions that might otherwise occur and change the chemical make-up of the leaves base. The dried leaves were crushed into small particles using a laboratory grinder/mill to give a fine powder. This powdered form was kept in the tightly closed and aprotic bottles, at room temperature, away from

light and humidity, for subsequent examinations. This preparation made the samples homogenized and in a ready state for proximate and heavy metal analysis.

2.5 Heavy Metal Analysis

The concentrations of heavy metals such as lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg) in the *M. oleifera* leaves were analyzed to assess potential contamination from environmental sources[4]. The powdered leaf samples were digested using microwave assisted acid digestion with 9ml of extraction solution of 6ml of nitric acid (HNO3), 1ml of sulfuric acid (H2SO4) and 2ml of hydrogen peroxide (H2O2)[5]. This procedure provided for optimum disintegration of the plant matrix and improved mobilization of the heavy metals in to a form that could be easily analysed.

Samples subjected to digestion were analysed using Atomic Absorption Spectrophotometry or Inductively Coupled Plasma Mass Spectrometry[6]. These techniques are very selective and suitable to determine the very low concentrations of heavy metals. To calibrate the electrode, a standard solution of the heavy metals was prepared with aspiration to measurement[7].

2.6 Proximate Analysis: Moisture Content

Moisture content was obtained by drying a known weight of the leaf powder at 105°C in an oven until constant weight, was obtained. This method made it easy to get the right measurement of water content which enable the determination of the dry weight of the samples.

2.7 Crude Fat

Interpolations of the crude fat content of the feed samples were done using the Soxhlet extractor with a suitable solvent like petroleum ether. The fat obtained in the process was weighed and the fat content expressed as a percentage of the dry weight was also calculated.

2.8 Crude Protein

The crude protein estimation, the Kjeldahl method was adopted following digestion of the sample with sulfuric acid, neutralization and titration for nitrogen. Following that, they used this conversion factor to come up with the protein content from the existing nitrogen content.

2.9 Ash Content

The ash content was ascertained by combusting a known mass of the plant leaf powder, in a muffle furnace at 550°C until a constant weight of the inorganic minerals, in form of ash was obtained. This offered a quantitative assessment of the overall minerals in the leaves[8].

2.10 Brine Shrimp Lethality Test

Cytotoxicity of the *M. oleifera* extracts were tested employing the brine shrimp lethality bioassay which is a potent bioassay in determining preliminary toxicity[7]. Artemia salina or brine shrimp was cultured in artificial seawater with light being on all the time and aeration. The nauplii (larvae) was harvested after 24-48h for use in the assay. *M. oleifera* fresh leaves were collected and dried to produce powder which was then dissolved in solvent ethanol. In order to study the effect of the different concentrations of the extracts, it is necessary to determine the optimal concentration, so the solutions with different concentrations were prepared for the experiment. Various concentrations of samples were then added into test tubes with brine shrimp larvae. To test the validity of the assay a non-toxic control solution (seawater) and a toxic reference substance (potassium dichromate) was used. Counts of the number of surviving larvae in each test tube or well were performed after 24 hours using a stereomicroscope. Percentage of mortality was also recorded on the basis of different concentrations and the LC_{50} (Lethal Concentration for 50% of the larvae) was estimated[9].

LC_{50} Calculation

$$Percentage\ of\ Lethality = \left(\frac{Number\ of\ Dead\ Larvae}{Total\ Number\ of\ Larvae} \right) \times 100$$

3. Results

The heavy metal contents of arsenic (As), cadmium (Cd), lead (Pb), and chromium (Cr) were measured by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The following concentrations were observed in the organic *Moringa* powder (designated as "I"): 1.76 milligramme per kilogramme of 0.02 mg/kg of cadmium, 0.48 mg/kg of lead, 0.06 mg/kg of arsenic, and chromium. The amounts of lead, arsenic, cadmium, and chromium in the inorganic *Moringa*

powder (designated as "II") were noticeably higher at 2.05 mg/kg, 0.21 mg/kg, chromium, and 12.10 mg/kg, respectively[10] (Table 1). The permissible limits for heavy metals for edible plants set by the WHO/FAO regulatory bodies are 0.3mg/kg Lead; 0.1mg/kg Cadmium; 0.1mg/kg Arsenic; 0.03mg/kg Mercury; 2.3mg/kg Chromium.

Chromium is an essential trace element required for normal metabolism, but excessive intake can lead to toxicity. The inorganic sample's Chromium level is alarmingly higher (12.10 mg/kg) compared to the organic sample (1.76 mg/kg). Prolonged exposure to high levels of Chromium can cause respiratory problems, weakened immune response, and increased risk of lung cancer[11].

Cadmium is a toxic metal with no known biological function in humans. It can cause kidney damage, bone demineralization and is classified as human carcinogen. (0.20mg/kg) found in the inorganic sample. Chronic exposure to even low levels of Cadmium can have severe health implications, making the lower levels in the organic sample (0.02mg/kg) more desirable. Increased Cd levels can cause neurological, hepatic, or renal problems. When people are exposed to moderate to high levels of Cd, it primarily builds up in the kidney, producing renal illness that can result in end-stage chronic kidney disease or even death[12].

Lead is a highly toxic metal that can affect almost every organ and system in the body, particularly the nervous system. The organic *Moringa* powder contains 0.48 mg/kg of lead, whereas the inorganic sample contains 2.05 mg/kg. Lead exposure is particularly harmful to children, causing developmental issues and cognitive deficits. The substantially lower Lead content in the organic sample underscores its safety[13].

One well-known poisonous element that can lead to a number of health issues is arsenic. These issues include cancer, cardiovascular illness, and skin sores[14]. The Arsenic content in the organic sample is 0.06 mg/kg, compared to 0.21 mg/kg in the inorganic sample. Reducing Arsenic exposure is crucial for health, and the lower levels in the organic *Moringa* powder make it a safer option[15,17].

Crude protein was analyzed following the IS 7219 method, revealing similar levels in both samples. The organic *Moringa* powder contains 23.88 g/100g of crude protein, while the inorganic sample contains 23.81 g/100g. Protein is essential for growth, repair, and overall health, and these values indicate that both samples provide substantial protein content. However, given the lower heavy metal content, the organic sample is preferable for safe consumption without compromising nutritional benefits[16]. Fat content was determined using the AOAC 996.06 method. The organic *Moringa* powder contains 6.36 g/100g of fat, and the inorganic sample contains 6.28 g/100g. Fat is a vital macronutrient necessary for energy storage, hormone production, and cell structure. The comparable fat content in both samples suggests that consumers can obtain similar nutritional benefits from the organic sample, with the added advantage of lower heavy metal exposure (Table 2).

Moisture content was determined for both organic and conventional *Moringa* powder and it was found to be 4.95 % of moisture content in organic powder whereas 4.81 % of moisture content in conventional powder

Table 1. Test results of heavy metal analysis

Test\Parameter	Method	Result(Organic)	Unit	Result(Inorganic	Unit
Chromium as(Cr)	SO-INMUL-TE-063 A by ICPMS	1.76	mg\kg	12.10	mg\kg
Cadmium as (cd)	SO-IN-MUL-TE-063 A by ICPMS	0.02	mg\kg	0.20	mg\kg
Lead as (pb)	SO-IN-MUL-TE-063 A by ICPMS	0.48	mg\kg	2.05	mg\kg
Arsenic as (As)	SO-IN-MUL-TE-063 A by ICPMS	0.06	mg\kg	0.21	mg\kg

Table 2. Test report on proximate analysis

Test	Method	Results (organic)	Unit	Results (inorganic)	Unit
Crude protein	IS 7219	23.88	g/100g	23.81	g/100g
Fat	AOAC 996.06	6.36	g/100g	6.28	g/100g

(Table 3). Ash value was determined for both organic and conventional *Moringa* powder and it was found to be 8.52 % of ash value in organic powder whereas 9.38 % of ash value in conventional powder (Table 3). Comparing the organic extracts to the conventional extracts, the organic extracts showed higher LC_{50} values, which indicated decreased toxicity. This lower toxicity may be explained by the fact that organic farming uses fewer or no synthetic chemicals, which lessens the possibility that the finished product would include hazardous residues. Conventional *Moringa* exhibited marginally increased toxicity, possibly as a result of residual pesticides and other agrochemicals, even if its LD_{50} values were still rather safe (Tables 4 and 5) (Figure 1).

Table 3. Moisture content and ash value

Sample	Moisture content	Ash value
Organic Powder	4.95 %	8.52 %
Conventional Powder	4.81 %	9.38 %

Table 4. Brine shrimp lethality

Concentration (µg/ml)	Conventional(%)	organic (%)
1000	60	50
500	70	50
100	70	60
10	80	80
1	90	80

Table 5. Comparison of organic vs conventional mortality values

	LC_{50}– Conventional	LC_{50}– organic
Log(agonist)vs.response		
Bestfit-values		
Bottom	= 0.000	= 0.000
Top	33.85	48.58
$LogEC_{50}$	0.7167	1.011
EC_{50}	5.208	10.26
Span	33.85	48.58
Std. Error		
Top	3.264	6.479
$LogEC_{50}$	0.2861	0.3632
Span	3.264	6.479
95%CI (asymptotic)		

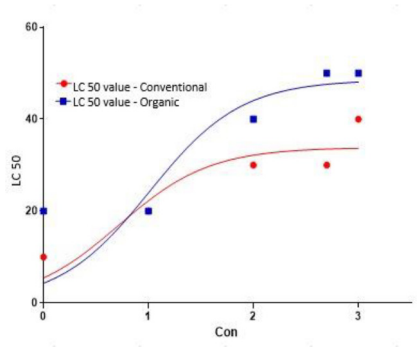


Figure 1. LC_{50} Comparison graph (organic vs conventional).

4. Discussion

This study compares the lethality tests with brine shrimp and determines the proximate analysis and concentration of heavy metals in whole-leaf powder of conventional and organic *M. oleifera* leaves collected from the Chennai district. Both *Moringa* varieties showed significant differences in their heavy metal content, with organic samples displaying markedly lower levels of lead (Pb), Arsenic (As), Chromium (Cr), and cadmium (Cd) compared to conventional samples. This observation aligns with recent studies that highlight the health safety advantages of organic farming practices, which reduce the application of artificial chemical-based pesticides and thereby significantly lower the potential buildup of toxic heavy metals in crops.

The heavy metal content in organic *Moringa* leaves was notably lower compared to findings from earlier research, as outlined in (Table 3). For instance, a study revealed that organic plants generally exhibit reduced heavy metal content compared to conventionally cultivated ones, primarily due to the absence of synthetic herbicides and fertilizers that contribute to heavy metal accumulation in agricultural products[18]. Similarly, organic farming practices are associated with lower environmental contamination and reduced heavy metal uptake by plants, which corroborates our findings of lower metal concentrations in organic *Moringa* samples[19].

The proximate analysis revealed no significant differences in the crude protein, fat, moisture content, and ash value between conventional and organic *Moringa* samples. The nutritional value of both

Moringa types appears to be comparable, although organic samples are safer due to their lower heavy metal content. This result is supported by the work which found that while the heavy metal content varies across different samples, the overall nutritional profiles, such as protein and fat content, were similar in organic and conventional *M. oleifera* leaves[20].

Conventional *Moringa* extracts were found to be comparatively more toxic than organic extracts, as indicated by higher LC_{50} values obtained from the brine shrimp lethality assay. This finding is consistent with a recent study, which reported that organically grown plants tend to exhibit lower levels of toxicity. It suggested that the lower cytotoxicity observed in organic plant extracts is likely due to the absence of pesticide residues and fewer environmental pollutants compared to conventionally cultivated plant counterparts. This suggests that organic *Moringa* could be a safer option for regular consumers, as it reduces exposure to potentially dangerous compounds[21]. Conversely, the high levels of heavy metals found in conventional *Moringa* samples pose serious health risks. Chronic exposure to heavy metals such as lead and cadmium has been associated with adverse health effects, including organ dysfunction, developmental issues, and an increased risk of cancer[22]. The challenges posed by conventionally cultivated *Moringa* may be mitigated by the reduced health risks associated with organic cultivation, as indicated by the lower levels of harmful metals in the plant. Furthermore, the importance of minimizing heavy metal exposure to prevent long-term health impacts, a goal that could be achieved through the consumption of organically grown *Moringa*[23].

4. Conclusion

The study on heavy metals and nutritional components in *Moringa* powder reveals significant differences between organic and conventional samples. This is due to the use of fewer synthetic chemicals in organic farms. Organic *Moringa* powder shows lower levels of Chromium, Cadmium, Lead, and Arsenic compared to conventional samples, reducing potential health risks associated with these metals. Higher heavy metal content in the conventional sample poses risks such as respiratory issues, kidney damage, developmental problems, and cancer. These two samples provide nearly identical results in assessing crude protein and fat that play an essential role in muscles' build, repair, and health. That said, owing to reduced heavy metal concentrations, Organic *Moringa* is recommended for regular consumption due to its reduced exposure to agrochemical residues and heavy metals.

Essentially, this comparative analysis successfully shows that there is a substantial difference in the heavy metal content, nutritional profile, and toxicity of the organic and conventional extracts of *M. oleifera*. In addition, organic *Moringa* samples had slightly higher nutritional values for proteins, vitamins, minerals, and a trustworthy level of phytochemicals to endorse probable health advantages for consumers. Thus, the brine shrimp lethality assay proved the less toxic effects of organic extract which shows the better benefit to use in organic farming. Though both organic and conventional *Moringa* provide essential nutrients that can help improve the health of the consumers, going for the former can be safer and better, especially in the light of the global trend towards healthy foods. Further research should be of larger sample size and should include follow up on health effects due to food choices to further substantiate these conclusions for healthy eating.

5. Author Contribution

The contributors to this project were Sermugapandian Nithya, review, editing, supervision, conceptualization, validation, project administration, and formal analysis. R. Dinesh Kumar and Sajan Muruganandhan contributed to the study's conceptualization and design. Methodology S. Abdul Kadar Sajith and Mithun Edwinraj Rickslin Mary. Pradeep Srinivasan data analysis and interpretation. Shruthikaa Kasi Vishwanathan original draft. G. Jeevadharsana graphical abstract, while M. Durga, G. Ragavendhar writing review and editing. All authors have read and agreed to the published version of the manuscript.

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