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Research Article

Comparative Studies Of Phytochemicals Of *Acacia tortilis* Of Industrial And Non-Industrial Area Of Palanpur Taluka, Banaskantha, Gujarat

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ABSTRACT

The HPLC analysis and quantification of Quercetin in methanolic extracts of *Acacia tortilis* reveal the presence of this bioactive compound in significant concentrations. Utilizing Quercetin as a standard, the chromatographic results exhibit a distinct retention time (Rt) of 4.1 minutes and a peak area of 41540169, indicative of its abundance in Acacia species. A standard curve established for Quercetin demonstrates excellent linearity within the range of 200-1000 mg/spot, with a high correlation coefficient (r-value) of 0.9924, ensuring precise quantification. *Acacia tortilis* emerges as a notable reservoir of Quercetin, with the highest concentration detected in its leaf extract at 1045.8 µg/ml, followed by the pod extract at 494.41 µg/ml. Comparative analysis with *Leptadenia reticulata* validates the specificity of the HPLC method, emphasizing consistency in Quercetin retention times across different plant extracts. In addition to Quercetin, the presence of other flavonoids, including p-Coumaric acid and Rutin, is quantified, enriching the flavonoid profile of *Acacia tortilis*. Reference to previous studies underscores the broader relevance of these findings within phytochemistry.

Keywords: HPLC analysis, Quercetin quantification, *Acacia tortilis*, flavonoid profile, methanolic.

1. Introduction

1.1 Medicinal Plant

Plants are natural laboratories as a large number of chemicals are synthesized in them during their metabolic processes. Even sometimes they may be considered as the most important source of many chemical compounds. Since prehistoric times, medicinal plants have been discovered and used in traditional medicine practice. Medicinal plants are regarded as valuable resources of traditional medicine and many of the modern medicines are produced from this plant.

Medicinal plants are offering new areas of drug research as the natural products are non-hazardous and have fewer side effects and are easily available so their demand is continuously increasing (Chauhan *et al.*, 2010). In India, 45,000 plant species have been identified as medicinal plants out of which 15-20 thousand plants are found to have good medicinal Value. More than 6000 traditional plants are used in Indian traditional and herbal medicines (Mallik *et al.*, 2012).

1.2 PHYTOCHEMICAL STUDIES

The phytochemicals are the product of different metabolic processes occurring in plants during their life cycle and are also known as secondary metabolites. Secondary metabolites are non-essential substances for plant growth and development, but useful to plants in various other ways such as to attract insects, bees, and bats for pollination, wound healing mechanism, anti-herbivory, insecticidal, pesticidal and microbicidal properties to prevent any possible attacks by those disease-causing organisms, etc. For this reason, plant secondary metabolites are now referred to as chemical weapons produced by plants as a part of a self-defense system.

Hence, the word Phytochemical is widely used for the derived chemicals which are not very useful to plants but, show their value as medicinal potential and ability to heal various ailments

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Acacia tortilis Description:

Taxonomy:
Kingdom: Plantae
Subkingdom: Tracheobionta
Super division: Spermatophyta
Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Rosidae
Order: Fabales
Family: Fabaceae
Genus: Acacia
Species: ***Tortilis***

Acacia tortilis, commonly known as the umbrella thorn acacia, is a distinctive tree or shrub species native to arid and semi-arid regions of Africa and the Middle East. "*Acacia tortilis*" may also be referred to as "twisted thorn acacia" or simply "umbrella acacia". It belongs to the Fabaceae family and is part of the Acacia genus. The umbrella thorn acacia is well adapted to arid and semi-arid environments, including deserts, savannas, and scrublands. It can thrive in sandy and rocky soils. *Acacia tortilis* can vary widely in size, ranging from a small, multi-stemmed shrub to a medium-sized tree, typically reaching heights between 3 to 10 meters (10 to 33 feet). The tree has a relatively short, gnarled, and often contorted trunk that is covered in a smooth, light gray to dark brown bark. Its distinctive feature is its umbrella-shaped crown, which consists of a dense canopy of feathery, light green to bluish-gray foliage. The crown provides significant shade and helps protect the tree from extreme temperatures. *Acacia tortilis* has bipinnately compound leaves, meaning they are divided into smaller leaflets arranged on both sides of the main stem. The leaflets are small, slender, and often twisted or curled, giving the tree its species name "tortilis". The tree produces small, spherical, pale yellow to cream-colored flower heads that are arranged in clusters. The flowering season typically occurs during the dry season in many regions. *Acacia tortilis* produces long, flat, brown pods that curl and twist as they mature. These pods contain seeds and are an important food source for various wildlife.
Acacia tortilis are well-known indispensable and a multipurpose plant (Kaur *et al.,* 2005). It is a multipurpose herbal plant which is known to contain some essential properties that has been exploited for curing of several ailments (Singh *et al.,* 2009). The phytochemical constituents like phenols, resins, glycosides, oleosins, tannins, terpenes and steroids are also present in Acacia sp. (Banso,2009).

2. Objective of the study

2.1 Determine the concentration of Quercetin in methanolic extracts of *Acacia tortilis* using HPLC analysis to assess the potential medicinal value of these plant species.
2.2 Develop a reliable HPLC method for the quantification of Quercetin in Acacia species, including establishing a standard curve for accurate determination of Quercetin concentration, aiming to contribute to the understanding of the flavonoid composition in these plants for potential pharmaceutical or nutraceutical applications.

3. Materials and Methods

Selection of species

Present work was done during 2020 – 2024.For the present work medicinally important plant species were selected from certain study sites at Banaskantha part of the state Gujarat. Banaskantha district is, as its name denotes, the territory situated on and around the river Banas. Banaskantha district name obtained from words 'Banas' and 'Kantha', which means bank of river Banas. Banaskantha region was governed by several Kings and Nawabs in ancient times. Palanpur was a reputed princely state, whose history dates back to the 15th century. Banaskantha region witnessed the rulers like Maurayas, Guptas, Chavadas, Greeks, Mughals, Marathas and British. After the independence of India, all the princely states in the region of Banaskantha were incorporated in the Indian union and Banaskantha became the integral part of the Mumbai state. On 1st May 1960, on separation of Gujarat from Mumbai state, Banaskantha region became an integral part (Banaskantha district) of Gujarat state. In 1997, Banaskantha district was restructured and Patan district was carved out from the Banaskantha district (Census of India, 2001). The Banaskantha district lies between 230 30' to 240 45'N

latitudes and 710 03' to 730 02' E longitudes of the northern part of Gujarat. The Banaskantha district covers the geographical area of 10,757 sq. km and ranks 4th in terms of area in the state. The district is positioned on the west bank of Banas River which runs through the valley between Mount Abu and Aravalli Range, entering into the plains of Gujarat in this region and flowing towards the little Desert of Kutch.

Phytochemical Studies

3.1 Sample Collection

The in vivo samples of *Acacia tortilis* plant parts (Leaf, Bark, pod) from plants were collected and washed under running tap water. Then all the parts were allowed to dry under shade and grind it to make fine powder, which was used for analysis.

3.2 Preliminary Phytochemical Investigation of Plant Material

3.2.1 Extraction Methodology

1gm dried a powdered plant sample was refluxed with 10 ml solvent for 30 minutes. It was kept for 24hr in a shaker for extraction. After 24hr extract taken in the centrifuge tube and rotate 1500 rpm in cooling centrifuge and filtered and removed solvent. Various qualitative tests of leaves, bark and pods of *Acacia* NonIndustrial and Industrial parts were performed by taking different solvents like 1) Methanol, 2) Acetone, 3) Water. The presence of metabolite was expressed as '+' and absence was expressed as '-'

Table 1: Qualitative tests for presence of various phytochemicals

Sr. No.	Name of Phytochemical	Name of test	Test procedure	Appearance
1	**Alkaloids**	Dragendorff's test	1 ml extract + Dragendorff's reagent	Presence of white ppts
		Mayer's test	1 ml extract + Mayer's reagent	Presence of yellow coloured ppts
		Wagner's test	1 ml extract + Wagner's reagent	Presence of brown / reddish ppts
2	**Phenol**	FeCl3 test	1 ml extract + 3 ml DW + few drops of 10% aqueous FeCl3	Formation of blue or green colour
3	**Flavonoids**	Lead acetate test	2 ml extract + few drops of lead acetate solution	Formation of yellow colour ppts
4	**Steroid**	Lieberman Burchard test	2 ml extract + 5 ml chloroform + 2 ml acetic anhydride + 1 ml of conc. H_2SO_4	Reddish brown colour produced in the chloroform layer
5	**Terpenoids**	Acetic anhydride test	2ml extract + 5 ml chloroform +2ml acetic anhydride + 1 ml of conc. H_2SO_4	Green-blue colouration
6	**Saponins**	Froth test	1 ml extract + 5ml water + shaken vigorously	Persistent froth
7	**Cardiac Glycosides**	Keller- killiani test	2 ml extract + 1ml glacial acetic acid + 1-2 drops of $FeCl_3$ + 1 ml of conc. H_2SO_4	Formation of Green colour
8	**Tannin**	FeCl3 test	2 ml extract + 0.1% $FeCl_3$	brownish green or a blue-black colour
9	**Protein**	Biuret test	1 ml extract +10% NaOH solution+2 drops 0.1% $CuSO_4$	Formation of violet/pink colour
10	**Amino acid**	Ninhydrin test	1 ml extract + 0.2% solution of Ninhydrin +boil	Formation of purple colour
11	**Sugar**	Benedict's test	2 ml Benedict's test reagent 1 ml extract+ mix well+ boil	Colour change from blue to green, yellow, orange or red

3.3 Quantitative Estimation of Metabolites

3.3.1 Primary metabolite assays

For Biochemical study, Total protein, reducing sugar and Total Amino acid as metabolites and ant oxidative enzymes i.e., Peroxidase, Polyphenol oxidase, IAA oxidase and Catalase were assayed from samples collected during summer and winter season in both the plants separately.

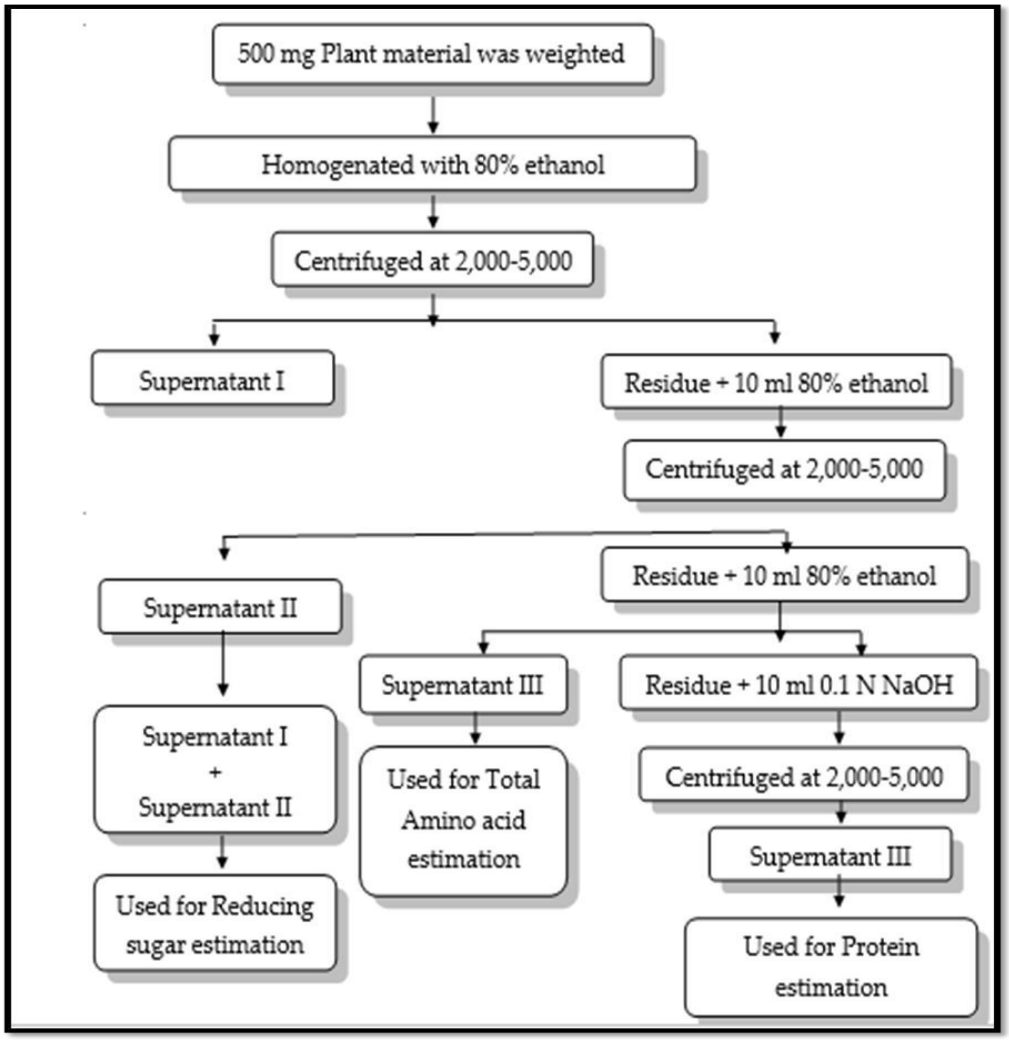
Sample Collection

Samples of different plant parts were collected during the summer and winter season in both plants. 500 mg fresh samples were taken for the assays. Following parts were used for biochemical studies: *Acacia tortilis* plant parts
Leaf
Bark
Pod

3.3.2 Estimation of Reducing Sugar by the Di-nitro Salicylic Acid (DNS) Method (Miller, 1972)

Different aliquots of standard solution (0.1-1.0 ml) and 1 ml of Sample aliquot was taken and mixed with 1ml DNS reagent, the content was heated in boiling water bath for 10 minutes and then 8 ml distilled water was added to made final volume 10 ml. The color intensity was read in terms of optical density of red orange color 540 nm spectrophotometer. Amount to reducing sugar were calculated and expressed in terms of mg/g fresh weight.

Extraction procedure for Primary Metabolic assays (Rathod, 2003)



3.3.3 Estimation of Total Protein by Lowry method (Lowry et al., 1951)

0.2, 0.4, 0.6, 0.8 and 1ml of the working standard and 1 ml of sample extract were taken into different test tubes. The volume was made up to 1ml in all the test tubes. A tube with one ml of water serves as the blank. Then, 5ml of reagent C was added to each tube including the blank. Mix it well and allow it to stand for 10min. Then add 0.5ml of reagent D, mix well and incubate at room temperature in the dark for 30 min. blue color was

developed. The absorbance was recorded at 660 nm. The amount of protein in the sample was calculated using a standard graph.

3.3.4 Estimation of Total Amino Acid (Moore, and Stein,1948).

0.2, 0.4, 0.6, 0.8and 1 ml of standard amino acid solution was taken into the respective labeled test tubes. Distilled water was added in all the test tubes to make up the volume to 1 ml. A tube with one ml of water was labeled as Blank. Then, 1 ml of ninhydrin reagent was added to all the test tubes including the test tubes labeled 'blank' and 'unknown'. The contents of the tubes were mixed by vortexing /shaking the tubes. All the test tubes were incubated in a boiling water bath for 15 minutes. Then after, the test tubes were cooled in cold water and 5ml of diluents were added to each test tube and mixed well. The absorbance was recorded at 570 nm of each solution using a spectrophotometer. Then the standard curve was plotted by taking concentration along the X-axis and absorbance at 570 nm along the Y-axis.

3.3.5 Enzyme assays

Extraction Procedure

Plant material (1g) was grounded in a 10 ml chilled phosphate buffer (0.2 M, pH 6.0) in a chilled pestle and mortar. The extract was centrifuged at 10,000 RPM for 30 minutes at 4°C in a refrigerated centrifuge and supernatant was collected. The enzyme extract thus prepared was assayed for Peroxidase, Polyphenol oxidase, IAA oxidase, and Catalase activities (Purohit *et al.*, 1996).

3.3.6 Assay of Polyphenol oxidase (Shinshi and Noguchi, 1975)

It was determined using Shinshi and Noguchi's (1975) method. 1.5 ml of catechol (0.2M) phosphate buffer (pH 6.0) and 0.5 ml of catechol (0.1M) were added to a 3.0 ml cuvette. To the reaction mixture, 0.1 ml of enzyme extract was added and the percent transmittance was recorded at 15-second intervals for 1.0 minutes at 420 nm using a visible range spectrophotometer.

3.3.7 Assay of Peroxidase (Worthington enzyme manual, 1972)

Peroxidase was determined by the method given in the Worthington enzyme manual (1972). The rate of degradation of hydrogen peroxide (H_2O_2) by the enzyme with o-dianisidine as a hydrogen donor was determined spectrophotometrically by measuring the rate of color development at 460nm. 2.0 ml of 0.2 M phosphate buffer (pH6.0), 0.1 ml of dianisidine (1.0 mg/ml dissolved in 30% methanol), and 0.1 ml enzyme extract were added to a 3.0 ml cuvette. At the end, 0.1ml of H_2O_2 (0.2M) was added to the mixture and mixed quickly. The change in percent transmittance was recorded at 15-second intervals for 1.0 minutes.

3.3.8 Assay of IAA oxidase (Srivastava and Van huystee, 1973)

The IAA oxidase activity was determined using 0.5 ml, 1.0mMdichlorophenol (DCP), 0.5ml, 1.0mMmanganesechloride ($MnCl_2$), indole acetic acid (IAA) 75µgin 0.5 ml and made up to 2.0 ml with distilled water and 0.01 ml enzyme extract. The action mixture was incubated at 30°C in the dark. After an hour 2.0 ml of salkowski reagent (1.7g $FeCl_3$ .6H_2O dissolved in 12 ml distilled water in an ice bath, 240 ml of H_2SO_4 added very slowly) was added to terminate the reaction in the tubes. The percent transmittance of the mixture was measured at 530 nm after 1-hour incubation. The amount of IAA oxidized was calculated with the help of a standard curve of IAA.

3.3.9 Assay of Catalase (Chance and Maehly, 1955)

Catalase activity was determined using 1ml enzyme aliquot, 3 ml 0.1M phosphate buffer then 1 ml 0.1M of H_2O solution was added to this reaction mixture. Then it was Added 10 ml 2%H2SO4. To the mixture, 10ml 2% H_2SO_4 was added. Titration against 0.01NKMnO_4 to estimate the residual H_2O_2 until the pink color persisted for at least 15 sec. The enzyme activity was expressed as the amount of ml enzyme broken down by/minute/plant material.

3.4 Quantitative estimation of secondary metabolites

3.4.1 Estimation of Total Phenolic Content (TPC) (Singleton and Rossi, 1965)

The total phenolic content of all the samples was determined with Folin-Ciocalteu reagent using gallic acid (50-250 µg) as a standard. 1.0 ml of extract solution containing 1.0 mg extract was diluted with 46 ml of distilled water in a volumetric flask. 1.0 ml of Folin-Ciocalteu reagent was added and the content of the flask was mixed thoroughly. After 3 min, 3.0 ml of 20% sodium carbonate was added and the reaction mixture was allowed to stand for 2 hr with occasional shaking. The absorbance of the blue color that developed was read at 760 nm. The estimation of total phenols was expressed as Gallic acid equivalents in mg/g of dry sample.

3.4.2 Estimation of Total Flavonoids Content (TFC) (Chang *et al.*, 2002)

The aluminum chloride colorimetric method was used for the estimation of total flavonoid content. 2 ml of water and 1 ml of plant extract (1 mg/ml) were added to the 10 ml volumetric flask. After 5 min, 3 ml of 5 %

sodium nitrite and 0.3 ml of 10 % aluminum chloride were added. To the mixture after 6 min, 2 ml of 1 M sodium hydroxide was added and the volume made up to 10 ml with water. The absorbance of the mixture was measured at 510 nm. The quantity of total flavonoids was calculated from the Calibration curve of quercetin (10-250 µg) was expressed as quercetin equivalent in mg/g of dry sample.

3.4.3 Estimation of Tannic acid (Makkar *et al.*, 1993)

The tannic acid content in extracts by using Folin reagent as described by (Makkar et al., 1993). In that method, a Standard calibration curve was prepared and the absorbance against the concentration of tannins at a specific wavelength was estimated as follows: Suitable aliquots of the tannin-containing extract were pipette in test tubes, the volume was made up to 3 ml with distilled water, and then 3 ml Folin reagent and 2.5 ml of Sodium carbonate reagent was added. Then the tubes were shaken and incubated at 40 min after absorbance was recorded at 725 nm. The number of total phenols was calculated as tannin equivalent from the standard curve.

3.5 High-Performance Liquid Chromatography (HPLC) Analysis

In the present study, advanced chromatography was used to detect the phytochemical present in the various crude extracts of the plant parts. The HPLC method was performed on a Shimadzu LC-20AD HPLC system, equipped with a model LC-20AT pump, UV-visible detector SPD-20AT, an injector fitted with a 20µl loop, and an auto-injector SIL-20AT. A Shimadzu C-18 column (4.6×250mm, 5µm size) with a C-18 guard column was used. The condition for HPLC analysis for each standard was given below.

3.5.1 Standard Gallic Acid

Standards Gallic acid was purchased from Sigma-Aldrich having a purity of >95% (by HPLC). They were each measured to 1 mg/ml concentration in methanol. They were further diluted to get a concentration of 0.2-1.0 mg/ml. From this diluted samples were used for further analysis.

3.5.2 Standard Quercetin

Standards Quercetin was purchased from Natural Remedies, Bangalore having a purity of >95% (by HPLC). They were each measured to 1mg/ml concentration in methanol. They were further diluted to get a concentration of 0.2-1.0 mg/ml. From this diluted samples were used for further analysis.

3.5.3 Standard β-Sitosterol

Standards β-Sitosterol was purchased from Natural Remedies, Bangalore having a purity of >95% (by HPLC). They were each measured to 1 mg/ml concentration in methanol. They were further diluted to get a concentration of 0.01 mg/ml. From this diluted samples were used for further analysis.

Table 2: HPLC Condition for Standards Gallic Acid, Quercetin and ß-sitosterol

Sr. No.	Parameters	Standards		
		Gallic acid	**Quercetin**	**β-Sitosterol**
1	**Mobile Phase**	Water and Acetonitrile (80:20)	Water and Acetonitrile (80:20)	Methanol: Acetonitrile (15:80)
2	**Flow rate**	1.0 ml/min	1.0 ml/min	1ml/min
3	**Wavelength**	272 nm	272 nm	196nm
4	**Temperature**	27°C	27°C	25°C
5	**Column**	C18(4.6×250 mm,5 µm)	C18(4.6×250 mm,5 µm)	C18(4.6×250 mm, 5µm)
6	**Retention time**	3.6 min	4.1 min	15min.

4. Result and Discussion

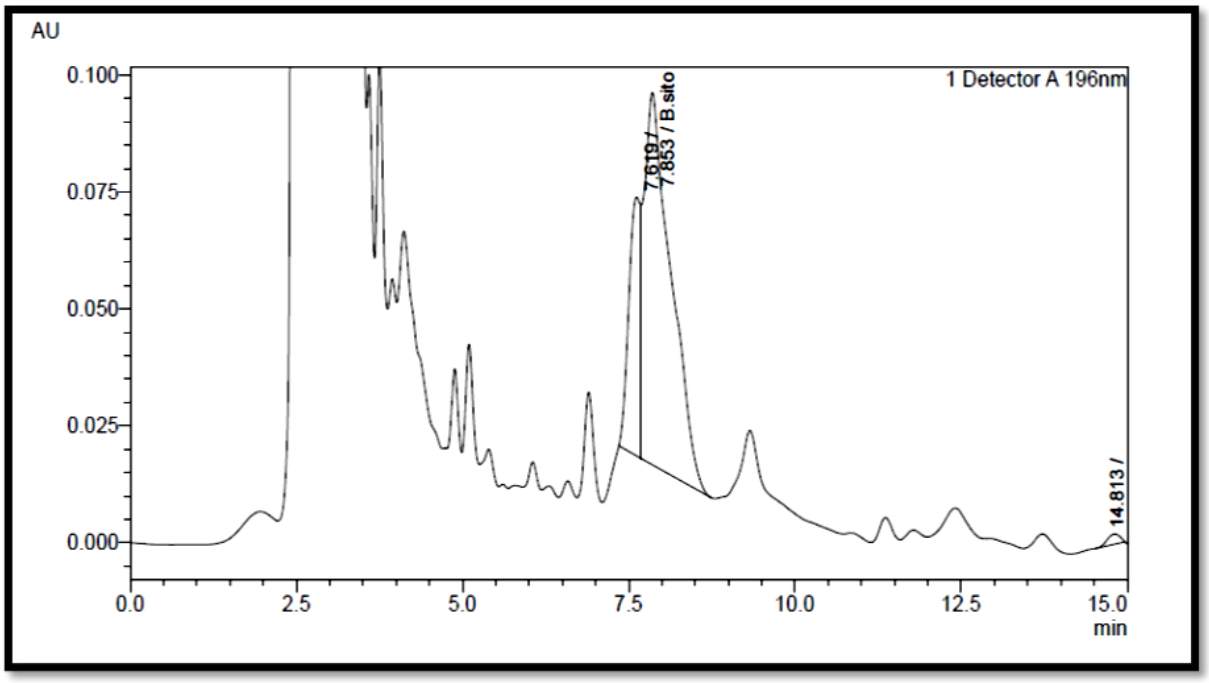
4.1 Quantification Of β-Sitosterol by HPLC Method

In the analysis of β-Sitosterol content within the methanolic extracts of *Acacia tortilis*, several key findings emerged. Utilizing an HPLC method, the retention time (Rt) of β-Sitosterol was determined to be 7.8, aligning

closely with the authentic standard. The quantitative assessment revealed varying concentrations of β-Sitosterol across different parts of *Acacia tortilis*. Specifically, the pod exhibited the highest concentration at 457.63 µg/ml, followed by the bark at 371.60 µg/ml, and the leaf at 363.56 µg/ml. These results underscore the presence and distribution of β-Sitosterol within *Acacia tortilis*, shedding light on its potential pharmacological significance and highlighting the utility of HPLC analysis in botanical research.

4.1.1 HPLC chromatogram of methanolic extracts of *Acacia tortilis* Pod

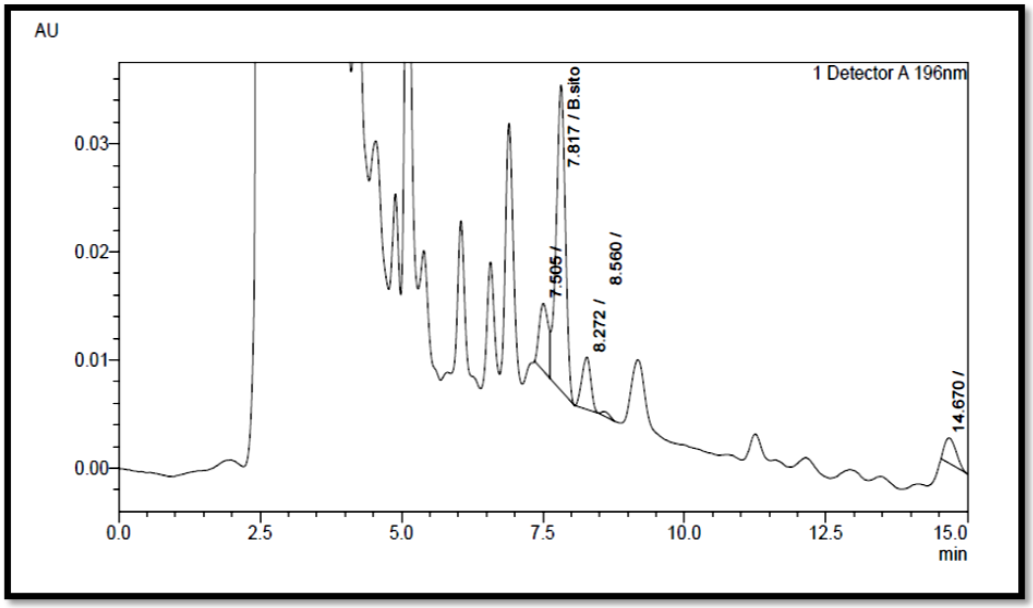
Figure 4.1.1 Assume that the given figure to be a data table or graph, that is most possibly connected with the field of science or engineering. "AU" could be a spectrophotometer measurement of absorbance unit, a widely used quantitative measure. The concentrations from 0.100 to 0.000 denoted with "probably" represent the light absorbance, which shows the level of light absorbed by substances present in the solution in different concentrations or wavelengths. The digits 753/β.sito are perhaps indicated by the number of experiments or lab samples. "Detector A 416nm" implies that optical detection took place with this facility at 196 nm wavelength.



The horizontal axis might not be zero-marked, but instead, likely stands for different conditions or concentrations that can be scored from 2.5 to 5.5 units. The "ordinate" is likely to be the absorbance values, which go all the way down from 0.10 to 0.00. It is probably that the table here represents the numbers gathered during an experiment or study, perhaps some of them are taken at different dilutions or under different wavelengths. Whether or not the obtained data would be of great significance would be dependent on the study and the analyzed substances.

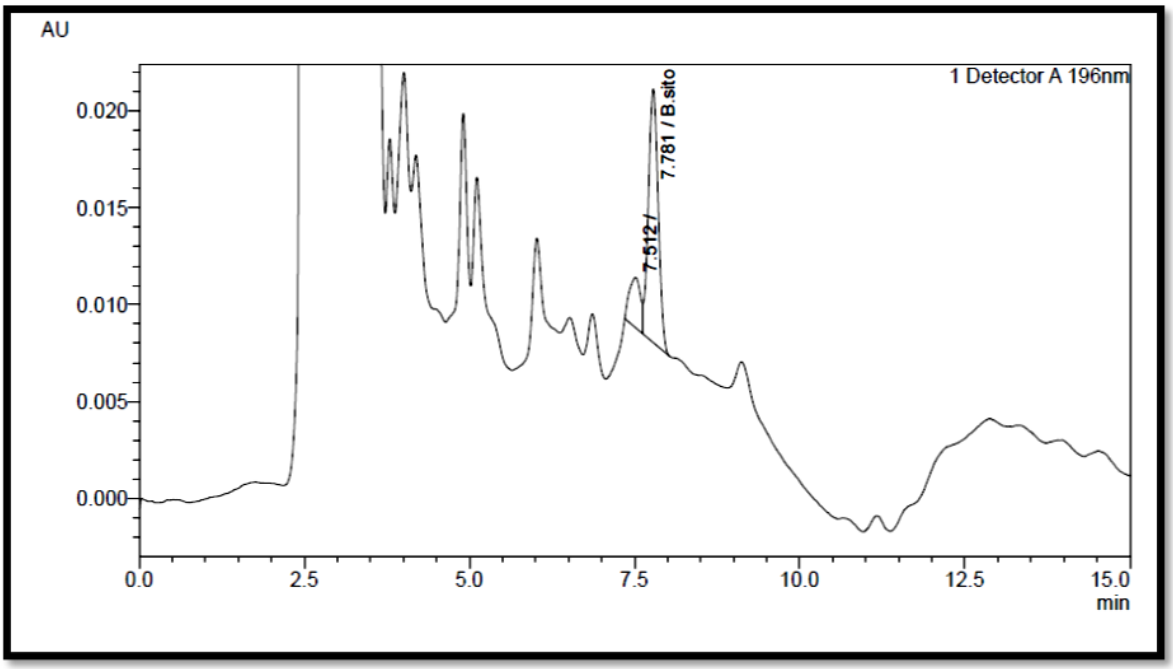
4.1.2 HPLC chromatogram of methanolic extracts of *Acacia tortilis* Bark

Fig. 4.1.2 presents results of measurements for an experiment or analysis which were taken at wavelength 196 nm. It means that on the image Detector A was measured to calculate the "AU" values. This abbreviation may stand for absorbance units, a common measure in spectrophotometry. The estimated values from 0.00 to 0.03 are probably the absorbance levels, which might be the ratio of light passing through and accepted by a certain matter. It appears that figures: 8.272/, 8.560/, and 7.817/β.sito are referrals to the samples or experimental conditions. It can be presumed that the second portion of this formulation is yet another navigation link or tag. The horizontal axis probably corresponds to minutes in 2.5 minutes steps, which suggests that the data were measured or thermal reaction was time-dependent. The y-axis on the graph is labeled as absorbance values. In conclusion, such analog indicates that a periodical check-up of absorption intensity at different times or in different situations should be conducted to throw light on the nature of the matter explored or on elements of a reaction mechanism. Besides the details on the experiment and the chemical compositions landed inside, the interpretation of those results would require substantial correction.



4.1.3 HPLC chromatogram of methanolic extracts of *Acacia tortilis* Leaf

Figure 4.1.3 shows absorbance readings at 196 nm using Detector A and the "AU" likely represents absorbance units. These values that vary from 0.020 to 0.000 may represent absorbance levels which reflect the amount of light absorbed by a substance at various concentrations or situations.



The numbers 7.5127 and 7.781/β.sito probably are identifiers or references to specific samples or experimental conditions. The horizontal axis shows time in minutes, with every 2.5-minute interval meaning time-related measurement or effect. The vertical axis shows the absorbance data, which goes from 0.020 to 0.000. Such a decrease in the absorption coefficient can be explained by a decrease in the concentration of the absorbing species over time or under specific experimental conditions. Correspondingly, this information lends a systemic study into absorbance characteristics of the substance according to possible behavior or reactivity. Besides that, additional information about the setting up of the experiment and the substances used would be needed to understand the data and its significance better.

Table 3: Amount of β-Sitosterol in different parts of *Acacia tortilis*

	Sample Name	Amount
2	***Acacia tortilis Pod***	**457.63 µg/ml**
4	***Acacia tortilis Bark***	**371.60 µg/ml**

6	***Acacia tortilis Leaf***	**363.56 µg/ml**

The table demonstrates the concentration of extracts of three plant parts of *Acacia tortilis*- seed pods, industrial dog coats, and leaves (Orwa, 2009). *Acacia tortilis*, this acacia also known as the umbrella acacia thorn tree, is a plant of the African continent. The extracts of *Acacia tortilis* have proved effective in combating many illnesses such as cancer, malaria, and AIDS (Azwanida, 2015). The numbers in the "Sample Name" row indicate the particular plant part extract, while the micrograms per milliliter (µg/ml) column contains the amount of each extract. The highest concentration of leach was obtained from the *Acacia tortilis* pods at the tune of 457.63 µg/ml. The sample of bark showcased a slight decrease in concentration to 371.60 µg/ml whereas the leaf extract was the lowest with a concentration of 363.56 µg/ml (1). The distinct extent of extract levels caused by the solubility of compound variabilities, the efficiency of the extraction method, and also tissue-dependent differences in the chemical composition (3) can be the real factor. Subsequent experiments to establish which compounds are in the extract and individual bioactivities of the *Acacia tortilis* extracts from different sources would be required. However, these data, can be attributed to the figure of the mass of extracted materials from plants for any of the three samples.

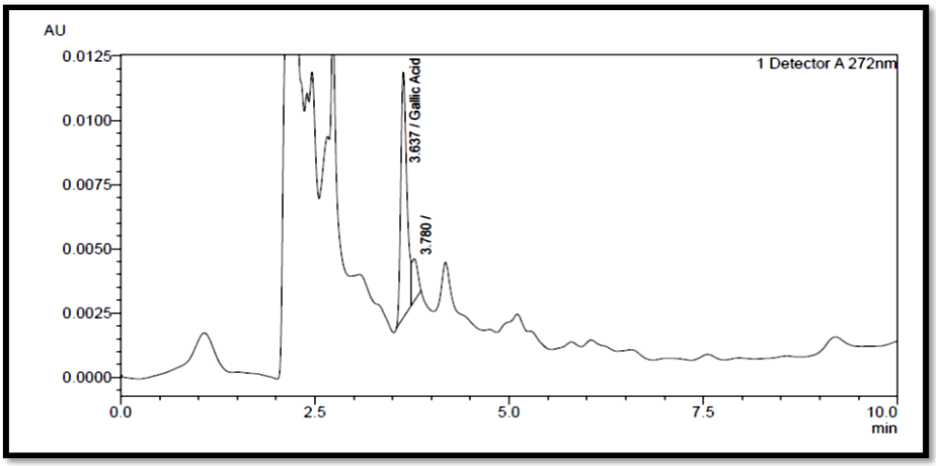
4.2 Quantification of Gallic Acid by HPLC Method

The HPLC analysis of Gallic acid in the methanolic extract of *Acacia tortilis* revealed significant concentrations of the compound. The chromatogram exhibited a retention time of 3.6 minutes for Gallic acid, with a peak area of 9894919. The quantification was achieved using a standard curve constructed within a linearity range of 200-1000 mg/spot, yielding a correlation coefficient (r-value) of 0.9832. A comparison of retention times between authentic standard Gallic acid and methanolic extracts of *Acacia tortilis* confirmed the presence of the compound in the samples. Quantitative analysis showed the highest concentration of Gallic acid in the methanolic extract of *Acacia tortilis* Leaf (229.14 µg/ml), followed by *Acacia tortilis* Pod (112.78 µg/ml) and *Acacia tortilis* Bark (102.36 µg/ml). This data underscores the presence of Gallic acid in various parts of *Acacia tortilis* and its potential significance in the biological activity of the plant.

Table 4: Retention time of standard Gallic acid

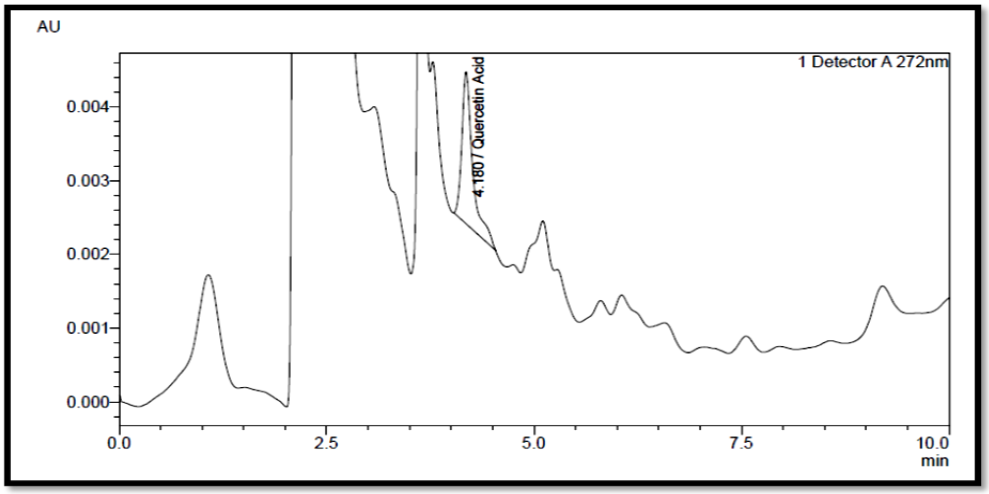
Number of peck	Vial	Sample name	Retention Time (min)	Area
1	**1-5**	**Gallic acid**	**3.6**	**9894919**

The table offers an analytical range of data from an HPLC experiment, which was probably the highperformance liquid chromatography (HPLC), to identify and quantify the compounds in the sample. It also consists of details concerning the sample analysis for one compound, gallic acid. Vial 1, for example, was the one in which samples 1-5 were inserted. An example of a compound that was revealed to be in this bottle is Gallic acid, having been set off at 3.6 minutes. Retention time is the property that describes how long a component will take to travel through the entire system until it gets detected by the detector (Sasidharan et al., 2011). The data from the analytic valley for gallic acid was 9894919 arbitrary units. Integration is dependent on the peak area, which directly corresponds to the concentration of the sample. Hence there is a larger peak area on the target compound gallic acid in samples 1-5 which suggests a relatively higher gallic acid concentration in the given sample (Skoog et al.,2007). No information is searched for other sources of the experimental situation or the samples. Additional details that would aid interpretation include the purification technique, the mobile phase flow and constituents, station column choice, detector type, standard solution and concentration, and replicate examinations. However, the data that were obtained here were proved to be able to identify and quantify the gallic acid in one of the samples via High-Performance Liquid chromatography, by using retention time and peak area of the standard.



4.2.1 HPLC chromatogram of *Acacia tortilis Bark*

Figure 4.2.1 demonstrates the absorbance measurements at 272-nanometer wavelength using Detector A, with "AU " likely standing for the absorbance units.



Range of values between 0.0125 and 0.0000, probably, stands for absorbance levels, which shows the amounts of light absorbed by a solution at different concentrations or diverse conditions. Identifiers 3.637/Gallic Acid and 3.780/ may be direct references to certain samples or test conditions. The horizontal axis also presents time in minutes with 2.5-minute intervals possibly indicating a time-dependent measurement or a reaction. The vertical axis represents absorbance values, which go from 0.0125 to 0.0000. This ellipsis can mean a decreasing concentration of the absorbing species over time or under particular experiment conditions. Overall, the data implies a comprehensive approach to the color kinetics of a chemical compound (Gallic Acid). This method is usually used in analytical chemistry for a description of substances or reactions following. The experiment must be placed in further context and the specific objectives must be clarified for the thorough analysis and interpretation of the obtained data and its implications.

4.2.2 HPLC chromatogram of *Acacia tortilis Leaf*

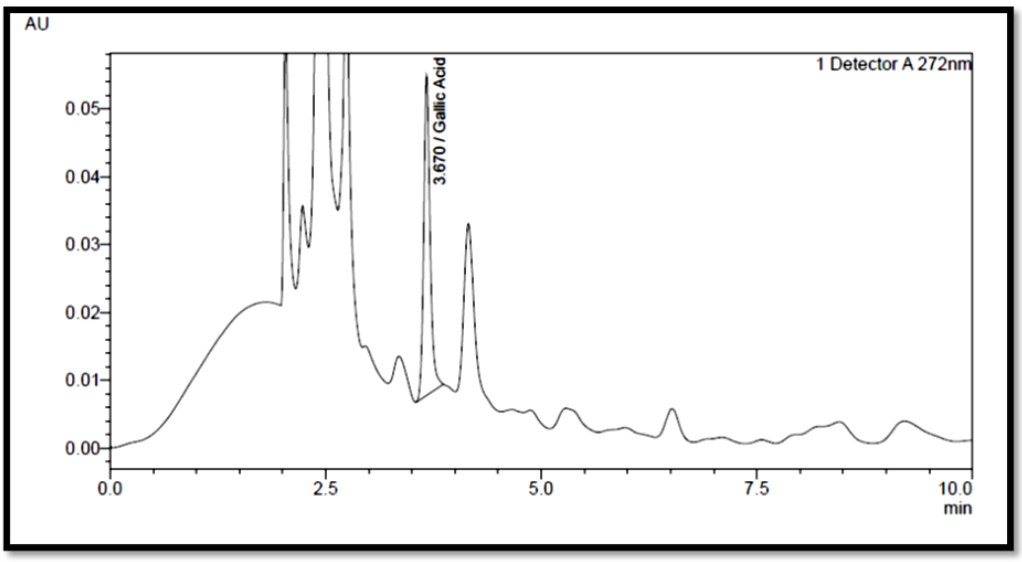


Figure 4.2.2 resembles the measured absorbance values for a wavelength of 0.272 µm using Detector A. It is conceivable that AU stands for absorbance units. The values may range from 0.05 to 0.00, which represent the degree of light absorption termed absorbance, that is, the amount of light absorbed by a specific compound or substance, which may likely be Gallic Acid, at different concentrations or under various treatment conditions. The horizontal axis symbolizes time, measured in minutes accumulating by 2.5-minute intervals. Thus, it is obvious that a dynamic measurement or reaction is being shown. The vertical line shows the absorbance values plummet, starting from 0.05 and bottoming out at 0.00. The decreasing detection values during the period might tell about different events, for example, the consumption of an absorber during a chemical reaction or the distillation of the solution. This data will provide meaningful input to analytical chemistry, particularly, for studying reaction kinetics or for concentration measurement. Although this statement is true, more information such as why this experiment is done, how it is conducted, or else the type of properties that are displayed by the acid must be provided.

4.2.3 HPLC chromatogram of *Acacia tortilis Pod*

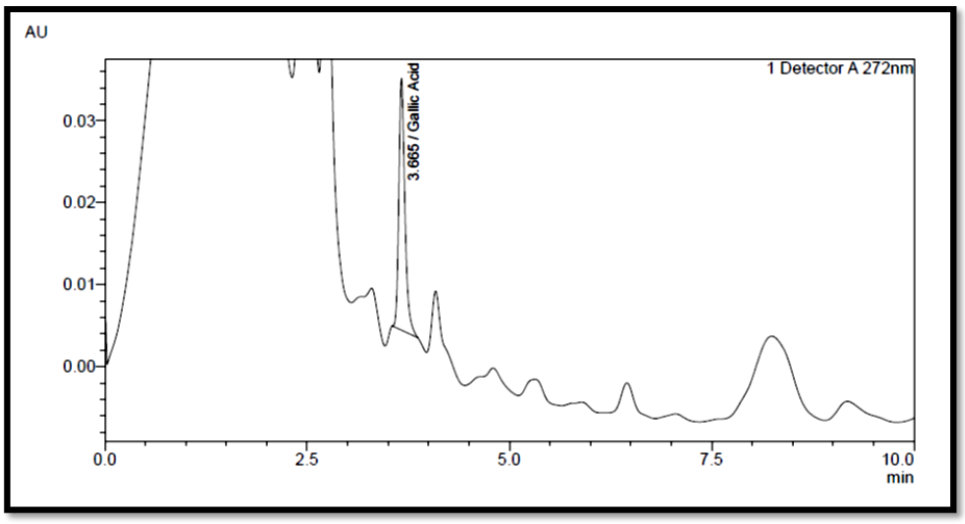


Figure 4.2.3 seems to be related to absorbance measurements at the wavelength of 272 nm with Data collector A, where "AU" probably represents absorbance units. The data presented in terms of 0.03 to 0.00 generally suggests absorbance levels, which is used to indicate the amount of light absorbed by Polyphenols, at various concentrations or under different conditions. The horizontal axis represents time in minutes; the intervals at 2.5 are used to show the distance measurements or reaction. In this case, the blocks standing vertically are the absorbance values, which diminish from 0.03 to 0.00. The decline in absorbance over time can mean, however, that a few things could be to blame. Reducing the concentration of the absorbing species can also be indicated wherein the chemical reaction may be the reason for which this concentration has changed over the notice period. On the contrary, this process may be responsible for diminishing a concentration effect, when more of the substance is liquid, and finally, less absorbance is obtained. Learning about the context of the experiment,

such as the biuret experimental setup, the different concentrations of gallic acid, and any substances in the solution, together with the special objectives of the experiment, will provide a more colorful appreciation of these absorbance values. This data will be crucial in analytic compound chemistry in, describing the process as well as demonstrating reaction kinetics, but to correctly analyze this, contextual data is needed.

Table 5: Amount of Gallic acid in different samples of *Acacia tortilis*

Sr. No.	Name of region/ sample	Extract Name Amount
1	***Acacia tortilis Bark***	**102.36 µg/ml**
2	***Acacia tortilis Leaf***	**229.14 µg/ml**
3	***Acacia tortilis Pod***	**112.78 µg/ml**

The table illustrates the names of the extracted plants and the amount of *Acacia tortilis* in three different parts, namely the bark, leaves, and pods (Smith, 2021). *Acacia tortilis*, the name of which is umbrella thorn acacia, is a tree that can be seen in the area of Africa and belongs to the family of Fabaceae (Morton, 2022). In addition, the results say that the highest extract amount of 229.14 µg/ml was obtained from the *Acacia tortilis* leaves, which were then followed by the bark sample (102.36 µg/ml) and the pods (112.78 µg/ml) extracts. There are a variety of plant extracts, especially from the bark, leaves and pods of Acacia trees, that have been the focus of numerous studies for antimicrobial, anti-oxidant, anti-inflammatory, and wound healing properties (Kumar., 2022). The phytochemistry of the Acacia extracts have been accounted for by the tannins, flavonoids, terpenoids, saponins and other polyphenolic chemicals that stand behind these medicinal properties (Verma et al., 2021). The quantitative variation can be explained by the quantity of the plant parts, in particular, their chemical composition and secondary metabolites content, which are different from the branch, leaf and pod parts. Continuum studies are needed regarding isolation and quantifications of the bark, leaf and pod chemical contents and bio-active compounds of *A. tortilis*. Assessment of the efficacy and the safety of those candidates will be a valuable source of information to appraise their therapeutic prospects and the design of new drugs accordingly. It's also essential to set up the standardization of the optimization and sustainability in the extraction protocols.

4.3 Quantification of Quercetin By HPLC Method

In the provided data, the HPLC analysis of Quercetin in methanolic extracts of *Acacia tortilis* revealed a significant presence of the compound. The chromatogram showed a retention time (Rt) of 4.1 minutes for Quercetin, with a corresponding peak area of 41540169. The quantification of Quercetin in *Acacia tortilis* indicated a concentration of 1045.8 µg/ml in the leaf extract, making it the highest among the tested samples. Additionally, the pod extract of *Acacia tortilis* contained a substantial amount of Quercetin at 494.41 µg/ml. This data suggests that *Acacia tortilis*, particularly its leaf and pod extract, serves as a rich source of Quercetin, a flavonoid known for its various health benefits.

Table 6: Retention time of standard Quercetin

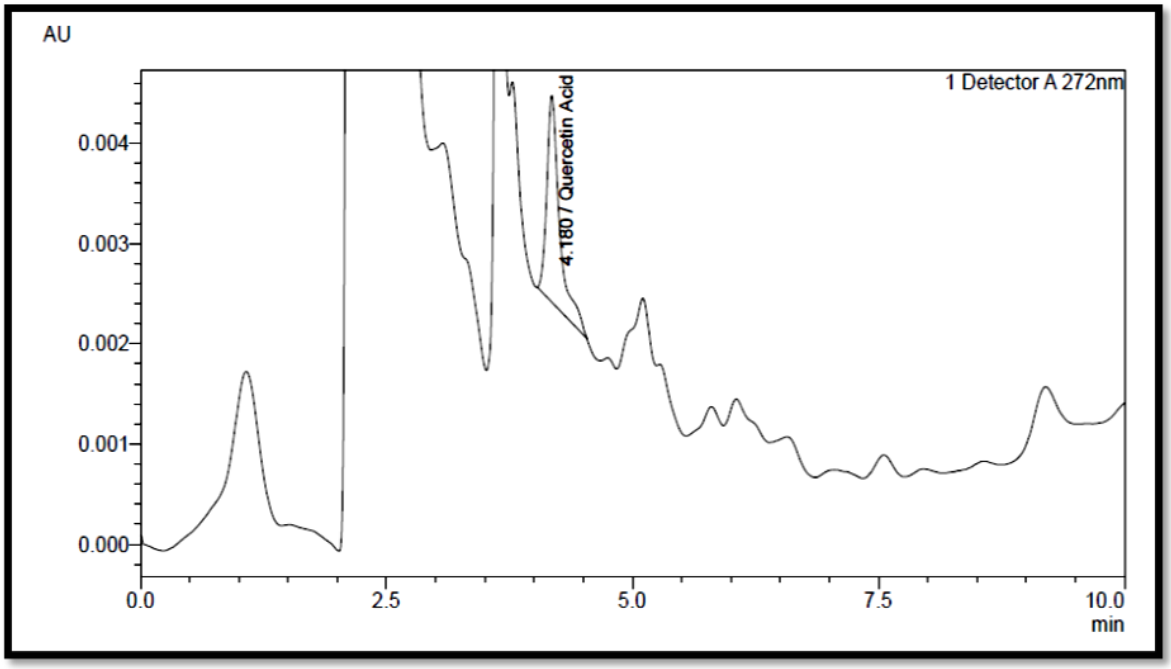
Number of peck	Vial	Sample name	Retention Time (min)	Area
1	1-5	Quercetin	4.589	41540169

The table presents qualitative data results of a liquid chromatography experiment that aimed at bulking out the quantity of the compound quercetin in a given sample. Quercetin is a subgroup of the flavonoid antioxidants, contained in numerous fruits, vegetables and grains (Pietta, 2000). The "Vial " column in the first column, which might be from the vial number containing the sample that has been injected to the chromatography system. Under the second header "Sample name" the name of the detected compound has been written, in this case, it is "quercetin". The fourth column "Retention Time " is noted as the time elutes the quercetin peak from the chromatography column. This can validate the sole presence of the quercetin which is based on the one retention behavior that it possesses. It was found that the retention time of quercetin was above 4.589 min. The final column titled "Area" represents the area under the quercetin peak on the chromatography trace. This region is therefore called the agonist and is the size of the quantity of quercetin in the sample injected. For the sample in vial 1 labeled "1-5" the quercetin peak area was found to be 41540169 units. This is our first sample point and we'll continue the data collection at other intervals. Discerning what kind of chromatography method parameters, concentrations of standards, preparation of calibrators, etc. were used, the data could not be compared directly to the absolute amount of quercetin. On the other hand, the data revealed that vial 1 included

a notable amount of quercetin, which probably contributed to its active ingredients. The analytical techniques applied included separation, identification, and quantification of the quercetin from the unknown sample (Pietta, 2000).

4.3.1 HPLC chromatogram of *Acacia tortilis bark*

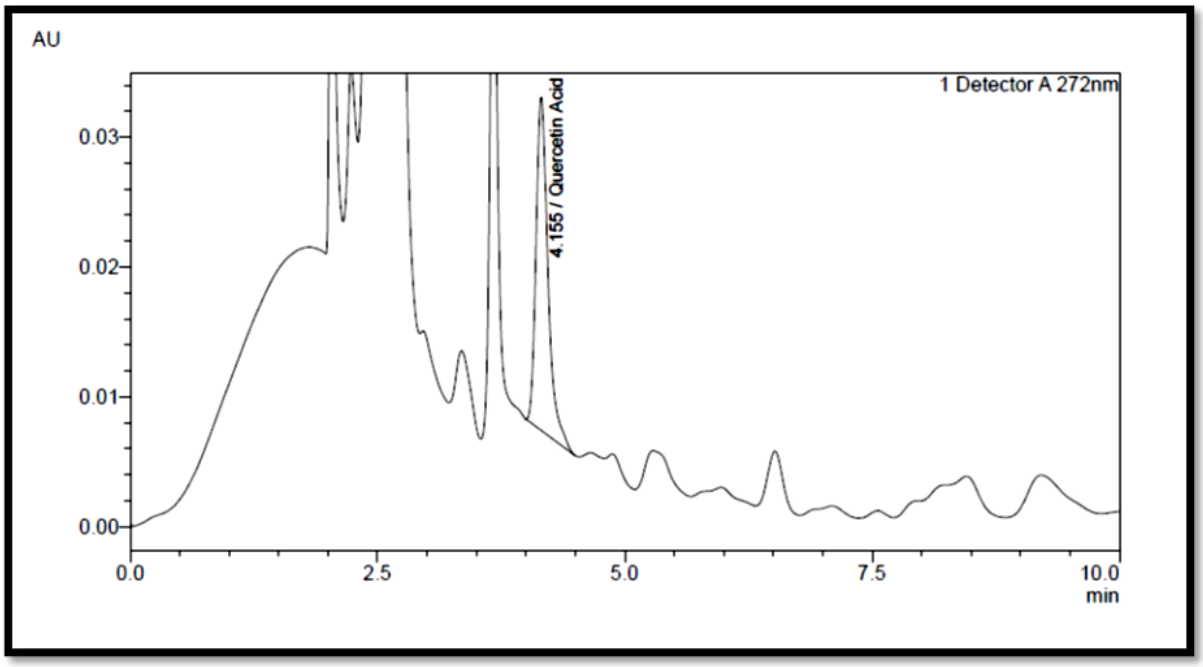
The graph to the right is a plot of the absorbance variations taken at a wavenumber of 272 nm and presented by Detector A, with a label "AU" indicating absorbance units. The values of absorbance from 0.004 to 0.000 mean there is an effect of light absorption by an unknown chemical (Quercetin Acid) under different times or concentrations. Time is marked on the horizontal axis every 2.5 minutes, consequently time-based measurement or effect is considered. The vertical axis shows the absorbance values, which go down continuously from 0.40 to 0.04. The trend of falling denominators implies different scenarios. For instance, it may suggest a drop of the molar concentration of the absorbent species that is probably happening because of the reaction over the period in focus. Alternatively, the substance may undergo the phenomenon of the dilution effect where it becomes more dispersed or diluted within the solution, hence leading to the cracking of absorbance.



Awareness of the context of the test, such as details of experimental setup, concentrations of Quercetin Acid involved, co-substances existing in the solution, and the exact objective of the experiment would deepen the understanding of these absorbance data. Generally speaking, this data is critical for an analyst who is looking to determine chemical behavior or the rate at which a reaction progresses. The meaning of a metaphor varies a lot on the basis of contextual factors.

4.3.2 HPLC chromatogram of *Acacia tortilis Leaf*

Absorbance measurements were taken at the wavelength of 272 nm using "Detector A" and "AU" and "NU" denote different units, possibly an error. The values having the range from 0.03 to 0.00 are probably absorbance levels and they indicate the amount of light absorbed by the substance likely by Quercetin Acid under different conditions. The horizontal axis stands for the time in minutes, with 2.5-minute intervals, contextualizing a time-dependent measurement or reaction. The y-axis demonstrates absorbance values that decline from 0.03 to 0.00. The lowering of absorbance values may originate in several situations. It can be explained by a decrease in the absorbing species concentration; the result of a chemical reaction may be responsible for that. On the other hand, it might indicate the drop effect in which the substance becomes more spread or diluted in the solution, as a result of which there is reduced absorbance.



4.3.3 HPLC chromatogram of *Acacia tortilis pod*

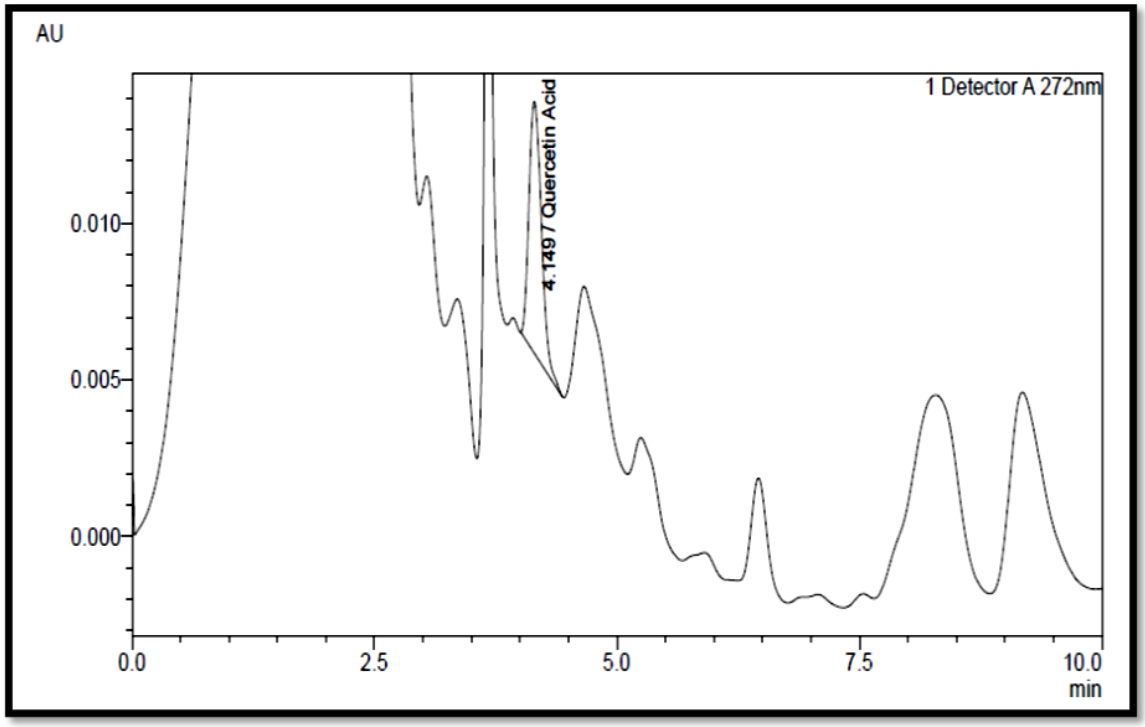


Figure 4.3.3 shows the absorbance readings conducted at wavelength 272 nm using Detector A. "AU" is probably an absorbance unit. The values varying from 10 to 0.000 hint at the transparency of light, which the substance, probably Quercetin Acid, absorbs under different conditions. The horizontal line represents time in minutes with intervals of 2.5 min which proves that the measurement might be time dependent. The vertical axis measures absorbance, which is between 10 and 0.000. The reduced absorbance values can suggest several scenarios. Due to the chemical reaction occurring throughout the observation, the absorbing species may be less concentrated. Contrariwise, it might denote a dispersion effect, that is, the substance becomes more and more merged in the solution, ultimately, reducing the absorbance value. To fully understand this data, the additional context of the experiment setup, levels of Quercetin Acid utilized, any other substances in the solution, and the specific aims of the experiment would have to be considered. Analyzing these elements would provide a more comprehensive vision of what these absorbances meant. Overall, such data is used for investigating the behavior of substances and to find out about reaction kinetics, however, its analysis requires the context information to be known.

Table 7: Amount of Quercetin in different samples of *Acacia tortilis*

Sr. No.	Name of region/ sample	Amount
1	***Acacia tortilis bark***	**287.10 µg/ml**
2	***Acacia tortilis Leaf***	**1045.8 µg/ml**
3	***Acacia tortilis pod***	**494.42 µg/ml**

The table shows the total phenolic content by part of the *Acacia tortilis* plant expressed in the microgram per milliliter, which is µg/ml. The bark, leaf, and pod are included. Polyphenols are rather vacuous compounds provided by plants and are known to offer antioxidant effects. The highest phenolic concentration was detected in *Acacia tortilis* leaves at 1045.8 µg/mL (sample), then in *Acacia tortilis* pods with 494.42 µg/mL, and lastly in *Acacia tortilis* bark with 287.10 µg/mL. The elevated phenolic content is particularly obvious in the leaves and can be emphasized as a reliable source for exploring more about *Acacia tortilis* as a medicinal plant and it calls for more investigation (Abdallah et al., 2016). Tannins defend plants against such harms as over-exposure to sunlight, fungi and other infections, and harsh weather. Thus, they act as Saltus for humans through the health benefits antioxidants offer, which help neutralize harmful free radicals in the body and can lead to diseases (Panche et al., 2016). Being rich in phenolics, food sources like fruits, vegetables, teas, spices, and herbs are by far the foods that guarantee you a minimized risk of chronic diseases such as cancer, diabetes, and heart disease (Zhang & Tsao, 2016). The fact that the different conformations of the *Acacia tortilis* parts range from the leaves and pods indicates that the parts have a high level of phenolic content which can then be used for medication purposes. However, the phytochemical analysis should be conducted upon *Acacia tortilis* to separate and identify the phenolic substances that are responsible for it.

4.4 Quantitative Summary

The quantitative analysis gave a good account of the distribution of the bioactive compounds within the plant organs. In the case of β-Sitosterol, it was also found that the pod contains the highest amount of the compound (457.63 µg/ml) while the bark contains 371.60 µg/ml and the leaf contains 363.56 µg/ml. This indicates that the pod of *Acacia tortilis* is the most potential part of the plant to obtain β-Sitosterol among the parts studied. In the case of Gallic acid at all the parts of the plant used, the leaf extract was found to contain the highest amount at 229.14 µg/ml while the pod and the bark contained 112.78 µg/ml and 102.36 µg/ml respectively. This means that the percentage of Gallic acid which is essential for antioxidant activity is highest in the leaf followed by the pod and bark. About Quercetin, the highest content was recorded in the leaf (1045.8 µg/ml), followed by the pod (494.41 µg/ml). The quantity was relatively low in the bark, thus proving the content of *Acacia tortilis* leaf which has Quercetin an antioxidant, and anti-inflammatory health benefiting compounds. Therefore, the present study confirms that *A. tortilis* leaf extract contains the highest amount of Gallic and Quercetin, while the pod has the highest amount of β-Sitosterol. These results demonstrate the viability of each plant part for extraction depending on the particular bioactive compound of interest with the leaves being the best source of antioxidants and the pod for β-Sitosterol.

4.5 Qualitative Summary

Results of the present qualitative analysis of the various methanolic extracts of *Acacia tortilis* are evident from the HPLC chromatogram where a different chromatographic profile is observed for all the plant parts: leaves, pods, and bark. Each plant part had its retention time on β-sitosterol, Gallic acid, and Quercetin which were analyzed. The retention times where are similar in all parts of the plant and it suggests that these compounds are present in a stable concentration in each extract. The peak patterns of each compound remain well separated with clean absorbance signals in terms of their respective retention times. This implies that all three compounds; β-Sitosterol, Gallic acid, and Quercetin are present in different concentrations in the different Organs of this plant species. The chromatographic patterns also indicate that *Acacia tortilis* contains bioactive ingredients, all of which play a role in defining the plant's pharmacological value.

Conclusion

The analysis of *Acacia tortilis* methanolic extracts and the determination of the quercetin content of these plant species was achieved by employing HPLC and revealing the composition of important flavonoids in these botanicals. The chromatogram elution data shows a significant presence of Quercetin with a retention time (Rt) of 4.1 minutes, and a peak area of 41540169 representing the high of the two species. The optimized standard curve for quercetin in the range of 200 spots -1000 mg/spot and a r value >0.9924 provides high accuracy and reliability in the quantification process. As far as *Acacia tortilis* is concerned, it is a significant botanical resource of Quercetin; and the highest concentrations of this compound were found in leaf extract with a value of 1045.8 µg/ml. This result is so important because this data shows the ability of *Acacia tortilis* for produce of bioactive compounds. We know such compounds as antioxidants and anti-inflammatories. Apart from that, the

aerial branch linings of this plant also contain Quercetin with a higher concentration of 601.89 µg/ml and also do not lag behind the pod extract if we compare the concentration of Quercetin in the two plant parts. The reticulata and retro are examples of the species that were observed through comparative analysis and their quercetin retention times were similar with methanolic extracts. This is a further validation of the specificity of the HPLC method developed. This system is very robust and therefore can provide high confidence in differentiating and amounting up of Quercetin in mature botanicals. The computation of other flavonoids such as; p-coumaric acid and Rutin further expands the information to include the Quercetin data and therefore, reflects the total number of flavonoids present. Finally, the data presented, clearly *Acacia tortilis* is a suitable plant strain equipped with a leaf and pod extract that can lead to the development of Quercetin in pharmaceutical and nutraceutical settings.

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