Short Communication

Phytochemical screening, antibacterial and anti-diabetic activities of *Moringa oleifera* cultivated in east region of Libya

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Abstract

Moringa oleifera, a very important healthful plant, is one amongst the foremost wide cultivated species of the family *Moringaceae*. It's extremely valued from past as a result of its large healthful properties. *Moringa oleifera*, native to India, grows within the tropical and climatic zone regions of the globe. It's commonly called drumstick tree or horseradish tree. *Moringa* will face up to each severe drought and gentle frost conditions and, therefore wide, cultivated across the globe. With its high alimentary values, each part of the tree is appropriate for either nutritional or commercial purposes. The leaves are rich in minerals, vitamins and alternative essential phytochemicals. Extracts from the leaves are useful in treatment of anemia, enhance breast milk in wet mothers. It's used as a potential antioxidant, anti-cancer, anti-inflammatory, anti-diabetic and antimicrobial agent. Thus, this study was aim to investigate the phytoconstituents from ethanolic and aqueous extracts of the *Moringa oleifera* cultivated in Libya and to assess its anti-bacterial and anti-diabetics activity. The phytochemical investigations of these extracts of *Moringa oleifera* revealed the presence of alkaloids, carbohydrates, flavonoids, saponins, steroids, tannins and phenolic compounds. The antibacterial activity of cold and hot extraction of methanolic and water extracts of *Moringa oleifera* by using the well diffusion method were assessed. Results revealed that there was a profound antibacterial activity of the studied *Moringa oleifera* against representatives of Gram-positive bacteria. After *in vitro* evaluation of anti-hyperglycemic properties of the ethanolic extract of *Moringa oleifera* by testing its capacity to inhibit α-amylase activity, the methanolic extract exhibited inhibitory effect on the α-amylase enzyme.

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Introduction

Over the last decades, there was an exponential growth in the field of herbal medicine. It is getting popularized in developing and developed countries owing to its natural origin and lesser side effects [1]. The evaluation of various plant products according to their traditional uses and medicinal value based on their therapeutic efficacy which leads to the discovery of newer drugs for treating various diseases and disorders. This fact forms the basis for the development of new pharmaceutical products from various plant sources [2]. One of such plants of medicinal value is *Moringa oleifera*, belonging to the family *Moringaceae*, commonly known as Sahajan in Hindi, Horse radish in English. The genus *Moringa* comprises of 14 different species distributed in Africa, Madagascar, Western Asia and Indian subcontinent. This species is introduced intentionally into Libya for its nutritional and medicinal value. This is because almost all parts of the tree are utilized as natural medicine for several diseases [3]. Various properties of *Moringa oleifera*, are attributed to various parts as antispasmodic, diuretic, expectorant and abortifacient [4]. The plant is used as antispasmodic, stimulant, expectorant and diuretic [5]. The limit of

microbiological illnesses and infections has severely been exceeded in recent time. The rising frequency of antibiotic resistance is an issue in antimicrobial chemotherapy [6]. Continued antibiotic use and hence antibiotic selection pressure is regarded to be the most important factor contributing to the emergence of various types of resistant bacteria [7, 8]. The development of new antimicrobial drugs and herbal medicines is critical for the control of harmful bacteria, particularly in the treatment of diseases caused by resistant microbes. Antimicrobial-active medicinal plants are thought to be a good source of new antimicrobial function [9].

Diabetes is chronic metabolic disease that can be treated, but as yet it cannot be cured. Type II diabetes mellitus is complex disease that involves impaired insulin secretion by pancreatic beta cells and defects in action on its target tissues. Type II diabetes is a global public health problem that can lead to serious complications if controlled poorly [10]. Keeping blood sugar along with body weight under control through a combination of healthy lifestyle and standard treatment is an important part of diabetes management [11, 12]. Depending on the root cause, diabetes can be treated by administration of insulin or the inhibition of enzymes as α-amylase, α-glucosidase, dipeptidyl peptidase IV, protein tyrosine phosphatase etc. Complications and side effects associated with the use of conventional drugs lead to the search for alternative anti-diabetic drugs from plant sources [13]. *Moringa oleifera* has been used in traditional herbal medicines for numerous years. The plant is believed to offer many benefits and its uses range from beauty to preventing and curing diseases as asthma, liver diseases, cardiovascular diseases, cancer and digestive issues [14]. Previous studies have shown that *Moringa oleifera* or drumstick contains beneficial antioxidants and bioactive compounds that can help treat and prevent diseases, including type II diabetes mellitus [15]. Olayaki et al. [16] and showed effects of *Moringa* leaves on lowering blood sugar levels in diabetes patients and animals indicating that the plant can be used as a natural way to combat the diseases. On study involving 30 women reported that consuming seven grams of *Moringa* leaf powder every day for three months helped reduce fasting blood sugar levels by about 10%. Similarly, another small study found that using *Moringa* leaves about 50 grams in a meal reduced the rise in blood sugar by about 20% [17]. Villarruel-López and others conducted a study to evaluate *Moringa* leaves powder concerning Type II Diabetes. The findings suggest that consumption of *Moringa*. *oleifera* powder leaves could be beneficial in the diabetes mellitus [18]. The aim of the current study is to determine the phytoconstituents of the ethanolic and water extracts of *Moringa oleifera* leaves and to investigate the antibacterial and anti-diabetics effects of the ethanolic extracts of *Moringa oleifera* leaves.

Materials and methods

Sample collection and preparations: Plant was purchased from local Botanist (Benghazi, Libya, 2021), washed and dried. The plant was classified by two independent Taxonomists at Department of Botany, Faculty of Science, University of Benghazi, Benghazi, Libya (2021). Then it was grounded using miller to fine powder and stored at a room temperature until used.

Preparation of the extracts: Dried powder of plant leaves was continuously extracted with ethanol for three hours using Soxhlet apparatus. The solvent was evaporated under vacuum using rotary vapor. The residue was then stored in air-tight containers at 4 ^{0}C for further use. The dried powdered plant was cold extracted by maceration with ethanol for 24 hours with frequent shaking. The extract was filtered and evaporated under reduced pressure using rotary evaporator. The obtained residue was dissolved in ethanol and kept in tightly closed container for further analysis. The aqueous extract of the plant was obtained by decoction of the dried leaves with water for about 30 min, the extract was decanted and concentrated under vacuum pressure [16].

Preliminary phytochemical screenings: Moringa oleifera extracts (aqueous and ethanol) were subjected to qualitative tests for the identification of various phytochemical constituents such as phenols, carbohydrate, flavonoids, tannins, alkaloids, coumarone, saponins, terpenoids, glycosides, steroids and proteins by using standard procedure [19].

Study of antibacterial properties: Agar well diffusion method was used to screen the antibacterial activities of the extracts as described by Baver and others with some modifications [20]. The antibacterial activity of *Moringa oleifera* extracts were assessed against four different bacterial strains (two Gram-negative bacteria: *Escherichia coli*, and *Pseudomonas aeruginosa* and two Gram-positive bacteria: *Staphylococcus aureus* and *Eterococcus feacalis*) which obtained from Sigma-Aldrich, Merch, Benghazi, Libya.

Screening for anti-diabetic properties using the Alpha-amylase inhibitory effect method: The anti-hyperglycemic activity of the plant extract was calculated by applying the α-amylase inhibition assay proposed by Kusano et al. [21]. The technique is based on the change in the intensity

of the blue color developed as a result of iodine binding to starch polymers, the difference in reading between the control (starch and iodine) and test sample (starch, alpha-amylase, the sample and iodine) was measured by spectrophotometric at 630 nm. The finding was taken as percentage inhibition of α-amylase; % inhibition = [(reading of control – reading of sample) per reading of control] x 100.

Results and discussion

Phytochemical screenings: The alcoholic and aqueous extracts of the *Moringa oleifera* were preliminary screened to determine various phytochemical compounds in the plant. The common phytochemicals from plant such as flavonoid, alkaloid, carbohydrates, phenols, tannin and saponins were identified (**Table 1**).

Table 1: Phytochemical screening findings of *Moringa oleifera*

Constituents	Methods	Alcoholic extract	Aqueous extract
Alkaloids	Mayer's test Drangdorff`s reagent	++ ++	--
Phenols	Ferric chloride solution ($FeCl_3$) add to aqueous extracts black – green developed	+	
Sterols	Libermaan's test	++	
Flavonoids	10% NaOH Lead acetate solution.	+ +	+
Carbohydrates	Molish's test	+	+
Tannins	Ferric chloride solution ($FeCl_3$) add to aqueous extracts black –green developed	-	+
Saponins	Foam test	-	+
+ weak & ++ strong			

Antibacterial activity: The ethanolic extracts (hot and cold) and hot aqueous extract of aerial part of *Moringa oleifera* plant were investigated to evaluate their antibacterial properties against four important pathogenic bacteria including two strains of Gram-positive bacteria (*Staphylococcus aeries and Enterococcus faecalis*) and two strains of Gram-negative bacteria (*Escherichia coli and pseudomonas aeruginosa*) using well diffusion method. All the experiments were repeated three times and the findings were expressed as mean values. Results ≤ 7 mm were considered to be resistant, 50 - 60 µl of the extract was applied. The diameters of inhibition zones of *Moringa oleifera* was recorded showed in Table 2.

Table 2: The antibacterial activity of *Moringa oleifera* extracts

Extract / Microorganism	Hot methanol	Cold methanol	Hot water
Escherichia coli	10 mm	09 mm	10 mm
Pseudomonas aeruginosa	12 mm	11mm	12 mm
Eterococcus feacalis	15 mm	13mm	13 mm
Staphylococcus aeurus	18 mm	15 mm	14 mm
Note: all the experiments were repeated three times. Results ≤ 7 mm were considered to be resistant.			

The antibacterial activity was screened because of their great medicinal properties towards the pathogenic organisms. The tested medicinal plant *Moringa oleifera* showed a good antibacterial activity against several organisms like *Staphylococcus aureus*, *Eterococcus feacalis*, *Pseudomonas aeruginosa* and *Escherichia coli* as supported by the previous studies.

The antibacterial activity of the extract was greater against Gram-positive bacteria species (*S. aureus* and *E. faecalis*) than against Gram-negative bacteria strains (*E. coli and P. aeruginosa*), further, similar effects have been reported for other medicinal plant extracts in previous published studies [22 - 24]. The antibacterial effect is attributed to the presence of saponine, tannic, phenolic and alkaloid phytoconstituents. Various studies have shown that crude extracts from *Moringa oleifera* exhibit antimicrobial activity against various pathogenic bacteria, fungi, viruses and parasites that affect man and his environment. Thus, it can be used in medicinal treatments to control the infection of pathogenic microbes [25 - 27].

The results indicated that *S. aureus* was found to be the most sensitive test organism to different extracts of *Moringa oleifera*. Looking to these results it may be concluded that *Moringa oleifera* may be a potential source for the treatment of different infections caused by the resistant microbes. The results showed that *E. coli* was the most resistance to all *Moringa* extracts used in this study. Similarly, using agar well diffusion method, Rajendrhan et al. [28] reported *E. coli* to be resistant to *Moringa* extracts.

Anti-diabetic effect

After *in vitro* evaluation of anti-hyperglycemic properties of the ethanolic extract of *M. oleifera* by testing its capacity to inhibit α-amylase activity, MOE exhibited inhibitory effects on the α-amylase enzyme (**Table 3**).

Table 3: Results of the in vitro α-amylase inhibitor assay of (MOE)

Sample	IC_{50} (µg/ml)
Moringa Extract	37 ± 3
Acarbose	25 ± 3

Diabetes is a devastating illness that has piqued the interest of people all over the world. In 2010, about 6.4% of adults were diabetic. In 2030, this is expected to increase to 7.7%. Between 2010 and 2030, an estimated increase of 69% and 20% among adults in developing and developed countries, respectively, is expected [29]. α-amylase is abundant in pancreatic juice, saliva and many other tissues. The pancreatic α-amylase hydrolyses α-(1,4)-glycosidic linkage haphazardly, producing dextrin, maltose and/or maltotriose [30]. A recent report reveals that aqueous leaf extract (2 g/100 ml) of *Moringa oleifera* inhibited both α-amylase and α-glucosidase with a corresponding IC_{50} of 6.49 and 4.73 mg/ml, respectively [31]. In other report, aqueous extract of *Moringa* leave was found to exhibit higher inhibitory effect on sucrose (IC_{50} = 0.98 mg/ml), in comparison to α-amylase and maltase whose IC_{50} values were greater than 5.00 mg/ml [32].

This study investigated the inhibitory effect of *Moringa oleifera* ethanolic extract cultivated in Benghazi city on α-amylase activity. *Moringa oleifera* extract exhibited a considerable α-amylase inhibitory effect with IC_{50} of 0.37 ± 0.003 mg/ml in comparison to the positive control acarbose (0.25 ± 0.003). The α-amylase inhibitory effect of *Moringa* may be attributed to its richness in varied amount of phytochemical that includes: sterols such as b-sitosterol, stigmasterol and campesterol [33]; flavonoids like quercetin, kaempferol and myricetin are also reported to be found in the leaves; phenolics and other compounds like tannins, alkaloids, saponins, terpenoids and ascorbic acid which are present in significant amounts in various components of the plant and they have shown to be beneficial in several chronic conditions, including hypercholesterolemia, high blood pressure, diabetes, insulin resistance, non-alcoholic liver disease, cancer and overall inflammation [34].

Conclusion

This study provides a scientific support for the traditional use of *Moringa oleifera* as a treatment for some medical conditions such as bacterial infections and Diabetes. The phytochemical testing indicated that the plant extract could be a good source for the isolation of bioactive secondary metabolites (phenols and flavonoids). The selected herb revealed marked antibacterial and anti-diabetic properties and may have a potential to be utilized for production of food supplements for postprandial hyperglycemia.

Ethical issues

Including plagiarism, Informed Consent, data fabrication or falsification and double publication or submission have completely been observed by authors.

Conflict of interest

The author declares no potential conflict of interest.

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