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Research Article

Research on HPLC-Based Flavonoid Fingerprinting and Quantitative Analysis of *Bauhinia purpurea* Linn. Flower Extract

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ABSTRACT

The idea of health consciousness is rapidly gaining popularity, leading to increased reliance on herbal remedies with fewer adverse effects compared to modern medicines. Bauhinia purpurea L. is a prominent medicinal species traditionally utilized for its antidiarrheal, anticancer, and thyroid gland-stimulating properties. The flowers of this plant are particularly rich in phytoconstituents, making them a valuable source for pharmaceutical applications. This study aimed to identify and quantify the flavonoids, specifically quercetin, present in the ethanolic flower extract of B. purpurea using Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC). The extraction was performed using Soxhlet, maceration, and sonication methods, with the Soxhlet extraction yielding 23.07% crude extract. Preliminary phytochemical screening confirmed the presence of flavonoids and phenolics. Thin Layer Chromatography (TLC) utilizing a mobile phase of toluene : chloroform : ethyl acetate (2:2:0.5) successfully separated quercetin, demonstrating an R_f value between 0.47 and 0.60. Further, an efficient RP-HPLC method was developed using a C18 column and a mobile phase of methanol : acetonitrile (7:3) with 0.1% formic acid. The standard quercetin exhibited a retention time (R_t) of 5.52 to 5.62 min, while the sample extract confirmed its presence with a matching R_t of 5.68 to 5.70 min. The study confirms that B. purpurea flowers are a promising natural source of bioactive flavonoids, supporting their potential therapeutic and nutraceutical use.

INTRODUCTION

The idea of health consciousness is currently gaining popularity among both urban and rural populations. In order to attain this level of health, people have been taking herbal remedies either by itself or in conjunction with other goods, keeping in mind the negative health impacts of modern medicine. There are significant financial gains linked to the growth and application of medicinal plants and their derivatives in indigenous remedies for illness, suggesting that the avenues for the

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herbal renaissance are expanding. According to World Health Organization (WHO) data, 70% to 80% of the world's population uses various plants for therapeutic reasons to meet their basic healthcare needs. Approximately 53,000 types of plants have been used since prehistoric times.[1]

Flavonoids are a significant class of natural products; in specifically, they are a class of secondary metabolites of plants with a polyphenolic structure that are commonly present in fruits, vegetables, and flowers. They have a variety of beneficial biochemical and antioxidant properties linked to a number of illnesses, including cancer, Alzheimer's disease, atherosclerosis, etc. . Flavonoids are an essential part of many pharmacological, nutraceutical, medical, and cosmetic uses and are linked to a wide range of health-promoting properties. This is due to their ability to regulate important cellular enzyme processes as well as their antioxidative , anti-inflammatory, anti-mutagenic, and anti-carcinogenic qualities.[2]

Plants produce flavonoids from the aromatic amino acids phenylalanine, tyrosine, and malonate. The flavan nucleus, which is made up of 15 carbon atoms organized in three rings (C6-C3-C6), is the fundamental structure of flavonoids. The flavan nucleus, which is made up of 15 carbon atoms organized in three rings (C6-C3-C6), is the fundamental structure of flavonoids. Typically found in plants as glycosylated derivatives, flavonoids give leaves, flowers, and fruits their vivid blue, red, and orange .In addition to a variety of fruits and vegetables, flavonoids may be found in seeds, nuts, grains, spices, and many medicinal plants.[3]

Antioxidant Activity: Although flavonoids have a variety of biochemical characteristics, their ability to function as antioxidants is the most well-described characteristic of nearly all flavonoid groups. The arrangement of functional groups around the nuclear structure determines the antioxidant activity of flavonoids; the configuration, substitution, and total number of hydroxyl groups significantly affect various mechanisms of antioxidant activity, such as radical scavenging and metal ion chelation ability. Although flavonoids have a wide range of biochemical characteristics, their ability to function as antioxidants is the most well-documented characteristic of nearly all flavonoid groups.[4]

Hepatoprotective Activity: A number of flavonoids, including catechin, apigenin, quercetin, naringenin, rutin, and venoruton, have been shown to have hapatoprotective properties .. Numerous flavonoids have been shown to have hapatoprotective properties, including catechin, apigenin, quercetin, naringenin, rutin, and venoruton Hepatic clinical symptoms can occur as a result of several chronic disorders, including diabetes.[4]

Antimicrobial action. Since plants are known to produce flavonoids in response to microbial infection, it should come as no surprise that they have been shown to be potent antimicrobial agents against a variety of microbes in vitro. Antibacterial activity has been demonstrated for flavonoid-rich plant extracts from several species . Strong antibacterial action has been demonstrated for a number of flavonoids, including apigenin, galangin, flavone and flavonol glycosides, isoflavones, flavanones, and chalcones. [4]

Figure No. 1: Flower of Bauhinia purpurea Linn.

Bauhinia purpurea:

There are around fifteen species in the genus *Bauhinia*_of the family *Cesalphiniacea* that are found in India; some are climbers, while others are trees or shrubs. There are around fifteen species of *Cesalphiniacea* found in India. While some of them are climbers, others are trees or shrubs. In the past, all valuable medicinal preparations were derived from plants, whether in the form of simple plant parts or more complex forms of crude extract. *Bauhinia purpurea* .L is one of the most important species used to treat several ailments in the traditional system of medicine. Bauhinia purpurea *L*was reported for its antidiarrheal, anticancer, and thyroid gland stimulating properties. Because the flowers are rich in phytoconstituents, it was considered a principle source in the pharmaceutical industries. Bauhinia purpurea *L* a most important species used to treat several ailments in traditional system of medicine. The antidiarrheal, anticancer, and thyroid gland-stimulating qualities of Bauhinia purpurea *L*_have been shown. The flowers were regarded as a primary source in the pharmaceutical and nutraceutical sectors due to their abundance of phytoconstituents. Historically, all valuable medicinal preparations were derived from plants, whether in the simple form of plant parts or more complex form of crude extract.[5] Bauhinia Purpurea also shows some other therapeutic uses in psychotic syndrome , burnind sensation, disorders of blood .[7]

Figure No. 2 : Tree of Bauhinia purpurea Linn.

Table No .1 : Table of Plant information

Parameter	Details
Plant Name	Bauhinia Purpurea
Family	Cesalphiniacea
Common Name	Kachnar
Part used	Flower
Collection Place	Wanadongri, Hingana
Collection time	December

HPLC :

High-performance liquid chromatography (HPLC) is now recognized as the most practical technique for separating and identifying flavonoids using a variety of detection systems.In terms of quantitative analysis, a lot of data has been published in recent years confirming the technique's suitability for simultaneous determination of flavonoid compounds in various samples, which provides an insight into the distribution of flavonoids in the studied material.Regarding the quantitative analysis, a lot of information has been released in recent years that attests to the technique's usefulness for the

simultaneous determination of flavonoid compounds in different samples, which provides insight into the distribution of flavonoids in the material under study.[6]

Literature Review

1. K. Marimuthu and R. Dhanalakshmi (2014)A study on Bauhinia purpurea leaf and flower revealed the presence of several phytochemicals such as carbohydrates, alkaloids, steroids, glycosides, saponins, flavonoids, tannins, phenolics, proteins, and amino acids. The plant samples were shade-dried, powdered, and extracted with water. The leaf extract showed a yield of 29.57%, while the flower extract yielded 20.20%. The study indicated that Bauhinia purpurea contains important bioactive compounds with potential medicinal value

2. Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: an overview. Journal of Nutritional Science, 5,Flavonoids are natural compounds with variable phenolic structures widely found in fruits, vegetables, grains, bark, roots, stems, flowers, tea, and wine. They are known for their significant health benefits and are widely used in nutraceutical, pharmaceutical, medicinal, and cosmetic applications. Flavonoids possess important biological properties such as antioxidant, anti-inflammatory, anti-mutagenic, and anti-carcinogenic activities, and they can modulate key cellular enzyme functions. Studies have also associated flavonoid consumption with reduced cardiovascular mortality and prevention of coronary heart diseases. Current research focuses on the isolation, identification, characterization, and biological functions of flavonoids, along with their potential use as therapeutic agents for preventing chronic diseases

3. Krishnaveni, M. (2015). Phytochemical study of Bauhinia purpurea Linn.Stem. Research Journal of Pharmacy and Technology Studies on Bauhinia purpurea stem revealed the presence of several phytochemicals including carbohydrates, proteins, alkaloids, saponins, phenols, flavonoids, fats, anthocyanins, terpenoids, and steroids. Nutritional analysis showed higher amounts of amino acids and proteins compared to carbohydrates. The stem extract also exhibited antioxidant activities such as metal chelating, nitric oxide scavenging, and hydrogen peroxide scavenging activity. Phenolic compounds were found in higher amounts than flavonoids, suggesting that the presence of these secondary metabolites contributes to its antioxidant potential and supports its pharmaceutical applications.

4. Claessens, H. A. (2001). Trends and progress in the characterization of stationary phases for reversed-phase liquid chromatography. TrAC Trends in Analytical Chemistry Reversed-Phase Liquid Chromatography (RPLC) columns have undergone significant developments in terms of characterization, stability, and performance. Studies have evaluated important parameters such as hydrophobicity and silanol activity that influence retention and selectivity. Improvements in column manufacturing have enhanced chemical stability, column lifetime, and reproducibility between batches and columns. These advancements have expanded the applicability of RPLC columns under a wide range of experimental conditions in chromatographic analysis.

5. Stafilov, T. (2004). HPLC analysis of flavonoids. Encyclopedia of Chromatography 2004 Update Supplement, 3(6), 113. Flavonoids are widely distributed plant secondary metabolites belonging to the C6–C3–C6 phenolic group. They are classified into several categories such as flavones, flavonols, flavanones, catechins, anthocyanidins, chalcones, and aurones based on their structural differences. These compounds often occur as aglycones or glycosides, where attachment of sugar molecules increases their solubility and transport within plants. Flavonoids exhibit characteristic ultraviolet absorption bands, which make UV Spectroscopy useful for their identification. However, High-Performance Liquid Chromatography (HPLC) is widely used for the separation, identification, and quantitative analysis of flavonoids in plant materials, fruits, beverages, honey, and other natural products. This technique provides reliable information on the distribution and concentration of flavonoids in different samples.

6. Swati M. Wakchoure*1, Ajay Kharate2, Bharavi Keni3, Abhilasha Karve4 and Neha Kasurde Bauhinia purpurea (family Fabaceae) has been studied for its pharmacological properties, including anthelmintic activity. Preliminary phytochemical screening of its extracts revealed the presence of phytosterols, alkaloids, glycosides, flavonoids, saponins, tannins, and phenolic compounds. Among the different extracts, ethyl acetate and methanolic extracts of the flowers showed significant anthelmintic activity, indicating the medicinal potential of the plant.

7. Tin Mar Htay, Kyi Kyi Sann, Hazwan Haini. Comparative Study on Phytochemical Screening and Antioxidant Activity of Aqueous Extract from Various Parts of Bauhinia purpurea. Bioactivities, 2023, 1 (1), pp.24-31. 10.47352/bioactivities.2963-654X.183. hal-04177101 A comparative study on different parts of Bauhinia purpurea showed the presence of phytochemicals such as alkaloids, flavonoids, saponins, carbohydrates, polyphenols, and phenolics. Among the plant parts studied, the flower extract exhibited the highest total phenolic and flavonoid content. The flower extract also demonstrated strong antioxidant activity in the DPPH radical scavenging assay, indicating that *Bauhinia purpurea* flowers are a potential natural source of antioxidant compounds.

8. Neeraj Kumar Pamita Bhandari Bikram Singh Ajai P. Gupta Vijay K. Kaul Natural Plant Products Division, Institute of Himalayan Bioresource Technology, Palampur, Himachal Pradesh, 176 061, India. Reversed phase-HPLC for rapid determination of polyphenols in flowers of rose species. For the purpose of determining polyphenols, gallic acid, catechin, epicatechin, rutin, m-coumaric acid, quercitrin, myricetin, quercetin, apigenin, and kaempferol in fresh flowers of Rosa bourboniana and R. brunonii as well as in both fresh flowers and marc of R. damascena, a quick, easy, sensitive, reliable, and enhanced HPLC method was created and validated. Gallic acid, rutin, quercitrin, myricetin, quercetin, and kaempferol were the six polyphenols that were found and measured in every extract. Using an RP-HPLC column and linear gradient elution of water and acetonitrile (with a flow rate of 1 mL/min at k 280 nm), the chromatographic separation of 10 polyphenols was accomplished in less than 16 minutes. In the range of 0.39 to 500 lg/mL,

standard calibration curves were linear. In terms of recovery and repeatability, good outcomes were attained (98.6–100.8%).

9. Abdul Qayoom Laghari1 , Shahabuddin Memon1*, Aisha Nelofar2 , Abdul Hafeez Laghari1,2. Extraction, Identification and Antioxidative Properties of the Flavonoid-Rich Fractions from Leaves and Flowers of Cassia angustifolia. The extracts from Cassia angustifolia leaves and flowers were analyzed for flavonoid identification, total flavonoid concentration, and antioxidant activity. Flavonoids were extracted from leaves and flowers using five different methods. The extracts' total flavonoid contents were determined using UV-visible spectrophotometry. High-performance liquid chromatography combined with photodiode array detection and electrospray ionization tandem mass spectrometry (HPLC-PDA-ESI-MS) was used to identify and quantify individual flavonoids. Aqueous ethanol (70%) fractions of C. angustifolia flowers and leaves have been shown to be extremely rich in flavonoids, and microwave extraction is the most effective technique for extracting both the total and individual flavonoid contents.

10. Ayyakkannu Purushothaman1 , Packirisamy Meenatchi 1 , Saravanan S 1 , Ramalingam Sundaram1 , Nallappan Saravanan 2, Quantification of Total Phenolic Content, HPLC Analysis of Flavonoids and Assessment of Antioxidant and Anti-haemolytic Activities of Hibiscus rosasinensis L. Flowers in vitro.Hplc analysis is studied from this article.

MATERIALS AND METHODS

1. Collection and Authentication

Fresh flowers of *Bauhinia purpurea Linn.* were collected from the Herbal Garden of Data Meghe Ayurvedic Medical College , Nagpur during the flowering season in the month of November and December .The plant is authenticated by recognized Botanist and the a voucher specimen was preserved for future reference The collected flowers were washed thoroughly with distilled water to remove dust and foreign particles. The flowers were shade dried at room temperature for 3 to 4 days and then pulverized using a mechanical grinder to obtain coarse powder. The powdered material was stored in an airtight container until further use.

Figure No. 3 : Authentication of Plant

2. Chemicals and Instruments

All chemicals and solvents used in the study were of analytical and HPLC grade.

- Methanol
- Acetonitrile
- Distilled water
- Formic acid

- Quercetin standard

All instrument used in the analytical study

- Soxhlet Apparatus
- Maceration Container
- Sonication Instrument
- Mechanical Grinder
- Heating Mantle
- Hot Air Oven
- HPLC System- Shimadzu LabSolution
- UV Visible Detector
- Analytical Balance
- Refrigerator

3. Preparation of Extract

Ethanolic Extraction:

A total yield of 130 g of coarse powder was obtained from the dried flower, which was subsequently utilized for extraction procedures including maceration, sonication-assisted extraction, and Soxhlet extraction.

1. In a Soxhlet apparatus the powder with 500 mL ethanol for 5–6hours.The extract obtained was filtered and concentrated using a Heating mentle at controlled temperature. The concentrated extract was dried to obtain a semisolid mass and stored in a refrigerator for further analysis.

Figure No. 4: Soxhlet Apparatus

2. In Maceration the powdered flower with 500ml ethanol kept for 7 -10 days with frequent shaking. The extract obtained was filtered and concentrated using a Heating mentle at controlled temperature. The concentrated extract was dried to obtain a semisolid mass and stored in a refrigerator for further analysis.

Figure No. 5 : Extraction by Maceration

3. In Sonication apparatus the powdered flower with 400ml ethanol kept for 5-6 hours . The extract obtained was filtered and concentrated

using a Heating mentle at controlled temperature. The concentrated extract was dried to obtain a semisolid mass and stored in a refrigerator for further analysis.

Figure No. 6: Sonication Instrument

4. Preliminary Phytochemical Screening

The ethanolic extract was subjected to preliminary phytochemical tests for identification of various phytoconstituents such as flavonoids and phenolic compounds using standard procedures.

Test for Flavonoids

- Shinoda Test

To the extract, a small quantity of magnesium turnings and concentrated hydrochloric acid were added. Formation of pink or reddish coloration indicated the presence of flavonoids.

- Zinc HCl Test

One or two drops of strong hydrochloric acid and zinc dust were added to the extract. A favorable outcome was signaled by the presence of red.

Test for Phenolic compounds

- Ferric Chloride Test

A few drops of a 5% ferric chloride solution were added to a tiny volume of the extract and thoroughly stirred.Phenolic chemicals are indicated by the development of bluish-black, dark green, or violet .

- Potassium Dichromate Test

Potassium dichromate solution was added to the extract. The production of brown precipitate indicated a successful outcome.

- Gelatin Test

1% gelatin solution with 10% sodium chloride was added to the extract. White precipitate production demonstrated a favorable outcome.

5. TLC Separation Parameters:

TLC (Thin Layer Chromatography) parameters you can use for flavonoid separation from flower extract of Bauhinia purpurea.

Stationary phase: Silica gel

Plate size**:** 10 × 10 cm or 20 × 20 cm

Plate type**:** glass plates

Use 5–10 µL for spotting.

Mobile Phase (Solvent System) : Toluene : Chloroform : Ethyl Acetate (2:2:0.5)

Detection / Visualization:

Observe plates under:

UV light 254 nm

UV light 366 nm

Rf Value :

- Quercetin 0.45 – 0.60

6. Preparation of Standard Solution:

A stock solution of quercetin standard was prepared by dissolving 10 mg of quercetin in 10 mL methanol to obtain a concentration of 1000 µg/mL. From the stock solution, working standard solutions of concentrations 10, 20, 30 and 40 ppm were prepared by suitable dilution with methanol.

7. Preparation of Sample Solution:

About 10 mg of dried ethanolic extract was dissolved in 10 mL HPLC-grade methanol and sonicated for 10 minutes for complete dissolution. Then further dilutions were prepared of concentrations of 10,20,30 and 40ppm with methanol .The solution was filtered through a 0.45 µm membrane filter before HPLC analysis.

8. HPLC Analysis:

Instrumentation: Shimadzu LabSolutions

HPLC analysis was carried out using an HPLC system equipped with UV-Visible detector, quaternary pump, auto sampler, and data acquisition software.

Chromatographic Conditions:

Column: Reverse Phase C18 column (250 mm × 4.6 mm, 5 µm)

Mobile Phase: Methanol : Acetonitrile (7:3), Formic acid 0.1% v/v in water .

Flow Rate: 1.0 mL/min

Detection Wavelength: 254nm

Injection Volume: 5 µL

Column Temperature: 26 °C

Run Time: 20 minutes

The mobile phase was filtered through 0.45 µm membrane filter and degassed before use.

9. Calibration Curve Preparation:

Different concentrations of standard quercetin solutions were injected into the HPLC system and chromatograms were recorded. Peak area obtained for each concentration was plotted against concentration to obtain the calibration curve.

The amount of flavonoid present in the sample extract was determined using the linear regression equation obtained from the calibration curve.

10. Identification and Quantification of Flavonoids:

Identification of flavonoids in the extract was carried out by comparing the retention time (Rt) of sample peaks with those of standard quercetin .

Quantification was performed based on peak area measurements using the calibration curve method.

11. Statistical Analysis:

All experiments were carried out in triplicate and results were expressed as mean ± standard deviation (SD).

Data obtained from HPLC analysis were analyzed statistically using suitable software such as Microsoft Excel .

12. Results and Discussion :

Extract Yield % = Weight of Extract Obtained / Weight of Raw Material used * 100

=30/130*100

Extract Yield %=23.07%

Table No. 2 : Percentage Yield of Extract

Weight of Powder	Extract Obtained	% Yield (w/w)
130g	30g	23.07%

Phytochemical screening results:

- Flavanoids

Table No. 3 : Phytochemical test for Flavanoid

Test Name	Reagents	Observation
Shinoda test	Magnesium turnings + concentrated HCl	Pink, red or orange color appears
Zinc Dust Test	Zinc Dust + Concentrated HCl	Red Color Develops

- Phenolic content

Table No. 4 : Phytochemical test for Phenolic content

Test Name	Reagents	Observation
Ferric Chloride test	5% ferric chloride solution	Blue,green ,purple, black coloration
Potassium Dichromate test	Potassium dichromate solution	Brown precipitate forms
Gelatin test	Gelatin solution +10%NaCl	White precipitate forms

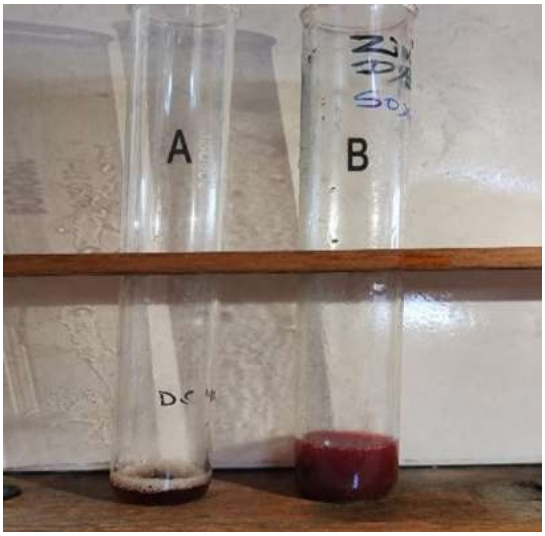

Figure No.7 : Test for Flavanoid
A: Shinoda test
B: Zinc test

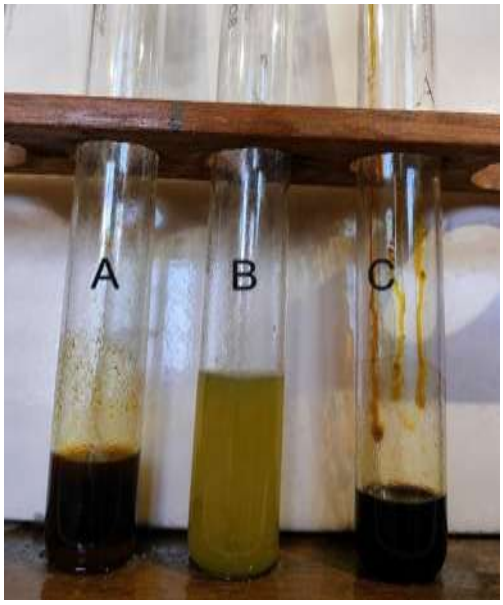

Figure No. 8 : Test for Phenolic content
A: Potassium Dichromate test
B: Gelatin test
C: Ferric Chloride test

TLC Separation with Results :

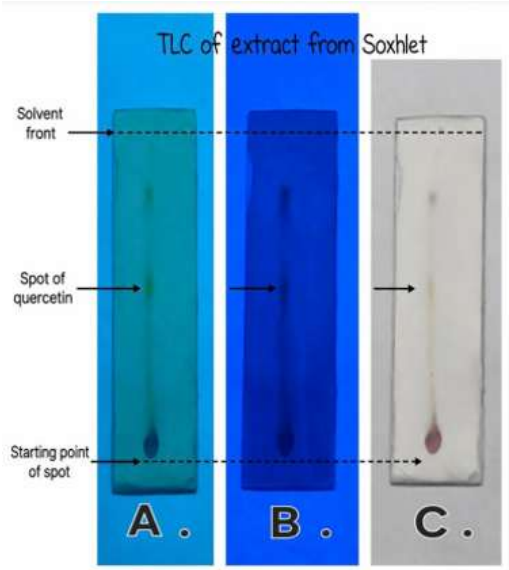

Figure No. 9 : TLC of extract from Soxhlet
A : TLC plate under shorter wavelength UV light
B : TLC plate under longer wavelength UV light
C : TLC plate under Visible light

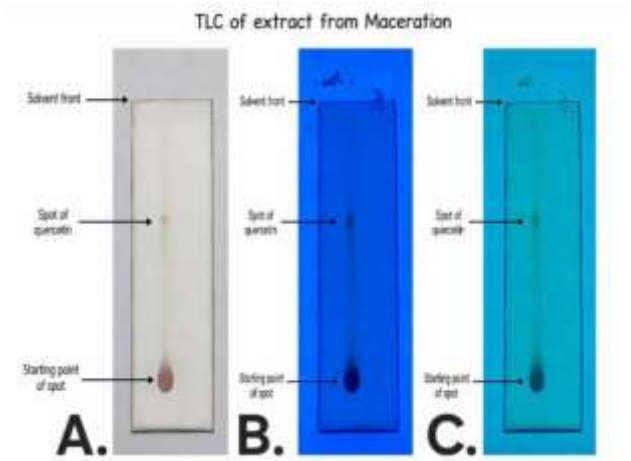

Figure No. 10 : TLC of extract from Maceration
A : TLC plate under Visible light
B : TLC plate under longer wavelength UV light
C : TLC plate under shorter wavelength UV light
Rf Value = 0.60

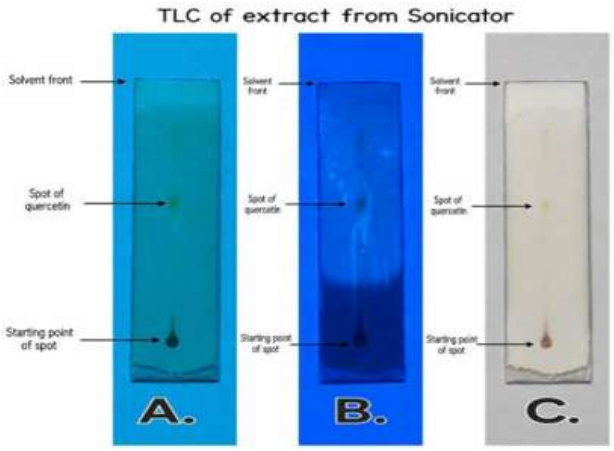

Figure No. 11 : TLC of extract from Sonicator
A : TLC plate under shorter wavelength UV light
B : TLC plate under longer wavelength UV light
C : TLC plate under Visible light
Rf Value = 0.47

HPLC Analysis:

- HPLC Standard Data:

Table No. 5 : HPLC Standard Calibration Data of Quercetin

Standard Concentration	Peak Area	Retention Time (Rt)
10ppm	826974	5.622 min
20ppm	2675575	5.569 min
30ppm	4112808	5.552 min
40ppm	5364659	5.526 min

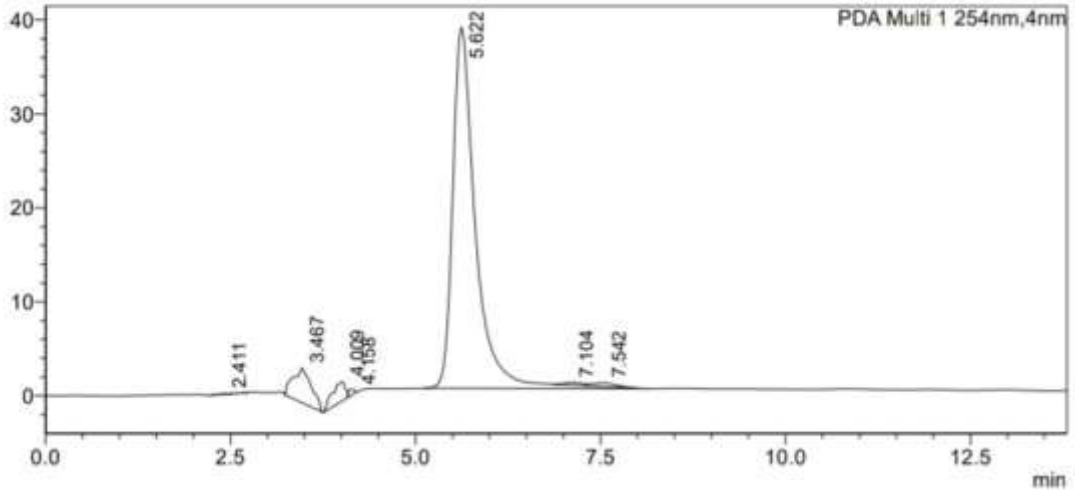

Figure No. 12 : Chromatogram of Standard 10ppm of Quercetin

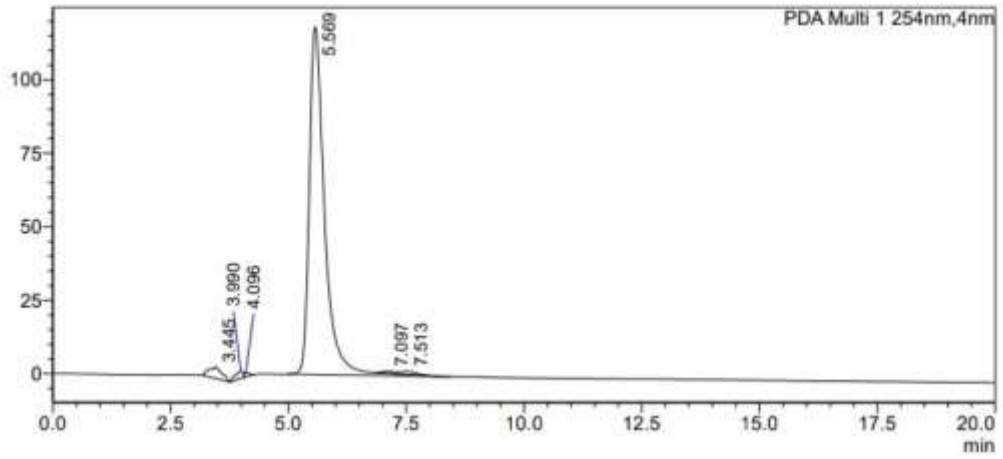

Figure No. 13 : Chromatogram of Standard 20ppm of Quercetin

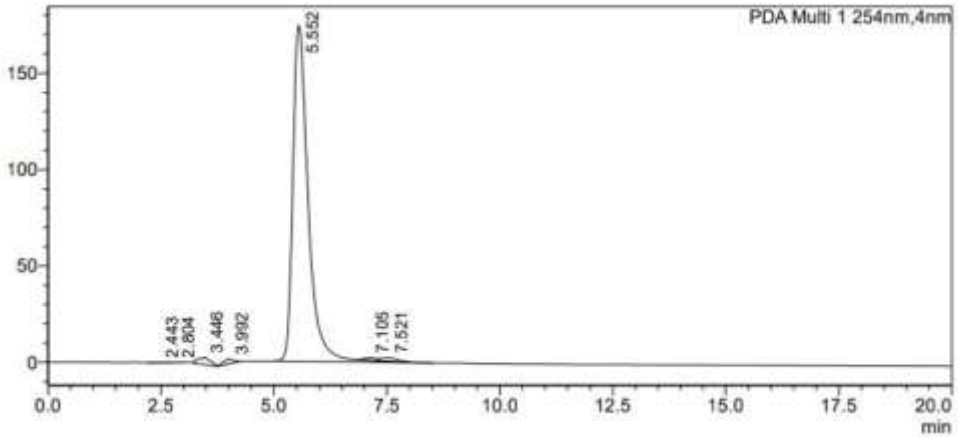

Figure No. 14 : Chromatogram of Standard 30ppm of Quercetin

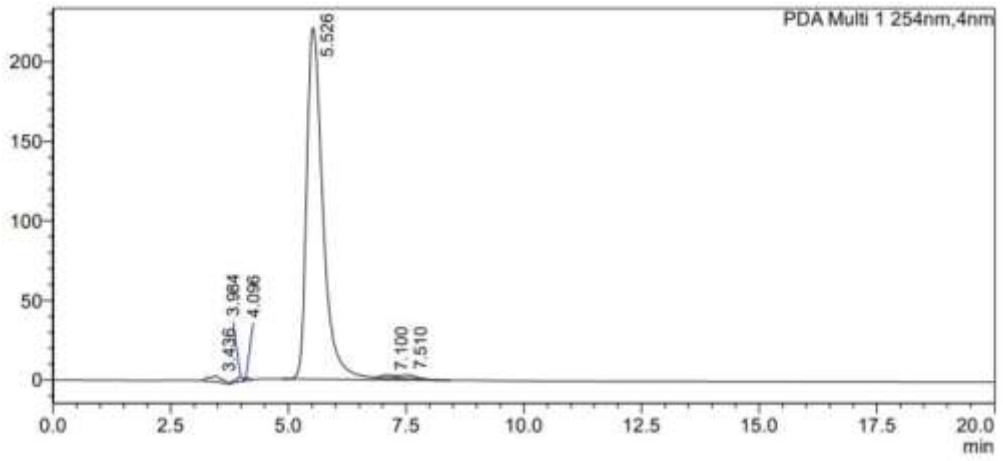

Figure No 15 : Chromatogram of Standard 40ppm of Quercetin

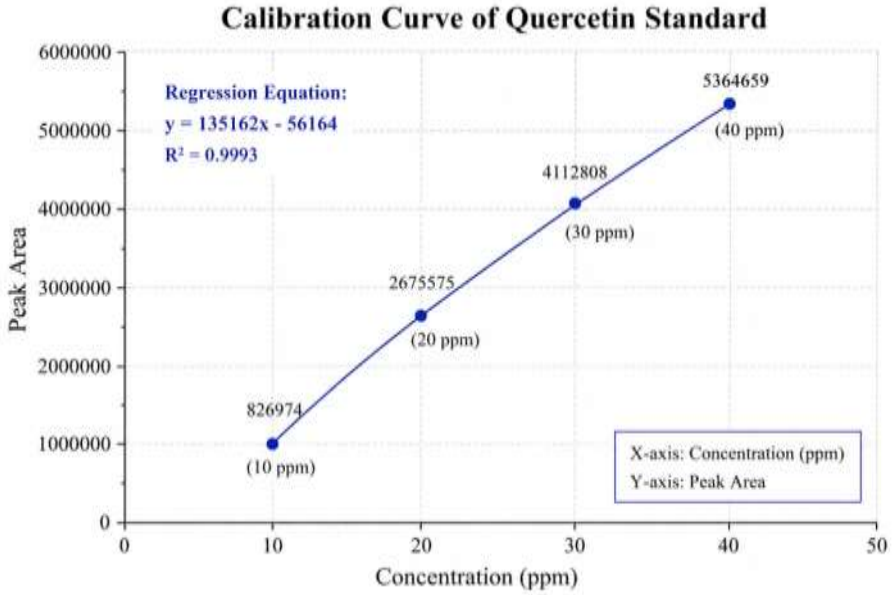

Figure No. 16: Calibration Curve of Quercetin Standard

Observation :

The calibration curve shows a linear relationship between concentration and peak area over the range of 10-40ppm.Detection wavelength: 370nm

Regression Equation :

y =133512x-58161

R2=0.9996

- HPLC sample data :

Table No. 6 : HPLC Sample Calibration Data of Quercetin

Sample Concentration	Peak Area	Retention time (Rt)
10ppm	48832	5.697
20ppm	66112	5.705
30ppm	35005	5.686
40ppm	51338	5.702

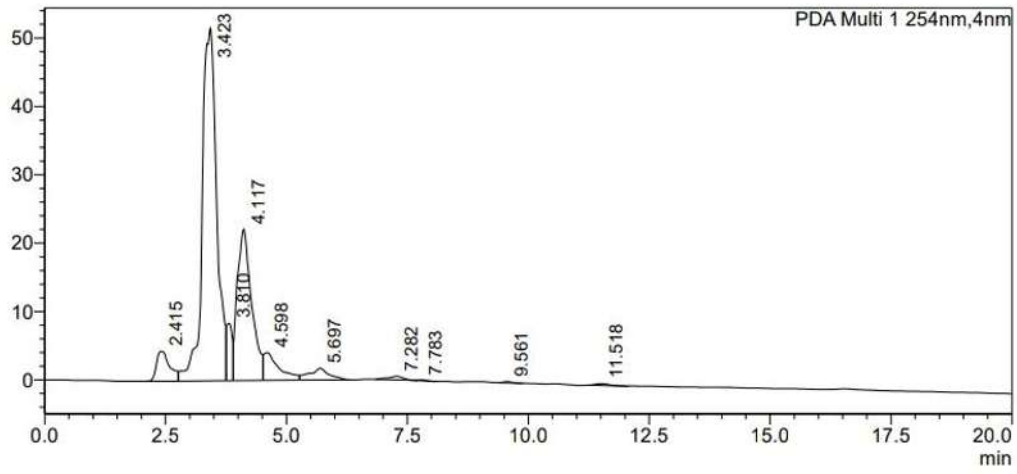

Figure No 17 : Chromatogram of sample of 10ppm concentration

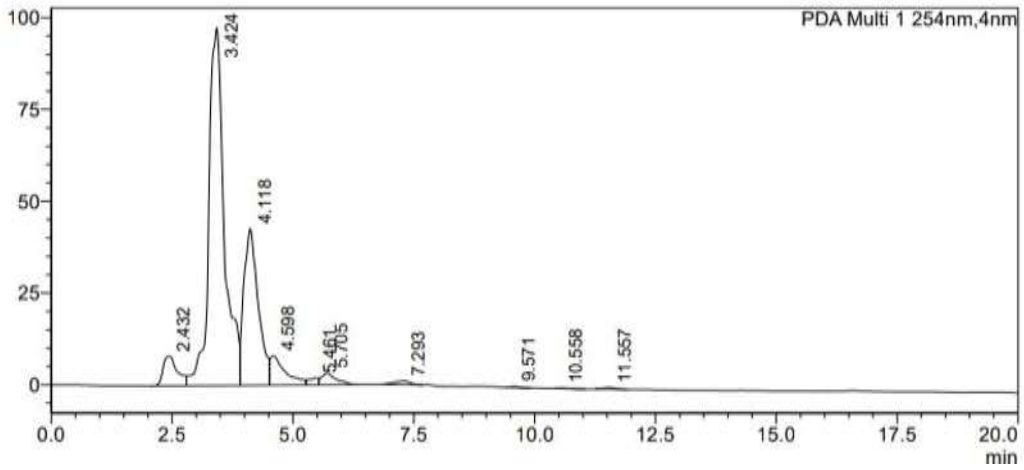

Figure No 18 : Chromatogram of sample of 20ppm concentration

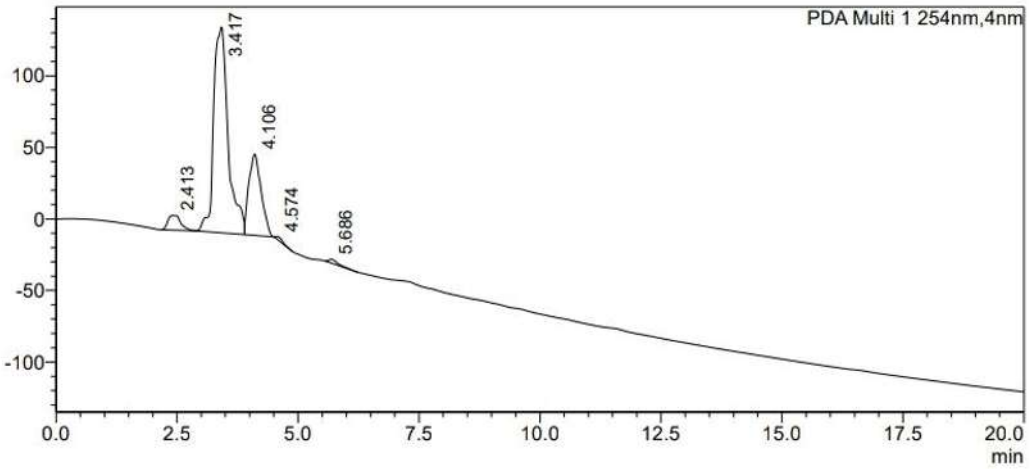

Figure No. 19 : Chromatogram of sample of 30ppm concentration

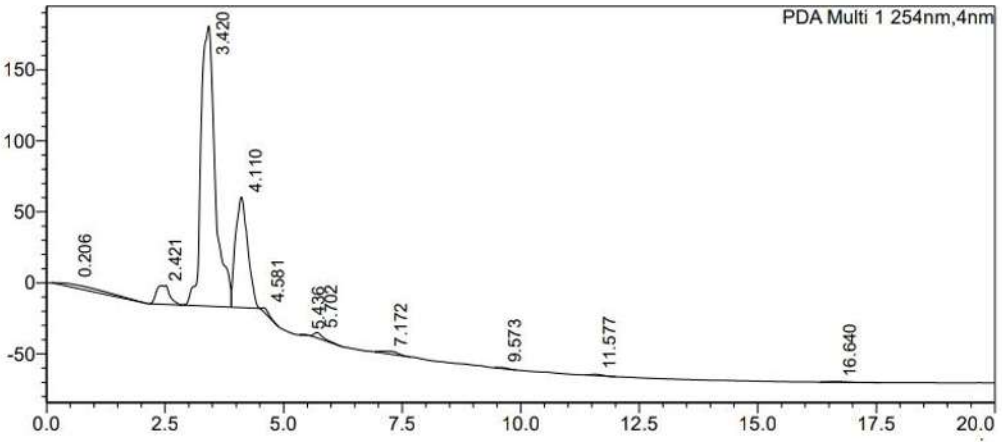

Figure No .20 : Chromatogram of sample of 40ppm concentration

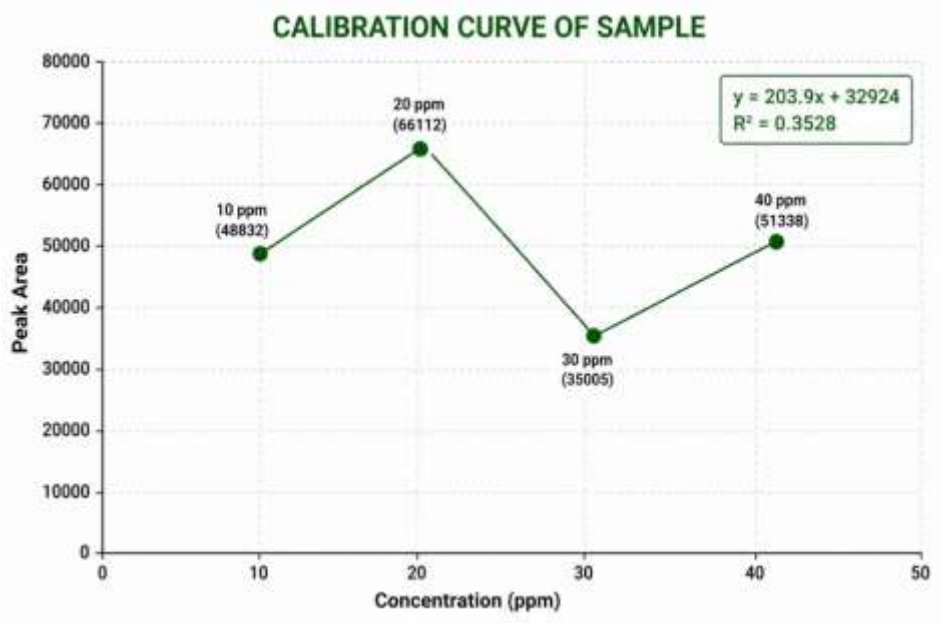

Figure No. 21 : Calibration curve of sample

Observation:

Sample chromatograms show peaks with retention times around 5.68-5.70 min, close to quercetin standard(near 5.5-5.6 min), confirming the presence of flavanoid compounds in the extract.

Regression Equation :

y= 203.9x+32924

R^2=0.3528

Result of HPLC Analysis :

The HPLC analysis of quercetin standard and sample was performed at 254 nm using Shimadzu LabSolutions. The standard showed a major peak at 5.622 min, while the sample showed a similar peak at 5.697 min, confirming the presence of quercetin in the sample of concentration 10ppm. The method showed good peak separation and was found suitable for quercetin identification and analysis.

In The standard chromatogram of concentration 20 ppm showed a prominent peak at retention time around *5.569 min*, confirming the characteristic peak of quercetin. In the sample chromatogram, major peaks were observed at different retention times, with significant peak intensity at 5.705 min, indicating the presence of quercetin and related phytoconstituents in the sample.The comparison between standard and sample chromatograms suggests that the prepared sample contains quercetin compound.

The standard Quercetin (30 ppm) showed a sharp, well-defined peak at RT 5.552 min with an area of 4,112,808, confirming its retention behaviour under optimized conditions. On comparison, the sample (30 ppm) showed a dominant peak at RT 3.417 min along with other minor peaks, indicating the presence of quercetin (peak at 5.686 min)with co-existing compounds in the sample matrix.

The standard Quercetin (40 ppm) showed a sharp, well-defined peak(5.526 min) confirming its retention behaviour under optimized conditions. On comparison, the sample chromatogram showed the presence of quercetin (at 5.702min) along with other co-existing compounds, indicating the

complex nature of the sample matrix. It can be concluded that the developed RP-HPLC method is accurate, precise, and reliable for the qualitative and quantitative estimation of Quercetin and can be successfully employed for routine quality control analysis of pharmaceutical and herbal formulations.

RESULT AND DISCUSSION:

The ethanolic extract of *Bauhinia purpurea* flowers was successfully produced using extraction techniques, with an extractive yield of 23.07% (30 g from 130 g powdered material).

Preliminary phytochemical screening and TLC separation confirmed the presence of flavonoids and phenolic compounds. The Shinoda, Zinc-HCl, Ferric chloride, Potassium dichromate, and Gelatin assays yielded positive findings. and providing Rf values in the range of 0.4 to 0.6. This suggests that quercetin, a flavanoid, is present.

The usual retention times of 5.5–5.7 minutes for both standard and sample chromatograms in the HPLC analysis using quercetin as the standard demonstrated the presence of flavonoid components in the extract. The calibration approach based on peak area and concentration assisted both the qualitative and quantitative assessment of flavonoids in the sample.

CONCLUSION:

The current study showed that Bauhinia purpurea flowers are a promising natural source of phenolic compounds and flavonoids.

Phytochemical screening verified the presence of significant bioactive components, and ethanolic extraction yielded a good result.

And by using TLC separation the presence of quercetin is verified .

Flavonoid chemicals in the floral extract were effectively identified by HPLC analysis using quercetin as a reference standard.

The results corroborate the traditional therapeutic significance of Bauhinia purpurea and imply that its floral extract may have beneficial pharmacological characteristics linked to flavonoids, including hepatoprotective, antibacterial, and antioxidant effects.

As a result, Bauhinia purpurea flowers may be used in medicinal, nutraceutical, and herbal applications.

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