

Phytochemical and Antibacterial Activity of *Moringa Oleifera* Available in the Market of Mekelle

Abstract

The aim of the present study was to evaluate the antibacterial activity of *Moringa oleifera* leaf extracts, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus vulgaris*, *Streptococcus mutans*, *Bacillus subtilis*, and *Staphylococcus epidermidis* bacteria were used. Phytochemical analyses of the leaf in solvents of varying polarity with aqueous, ethanol were also carried out. The phytochemical screening indicated the presence of flavonoids, tannins, steroid, alkaloid, saponins etc., in the both extracts. Well diffusion method was used to assess the antibacterial effect of the extracts on micro-organisms. The ethanolic and aqueous extract were active against all strains but the ethanol leaf extract showed maximum activity against *Streptococcus mutant* and aqueous extract shows maximum activity against *Proteus vulgaris*. The research showed that the higher reducing power of the aqueous extract could be due to the better solubility of the antioxidant components in water whereas the predominant antibacterial activity in organic solvent extracts as compared to aqueous extracts, indicates that the active components responsible for the bactericidal activity are more soluble in organic solvents. These studies provide an evidence to support traditional medicinal uses of the plant.

Keywords: Antibacterial activity; *Moringa oleifera*; Phytochemical screening

Research Article

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Introduction

Moringa oleifera is medium sized tree, about 10m height, found in the sub-Himalayan tract [1], which belongs to family *Moringaceae*, native to, Africa, Arabia, South Asia, South America, Himalaya region, India, Pakistan, the Pacific and Caribbean Islands. *Moringa oleifera* has been naturalized in many tropical and subtropical regions worldwide, the plant is referred to number of names such as horseradish tree, drumstick tree, oil tree, miracle tree, and "Mothers best friend" [2]. *Moringa oleifera* is commonly known as "Drumstick". *Moringa oleifera* is a small, fast-growing evergreen or deciduous tree that usually grows up to 10 to 12m in its height, open crown of drooping fragile branches, feathery foliage of trip innate leaves and thick corky, whitish bark [3]. The *Moringa oleifera* plant provides a rich and rare combination of zeatin, quercetin, kaempferol and many other phytochemicals [4]. The leaves are used as a source of vitamins A and C. They are also good sources of vitamin B and are also a source of minerals [5]. Ethanolic extract of *Moringa oleifera* leaves contain niazirin, niazirin, niazirinins A and B [6]. Benzoic acid, gallic acid, beta benzaldehyde have been isolated from methanolic extract of *Moringa oleifera* leaves [7]. The leaf of this plant has diverse biological activities, including hypocholesterolemic, antidiabetic, hypertensive agent [8-11], and regulate thyroid hormone [12], central nervous system, digestive system, nutrition and metabolism. This plant is also reported to be hepato protective against anti tubercular drug such as isoniazid and rifampicin [13,14], and being studied for its

anti-inflammatory, antimicrobial, diuretic [15-17], antibiotic [18], hypotensive [6], and antimicrobial properties [19]. An immune enhancing polysaccharide [20] and niaziminin, having structural requirement to inhibit tumor promoter induced Epstein Barr virus activation have been reported from the leaves [21]. The alcoholic extract of leaves of *Moringa oleifera* was reported to have analgesic activity [22]. Traditionally, the plant is used as antispasmodic, stimulant, expectorant and diuretic [23]. *Moringa oleifera* is used as a drug by many ayurvedic practitioners for the treatment of asthma and to evaluate the anthelmintic activity of methanolic extract of *Moringa oleifera* in adult Ethiopian earth worm's pheretima posthuma at different doses [24].

According to World Health Organization (WHO), more than 80% of the world's population relies on traditional medicines for their primary health care needs [16]. The medicinal value of plants lies in some chemical substances that produce a definite physiologic action on human body. The most important bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds. The phytochemical research based on ethno pharmacological information is generally considered an effective approach in the discovery of new anti-infective agents from higher plants [17]. Despite this array of uses to which parts of *Moringa* tree are put to, scanty literature is available on the uses of *Moringa oleifera* plants as sanitizers or preservatives in foods. However, a very important step in the screening of a plant material for sanitizing / preservative activity was to evaluate its phytochemical and antibacterial activity

Materials and Methods

Preparation of leaf extract

The experiment was conducted in the year 2015 in the department laboratory. Leaves were collected from the super market in mekelle. It was ensured that the plant was healthy and uninfected. The leaves were washed under running tap water to eliminate dust and other foreign particles and to cleanse the leaves thoroughly and dried.

Preparation of leaf extracts

20-30 grams of fresh leaves were boiled with 200 mL of solvent for 1 hour. The extract was filtered using Whatman filter paper No. 1 and then concentrated in vacuum at 40°-50°C using a rotary evaporator. Evaporation of solvent in the rotary evaporator affords a crude extract of the soluble components and these extracts were subjected to the qualitative phytochemical analysis and antibacterial studies.

Phytochemical analysis

Phytochemical constituent analysis of the leaf extract of *Moringa oleifera* was conducted to investigate the presence of alkaloids, flavonoids, steroid, and volatile oil, glycoside, reducing sugar, tannins and saponins was performed by the extracts. According to the procedure given by Talukdar AD 2010.

Alkaloid

The presence of alkaloid was illustrated by using Hager and Baljet test method.

Flavonoids

3ml of each extract was added to 10ml of distilled water and the solution was shaken and 1ml of 10% NaOH solution was added to the mixture. Then the result of yellow color was taken as indicator for the presence of flavonoid.

Saponins (Frothing test)

3ml of each extract diluted with 2ml of distilled water was added in a test tube, and the solution was shaken vigorously. A full mass of bubble formation observed as an indication for the presence of saponin.

Steroids (Salkowski test)

5 drops of concentrated H_2SO_4 were added to 1ml of each extracts in a separate test tube. The formation of a reddish brown color was taken as a positive reaction.

Tannins

2ml of each extract in a separate test tube were boiled gently for 2min and allowed to cool. 3 drop of ferric chloride solution was added to each extract.

Glycosides

25ml of dilute sulphuric acid was added to 5ml extract in a test tube and boiled for 15 minutes, cooled and neutralized with 10%

NaOH, then 5ml of Fehling solution added.

Reducing sugars

To 0.5ml of plant extracts, 1ml of water and 5-8 drops of Fehling's solution was added and heated over water bath.

Volatile oil

2ml of Extract was shaken with 0.1ml dilute NaOH and a small quantity of dilute HCl.

Source of microorganisms

The organisms used were *Escherichia Coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus vulgaris*, *Streptococcus mutans*, *Bacillus subtilis* and *Staphylococcus epidermidis*. The organisms were obtained from the Microbial Lab of Department of Microbiology mekelle university College, Health science mekelle, Tigray Ethiopia.

Determination of antibacterial activity

The antibacterial activity of *Moringa oleifera* leaf extracts was determined using agar well Diffusion method described by [25]. Following the known procedure. Nutrient agar was inoculated with the selected microorganisms, and wells of 6mm were punched in the agar and filled with plant extracts. Control wells with no plant extract were also run along with wells having plant extract in the same plate. The plates were incubated at 37°C for 24 hours and the antimicrobial activity was assessed by measuring the diameter of the zone of inhibition. The antibacterial activities of the different extracts were evaluated by comparing their zones of inhibition with standard antibiotic streptomycin.

Results and Discussions

The present study reveals that *Moringa oleifera* plant shows the presence of phytochemical constituents like alkaloids, flavonoids, carbohydrates, glycosides, proteins, saponins, tannins, terpenoids and anthrax quinones in different solvent extracts as shown in Table 1.

Antibacterial activity of *Moringa oleifera* was seen against several bacteria namely *Escherichia Coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus vulgaris*, *Streptococcus mutans*, *Bacillus subtilis* and *Staphylococcus epidermidis* [26]. The ethanol leaf extract showed maximum activity against *Streptococcus mutant* and aqueous extract shows maximum activity against *Proteus vulgaris* as shown in the Table 2.

Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms. They often have pharmacological effects and are used as medications and recreational drugs [27]. Flavonoids enhance the effects of Vitamin C and function as antioxidants. They are also known to be biologically active against liver toxins, tumors, viruses and other microbes [28]. Plant terpenoids are used extensively for their aromatic qualities.

They play a role in traditional herbal sonedies and are under investigation for Antibacterial, Antineoplastic and other Pharmaceutical functions [29].

Table 1: Qualitative Phytochemical Screening of Ethanol and Aqueous Leaf Extract of *Moringa Oleifera*.

Solvents Used for Extraction	Alkaloid	Flavonoid	Saponin	Steroid	Tannin	Glycoside	Reducing Sugar	Volatile Oil
Ethanol	+	+	+	+	+	±	-	-
Water	+	+	+	+	±	±	-	-

Table 2: Antibacterial Activity of Ethanol and Aqueous Leaf Extract of *Moringa Oleifera*.

Name of Microorganism	Zone of Inhibition (mm)		Standard (Streptomycin)
	Water Extract	Ethanol Extract	
<i>Escherichia Coli</i>	-	8	6
<i>Staphylococcus aureus</i>	5	-	8
<i>Proteus vulgaris</i>	6	3	8
<i>Pseudomonas aeruginosa</i>	-	5	7
<i>Bacillus subtilis</i>	5	4	6
<i>Staphylococcus epidermidis</i>	1	3	5
<i>Streptococcus mutans</i>	2	6	7

Tannins have shown potential Antiviral, Antibacterial and Antiparasitic effects. Saponins cause hemolysis of red blood cells [30]. The antibacterial activity was screened because of their great medicinal properties towards the pathogenic organisms. The medicinal plant *Moringa Oleifera* showed good antibacterial activity against several organisms like *Staphylococcus aureus*, *Pseudomonas*, *Bacillus*, *Klebsiella*, and *E.coli* as supported by previous studies.

Conclusion

Moringa oleifera an important medicinal plant is one of the most widely cultivated species of the family *Moringaceae*. Pharmacologically reported that Different parts of it have been used for different human ailments, extracts showed varying degrees of antimicrobial and antifungal activity on the microorganism tested.

Antibacterial activity of *Moringa oleifera* extracts on bacterial isolates showed that *Moringa oleifera* leaf ethanol (MLE) extract had the broadest spectrum of activity on the test bacteria. The result exhibited that MLE had antimicrobial activity against five bacterial isolates. *P. aeruginosa* (05nm), *P.vulgaris* (03nm), *B. subtilis* (04 nm), *S. epidermidis*, (03nm) and *S. mutans* (06nm). *Moringa oleifera* is a good source of various phytochemicals like alkaloids, flavonoids, glycosides, saponins, and tannins. The antibacterial activity *Moringa oleifera* was clearly shown by the present study against various test organisms like *Escherichia Coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus vulgaris*, *Streptococcus mutans*, *Bacillus subtilis*, and *Staphylococcus epidermidis*.

Moringa oleifera leaves to treat common medical conditions but a few use it for preventing and treating malnutrition. Presence of phytochemicals indicates possible preventive and curative properties of *M. oleifera* leaves. Further work is needed

to carry out more pharmacological from the extracts in order to support antimicrobial activity of the *M. oleifera*. Our study demonstrated that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms to carry out more pharmacological studies to support the use of *M. oleifera* as a medicinal plant [31].

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