Phytochemical screening, antioxidants properties and antibacterial efficacy of moringa leaves

Abid Mehmood[1], Khalid Naveed[1], Sami Ullah Khan[1], Naveed Ul Haq[3], Malik Faizan Shokat[2], Muzafar Iqbal[1], Rashid Ali[1], Shahbaz Nisar[1], Jawad Ahmad[1], Anees Ur Rehman[1], Saif Ur Rehman[1], Ahmad Ayub[2], Muhammad Usman[2], Qammer Shahzad[3] and Shujat Ali[2]

1. Department of Agronomy, The University of Haripur
2. Department of Horticulture, The University of Haripur
3. Department of Food Science and Technology, The University of Haripur

Abstract

Plants are unique source of food, medicinal artifacts, energy and shelter for both human and animal., many useful harvests obtained from plants directly or indirectly validate their importance to the human and other living organisms. Drug discovery, often known as "natural product screening," is the process of evaluating all available medications using pharmacological and phytochemical methods. The current investigation was carried out at Agronomy Laboratory and Microbiology Laboratory, The University of Haripur in order to investigate phytochemical analysis and antimicrobial activity of moringa leaves. Physiochemical and antioxidant evaluation of moringa leaves yielded 6.37% moisture content, 9.66% total ash, 6.41 pH (1% solution), 5.85 pH (10% solution), 0.08% acidity (1% solution), 0.34% acidity (10% solution), 2.59 mg GAE/100g total phenolic contents (TPC), 3.75 mg QE/100g total flavonoids contents and 72.02 DPPH radicals Scavenging activity. Phytochemical screening of water and ethanol extracts showed presence of coumarine, phytosterols, glycosides, tannins, flavonoids and alkaloids in water extracts. Ethanolic extracts showed positive presence of polyphenols, tennins. Antibacterial assay reviled that the highest antibacterial activity of 1mg/ml and 0.5 mg/ml of ethanolic extracts were found to be against E. Coli (17.6, 13.3 and 7.6 mm respectively) followed by S. Aurus (13.6, 8.2 and 7.6 mm), B. Subtilis (10.8, 8.3 and 6.3 mm) and P. aeruginosa (7.7, 5.7, 4.8 mm respectively). Moringa extract also inhibited the development of certain bacteria, demonstrating its anti-microbial potential. This study verifies the presence of important phytochemicals in moringa leaf extracts, leading to the conclusion that moringa leaves can be utilized to cure a variety of ailments.

Introduction

Drug discovery, often known as "natural product screening," is the process of evaluating all available medications using pharmacological and phytochemical methods (Asghar *et al*., 2022). Moringa belonging to the family Moringaceae. There are 13 species that are being the part of this family. Uses of moringa pods as vegetables has been known from 150 BC and used by higher hierarchy in making their skin beautiful (Mehmood *et al*., 2021a). It had been used in cultures of Romans, Greeks and Egyptian. At ancient Indian times, its juice was known as "Elixir Drink" believed to boost energy and also important pain and stress reliever (Fahal *et al*., 2018).

Moringa leaves pods, flower and seeds are used as vegetable, roots and spice and water purifier, seed oils in cosmetics and its use as a therapeutic plant is also evident (kumar, 2015). Its nutrition value which is attributed to presence of carbohydrates, fat and protein belong with vitamins (A, B and C) and minerals (Iqbal and Bhanger, 2015) proved it as medicinal plants. The leaves contain a wealth of nutrients, both macro- and micro, including a plenty of vitamins and protein. Therefore, it has been widely used in the Ayuvedic and Unani system (Mehmood *et al*., 2021a). Moringa commonly known as "Sohanjna" has been considered as an important plant in agro forestry of Pakistan (Maqsood *et al*., 2017). It is commonly belonging and cultivated in Punjab, Sindh, Baluchistan and Khyber Pakhtunkhwa province of Pakistan.

The hunt for natural antioxidants that are both safe and efficient is currently focusing on food plants, particularly spices and herbs (Mehmood *et al*., 2018). Moringa oliefera is regarded as one of the most beneficial trees in the world (Tang *et al*., 2018). Moringa is gaining popularity these days because of the presence of cytokinin, antioxidants, and macro and micronutrients in its leaves (Abdalla, 2013: Mehmood *et al*., 2021a). Furthermore, through activating the antioxidant defense system and plant secondary metabolites, moringa leaf extract (MLE) mitigated the harmful impacts of abiotic conditions such as salt and drought stress (Afzal *et al*., 2015). The current study was conducted to evaluate the phytochemicals and to find the antimicrobial activity of moringa leaves.

Materials and methods

Collection of moringa leaves

Moringa leaves were collected from Agricultural Research Farm, The University of Haripur.

Preparation of moringa leaf powder

Moringa leaves were separated from plants, cleaned and subjected to dry at room temperature in Agronomy Laboratory, The University of Haripur. After drying leaves were grounded into fine powder.

Physiochemical evaluation of moringa leaves

The following parameters were analyzed as described by AOAC, 2000.

Moisture (%)

Moisture content was analyzed by AOAC (2000) method.

$$\text{Moisture (\%)} = \frac{W1-W2}{W1} \times 100$$

Where W1 is weight of sample before drying and W2 is weight of sample after drying.

Total ash (%)

Following equation was employed to obtain total ash (%)

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

pH measurement

one percent and 10% (w/v) solution of moringa leaf powder was prepared and subjected to pH measurement using pH meter.

Titratable acidity

Acidity of moringa leaf powder was determined by using formula

$$\text{Titratable acidity (\%)} = \frac{\text{ml of NaOH x normality of NaOH x mileq factor}}{\text{Weight of sample}} \times 100$$

Antioxidants and radical scavenging activity

Extract preparation

One gram of moringa leaf powder was added in 40 ml of methanol (80%) and concentrated to 10 ml of its original volume using rotary evaporator at 55^{o}C. The concentrate was used to evaluate the total phenolic content, total flavonoids content, and DPPH- Radical scavenging activity.

Total phenolic content (mg GAE/100g DW)

Julkenen-Titto (1985) method was used to determine total phenolic content.

Total flavonoid contents (mg QE/100g DW)

0.3 mL sodium nitrate was added to one mL of already prepared sample (5%). For 5 minutes, this combination was left alone. After that, 0.6 mL of aluminium chloride was added. 2 ml of 1 M

NaOH was added after 5 minutes. Finally, using a spectrophotometer, the absorbance was measured at 510 nm. Quercetin was used as a reference to determine the total flavonoids content (Mehta *et al*., 2014).

Free Radical scavenging activity (%)

Radical scavenging activity expressed as the inhibition percentage and calculated using the following formula:

$$RSA\ (\%) = \frac{AC - AS}{AC} \times 100$$

Where AC and AS are the absorbance of control and sample solutions respectively.

Phytochemical analysis

The Leaves extracts of all solvents were subjected to qualitative determination of flavonoids (Trease & Evans, 1996; Shahzad *et al*., 2018), saponins (Tyler, 1994; Mehmood *et al*., 2021b), alkaloids (Tyler, 1994; Hussain *et al*., 2011), phenols (Roopashree *et al*., 2008) and tannins (Roopasheer *et al*., 2008).

Antibacterial activity assay

Antibacterial assay of both ethanolic and aqueous extracts were performed by following Mehmood *et al*., (2021b). Four different strains of bacteria were selected on the basis of their suitability and popularity, which were made up of two gram positive bacteria (*Staphylococcus aureus and bacillus subtitis*) and two gram negative bacteria (*Ecoli and Klebsiella pneumonia*). These were cultured on McConky agar, while antibacterial assay was carried out on nutrient broth. The microorganisms used were collected from Microbiology Lab, The University of Haripur. Inoculation of the culture was carried separately in each nutrient broth and agar. Wells (5mm in diameter) were prepared on inoculated agar and filled with different concentrations of extract. It was then incubated at 37^{o}C for 24 hours and the clear zone of inhibition was measured after 24 hours.

Results and discussion

Physiochemical analysis of moringa leaves

Physiochemical analysis merits the identification and quality of the plant material especially in concern with food and pharmacological application. These parameters are said to be important in articulate preparation of quality standards attributes of plant for monograph (Bekoe *et al*., 2020). The results presented in table 1 showed a decline in pH value (6.41 to 5.85) and increase in acid contents (0.08% to 0.34%) with increasing concentration of moringa in aqueous solution. The

moisture content of moringa was analyzed as 6.45% on average basis which is facilitated by the average temperature during drying around 23 ± 3°C.

Table 1 Physiochemical attributes of moringa leaves

Physiochemical parameters	**Quantitative analysis**
pH (1% solution)	6.41 ± 0.02
pH (10% solution)	5.85 ± 0.03
Titratiable acidity (1%)	0.08 ± 0.005
Titratiable acidity (10%)	0.34 ± 0.09
Moisture content (%)	6.37 ± 0.4
Ash (%)	9.66 ± 0.35
*the values are given as mean (n = 3) ± standard error (SE)	

Moringa is considered as best source of mineral and exhibited 9.66% ash content in this study. The value of ash content in this study is somewhat lower than that of study conducted by Wiltshire *et al*., (2022) which might be due to the variation of geographical and growing conditions.

Antioxidant properties of *moringa oliefera* leaves

The data presented in table 2 indicated that concentrations of 100mg/ml ethanolic extracts contained higher amount of TPC (2.59 mg GAE/ml) as compared to aqueous extracts (1.49 mg GAE/ml) while no market difference was observed in TFC of both ethanolic (3.82 mg QE/ml) and aqueous extracts (3.75 mg QE/ml). RSA of both extracts were determined by using DPPH which acts as a free radical which reduces in presence of antioxidants and become colorless from violet. As moringa extracts at the concentration of 100mg/ml were green colored complex due to the presence of chlorophyll, a notable anomaly in determination of RSA was observed. The original green color masked the reduction in DPPH color complex, therefore RSA (%) at this concentration was not determined. However, when the concentration of moringa extracts were reduced considerably to 5 mg/ml (colorless solution obtained), DPPH-RSA was determined as 72.034% and 72.31% for ethanolic and aqueous extracts respectively.

The results from this study support the greater antioxidant potential of moringa leaves to reduce the oxidative damage. The results of TPC in this study were lower than the study of Iqbal and Bhanger, (2006). Highest TPC in ethanolic extract was observed due to the ability of ethanol to extract not only polar substances but also weak polar and non-polar compounds (Sun et al., 2015). Flavonoids are said to be soluble in many different type of solvents viz water and organic solvents (Shahzad *et al*., 2018). Although antioxidant activity is said to be dependent on TPC, however, in case of moringa ascorbic acid and flavonoids also contributed to the antioxidant activity (Siyanbola *et al*., 2015).

Table 2. Assessment of antioxidant potential of moringa leaves

Antioxidants	**Ethanolic extracts**		**Aqueous extracts**	
	100 mg/ml	**5 mg/ml**	**100 mg/ml**	**5 mg/ml**
TPC (mg GAE/100g DW)	2.59 ± 0.168	0.168 ± 0.06	1.49 ± 0.29	0.078 ± 0.026
TFC (mg QE/100g DW)	3.75 ± 0.12	1.2 ± 0.025	3.82 ± 0.16	0.88 ± 0.07
RSA (%)	--	72.03 ± 0.92	--	72.31 ± 2.2
*the values are given as mean (n = 3) + standard error (SE)				

Phytochemical analysis

It is clear from the data that major phytochemicals present in moringa leaves are water soluble in nature. Aqueous extracts of moringa leaves exhibited positive results for coumarine, diterpenses, phytosterols, carbohydrates, glycosides, tannins, terpeniods, flavonoids, and alkaloids. Chloroform extracts of moringa leaves showed positive for cardiac glycosides and steroids while ethanolic extracts of moringa leaves were deemed positive for polyphenols, glycosides, tannins and terpenoids. It is further concluded that tests adopted for leucoantocyanins, amino acids, anthocyanins, proteins and saponins failed to show any positive result.

Suresh *et al*., (2020) also concluded that aqueous extract of moringa leaves showed presence of phenols, alkaloids, flavonoids, tannins, carbohydrates, and saponins, while chloroform extracts

only showed positive results of carbohydrates. The difference might be due to the geographical variation and growing conditions, which played a significant role in difference of bioactive compounds in moringa samples (Iqbal and Bhanger, 2006). Variation in type of phyto-constituents with respect to solvent used is usually observed as Leone *et al*., (2015) mentioned such variation in different extracts of moringa leaves especially with regard to alkaloids, saponins and phenols. The probable reason can be attributed to difference in polarity of the extraction mediums, method of extraction and techniques of extractions applied as mentioned by Elangovan *et al*., (2015). A highly diversified profile of secondary metabolites was exhibited by the polar fraction when compared to those of the other fractions of moringa extract (Lawan *et al*, 2020). It was already suggested that the use of polar solvent for extraction through maceration released such diversified secondary metabolites. Therefore, it can be suggested that sonication of aqueous samples influences the extraction of phytochemicals. All above mentioned factors impacts the ability of aqueous extracts of moringa leaves to exhibit better phytochemical profile followed by ethanol as solvent. However, chloroform (although it is less polar) facilitated the extraction of cardiac glycosides and steroids.

Table 2. Phytochemical Analysis of moringa leaf extracts

Phytochemicals	**Extracts**	
	Aqueous	**Ethanol**
Alkaloids	+	++
Steroids	-	-
Flavonoids	+++	+++
Terpenoids	-	-
Tennins	++	++
Saponins	+	+++
Cardiac glycoside	-	-
Anthocyanins	-	-

Coumarins	+	+++
Phytosterols	+	+
Polyphenols	-	+

+ = weakly present, ++ = Moderately present, +++ = Highly present, - = Not Present

Antibacterial properties of moringa leaf extract

Data regarding antibacterial properties of ethanolic and aqueous extracts of moringa leaves is presented in table 3. It was observed that higher the concentration of extract used the greater is the antibacterial activity which might be due to the difference in concentration of secondary metabolites among tested concentration. Moreover, it can also be depicted that ethanolic extracts showed higher activity in case of *E. coli* and *B. subtilis* which might be due to the presence of high levels of terpenoids and tannins in ethanolic extract as explained by (Manilal *et al*., 2020). This antibacterial activity was attributed the ability of tannins to inhibit cell was synthesis (Falowo *et al*., 2018) as well as ability of terpenoids to weaken the membranous tissues creating dissolution of cell was of microorganism (Putri *et al*., 2021). The highest antibacterial activities of 1 mg/µl and 0.5mg/µl of ethanolic extracts were found to be against *E. coli* (17.6, 13.3 and 7.6 mm respectively) followed by S. aureus (13.6, 8.2 and 7.6mm), *B. subtilis* (10.8, 8.3 and 6.3mm) and *P. aeruginosa* (7.7, 5.7, 4.8 mm) respectively.

It can be concluded that aqueous extracts failed to show any antibacterial activity on S. aureus. Moreover, 0.5 mg/µl aqueous extracts were unable to show any prominent zone of inhibition against B. subtilis, P. aeruginosa and E. coli which might be due to the fact that these bacteria overcome the antibacterial power of extract within 24 hours. Moreover, aqueous extracts were proven to have better antibacterial activity against *P. syringica* as compared to ethanolic extracts which might be due to the presence of diterpenses which can damage the bacterial cell wall including cell wall of gram negative bacteria in which phospholipids and lipopolysaccharides acts as barriers (Syeda and Riazunnisa, 2020). *Micrococci, styphylococci, bacilli, lactobacilli, pseudomonas* and *coliforms* are the most common bacteria found in food items. Therefore, aqueous extract seems to be promising agent to deal with *pseudomonas* species such as *P. aeruginosa.*

Table 3. Antibacterial activity of ethanolic and aqueous extracts of moringa leaves

Microorganism	Concentrations/ ml of ethanolic extract	Zone of inhibition (mm)	Concentrations/ ml of aqueous extract	Zone of inhibition (mm)
Bacillus subtilis	1 mg	10.8 ± 0.7	1 mg	8.8 ± 0.78
	0.75 mg	8.3 ± 0.61	0.75 mg	5.7 ± 0.57
	0.5 mg	6.3 ± 0.59	0.5 mg	---
Staphylococcus aureus	1 mg	13.6 ± 1.55	1 mg	---
	0.75 mg	8.2 ± 0.36	0.75 mg	---
	0.5 mg	7.52 ± 0.55	0.5 mg	---
Pseudomonas aeruginosa	1 mg	7.7 ± 0.66	1 mg	16.6 + 1.15
	0.75 mg	5.7 ± 0.57	0.75 mg	12.9 ± 0.55
	0.5 mg	4.8 ± 0.78	0.5 mg	---
Escherichia coli	1 mg	17.6 ± 1.55	1 mg	11.3 + 1.54
	0.75 mg	13.3 ± 0.71	0.75 mg	10.7 ± 0.79
	0.5 mg	7.6 ± 0.55	0.5 mg	
*the values are given as mean (n = 3) + standard error (SE)				

Conclusion

Moringa is a highly medicinal plant. The phytochemical analysis revealed it is rich in alkaloids, saponins, phenolics and flavonoids. The ethanol is the best solvents to isolate phytochemicals and their extracts are most effective against Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli.

References

Abdalla, M.M., 2013. The potential of Moringa oleifera extract as a biostimulant in enhancing the growth, biochemical and hormonal contents in rocket (*Eruca vesicaria subsp. sativa*) plants. *International Journal of Plant Physiology and Biochemistry*, *5*(3), pp.42-49.

Afzal, M.I. and Iqbal, M.A., 2015. Plant nutrients supplementation with foliar application of allelopathic water extracts improves wheat (*Triticum aestivum* L.) yield. *Advance in Agriculture and Biology*, *4*, pp.64-70.

Asghar, N., Aziz, A., Azhar, M.F., El-Sharnouby, M., Irfan, U., Rafiq, I., Farooq, H., Rehmani, M.I.A., Sakran, M., Altalhi, A. and Alzuaibr, F.M., 2022. Assessment of Phytochemical Analysis, Nutritional Composition and Antimicrobial Activity of Moringa oleifera. *PHYTON-INTERNATIONAL JOURNAL OF EXPERIMENTAL BOTANY*, *91*(8), pp.1817-1829.

Bekoe, E.O., Jibira, Y. and Agyei, G.A., 2020. Physicochemical, phytochemical, heavy metal and microbiological analysis of Moringa oleifera Lam. leaves. *Pharmacognosy Journal*, *12*(6s).

Elangovan, N.M., Dhanarajan, M.S. and Elangovan, I., 2015. Preliminary phytochemical screening and HPTLC fingerprinting profile of leaf extracts of Moringa oleifera and Phyllanthus emblica. *International Research Journal of Pharmaceutical and Bioscience*, *2*(2), pp.32-40.

Fahal, M.E., Rani, B.M.A., Aklakur, M.D., Chanu, T.I. and Saharan, N., 2018. Qualitative and quantitative phytochemical analysis of Moringa oleifera (Lam) Pods. *International journal of current microbiology and applied sciences*, *7*(5), pp.657-665.

Falowo, A.B., Mukumbo, F.E., Idamokoro, E.M., Lorenzo, J.M., Afolayan, A.J. and Muchenje, V., 2018. Multi-functional application of Moringa oleifera Lam. in nutrition and animal food products: A review. *Food research international*, *106*, pp.317-334.

Iqbal, S. and Bhanger, M.I., 2006. Effect of season and production location on antioxidant activity of Moringa oleifera leaves grown in Pakistan. *Journal of food Composition and Analysis*, *19*(6-7), pp.544-551.

Iqbal, S. and Bhanger, M.I., 2006. Effect of season and production location on antioxidant activity of Moringa oleifera leaves grown in Pakistan. *Journal of food Composition and Analysis*, *19*(6-7), pp.544-551.

kumar Bargah, R., 2015. Preliminary test of phytochemical screening of crude ethanolic and aqueous extract of Moringa pterygosperma Gaertn. *Journal of pharmacognosy and phytochemistry*, *4*(1).

Lawan, F.A., Shehu, B.B., Benisheikh, A.A.G., Dige, A.M., Jidda, H.A. and Abdulkadir, A., 2020. Evaluation of antimicrobial potential and phytochemical screening of Moringa oleifera leave extract: An Emerging folkloric medicinal plant in Maiduguri, Borno state, Nigeria. *World Journal of Biology Pharmacy and Health Sciences*, *4*(3), pp.043-047.

Leone, A., Spada, A., Battezzati, A., Schiraldi, A., Aristil, J. and Bertoli, S., 2015. Cultivation, genetic, ethnopharmacology, phytochemistry and pharmacology of Moringa oleifera leaves: An overview. *International journal of molecular sciences*, *16*(6), pp.12791-12835.

Manilal, A., Sabu, K.R., Shewangizaw, M., Aklilu, A., Seid, M., Merdekios, B. and Tsegaye, B., 2020. In vitro antibacterial activity of medicinal plants against biofilm-forming methicillin-resistant Staphylococcus aureus: efficacy of Moringa stenopetala and Rosmarinus officinalis extracts. *Heliyon*, *6*(1), p.e03303.

Maqsood, M., Qureshi, R., Arshad, M., Ahmed, M.S. and Ikram, M., 2017. Preliminary phytochemical screening, antifungal and cytotoxic activities of leaves extract of Moringa oleifera Lam. from Salt range, Pakistan. *Pak. J. Bot*, *49*(1), pp.353-359.

Mehmood, A., Naveed, K., Ayub, Q., Alamri, S., Siddiqui, M.H., Wu, C., Wang, D., Saud, S., Banout, J., Danish, S. and Datta, R., 2021a. Exploring the potential of moringa leaf extract as bio stimulant for improving yield and quality of black cumin oil. *Scientific reports*, *11*(1), pp.1-10.

Mehmood, A., Naveed, K., Azeem, K., Khan, A., Ali, N. and Khan, S.M., 2018. Sowing time and nitrogen application methods impact on production traits of Kalonji (*Nigella sativa* L.). *Pure and Applied Biology (PAB)*, *7*(2), pp.476-485.

Mehmood, A., Naveed, K., Jadoon, N., Ayub, Q., Hussain, M. and Hassaan, M., 2021b. Phytochemical screening and antibacterial efficacy of black cumin (*Nigella sativa* L.) seeds. *FUUAST Journal of Biology*, *11*(1), pp.23-28.

Putri, G.E., Rilda, Y., Syukri, S., Labanni, A. and Arief, S., 2021. Highly antimicrobial activity of cerium oxide nanoparticles synthesized using Moringa oleifera leaf extract by a rapid green precipitation method. *Journal of Materials Research and Technology*, *15*, pp.2355-2364.

Shahzad, Q., Sammi, S., Mehmood, A., Naveed, K., Azeem, K., Ahmed Ayub, M.H., Hussain, M., Ayub, Q. and Shokat, O., 2020. Phytochemical analysis and antimicrobial activity of adhatoda vasica leaves. *Pure and Applied Biology (PAB)*, *9*(2), pp.1654-1661.

Siyanbola, T.O., Ajanaku, C.O., Akinsiku, A.A., Adedapo, E.A., Aladesuyi, O., Olanrewaju, I.O. and Jokotagba, O.A., 2015. Nutritional and Physico-Chemical Evaluations of Moringa oleifera Seedlings and Oil. *Journal of Advance Research in Applied Science*, *2*(7), pp.01-04.

Syeda, A.M. and Riazunnisa, K., 2020. Data on GC-MS analysis, in vitro anti-oxidant and anti-microbial activity of the Catharanthus roseus and Moringa oleifera leaf extracts. *Data in brief*, *29*, p.105258.

Tang, X., Xu, C., Yagiz, Y., Simonne, A. and Marshall, M.R., 2018. Phytochemical profiles, and antimicrobial and antioxidant activities of greater galangal (*Alpinia galangal*) flowers. *Food chemistry*, *255*, pp.300-308.

Wiltshire, F.M.S., de França Santos, A., Silva, L.K.B., de Almeida, L.C., dos Santos Freitas, L., Lima, A.S., Fricks, A.T., Dariva, C. and Soares, C.M.F., 2022. Influence of seasonality on the physicochemical properties of Moringa oleifera Lam. Seed oil and their oleochemical potential. *Food Chemistry: Molecular Sciences*, *4*, p.100068.