

PHYTOCHEMICAL ANALYSIS AND ANTIFUNGAL ACTIVITY OF *MORINGA OLEIFERA*

PINAL PATEL*¹, NIVEDITA PATEL¹, DHARA PATEL¹, SHARAV DESAI², DHANANJAY MESHAM¹

¹Department of Quality Assurance, Pioneer Pharmacy Degree College, Sayajipura, Vadodara, Gujarat, India, ²Department of pharmaceutical Microbiology and Biotechnology, Pioneer Pharmacy Degree College, Sayajipura, Vadodara, Gujarat, India.
Email: pinal020210@gmail.com

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ABSTRACT

Objective: The aim of the present study was to carried out phytochemical analysis of aqueous and ethanolic extract of *Moringa oleifera* and to find out antifungal property of *Moringa oleifera*.

Methods: *Moringa oleifera* leaf extracts was used for plant component analysis and for determination of antifungal activity. *Saccharomyces cerevisiae* (MTCC No.170), *Candida albicans* (MTCC No.183), *Candida tropicalis* (MTCC No.1000) strain were used for experimental purpose. Well diffusion method was used to assess the antifungal effect of the extracts on micro-organisms.

Results: The phytochemical screening indicated the presence of flavonoids, tannins, steroid, alkaloid, saponins etc., in the both extracts. Antifungal activity of ethanolic and aqueous extract of *Moringa oleifera* leaf was highly active against *Saccharomyces cerevisiae* and active against *Candida tropicalis* and not showing activity against *Candida albicans*.

Conclusion: The present study conclusively demonstrates that *Moringa oleifera* is a good source of various phytochemicals like alkaloids, flavonoids, carbohydrates, glycosides, saponins, tannins, Terpenoids. The antifungal activity *Moringa Oleifera* was clearly shown by the present study against various fungi like *Saccharomyces cerevisiae*, *Candida albicans* and *Candida tropicalis*. All these preliminary reports warrant an in depth analysis of the usefulness of *Moringa oleifera* as miracle drug against various ailments.

Keywords: Antifungal activity, *Moringa oleifera*, Phytochemical Screening

INTRODUCTION

Moringa oleifera is one of the species of 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 tropic and subtropics regions worldwide, the plant is referred to number of names such as horseradish tree, drumstick tree, ben oil tree, miracle tree, and "Mothers best friend" [1]. *Moringa oleifera* is commonly known as "Drumstick". It is a small or medium sized tree, about 10m height, found in the sub-Himalayan tract [2]. *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* plant provides a rich and rare combination of zeatin, quercetin, kaempferol and many other phytochemicals [4]. The leaves are outstanding as a source of vitamins A when raw as a source of vitamin C. They are also good sources of vitamin B and are among the best plant sources 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]. Leaves of this plant are reported to possess various biological activities, including hypocholesterolemic, antidiabetic, hypertensive agent and [8,9,10,11], regulate thyroid hormone [12], central nervous system, digestive system, nutrition and metabolism eye, ear nose throat genito-urinary system [13], to treat gastric ulcers [14] and scurvy [15]. Reports have also described the plant to be highly potent anti-inflammatory agent [16] and antitumour activity [17]. The plant has also been reported to be hepato protective against antitubercular drug such as isoniazid and rifampicin [18, 19]. *Moringa oleifera* is also being studied for its anti-inflammatory, antimicrobial, diuretic [20, 21, 22], antibiotic [23], hypotensive [10], and antimicrobial properties [24]. An immune enhancing polysaccharide [25] and niaziminin, having structural requirement to inhibit tumour promoter induced Epstein Barr virus activation have been reported from the leaves [17]. The alcoholic extract of leaves of *Moringa oleifera* were reported to have analgesic activity [26]. Traditionally, the plant is used as antispasmodic, stimulant, expectorant and diuretic [27]. *Moringa oleifera* is used as

drug many ayurvedic practitioners for the treatment of asthma and evaluate the anthelmintic activity of methanolic extract of *Moringa oleifera* in adult Indian earthworms *Pheretima posthuma* at different doses [28].

MATERIALS AND METHODS

Collection of plant materials

The experiment was conducted in the year 2013 in the college laboratory. Leaves were collected from the *Moringa oleifera* plant (Figure.1 A, B) from the herbal garden. 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.



Fig. 1(A): *Moringaoleifera*

Preparation of leaf extracts

Fresh leaves (20-30 gm) of *Moringa Oleifera* were shade dried at room temperature (32 – 35 °C) to constant weight over a period of 5 days. The dried leaves were ground into powdered using a mortar and pestle. 25 g of the powdered leaves were separately extracted in 500ml conical flasks with 90% ethanol (Ethanolic extraction) and water (Aqueous extraction). The conical flasks were plugged with rubber corks, then shaken at 120 rpm for 30 min and allowed to stand at room temperature for 5 days with occasional manual

agitation of the flask using a sterile glass rod at every 24 hour. The extracts were separately filtered using sterile Whatman no. 1 filter paper. These extracts (Ethanol and aqueous) were used in further process.



Fig. 1(B): *Moringa oleifera*

Phytochemical Analysis

Phytochemical analysis of extract for qualitative detection of alkaloids, flavonoids, steroid, volatile oil, glycoside, reducing sugar, tannins and saponins was performed by the extracts.

Alkaloids

Wagner's test-Drug solution + few drops of Wagner's reagent (dilute Iodine solution).

Dragendroff's test-Drug solution + Dragendroff's reagent (Potassium Bismuth Iodide).

Hager test-Drug solution + few drops of Hagers reagent (Saturated aq. Solution of Picric acid).

Mayer's Test: Drug solution + few drops of Mayer's reagent (K₂HgI₄).

Flavonoids

3ml of each extract was added to 10ml of distilled water and the solution was shaken. 1ml of 10% NaOH solution was added to the mixture.

Saponins

Frothing test - 3ml of each extract and dilute with 2ml of distilled water was added in a test tube. The mixture was shaken vigorously.

Steroids

Salkowski Test - 5 drops of concentrated H₂SO₄ were added to 1ml of each extract in a separate test tube.

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 were 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 *Saccharomyces cerevisiae*(MTCC No.170), *Candida albicans*(MTCC No.183), and *Candida tropicalis*(MTCC No.1000). The organisms were obtained from MTCC Chandigarh and maintain according to specification. Sub culturing was done at the interval of 15 days.

Determination of Antifungal Activity

The antifungal activity of the *Moringa oleifera* leaf extracts was determined using agar well diffusion method by following the known procedure. Small amount of diluted fungal suspension were poured over the media to spread uniformly on the surface. Later when the surface was little dried wells of 8mm were punched in the agar with stainless steel borer and filled with 300µl of plant extracts. Control wells containing neat solvents (negative control) were also run parallel in the same plate. The plates were incubated at 28°C for 72 hours and the antifungal activity was assessed by measuring the diameter of the zone of inhibition at the interval of every 24hrs. The antifungal activity of the different extracts were evaluated by comparing their zones of inhibition with standard antibiotic amphotericin B.

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: Antifungal activity of ethanol and aqueous leaf extract of *Moringa oleife*

Name of microorganism	Zone of inhibition ±SD (mm)		Standard
	Water extract	Ethanol extract	
<i>Saccharomyces cerevisiae</i>	12± 0.012	15 ± 0.013	15± 0.002
<i>Candida albicans</i>	-	-	6 ± 0.084
<i>Candida tropicalis</i>	5± 0.034	4 ± 0.0065	9 ± 0.069

RESULTS AND DISCUSSION

The present study reveals that *Moringa oleifera* plant shows the presence of phytochemical constituents like alkaloids, flavonoids, carbohydrates, glycosides, proteins, saponins, tannins and terpenoids in different solvent extracts as shown in Table 1. Antifungal activity of *Moringa oleifera* was studied against several fungi namely *Saccharomyces cerevisiae*, *Candida albicans* and *Candida tropicalis*. The ethanol and aqueous leaf extract showed maximum activity against *Saccharomyces cerevisiae* as shown in the Table No. 2. Figure 2 shows zone of inhibitions produced by ethanol

and water extract of *Moringa oleifera* against the *Saccharomyces cerevisiae* and *Candida tropicalis*. The largest zone of inhibition was produced by water and ethanol extract of *Moringa oleifera* against *Saccharomyces cerevisiae*.

Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms. They often have pharmacological effects and are used as medications and recreational drugs [29]. 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 [30]. Plant terpenoids are used

extensively for their aromatic qualities. They play a role in traditional herbal medicines and are under investigation for Antibacterial, Antineoplastic and other Pharmaceutical functions [31]. Tannins have shown potential Antiviral, Antibacterial and Antiparasitic effects. Saponins cause hemolysis of red blood cells[32]. The antifungal activity was screened because of their great medicinal properties towards the pathogenic organisms. The medicinal plant *Moringa Oleifera* showed good antifungal activity against several organisms like *Saccharomyces cerevisiae*, *Candida tropicalis* as supported by previous study.

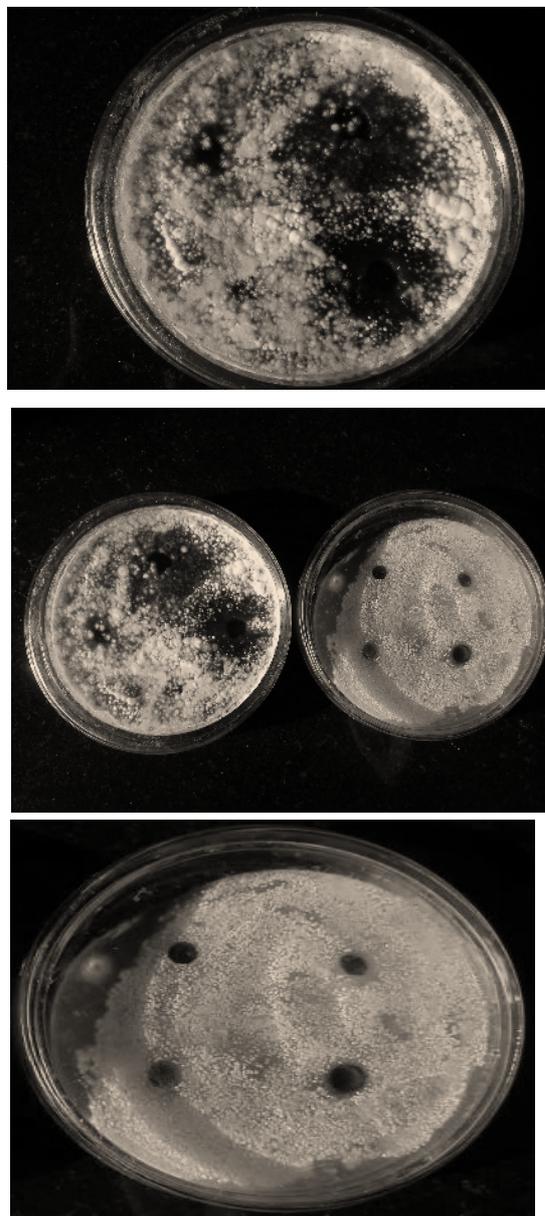


Fig. 2: Plates showing zone of inhibition of ethanol and aqueous leaf extract of *Moringa oleifera*

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