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# Phytochemical screening of *Moringa oleifera* Lam

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**Abstract**
The present study is aimed at the development of phytochemical parameters and to investigate the medicinally active substances present in methanolic extract obtained from leaves of *Moringa oleifera* Lam. Preliminary phytochemical screening of the extracts revealed the presence of alkaloids, terpenoids, steroids, flavanoids, tannins, carbohydrates, saponins and phenolic compounds. For the TLC new solvent system developed for the best separation of the phytoconstituents present in the extract. The solvent system selected for the best results of TLC was the ratio of Toulene: Aceteic acid: Formic acid 5 : 3.5 : 0.5 ml for methanolic extract. The five spots found in the methanolic extract. The R*f* values of the extract were 0.08, 0.39, 0.44, 0.7, and 0.73.The study will provide referential information for the correct identification of the methanol crude extract of *Moringa oleifera* Lam.

**Keywords:** *Moringa oleifera* Lam, phytochemical screening and TLC

## Introduction
The intensity of life-threatening microbe infections brought on by pathogenic microbes has elevated world-wide and it is becoming a significant cause of morbidity and mortality within immune sacrificed affected individuals within acquiring places [1]. Totally free radicals play a vital function in the pathogenesis involving various human diseases, for instance cancers, rheumatoid arthritis, as well as cardiovascular diseases [2]. Natural antioxidants contained in foods from plant origin involving control most of these radicals and so are therefore essential methods inside acquiring as well as preserving a healthy body [3-4]. The "Moringa" tree is considered one of the world's most useful trees, as almost every part of the Moringa tree can be used for food or has some other beneficial properties [5]. *Moringa oleifera* L. also known as drumstick in India belongs to family Moringaceae is a well-documented world renowned plant herb for its extraordinary nutritional and medicinal properties. It is a natural antihelmintic, antibiotic, detoxifier, outstanding immune builder and is used in many countries to treat malnutrition and malaria. Moringa leaves are known to have a high content ofs essential amino acids, proteins, minerals and vitamins, hence an ideal nutritional supplement [6, 7]. Many evidences exposed in which *Moringa oleifera* Lam experienced several drug actions including antibacterial [8], antifungal, anti-inflammatory and diuretic activities [9]. The antioxidant activity of various extracts of *Moringa oleifera* Lam leaf has been reported by several other authors [10-12].

## Material and Methods
The Plant material *Moringa oleifera* L. was collected from the road sides of Alirajpur District M.P. and major quantity of the plant material was collected from a fields of local village of Alirajpur namely Udaigarh some about 45 kms from Alirajpur in the month of December – January, 2017. The plant was then identified and authenticated by Dr. Jagrati Tripathi, Asst. Prof. of Botany Govt. L.B.S. College Sironj Vidisha, (M.P.) and Specimen Voucher No. 267 and the specimen was put in the P.G. Department of Chemistry Govt. Geetanjali Girls Autonomous P.G. College, Bhopal Madhya Pradesh.

**Scientific Classification of *Moringa oleifera* L.**
**Kingdom:** *Plantae*
**Division:** Magnoliophyta
**Class:** Magnoliopsida
**Order:** Brassicales
**Family:** Moringaceae
**Genus:** *Moringa*
**Species:** *oleifera*
**Botanical name:** *Moringa oleifera*

**Fig 1:** Showing the plant material *Moringa oleifera* L.

**Extraction and Preliminary Phytochemical Screening**
*Preparation of Crude Methanol Extract:* The dried plant leaves of *Moringa oleifera* Lam. was grounded by electrical grinder. Till the fine powder in a mixer grinder and weighed accurately. The powdered material was subjected to solvent extraction with methanol by Soxhlet apparatus at room temperature for 48 hours. The resulting mixture was filtered and evaporated in a shaker water-bath; temperature maintained at 55-65 $^0$C the obtained dried crude extract was used for phytochemical analysis.

***Phytochemical evaluation:*** The phytochemical evaluation of the plant is carried out by testing of different class of compounds using standard methods [13].
The preliminary phytochemical investigations were carried out with the methanolic extract of *Moringa oleifera* Lam. Leaves of plant for qualitative identification of phytochemical constituents using standard conventional protocol. All the chemicals and reagents used were of analytical grade [14] (Table 1).

**Tests for Alkaloids**
**Mayer's Reagent Test:** 1.36 gm of mercuric chloride and 3 gm of potassium iodide were dissolved in water to make 100 ml. To a little of each extract taken in dilute hydrochloric acid in a watch glass, few drops of the reagent was added, formation of cream coloured precipitate shows the presence of alkaloid.

**Hager's Reagent Test:** It is a saturated solution of picric acid in water. When the test filtrate was treated with this reagent, yellow precipitate was obtained indicating the presence of alkaloids.

**Wagner's Reagent Test:** It is a solution of potassium triiodide in water which was prepared by dissolving 1.3 gm iodine in a solution of potassium iodide (2 gm) in water to make 100 ml. Formation of brown precipitate after addition of this reagent in extract indicates the presence of alkaloids.

**Test for Flavonoids**
**Shinoda Test:** Crude extract was mixed with few fragments of magnesium ribbons and conc. hydrochloric acid was added drop wise. Pink scarlet color appears after few minutes, indicated the presence of flavonoids.

**Zinc Hydrochloride Test:** To the test solution add a mixture of Zinc dust and conc. hydrochloric acid. It gives red color after few minutes confirms flavonoids.

**Tests for Carbohydrates**
**Molish test:** About 0.1 gm of the sample was dissolved in 2 ml of water and added 2-3 drops of 1% ethanolic solution of alpha napthol and then carefully poured 2 ml of concentrated sulphuric acid down the side of the test tube so that it forms a heavy layer at the bottom. A deep violet colour is produced if carbohydrates are present.

**Fehling's Test:** 1 ml of Fehling's A and 1 ml of Fehling's B solutions were mixed and boiled for 1 minute. Equal volume of sample was then added, heated in boiling water bath for 5-10 minutes. First a yellow then brick red color shows the presence of carbohydrates.

**Test for Saponins**
**Foam Test:** About 2 gm of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously. Persistent froth indicated the presence of saponins.

**Hemolytic Test:** Sample was added to one drop of blood placed on glass slide. Hemolytic zone indicated the presence of saponins.

**Test for Tannins:** Sample was taken separately in water, warmed and filtered. Tests were carried out with the filtrate using following reagents;

**$FeCl_3$ Solution Test:** A 5% w/v solution of ferric chloride in 90% alcohol was prepared. Few drops of this solution were added to a little of the above filtrate. Dark green or deep blue color shows the presence of tannins.

**Lead acetate Test:** A 10% w/v solution of basic lead acetate in distilled water was added to the test filtrate. Precipitate indicates the presence of tannins.

**Test for Amino acids**

**Ninhydrin test:** To the 3 ml of crude sample 3 drops 5% ninhydrin was mixed and heated for 10min in boiling water bath. Purple or bluish colour indicated presence of amino acids.

**Test for anthraquinone glycosides**

**Borntrager's Test:** To the 3ml of the sample, dilute sulphuric acid was added, boiled and flittered. To the filtrate equal volume of chloroform was added and shaken. After separating the organic layer, ammonia was added. Turning pink of ammonical layer indicates the presence of said glycosides.

**Tests for sterol/steroids**

**Salkowski Test:** Few mg of the sample was taken in 2 ml of chloroform and 2 ml of concentrated sulphuric acid and shaken. The development of red colour in the chloroform layer indicates the presence of sterols/steroids.

**Test for Terpenoids**

**Liebermann–Burchard Test:** Few mg of the sample was dissolved in 1ml of chloroform and few drops of acetic anhydride. Concentrated sulphuric acid was added by the side of the test tube. Production of purple color indicates the presence of triterpenoids and blue–green color indicates the presence of sterols.

**Test for Proteins**

**Biuret Test:** To the sample 4% NaOH and few drops of 1% $CuSO_4$ were added. Violet or pink colour indicates the presence of proteins.

**Xanthoproteic Test:** Sample was mixed with 1ml of concentrated sulphuric acid, formation of precipitate shows positive test.

**Millon's Test:** 3ml of sample was mixed with Millon's reagent, formation of precipitate indicates the presence of proteins.

**Separation of Chemical Constituents**

The purity of each eluted sample was tested by using TLC method. It is a technique used to separate wide range of compounds of biochemical interest. It can be utilized to quantitative as well as qualitative (Stahl, 1965). The methanol extract was subjected to thin layer chromatography about 0.1-0.2 ml of conc. Methanolic extract was loaded on the plate by using capillary tube. During spotted plates were carefully dried and used for elution purpose. Initially various solvents such as benzene, pet ether, chloroform ethanol were tested alone. Later different combinations of solvents were tested depending on polarity basis. The spotting was done at the center of plate three spots were appeared on the plate. The spotting plate was carefully dried and used for elution purpose. Different solvent systems ranging from lower polarities to higher polarities were tested for the separation of bioactive components. The TLC plates were observed under UV light and the separated spots were marked [15, 16]. The R*f* values of cleared sport were calculated & proper solvent system was identified R*f* values determined are shown in Table 2.

*The Column Chromatography:* 50 ml of concentrated petroleum ether were dissolved in 10 ml of benzene. The activated silica gel H is added slowly to benzene solution and absorbs pet ether extract. The chromatograms are allowed to develop Elution was started after, the formation of complete bands and it was adjusted to 12-15 drops per mm. Nearly 10 ml of eluted solvent was collected in a clean bottle of 50 ml capacity and was labeled by given number 6. [17]

**Table 1:** Preliminary Phytochemical screening of Extract of leaves of *Moringa oleifera* Lam.

| S. No. | Tests | Plant type | Observation for extracts | |
|---|---|---|---|---|
| | | | Petroleum Ether | Methanol |
| 1 | **Carbohydrates** | | | |
| 1.1 | Molish test | *M. oleifera* | Negative | Positive |
| 1.2 | Fehling | *M. oleifera* | Negative | Negative |
| 1.3 | Barfoed's Test | *M. oleifera* | Negative | Positive |
| 2. | **Proteins and amino acids** | | | |
| 2.1 | Biuret's test | *M. oleifera* | Negative | Negative |
| 2.2 | Ninhydrin test | *M. oleifera* | Negative | Negative |
| 3 | **Glycosides** | | | |
| 3.1 | Legal's test | *M. oleifera* | Positive | Positive |
| 3.2 | Keller-Killani test | *M. oleifera* | | Positive |
| 4 | **Saponins** | | | |
| 4.1 | Froth test | *M. oleifera* | Negative | Positive |
| 5 | **Alkaloids** | | | |
| 5.1 | Mayer's test | *M. oleifera* | Negative | Positive |
| 5.2 | Hager's test | *M. oleifera* | Negative | Positive |
| 5.3 | Wagner's test | *M. oleifera* | | Positive |
| 6 | **Flavonoids** | | | |
| 6.1 | Lead acetate test | *M. oleifera* | Positive | Positive |
| 6.2 | Alkaline reagent test | *M. oleifera* | Positive | Positive |
| 7 | **Triterpenoids and Steroids** | | | |
| 7.1 | Libermann- burchard,s test | *M. oleifera* | Positive | Negative |
| 7.2 | Salkowski,s test | *M. oleifera* | | Positive |
| 8 | **Tannins and Phenols** | | | |
| 8.1 | Ferric chloride test | *M. oleifera* | Positive | Positive |
| 8.2 | Lead acetate test | *M. oleifera* | Positive | Positive |
| 8.3 | Gelatin test | *M. oleifera* | | Positive |

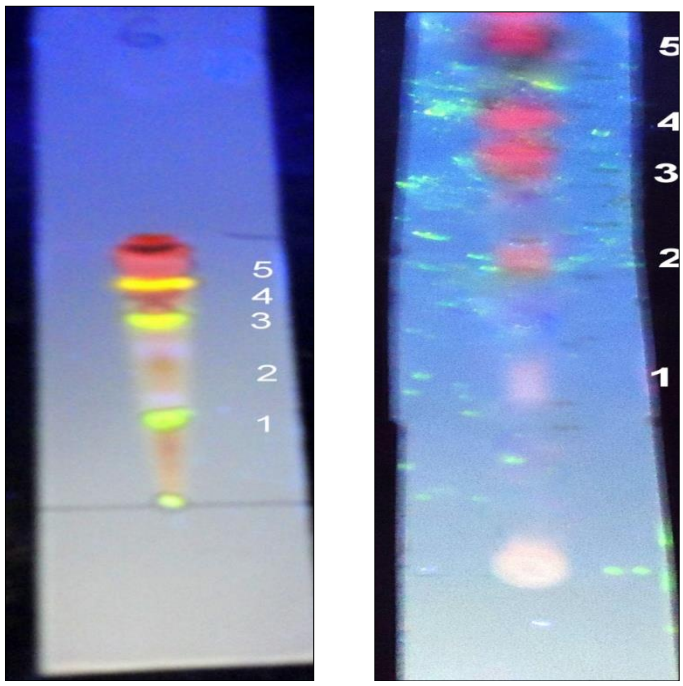
**Table 2:** Showing *Rf* values of methanolic extract of *Moringa oleifera* L.

| Solvent System | Spot No. | R*f* Values | Colors of Peaks |
|---|---|---|---|
| Toulene : Aceteic acid : Formic acid (5 : 3.5 : 0.5) | 1 | 0.08 | Blue |
| | 2 | 0.39 | Yellow |
| | 3 | 0.44 | Brown |
| | 4 | 0.7 | Green |
| | 5 | 0.73 | Green |

Stationary Phase: Silica gel. 60-120 mesh size (Merk).

**Results and Discussions**

Table (1) show the results of preliminary phytochemical screening of methanol extract leaves of *Moringa oleifera* Lam of shows that the leaves extract was rich in chemical know as Phytoconstituents, such as terpenoids, tannins, saponins and steroids petroleum ether extract was subjected to TLC in order of separate and identify the bioactive compounds present in the leaves of methanolic extract of *Moringa oleifera* Lam. In the present study the most suitable TCL system for analysis was shown to be alkaloids, carbohydrates, glycosides, terpenoids, saponins, tannins and steroids with the largest discriminating power TLC plates shown in the fluoroscence light under UV at 254-365 nm wave length and find these active spots in TLC plate with following $R_f$ values (0.08, 0.39, 0.44, 0.7 and 0.73).




1. TLC of Methanol fraction 2. TLC of ethyl acetate fraction

**Fig 2:** Showing TLC plate of Methanol extract

**Conclusion**

In the present study the methanolic leaves extracts obtained through solvent extraction by Soxhlet apparatus. The *Moringa oleifera* Lam plant have been raw material for the synthesis of many drugs and thus remain an important source of new therapeutic agent. It is found that of *Moringa oleifera* Lam has a beneficial effect on the learning and memory process in mentally retarded children. The methanolic extract obtained from *Moringa oleifera* Lam. Though successive solvent extraction in order of prove that the ethno pharmacological applications of the plant in Indian folk medicines. Phytochemical screening of *Moringa oleifera* Lam. Preliminary and important aspect. It is concluded from the data that methanolic extracts of *Moringa oleifera* Lam. leaves exhibited significant role in medicinal chemistry for formulation of life saving drugs.

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