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

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Abstract

The phytochemical composition of several medicinal plants has been examined. One of these plants is Moringa oleifera. It is widespread in tropical and subtropical regions. It has vital medical applications as well as a high nutritional value. The primary goal of this work was to conduct a preliminary phytochemical screening to determine the key classes of bioactive chemicals found in Moringa oleifera dry leaves. Many solvents (Methanol, ethanol, ethanol acetate, and water) utilized to select the optimal solvent can be employed for extraction and TLC profiling of all sequential extracts. TLC was performed in silica gel plates using various mobile phase methods, including chloroform: methanol: ethanol (1:1:1), (2:2:0.5), and chloroform: glacial acetic acid: methanol (4:5:1) chloroform:methanol:ethyl acetate(1:1:1), trichloro acetic acid:ethyl acetate:methanol(1:7.5:1.5). All solvent extracts contained alkaloids, flavonoids, tannins, and phenols, according to phytochemical analysis of four extracts. The concentration of these chemicals, however, is higher in ethanol and ethanol acetate extracts (+++).Thin layer chromatographic investigations of Moringa olifera leaf extract (methanol, ethanol, ethanol acetate, and water) revealed many colored phytochemical substances with varying Rf values. Methanol and ethyl acetate extracts performed best, yielding four bands with Rf less than one, whereas ethanol extract yielded five bands with Rf less than one and water extract yielded none.

Furthermore, with various solvent extracts, the other mobile phases detect less bands. The current study found that employing ethanol and ethyl acetate as solvents increased the content of bioactive components in Moringa oleifera leaf extracts. The solvent is more expensive than the other solvents. TLC, on the other hand, revealed four bands with higher Rf when methanol was used. Extract of ethyl acetate. In conclusion, methanol and ethyl

acetate extracts of Moringa oleifer leaves possess a greater concentration ofBioactive component content, which can be employed in future research on this plant.

Key words -TLC, M.oleferia, phytochemical analysis,RF value.

INTRODUCTION

The best source for conventional agents has been nature for thousands of years [1]. Approximately 20% of all plants have been used in research on pharmaceutical medication discovery and the impact of bioactive chemicals on diseases like cancer and harmful chronic illnesses on the health care system [2]. A tropical plant known for its therapeutic properties, moringa is used to make oil, medicine, and food for humans [3].

Thin layer chromatography (TLC) is an important analytical technology for separation, identification, and quantification. estimate of several phenolics and flavonoids have been discovered [5].bioactive molecule types [4] Moriga Oleifera leaves, in particular, have These chemicals exhibit a wide range of biological characteristics. antioxidant, anticarcinogenic, and so forth anti-diabetic, anti-atherogenic, and immunomodulatoryHepatoprotective properties [6].

These phytoconstituents provide plants unique features and properties. As a result, the investigation of these bioactive compounds in Plants would aid in determining various biological processes. Plant activities [7]. Nonetheless, the biological The significance of Moringa Oliefera has been discovered. more information is still safe. As a result, in recent years, More emphasis has been placed on the discovery of plants having antioxidant properties that could be exploited for human consumption [8]. These phytoconstituents provide plants distinct features and properties. Although much has been known about the biological characteristics of M. oleifera, more research is needed. As a result, much research has been focused in recent years on identifying plants having antioxidant properties that could be used for human consumption. Thus, the goals of this study are to investigate the phytochemical profile and thin layer chromatography (TLC) studies of M. oleifera leaves grown in Libya using various solvents in order to learn about the various constituents present in the plant and, as a result, to evaluate the medicinal potential of the plant and justify its traditional use.

MATERIALS AND METHODS

The experimental plant M.oleifera was collected from lohagaon forest.

A powder extract of dried leaves of moringa olifera is disolved in 4 different solvents

1. Methanol
2. Chloroform
3. Ethyl acetate
4. Water

After 24 hours filter the solution.

The dried M. oleifera leaves were powdered using a mixer grinder. The powdered leaves were then extracted for 24 hours using a series of solvents including pet methanol, ethanol, ethyl acetate, and water. Whitman's no. 1 filter paper was used to separate the extract.

Initial Phytochemical Examination of Succeeding M. oleifera Pod

Extracts

Using established protocols, a qualitative phytochemical examination of M. oleifera leaves was conducted to determine the constituents, namely Alkaloids, Flavonoids, Tannin, and Phenol, as reported by [10].

Test for alkaloids

The Moringa oleifera extract in the test tube was heated for 20 minutes while being gently shaken, and then 1% of HCL was made and added. It was then allowed to cool somewhat. After adding a few drops of Wagner's reagent to one milliliter of the extract, you should see a creamy brown color that indicates the presence of alkaloids.

Test for flavonoids

After thoroughly mixing 3 milliliters of Moringa oleifera extract with 10 milliliters of distilled water, a yellow hue that indicates the presence of flavonoids is visible.

Test for tannins

Put two milliliters of Moringa oleifera extract in a test tube and warm it gently for two minutes. three drops of ferric chloride were added, and the orange color revealed the presence of tannin.

Test for phenols

A few drops of 5% ferric chloride are added to 3 milliliters of Moringa Oleifera extract and 5 milliliters of distilled water. The dark green color indicates the presence of phenol.

Chromatographic purification

The main ingredients found in the most potent plant extracts were isolated using TLC, which was performed using various solvent systems.

Solvent phase

Chloroform:methanol:ethyl acetate in ratio 1:1:1, and trichloro acetic acid:ethyl acetate:methanol in ratio 1:7.5:1.5 .

Methods

Using capillary tubes, the plant extracts were administered in four different solvents on pre-coated TLC plates. To identify the location of each extract placed to the plate, draw a thin line and dots on it. Following the use of each mobile phase, the TLC plate was allowed to air dry before being examined under an ultraviolet light source. They were then sprayed with iodine vapour to promote the separation of the bands. The movement was measured by the retention factor (Rf), and values were computed for several samples

RF = Distance traveled by the solute / Distance traveled by the solute front TLC plate

Analyte detection

All plates were seen using a UV lamp after being dried and exposed to iodine vapour, and each distinct spot that was noticed was calculate

Result and Discussion

Phytochemical Screening of Sequential Extracts of M. oleifera leaves
The sequential extract of M. oleifera leaves underwent phytochemical screening, which revealed the presence of several bioactive substances, the most notable of which are tannin, phenol, alkaloids, and flavonoids.

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

Table-1: Qualitative Photochemical Screening of Sequential Extracts of Moringa Oleifra Leaves

Table 1 displays the components and the phytochemical test result. In these phytochemical studies, it was discovered that, when compared to other solvents, ethanol and ethyl acetate extract contained the highest concentrations of alkaloids, flavonoids, tannin, and phenol. Each of these phytochemicals has been shown to have strong antioxidant properties and to have a variety of biological effects, such as anti-inflammatory and anticancer properties.

Fig 1 Separation of Compounds A,B,C,D in Methanol Extract (cholroform : Methanol :Ethyl Acetate) (1:1:1)

- **Chromatographic Purification:TLC**

By using different solvent system as trials because various phytochemicals give different RF values in different solvent systems.

The variation in RF values of phytochemicals provides important clue in understanding of their polarity.

e.g

Chloroform:ethanol:methanol(1:1:1)

Chloroform:glacial acetic acid:methanol(4:5:1)

Chloroform:ethanol:methanol(2:2:0.5)

Hence, by studying these selection of appropriate solvent for further separation of compound from these plant extracts are,

chloroform:methanol:ethyl acetate(1:1:1)TLC of pet methanol extract of M.oleifera leaves revealed the presence of 4 compounds having RF values of 0.01 ,0.89 ,0.92 ,0.93.repectively when solvent phase of was used . with ethyl acetate extract of M.oleifera leaves also revealed the presence of 4 compounds having RF values0.67, 0.65, 0.63, 0.6 .

Trichloro acetic acid:ethyl acetate:methanol(1:7.5:1.5) of methanol extract shows 4 different bands having RF values of 0.75, 0.58, 0.51, 0.48 .and with ehyl acetate shows no bands.

Fig 2 Separation of Compounds A,B,C,D, In Ethyl Acetate Extract (cholroform : Methanol :Ethyl Acetate) (1:1:1)

Conclusion

According to the findings, M. oleifera leaves are a rich source of bioactive substances. These results implied the possibility of a natural antioxidant source in M. oleifera leaves, which would be valuable as a therapeutic agent for numerous chronic illnesses. Further studies on this plant can benefit from the increased concentration of bioactive components found in the methanol and ethyl acetate extracts of Moringa oleifera leaves.

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