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In-vitro Phytochemical Screening and Bioactivity of *Moringa oleifera* Accessions

Nikita Patel and Ramar Krishnamurthy

C. G. Bhakta Institute of Biotechnology, Uka Tarsadia University, Bardoli, Gujrat India

ABSTRACT

There is a huge demand of plant with substantial phytocompounds due to their health benefits. *Moringa oleifera* is an important and fast-growing plant species with beneficial immuno-modulating properties. *Moringa oleifera* can withstand high temperatures, drought and mild frost conditions and hence it can be widely grown across the world. Large numbers of reports are available on nutritional properties of this plant however; research work on bioactivity of wild *Moringa oleifera* in India is still scanty. Hence, the present study was aimed to identify and quantify important phytochemicals with potent bioactivity as nowadays microorganism is adopting multidrug resistance and to cope up with these difficulties there is a need to identify plant-based drug for the betterment of society. *Moringa oleifera* accessions were collected from different regions of Gujarat and they were screened for preliminary phytochemicals by using standard protocols. It was revealed that all the accessions were showing presence of important phytochemicals and the total phenolic content ranged from 0.0076 mg/g to 2.98mg/g GAE equivalent. The total flavonoids ranged from 1.12mg/g to 1.59mg/g QE equivalent and the total tannins were found in the range of 0.66mg/g to 1.35mg/g tannic acid equivalent. The antibacterial activity of *Moringa oleifera* accessions was carried out by implying agar well diffusion assay and it was found that MONV, MOVR and MOAN showed potent inhibitory activity against all the test bacteria in except *Vibrio cholerae*. Hence, these accessions could be further explored for *in vivo* studies with pure form of extracts for enhanced applications in pharmaceutical industries.

KEY WORDS: MORINGA OLEIFERA ACCESSIONS, PHYTOCHEMICALS, BIOACTIVITY, MIC.

INTRODUCTION

Moringa oleifera (Moringaceae family) is native to the Indian subcontinent and Africa and is also known as the Miracle tree. It is multipurpose tree species with multifarious pharmacological and nutraceutical property (Thurber and Fahey, 2009). Each and every part of *Moringa oleifera* possesses potential phytopharmacological properties. Apart from its use as a food product it also possesses Medicinal and Industrial application (Moyo et al., 2011). The leaf material of this plant is well-known for its high mineral content as it is rich in essential amino acid, vitamins and minerals (Tahiliani and Kar, 2000; Amabye and Tadesse, 2016). Owing to the multipurpose properties of *M. oleifera*, this plant is also rich in important antioxidants that can quench free radicals (Rockwood et al., 2013). It was also reported that *M. oleifera* is rich in important phytocompounds such as phenolics, flavonoids, tannins, and saponins with superior bioactivity (Mishra et al.,2011; Patel et al., 2014; Zainab et al., 2020).

The incidence of UTI (Urinary tract Infections) infections and Staphylococcal infection is still high in many developing countries and this is because of the lack of information and knowledge regarding multi-drug

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resistance (Arumugam et al., 2011). To overcome the problems associated with synthetic drugs, researchers are now focusing on plant-based materials as they are readily available with no known side effects (Ncube et al., 2008; Al_husnan and Alkahtani,2016). Various portions of this plant including gum, pod extract, flowers and roots, have shown potential effects as a source of indigenous medicine (Odebiyi and Sofowora, 1978; Anwar et al., 2007; Sandeep et al., 2019). Therefore, the objective of this study is to evaluate the antibacterial efficacy and to screen *Moringa oleifera*'s substantial phytochemicals to imply a natural plant-based system as an alternative to the synthetic drug (medicine) system.

MATERIAL AND METHODS

Moringa oleifera accessions were collected from different regions of Gujarat such as Navsari, Anand, Bardoli and Vadodara (Table 1) and were maintained at study farm of Uka Tarsadia University, Bardoli, Gujarat, India. The voucher specimens were deposited at herbarium of C.G.Bhakta Institute of Biotechnology, Uka Tarsadia University. Disease free and healthy *Moringa oleifera* leaves of all the accessions were thoroughly washed under running tap water and dried separately at room temperature for three weeks until completely dried. The powdered samples (5g) were extracted by double distilled water (150 ml) using soxhlet apparatus and were dried and preserved at 4 °C prior to further use (Patel et al., 2020). Preliminary phytochemical screening was carried out by following standard protocols as defined by (Harborne, 1984; Patel et al., 2020).

Table 1. *Moringa oleifera* **accessions collected from different regions of Gujarat (Tables were prepared by us according to the area of collection).**

Sr. no	Accession code	Area of Collection
1	MONV	Navsari
2	MOBL	Bardoli
3	MOVR	Vadodara
4	MOAN	Anand

Table 2. Standard bacterial strains used in the study

Sr.no	Bacterial strain	NCIM Accession no
1	*Escherichia coli*	NCIM 2931
2	*Vibrio cholerae*	NCIM 5316
3	*Bacillus subtilis*	NCIM 2921
4	*Staphylococcus aureus*	NCIM 5345

The quantitative estimation of phenolic and flavonoid contents of *Moringa oleifera* accessions was determined by following Folin–Ciocalteu and Aluminum chloride colorimetric method respectively as reported by (Patel et al., 2020). The presence of total tannins was determined by using the method reported by (Patel et al., 2019). The bacterial strains used in the study were procured from National Collection of Industrial Microorganisms (NCIM), Pune, India (Table 2). The standard bacterial strains were maintained on nutrient agar medium at 37°C prior to further use (Balouiri et al., 2016; Patel et al., 2019).

Agar well diffusion method was employed for monitoring the antibacterial activity of *Moringa oleifera* extracts where streptomycin (10µg/ml) was used as positive control and zone of inhibition was recorded in mm (Balouiri et al., 2016). Broth Macro-dilution method was employed in investigating the Minimum inhibitory concentration (MIC) of *Moringa oleifera* extracts (Rahman et al., 2009; Adamczak, et al., 2020).

RESULTS AND DISCUSSION

The present study was carried out to analyze the preliminary phytochemicals present in *Moringa oleifera* accessions collected from different regions of Gujarat. Preliminary phytochemical screening revealed the presence of Alkaloids, Phenols, Flavonoids, Tannins, Carbohydrates and Amino acids in all the accessions except alkaloids, it was absent in the accession collected from Bardoli and Valsad (Table 3). The quantitative analysis of phenolic, flavonoids and tannin content are as shown in (Table 4). The total phenolic content ranged from 0.0076 mg/g to 2.98mg/g GAE equivalent in the order of MONV>MOAN>MOVR>MOBL. The total flavonoids ranged from 1.12mg/g to 1.59mg/g QE equivalent in the order of MONV>MOAN>MOVR>MOBL. Total tannins were found in the range of 0.66mg/g to 1.35mg/g tannic acid equivalent in the order of MOBL>MOVR>MOAN>MONV.

Table 3. Preliminary Phytochemical Screening of Aqueous extract of *Moringa oleifera* **accessions + indicates present; - indicates Absent**

Phytochemical Constituent	Accessions			
	MONV	MOBL	MOVR	MOAN
Alkaloid	+	-	-	+
Phenol	+	+	+	+
Flavonoids	+	+	+	+
Tannins	+	+	+	+
Carbohydrates	+	+	+	+
Amino acid	+	+	+	+

Antibacterial activity of *Moringa oleifera* accession MONV, MOVR and MOAN showed potent inhibitory activity against all the test bacteria in dose dependent manner except Vibrio cholerae (Table 5 and Table 6). Previous studies also reported good antibacterial activity of *Moringa oleifera* leaf extracts against various human pathogens (Rahman et al., 2009; Amabye and Tadesse, 2016).

The antibacterial property attributed to any plant material is because of the presence of important

phytochemicals such as Phenols, Flavonoids, Alkaloids, Triterpenoids and Saponins (Chandra et al., 2014; Patel et al., 2020). Phytochemicals play a major role in preventing various diseases. They possess immunomodulating and cardioprotective properties. Several researchers have reported that flavonoids and phenols play a major role in showing inhibitory activity by modifying or preparing complex with bacterial cell wall (Olowosulu and Ibrahim, 2006). In a study conducted on antibacterial activity of different extracts of *Moringa oleifera* leaf against pyogenic bacteria the ethanolic extract showed highest bioactivity when compared to hot water extract (Fouad et al., 2019).

Table 4. Quantitative Phytochemical Analysis of Aqueous extract of *Moringa oleifera* accessions n=3, Value indicates Mean ±SEM

Phytochemical Constituent mg/g	Accessions			
	MONV	MOBL	MOVR	MOAN
Phenol	2.98±0.02	0.0076±0.003	0.14±0.031	0.35±0.09
Flavonoids	1.59±0.29	1.12±0.088	1.43±0.18	1.54±0.29
Tannins	0.66±0.008	1.35±0.16	0.97±0.013	0.74±0.03

Table 5. Antibacterial activity of *Moringa oleifera* accessions against test organism at the concentration of 50 mg/ml n=3, Value indicates Mean ±SEM

Test Organisms	Accessions (zone of inhibition in mm) Concentration (50mg/ml)				Streptomycin
	MONV	MOBL	MOVR	MOAN	
E.coli	0.66±0.33	-	-	4.66±0.33	8.6±0.33
S.aureus	7.66±0.33	-	-	-	10.66±0.33
V. cholerae	-	-	-	-	7.25±0.08
B. subtilis	1.66±0.33	-	4.66±0.33	-	9.34±1.3

Table 6. Antibacterial activity of *Moringa oleifera* accessions against test organism at the concentration of 100 mg/ml n=3, Value indicates Mean ±SEM

Test Organisms	Accessions (zone of inhibition in mm) Concentration (100mg/ml)				Streptomycin
	MONV	MOBL	MOVR	MOAN	
E.coli	*4.33±0.33*	-	-	*10.33±0.33*	*8.6±0.33*
S.aureus	*12.33±0.33*	-	-	-	*10.66±0.33*
V. cholerae	-	-	-	-	*7.25±0.08*
B. subtilis	*3.33±0.66*	-	*9.33±0.33*	-	*9.34±1.3*

Table 7. Minimum Inhibitory Concentration against test organism

Test Organisms	Accessions		
	MONV	MOVR	MOAN
E.coli	37.5mg/ml	-	75mg/ml
S.aureus	18.75mg/ml	-	-
B. subtilis	9.37mg/ml	18.75mg/ml	-

However, the present study showed good antibacterial activity of aqueous extract of *Moringa oleifera* against the pathogenic bacteria. The minimum inhibitory activity of the extracts ranged from 9.37mg/ml to 75mg/ml against *E. coli, S. aureus* and *B. subtilis* (Table 7). Thus, in the present study it was revealed that there is a varietal response in bioactivity of *Moringa oleifera* accessions because of broad spectrum of antibiotics present in these extracts. Hence, *Moringa oleifera* can serve as good and natural immunobooster to treat various ailments (Fouad et al., 2019).

CONCLUSION

Owing to the issue of drug resistance caused by microbial mutation over the years, certain antibiotics have become almost ineffective. Thus, it can be inferred from the present analysis that there is a wide variability in phytochemical constituents of *Moringa oleifera* accessions collected from different regions of Gujarat and in order to establish the relationship between the MIC's obtained in this study and the active doses at which the herbs can be used in conventional practice these accessions could be further explored for in vivo studies with pure extracts for enhanced applications in pharmaceutical as well as herbal industries.

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Conflict of Interest: The authors have no conflict of interest to declare.

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