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RESEARCH ARTICLE
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QUANTITATIVE ANALYSIS OF HYDROETHANOLIC EXTRACT OF *MORINGA OLIEFERA* BY HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC)

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ABSTRACT:
Objective: *Moringa oliefera* is well known for its therapeutic properties because of presence of bioactive compounds. Therefore, in the present study, the High-Performance Thin Layer Chromatography (HPTLC) analysis of hydroethanolic extract of *Moringa oliefera* was performed for its phytochemical profiling.
Methods: Preliminary phytochemical screening was done. HPTLC studies were carried out. CAMAG make HPTLC system equipped with Linomat 5 applicator, TLC scanner 3, Reprostar 3 and WIN CATS were used. The HPTLC densitometric analysis of the hydroethanolic extract of *Moringa oliefera* was carried out using CAMAG HPTLC system, and the results were obtained in the form of chromatograms (scanned at the wavelength of 254 nm and 366 nm) representing several peaks.
Results: Preliminary phytochemical screening of the extract showed the presence of alkaloids, flavonoids, saponins, tannins, terpenoids, phenolic compounds, proteins. HPTLC finger printing of hydroethanolic extract of *Moringa oleifera* under white light reveal presence of seven components 0.04, 0.05, 0.25, 0.29, 0.40, 0.44, 0.96. Component number 2,3 and 5 at Rf 0.05, 0.25 and 0.40 respectively showed maximum concentration.
Conclusion: The study came to the conclusion that *Moringa oliefera's* hydroethanolic extract includes a wide range of phytochemicals that may be responsible for its medicinal potential, supporting the plant's traditional use in India.

Keywords: HPTLC, Hydroethanolic extract, Phytochemical profiling, *Moringa oliefera*

INTRODUCTION:

A vital part of medicinal plant's significance in human health maintenance is attributed to their bioactive phytochemical content. Numerous natural products are utilised as phytotherapy to treat a wide range of ailments. The use of medicinal plants to cure infections is a long-standing tradition.[1] The search for a newer source of antibiotics is a global challenge, since many infectious agents are becoming resistant to synthetic drugs.[2] It is known that thousands of medicinal plants have been used for a very long time to treat a wide range of illnesses and conditions.[3,4]
Moringa oleifera Lam. also called as drumstick tree or horseradish tree (Moringaceae) is an indigenous tree from northwestern India, often cultivated in hedges and home backyards. *Moringa oleifera* and thus the leaves can be a good source of natural antioxidants.[5]

The World Health Organisation has emphasised the importance of establishing, developing, and using sound science as well as the necessity for herbal medications to be scientifically credible.[6] Numerous methods exist for the qualitative and quantitative assessment of phytochemicals found in plants. These days, new technology makes it possible to separate, identify, and test these active ingredients.[7]
HPTLC is semiautomatic instrumental TLC. HPTLC is different from conventional TLC due to smaller particles (<10 μm) of adsorbent and less thickness of applied layer (<150 μm). The automatic sample applicators of HPTLC applies sample in the form of bands and hence it gives better separation and resolution in close Rf value components. Simple spot application in TLC causes mixing of close Rf value components and look like tailing. HPTLC enables simultaneous analysis of many samples in less time with better analytical accuracy and precision. There is no need of filtration and degassing for solvents as required in HPTLC. HPTLC plates give the faster development and less mobile phase is required per sample. There are no chances of contamination from previous analysis as fresh stationary and a mobile phase has to use for each sample. Separated samples can be analysed visually by simple UV detector. By pre or post-chromatographic derivatization even non UV absorbing compounds can be detected.

MATERIAL AND METHODS

Collection of plant material: The plant material was collected from local market, Akola. The plants were authenticated from Dr. P. P. Umale (Kokate) Professor and Head Department of Botany, Shri Shivaji College of Arts, Commerce and science, Akola Maharashtra India.
Extraction: Leaves were collected manually washed thoroughly under running tap water and shade dried at room temperature (25-30°C) for 7 days. The dried leaves were subjected to pulverized using hammer mill to a coarse powder. Powdered mass (500 g) was extracted with hydroethanolic solvent [ethanol and water solvent (60:40)] at 45-65°C with the help of Soxhlet apparatus for 72 hours. Excess solvent was then evaporated in a water bath at 50–100°C to obtain the crude and stored in airtight containers.
Chemicals and solvents: All the solvents used were of chromatography grade, and all the chemicals used were of analytical reagent grade.
Phytochemical methods: Preliminary phytochemical screening for alkaloids, steroids, carbohydrates, tannins, fixed oils, proteins, terpenoids, flavonoids and glycosides were carried out according to the official procedures to know the presence of different phytoconstituents in the plant extracts.[8-12]

THIN LAYER CHROMATOGRAPHY [13-16]

Preparation of TLC plates: 25g of silica gel-G (Hi media, Manufactured, India) was uniformly spread over TLC plates with a thickness of 0.25 mm using the spreader. The plates were allowed to dry at room temperature and heated in an oven at 100°C for 2 h.
Standardisation of solvent system: Each sample of the crude extract of *Moringa oleifera* leaves was diluted in ethanol. The prepared TLC plates were marked 1 cm from bottom and 10 μl each sample was applied on TLC plates at equal distance with the help of capillary tubes. For separation of maximum bands on TLC plates different solvent systems were used according to polarity. Toluene: Ethyl acetate: Methanol (7:2:1) was selected as standard solvent system. *Moringa oleifera* extract was screened for preliminary phytochemical analysis. Observations were recorded for presence or absence of phytochemicals, number and Rf values of bands (compounds) present in extract.

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY [17-20]

Instrumentation: Analysis was performed on a CAMAG HPTLC system equipped with a sample applicator Linomat V, 100 ml syringe (Hamilton, Bonaduz, Switzerland), twin trough development chamber (20 cm x10 cm) size, TLC Scanner III linked to Win Cats software (CAMAG), 0.2 mm thickness pre-coated with silica gel 60 F254 (Merck) were used in this study.

Procedure: Analysis was performed on 20 cm × 10 cm HPTLC silica gel G60 F254 plates with fluorescent indicator. Before starting the analysis, HPTLC plates were cleaned by predevelopment with methanol by ascending method. HPTLC plate was immersed in a CAMAG glass chamber (20 cm × 10 cm), containing Toluene: Ethyl acetate: Methanol (7:2:1) (HPTLC grade) as solvent system. The chamber was covered with glass lid and left till development of the plate to the top with methanol. After complete development, the plate was removed from TLC glass chamber and dried in an oven. Sample spots of 10 µl of sample preparation as the bands on the plate by means of a CAMAG Linomat 5 (automated spray-on applicator equipped with a 100 µl syringe and operated with the settings band length 6 mm, distance between band 15 mm, distance from the plate side edge 15 mm and distance from the bottom of the plate 15 mm).

TLC Development and Scanning (Chromatographic Conditions): Samples of extract was spotted on a Precoated TLC aluminium sheets silica gel 60 F254 (10x10cm, 0.2 mm thickness) as 8 mm wide band width by using automatic TLC applicator Linomat V,10 mm from the bottom. The Mobile phase used was Toluene: Ethyl acetate: Methanol (7:2:1) for extract. The plates were kept for saturation in twin trough chamber for 15 min. After development the plates were dried in oven and scanned at 254 nm and 366nm by using CAMAG Scanner III.

Result

Preliminary Phytochemical Screening: The early phytochemical screening revealed the presence of a variety of phytochemicals as shown in the Table 1 (+ Present, - Absent).

Table 1: Phytochemical screening of extracts

Test	Moringa
Alkaloids	+
Glycosides	+
Flavonoids	+
Tannins	+
Proteins	+
Fixed oils	-
Steroids	+
Terpenoids	+
Carbohydrates	-
Cardiac Glycoside	-

TLC: TLC is a fundamental technique for separating and identifying several classes of natural products. This method allows the identification of distinct components by differential solute migration amongst two phases, one stationary and one migratory. The adsorbent (Silica) functions as a stationary phase while the solvent system function as mobile phase. Polar compounds have lower affinity for the solute, adhere to it and gradually ascends as a mobile phase advances. Compounds will travel relatively fast up the plates, resulting in increased Rf values. Mixture of substances will be separated according to their polarities.

Table 2: Thin Layer Chromatography of hydroethanolic of *Moringa oleifera*

Plant	Extract	Solvent system	Rf value
Moringa oleifera	Hydroalcoholic extract	Toluene: Ethyl acetate: Methanol (7:2:1)	0.05, 0.23, 0.41

High Performance Thin Layer Chromatography: HPTLC analysis of *Moringa oleifera* extract was performed using a particular solvent system Toluene: Ethyl acetate: Methanol (7:2:1) which was detected under UV 254. HPTLC finger printing of hydroethanolic extract of *Moringa oleifera* under

white light reveal presence of seven components 0.04, 0.05, 0.25, 0.29, 0.40, 0.44, 0.96. Component number 2,3 and 5 at Rf 0.05, 0.25 and 0.40 respectively showed maximum concentration.

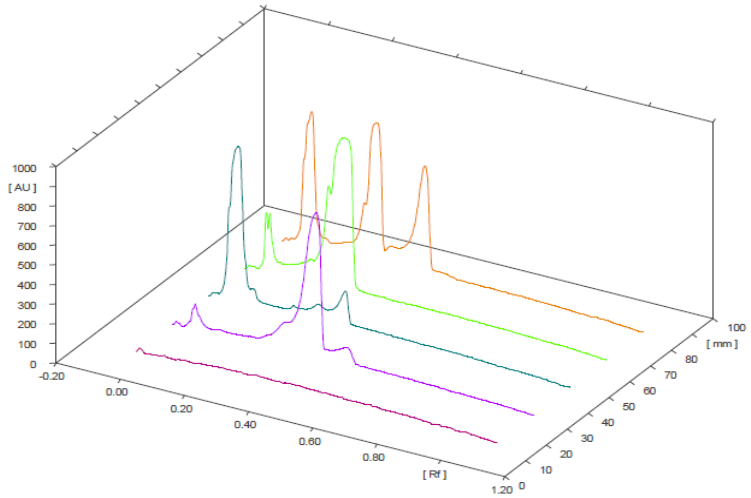


Fig 1: 3D Overlay of HPTLC chromatogram of *Moringa oleifera* of all tracts, at all wavelengths

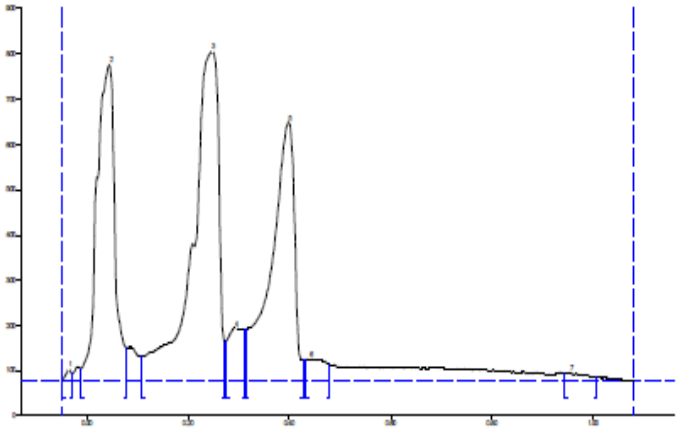
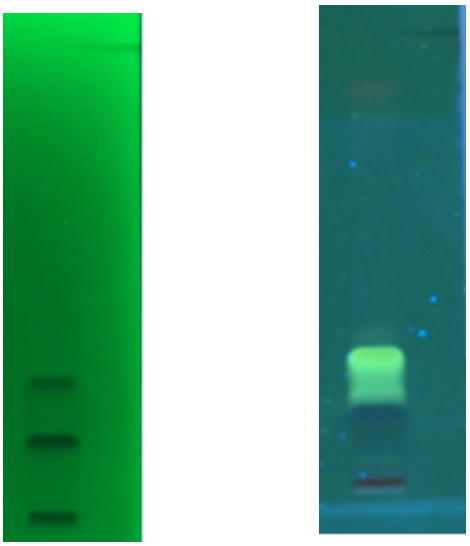


Fig 2: HPTLC chromatograms of *Moringa oleifera*



(a) **(b)**

Fig. No.3: HPTLC chromatograms of *Moringa oleifera* visualized under a. UV 254 nm, and b. UV 366 nm

Table 3 HPTLC peak table of hydroethanolic extract of *Moringa oleifera*

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.05	1.6	0.04	24.0	1.09	0.03	14.8	189.7	0.28
2	0.01	25.6	0.05	696.8	31.69	0.08	71.4	17631.5	25.96
3	0.11	51.5	0.25	726.2	33.03	0.27	84.2	26895.9	39.59
4	0.28	85.5	0.29	116.0	5.28	0.31	112.3	2595.2	3.82
5	0.31	112.4	0.40	570.0	25.92	0.43	45.6	18802.1	27.68
6	0.43	46.2	0.44	47.9	2.18	0.48	35.5	1318.6	1.94
7	0.95	14.6	0.96	17.9	0.82	1.01	7.6	494.8	0.73

Discussion

Herbal medicines are composed of various phytoconstituents and are therefore shows variability in the chemical nature. Hence it is very important to obtain to study these phytoconstituents by using reliable chromatographic techniques which represent pharmacologically active and chemically characteristic components of the herbal medicine. For the correct identification of medicinal plants, the HPTLC fingerprinting profile is a crucial component of the standardisation of herbal drugs.

The HPTLC performed on the hydroethanolic extract of *Moringa oliefera* showed the presence of various phytoconstituents in different concentrations as illustrated in figures 1,2,3 and table 1. The number of peaks indicates the presence of different phytoconstituents present in the sample. The Rf values (Tables no. 3) calculated for the phytoconstituents present in the tested sample would be helpful in the identification of the unknown compounds by comparing them with the reference standards, and from the values of peak area, the concentration of the compounds can be determined.

Conclusion

The results of this study supported the use of *Moringa oliefera* as a treatment for a variety of illnesses by revealing the presence of many phytochemicals in the hydroethanolic extract of the plant, which may be the source of its therapeutic qualities. It is necessary to isolate and identify significant phyto-compounds with pharmacological qualities in order to formulate new drugs. By comparing the Rf values of the compounds with the reference standards, the HPTLC study conducted for the hydroalcoholic extract of *Moringa oliefera* chemical profiling would be useful in the identification of bioactive chemicals.

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