

Research Article

ANTIMICROBIAL, PHYTOCHEMICAL AND QUANTITATIVE HPLC ANALYSIS OF *MORINGA OLEIFERA* ROOT

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ABSTRACT

Objective: Phytochemical, in-vitro antibacterial activity, qualitative and quantitative phytochemical analysis of *Moringa oleifera*, belonging to family Moringaceae was taken to identify active compound. **Methods:** Dried root powder was extracted with ethanol and subjected to phytochemical analysis and identified phytoconstituents by HPLC and also using standards Gallic acids, benzoic acids, quercetin as well as their antimicrobial activity was tested on *Escherichia coli* and *Staphylococcus aureus*. **Results:** *Moringa oleifera* root extract (ethanolic) showed antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*. Qualitative and quantitative analysis detects the presence of phenolic compounds and flavonoids which confers the bioactivity to the extract. **Conclusion:** From the studies, we conclude that ethanol extract of *Moringa oleifera* root showed evidence that it could be a good therapeutic agent

Key words: *Moringa oleifera*, HPLC, antimicrobial activity, the phytochemical analysis.

INTRODUCTION

Moringa oleifera is found in India Africa, Himachal Pradesh, Pakistan and mostly all over tropic and subtropic regions plant have many alternative names such as drumstick tree, miracle tree and ben oil tree etc. [1]. *Moringa oleifera* is commonly known as "Drumstick". It is found in the Himalayas, small tree growing upto 10m height [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 tripinnate leaves and thick corky, whitish bark [3]. Phytochemicals such as Zeatin, quercetin, kaempferol were found in *Moringa oleifera* [4]. Minerals such as vitamin A, B and C are present in leaves [5]. Analgesic activity was also found in the alcoholic extract of *Moringa oleifera* [6]. The plant is used as anti-inflammatory, antimicrobial, diuretic antispasmodic. Moringine and Moringinine were The two alkaloids Moringinine and Moringine were isolated from the stem bark of *Moringa oleifera* (Lam.) [7]. Aqueous extract of root and bark reported postcoital anti-infertility and induced foetal resorption at late pregnancy in the rat. [8]. Leaves of *Moringa* showed antidiabetic activity in blood [9]. Retinoprotective effects of *Moringa oleifera* via antioxidant, anti-inflammatory, and anti-angiogenic mechanisms in streptozotocin-induced diabetic rats showed that it is a good therapeutic agent. [10].

MATERIALS AND METHODS

Collection of Plant Materials

Roots of *Moringa oleifera* were collected from the garden of the plant from the herbal garden of Maduravoyal Chennai. Roots were washed under running tap water to eliminate dust and other foreign particles. Dried it in shade and made it into corrosive. *Moringa oleifera* root was authenticated by Prof.P.Jayaramam, Director, Institute of Herbal Botany, Plant Anatomy and Research Centre, Chennai-45.

Preparation of root extracts

100 gram of *M. oleifera* were shade dried at room temperature (32 – 35 °C) to constant weight over a period of 15 days. By using mortar and pestle the dried roots were ground to a powdered form

and mixed with 200 ml ethanol in a conical flask. The conical flasks were plugged with rubber corks, then shaken at 120 rpm for 30 min and allowed to stand at room temperature for 5-6 days.

Phytochemical Analysis (Qualitative Analysis)

Tannins

3-4 drops of 10% ferric chloride solution was added to the portion of extract. The blue colour was observed for Gallic tannins and green colour indicates for catecholic tannins.

Reducing Sugars

To 0.5ml of plant extract, 1ml of water and 5-8 drops of Fehling's solution was added and heated over the water bath. Brick red precipitate indicates the presence of reducing sugars.

Glycosides

25ml of dilute sulphuric acid was added to 5ml extract in a test tube and boiled for 15 minutes, cool and neutralized with 10%NaOH, then 5ml of Fehling solution added. Glycosides are indicated by a brick red precipitate.

Alkaloids

2ml of the extract was measured in a test tube to which picric acid solution was added. An orange colouration indicated the presence of alkaloids.

Flavonoids

4ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid were added and the red colour was observed for flavonoids and orange colour for flavones.

Terpenoids

Four milligrams of the extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then the concentrated solution

of sulphuric acid was added slowly and the red violet colour was observed for terpenoid.

Volatile Oil

2ml of the extract was shaken with 0.1ml dilute NaOH and a small quantity of dilute HCl. A white precipitate is formed if volatile oils are present.

Saponins

Saponins were detected using the froth test. 1g of the sample was weighed into a conical flask in which 10ml of sterile distilled water was added and boiled for 5 minutes. The mixture was filtered and 2.5 ml of the filtrate was added to 10ml of sterile distilled water in a test tube. The test tube was stoppered and shaken vigorously for about 30 seconds. It was then allowed to stand for an hour. Honeycomb froth indicated the presence of saponins. The appearance of a greenish yellow colour, confirms the presence of phenol.

Determination of Antimicrobial activity

Anti-microbial effect of *Moringa oleifera* was determined against *Escherichia coli* and *Staphylococcus aureus*. These organisms were collected from the microbiology department of Dr.MGR Medical University, Chennai. The prepared ethanolic extracts of the roots of *Moringa oleifera* were tested for antimicrobial activity against the test organism using the agar diffusion method of [11]. Muller Hinton agar was inoculated with the given microorganisms by spreading the bacterial inoculums on the media. Wells of 5mm was punched in the agar using sterile borer [12]. and filled with plant extracts. Control wells containing neat solvents (negative control) were also run parallel 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.

Phytochemical Analysis through HPLC

Content of Flavonoids

Extraction solvent— Prepare a mixture of alcohol, water, and hydrochloric acid (50:20:8).

Mobile phase— Prepare a mixture of methanol, water, and phosphoric acid (100:100:1). Make adjustments if necessary (see System Suitability under Chromatography á 621 ñ).

Standard solutions— Transfer accurately weighed quantities of USP Quercetin RS, kaempferol, and isorhamnetin to separate volumetric flasks, dissolve each in methanol, and dilute quantitatively, and stepwise if necessary, with methanol to obtain Standard solutions 1 mg per mL, respectively.

Test solution— Transfer about 10.0 g of the sample given finely powdered and accurately weighed, to a 250-mL flask fitted with a reflux condenser. Add 78 mL of Extraction solvent, and reflux on a hot water bath for 135 minutes.

Allow cooling at room temperature. Decant to a 100-mL volumetric flask. Add 20 mL of methanol to the 250-mL flask, and sonicate for 30 minutes. Filter, collect the filtrate in the 100-mL volumetric flask, wash the residue on the filter with methanol, collect the washing in the same 100-mL volumetric flask, dilute to volume, and mix.

Chromatographic system (see Chromatography á 621 ñ) — The liquid chromatograph is equipped with a 270-nm detector and a 4.6-mm × 25-cm column that contains packing L1. The flow rate is about 1.5 mL per minute. Chromatograph Standard solution 1, and record the peak responses

Procedure— Separately inject equal volumes (about 20 µL) of each of the Standard solutions and the Test solution into the chromatograph, record the chromatograms, and measure the areas for the major peaks. Calculate the percentage of each flavonoids in sample

Content of total phenols— Solvent, Solution A, Solution B, Mobile phase, Standard solution 2, and Chromatographic system— Proceed as directed for Content of total phenols under Echinacea

Angustifolia. Standard solution 1— Dissolve an accurately weighed quantity of USP Powdered Echinacea purpurea Extract RS in Solvent, shaking for 1 minute, and dilute with Solvent to obtain a solution having a known concentration of about 5 mg per mL. Pass through a membrane filter having a 0.45-µm or finer porosity.

Test solution— Transfer about 60 mg of Powdered Extract, accurately weighed, to a 50-ml centrifuge tube. Add 25 mL of Solvent, and shake by mechanical means for 15 minutes. Centrifuge, or pass through a membrane filter having a 0.45-µm or finer porosity.

RESULTS AND DISCUSSION

Table 1 showed the presence of phytochemical constituents like flavonoids, carbohydrates, glycosides, saponins, tannins in ethanolic extracts of *Moringa oleifera* root. It also possesses antimicrobial activity and studied against *E.Coli* and *Staphylococcus aureus*. Ethanolic extract showed maximum activity against *E.coli* as shown in Table No: 4. Figure 4 and Figure 5 shows the zone of inhibitions produced by ethanolic root extract. Hence it could be an excellent herbal medicine against bacteria, microbes and used in pharma companies [13]. Tannins have shown potential antiviral, antibacterial and antiparasitic effects. Saponins cause hemolysis of red blood cells [14]. The effects of Vitamin C was enhanced by flavonoids and it functions as good antioxidants. They are also known to be biologically active against liver toxins, tumours, viruses and other microbes

HPLC analysis of the ethanolic extract of *Moringa oleifera* is done. The HPLC chromatograph will help as standard chromatogram in future studies, comparing the retention time of isolated compounds with given literature. The good separation of the peaks which could be identified in the chromatogram, as flavonoid (quercetin)(RT=4.137) kaempferol (RT=10.507) and phenolic compounds, gallic acid (RT=2.663) and benzoic acid (RT=3.747). Both Quercetin and kaempferol are flavonoids. Quercetin is found in fruits like grapes, red wine etc. It helps to inhibit the oxidation of other molecules and scavenge free radicals that cause oxidative chain reactions. Kaempferol is another natural flavonoid found in apples, onions, grapes and citrus fruits. It also scavenges free radicals and an anticancer agent. Further research has to be done to make powder form of moringa root in tablet form so that it could be used as a good natural antioxidant.

CONCLUSION

Medicinal properties of *Moringa oleifera* was studied and HPLC chromatograph reveals the presence of compounds isolated and can be used as a fingerprint.

Table 1: Phytochemical screening of ethanolic extract of by *Moringa oleifera* root

CONSTITUENTS	RESULTS
Flavonoids	++
Saponins	++
Steroids	++
Tannins	++
Glycosides	++
Reducing Sugars	--
Volatile Oil	--

++ Presence, Absent --

The curative properties of medicinal plants are due to the presence of various secondary metabolites such as Flavonoids, steroids, Tannins Glycosides, Tannins etc. The successive extraction of *Moringa oleifera* in ethanolic solvent system revealed the presence of Flavonoids, steroids, Tannins Glycosides, Tannins etc. as shown in Table 1

Table 2

Compound	Retention Time
Quercetin	4.137
Kamferol	10.507
Rutin	15.137

Luteolin	38.713
Galagin	52.903

Table 3

Compound	Retention Time
Gallic acid	2.663
Benzoic acid	3.747

Table 4: Quantification of phytochemicals for 1 ml of ethanolic root extract of *Moringa oleifera*

Compound	Weight
Tannins	3.24
Glucosides	9.45
Steroids	2.11
Quinones	0.34
Alkaloids	7.35
Saponins	12.98
Quercetin	1.04
Kamferol	0.025
Gallic acid	0.544
Benzoic acid	0.468

Table 5: Antimicrobial activity of *Moringa oleifera*

Microorganisms	Zone of Inhibition (mm) <i>Moringa oleifera</i>
<i>E. coli</i>	35
<i>S. aureus</i>	15



Fig. 1: Fine powder of *Moringa oleifera* root and *Moringa oleifera* root

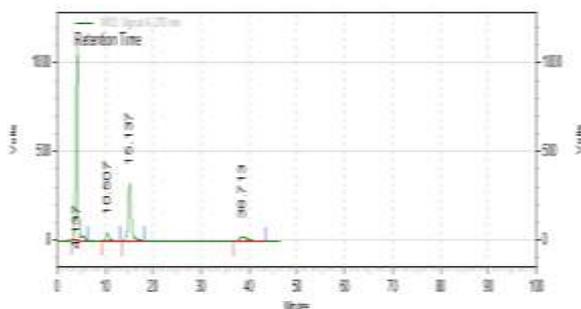


Fig. 2: HPLC Chromatogram of flavonoid (Quercetin)

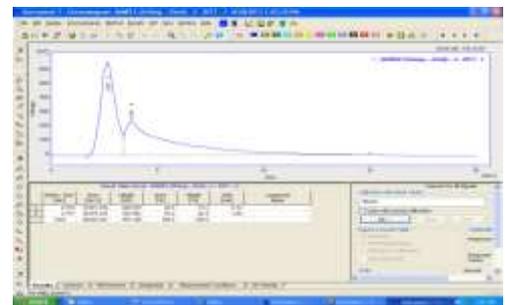
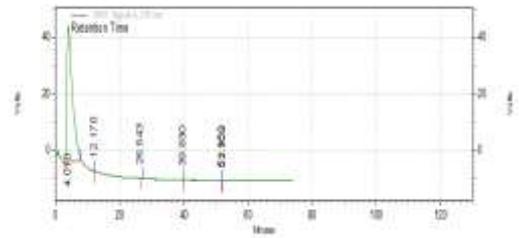


Fig. 3: HPLC Chromatogram of Phenols

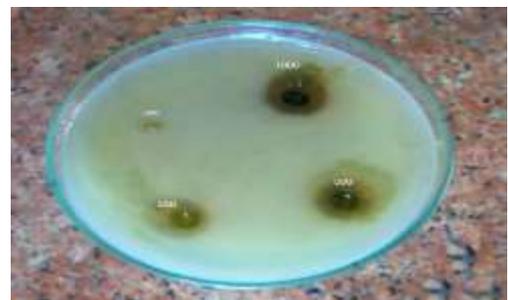


Fig 4. Antimicrobial Activity of *Moringa oleifera* against *E. coli*

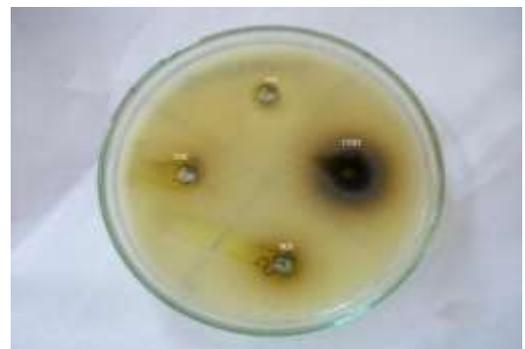


Fig. 5: Antimicrobial Activity of *Moringa oleifera* against *S. aureus*

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