RESEARCH PAPER

STANDARDIZATION AND PHYTOCHEMICAL EVALUATION OF HERBAL FORMULATIONS FOR ANTIDIABETIC THERAPY

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Abstract

This study aimed to assess the physicochemical properties, phytochemical contents, and standardization parameters of *Mangifera indica* leaf extract for potential use in antidiabetic herbal formulations. The extraction of plant material utilized several solvents, with ethyl acetate and aqueous solvents yielding the most extractive results, while chloroform exhibited the lowest yield. The ethyl acetate extraction of Mangifera indica demonstrated a markedly superior yield of 45.48% (w/w), whereas the chloroform extraction produced merely 0.28%. The physicochemical assessment of the plant extract was conducted to ascertain foreign matter, ash values, extractive values, and loss on drying (LOD). The chosen plant exhibited significant ash values, encompassing total ash, water-soluble ash, acid-insoluble ash, and sulphated ash, signifying the purity and quality of the crude medicine. The alcohol-soluble and water-soluble extractive values were determined to be 19.2 ± 0.68% and 19.1 ± 0.42% (w/w), respectively. The loss on drying value was measured at 1.51 ± 0.52%, signifying reduced moisture content and enhanced stability of the extract. Qualitative phytochemical analysis identified significant bioactive compounds including flavonoids, glycosides, phytosterols, alkaloids, terpenoids, and tannins, however saponins were not detected. Quantitative phytochemical study indicated tannin levels between 2.1% and 2.5%. The total phenolic content displayed an absorbance of 0.99 at 790 nm, while the total flavonoid content shown an absorbance of 0.9969 at 425 nm, signifying the abundance of phenolic and flavonoid chemicals in the extract. Additional standardization of the extract was conducted utilizing HPLC and HPTLC methodologies. The HPLC chromatographic examination revealed a prominent peak at Rt 1.543, with a peak area occupancy of 97.12%, so affirming the existence of substantial phytoconstituents. The HPTLC analysis revealed an Rf value of 0.66 ± 0.03 for the Mangifera indica extract. The research concludes that Mangifera indica has significant phytochemical compounds and may be a viable source for creating standardized antidiabetic herbal medicines.

Keywords: *Mangifera indica*, Qualitative and Quantitative phytochemical study, standardization parameters

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1. INTRODUCTION

Diabetes has emerged as the world's most significant issue in the modern era. The metabolisms could be affected. This imbalance may impair insulin secretion and function. Diabetes mellitus manifests difficulties and can have an impact on people's health as well as socioeconomic issues in the majority of countries. A quarter to a third of the population could be impacted. Diabetes can be caused by environmental and genetic factors.[1,2] The sugar metabolism is halted and insulin is not produced in the early stages of diabetes. Lack of insulin could arise from this, and it might also control blood sugar levels. The fat, protein, and glycogen may be broken down in this way, producing sugar. It caused the blood sugar to be high, and the liver could generate ketones. Chronic hyperglycemia has been linked to a disruption in macromolecule metabolism. As a result, insulin's ability to secrete and function is compromised.[3,4] Long-term damage could result from this. Heart, eye, and nerve damage or failure may potentially result from this illness. Death and disability could result. The organ system may suffer harm from hyperglycemia, which may also be related to the duration and management of other disorders. Additionally, diabetes can result in polyuria, weight loss, and thirst.[5,6]

Classification of Diabetes mellitus (DM)

Patients with diabetes are unable to create or properly use insulin due to improper insulin digestion. There are a few different types and descriptions of diabetes depending on the cause, and many of them exhibit insufficient insulin production or digestion. Diabetes comes in two basic varieties: -

Type 1 diabetes

In this diabetes mellitus the patients may be depend on insulin. These are autoimmune disorder. It may develop when the pancreases cell which produces insulin may be damage or destroyed. By this insulin may be produced in little amount or no insulin. If this condition occurs the patient may take insulin regularly whenever it been needed.[7,8]

Type 2 diabetes

Enough insulin is produced in this beta-cell. However, the problem arises when the objective organs' insulin receptors recognise it (insulin resistance), disrupting the intracellular signalling cascades for insulin. Insulin resistance (IR), which slows down the rate at which normal fuel is digested.[9,10]

According to general consensus, IR is the primary obsessive malformation seen in T2 DM, followed by beta cell death as a consequence brought on by the pancreas' compensatory response mechanism.

This type of diabetes has multiple contributing factors, many of which revolve around inherited changes and environmental factors. If ignored, hyperglycemia can lead to long-term microvascular and macrovascular consequences such atherosclerosis, neuropathy, and retinopathy. Nephropathy accounts for 60% of nontraumatic lower-limb amputations.

Gestational diabetes

It may occur due the intolerance of glucose. It may recognize in pregnancy period of second or third trimester. These causes due the pregnancy hormones and low amount of insulin secretion. During pregnancy, this diabetes are most common disorders.[11,12]

2. METHODOLOGY

2.1 Collection of material

The plants raw materials were gathered from regional farmers.

2.2 Preparation of extract

Leaves were rinsed with water to remove any pollution. Then 80% ethanol was used to wash the leaves. These leaves are then placed in an oven set at 50°C for 24 to 30 hours before being pulverised into a fine powder. 200ml of solvents are used to dissolve 50gm of the samples. Keep this for 48 to 50 hours while it is 40°F outside and being shaken at 150RPM. Once the solvent has evaporated, the sample is then transferred to a rotatory vacuum evaporator set at 400°C. These samples were then placed in a 40°C refrigerator and used for the phytochemical analysis.

2.3 Physico-chemical evaluation

Physico-chemical evaluation of the plant extract was performed to determine its purity, quality, and standardization parameters. Ash value determination was carried out using a silica crucible. Approximately 3 g of powdered sample was accurately weighed, incinerated, cooled in a desiccator, and weighed to calculate the total ash content and percentage ash value.

For acid-insoluble ash determination, the obtained ash was boiled with 30 ml of hydrochloric acid for about 5–6 minutes. The insoluble matter was collected using ashless filter paper, washed with hot water, ignited in a crucible, cooled, and weighed. The remaining residue represented the acid-insoluble ash value.

Water-soluble ash was determined by boiling the total ash with approximately 30 ml of water for 5 minutes. The insoluble matter was filtered, washed with hot water, ignited, and weighed. The difference between total ash and insoluble residue provided the water-soluble ash value.

Loss on drying (LOD) was determined by heating the weighed sample at 105°C until a constant weight was obtained. The percentage weight loss indicated the moisture and volatile matter content of the sample.

Extractive values were determined by macerating 5 g of dried extract in 100 ml of solvent for 24 hours. The mixture was shaken intermittently during the first 6 hours and kept undisturbed for the remaining 18 hours. The filtrate was collected, evaporated to dryness, and weighed to determine the extractive value.

2.4 Preliminary phytochemical studie

Qualitative phytochemical screening was performed to identify the major bioactive constituents present in the plant extract using standard chemical tests. Carbohydrates were identified by Molisch's, Fehling's, and Benedict's tests. Formation of a purple ring in Molisch's test and brick-red precipitate in Fehling's and Benedict's tests confirmed the presence of carbohydrates.

Alkaloids were detected after acidification and filtration of the extract using Mayer's, Hager's, and Wagner's reagents. Cream, yellow, and reddish-brown precipitates obtained in these tests indicated the presence of alkaloids. Terpenoids and steroids were evaluated by Salkowski and Libermann–Burchard tests. Development of red and bluish coloration confirmed steroidal and terpenoid compounds.

Flavonoids were identified using lead acetate and alkaline reagent tests. Formation of yellow precipitate and yellow coloration indicated the presence of flavonoids. Phenolic compounds and tannins were detected using ferric chloride, lead acetate, and gelatin tests, which produced blue-black coloration and white precipitates.

Saponins were confirmed by the froth test, where persistent foam formation indicated their presence. Proteins and amino acids were identified using

Ninhydrin, Biuret, and Millon's tests. Appearance of violet, pink, or brick-red coloration confirmed proteinaceous compounds.

Glycosides were detected using Borntrager's, Legal's, and Keller–Killiani tests. Development of pinkish-red and characteristic color changes confirmed the presence of anthraquinone and cardiac glycosides. Fixed oils and fats were identified by the spot test, where a permanent oily stain on filter paper indicated the presence of lipid constituents.

These qualitative phytochemical tests confirmed the presence of various secondary metabolites such as alkaloids, flavonoids, tannins, glycosides, terpenoids, phenolic compounds, proteins, and fixed oils in the plant extract. Such phytoconstituents are known for their diverse pharmacological activities, including antioxidant, antimicrobial, anti-inflammatory, and antidiabetic properties. Therefore, phytochemical screening provides important preliminary information regarding the therapeutic potential and medicinal value of the selected herbal extract.

2.5 Quantitative Analysis

Quantitative phytochemical analysis was carried out to estimate the tannin, total phenolic, and total flavonoid contents present in the plant extract. For tannin determination, 6 g of powdered plant extract was mixed with 96% ethanol and allowed to stand for 3 hours with intermittent shaking, followed by filtration. The filtrate was treated with ferric chloride solution, producing a violet coloration indicating tannin presence. Another portion of powdered extract was boiled with water, filtered, and examined for characteristic color formation.

Total phenolic content was estimated using methanolic leaf extract (10 mg/ml). The reaction mixture was prepared and kept in the dark for 30 minutes before measuring absorbance at 790 nm using a UV spectrophotometer. Quantification was carried out using a gallic acid standard calibration curve, and results were expressed as gallic acid equivalents (GAE).

Total flavonoid content was determined by treating the methanolic extract with aluminium chloride and potassium acetate. After incubation for 30 minutes, absorbance was measured at 425 nm. The flavonoid content was quantified using a rutin standard curve and expressed as rutin equivalents.

2.6 Standardization of Extract

Standardization of the plant extract was carried out using HPLC and HPTLC techniques. HPLC analysis was performed to identify and standardize the phytoconstituents present in the extract. Formic acid and isopropanol were used in the chromatographic system, while ethanol served as the mobile phase. The extract sample was prepared in HPLC-grade methanol and analyzed for 30 minutes. Standardization was achieved by comparing the retention time (Rt) of the sample with that of the standard compound.

HPTLC fingerprint analysis was also conducted using methanolic leaf extracts. The samples were applied using a 100 µL syringe, and toluene:ethyl acetate (7:4) was used as the mobile phase. Anisaldehyde sulfuric acid served as the spraying reagent for visualization of separated compounds.

3. RESULTS

3.1 Extraction of plant material:

When the plant material was extracted, ethyl acetate and water produced the maximum yield. Chloroform yielded the least amount of product. Mangifera indica produced ethyl acetate in a yield of 45.48 percent (w/w), compared to 0.28 percent for chloroform.

Plant	Solvents	Total yield in gm	% Yield in gm
Mangifera indica	Petroleum ether	1.74gm	0.25
	Chloroform	0.68gm	0.28
	Ethyl acetate	45.89gm	25.48
	Butanol	40.82gm	26.48
	Water	19gm	13.8
	Methanol extract	10.85gm	8.58

Table 1.Extraction of plant material

3.2 Physico-chemical evaluation

3.2.1 Determination of foreign matter

The chosen Mangifera indica plants displayed the greatest content.

S.no	*Mangifera indica*
1	1.6±0.75

Table 2. Plants foreign substances

3.2.2 Ash value determination

Mangifera indica, a group of chosen plants, has the greatest ash level (Total ash, water soluble, acid insoluble and sulphated ash value).

Plant name	Total ash	Water soluble	Acid insoluble	Sulphated
Mangifera indica	0.7±0.42	1.8±0.48	0.55±0.21	0.8±0.55

Table 3. Calculation of the Ash value

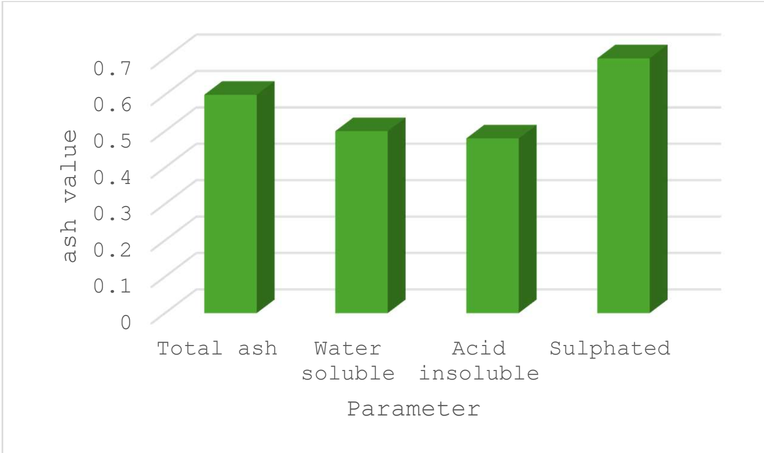

Figure 1.Ash content of selected plants

3.2.3 Extractive value

When evaluating crude drugs, the extractive value that is water-soluble is very important. A lower extractive value suggests the use of used up ingredients, adulteration, or improper drying, storing, or formulating techniques. The extractive values of a particular leaf extract were established.

Plants	***Mangifera indica***
Alcoholic (% w/w)	19.2±0.68
Aqueous (% w/w)	19.1±0.42

Table 4. Extractive value

3.2.4 Loss on drying (LOD)

Although occasionally it may refer to the loss of any volatile matter from the sample, loss on drying is a commonly used test method to determine the moisture content of a sample. LOD of a particular plant extract was established.

Plants	***Mangifera indica***
LOD	1.51±0.52

Table 5. LOD (Loss on drying)

3.2.5 Yield percentage

All of the herbs' plant materials were removed, and yield percentage was computed.

Plants	***Mangifera indica***
Percentage yield(w/w)	3.1

Table 6. Ratio of yield

3.3 Phytochemical screening

3.3.1 Qualitative phytochemical screening

S.no	**Phytochemicals**	**Presence (+)/absence (-)**
1	Flavonoids	+
2	Glycosides	+
3	Phytosterols	+
4	Alkaloids	+
5	Terpenoids	+

6	Saponins	-
7	Tannins	+

Table 7. Mangifera indica qualitative phytochemical screening

3.4 Quantitative analysis:

3.4.1 Estimation of tannins

Plant extract	Tannins
Mangifera indica	2.1-2.5%

Table 8. Tannins of the selected plants

3.4.2 Estimation of total phenolic content

The extract's estimated total phenolic content had an absorbance of 0.99 at 790 nm.

Table 9.

Sr. No.	Concentration (µg/mL)	Absorbance
1	0	0
2	0.5	0.07
3	2	0.137
4	4	0.21
5	6	0.282
6	8	0.35
7	10	0.45
8	12	0.55
9	14	0.62
10	16	0.72
11	18	0.825
12	20	0.999

Calibration curve of Gallic acid

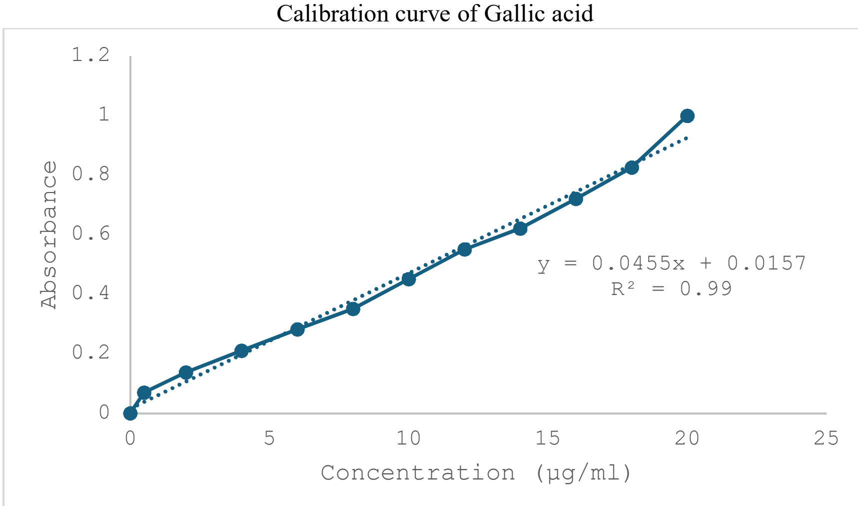

Figure 2. Calibration curve of Gallic acid

Solvents	*Mangifera indica*
Petroleum ether	71.66±5.04
Chloroform	35.06±5.41
Ethyl acetate	250.01±6.59
Butanol	98.58±2.79
Water	38.59±2.79
Methanol Extracts	26.67±2.78

Table 10. Results of Total phenolic contents of selected plants

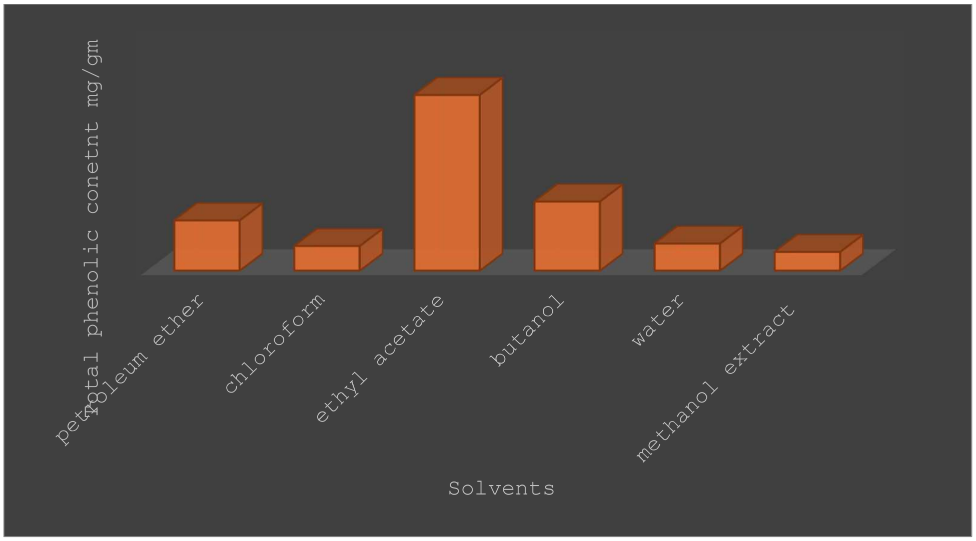

Figure 3. Total phenolic content of selected plant

3.4.3 Estimation of total flavonoid

The methanol extract's total flavonoid concentration displayed an absorbance of 0.9969 at 425 nm.

Concentration (μg/ml)	Absorbance
0	0
0.5	0.11
2	0.21
4	0.298
6	0.45
8	0.55
10	0.711
12	0.825
14	0.946
16	1.064
18	1.185
20	1.29

Table 11. Calibration curve of Rutin

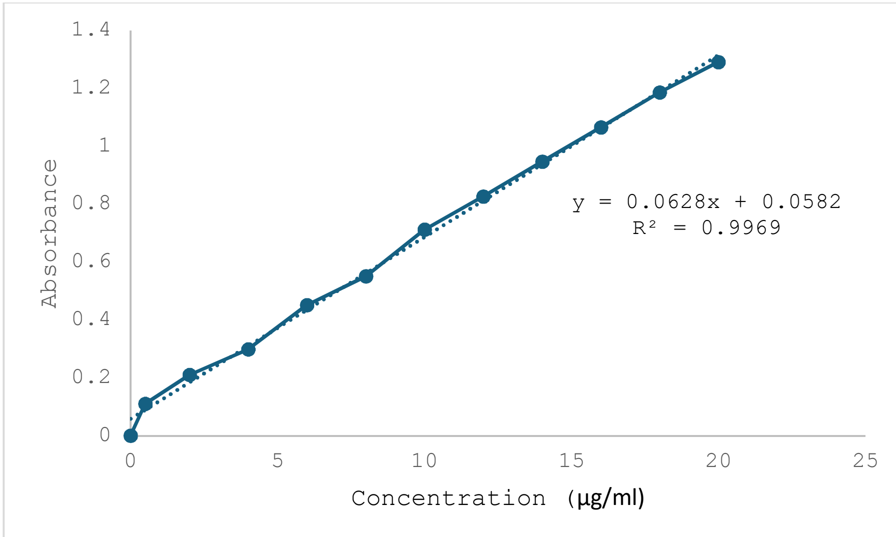

Figure 4. Calibration curve of Rutin

Fractions	***Mangifera indica***
Petroleum ether	91.66±5.04
Chloroform	55.06±5.41
Ethyl acetate	310.01±6.59
Butanol	99.58±2.79
Water	40.59±2.79
Methanol extract	35.67±2.78

Table 12. Results of Total flavonoid contents of selected plant

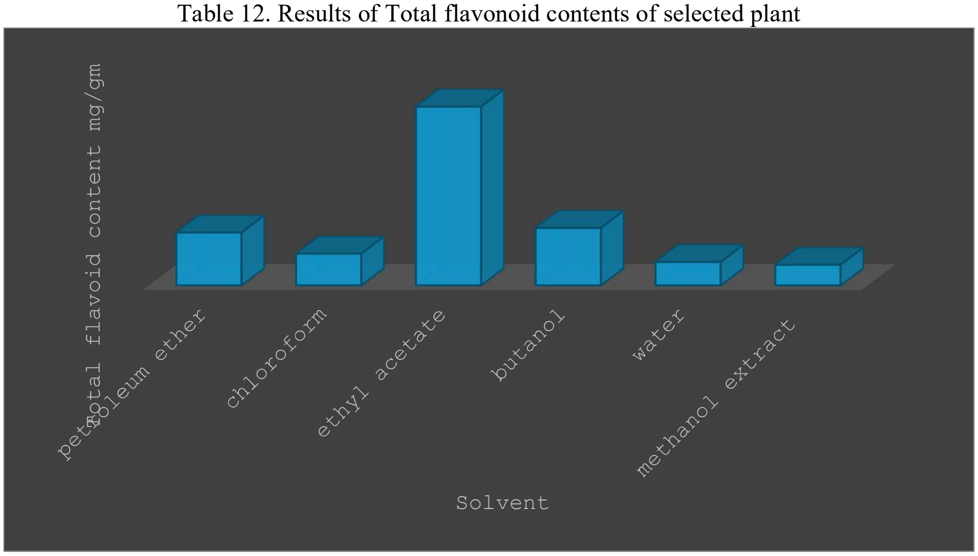

Figure 5. Total flavonoid content of selected plant

3.5 STANDARDIZATION OF EXTRACTS

3.5.1 HPLC

On plant extract, standardisation was carried out. An extract's HPLC chromatogram showed a peak at Rt1.543 with a 97.12 percent occupied peak area.

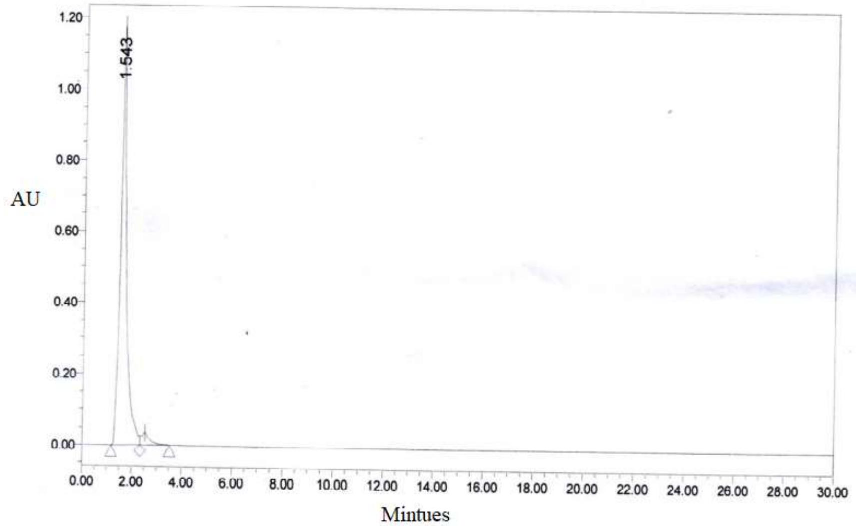

Figure 6. Chromatogram of mixture of select plants

4.2.2 Standardization of selected plant extract by HPTLC

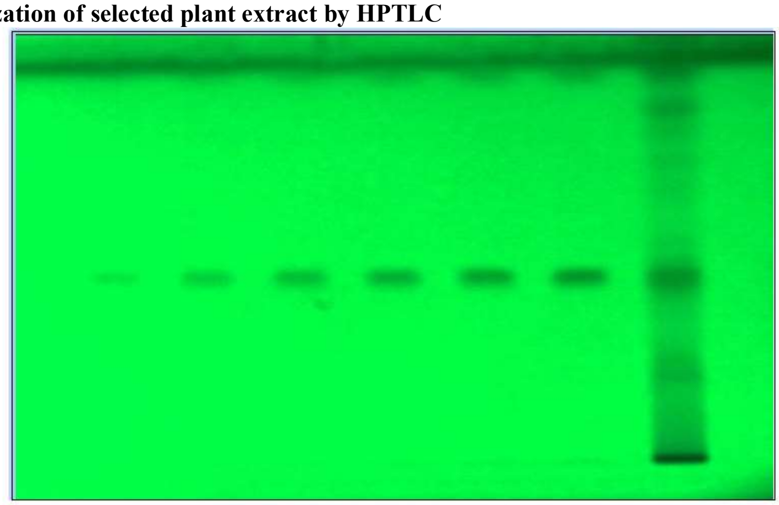

Figure 7. HPTLC plate of *Mangifera indica* (200 - 700ng/spot)

R_f value of *Mangifera indica* 0.66 ± 0.03

4. SUMMARY & CONCLUSION

The present study was conducted to evaluate the physicochemical characteristics, phytochemical constituents, and standardization parameters of Mangifera indica leaf extract for its potential application in antidiabetic herbal formulations. Different solvents were employed for extraction, among which ethyl acetate produced the highest extractive yield, while chloroform showed the lowest yield. The physicochemical evaluation confirmed the quality and purity of the extract through determination of ash values, extractive values, and loss on drying. The low moisture content indicated better stability and storage properties of the extract.

Qualitative phytochemical screening revealed the presence of several important bioactive constituents such as flavonoids, glycosides, phytosterols, alkaloids, terpenoids, and tannins. Quantitative analysis further demonstrated appreciable amounts of tannins, phenolic compounds, and flavonoids, which are known for their antioxidant and antidiabetic properties. The high phenolic and flavonoid contents suggest that the extract possesses significant therapeutic potential.

Standardization studies using HPLC and HPTLC techniques confirmed the presence of major phytoconstituents and provided characteristic chromatographic fingerprints for the extract. The prominent HPLC peak and specific HPTLC Rf value indicated the consistency and reliability of the herbal extract.

Overall, the findings of this study demonstrate that *Mangifera indica* leaf extract possesses valuable phytochemical constituents and satisfactory physicochemical characteristics. Therefore, it can be considered a promising natural source for the development of standardized and effective antidiabetic herbal formulations with potential therapeutic benefits.

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