

Thin Layer Chromatography (TLC) and Phytochemical Analysis of Moringa Oleifera Methanol, Ethanol, Water and Ethyl Acetate Extracts

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Abstract

Many medicinal plants were studied for their phytochemical contents. Moringa oleifera is one of these plants. It is widely distributed in tropical and subtropical areas. It has an important medical uses with high nutritional value. This study, primarily aimed to carry out a preliminary phytochemical screening to detect the major classes of bioactive compounds presented in Moringa oleifera dry leaves. Many solvents (Methanol, ethanol, ethanol acetate and water) used to determine the best solvent can be used for extraction and to perform thin layer chromatography (TLC) profiling of all sequential extracts. TLC was carried out in silica gel plates using many mobile phase protocols, chloroform: methanol: ethanol (1:1:1), (2:2:0.5) and Chloroform: Glacial acetic acid: methanol (4:5:1) and use four mentioned solvents. Phytochemical screening of four extracts showed the presence of alkaloids, flavonoids, tannins, and phenols in all solvent extracts. However, the concentration of these compounds is more with ethanol and ethanol acetate extracts (+++). As well as thin layer chromatographic studies of the Moringa olifera leave extract (methanol, ethanol, ethanol acetate and water) constituted different colored phytochemical compounds with different Rf values. Methanol and ethyl acetate extracts were the best which show 4 bands with Rf less than 1 while with ethanol extract 5 bands with Rf less than 1 and with water extract no band appeared by using chloroform: methanol: ethanol (1:1:1). Furthermore with the other mobile phases detect fewer bands with different solvent extracts. The results obtained in the present study revealed that the concentration of the bioactive compounds of Moringa oleifera leaves extracts by using ethanol and ethyl acetate as a solvent is more than the other solvents. On the other hand, TLC showed 4 bands with better Rf by using methanol and ethyl acetate extract. Conclusion, the methanol and ethyl acetate extracts for Moringa oleifer leaves contain a higher content of bioactive compounds, which can be used for further researches on this plant.

Keywords: Moringa oleifera Thin Layer.

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INTRODUCTION

For thousands of years the nature is the best origin for the traditional agents [1]. About 20% of known plants have been used in pharmaceutical drug discovery and study their effectiveness of the bioactive compounds on the health care system such as harmful chronic diseases and cancer [2]. Moringa is a medicinal plant, which grow in tropical area and used as human food, medicine and in oil production [3]. The Thin layer chromatography (TLC) method is an important analytical tool for the separation, identification and estimation of different classes of bioactive compounds

[4]. The leaves of Moriga Oleifera, in particular, have been found to contain phenolics and flavonoids [5]. These compounds have various biological properties, such as antioxidant, anticarcinogenic, immunomodulatory, antidiabetic, antiatherogenic, and hepatoprotective functions [6]. These phytoconstituents give special characteristics and properties to plants. Therefore, the analysis of these bioactive compuonds in plants would help in determining various biological activities of plants [7]. Although, the biological importance of Moringa Oliefera has been learned additional knowledge still secured. So, in recent years,

more attention has been pointed towards identification of plants with antioxidant ability which could be used for human consumption [8]. These phytoconstituents confer specific characteristics and properties to plants. Although, much has been learned about the biological aspects of *M. oleifera* additional knowledge remains to be secured. Therefore, in recent years; considerable attention has been directed towards identification of plants with antioxidant ability that may be used for human consumption. Thus, the aims of present study are to investigate the phytochemical profile and thin layer chromatography (TLC) studies of *M. oleifera* leaves grown in Libya by using many types of solvents so as to know various constituents present in it and hence to evaluate the medicinal potential of the plant and justify its traditional use.

MATERIALS AND METHODS

Procurement of Experimental Plant

The experimental plant *M. oleifera* was collected from a farm at Tripoli Libya *Moringa Oleifera* leaves was healthy and uninfected. The leaves were air dried at room temperature and grinded in powder form [9]. 5gm of the powdered leaves were separately extract with 4 type of solvents 50 ml each

Sequential Extraction of *M. oleifera* leaves

The dried *M. oleifera* leaves were powdered with the help of mixer grinder. The powdered leaves were then extracted by using sequential solvents that were pet methanol, ethanol, ethyl acetate and water for 24 h. The extract was separately filtered using Whitman's no1 filter paper.

Preliminary Phytochemical Screening of Successive

Extracts of *M. oleifera* Pods

Qualitative phytochemical analysis of *M. oleifera* leaves was carried out using standard procedures to identify the constituents, Alkaloids, Flavonoids, Tannin and Phenol as described by [10].

Test for alkaloids

1% of HCL prepared and added to the *Moringa Oleifera* extract in test tube and heat it for 20min with gently shake then leave it to cool a bit. Take 1 ml of the extract and added few drops of Wagner's reagent notice a creamy brown indicate the presence of Alkaloids.

Test for flavonoids

3 ml of *Moringa Oleifera* extract added to 10ml of distilled water and mix it well notice a yellow color indicate the presence of Flavonoid.

Test for tannins

2ml of *Moringa Oleifera* extract and put it in test tube and gently heat it for 2min .added 3 drops of Ferric chloride notice orange color indicate the presence of Tannin.

Test for phenols

3ml of *Moringa Oleifera* extract added to 5ml distilled water added few drops of 5% Ferric chloride notice dark green indicate the presence of phenol.

Chromatographic purification

TLC was carried out to isolate the principle components that were presents in most effective extracts of plants the TLC was used by different solvent systems

Solvent phase

Chloroform: methanol :ethanol (1:1:1), Chloroform: methanol: ethanol (2:2:0.5) Chloroform : Glacial acetic acid :methanol (4:5:1) [11].

Methods

The plant extract were with 4 different solvent each extract applied on – pre coated TLC plate by using capillary tubes. Drawing a light line on the plate and dots to know the place of each extract applied on the plate. After using each mobile phase the TLC plate were air dried and observed under ultra violet light. They were later sprayed with iodine vapours for the development of the separated bands the movement was expressed by its retention factor (R_f) values were calculated for different sample.

$$R_f = \frac{\text{Distance traveled by the solute}}{\text{Distance traveled by the solute front TLC plate}}$$

Analyte detection

After drying the plate and exposed with iodine vapours all plates were visualized with the help of UV and all different spots that were observed was calculated.

RESULT

Phytochemical Screening of Sequential Extracts of *M. oleifera* leaves

Phytochemical screening of the sequential extract of *M. oleifera* leaves showed the presence of various bioactive components of which phenol, alkaloids, flavonoids and tannin are the most prominent

components and the result of phytochemical test is presented in Table 1. Among these phytochemical tests, ethanol and ethyl acetate extract were found to contain maximum of alkaloids, flavonoids, tannin and phenol in

comparison with other solvents. All these phytochemicals possess good antioxidant activities and has been reported to exhibit multiple biological effects including anti-inflammatory and antitumor activities.

Table-1: Qualitative phytochemical screening of sequential extracts of *Moringa oleifera* leaves

Solvent used	Alkaloids	Flavonoids	Tannin	Phenol
Methanol	++	+++	++	+++
Ethanol	+++	+++	+++	+++
Ethyl acetate	+++	+++	+++	+++
Water	++	+++	+++	+++

Chromatographic Purification: TLC

Chloroform: Methanol: Ethanol (1:1:1) TLC of pet methanol extract of *M. oleifera* leaves revealed the presence of 4 compounds having R_f values of 0.01, 0.89, 0.92 and 0.93 respectively when a solvent phase of was used. With ethanol extract by same solvent showed 5 bands having R_f values of 0.046, 0.66, 0.83, 0.90 0.93 respectively. With ethyl acetate extract shows 4 bands having R_f of 0.046, 0.50, 0.56, 0.89. However, with water extract shows no bands.

Chloroform: methanol: ethanol (2:2:0.5) Methanol extract shows 3 bands having R_f values of 0.090, 0.090, 0.81. With ethanol extract shows 3 bands having R_f values of 0.090, 0.8, 0.81. With ethyl acetate shows no bands with water extract show 2 bands having R_f values of 0.63, 0.76

Chloroform: Glacial acetic acid: methanol (4:5:1) with methanol extract shows 2 bands having R_f values of 0.45, 0.78. while, Ethanol extract show no bands and with ethyl acetate extract shows 3 bands having R_f values of 0.4, 0.76, 0.83. However with water extract show no bands

DISCUSSION

By using the qualitative phytochemical screening of plant extracts the chemical constituents can be provided for pharmacological and pathological discovery of the pharmaceutical agents [11]. In the present study, qualitative tests for all four extracts showed significant indication about the presence of metabolites. Alkaloids, phenols, tannins and Flavonoids were found to be present in the all the sequential extracts of *M. oleifera* leaves, with a maximum concentration by using ethanol and ethyl acetate solvents. TLC profiling of all 4 extracts gives an impressive result that directing towards the presence of number of phytochemicals. Various phytochemicals gives different R_f values in different solvent system. This variation in R_f values of the phytochemicals provides a very important clue in understanding of their polarity and also helps in selection of appropriate solvent system for separation of pure compounds by column chromatography. Mixture of solvents with variable polarity in different ratio can be used for separation of pure compound from plant extract. The

selection of appropriate solvent system for a particular plant extracts can only be achieved by analyzing the R_f values of compounds in different solvent system [8, 12]. Different R_f values of the compound also reflect an idea about their polarity. This result will help in selection of appropriate solvent system for further separation of compound from these plant extracts.

CONCLUSION

The results revealed that *M. oleifera* leaves as a rich source of bioactive compounds. These findings suggested that *M. oleifera* leaves have a potential source of natural antioxidant which have great importance as therapeutic agent for many chronic diseases. In addition, the methanol and ethyl acetate extracts for *Moringa oleifera* leaves contain a higher content of bioactive compounds, which can be used for further researches on this plant.

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