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Studies on Phytochemical Properties and Antibacterial Activity of *Moringa oleifera* Flower Extract

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ABSTRACT

The *Moringa oleifera* plant is a well-known and commonly utilized herbal remedy for a variety of contagious and non-contagious medical disorders. In this study, results revealed the presence of various phytochemicals: alkaloids, phenolic and flavonoid compounds, tannins, saponins and terpenoids. HPLC analysis revealed the presence of various phytochemicals such as gallic acid, quercetin and rutin. The goal of this study was to analyze the phytochemicals and to assess the antibacterial effects of *M. oleifera* flower extract (MOFE) against two common microorganisms: *E. coli* ATCC25922 and *S. aureus* ATCC33591. *M. oleifera* flower extract exhibited antibacterial effects against the two tested bacteria *E. coli* and *S. aureus* with the diameter of the zone of inhibition 11 and 9 mm, respectively. Five different concentrations (1000, 500, 250, 125, 62.5 mg/ml) of *M. oleifera* flower extract were prepared in 5% DMSO (Dimethyl sulfoxide) and tested for the antibacterial effect by using the agar disc diffusion method. Minimum inhibitory concentrations were found to be 250 mg/ml. *M. oleifera* had high antibacterial action against bacterial pathogens that were pathogenic and virulent, and it may be a source of yet another efficient and practical antibacterial antibiotic.

Key words: Contagious, phytochemicals, dimethyl sulfoxide, minimum inhibitory concentration, zone of inhibition

INTRODUCTION

Plants have played a significant role in the treatment of sickness since ancient times and throughout history. All across the world, people continue to use plants for various purposes and according to estimates from the World Health Organization (WHO), 80% of the population in some poor nations uses herbal medicine for part of basic healthcare (Sharma *et al*., 2022). For the vast majority of the impoverished, there are no other treatment options outside indigenous cures, whereas only 11% of the population, according to estimates, has access to formal health care. *Moringa oleifera* is one out of the 14 species in the Moringaceae family. According to literature, the Moringa tree was brought from India to Africa at the beginning of the 20th century to be utilized as a dietary supplement (Kashyap *et al*., 2022). *M. oleifera* is sometimes referred to as the kelor tree, the drumstick tree, or the horse radish tree. The tree's name in the Nile Valley (Sudan) is Shagara al Rauwaq, which stands for tree for cleansing (Kaur *et al.,* 2023). Due to *M. oleifera's* antibacterial activity, it has been widely utilized to treat various ailments. It is abundant in substances that include the simple sugar rhamnose as well as a special class of substances known as glucosinolates and isothiocyanates. It is known for its antipyretic, antibacterial, anti-ulcerative, hepatoprotective and anti-inflammatory properties along with antioxidant and cholesterol-lowering effects (Maisto *et al.,* 2022). *M. oleifera* flower had been tested for various health benefits but the bioactive compounds may vary according to the various geographical sites (Penalver *et al.,* 2022). It is rich source of phenolic compounds and alkaloids. A diet high in antioxidant fruits and vegetables, according to epidemiological research, dramatically lowers the risk of several oxidative stress-related diseases, such as cancer, diabetes and cardiovascular. The majority of dietary antioxidants come from polyphenols, which are easily absorbed in the intestine (Sehrawat *et al*., 2022). Alkaloids are a potentially new class of natural antibiotics with a broad antibacterial range, with few side

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effects, and a low propensity to induce drug resistance; as a result, major research efforts are concentrated on them (Yan *et al.*, 2021).
Establishing the antibacterial activity of *M. oleifera* flowers requires scientific proof of their antimicrobial potential because the majority of earlier attempts to pinpoint the antibacterial properties of *M. oleifera* utilized tests against human clinical bacterial isolates rather than approved standard bacterial species (Mohammed *et al.,* 2022). Given the worldwide difficulty in treating infections brought on by superbugs, the multidrug resistance of the bacteria utilized in some of these assays was not clearly established. Additionally, numerous researches looked at the effectiveness of *M. oleifera* against bacteria originating from plants and animals as well as those from other environmental sources including water and sewage, perhaps because some of these bacteria can be acquired by humans and may cause diseases (Adji *et al.*, 2022). Although these investigations suggested that *M. oleifera* had antibacterial capabilities, it would be helpful to know how it performs in the presence of pathogenic, virulent and multi-drug resistant bacteria, for which effective therapeutic alternatives are urgently needed. The purpose of this study was to assess the phytochemical properties, antimicrobial activity and minimal inhibitory concentration of *M. oleifera* flowers.

MATERIALS AND METHODS

Moringa oleifera flowers were collected from Karnal, Haryana, and were taxonomically authenticated from the Department of Botany, Lovely Professional University, Phagwara, Punjab. Flowers were washed twice with regular tap water and afterward once with distilled water and left in a larger strainer to remove extra water from flowers. The flowers were dried in a tray drier at 40° for four days (96 h) and after that Sujata Powermatic plus grinder was used to make powder and sieved with a 150 µm sieve by using an electric sieve shaker. The powder was then stored in a glass container at room temperature.
The sample was prepared by extraction process where 10 g of flower powder was dissolved in 100 ml of methanol for 72 h and then filtered by using Whatman filter paper No. 1 and afterward dried by using a rotary evaporator. The total yield in percentage was found to be 8.5 (Bhattacharya *et al.*, 2018).
Methanolic extract of the *M. oleifera* flower was taken for the qualitative estimation of various classes of phytochemicals present in the flower. For testing phenols, 3 ml of the distilled water was added in 1 ml of flower extract. 3 to 4 drops of 10% aqueous ferric chloride was added. The appearance of green or blue colour indicated the presence of phenols (Kandeepan *et al.*, 2022). For testing flavonoids, 1 ml methanolic extract of *M. oleifera* flower was heated with 10 ml of the methyl acetate in a water bath at 80° for 3 min and then filtered. 1 ml of 1% ammonium solution was added. Presence of yellow colour confirmed the presence of the flavonoids (Al-Reza *et al.*, 2022). For testing alkaloids, 1 ml of *M. oleifera* flower extract was added to 2 ml of Wagner's reagent and a reddish-brown colour appeared indicating the presence of alkaloids (Kandeepan *et al.*, 2022). For testing tannins, 1 ml of flower extract and 1 ml of 0.008 M potassium ferricyanide were mixed and then 1 ml of ferric chloride (0.02 M) containing 0.1N HCl was added to it. The appearance of a blue-black colour showed the presence of tannins (Kandeepan *et al.,* 2022). For testing saponins, 2 ml of flower extract was diluted in 5 ml distilled water and shaken vigorously. The formation of the stable foam indicated the presence of saponins but there was no such foam which meant saponins were not present in the flower extract (Vasanth *et al.,* 2022). For testing steroids, 1 ml of the flower extract and 5 ml of the acetic anhydride were mixed with 5 ml of the sulphuric acid. The violet colour did not change to green determined the absence of the steroids (Vasanth *et al.*, 2022).
M. oleifera flower extract was analyzed with two standardized Rutin (Tokyo Chemical Industry Co. Ltd.) and Quercetinhydrate (Loba ChemiePvt. Ltd., Mumbai). The flower extract was quantified by using HPLC (Shimadzu Prominence i-Series LC-2030 Plus, Kyoto, Japan) consisting of an LC-20AD pump, an SPD-M20Aultraviolet-visible(UV-vis) detector along with an autosampler SIL-20AC, column oven CTO-10A. Before quantification, the method was validated for precision, specificity, accuracy and linearity (Çaglar *et al.*, 2022). The pump was set at a low-pressure gradient; mixing was done before the pump and the sample was run at 1 ml/min at a pressure of

5400 psi. The sample rack was 1.5 ml with 105 vials and a rising volume of 500 µl. The oven temperature was set at 30-85°C, whereas the wavelength was set at 190 to 800 nm (Ntshambiwa *et al.*, 2023).

The antibacterial activity of *M. oleifera* flower powder extract was checked against one gram-negative bacteria *E. coli* (ATCC25922) and the second gram-positive bacteria *S. aureus* (ATCC23235). Both of the bacterial strains were collected from the Microbiology Department at Lovely Professional University, Punjab. Antibiotic streptomycin (Hi-Media, Mumbai, India) was used as a control to check the activity of the bacteria (Talath *et al.,* 2022).

For preparation of stock solution, 1000 mg of *M. oleifera* extract powder was dissolved in 1 ml of the 3% DMSO (Dimethyl sulfoxide) because DMSO had no activity at 5% concentration. *M. oleifera* flower extract stock solution was serially diluted in saline to make the working concentrations of 62.5, 125, 250, 500 and 1000 mg/ml (Das *et al.,* 2020).

Antibacterial assay of *M. oleifera* flower methanolic extract against *E. coli* and *S. aureus* was performed by the Agar disc diffusion method. The DMSO-soaked disc was used as a negative control, the streptomycin-soaked disc as a standard antibiotic and various concentrations of extract discs were used to check the antimicrobial effect against the bacteria (Talath *et al.,* 2022). The zone of inhibition was measured to check the antibacterial activity of the flower extract (Wali *et al.,* 2020).

Minimum inhibitory concentration (MIC) of *M. oleifera* flower extract was the minimum concentration of the sample flower extract which was required to inhibit the growth of the microbes. The minimum inhibitory concentration was determined by using a 96 well microplate. 10 µl of nutrient broth (bacterial culture broth) was taken in each well and 200 µl of flower extracts (concentrations ranging from 1000 to 62.5 mg/ml) were added to the wells. The wells were then incubated for 6 h and then 0.4 mg/ml (40 µl) of p-iodonitrotetrazolium violet (INT) was added to the wells and again incubated for 12 h at a temperature of 37°C. During this time, the change in colour was noticed from yellow to red and then to purple which signified the presence of bacteria. This is the minimum concentration of the extract which did not alter the colour of the sample (Wali *et al.*, 2019).

RESULTS AND DISCUSSION

Phytochemicals are plant compounds with nutritional value and are capable of preventing diseases. The analysis of *M. oleifera* flower extract in methanol indicated various types of phytoconstituents. It was observed that the phenols, flavonoids, alkaloids and tannins were present in *M. oleifera* flower extract, whereas saponins and steroids content were not reported. Similar study was carried out by Al-Reza *et al.* (2022) who confirmed the presence of alkaloids, flavonoids and tannins, whereas the absence of saponins, terpenoids and steroids in different extracts of moringa.

The isolation of various natural compounds is accomplished by using the HPLC analytical technique (Fig. 1). The X-axis represents retention time, whereas Y-axis represents µAu (milli absorbance unit). Retention time is the

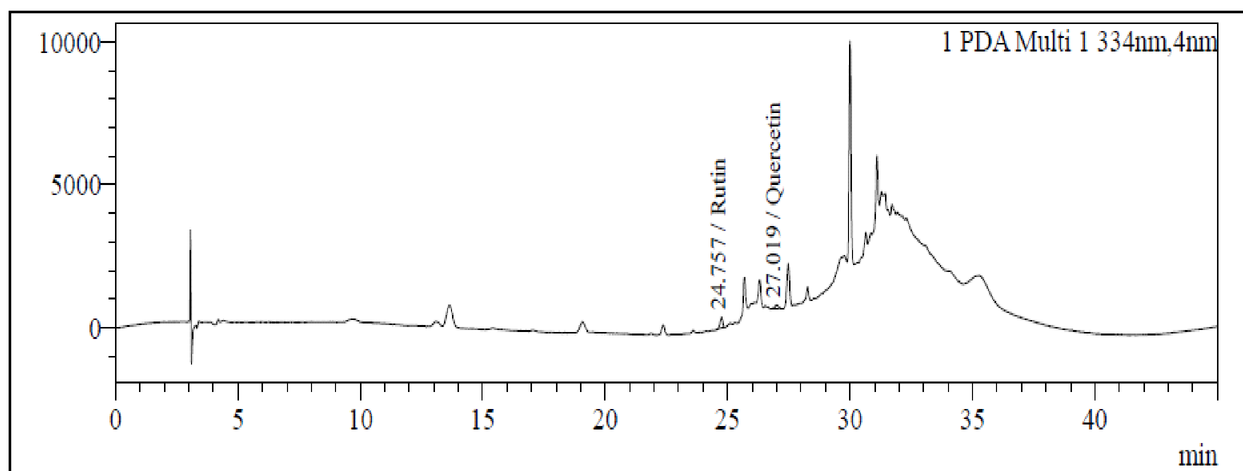

Fig 1. HPLC chromatogram *M. oleifera* flower extract.

time between the injection of the analyte and the detection of the analyte and is used as a tool for the identification of the compounds/ phytochemicals. The peaks represent the concentrations of the compounds, the wider and larger the peaks higher would be the concentration of a particular compound because the peak area determines the amount of the compounds that have passed through the detector. Peak area is used for the calculation of the concentration of the compound, for example, here quercetin had a peak area of 1974515 so the concentration of quercetin was 1974.515 mg/l, whereas the peak area of rutin was 1366452 so the concentration was 1366.452 mg/l. HPLC results showed that *M. oleifera* flower extract contained rutin with a retention time of 24.75 and quercetin at 27.01 with reference to standards and extracted samples of flowers (Fig. 1). There were peaks in the chromatogram for quinol (3.31), gallic acid (3.65), chlorogenic acid (9.67) and naringenin (22.43).

The presence of an alleviated concentration of phenolic compounds in result might be associated with the high percentage of inhibition of the flower extract. The free radical scavenging and antioxidant properties of plants are due to phytochemicals, particularly polyphenols such as phenolic acids, flavonoids and phenylpropanoids which may be attributed to the antioxidant activity of the *M. oleifera* flower extracts (Ahmed *et al.*, 2021).

Almost the same results were shown by Ahmed *et al.* (2021) where multiple standards were used for the estimation of compounds, and catechin hydrate was found to be 20.19 mg/g, rutin 60.38 mg/g and quercetin 137.81 mg/g. However, the results given by Ademiluyi *et al.* (2018) depicted the difference in concentration of compounds such as catechin 6.08 mg/g, rutin 91.05 mg/g and quercetin 17.83 mg/g.

The antibacterial assay, to check the activity of *M. oleifera* flower extract, exhibited excellent potential against both bacteria: *E. coli* and *S. aureus* (Table 1 and Fig. 2). *M. oleifera* flower extract exhibited an antibacterial effect against *E. coli* with an inhibition zone of 11 mm, whereas it showed activity against *S. aureus* with an inhibition zone of 9 mm. Antibiotic streptomycin showed the maximum zone of inhibition by 21 and 19 mm, respectively, whereas negative control DMSO showed no activity against any of the bacteria (Table 1). In a similar study carried out by Talath *et al.* (2022), the inhibition zone of 14.5 and 12.7 mm, respectively, was reported when activity was checked with *M. oleifera* leaf extract. The maximum zone of inhibition was shown at a concentration of 1000 mg/ml in both the cases, whereas there was no zone of inhibition in concentrations below 125 mg/ ml. The antibacterial potential of *M. oleifera* flower extract could be because of the phytochemicals present in the flower extract as reported by Munevar and Zambrano Orozco (2019). The isothiocyanates present in the moringa are responsible for the antibacterial properties of the plant.

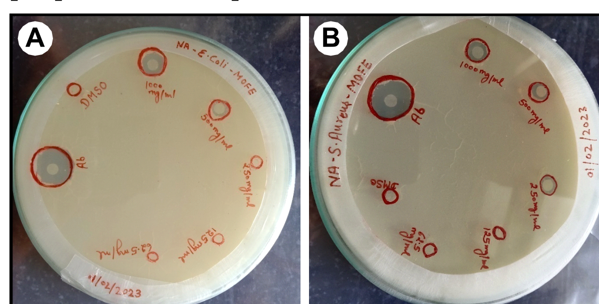

Fig 2. Zone of inhibition against (A) *E. coli* and (B) *S. aureus*.

The minimum inhibitory concentration against both bacteria was found to be 250 mg/ ml (Table 1). These findings also match the results reported by Farhan *et al.* (2021) where minimum inhibitory concentrations for food-borne pathogens were found to be 200 mg/ml. The study on various properties of Moringa flower powder (Table 2) confirmed high symmetry in data values (skewness -0.5 to 0.5) for most of the properties except water solubility index (-0.83), bulk density (0.57), Hausner ratio

Table 1. Antibacterial activity of *Moringa oleifera* flower extract

Bacteria	Concentrations of *M. oleifera* flower extract					DMSO	Antibiotic streptomycin
	1000 (mg/ml)	500 (mg/ml)	250 (mg/ml)	125 (mg/ml)	62.5 (mg/ml)		
E. coli	11±0.65	9±0.45	3±0.67	Resistant	Resistant	Resistant	21±0.85
S. aureus	9±0.72	6±0.64	2±0.71	Resistant	Resistant	Resistant	19±0.64

(0.91), foaming capacity (-1.96), foaming stability (0.58) and IC 50 (-0.81). The kurtosis for most of the properties indicated symmetric distributions except water absorption index (-3.13), moisture content (-3.30), angle of repose (-3.22), foaming capacity (4.17) and oil absorption capacity (-3.32).

The correlation study for various active properties of moringa flower powder (Table 3) and defatted Moringa flower powder (Table 4) with IC 50 values was estimated to establish relation between active composition of moringa powder and the effectiveness of moringa flower extracts. The observation confirmed the strong and positive correlation of IC 50 values with porosity (1.00) and oil absorption capacity (0.87), while strong and negative correlation with moisture content (-0.86) and Hausner ratio (-0.72). Thus, the potential of moringa flower extracts can be improved by improving porosity and oil absorption capacity and reducing moisture content and Hausner ratio. However, the properties of defatted moringa flower powder reflected a strong correlation of IC 50 value with most of the properties (0.75 to 1.00) confirming superiority moringa flower extracts from defatted moringa flower powder. These properties of moringa flower powder confirmed a high IC 50 value and so the high degree of efficacy of moringa against microbial growth (Starzak *et al.,* 2019).

CONCLUSION

A thorough examination of a plant's bioactive secondary metabolites can help us better understand its therapeutic actions. *M. oleifera* flowers continue to be a prime source of phytochemicals and micronutrients that can be used to create nutraceuticals and functional foods. The phytochemicals included in *M. oleifera* flowers make this plant a perfect source of BASM. Food items made from flowers, therefore, have higher levels of protein, dietary fiber, minerals and antioxidants. Moringa's bioactive secondary metabolites provide motivation for additional research to find innovative lead compounds for drug discovery

Table 2. Descriptive statistics for various properties of *M. flower* powder

Statistics	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}
Mean	11.06	8.94	5.59	0.30	0.40	0.63	0.17	22.78	15.14	56.32	6.99	46.58
Standard error	0.18	0.14	0.55	0.03	0.02	0.01	0.01	0.22	0.05	0.03	0.38	0.16
Median	11.08	9.06	5.56	0.31	0.39	0.62	0.18	22.78	15.18	56.32	7.00	46.67
Standard deviation	0.44	0.34	1.35	0.08	0.05	0.03	0.02	0.55	0.12	0.08	0.93	0.38
Sample variance	0.20	0.12	1.81	0.01	0.00	0.00	0.00	0.30	0.01	0.01	0.87	0.15
Kurtosis	-3.13	-0.73	-3.30	-1.65	0.03	0.12	0.00	-3.22	4.17	-0.47	-3.32	-0.61
Skewness	-0.03	-0.83	0.01	-0.32	0.57	0.91	0.30	0.01	-1.96	0.58	0.00	-0.81
Confidence level (95.0%)	0.47	0.36	1.41	0.08	0.05	0.03	0.03	0.57	0.12	0.09	0.98	0.40

Where, X_1: Water absorption index (%), X_2: Water solubility index (%), X_3: Moisture content (%), X_4: Tapped/true density (g/m^3), X_5: Bulk density (g/m^3), X_6: Hausner ratio (g/m^3), X_7: Porosity (g/m^3), X_8 : Angle of repose (°), X_9: Foaming capacity (ml/l), X_{10}: Foaming stability (ml/l), X_{11}: Oil absorption capacity (%) and X_{12}: IC 50 (M).

Table 3. Correlation between various properties of Moringa flower powder

Statistics	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}
X_1	1.00											
X_2	-0.99	1.00										
X_3	-0.83	0.72	1.00									
X_4	0.99	-1.00	-0.72	1.00								
X_5	-0.98	1.00	0.69	-1.00	1.00							
X_6	0.32	-0.47	0.27	0.47	-0.52	1.00						
X_7	0.50	-0.35	-0.90	0.35	-0.3	-0.66	1.00					
X_8	-0.86	0.93	0.42	-0.93	0.95	-0.76	0.01	1.00				
X_9	1.00	-0.98	-0.86	0.98	-0.96	0.27	0.54	-0.83	1.00			
X_{10}	-0.48	0.62	-0.10	-0.62	0.66	-0.98	0.52	0.86	-0.43	1.00		
X_{11}	0.81	-0.70	-1.00	0.7	-0.67	-0.29	0.91	-0.4	0.84	0.12	1.00	
X_{12}	0.42	-0.27	-0.86	0.27	-0.22	-0.72	1.00	0.1	0.47	0.59	0.87	1.00

Where, X_1: Water absorption index (%), X_2: Water solubility index (%), X_3: Moisture content (%), X_4: Tapped/true density (g/m^3), X_5: Bulk density (g/m^3), X_6: Hausner ratio (g/m^3), X_7: Porosity (g/m^3), X_8: Angle of repose (°), X_9: Foaming capacity (ml/l), X_{10}: Foaming stability (ml/l), X_{11}: Oil absorption capacity (%) and X_{12}: IC 50 (M).

Table 4. Correlation between various properties of defatted Moringa flower powder

Statistics	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}
X_1	1.00											
X_2	-0.50	1.00										
X_3	0.68	0.29	1.00									
X_4	-0.44	1.00	0.35	1.00								
X_5	0.19	-0.94	-0.59	-0.96	1.00							
X_6	-0.65	0.98	0.11	0.97	-0.87	1.00						
X_7	1.00	-0.5	0.68	-0.44	0.19	-0.65	1.00					
X_8	0.99	-0.38	0.77	-0.32	0.06	-0.55	0.99	1.00				
X_9	-0.79	0.93	-0.08	0.90	-0.76	0.98	-0.79	-0.70	1.00			
X_{10}	0.98	-0.65	0.53	-0.60	0.37	-0.79	0.98	0.95	-0.89	1.00		
X_{11}	0.97	-0.69	0.49	-0.65	0.42	-0.82	0.97	0.93	-0.91	1.00	1.00	
X_{12}	1.00	-0.41	0.75	-0.35	0.09	-0.58	1.00	1.00	-0.72	0.96	0.94	1.00

Where, X_1: Water absorption index (%), X_2: Water solubility index (%), X_3: Moisture content (%), X_4: Tapped/true density (g/m^3), X_5: Bulk density (g/m^3), X_6: Hausner ratio (g/m^3), X_7: Porosity (g/m^3), X_8: Angle of repose (°), X_9: Foaming capacity (ml/l), X_{10}: Foaming stability (ml/l), X_{11}: Oil absorption capacity (%) and X_{12}: IC 50 (M).

and the creation of novel herbal pharmaceuticals that meet GRAS (Generally Recognised as Safe) standards. For research where quality is the main priority, HPLC is very helpful in isolating and purifying plant-based products such as secondary metabolites and proteins. HPLC results showed that the sample contained gallic acid, quercetin and rutin. In conclusion, results for antibacterial activity againsttest microorganisms revealed that *M. oleifera* flower extract was a potential source for antibacterial activity. In addition, this study also revealed an alternative source of treatment for infectious diseases along with minimum inhibitory concentration.

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