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Phytochemical Constituents and Antibacterial Activity of Different Plant Parts Extracts of Wild *Moringa oleifera* Lam.

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ABSTRACT

Numerous studies have documented the diverse and interesting pharmacological properties of *Moringa oleifera* using its leaves, fruits/pods and seeds, however, majority of these findings have failed to offer conclusive link between the relationships of *Moringa* to any particular clinical benefit. The present study investigated the phytochemical and antibacterial action of different parts of *Moringa* viz, leaf, seeds and pulp against pathogenic strains of *E. coli* and *S. aureus*. It was observed that leaves, seeds and pod pulp were diverse and abundant in phenols, flavonoids, alkaloids and tannins in the range of *Moringa* pulp > *Moringa* seeds > *Moringa* leaves against both *E. coli* and *S. aureus*, respectively.

Key words: *Moringa* seeds, *Moringa* pulp, *Moringa* leaves, phytochemicals, antibacterial

INTRODUCTION

Moringa oleifera (Lam.), which is widely known as Drumstick tree, belongs to family Moringaceae. This perennial tropical plant is rich in bioactive compounds with multifunctional properties. Recently, plants with medicinal properties are used to improve immunity and human health. One such plant with both medicinal and nutraceutical property is *Moringa.* This genus *Moringa* comprises total 13 species which are distributed across the globe (Abd-Rani *et al.,* 2018). During 1900-90's, it gained much of the popularity in underdeveloped and emerging countries owing to the multifaceted properties of each and every part of this plant. This is because of the fulfilment of nutrient requirement from pods, leaves, seed kernels and flowers where hunger and malnourishment is a major concern (Kou *et al.,* 2018). Recent advances in the field of sustainable diet have been increased emanating from the large and widespread use of this plant in ethnomedicine, cuisine and herbal remedy (Leone *et al.,* 2015).

Drug discovery or formulation dealing with screening and identification of pure natural compounds targets both bioactives and pharmacological action of herbal products. Use of the pulp and pods of *Moringa* as food and vegetable dates back to 150 BC (Mehmood *et al.,* 2021). There are majorly five classes of phytochemicals present in any aromatic and medicinal plants and these classes are present in all the parts of *Moringa* which contribute towards the therapeutic and disease preventive property of this plant (Saini *et al.,* 2016; Suresh *et al.,* 2020; Patel and Krishnamurthy, 2021). Numerous studies have documented the diverse and interesting pharmacological properties of *M. oleifera* using its leaves, fruits/pods and seeds, however, majority of these findings have failed to offer conclusive link between the relationships of *Moringa* to any particular clinical benefit. This could be due to the lack of high throughput human intervention studies that glanced into the effects on human health, similar to other plants. Therefore, the present study dealt with the phytochemical and antibacterial activity of different parts of *M. oleifera* viz, leaf, seeds and pulp against pathogenic strains of *E.coli* and *S. aureus.*

MATERIALS AND METHODS

Leaves, seeds and fruit pulp of *M. oleifera* were procured from Vedchhi (21.0473° N, 73.2854° E), Surat district, Gujarat, India. Plant material was authenticated and identified at C. G. Bhakta Institute of Biotechnology, Uka Tarsadia University. A voucher specimen with voucher number (MOVD1) was submitted to the department for further reference.

Plant material including seeds, fruit pulp and leaves were shade-dried for 3-4 days, powdered

and stored for further use. Twenty grams of plant material was collected in 100 ml of solvent (distilled water and methanol) and kept overnight for two days for extraction. Subsequently, fresh material of seeds, leaves and fruits were also subjected to solvent extraction using methanol and distilled water as solvent. After incubation period, the extracts were filtered and dried using rotary evaporator. Later, the extracts were maintained at 4°C prior to further use.

The major classes of phytochemicals viz, phenols, flavonoids, tannins, sterol and alkaloids were analyzed through standard protocols.

The total tannin content was determined by Folin–Ciocalteu method where known amount of sample extract was incorporated into a test tube containing 500 µl of folin-ciocalteu reagent along with 7500 µl distilled water and 1000 µl of 35% sodium carbonate solution. The final volume of the solution was made up to 10 ml with distilled water and it was kept for 30 min at room temperature. Absorbance was measured at 700 nm against the standard tannic acid solution and was expressed as mg/g of tannic acid equivalent.

The total flavonoid content was determined by aluminium trichloride method where known amount of sample extract was mixed with 75 µl of 5% sodium nitrite and 150 µl of 10% AlCl3 and was allowed to stand for 5-6 min. To that 750 µl of 1 molar NaOH was added and the final volume was adjusted to 2.5 ml with distilled water. Absorbance was measured at 510 nm against standard quercetin solution and was expressed as mg/g equivalent of quercetin.

The total alkaloids were quantified by method employed by where, 5 g of powdered sample was mixed with 200 ml of 10% of acetic acid in ethanol. It was properly covered and allowed to stand for 4 h. After the incubation period, it was filtered and concentrated to $1/4^{th}$ part of the original volume and preceded by addition of concentrated ammonium hydroxide solution until complete precipitation. Later, the precipitates were collected and washed with diluted solution of ammonium hydroxide and weighed to a constant mass. The alkaloid content was calculated using following formula:

$$\text{Alkaloid content} = [(B - A) \times 100]/S$$

Where, B–Weight of Whatmann filter paper
S–Sample weight
A–Weight of Whatmann filter paper after drying

Saponins were quantified following Arwani *et al.* (2019). 50 ml of 20% ethanol was mixed with 5 g of powdered samples and heated over a hot plate at 55° for about 4 h. The residues obtained were again re-extracted using the same procedure. The extracts obtained were evaporated at 90° using water bath. The concentrated extract was mixed with 10 ml of diethyl ether and constantly agitated in a separating funnel to remove the ether layer. The purification process was repeated twice and the recovered aqueous layer was mixed with 30 ml of butanol along with 5% of sodium chloride. The remaining solution was evaporated and dried to a constant weight. The percentage of saponin was calculated by using following equation:

$$\text{Saponin content \%} = [(B\text{-}A) \times 100]/S$$

Where, B–Weight of Whatmann filter paper
S–Sample weight
A–Weight of Whatmann filter paper after drying

Total phenolics were quantified by the standard method where, 2 ml of folin ciocaltaeu reagent was mixed with 0.5 ml of *Moringa* samples. To that 4 ml of sodium carbonate was incorporated at the rate of 7.5%. The samples were kept at room temperature (RT) for 30 min with intermittent shaking. Absorbance was recorded at 765 nm by using double beam spectrophotometer. Gallic acid was used as reference standard for calibration curve.

The antibacterial activity of leaf, seed and pulp extracts of *M. oleifera* was carried out by agar well diffusion assay. Initially, the nutrient agar medium was inoculated with test microorganisms which were procured from NCIM, Pune (*E. coli* NCIM accession number: 5033; *S. aureus* NCIM accession number: 2079). The wells were filled with *Moringa* samples where distilled water served as control. The nutrient agar medium was incubated at 37° for 24 h and the zone of inhibition was measured in mm.

RESULTS AND DISCUSSION

The medicinal or therapeutic importance of any plant is dependent on the bioactive compounds present in them. These bioactives

Table 1. Quantitative phytochemical estimation of leaf, seed and pulp sample of *Moringa oleifera* (N=3)

Phytochemical	Leaf	Seed	Pulp
Total phenols (mg/g)	3.3±0.30	3.46±0.31	2.96±0.46
Total flavonoids (mg/g)	3.06±0.14	4.56±0.29	3.76±0.31
Total tannins (mg/g)	1.9±0.23	2.4±0.15	6.86±0.14
Total alkaloids (%)	2.16±0.14	1.2±0.11	3.1±0.15
Total saponins (%)	2.10±0.17	1.4±0.28	4.9±0.40

are accumulated in different parts of the plants as secondary metabolites in varied concentrations (Balouiri *et al.,* 2016; Patel and Krishnamurthy, 2021). Different plant parts i.e. leaves, seeds and pulp indicated varied levels of phytocompounds. It was observed that the total phenolic compounds were in the range of 2.96 to 3.46 mg/g. The total flavonoid contents were in the range of 3.06 to 4.56 mg/g, whereas the total tannin content was in the range of 1.9 to 6.86 mg/g as tannic acid equivalent. This indicated that *Moringa* seeds, leaves and pulp had wide range of phytochemicals including saponins and alkaloids (Table 1). It was believed and observed that phytocompounds such as flavonoids, phenols and tannins imparted protection against various allergic reactions, ulcers, inflammation, tumors along with the pain reliving antioxidant and spasmolytic property (Adekanmi *et al.,* 2020). Phytochemicals in form of secondary metabolites gets accumulated in higher amount in both medicinal and aromatic plants. However, they do not play any crucial role in development of plants. More than 80% of global population relies on these phytocompounds for their day to day healthcare system as every part of *Moringa* serves as a vital source of nutrients (Krishnamurthy *et al.,* 2018; Ma *et al.,* 2020).

The antibacterial profile of leaves, seeds and pulp was also carried out against one gram positive and one gram negative bacteria viz, *E. coli* and *S. aureus,* respectively. These strains are known to be responsible for UTI (Urinary tract infection) and skin diseases. A dose of 50 and 100 mg/ml was selected for the study and it was observed that *Moringa* pulp, leaves and seeds were able to inhibit both the pathogenic strains in dose dependent manner (Figs. 1 and 2). The data pertaining to antibacterial activity of different plant parts of *Moringa* are depicted in Table 2. It was observed that *M. oleifera* pulp showed potent inhibitory activity against *E. coli* (4.76±0.40) and *S. aureus* (5.81±0.30) followed by *Moringa* seed (0.41±0.012 and 0.50±0.44) and *Moringa* leaf (0.22±0.013 and 0.33±0.01) at the dose of 100 mg/ml, respectively. However, the trend was same in 50 mg/ml but it was pretty low. The immune-

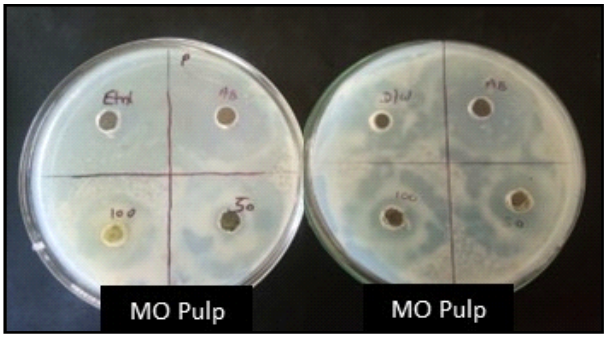

Fig. 2. Antibacterial activity of *Moringa* pulp against *E. coli* and *S. aureus* at the dose of 50 and 100 mg/ml.

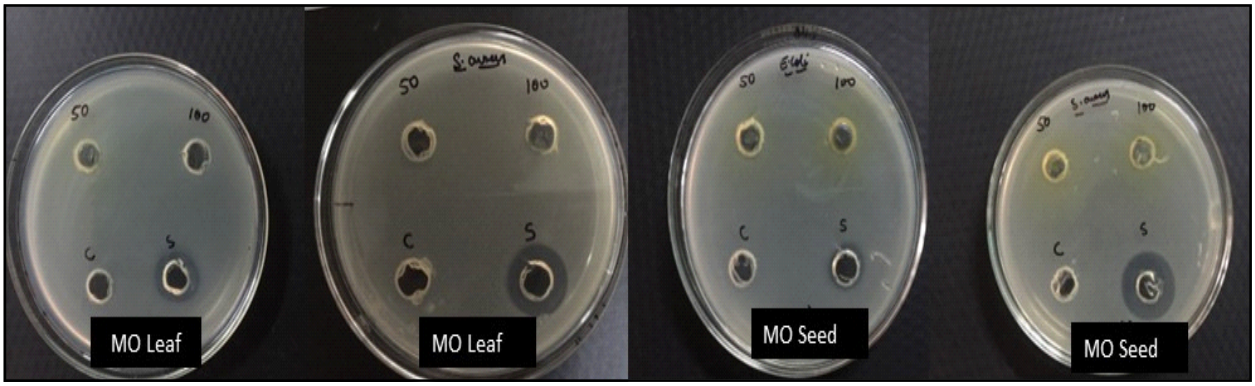

Fig. 1. Antibacterial activity of *Moringa* leaf and seed against *E. coli* and *S. aureus* at the dose of 50 and 100 mg/ml.

Table 2. Antibacterial activity of *Moringa* leaf, seed and pulp sample against *E. coli* and *S. aureus* at 50 and 100 mg/ml (N=3)

Test strains	*Moringa* leaf	*Moringa* seed	*Moringa* pulp	Streptomycin (1 mg/ml)
Concentration: 50 mg/ml				
E. coli	No zone	0.06±0.02	0.96±0.015	1.66±0.21
S. aureus	No zone	0.26±0.020	1.06±0.015	2.4±0.05
Concentration: 100 mg/ml				
E. coli	0.22±0.013	0.41±0.012	4.76±0.40	1.66±0.21
S. aureus	0.33±0.01	0.50±0.44	5.81±0.30	2.4±0.05

modulating and cardio-protective activity along with potent antibacterial activity was due to the presence of saponins, triterpenoids and other bioactives present in the *Moringa.* This is in analogy with the findings on *Moringa oleifera, Solanum*, *Ocimum,* turmeric, lime and beetroot (Patel *et al.,* 2019; Patel and Krishnamurthy, 2021).

CONCLUSION

The bioactive compounds in the form of phytochemicals in *Moringa oleifera* exhibited multitude therapeutic benefits, though the exact mechanism underlying these effects is still unknown. Hence, further investigation must be focused on examining the mechanistic action of these phytocompounds with potential antibacterial activity to prevent and manage various ailments.

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