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Estimation of phytoconstituents and screening of antibacterial and antioxidant activity of different extracts from *Moringa oleifera*

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Abstract

The present study deals with the preliminary phytochemical screening and determination of antibacterial and antioxidant activity of various extracts from leaves and flowers of *Moringa oleifera*. The results revealed the presence of phenolic compounds, flavonoids, terpenoids, alkaloids, saponins, tannins and steroids in the extracts. All the extracts exhibited antibacterial effect against the tested bacteria with the diameter of inhibition zone ranging from 7 to 22 mm. However, highest antibacterial activity was observed by methanol and ethyl acetate extracts. The scavenging effect of the n-hexane extracts of leaf and flower on DPPH radicals increased with increasing concentration and highest activity was 70.94% and 73.50%, respectively while ascorbic acid was the highest DPPH radical scavenging activity (82.91%) at a concentration of 180 µg/ml. These results indicated that n-hexane extracts of flower and leaf of *Moringa oleifera* have a noticeable effect on scavenging free radical, however, it can be categorized as a good and potential antioxidant agent and could be one of the areas which attached a great deal of attention is the possible therapeutic potential of antioxidants in controlling degenerative diseases associated with marked oxidative damage. The preliminary studies on *Moringa oleifera* extracts exhibited their antibacterial and antioxidant potential which could be exploited further as future antimicrobials for pharmaceutical treatment, natural therapies, food preservation and cosmetic applications.

Keywords: *Moringa oleifera;* Phytochemicals*;* Antibacterial activity*;* Antioxidant

1. Introduction

Plants are important sources of potentially useful substances for the development of new therapeutic agents. Various phytochemical compounds as secondary metabolites have been implicated in plants as the conferment of antibacterial activities [1,2]. Now a days, there is a renewed interest in traditional medicine. This revival of interest in plant-derived drug is mainly due to the current wide spread belief that the 'green medicine' is safe and more dependable than the costly synthetic drugs, many of which have adverse side effects. The medicinal action of plants is unique to a particular plant species, consistent with the concept that the combination of secondary metabolites in a particular plant is taxonomically distinct to other species [3]. Medicinal plants have provided the modern medicine with numerous plant derived therapeutic agents [4].

In Bangladesh, about 500 plant species have been identified as medicinal plants because of their therapeutic properties. Approximately hundreds of traditional medicines have been developed in the form of Ayurvedic and Unaani formulations in Bangladesh. About 400 herbal industries have been established in this country for producing Ayurvedic and Unani medicines and marketed herbal products of 500-crore taka worth annually [5]. Proper scientific evaluation of the pharmacological properties of these plants, used in different formulations, would carry enormous potential and promise for the 21st century.

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Moringa oleifera is the most widely cultivated species of a monogeneric family, the Moringaceae that is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. It is a perennial softwood tree with timber of low quality, but which for centuries has been advocated for traditional medicinal and industrial uses. All parts of the Moringa tree are edible and have long been consumed by humans. *Moringa oleifera* is called 'miracle vegetable' because it is not just a food, but also a medicine and it may therefore be a functional food [6]. Phytochemicals such as vanillin, omega fatty acids, carotenoids, ascorbates, tocopherols, beta-sitosterol, kaempferol and quercetin have been reported from the flowers, roots, fruits and seeds [7]. The leaves are highly nutritious, being a significant source of beta-carotene, vitamin C, protein, iron and potassium [8]. Leaves of this plant have been studied extensively for various biological activities including hypolipidemic, antiatherosclerotic, immune-boosting agents, and tumor-suppressive effects [9,10]. Due to the wide uses of *Moringa oleifera*, the present study was focused to evaluate their phytoconstituents and screening of antibacterial and antioxidant activities.

2. Material and methods

2.1. Plant materials

Fully matured fresh flowers and leaves were collected from Islamic University campus, Kushtia, Bangladesh. The plant samples were then grinded in a fine powder form and then stored in air-tight container with marking for identification and kept in cool, dark, and dry place for future use.

2.2. Preparation of extracts

100 g of each powdered plant materials is submerged in suitable solvents of increasing polarity as n-hexane, ethyl acetate and methanol subsequently in an air-tight separating funnel for 5 days at room temperature with occasional shaking and stirring. The obtained extract was filtered by using Whatman No.1 filter paper. Each filtrate was concentrated under reduced pressure on a rotary evaporator till a viscous mass was obtained. Finally, the prepared extracts were stored at 4^{o}C for further analyses.

2.3. Phytochemical screening

The extracts of *Moringa oleifera* (20 mg) were subjected to qualitative analysis to detect the presence of different classes of chemical constituents in the plant.

2.3.1. Test for Alkaloids

n-Hexane, ethyl acetate, methanol extract of each part of *Moringa oleifera* were warmed separately with 2% H_2SO_4 for two minutes. It was filtered and few drops of Dragendroff's reagent were added and a red precipitation indicated the presence of alkaloids.

2.3.2. Test for Flavonoids

A small quantity of the extract was heated with 10 ml of ethyl acetate in boiling water for 3 minutes. The mixture was filtered and shaken with 1 ml of dilute ammonia solution (1%). The layers were allowed to separate. A yellow coloration confirmed the presence of flavanoids.

2.3.3. Test for Saponins

A small quantity of different extracts was diluted with 4 ml of distilled water. The mixture was shaken vigorously and then observed on standing for stable foam indicated the positive test.

2.3.4. Test for Steroids

2 ml of acetic anhydride and 2 ml H_2SO_4 were added to the extracts. The color changed from violet to blue or green indicated the presence of steroids.

2.3.5. Test for Terpenoids

Each extract was mixed with 2 ml of chloroform followed by concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown coloration in the interface indicated positive result for the presence of terpeniods.

2.3.6. Test for Tanins

About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration confirmed the presence of tannins.

2.4. Bioactivity screening

2.4.1. Microbial strains

The antimicrobial properties of *Moringa oleifera* were investigated against two gram positive bacterial strain*, Bacillus subtilis, Staphylococcus aureus* and two gram negative bacterial*, Escherichia coli, Pseudomonas aeruginosa*. The strains were collected from the Department of Applied Nutrition and Food Technology, Islamic University, Kushtia. Active cultures for experimental use were prepared by transferring a loopful of cells from stock cultures to flasks and inoculated in Luria-Bertani (LB) broth medium at 37°C for 24 h. Cultures of each bacterial strains were maintained on LB agar medium at 4°C.

2.4.2. Antibacterial activity assay

The dried extracts were dissolved in the same solvent used for their extraction to a final concentration of 30 µg/µL and sterilized by filtration by 0.45 µm Millipore filters (Millipore Corp., Bedford, MA, USA). The antibacterial test was then carried out by agar disc diffusion method [11] using 100 µL of standardized inoculums suspension containing 10^7 CFU/mL of bacteria. 10 µL of 30 µg/µL of each organic extract (300 µg/disc) was applied on the filter paper discs (6 mm diameter) and placed on the inoculated LB agar. Negative controls were prepared using the same solvents employed to dissolve the samples. Standard reference antibiotics streptomycin (10 µg/disc, each from Sigma-Aldrich Co., St. Louis, MO, USA) was used as positive controls for the tested bacteria. The plates were incubated at 37°C for 24 h. Antibacterial activity was evaluated by measuring the diameter of the zones of inhibition against the tested bacteria.

2.4.3. Assay of free radical scavenging activity

DPPH radical scavenging activity of the extracts was measured on the basis of the scavenging activities of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich, St. Louis, MO) free radical [12]. Various concentrations of test extracts (0.1 ml) were added to 2.9 ml of a 0.004% (w/v) methanol solution of DPPH. After 30 min of incubation period at room temperature, the absorbance was measured against a blank at 517 nm. Inhibition free radical DPPH in percent ($I\ \%$) was calculated in following way:

$$I\% = (A_{blank} - A_{sample}/A_{blank}) \times 100$$

Where, A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. Ascorbic acid was used as reference positive controls and all tests were carried out in triplicate.

3. Results and discussion

3.1. Phytochemical screening

Phytochemicals are non-nutritive plant chemicals that have disease preventive properties [13]. The investigation of n-hexane, ethyl acetate and methanol extracts of *Moringaoleifera* revealed differences in their phytoconstituents. According to the Table-1 and 2, the preliminary phytochemical screening was carried out on *Moringa oleifera* indicates the presence of alkaloids in methanol and ethyl acetate extract of leaf while all extracts of flower (n-hexane, methanol and ethyl acetate) contain alkaloids. Alkaloids have been reported to possess analgesic, antispasmodic and bactericidal, antimalarial and analgesic activities [14]. Saponins and terpenoids are found only in n-hexane extracts of various parts of *Moringa oleifera*. Saponin was found to be present in *Moringa oleifera* extracts, which are steroid or triterpenoid glycosides characterized by their bitter or astringent taste, foaming properties and their haemolytic effect on red blood cells [15]. Terpenoids are aromatic compounds found in plant species, which is responsible for flavour and fragrance. Plant terpenoids play vital role in the herbal remedies [16]. On the other hand, tanins and flavonoids are commonly found in methanol and ethyl acetate extracts of both leaf and flower. Flavonoids belong to the group of polyphenolic compounds and are typically known for health promoting properties such as antioxidant, anti-allergic, anti-inflammatory, antimicrobial and anticancer properties [17]. Tannins possess biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial ftinction, as well as inhibition of angiogenesis and cell proliferation activities [18]. Steroids

found in all the extracts of leaf but absent in flower. Steroids derived from plants are known to have cardiotonic effect and also possess antibacterial and insecticidal properties [19].

Table 1 Phytochemicals analysis of *Moringa oleifera* leaf with different solvents

Sl. No.	Name of the phytochemicals tests		Solvents		
			Methanol	Ethyl acetate	n-Hexane
1	Alkaloids	Dragendroff's	+	+	-
		Mayer's	+	+	-
2	Flavonoids		+	+	-
3	Saponins		-	-	+
4	Steroids		+	+	+
5	Terpenoids		-	-	+
6	Tanins		+	+	-

Table 2 Phytochemicals analysis of *Moringa oleifera* flower with different solvents

Sl. No.	Name of the phytochemicals tests		Solvents		
			Methanol	Ethyl acetate	n-Hexane
1	Alkaloids	Dragendroff's	+	-	+
		Mayer's	+	+	+
2	Flavonoids		+	+	-
3	Saponins		-	-	+
4	Steroids		-	-	-
5	Terpenoids		-	-	+
6	Tanins		+	+	-

3.2. Antibacterial activity

Table 3 Antibacterial activity of leaf extracts of *Moringa oleifera*

Microorganism	Zone of Inhibition in mm			
	Various extracts			Antibiotics
	n-Hexane	MeOH	EtOAc	Streptomycin
Bacillus subtilis	12±1.2	16±0.4	20±0.5	21±1.7
Pseudomonas aeruginosa	14±1.1	21±0.4	19±0.8	23±1.4
Escherichia coli	11±0.4	15±0.4	17±1.5	25±0.5
Staphylococcus aureus	14 ±1.1	21±0.6	22±1.1	22±0.6

Values are given as mean ± S.D of triplicate experiment,Values in the same column with different superscripts are significantly different ($p < 0.05$).

The antibacterial activities of extracts obtained from spices, herbs, and other aromatic plants or parts thereof using organic solvents or steam distillation have been recognized for many years. Plants and plants extracts have been used since antiquity in folk medicine and food preservation, providing a range of compounds possessing pharmacological activity [20]. The *in vitro* antibacterial activity of various extracts (n-hexane, ethyl acetate and methanol) of *Moringa oleifera* against the employed bacteria was qualitatively assessed by the presence or absence of inhibition zones. According to the results given in Table 3, different leaf extracts showed their respective diameter zones of inhibition of

from 11.0~22.0 mm. Ethyl acetate extract showed the strongest effect against *S. aureus* (inhibition zone: 22 mm), compared to standard drug streptomycin. On the other hand, hexane and methanol extracts showed noticeable antibacterial effect with inhibition zones in the range of 11.0~14.0 and 15.0~21.0 mm, respectively. The flower extracts showed their respective diameter zones of inhibition of from 7-19 mm as shown in Table 4. Methanol extract showed the strongest effect against *Pseudomonas aeruginosa* (inhibition zone: 19 mm), whereas, hexane and ethyl acetate extracts showed moderate antibacterial effect with inhibition zones in the range of 7.0~11.0 and 9.0~12.0 mm, respectively. The blind control did not inhibit the growth of the tested bacteria.

Table 4 Antibacterial activity of flower extracts of *Moringa oleifera*

Microorganism	Zone of Inhibition in mm			
	Various extracts			Antibiotics
	n-Hexane	MeOH	EtOAc	Streptomycin
Bacillus subtilis	7±1.2	12±0.4	10±0.5	21±1.7
Pseudomonas aeruginosa	11±1.1	19±0.4	9±0.8	23±1.4
Escherichia coli	8±0.4	15±0.4	12±1.5	25±0.5
Staphylococcus aureus	10 ±1.1	13±0.6	11±1.1	22±0.6

Values are given as mean ± S.D of triplicate experiment,Values in the same column with different superscripts are significantly different ($p < 0.05$)

3.3. Antioxidant activity

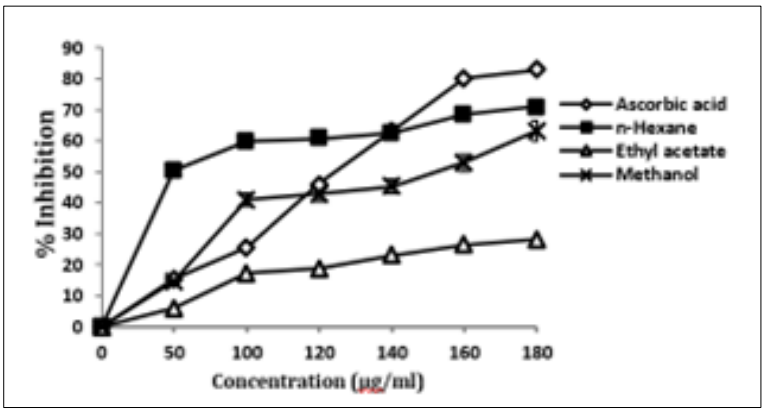

Figure 1 Antioxidant activity of leaf extracts of *Moringa oleifera* with standard ascorbic acid

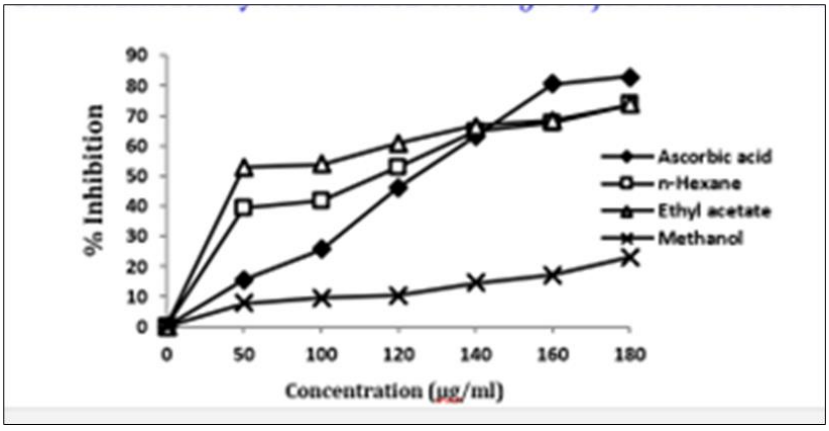

Figure 2 Antioxidant activity of flower extracts *of Moringa oleifera* wit*h* standard ascorbic acid

The DPPH assay measures the ability of the plant extract to donate hydrogen to the DPPH radical resulting in bleaching of the DPPH solution. The antioxidants in extracts react with the stable free radical DPPH and convert it to 1,1-diphenyl-

2-picrylhydrazine with decolorization of purple color of DPPH to pale yellow coloration. The extracts exhibited concentration dependent radical scavenging activity. That is higher the concentration, more is scavenging potential. The DPPH is a stable free radical, which has been widely accepted as a tool for estimating free radical scavenging activities of antioxidants. The DPPH radical scavenging activities of the organic extracts are shown in Figure 1 and 2 for leaf and flower, respectively. The scavenging effect of the n-hexane extracts of leaf and flower on DPPH radicals increased with increasing concentration and highest activity was 70.94% and 73.50% at a concentration of 180 µg/ml, respectively, and methanol extracts of them was 63.25% and 23.08% at a concentration of 180 µg/ml, respectively while ascorbic acid was the highest DPPH radical scavenging activity (82.91%) at a concentration of 180 µg/ml only. The ethyl acetate extracts of leaf and flower showed little and moderate scavenging effect on DPPH radicals and their activity was 28.20% and 73.50% at a concentration of 180 µg/ml. This may be due to the presence of high bioactive compounds in polar n-hexane fraction.

4. Conclusion

From the study, it could be concluded that plants are a great source of phytochemicals that could be utilized in curing various ailments. Phytochemical screening played an important role in identifying various phytoconstituents present in plant extracts. The study provided an important basis for further investigation into the isolation and characterization of phytoconstituents from *Moringa oleifera* for the development of drugs. The study also showed that various organic extracts of *Moringa oleifera* possess antibacterial and antioxidant activities that might be a natural potential source of preservative used in food and other allied industries.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that no conflict of interest exists.

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