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DEVELOPMENT AND VALIDATION OF UV AND HPLC METHOD FOR ESTIMATION OF POLYPHENOL IN MANGIFERA INDICA L.

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Abstract:

Mangifera indica L., commonly known as mango, is a tropical fruit tree with widespread cultivation for its delicious fruit and medicinal properties. The therapeutic potential of mango is attributed to bioactive compounds, particularly polyphenols, known for their antioxidant, anti-inflammatory, and anti-cancer properties. This research addresses the need for reliable analytical methods to estimate polyphenols in Mangifera indica L.

Two complementary methods, ultraviolet (UV) spectrophotometry and high-performance liquid chromatography (HPLC), were developed and validated.

The research involves optimizing extraction procedures for polyphenols from different mango parts, validating UV and HPLC methods, and applying them to quantify total and specific polyphenol content in leaves, stem bark, and roots. The study compares the performance of both methods, highlighting their strengths and limitations.

The findings provide insights into the polyphenolic profile of Mangifera indica L., demonstrating the suitability of UV and HPLC methods for polyphenol estimation. The validated methods contribute to quality control and standardization of herbal preparations from mango. By establishing reliable analytical methods, this research enhances the scientific understanding and utilization of Mangifera indica L. for human health.

Extraction involves soxhlet and aqueous decoction methods for methanol and water extracts, respectively. Total flavonoids and phenolic content are determined using UV spectrophotometry. HPLC solubility studies, stationary phase selection, mobile phase optimization, and validation of analytical parameters are conducted.

Methanol extraction yields for leaves, stem bark, and roots are 5.25%, 6.25%, and 3%, respectively. Aqueous extraction yields are 3.7%, 4%, and 2.1%, respectively. Total flavonoids and phenolic content determination shows varying levels across extracts. HPLC validation demonstrates precision, sensitivity, and accuracy. The robustness of the method is confirmed by recovery studies.

Keywords: Mangifera indica L,Polyphenols, UV, HPLC, Flavonoids.

Introduction

Mangifera indica L., commonly known as the mango, is a tropical fruit tree widely cultivated for its delicious fruit and diverse medicinal properties. The leaves, bark, and seeds of the mango tree have been used in traditional medicine for centuries to treat various ailments, including diabetes, wound healing, and inflammatory disorders. This therapeutic potential is largely attributed to the presence of a wide range of bioactive compounds, including polyphenols.

Polyphenols are a diverse group of secondary metabolites found in plants, known for their antioxidant, anti-inflammatory, and anti-cancer properties. They have gained significant scientific interest due to their potential health benefits and increasing consumer demand for natural products. However, accurately quantifying polyphenols in complex matrices like plant extracts is crucial for

understanding their biological activities and ensuring the quality and efficacy of herbal preparations. [1]

Therefore, the development and validation of reliable analytical methods for polyphenol estimation in *Mangifera indica* L. is essential. This research aims to address this need by developing and validating two complementary methods for polyphenol estimation in different parts of the mango tree

Ultraviolet (UV) spectrophotometry: This is a simple, rapid, and cost-effective technique for measuring total polyphenol content based on their absorbance at specific wavelengths.

High-performance liquid chromatography (HPLC): This is a more sophisticated technique that provides separation and identification of individual polyphenol compounds, enabling quantitative analysis of specific bioactive components. [2]

This research will involve:

Optimizing extraction procedures to efficiently isolate polyphenols from different parts of the mango tree.

Developing and validating UV and HPLC methods according to international guidelines for linearity, precision, accuracy, limit of detection, limit of quantification, and robustness.

Applying the validated methods to quantify total and specific polyphenol content in various mango samples (e.g., leaves, Stem bark and Root).

Comparing the performance of both methods and highlighting their strengths and limitations.

The findings of this research will provide valuable insights into 0the polyphenolic profile of different parts of *Mangifera indica* L.

The suitability of UV and HPLC methods for polyphenol estimation in the mango tree.

The potential application of these methods for quality control and standardization of herbal preparations derived from the mango tree.

By establishing reliable and validated analytical methods, this research will contribute to the scientific understanding and utilization of the therapeutic potential of *Mangifera indica* L. for human health. [3]

Objective:

Standardization means verified those active ingredient are believe to present in the plant material and that the potency and amount of active ingredient. Since number of phytoconstituents remains present in the plant material, it is much tedious to establish the method of qualitative and quantitative for specific phytoconstituents. So, modern methodologies are needed to verify the interested phytoconstituents[4,5]**.**

In present status, it is need to evaluate and validate the plant medicine with known kind of marker with specific method. The literature survey revealed that HPLC [6], HPTLC [7], GC-MS [8]and HPLC-MS [9] method was reported for the determination of rutin, quercetin and gallic acid. But no method reported till to estimates rutin, gallic acid and quercetin in *Mangifera indica* L. different parts (Leaves, Stem Bark and Root) by a simultaneous method. Hence, taking this point in consideration present study was to develop and validate chromatographic method for determination of rutin, quercetin and gallic acid in *Mangifera indica* L. by using HPLC and UV spectroscopy.

MATERIAL

Chemicals and Reagents

Rutin (S D Fine Chem. Mumbai), Gallic acid (Loba Chem.), Mumbai, Quercetin (S D Fine Chem. Mumbai), Methanol (HPLC grade) (Qualigens, Mumbai, India), Water (HPLC grade) (Qualigens, Mumbai, India.) were procured from respective vendors.

Equipments

UV experimentation was perform on Shimadzu 1800 UV-visible spectrophotometer equipped with photo diode array detector, with 1 cm quartz cell. Chromatographic experimentation were performed using YOUNGLIN ACME 9000 HPLC system (South Korea) equipped with 8600 HPLC pump and 8600 dual wavelength detector, data acquisition and processing was processing was performed using Autochoro 3000 system software. The mobile phase was prepared freshly, filtered through 0.45µm membrane filter paper (Millipore, USA) and sonicated for 30 min.

Plant Materials

Fresh leaves, stem bark, root of *Mangifera indica* L. (FamilyAnacardiaceae) were collected from agricultural area of village Vadri, TalYawal, Dist-Jalgoan, Maharasshtra.

Identification and Authentication of Plant Material

Confirmation of identity and authentification, on the basis of organoleptic character, exomorphology and pharmacognostic study of plant was carried out by J. Jayanthi, Head, Botanical Survey of India, Government of India, Ministry of Environmental and Forest, Pune, India. The certificate for the authentification is enclosed (Voucher No. GSP-1)

METHOD

Extraction

The extractions of plant materials were carried out by soxhlet and then successively by aqueous decoction method.

Methanol extraction of *Mangifera indica* L. leaves, stem bark and root The leaves, stem bark and roots were washed with water and allowed to air dry at room temperature. Then they were placed in an oven at 40^0c until dried, after which they were powder using a blender. Then a sample of 400 gm of each powder was subjected to successive extraction with methanol in soxhlet extractor at temperature 45-50^0c.The extraction was continued until the solvent in the thimble becomes clear indicating complete extraction. The methanolic extracts obtained was filtered and collected, and then solvent were distilled off and the concentrated extracts transferred to previously weighed petri dish and evaporated to dryness at room temperature 45-50^0c to obtained dried extracts. The yield of extracts were calculated and represented as per percentage yield.

Aqueous extraction of *Mangifera indica* L. leaves, stem bark and root the marc of leaves, stem bark and roots after methanolic extraction were dried in air. The dried marc was extracted using water as solvent. The marc was boiled with sufficient quantity of water for 30 min, the aqueous extracts obtain were filter and collected. Collected extract then subjected to distillation to remove water. The concentrated extracts were transferred to previously weighed petri dish and evaporated to dryness at room temperature 45-50^0c to obtained dried extract. The yield of extracts were calculated and represented as per percentage yield [10].

Determination of Total Flavonoids Content

The amount of total flavonoids was determined according to the method described by, briefly, (10-50 µg/ml) of standard and (100 µg/ml) of sample 1.0 ml of solution of aqueous and methanol extract was mixed with 1 ml aluminium chloride (2% in methanol). After shaking, the mixture was incubated at room temperature for 15 min and the absorption was measured at 430 nm using a

Shimadzu UV spectrometer. Total flavonoids content was expressed as rutin equivalents in milligram per gram extract[8].

Determination of Total Phenolic Content

The amount of total phenolics was determined according to the folinciocalteu procedure, briefly, 1.0 ml folin-ciocalteu,s reagent (50 %) and 0.8 ml 7.5 % (w/v) Na2CO3, were added to (20-100 µg/ml) of standard and (100 µg/ml) of sample (100 µg/ml) 0.2 ml of solution of aqueous decoction and methanol extract. After shaking, the mixture was incubated at room temperature for 30 min. Absorption was measure at 765 nm using a Shimadzu UV spectrometer. Total phenolic was expressed as gallic acid equivalent in milligram per gram extract [8]

HPLC

Solubility study

Various solvent were used for solubility studies of Gallic Acid, Quercetin, and Rutin.

Selection of stationary phase

On the basis of nature of analyte C18 (Grace) was selected.

Selection and optimization of mobile phase

Gallic Acid, Rutin, Quercetin was freely soluble in Methanol, Acetonitrile, ethanol, poorly soluble in water. But this entire chemical soluble in methanol and water mixture hence the mixture proportion optimized and used as mobile phase for initial separation.

Preparation of mobile phase:

Methanol: water in ratio of 50:50 (0.1 ml T.E.A mix pH 3.0 with ortho phosphoric acid.

Preparation of standard stock solution

10 mg of Gallic acid, Rutin, Quercetin were accurately weighed and transfers to 100 ml volumetric flask and dissolved in 50 ml of methanol. The flask was degassing for 15 min and volume was made up to mark using methanol to get a solution 100 µg/ml.

Selection of analytical wavelength

Stock solutions of drugs were prepared in methanol and UV spectrums of rutin, gallic acid and quercetin solutions (10 µg/ml) were taken. The solution was scan in UV visible range of 200-400 nm at which drug shows significant absorbance.

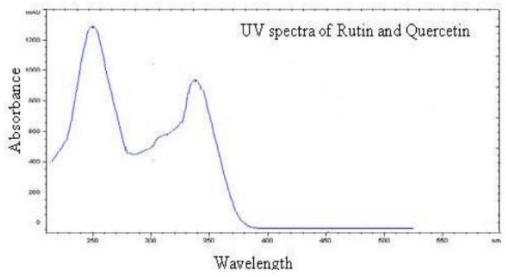


Fig. 1 UV Spectra of Rutin and Quercetin

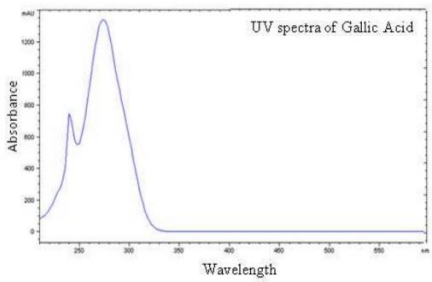


Fig. 2 UV Spectra of Gallic Acid

Optimization of HPLC parameters

Optimization of HPLC parameters was carried out to find a set of condition that adequately separate and enable the quantification of the analyte from the endogenous material with acceptable accuracy, precision, ease and speed.

Linearity and calibration

The solutions in different concentration rutin and quercetin (1-5µg/ml) and gallic acid (5-25µg/ml) were prepared from standard stock solution. The solutions (20 µL) were injected into column with

the help of Hamilton syringe. All measurements were repeated six times for each concentration. The calibration curve of the area under curve Vs concentration was recorded for the each drug. [11,12]

Method for Estimation of Rutin, Gallic acid and Quercetin in Mangifera indica L. Extracts

Methanol extract

100 mg of methanol extract of leaves, stem bark and roots was weighed accurately and diluted up to 100 ml methanol. (1 mg/ml) The solutions were sonicated for 10 min, and solutions were filter through Whatman filter paper no.1. A sample solution of each extract was injected 20 µL into HPLC separately.

Aqueous extract

100 mg of aqueous extract of leaves, stem bark and roots was weighed accurately and diluted up to 100 ml methanol. (1 mg/ml) The solutions were sonicated for 10 min. and solutions were filter through Whatman filter paper no.1. A sample solution of each extract was injected 20 µL into HPLC separately [13].

Validation of HPLC Method

The method was validated according to ICH guidelines. The following validation characteristics were addressed linearity, accuracy, precision, limits of detection and limit of quantification; and robustness. [14]

RESULT

Extraction of Plant Material

The percentage yield of methanol extract of *Mangifera indica* L. leaves, stem bark and root extract were found to be **5.25%**, **6.25%** and **3%** respectively. The percentage yield of aqueous extract of *Mangifera indica* L. leaves, stem bark and root extract were found to be **3.7%, .4% and 2.1%** respectively.

Table 5 Estimation of Total Phenolic Content and Flavonoids Content in *Mangifera indica* L. Extracts

Table 1 Total Flavonoids Content Determination

Sr. No	Concentration Rutin (ug/ml)	Absorbance (nm)	% RSD
1	10	0.065	0.13
2	20	0.120	0.27
3	30	0.215	0.08
4	40	0.300	0.15
5	50	0.350	0.34

Extract	Absorbance	TPC Gallic Acid mg/gm in extract	Absor bance	TFC Rutin mg/gm in extract
Leaves methanol	0.353	380	0.0845	130
Leaves aqueous	0.302	301	0.0325	50
Stem bark methanol	0.321	350	0.0260	42
Stem bark aqueous	0.172	160	0.052	80

Root methanol	0.165	146	0.058	81
Root aqueous	0.204	193	0.042	66

Linearity and Calibration:

Calibration curve were constructed from five calibration standard solutions of rutin, and quercetin (1-5 qg/ml), gallic acid (5-25 qg/ml). The results, summarized in Table 5.1.10 to 5.1.15 showed good correlation between analytes peak area and concentration with (r2= 0.9994, 0.9993, 0.9993) of rutin, quercetin and gallic acid respectively.

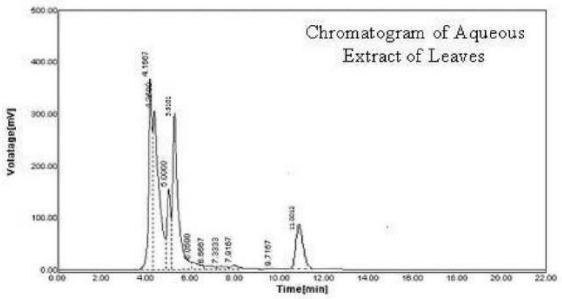
Table 10 Linearity Data of Rutin

Sr. No	Concentration of Rutin (µg/ml)	Retention Time (Rt)	Peak Area	% RSD
1	1	5.73	177.59	0.07
2	2	5.70	366.28	0.26
3	3	5.68	584.90	0.22
4	4	5.81	811.75	0.16
5	5	5.85	1003.30	0.39

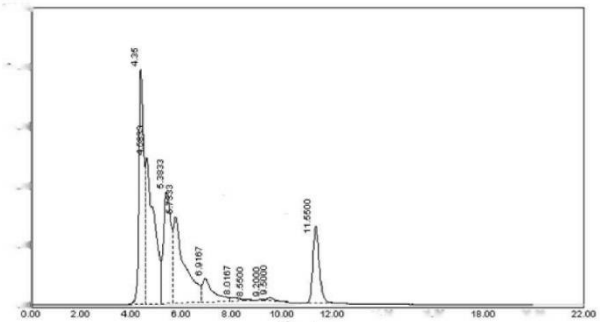
Estimation of Rutin, Quercetin and Gallic Acid in *Mangifera indica* L. Extracts The % of rutin, gallic acid and quercetin were found to as shown

Table 11 Estimation of Rutin, Quercetin and Gallic Acid in *Mangifera indica* L. Extracts

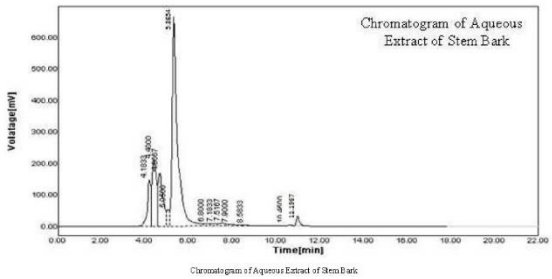
Extract	Peak Area of Rutin	(Rt) of Rutin	Rutin %	Peak Area of Quercetin	(Rt) of Quercetin	Quercetin %	Peak Area of Gallic acid	(Rt) of Gallic Acid	Gallic acid %
Leaves methanol	4629.58	5.73	2.87	5613.95	11.55	0.50	6036.80	4.58	7.8
Leaves aqueous	4047.25	5.91	1.93	2012.23	11.00	0.179	5843.48	4.35	7.5
Stem bark methanol	3784.06	4.45	0.037	1250.54	11.21	0.11	7216.25	4.45	9.32
Stem bark aqueous	9165.85	5.85	4.37	766.98	11.19	0.067	2995.82	4.40	3.87
Root methanol	6543.57	5.81	3.12	2982.12	11.22	0.267	2870.44	4.45	3.71
Root aqueous	5317.40	5.84	2.53	1027.23	11.10	0.091	2596.71	4.38	3.36



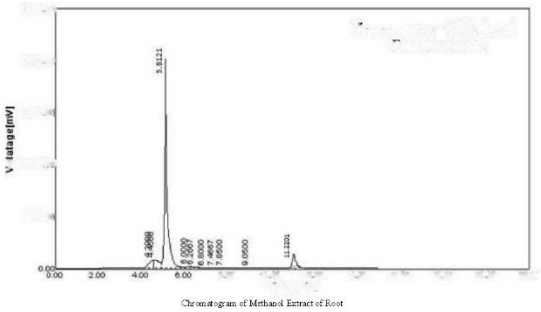
Chromatogram of Aqueous Extract of Leaves



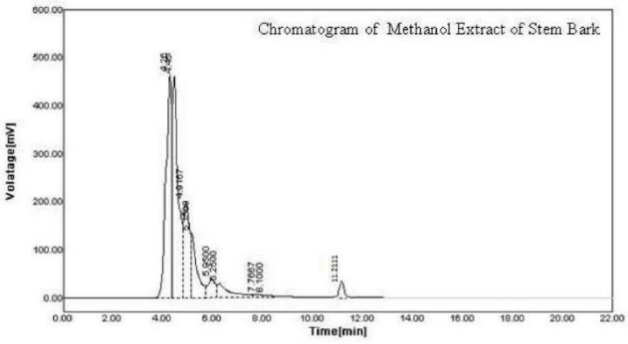
Chromatogram of Methanol Extract of Leaves



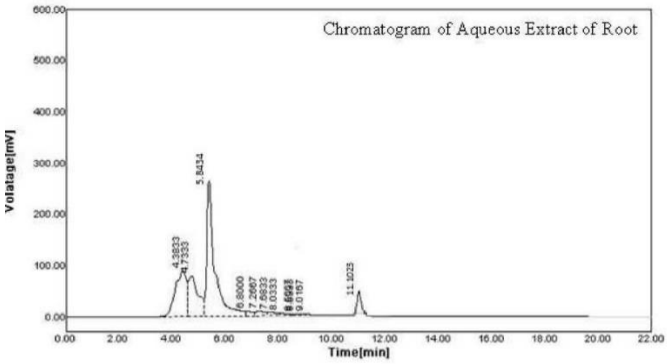
Chromatogram of Aqueous Extract of Stem Bark



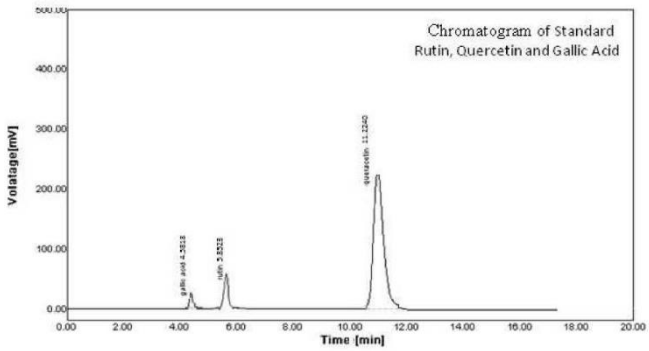
Chromatogram of Methanol Extract of Root



Chromatogram of Methanol Extract of Stem Bark



Chromatogram of Aqueous Extract of Root



Chromatogram of Standard Rutin, Quercetin and Gallic Acid

Validation of Chromatographic Method:

Precision:

Precision study was carried out using parameter like method repeatability and intra-day and inter-day precision study which showed that results were within acceptable limit i.e., % below 2.0 indicating that the method is reproducible.

LOD and LOQ:

The limits of detection and limits of quantification decided about the sensitivity of the method. The results are shown in following Table

Result of LOD and LOQ

Sample	LOD (^g/ml)	LOQ (^g/ml)
Rutin	0.00303	0.0093
Quercetin	0.0365	0.1109
Gallic Acid	0.3526	1.0685

The evaluation of robustness involves assessing a method's ability to endure slight variations in its operating parameters, such as flow rate, wavelength, and the percentage of mobile phase composition. In this study, the robustness of the current method was explored by measuring the recovery percentage of phenolic compounds at three concentration levels. The method was deliberately modified in each instance by altering a single chromatographic condition. Specifically, variations were introduced in flow rate (0.6 and 0.8 as opposed to 0.7 ml/min), the volume fraction of methanol and water at 50:50 (changes of -2:+2 and +2:-2), and wavelength (339 and 341nm compared to the original 340 nm). The findings indicated that the separation remained unaffected by slight changes in chromatographic conditions, with good resolution maintained between adjacent peaks. Recovery studies were carried out by adding a known amount of pure drug rutin, quercetin and gallic acid to a pre analysed sample solution. These studies were carried out at 80%, 100% and 120% level and percent recovery of Rutin, Gallic acid and Quercetin was found above 96 %.

The linearity of analytical method is ability to elicit test results that are directly proportional to concentration of analytes in the sample within given range. The range of analytical methods is the interval between upper and lower level of analytes that have been demonstrated to be determined within suitable level of precision, accuracy and linearity. Results are shown in Table

Linearity and Range

Parameter	Rutin	Quercetin	Gallic Acid
Linear Range (gg/ml)	1-5 gg/ml	1-5 gg/ml	5-25 gg/ml
Slope	209.68	1111.39	77.45
Intercept	0.1950	12.32	-8.27
r^2 Correlation Coefficient	0.9994	0.9993	0.9993

DISCUSSION

Extensive literature survey revealed that very rare spectrophotometric and chromatographic method was reported for comparative estimation of rutin, gallic acid and quercetin in a plant. Taking this point in to consideration, an attempt was made to develop a simple, accurate and precise HPLC method, with determination of total phenolic and total flavonoids content in different parts of Mangifera indica L. Separations were achieved by using methanol: water (50:50) 0.1 ml T.E.A mix pH 3.0 with ortho phosphoric acid as mobile phase. The detection was carried out at 340 nm. Linear regression data for calibration curve of rutin, quercetin and gallic acid is shown in table. The value were found to be linearity range of 1-5 µg/ml for rutin and quercetin, 5-25 µg/ml for gallic acid with correlation value (r2= 0.9994, 0.9993, 0.9993) for rutin, quercetin and gallic acid respectively. The total phenolic content and total flavonoids content determination was showed good content of phenolic and flavonoids compounds. Methanol extract of Leaves of Mangifera indica L. showed the high content of total phenolic content and methanol extract of root of Mangifera indica L. showed high content of total flavonoids content as compared to other extracts, (380 Gallic Acid /gm TPC, 130 Rutin /gm TFC respectively) and lowest content of total phenolic content shown methanol extract of root of Mangifera indica L. and methanol extract of stem bark of Mangifera indica L. showed low

Content of total flavonoids content (146 Gallic Acid /gm TPC, 42 Rutin /gm TFC respectively).

On the basis of parameter fixed, the method of estimation was validated

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