

Comparative Analysis of Polyphenolic and Flavonoid Content, Antimicrobial and Antioxidant Activities of *Moringa Olifeira* Varieties from Mali and Algeria

Assia Keniche¹, Asma Khelassi-Sefaoui², Fatoumata Smake¹, Abderrahmane Khechkhouché³, Aboura Amina¹ and Soltani Yassemine Ikram¹

¹Laboratory of Organic Chemistry Natural Products and Analysis, University of Tlemcen, Algeria.

²Department of Hydraulic, Institute of Sciences and Technology, University Centre of Maghnia, Tlemcen, Algeria.

³Technology faculty, University of El Oued, Algeria.

Corresponding author: KHELASSI-SEFAOUI Asma, Affiliation: ²Department of Hydraulic, Institute of Sciences and Technology, University Centre of Maghnia, Tlemcen, Algeria.

Email: Khelassi_asma@yahoo.fr

Received 03 /04/ 2023; Accepted 11 /09/ 2023

Abstract

This study presents a thorough examination of the nutritional, medicinal, and industrial benefits of *Moringa oleifera*, specifically focusing on the total phenolic content (TPC) and total flavonoid content (TFC) in diverse extracts from leaves collected in Mali and Algeria. Utilizing ethanol extraction (EE-MO), the TPC and TFC were analyzed by UV spectroscopy, revealing a TPC range of 1 to 2.35 mg/g, with the highest value observed in leaves from Algeria (LA-MO). Total flavonoids varied from 0.72 to 1.9 mg/g, and Mali's powder and leaves (PM-MO and LM-MO) exhibited significantly higher TF results. The study establishes a linear relationship between TPC, TFC, and antioxidant activity, demonstrating the impact of agroclimatic conditions. Ethanol extracts from *Moringa oleifera* exhibited potent antibacterial activity, with Mali's leaves (LM-MO) demonstrating superior inhibition against specific bacteria. This research highlights geographical variations in *Moringa oleifera* properties and underscores its potential as a source of natural antioxidants for diverse industries, providing valuable insights for nutrition, medicine, and antimicrobial applications.

Keywords: Total phenolic content (TPC), total flavonoid content (TFC), ethanol extraction, antioxidant activity, medicinal benefits.

Tob Regul Sci.™ 2023;9(2): 2849 - 2863

DOI: doi.org/10.18001/TRS.9.2.186

Introduction

Belonging to the Moringaceae family, *Moringa oleifera* (MO), colloquially known as the "miracle tree," originates from the sub-Himalayan regions of Northern India and finds widespread cultivation in tropical and subtropical climates [1]. Esteemed for its medicinal attributes, this genus of plants has a rich traditional history in treating wounds, colds, and diabetes, alongside its utilization as a nutritional source and water purifier [2]. Various parts of the multipurpose *Moringa oleifera* tree have been associated with medicinal and therapeutic properties, including the treatment of conditions like ascites and rheumatism, acting as cardiac and circulatory stimulants [3, 4]. Notably, almost every part of the tree holds high nutritional value and is utilized as food. Moreover, the plant is recognized for its antimicrobial properties, contributing to its extensive use in the treatment of human diseases [5, 6].

A significant reduction in the growth of test bacteria was observed with the distillate of *Moringa oleifera*, suggesting an antibacterial effect. Among the tested bacteria, greater inhibition was observed in the case of *Escherichia coli*, followed by *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*. Fungal inhibition was also noted, as evidenced by a reduced colony diameter in plates treated with the distillate compared to control plates. Specifically, more inhibition of *Aspergillus niger* was observed, followed by *A. oryzae*, *A. terreus*, and *A. nidulans* [7]. The reported components of *Moringa oleifera* preparations with antibacterial activity include 4-(4'-O-acetyl- α -L-rhamnopyranosyloxy) benzyl isothiocyanate, 4-(α -L-rhamnopyranosyloxy)benzyl isothiocyanate, niazimicin, pterygospermin, benzyl isothiocyanate, and 4-(α -L-rhamnopyranosyloxy) benzyl glucosinolate [8].

The antioxidant property of *Moringa oleifera* may be attributed to the presence of phenolic compounds, as confirmed by phytochemical screening of the hydro-ethanolic extract. *Moringa oleifera* pods contain important bioactive compounds, including glucosinolates, isothiocyanates, thiocarbamates, and flavonoids [9-11]. Antioxidants have been identified in both plant materials and supplements. Due to their natural origin, antioxidants obtained from plants are of greater benefit compared to synthetic ones. The use of natural antioxidants from plants does not induce side effects, while synthetic antioxidants were found to have genotoxic effects. Therefore, numerous investigations into the biological activity and chemical composition of medicinal plants as a potential source of natural antioxidants are extensive [12, 13].

This study aims to comprehensively analyze and compare the biochemical composition of *Moringa oleifera* from Mali and Algeria, specifically focusing on total phenolic content (TPC) and total flavonoid content (TFC) in different extracts. It also aims to evaluate the antioxidant and antibacterial activities, to establish a correlation between TPC, TFC and bioactivities, and to highlight the geographical variations in the properties of *Moringa oleifera*. Additionally, it aims

to explore the industrial potential of *Moringa oleifera* as a source of natural antioxidants for applications in various industries, including food, health and medicine.

Experimental Section

2.1. *Moringa oleifera*

Moringa oleifera as shown in Figure 1, commonly known as the drumstick tree, horseradish tree, or simply Moringa, is a versatile and fast-growing tree native to parts of Africa and Asia. It belongs to the family Moringaceae and is recognized for its nutritional, medicinal, and industrial significance. Almost all parts of the Moringa tree are utilized for various purposes. The leaves are rich in essential nutrients, including vitamins, minerals, and proteins, making them a valuable dietary supplement. The seeds contain oil with potential industrial applications, and the roots are used in traditional medicine. *Moringa oleifera* is known for its adaptability to diverse climatic conditions, making it a resilient and sustainable plant. It has gained global attention for its potential to address malnutrition and provide sustainable solutions in agriculture. Additionally, *Moringa oleifera* is studied for its antioxidant, anti-inflammatory, and antimicrobial properties, contributing to its role in traditional medicine and potential applications in the pharmaceutical and health industries.



Figure 1. *Moringa Oleifera*

2.1. Extraction protocol

Dry leaves and powder of *Moringa oleifera* leaves were collected from Bamako, Mali. The leaves from Mali were harvested in October, while those from Béchar, South Algeria, were collected in February 2019. Both sets of samples were dried at room temperature (in the shade) for a period of 3 days. Ethanol extracts of *Moringa oleifera* leaves (EE-MOL) and *Moringa oleifera* powder (EE-MOP) were prepared by adapting a method previously described by Kothari et al[8], with some modifications. The collected samples were ground, and 20 g of resulting powder was dissolved in 96% ethanol (270 mL) in sealed bottles, with frequent stirring at room temperature over a 3-day period. After filtration, the filtrate was concentrated under reduced pressure using a rotary evaporator with a bath maintained at 40 °C.

Table 1 presents data on ethanol extracts of *Moringa oleifera* leaves (EE-MOL) from Mali and Algeria, along with *Moringa oleifera* powder (EE-MOP) from Mali, specifying the extraction yields. The ethanol extract from *Moringa oleifera* leaves in Mali (EE-MOL: Mali) exhibited a yield of 17%, indicating the proportion of extracted material relative to the initial amount. Similarly, the ethanol extract from *Moringa oleifera* leaves in Algeria (EE-MOL: Algeria) showed a yield of 11.45%, while the *Moringa oleifera* powder from Mali (EE-MOP: Mali) had a yield of 20%.

Table 1. Yield of the extraction process of *Moringa Oleifera*

Extracts	Yield
Ethanol extracts of MO Leaves (EE-MOL) :(Mali)	17%.
Ethanol extracts of MO Leaves (EE-MOL) :(Algeria)	11.45%
MO powders (EE-MOP) :(Mali)	20%.

2.2. Total Polyphenols Contents from EE-MO

Total polyphenol content (TPC) is the overall concentration of polyphenolic compounds in a sample, measured in milligrams of gallic acid equivalents (GAE) per gram. Polyphenols, found in plants, are known for their antioxidant properties, contributing to potential health benefits. The TPC indicates the quantity of these bioactive compounds in a given substance, with higher levels suggesting increased antioxidant potential.

The determination of total polyphenol contents in the extracts followed the method of Singleton et al [14] using a Folin-Ciocalteu solution. A mixture was prepared by combining the sample (200 μ L), Folin-Ciocalteu reagent (1 mL), an aqueous sodium carbonate solution (75g/L) (0.8 mL), and water (1 mL). After incubating for 1 hour at room temperature, the characteristic blue color developed. The absorbance of the clear supernatant was measured at 760 nm. The total polyphenol content was calculated using a calibration curve previously constructed with Gallic acid solutions and expressed as milligrams of Gallic acid equivalents (GAE) per gram of the sample.

2.3. Total Flavonoids Contents of EE-MO:

Total flavonoid content (TFC) refers to the concentration of flavonoid compounds in a sample, measured in milligrams of Rutin equivalents (RE) per gram. Flavonoids are a subgroup of Polyphenolic compounds found in plants, known for their antioxidant and anti-inflammatory properties. TFC provides insight into the quantity of these bioactive compounds in a substance,

with higher levels indicating increased antioxidant potential and potential health benefits. The measurement of TFC is commonly performed using methods like UV spectroscopy.

The determination of total flavonoid contents followed the method outlined by Lamaison et al [15]. Specifically, 1 mL of the extract was added to a solution containing 2% methanol AlCl_3 (0.5 mL) and methanol (1.5 mL). After incubating for 1 hour at room temperature, the absorbance was measured at 420 nm. The total flavonoid contents were calculated in the standard manner (mg/g) using a calibration curve constructed with Rutin. The amount of flavonoids was determined as usual, expressed in equivalent milligrams per gram (mg Eq R/g ES) from a calibration curve.

2.4. Antioxidant activity of EE-MO by FRAP Method

The Ferric Reducing Antioxidant Power (FRAP) method is a laboratory technique used to measure the antioxidant capacity of a substance. It assesses the substance's ability to reduce ferric ions (Fe^{3+}), resulting in a color change that is proportional to its antioxidant strength. This method is commonly applied to analyze the total antioxidant capacity of biological samples, foods, and plant extracts, providing valuable insights into their potential health-promoting properties based on their ability to donate electrons and act as antioxidants.

The power of reduction of the ethanol solution of EE-MO was determined following the method of Lugasi et al [16] with some modifications. A solution of the sample (1 mg/mL, 1 mL) was mixed with a phosphate buffer solution (2.5 mL, 0.2 M, pH 7) and 1% potassium ferricyanide solution (2.5 mL). The resulting mixture was incubated at 35 °C for 20 min. Subsequently, a 10% aqueous trichloroacetic solution (2.5 mL) was added to the mixture, followed by centrifugation at 1500 rpm. The solution (2.5 mL) was then mixed with distilled water (2.5 mL) and a freshly prepared 0.1% FeCl_3 solution (0.5 mL). The absorbance was read at 700 nm, and the reduction power was expressed in Trolox equivalents (TEAC, g-1). Trolox equivalents signify the amount of Trolox (mg) that exhibits the same reducing power as 1 g of the sample, indicating a high reduction power [17].

2.5. Antioxidant activity using DPPH method

The DPPH (2,2-diphenyl-1-picrylhydrazyl) method is a laboratory assay used to measure the antioxidant capacity of a substance. It involves reacting a stable free radical, DPPH, with the substance under investigation. As the substance acts as an antioxidant, it donates electrons to the DPPH radical, resulting in a color change from purple to yellow. The extent of color change is measured spectrophotometrically, providing information about the substance's ability to neutralize free radicals and indicating its antioxidant strength. This method is frequently employed to assess the radical-scavenging activity of various compounds, including plant extracts, foods, and beverages, offering insights into their potential health-promoting properties.

The measurement of radical scavenging activity was adapted from Kikuzaki's method [18]. The reaction mixture consisted of methanol (2.5 mL), DPPH (125 µM), and the samples to be tested. After 45 minutes of incubation at room temperature, the absorbance was recorded at 517 nm. The results were expressed as a percentage decrease from the control values. The EE-MO was evaluated using decreasing assay concentrations, and the antioxidant activity was calculated using the following relation (1):

$$AA\% = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (1)$$

2.6. Antimicrobial activity

Antimicrobial activity refers to a substance's capacity to inhibit or kill microorganisms such as bacteria, viruses, fungi, or parasites. This property is assessed in laboratory settings by observing the substance's impact on microbial growth and viability. Antimicrobial agents can disrupt microbial cells or interfere with essential processes, making this evaluation crucial in medicine, drug development, and various industries for ensuring the safety and efficacy of products like antibiotics, antivirals, and preservatives. Commonly tested substances include plant extracts, essential oils, and synthetic compounds.

The antimicrobial activity of the samples was investigated using the disc diffusion method, as per Bauer and Col[19], on Mueller Hinton agar. Four reference strains, including *S. aureus* (ATCC29213), *Escherichia coli* (ATCC25922), *P. aeruginosa* (ATCC 27853), *B. subtilis* (ATCC 6633), and *K. pneumonia* (ATCC 70603), were provided by Institut Pasteur d'Alger/Algeria. The extracts were weighed using sterile volumetric flasks and dissolved with 70% ethanol to achieve a concentration of 0.1 mg/mL. Microorganism suspensions (0.5 McFarland scale) were spread onto solid media plates. Filter paper discs (6 mm diameter) were impregnated with 20 µL of each EE-MO sample and with ethanol as a control. Each disc was inoculated with a strain, swabbed, and then placed in Petri dishes containing Muller-Hinton agar medium for bacteria and PDA for yeasts. After 2 hours, the Petri dishes were incubated at 37 °C, and the diameters of the inhibition zones were measured in millimeters.

3. Results And Discussion

3.1. Total polyphenols and flavonoid contents

The total polyphenols TP (mg/g), determined from regression equation of calibration curve (2) and expressed in GAE, varied between 1 and 2.35 mg/g. TP was in order (Leaves MO Algeria) LA-MO > (Powder MO Mali) PM-MO > (Leaves MO Mali) LM-MO which was well correlated with previous studies about content of phenolic compounds in MO from other countries such as India, Nigeria and Nicaragua reported by Becker and Col [20].

$$(y = 12,844x + 0,3301) \quad (2)$$

Figure 2 represents the total polyphenol content of different test extracts MO *Moringa Oleifera*. The extracts exhibited varying total polyphenol (TP) content, with the highest value of 2.35 mg/g in GAE. In contrast, *Moringa oleifera* (MO) leaves from Algeria (LA-MO) and the extract of MO leaves from Mali (LM-MO) displayed lower values at 1 mg/g in GAE. The observed difference in TP content between Algerian and Malian MO extracts may be attributed to the timing of harvest, with Algerian MO collected in February 2019 and Malian MO in October 2018. Notably, fresh MO leaves are known to contain a substantial amount of polyphenols[21]. Furthermore, the powder form (PM-MO), obtained through trade, exhibited higher polyphenol content, likely due to the conservation method employed.

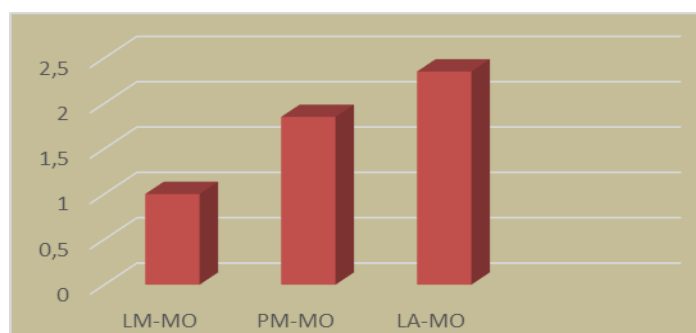


Figure 2 .Total polyphenol content of different test extracts MO *Moringa Oleifera*; LM-MO Leaves Mali MO; PM-MO powder Mali MO; LA-MO Leaves Algeria MO

Figure 3 shows the total flavonoid content of different test extracts MO *Moringa Oleifera*. The TF in (mg/g) determined from regression equation (3) of calibration curve and expressed in RE, varied between 0.72 and 1.9 mg/g. Significantly higher results were found in powder PM-MO and LM-MO extracts followed by LA-MO extracts.

$$(y = 23,55x + 0,22)$$

(3)

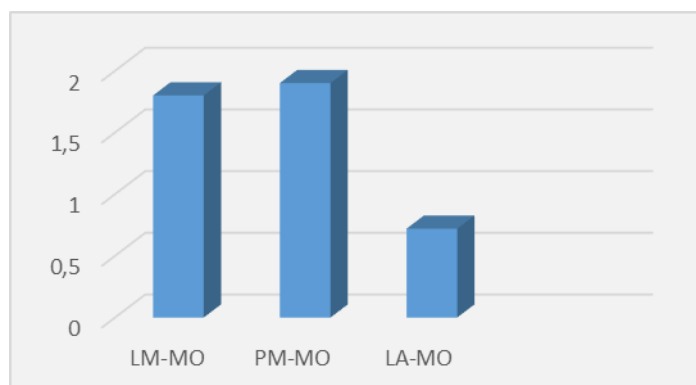


Figure 3. Total flavonoid content of different test extracts.MO *Moringa Oleifera*; LM-MO Leaves Mali MO; PM-MO powder Mali MO; LA-MO Leaves Algeria MO.

3.2. Antioxidant activity by DPPH

Figure 4 represents the Antioxidant activities of various extracts of *Moringa Olifeira* by DPPH method. The higher absorbance of the reaction mixture indicated superior reducing power, affirming a linear relationship between total polyphenol content (TPC), total flavonoid content (TFC), and antioxidant activity. Notably, the powder from Mali demonstrated enhanced antioxidant activity compared to leaves from both Algeria and Mali. The scavenging activity of various *Moringa oleifera* (MO) extracts exhibited concentration-dependent variations. Generally, extracts with higher polyphenol content displayed increased antioxidant activity, attributed to the synergistic effects of substances at varying concentrations and their potent hydrogen atom donating abilities. This aligns with previous reports establishing a linear correlation between DPPH• radical scavenging activity and polyphenolic extracts in diverse vegetables and fruits [22, 23]. Moreover, the presence of glycosides in flavonoids might influence radical scavenging ability by affecting hydrogen donation [24].

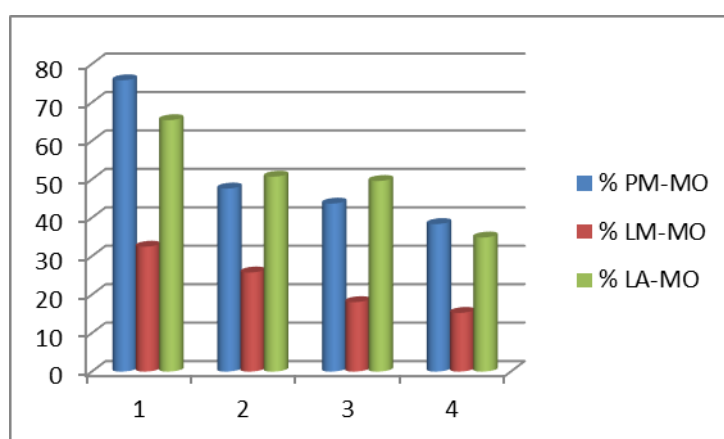


Figure 4. Antioxidant activities of various extracts of *Moringa Olifeira* by DPPH method.

Figure 5 represents the Comparison of IC₅₀ of *Moringa Olifeira* extracts with the reference ascorbic acid. These compounds quench ROS, chelate metal ions and regenerate membrane-bound antioxidants. The biochemical basis of the chemo-preventive potency of *Moringa Olifeira*, extract may be attributed to the synergistic action of the constituents of the extract and the induction of Phase- II enzymes (GSTs) and antioxidant enzymes, which might be implicated in activity [9].

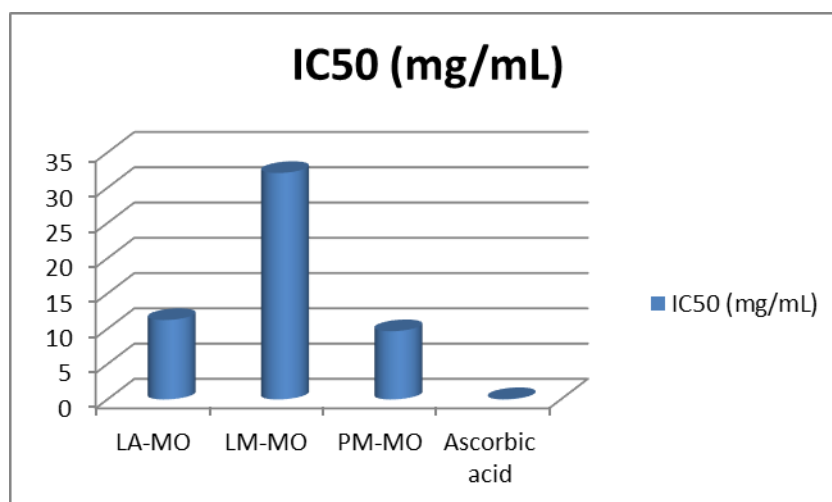


Figure 5: Comparison of IC50 of *Moringa Olifeira* extracts with the reference ascorbic acid.

3.3. Antioxidant activity by FRAP

Figure 6 represents the Antioxidant activities of various extracts of *Moringa Olifeira* by FRAP method. The amount of polyphenols and flavonoids was correlated with the Ferric-Reducing Antioxidant Power (FRAP) method. PM-MO exhibited high ion reduction activity, reaching a maximum optical density of 0.396 at a concentration of 0.2 mg/mL, surpassing the respective values of leaves from Mali and Algeria, which were 0.294 and 0.389 for the same concentration. Notably, the activity of all three samples was significantly lower than that of Trolox ($A = 1.36$). Polar molecules in plant extracts, as suggested by Kang et al[25], contribute to increased antioxidant activity. Natural extracts' ability to trap free radicals depends on various parameters, including dose, structure, substituent, and the degree of polymerization of the molecules [26]. Comparisons with previous studies on *Moringa Oleifera* from Nicaragua, India, and Niger indicate variations in total phenolics concentration. Nicaraguan leaf samples exhibited a higher phenolic concentration (4.25%) than those from India (2.94%) and Niger (3.66%). Despite being lower than earlier reported values from Nicaraguan *Moringa* leaves, these samples are considered more potent, containing nutraceutical phenolic constituents such as total flavonoids. Notably, freeze-dried leaf samples from various locations contained both quercetin and kaempferol in concentration ranges of 633.5 to 926 mg/100g and 104.7 to 225.4 mg/100 g, respectively [20]. The variations in these parameters may be attributed to agroclimatic conditions, including season, climate, altitude, temperature, annual precipitation, and wind, influencing secondary metabolite content and subsequent antioxidant and antibacterial activities [27].

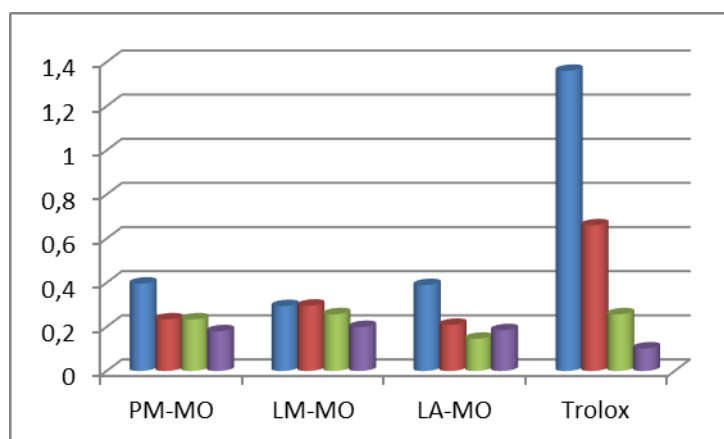


Figure 6: Antioxidant activities of various extracts of *Moringa Oleifera* by FRAP method.

3.4. Antibacterial activity assay

Figure 7 shows the histogram of antibacterial activity of MO samples. The Ethanol extracts of MO (EE-MO) were examined for their antibacterial activity using disc diffusion to measure inhibition zone diameters. Gentamycin, Ciprofloxacin, and Tobramycin served as references, with the same strains used throughout the study. According to the results, the antimicrobial activity of EE-MO samples varied based on the MO's origin. Leaves from Algeria (LA-MO) showed no zone of inhibition against all tested bacteria. The leaves from Mali (LM-MO) exhibited an 11 ± 0.05 mm diameter inhibition zone with *B.subtilis* and 8 ± 0.00 mm against *P. aeruginosa*. For the powder from Mali (PM-MO), an inhibition zone of 11 ± 0.07 mm on *B.subtilis* and 7 ± 0.00 mm on *P.aeruginosa* was observed, while *E.coli* and *S.aureus* were not inhibited by any MO samples. Consequently, leaves from Mali demonstrated the best activity among all samples.

The literature on *Moringa Oleifera* from various locations around the world has reported a considerable reduction in the growth of test bacteria, suggesting an antibacterial effect. Among the tested bacteria, more inhibition was observed in the case of *Escherichia coli*, followed by *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Bacillus subtilis* [28]. *Moringa Oleifera* extracts exhibited a potent inhibitory effect against multidrug-resistant methicillin-resistant *S.aureus* [29, 30]. According to Ojiako and colleagues [31], the extracts showed a wide range of activities. For example, the ethanolic extract of MO from Nigeria exhibited effectiveness against *Staphylococcus* (9 mm) and *E.coli* (4 mm). This evidence supports the potential use of MO leaves in the treatment of infections caused by these pathogens [32-35].

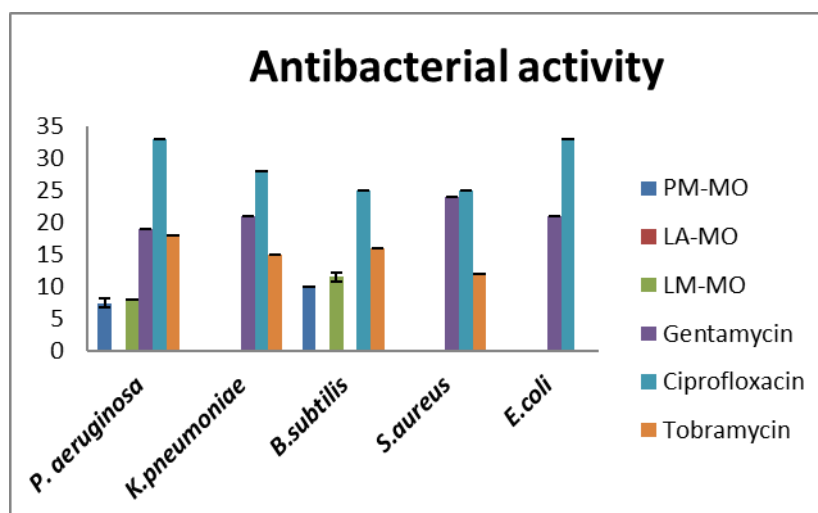


Figure 7. Histogram of antibacterial activity of MO samples.

Conclusion

This comprehensive investigation sheds light on the diverse benefits of *Moringa oleifera*, focusing on nutritional, medicinal, and industrial aspects. Utilizing ethanol extraction (EE-MO) and UV spectroscopy, the study meticulously analyzed total phenolic content (TPC) and total flavonoid content (TFC) in leaves collected from Mali and Algeria, yielding significant numerical results.

- The TPC ranged from 1 to 2.35 mg/g, with the highest value observed in Algerian leaves (LA-MO), while total flavonoids ranged from 0.72 to 1.9 mg/g, showing notably higher TFC in Mali's powder and leaves (PM-MO and LM-MO).
- The investigation established a linear relationship between TPC, TFC, and antioxidant activity, providing quantitative insights into the impact of agroclimatic conditions on *Moringa oleifera*'s bioactive compounds. Additionally, ethanol extracts exhibited potent antibacterial activity, with Mali's leaves (LM-MO) demonstrating superior inhibition against specific bacteria.
- The geographical variations highlighted underscore the potential of *Moringa oleifera* as a rich source of natural antioxidants for diverse industries, including nutrition, medicine, and antimicrobial applications.
- The inclusion of numerical data enhances the robustness of the conclusions, providing valuable quantitative information for further exploration and utilization of *Moringa oleifera*'s potential.
- The variability in secondary metabolites' levels due to factors such as geographic origin, drying, storage, and plant age, showcasing the plant's importance as a medicinal resource.
- The antioxidant and antimicrobial activities further support the presence of active phytochemicals in *Moringa oleifera*.

Acknowledgment

We are indebted to General Directorate for Scientific Research and Technological Development (DGRS-DT), Ministry of Higher Education and Scientific Research (Algeria) for the financial support of this work.

References

- [1] Singh A., 2018, Ethnomedicinal, Pharmacological and Antimicrobial Aspects of Moringa oleifera Lam.: A review, The Journal of Phytopharmacology, 7: 45-50.
- [2] Reetu Bhargavi K., Tomar M., Subha K., 2020, Moringa oleifera : a health food for animal and human consumption, Food and Scientific Reports., 1, 11-14.
- [3] Godinez-Oviedo A., Guemes-Vera N., Acevedo-Sandoval O.A., 2016, Nutritional and Phytochemical Composition of Moringa oleifera Lam and its Potential Use as Nutraceutical Plant: A Review, Pakistan Journal of Nutrition., 15: 397-405.
- [4] Al_husnan L., Alkahtani M., 2016, Impact of Moringa aqueous extract on pathogenic bacteria and fungi in vitro, Annals of Agricultural Science., 61:247–250.
- [5] Anwar F., Latif S., Ashraf M., Gilani A.H., 2017, Moringa oleifera: A food plant with multiple medicinal uses. Phytother Res., 21: 17-25.
- [6] Chuang P.H., Lee C.W., Chou J.Y., Murugan M., Shieh B.J., 2007, Anti-fungal activity of crude extracts and essential oil of Moringa oleifera Lam. Bioresour Technol., 98: 232-236.
- [7] Kekuda T.P., Mallikarjun N., Swathi D., Nayana K.V., Meera B.A., Rohini T.R., 2010, Antibacterial and Antifungal efficacy of steam distillate of Moringa oleifera Lam, J. Pharm. Sci. and Res.,2:34-37.
- [8] Vyas S., Kachhwaha S., Kothari S.L., 2015, Comparative analysis of phenolic contents and total antioxidant capacity of Moringa oleifera Lam., Pharmacognosy Journal., 7:44-54.
- [9] Bharali R., Tabassum J., Azad M.R.H., 2003, Chemomodulatory effect of Moringaoleifera, Lam. on hepatic carcinogen metabolizing enzymes, antioxidant parameters and skin papillomagenesis in mice, Asian Pacific Journal of Cancer Prevention., 2, 131 -139.
- [10] [10] Kumar A., Pari L., 2003, Antioxidant action of Moringa oleifera Lam (drumstick) against antitubercular drugs induced lipid peroxidation in rats, Journal of Medicinal Food.,6:255-259.

- [11] Paliwal R., Sharma V., Pracheta S., Sharma S., Padma P.R., 2009, Antioxidant activity and total phenolic content of Moringa oleifera leaves in two stages of maturity, Plant Foods Hum Nutr., 64:303-11.
- [12] Lockett C.T., Calvert C.C., Grivetti L.E., 2000, Energy and micronutrient composition of dietary and medicinal wild plants consumed during drought. Study of rural Fulani, northeastern Nigeria, Int J Food Sci Nutr., 51: 195-208.
- [13] Nikkon F., Saud Z.A., Rahman M.H., Haque M.E., 2003, In-vitro antimicrobial activity of the compound isolated from chloroform extract of Moringa
- [14] oleifera Lam, Pakistan J Biol Sci., 6:1888-1890.
- [15] Singleton V.L., Orthofer R., Lamuela-Raventos R.M., 1999, Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol., 299:152-178.
- [16] Lamaison J.L.C., Carnet A., Teneurs en principaux flavonoïdes des fleurs de Crataegus monogyna Jacq et de Crataegus laevigata (Poiret D. C) en fonction de la végétation, Plants, MedPhytother., 25 :12-16.
- [17] Lugasi A., 2003, the role of antioxidant phytonutrients in the prevention of diseases”, Acta Biologica Szegediensis., 47:119-125.
- [18] Berker K.I., 2007, Comparative evaluation of Fe (III) reducing power-based antioxidant capacity assays in the presence of phenanthroline, batho-phenanthroline, tripyridyltriazine (FRAP), and ferricyanide reagents”, Talanta., 72: 1157–1165.
- [19] Kikuzaki M., Hisamoto K., Hirose K., Akiyama H., 2002, Antioxidant properties of ferulic acid and its related compounds, Journal of Agricultural and Food Chemistry., 50: 2161– 2168.
- [20] Bauer A. W., Kirby M.D., Sherris J.C., Turck M.D., 1996, Antibiotic Susceptibility Testing by a Standardized Single Disk Method American Journal of Clinical Pathology., 45: 493–496.
- [21] Siddhuraju P., Becker K., 2003, Antioxidant Properties of Various Solvent Extracts of Total Phenolic Constituents from Three Different Agroclimatic Origins of Drumstick Tree (Moringa oleifera Lam.) Leaves, J. Agric. Food Chem., 51: 12-22.
- [22] Hertog M. G. L., Hollman P. C. H., Venema D. P., 1992, Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. J. Agric. Food Chem., 40: 1591 -1598.

- [23] Robards K., Prenzeler P. D., Tucker G., Swatsitang P., Glover W., 1999, Phenolic compounds and their role in oxidative processes in fruits. *Food Chem.*, 66: 401 -436.
- [24] Jimenez-Escrig A., Rincon M., Pulido R., Saura-Calixto F., 2001, Guava fruit (*Psidium guajava* L.) as a new source of antioxidant dietary fiber, *J. Agric. Food Chem.*, 49: 5489-5493.
- [25] Von Gadow A., Joubert E., Hansmann C. F., 1997, Comparison of the antioxidant activity of aspalathin with that of other plant phenols of rooibos tea (*Aspalathus linearis*), R-tocopherol, BHT and BHA, *J. Agric. Food Chem.*, 45: 632-638.
- [26] Kang W.Y., Wang J.M., 2010, In vitro antioxidant properties and in vivo lowering blood lipid of *Forsythia suspensa* leaves, *Med. Chem. Res.*, 19: 617-628.
- [27] Tumer T.B., Rojas-Silva P., Poulev A., Raskin I., Waterman C., 2015, Direct and Indirect Antioxidant Activity of Polyphenol- and Isothiocyanate-Enriched Fractions from *Moringa oleifera*, *J. Agric. Food Chem.*, 63: 1505 -1513.
- [28] Ndhlala A., Mulaudzi R., Ncube B., Abdelgadir H., 2014, Antioxidant, Antimicrobial and Phytochemical Variations in Thirteen *Moringa oleifera* Lam. Cultivars, *Molecules*, 19: 10480 -10494.
- [29] Siddhuraju P., Becker K., 2003, Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. *J. Agric. Food Chem.*, 51: 2144 - 2155.
- [30] Nikkon F., Saud Z.A., Rahman M.H., Haque M.E., 2003, In-vitro antimicrobial activity of the compound isolated from chloroform extract of *Moringa oleifera* Lam, *Pakistan J Biol Sci.*, 6: 1888-1890.
- [31] Esther Fayemi N.O., Ekennia A.C., Katata-Seru L., Ebokaiwe A.P., Ijomone O.M., Onwudiwe D.C., Ebenso E.E., 2018, Antimicrobial and Wound Healing Properties of Polyacrylonitrile *Moringa* Extract, *ACS Omega*, 3: 4791 -4797.
- [32] Ojiako E.N., 2014, Phytochemical Analysis and Antimicrobial Screening of *Moringa Oleifera* Leaves Extract, *The International Journal Of Engineering And Science*, 3: 32-35.
- [33] Adline J., Devi D., 2014, A study on phytochemical screening and antibacterial activity of *moringa oleifera*, *Int. J. Appl. Nat. Soc. Sci.*, 2: 169 -176.
- [34] Iqbal S., Bhanger M.I., 2006, Effect of season and production location on antioxidant activity of *Moringa oleifera* leaves grown in Pakistan, *J Food Comp Anal.*, 19: 544-551.

- [35] Lockett C.T., Calvert C.C., Grivetti L.E., 2000, Energy and micronutrient composition of dietary and medicinal wild plants consumed during drought, Study of rural Fulani, northeastern Nigeria, Int J Food Sci Nutr, 51: 195-208.
- [36] Abdulkadir I., Abdullahi Nasir I., Sofowora A., Yahaya F., Alkasim A., Adamu H.I., 2015, Phytochemical Screening and Antimicrobial Activities of Ethanolic Extracts of Moringa oleifera Lam on Isolates of Some Pathogens, J App Pharm., 7: 4-10.