Biomedical Letters ISSN 2410-955X DOI: https://doi.org/10.47262/BL/9.1.20230115

Research article
2023 | Volume 9 | issue 1 | Pages 7-15

ARTICLEINFO

Received
January 05, 2023
Revised
February 26, 2023
Accepted
March 31, 2023

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Keywords
Moringa oleifera
Chemical composition
Antibacterial activity
Antioxidant
Bioaccessibility

How to Cite
Waheed S, Shekh M, Anwar M, Tayyab M. Comparative analysis of nutritional, antioxidant, and antibacterial properties of *Moringa oleifera* extract. Biomedical Letters 2023; 9(1):7-15.

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Comparative analysis of nutritional, antioxidant, and antibacterial properties of *Moringa oleifera* extract

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Abstract

People are increasingly more concerned with their lifestyle and health due to the significant shift in socioeconomic level in the modern era. People are aware of the negative consequences of manufactured items. Natural compounds derived from plants with fewer adverse effects are getting more attention. *Moringa oleifera* is an example of a tree with significant nutritional and therapeutic advantages. In the present study, mineral and macronutrient content and antioxidant capacity were evaluated at two stages of maturity (mature and tender leaves). The chemical analysis revealed that the protein concentration was higher, and the lipid concentration was lower. Regarding mineral content, calcium (Ca) and iron (Fe) have exhibited a higher degree of bioaccessibility, with potassium (K), sulfur (S), Ca, and Fe constituting the most prevalent elements. Using an established *in vitro* model, the antioxidant activities of *Moringa oleifera* leaf extracts were evaluated to comprehend the mechanism of pharmacological action. At 900 µg/ml, the aqueous extract of *Moringa oleifera* showed significant antibacterial activity. Based on the results of this study, *Moringa oleifera* leaf extracts exhibit considerable antioxidant activity and substantial protection against bacterial infection due to the presence of phenolic and flavonoids. Based on *in vitro* experiments, we aimed to determine if *Moringa oleifera* may be used as a potential antibacterial in therapeutic applications in light of the rising incidence of antibiotic resistance.

Introduction

Moringa oleifera, often known as "The Miracle Tree," is the most well-known and extensively distributed species of the Moringaceae family, with a wide variety of therapeutic benefits and excellent nutritional value. *In addition to its native range in Pakistan, India, and Africa* [1,2], *Moringa oleifera is also widely distributed in other countries (the Philippines, the Caribbean, and America)* [3]. The ecological distribution and availability of *Moringa oleifera* make it more attractive for public health concerns worldwide. Among its many advantages are its ability to withstand a variety of temperatures and soil conditions, as well as its ability to tolerate drought. Furthermore, it is recognized in many regions for its medicinal properties. It is believed that people have consumed virtually every component of this highly respected tree and used it in various domestic applications, including fertilizer, green manure, blue dye, water purification, foliar nutrient, and animal forage, for a very long time [4]. Moringa's leaves contain most of the plant's macro- and micronutrients [5], the most commonly used component since they have a high protein level.

Moreover, Moringa leaves contain many antioxidants, with isothiocyanates as one of the critical sources of anti-cancer and antimicrobial capabilities [6]. Furthermore, it provides a wide variety of essential amino acids, as well as vitamins A and C, and various antioxidants [7]. Moreover, the tree may grow rapidly (up to 3-5 m per year) and withstand dry conditions without agricultural intervention. This final attribute and its cheap production costs make it excellent for growing in the African tropics' vast desert or semi-desert regions, where hunger, malnutrition, and lack of food are severe issues [5,8]. *Cultivating moringa in barren areas with excellent results is possible because it has a low demand for soil nutrients* [9]. So moringa could be a perfect option for preventing malnutrition, anemia, and other diseases in developing countries [10,11].

According to an estimation by WHO, in 14 countries worldwide, 8.7% of hospitalized patients get hospital-acquired infections [12]. It is because hospitals are often overcrowded and understaffed in these countries, which can lead to inadequate hygiene practices and a lack of medical supplies. Bacteria such as *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (S. aureus), *Pseudomonas*, and *Streptococcus* are the most commonly found bacteria in hospitals. Infections caused by these bacteria, including sepsis and pneumonia, can spread from patient to patient. *epidermidis* frequently cause the illness. The choice of antibiotics employed in infectious disease treatment has a crucial impact on patient recovery. The majority of these bacteria are human skin and mucous membrane flora. In the past, these microbes seldom caused severe infections.

Nonetheless, the growing use of catheters, implants, and prosthetics are higher risk of spreading bacterial infection [13]. Most isolates of *S. epidermidis* have developed resistance to penicillin, cefazolin, oxacillin, ciprofloxacin, and ciprofloxacin. Since antibiotics have been overused and the bacteria have developed genetic mutations that allow them to survive in the presence of antibiotics, *S. epidermidis* has become progressively more resistant to these treatments. Infections are difficult to treat due to the high rate of resistance [14]. Moringa leaves contain alkaloids, flavonoids, and saponins that can inhibit bacterial growth [18]. Plants produce these secondary metabolites to defend themselves against pathogens, and they can also inhibit bacterial growth. They act as antimicrobial agents by disrupting the cell membranes of bacteria, preventing their growth and reproduction. These compounds can also block the action of enzymes, interfere with the mechanisms bacteria use to attach to cells, and even prevent bacteria from taking in nutrients, causing them to starve. In this way, secondary metabolites provide adequate protection against pathogens and can even help prevent the spread of disease.

This study examines whether Moringa leaves' nutritional profile, bioaccessibility, and antioxidant activity make them suitable for a balanced diet. This study can inform food-based dietary guidelines and public health nutrition strategies. Their chemical composition and nutritional value must be understood to ensure moringa leaves provide essential nutrients as a dietary supplement. Moringa leaves' bioaccessibility and antioxidant activity may prevent chronic diseases. Finally, Moringa oleifera can be tested as an antibiotic substitute. This study showed that moringa leaf extract had antibacterial properties. More research needs to be done to find and isolate the active ingredients in the moringa leaf extract that make it work as an antibacterial.

Materials and Methods

Plant Material

Moringa powder (100% dried moringa leaves) was purchased from Macklin Biochemical Co., Ltd. (Shanghai, China).

Extract characterization

The moisture content was determined by comparing the weight of 3 g powder before and following drying for 24 hours at 110 °C in an oven with forced air. Total organic matter was determined by incinerating 1 g of extract in a muffle oven at 525°C for 24 hours. The total protein concentration was determined using the Kjeldahl methodology [15]. Weighing 3g of new sample, 7.5g of a catalytic mixture, and 15 mL of 97% sulfuric acid were then added. The tubes were put in a heating block, reaching 425 °C for 50 min. After cooling, the mixture was distilled for 5 minutes with the addition of 35% NaOH till neutralization. The distillation output included 0.1 N HCl. The following formula was used to compute the total percentage of protein content, with V being the total volume (mL) needed to neutralize the mixture and W being the sample's weight.

Raw protein (%) = V(HCl) × 0.1 × 1.4 × 5.7 W

Using a Tecator Soxhlet extractor [19], the total fat content of 1 g of dried material was measured. After placing the sample into cellulose cartridges, 50 mL of ether was poured. The ether heated to 80 °C eliminates the fat from the sample, which then falls into the metal cups that are already weighted.

Fiber determination was performed according to AOAC procedure 985.29 [15]. To accomplish this, 1 g of moringa powder and 60 mL of 0.05 M phosphate buffer pH six were weighed and added, respectively. In the following step, the samples were put into a bath at 95 °C for 35 minutes, and amylase (40,000 U/mL) was added. After adjusting the pH to 4.5, the amyl-glucosidase enzyme (6 U/mg) was added to raise the temperature to 60 °C for 30 minutes. When the digestion was complete, ethanol was added to precipitate the fiber before filtration. After filtration, the residue was placed in an oven to dry. When the residue was dried, the total dietary fiber (TDF) was calculated using the following equation:

TDF(%) = R1+R2 2 - (P + A) W1+W2 2 × 100 (2)

R1; R2: weight of the sample residue. P; A: proteins and ashes from the residue. W1; W2: sample weight.

Total phenolic content

Total phenolic content was measured using the Folin-Ciocalteu reagent and gallic acid as the standard, as per the previously reported procedure by Singleton and Rossi [16]. 0.4% Na_2CO_3 and 2% NaOH were added to the isolated material. The samples were gradually infused with the reagent, incubated for one hour in the dark, and then the absorbance at 750 nm was measured. The phenolic content was reported as mg of gallic acid equivalents (GAE) per gram of extract. In this study, the flavonoids were isolated and quantified by pipetting out an aliquot of the extract and evaporating it until it was dry [17]. A boiling water bath was used to heat 4.0 ml of the vanillin reagent for 15 minutes. This procedure was also applied to the standard. The optical density of the sample was measured at 342 nm. The results are reported as mg flavonoids/g leaf.

Determination of the mineral content

This study used inductively coupled plasma mass spectrometry to analyze the mineral composition. This technique detects and quantifies trace elements in samples by ionizing the sample with an inductively coupled argon plasma. The ions are then separated by their mass-to-charge ratio and detected by a mass spectrometer. It allows for precise measurements of the mineral content in the sample. We diluted the standards and used them to calibrate the inductively coupled plasma mass spectrometry for the mineral analysis by following the standard operating conditions [18].

Bioaccessibility of minerals

Minekus *et al*. [31] devised a method for making samples act as if they were being digested in the stomach to measure mineral solubility and dualizability. This method involves using a model of the digestive system to simulate the conditions of the stomach, such as pH and temperature. It enables researchers to accurately measure the solubility and dualizability of minerals in the sample, providing valuable insights into mineral bioavailability. There were three steps to the digestive process: the oral, gastric, and intestinal phases. Firstly, in the oral phase, 50:50 mixtures of moringa powder and simulated salivary fluids electrolyte stock solution were mixed, and the pH was adjusted to 7. The salivary beta-amylase was incubated with $CaCl_2$ 0.75 mM for two minutes at 37°C. During the gastric phase, the preceding mixture was combined with simulated gastric juices to create a final proportion of 50:50 (v/v), and 1 M HCl was added to reduce the pH to 3.0. A mixture of porcine pepsin and samples was incubated at 37°C for two hours. In the final stage, simulated gastrointestinal fluids were injected into the solvent mixture (50:50), and $CaCl_2$ was added to reach 0.3 mM in the ultimate digestion solution. Neutralization of pH was accomplished by adding 1

M NaOH and 100 U/mL of porcine pancreatin, and 10 mM of porcine bile salt. The mixtures were kept in an incubator at 37°C for two hours. The supernatant was filtered, and the mineral content was determined following the above procedure.

Preparation for the antibacterial test

The powdered moringa was put in a 24-well microtiter plate. It ensured that each well received the same quantity of powder and was exposed to the same number of nutrients and other elements throughout the experiment. Each well-containing moringa powder received one ml of an *S. aureus* and *E. coli* solution having a concentration of 10^5 CFU ml^{-1}. The inoculation plate was incubated at 37 °C for 24 h in a DNP 9272 incubator (Jinghong Company, Shanghai, China). All antibacterial tests were conducted at 37°C and 90% relative humidity. After 24 hours of incubation, 0.1 ml of diluted bacterial culture was scattered onto an agar plate (Aoboxing Biotech Corporation, Beijing, China). After 24 hours of incubation at 37 °C, the number of bacterial colonies on the plates was determined.

Statistical analysis

All data are provided as the mean standard deviation (SD). Duncan multiple comparisons and one-way analysis of variance (ANOVA) were utilized to conduct statistical analysis. Predetermined p values ≤ 0.05 were deemed statistically significant.

Results

Chemical composition of Moringa

Table 1 describes the approximate chemical nature of mature and tender moringa leaves. According to the data shown here, moringa leaves are an excellent provider of many different types of nutrients. The matured moringa leaves powder contained a humidity of 6%, and the tender leaves extract was 4.75%. The crude protein contents ranged from 28.25% in matured leaves powder and 24.50% in tender leaves extract. Similarly, the lipid content ranged from 5.50 to 4.20% between the matured and tender leaves powder. The carbohydrate contents were calculated as 26% in mature leaves and 22.5% in tender leaves.

High macromineral concentrations were collected, with potassium (K) being the most prevalent element. Mature leaves (1.92 mg/100g dry weight (DW)) contained a higher amount of K compared to tender leaves, followed by sulfur (S) (886.3 mg/100g DW) and calcium (Ca) (**Table 2**). In terms of microminerals, the leaves had the highest iron (Fe), boron (B), and zinc (Zn). In fact, moringa has the greatest Fe content (22.7 mg/100 g DW). B and Zn followed as the next most important microminerals and mature leaves had higher amounts (3.10 and 2.50 mg/100 g DW, respectively) (**Table 2**), offering healthcare benefits, including strengthing the immune system. Copper (Cu) and magnesium (Mn) are in lesser concentrations.

Total phenolic and flavonoid compounds

The phenolic content extracted from mature moringa leaves (173.98 ± 2.10 mg GAE/100 g) was higher compared to tender leaves extract (156.30 ± 1.60 mg GAE/100 g) (Table 3). The flavonoid content in mature moringa leaves and their crude extracts were determined as (25.33 ± 0.17 mg/RE/100 g), and the tender leaves extract was (23.88 ± 1.10 mg/RE/100 g) (**Table 3**). Several promising polyphenolic compounds exist in moringa leaves, including flavonoids and tannins.

Bioaccessibility of mineral content

The oral phase was determined to be the most effective for Na absorption, while Mg had the lowest absorption. However, Cu had the most absorption in the stomach phase, and Fe had the lowest. Ca was the mineral with the greatest bioaccessibility during the intestinal phase, whereas Fe had the lowest (**Fig. 1**). Ca bioaccessibility exhibited the opposite tendency in the intestinal phase compared to the stomach phase: it increased. Ca bioaccessibility increases in the intestinal phase because the proteins and amino acids help break down the mineral so the body can absorb it more easily.

Antibacterial efficacy of moringa leaf extract

The secondary metabolites compounds found in plants can help inhibit the growth of bacteria. The results indicated that different aqueous moringa leaf extract concentrations significantly differ the antibacterial growth. By using 300 µg/ml of aqueous moringa leaf extract, the antibacterial efficacy was 35% against *E. coli* and 45% against S. aureus, however by increasing the concentration to 900 µg/ml, the antibacterial efficacy was increased upto 75% against *E. coli* and 80% against S. aureus (**Fig. 2**). It indicates that the

Table1: *Moringa oleifera* leaf composition and mineral content (in g/100 g dry weight)

Parameters	*Moringa oleifera* matured leaves extract mean values	*Moringa oleifera* tender leaves extract mean values
Protein	28.25 ± 0.20	24.50 ± 0.40
Lipid	5.50 ± 0.37	4.20 ± 0.25
Carbohydrates	26 ± 3	22.5 ± 2.50
Ash	6.50 ± 0.25	4.70 ± 0.15
Moisture	6.75 ± 0.50	4.75 ± 0.30
Dietary fiber	30.50 ± 6.0	27.30 ± 4.8

Table 2: *Moringa oleifera* leaf composition and mineral content (in g/100 g dry weight)

Parameters	*Moringa oleifera* matured leaves extract mean values	*Moringa oleifera* tender leaves extract mean values
Protein	28.25 ± 0.20	24.50 ± 0.40
Lipid	5.50 ± 0.37	4.20 ± 0.25
Carbohydrates	26 ± 3	22.5 ± 2.50
Ash	6.50 ± 0.25	4.70 ± 0.15
Moisture	6.75 ± 0.50	4.75 ± 0.30
Dietary fiber	30.50 ± 6.0	27.30 ± 4.8
Sodium (Na)	152.11 ± 17.50	139.80 ± 13.0
Calcium (Ca)	1.15 ± 0.1	0.90 ± 0.05
Copper (Cu)	0.50 ± 0.05	0.42 ± 0.05
Iron (Fe)	22.75 ± 1.50	18.55 ± 1.10
Zinc (Zn)	2.50 ± 0.50	2.0 ± 0.34
Magnesium (Mg)	278.25 ± 5	247.30 ± 3.95
Manganese (Mn)	9.15 ± 1	8.15 ± 0.87
Phosphorus (P)	367.90 ± 3	355.25 ± 2.80
Sulphur (S)	886.30 ± 14.15	810.55 ± 13.0
Boron (B)	3.10 ± 0.1	3.05 ± 0.08
Potassium (K)	1.92 ± 0.08	1.81 ± 0.08

Table 3: The phenolic and flavonoid content and tender and mature moringa leave extract

Parameters	*Moringa oleifera* matured leaves extract Mean Values	*Moringa oleifera* Tender leaves extract Mean Values
Total Phenolic (mg/GAE/100 g)	173.68 ± 2.10	156.30 ± 1.60
Total Flavonoid (mg/RE /100 g)	25.33 ± 0.17	23.88 ± 1.10
IC50 Value of DPPH (mg/mL)	2.88 ± 0.15	2.82 ± 0.07

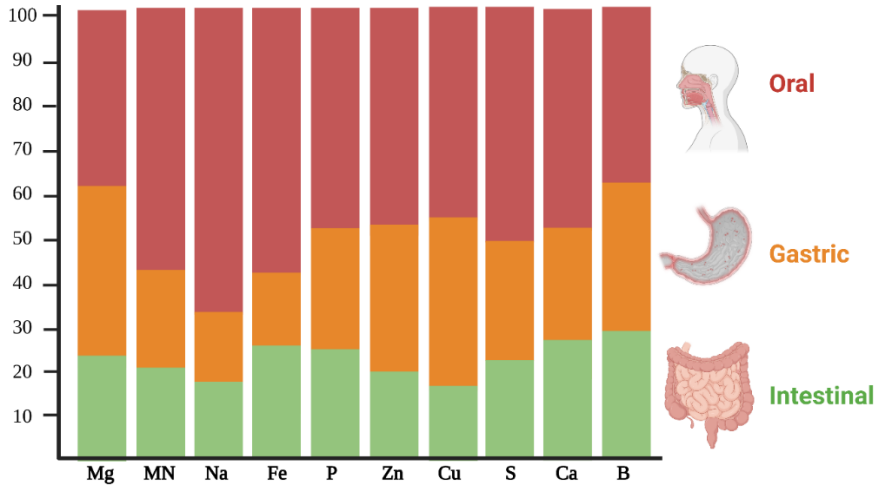

Fig. 1: The mineral content of *Moringa oleifera* was absorbed after being digested in a simulated Gastrointestinal environment. Sodium (Na), Calcium (Ca), Copper (Cu), Iron (Fe), Zinc (Zn), Magnesium (Mg), Manganese (Mn), Phosphorus (P), Sulphur (S), Boron (B), Potassium (K).

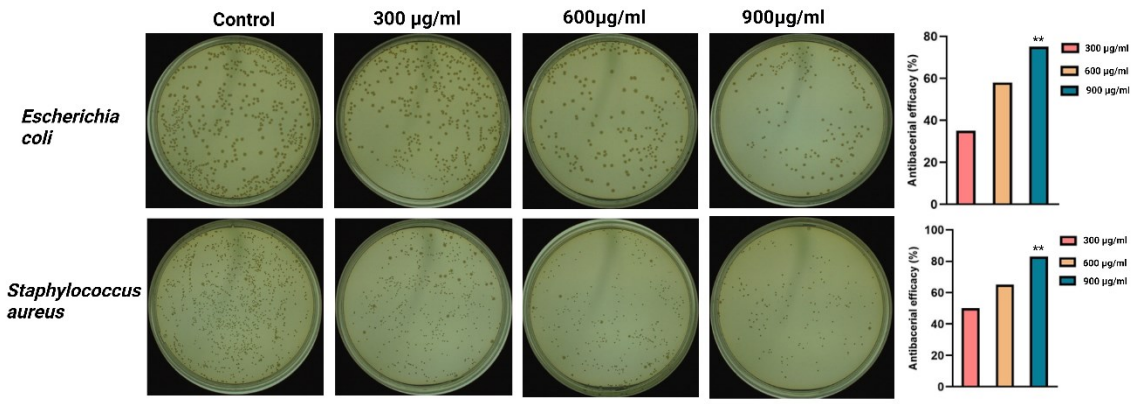

Fig. 2: Antibacterial efficacy of moringa leaf extract. Predetermined *p* values ≤ 0.05 were deemed statistically significant and are highlighted with **.

higher the concentration of moringa leaf extract, the greater the strength of its antibacterial activity.

Discussion

The present study examined and highlighted moringa's potential health benefits and practical applications. Table 1 provides an estimate of the chemical makeup of moringa leaves. Moringa leaves contain 25.30% of their dry weight as protein. The results are similar to those reported in the past, in which normal protein levels range between 19.15-28.8% [19]. Various factors affect the nutritional content of moringa, including the weather, crop management, cultivar, and post-harvest processing [20]. In previous studies, authors demonstrate moringa's ability to promote muscle recovery, which can also be attributed to its higher proteins and mineral composition (Fe, K, and Mg).

The extract is a rich source of essential vitamins and minerals for proper growth and development. Additionally, it contains compounds linked to improved brain function and improved immune response, both of which are important for populations at risk of malnutrition. The plant has vitamins, minerals, and other essential nutrients for healthy growth and development. Additionally, the plant has a low cost of production, making it an affordable and accessible supplement for those in need. *Moringa oleifera's* fat content can vary between 5 and 6% because vegetables are generally not highly lipid-rich [21], which is why *Moringa oleifera's* lipid content is relatively low. Factors such as nutrients, humidity, light, and temperature influence a plant's physiological processes. As a plant grows and matures, its lipid contents change due to environmental factors and the breakdown of lipids within the plant, as previously found by Oduro *et al*. [22], who reported 4.5%.

Regarding ash content, organic matter represents approximately 10 % of dry weight. These findings agree with those reported by Okiki *et al*. [23] and Oluduro [24]. Despite that, this value was lower than that of Mutayoba *et al*. [42] and Sanchez-Machado *et al*. [25]. It is a crucial factor to consider when assessing the nutritional benefits of moringa leaves. Carbohydrate concentration was high, although not as high as the values reported by Roco *et al*. [26] and Busani *et al*. [27], who found values between 52% and 65%. Consequently, to obtain their conclusions, these authors might add dietary fiber to the overall quantity of carbohydrates, which accounts for 25% of the overall weight.

In any case, the differences in the acquired data may be attributable to climate conditions, location, harvesting season, and plant nutrition [28,29]. Based on these results, fiber's hypoglycemic properties and the facilitation of easy digestion explain the anti-diabetic properties of moringa. In general, large amounts of macrominerals were found; however, K was the most prevalent element, followed by Ca and S, as demonstrated by other authors [23,30]. The high levels of macrominerals in fiber further suggest that it can help regulate blood sugar levels and improve digestion. It is because macrominerals are essential for the absorption of glucose and the breakdown of food. Therefore, the presence of these minerals in fiber could contribute to its anti-diabetic properties. It was discovered by Olusanya *et al*. [31] that it can be used to enhance the health and well-being of children and pregnant women by supporting the development of strong teeth and bones. In general, high levels were observed, with the exception of Na, which happened to be the least abundant macromineral compared to the

others. In contrast, the findings of the Mg research were lesser than those of earlier investigations [23,30,32].

It should be noted that the P content of this study was higher than those reported by Yameogo *et al*. [32] and Dhakar *et al*. [33]. The most abundant microminerals were Fe, B, Mn, and Zn. The macrominerals are essential in the human body for various functions, including energy production, nerve conduction, muscle contraction, and cell signaling. Therefore, consuming the macrominerals in the recommended amounts allows for the proper functioning of the body and can help prevent deficiency-related conditions. It can also provide the necessary microminerals, such as iron, to prevent anemia. B and Zn were the most important microminerals, providing health benefits such as immune support. As previously suggested by Moyo and others, Cu is present in lower levels, and higher values have been observed in other research [27]. Consequently, because of its chemical makeup, moringa may have anti-inflammatory capabilities.

As can be observed, Na had the highest absorption rate during the oral phase, while the mineral with the lowest absorption was Mg. Na is a mineral that dissolves easily in water and is easily absorbed by the body. At the same time, Mg is a mineral that is more difficult to dissolve and is not as easily absorbed. Nevertheless, in the gastric phase, Cu had the highest absorption, while Fe had the lowest. Due to this, prior research has demonstrated a significant loss of bioaccessibility between oral and gastric phases of Fe absorption in comparison to Zn, which exhibited the lowest oral bioaccessibility but experienced a significantly greater absorption loss in the gastric phase. It suggests that the Fe absorption rate is susceptible to the stomach's acidic environment. At the same time, Zn is more resistant to the acidic environment, resulting in less loss of bioaccessibility. Ca was the mineral with the highest bioaccessibility during the intestinal phase, whereas Fe had the lowest. Hence, the bioaccessibility of the Fe is reduced compared to prior studies. Recent research demonstrated that a daily intake of 25 grams of moringa extract for six months reduced the incidence of anemia in one-year-old infants. [34]. Ca bioaccessibility exhibited the opposite tendency in the intestinal phase compared to the gastric phase: it increased. Perhaps this results from interactions between the gut and digestive secretions and other dietary components, resulting in compounds with stable and solubilizing constants determined by the intestinal pH, facilitating absorption [35]. Additionally, specific proteins and amino acids have been shown to improve the bioavailability of calcium, further increasing its absorption in the intestines.

According to the current investigation, the phenolic content of moringa leaves is comparable to the values reported by do Nascimento *et al*. [36]; their results reveal that the active phenolic compound concertation is 171 ± 0.50 mg GAE/g. However, the crude moringa (*M. peregrine*) extracts showed a higher level of phenolics, according to Al-Owaisi *et al*. [37]. Many investigations have indicated that ethanolic extracts of moringa leaves include polyphenols, steroids, alkaloids, and terpenoids. Research on phenolic compounds has revealed that they may have biological features, including antioxidant, anti-diabetic, hepatoprotective, anti-inflammatory, antibacterial, and anti-cancer effects [38]. It means that moringa extracts have the potential to be used in drugs and other treatments to help reduce inflammation, fight cancer, and protect the liver, among other benefits. Additionally, polyphenols have been shown to offer antioxidant protection, helping to protect cells from damage caused by free radicals [39]. These medicinal plants' phenolic compounds are interesting due to their antioxidant and anticarcinogenic properties. The secondary metabolites of moringa, flavonoids, and tannins are regarded as potential polyphenolic substances [40].

Depending on the quantity of moringa leaf extract, antibacterial activity ranged from 35 to 80%. Higher leaf aqueous extracts of moringa had greater antibacterial activity. The higher the leaf extract concentration, the more powerful the antibacterial properties. Higher concentrations of the extract inhibited the growth of bacteria, leading to greater antibacterial activity. It supports the idea that moringa can damage the growth of *E. coli* bacteria [41]. It indicates that the antibacterial properties of the leaf extract can be optimized by increasing the concentration, which is a more cost-effective and efficient approach to combating bacterial infections. Olson and Fahey (2011) claimed that the antibacterial action of moringa might be a result of the chemical molecule 4-(4'-O-acetyl-Lrhamnopyranosyloxy)-benzyl isothiocyanate, which inhibits key cellular membrane enzymes [41]. This enzyme is found in high concentrations in M. oleifera and effectively inhibits the growth of various bacterial species. Evidence suggests that increasing the concentration of this molecule in the leaf extract could make it more effective at combating bacterial infections. The phytochemical components, such as alkaloids, tannins, and flavonoids found in the ethanolic extracts of M. oleifera, contributed to their antibacterial action

[42]. More research on this subject is required to identify suitable antibacterial compounds.

Conflict of interest

The authors declare no conflict of interest.

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