

Research Article



Estimation of Total Phenols, Flavonoids, Antioxidant and Quercetin Investigations in *Diospyros embryopteris* Pers. Barks

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ABSTRACT

The plant has been used for a long time, but it hasn't been thoroughly studied for phytochemical research, such as determining the amount of total phenols, flavonoids, and antioxidants. As a result, *D. embryopteris* will be tested for total phenol content, flavonoid content, antioxidant activity, quercetin content, and validation studies. Total phenols and total flavonoids were quantitatively determined using a standard curve for gallic acid and quercetin, respectively. With the exception of the methanol extract, none of the extracts displayed any total phenol or flavonoid content. It is clear that compared to all other extracts examined, the methanol extract of plant bark had a higher number of total phenols and flavonoids. This finding implies that the majority of polyphenols are extracted from plant bark using the solvent methanol. Preliminary phytochemical screening and the outcomes are closely related. The DPPH assay was used to assess the in vitro antioxidant activity of the different plant bark crude extracts. The common antioxidant was rutin. Standard plots were created between sample concentrations and the percentage of DPPH inhibition. The IC₅₀ values of the standard and samples were calculated using regression equations of standard plots. It quite clear that the plant bark methanol extract had the highest antioxidant activity, with an IC₅₀ value of 24.23 µg/ml, whereas normal rutin had an IC₅₀ value of 4.68 µg/ml. The other extracts other than methanol extract did not shown any sign of antioxidant activity. Plant bark was found to contain 0.780 ± 0.0003% w/w of quercetin.

Keywords: *Diospyros embryopteris*, Phenol, Flavonoid, Antioxidant, DPPH.

1. INTRODUCTION

Other botanical names for the plant under study, *Diospyros embryopteris*, include *Diospyros malabarica* Kostel and *Diospyros peregrina*. The plant, which is a member of the Ebenaceae family, is primarily known by several local names, including Black and White Ebony, Pale Moon Ebony, Gaub tree, Indian persimmon, Kalatendu, Tendu, Deshi gab, and Makurkendi. The wild areas of Eastern India, Sri Lanka, Burma, Cambodia, Laos, Vietnam, Thailand, and Indonesia are where the plant is primarily found¹. The plant has been used for a long time, but it hasn't been thoroughly studied for phytochemical research, such as determining the amount of total phenols, flavonoids, and antioxidants. As a result, *D. embryopteris* will be tested for total phenol content, flavonoid content and antioxidant activity studies.

2. MATERIALS AND METHODS

2.1 Plant materials

Diospyros embryopteris bark was procured from Himalaya Herbs Store, Madhav Nagar, Saharanpur, Uttar Pradesh, India in the month of January, 2026. The authentication of the plant material was confirmed on the basis of literature reported and various photographic pictures available online.

2.2 Preparation of extracts

Petroleum ether (3 L) was used in a Soxhlet device to extract 500 g of dried plant bark material. A sequence of solvents, beginning with medium polar solvents like chloroform (3 L)

and concluding with high polar solvents like methanol (3 L), were used to dry and extract the plant marc. Additionally, the reflux method was used to prepare the water extract. The solvent was then extracted from crude extracts using the distillation technique. Plant crude extract yields as a percentage (%) were noted. Various extracts of plant bark were subjected to phytochemical screening to visualize different groups of phytoconstituents present². The content of total phenols in various extracts of plant bark was estimated by following the standard procedure³. The content of total flavonoids in various extracts of plant bark was estimated by following the standard procedure⁴.

2.3 TLC densitometric method development

2.3.1 Test solutions

After being defatted by n-hexane, 10 g of coarsely powdered plant material was thoroughly extracted using methanol in a Soxhlet system. Reduced pressure was used to recover the solvent. After the extract was dried, methanol was added to a volumetric flask to get the volume down to 25 ml.

2.3.2 Preparation of standard plot

For the TLC densitometric investigations, a stock solution containing 1 mg/ml of the marker was made in methanol. Six dilutions of varying concentrations (10, 20, 30, 40, 50, and 60 µg/ml) were obtained by diluting the marker stock solution with methanol. On a pre-coated TLC plate, a volume of 10 µl from each dilution was applied in triplicate. The plate was developed at a distance of 8 cm in a chamber



that was saturated for 10 minutes using the toluene:ethyl acetate (6:4) solvent mixture. After being dried in a hot air current, the produced plate was scanned at 254 nm using a TLC scanner. For every track, the AUC of the peak associated with the marker compound was recorded.

2.3.3 Estimation of marker compound

On a pre-coated TLC plate (5 × 10 cm), test solutions (10 µl) of methanol extract were applied in triplicate. The same process used to prepare the standard plot was applied to the development and scanning of the plate. The test sample's average AUC of the peak that corresponded to the marker compound was found at 278 nm, and the standard plot was used to compute its concentration.

2.4 Validation of TLC densitometric method

The developed TLC densitometric method was validated as per ICH guidelines on the basis of following parameters such as linearity range, instrument precision, repeatability, intra-day and inter-day variation, limit of detection and limit of quantification, recovery studies, specificity, robustness and ruggedness⁵.

2.5 In vitro antioxidant activity studies using DPPH Method

2.5.1 Preparation of control

The DPPH test was used to measure the sample's capacity to scavenge free radicals⁶.

2.5.2 Statistics

The data were expressed as Mean ± S.D. The test groups were compared with the standard group using One way ANOVA followed by student Newman Keul's test.

3. RESULTS AND DISCUSSION

3.1 Percentage yields of various extracts

Table 1 listed the percentage yield (% w/w) of different plant bark extracts.

Table 1: Percentage yields of various extracts of plant barks

Name of extract	Percentage yield (w/w)
Petroleum ether extract	1.16
Chloroform extract	2.10
Methanol extract	5.26
Water extract	8.96

3.2 Phytochemical screening of various extracts

To identify different groups of phytoconstituents contained in the plant, a qualitative chemical test was performed on the various plant bark extracts. Table 2 displays the phytochemical screening results.

Table 2: Phytochemical screening of various extracts of plant barks

Class of Phytoconstituents	Petroleum ether extract	Chloroform extract	Methanol extract	Water extract
Carbohydrates	-	-	-	+
Proteins	-	-	-	+
Lipids	+	-	-	-
Alkaloids	-	+	-	-
Anthraquinone glycosides	-	-	-	-
Cyanogenetic glycosides	-	-	-	-
Cardiac glycosides	-	-	-	-
Flavonoids	-	-	+	-
Saponins	-	-	-	+
Tannins	-	-	+	-
Steroids/Triterpenoids	+/-	-/+	-/-	-/-
Coumarins	-	-	-	-

+ Present, - Absent

3.3 Estimation of total phenol and flavonoid contents in various extracts of plant barks

After defatting with n-hexane, the plant methanol extracts were made. The easily readable text reveals that phenolic compounds and flavonoids exhibit a variety of pharmacological activities. Flavonoids and phenolic chemicals have very important effects on human health and nutrition^{7,8}. A class of antioxidant experts known as flavonoids and phenolic chemicals works to eliminate free radicals. Phenols and flavonoids work by either scavenging or chelating.

Total phenols and total flavonoids were quantitatively determined using a standard curve for gallic acid (linearity: 20 to 120 mg/ml; $r^2 = 0.9934$) and quercetin (linearity: 30 to 180 mg/ml; $r^2 = 0.9978$), respectively. With the exception of the methanol extract, none of the extracts displayed any total phenol or flavonoid content. The methanol extract of plant bark had a higher amount of total phenols and flavonoids. This finding implies that the majority of polyphenols are extracted from plant bark using the solvent methanol. The preliminary phytochemical screening and the outcomes are closely related.



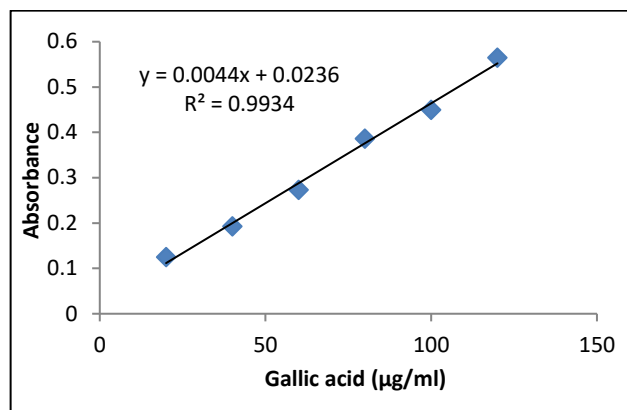


Figure 1: Standard curves of gallic acid vs. absorbance

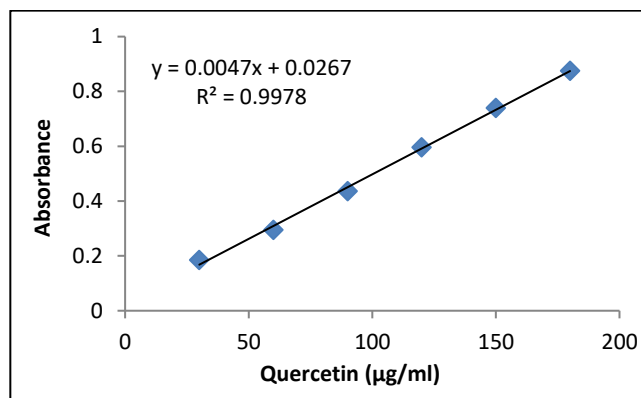


Figure 2: Standard curves of quercetin vs. absorbance

Table 3: Percentage content of total phenols and flavonoids in various extracts of plant barks.

Test sample	Total phenols content (% w/w) Mean ⁿ ± S.D.	Total flavonoids content (% w/w) Mean ⁿ ± S.D.
Petroleum ether extract	0.00 ± 0.00	0.00 ± 0.00
Chloroform extract	0.89 ± 0.01	0.35 ± 0.02
Methanol extract	12.14 ± 0.80	5.47 ± 0.25
Water extract	0.58 ± 0.14	0.41 ± 0.13

n=3

3.4 *In vitro* antioxidant activity of crude extracts of plant barks

Subjective chemical analyses of other plant bark extracts verified that the n-hexane extract had no flavonoids or phenolic chemicals, which are thought to be the main components employed as antioxidants. Water extract showed similar initial phytochemical screening results. Phytochemical screening tests revealed the presence of phenols and flavonoids in methanol extracts, thus these samples were subjected to an *in vitro* antioxidant evaluation. Natural antioxidants are frequently referred to be substances that prevent cancer. Due to the fact that plant-based foods are a good source of common cancer prevention agents, there has been an increase in their consumption in every year.

The effects of cancer preventive agents on human health are quite important. Natural antioxidants protect DNA from damage and eliminate free radicals. A straightforward, delicate method for estimating the antioxidant activity of plant materials is the DPPH measure. This procedure relies on estimating the DPPH tone (color) deficiency following test sample reaction at 517 nm, which was observed with the aid of a spectrophotometer.

The DPPH was a stable purple-toned free radical that became yellow when exposed to an antioxidant. Changes in shading indicate how adequate the antioxidant is. The percentage of scavenging power was calculated as the change in absorbance with respect to control. The value that results in a halving of the underlying clustering of DPPH is known as the IC₅₀.

Table 4: Antioxidant activity of various crude extracts of plant barks

Treatment	Concentration (µg/ml)	Mean ⁿ percentage inhibition of DPPH radical ± S.D.	IC ₅₀ Values (µg/ml)
Rutin	2	30.25 ± 0.125	4.68
	4	45.85 ± 0.241	
	6	58.97 ± 0.659	
	8	75.33 ± 0.350	
	10	86.78 ± 0.785	
	12	96.28 ± 0.987	
Petroleum ether extract	25	3.89 ± 0.098	3435.76 *
	50	4.12 ± 0.101	
	100	4.98 ± 0.125	
	200	6.50 ± 0.230	
	400	9.12 ± 0.159	
Chloroform extract	800	14.28 ± 0.190	627.67*
	25	9.25 ± 0.258	
	50	11.39 ± 0.412	
	100	14.60 ± 0.653	
	200	23.58 ± 0.745	
Methanol extract	400	35.67 ± 0.239	24.23
	800	60.70 ± 0.780	
	2	25.41 ± 0.320	
	4	28.65 ± 0.369	
	8	33.49 ± 0.452	
Water extract	16	41.89 ± 0.658	1122.69 *
	32	59.74 ± 0.789	
	64	90.58 ± 0.985	
	25	6.80 ± 0.005	
	50	8.46 ± 0.009	
	100	11.98 ± 0.105	
	200	15.90 ± 0.116	
	400	23.97 ± 0.258	
	800	36.78 ± 0.230	

n=3; *P<0.05 vs. Rutin; One way ANOVA followed by Student Newman Keul's test.



The DPPH assay was used to assess the in vitro antioxidant activity of the different plant bark crude extracts. The common antioxidant was rutin. Standard plots were created between sample concentrations and the percentage of DPPH inhibition. The IC_{50} values of the standard and samples were calculated using regression equations of standard plots. The plant bark methanol extract had the highest antioxidant activity, with an IC_{50} value of 24.23 $\mu\text{g/ml}$, whereas normal rutin had an IC_{50} value of 4.68 $\mu\text{g/ml}$. The other extracts other than methanol extract did not shown any sign of antioxidant activity.

3.5 Standardization of plant barks on the basis of quercetin using HPTLC

3.5.1 Preparation of methanol extract of plant barks

In order to defatten the plant material and avoid oily compounds interfering with the analytical tests, the plant's bark was extracted using n-hexane utilizing a Soxhlet equipment. In order to create methanol extract, the marc was further extracted using Soxhlet. The Soxhlet apparatus is utilized in current research because it offers a comprehensive extract of plant materials, allowing for the quantification of the marker compound's complete concentration in TLC densitometric analysis.

3.5.2 Confirmation of flavonoids presence in methanol extract of plant barks

To find the presence of flavonoids, a phytochemical technique called the Shinoda test was applied to the methanol extract of plant bark. The methanol extract contains flavonoids, according to the phytochemical test.

3.5.3 Estimation of quercetin in plant barks using TLC densitometer

A thorough analysis of the literature revealed the existence of a flavonoid called quercetin, which was scientifically isolated by natural product scientists. However, quercetin has never been used to standardize the plant. In order to standardize plant aerial parts, quercetin was employed as a marker chemical. Using an HPTLC method, the amount of quercetin in plant aerial parts was ascertained. ICH criteria were followed in the validation of the developed method using the HPTLC system.

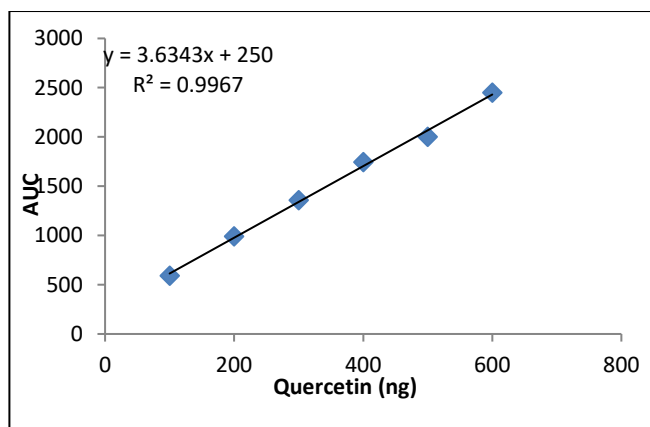


Figure 3: Standard plot of quercetin

Using a typical plot between the amount of quercetin (ng) loaded and the area under the curve obtained, the amount of quercetin in plant barks was determined. Plant bark was found to contain $0.780 \pm 0.0003\%$ w/w of quercetin.

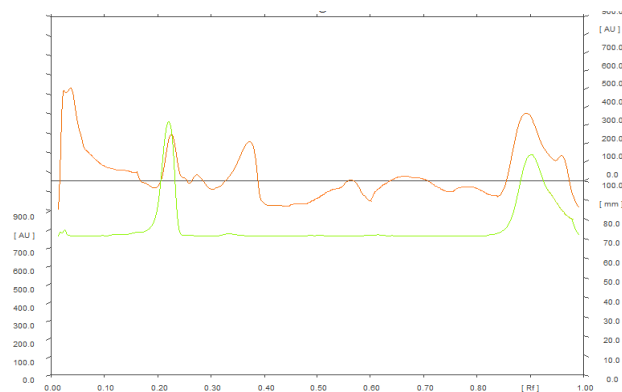


Figure 4: The thin layer chromatogram overlay of quercetin and methanol extract of plant aerial parts

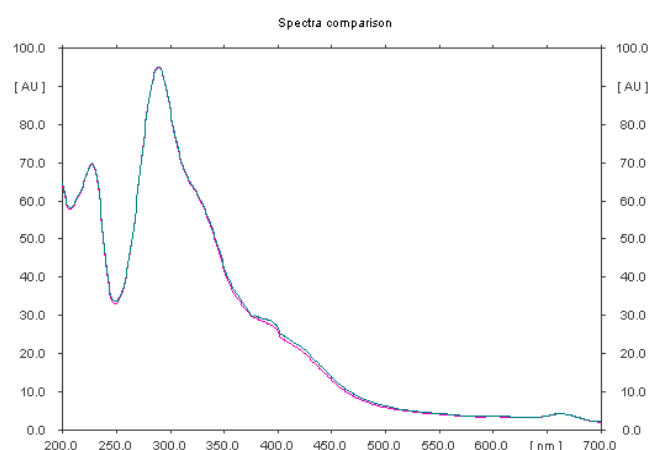


Figure 5: The thin layer spectra overlay of quercetin and methanol extract of plant aerial parts

3.6 Method validation studies of developed TLC densitometric method

TLC densitometry is a very useful subjective evaluation method that combines the art of chromatography with speed and affordability. It is a major advancement of the TLC principle that separates phytoconstituents more effectively and in less time. TLC densitometry is an excellent analytical tool for quantitative assurance of specific compound or compounds in complex combinations of inorganic, natural, and biomolecules. Its advantages include simple strategy improvement and approval, validation, filtering, full advancement, specific recognition of marker compound, less sample requirement, and so on⁵.

In the current investigation, a TLC-densitometric method was developed to measure the amount of quercetin in plant bark. Additionally, the developed technique was accepted for the ICH rules' bounds. Plant bark was found to contain $0.780 \pm 0.0003\%$ w/w of quercetin. The developed method for evaluating quercetin in plant bark was approved in accordance with ICH guidelines. The percentage coefficient of variation found in the created method's validation

parameters such as instrumental precision, inter- and intra-day precision, robustness, ruggedness, and repeatability complied with the specified limit. In accuracy studies, quercetin recovery exceeded 98%. Additionally, there was no difference between the sample and standard's thin layer chromatograms and ultraviolet spectra. These findings suggest that the established method for estimating quercetin in plant bark is specific, accurate, repeatable, and exact.

Table 5: Method validation parameters in TLC densitometric analysis of plant barks

Parameter	Quercetin
Instrumental precision (% CV, n=7)	0.85
Repeatability (% CV, n=5)	0.43
Linearity (Coefficient of correlation r^2)	0.9967
Linearity Range (ng)	100-600 ng
LOD (ng)	11
LOQ (ng)	37
Intra-day precision (%CV, n=9)	1.25
Inter-day precision (%CV, n=9)	1.56
Accuracy (average % recovery)	99.21
Specificity	Specific
Robustness (252 / 256 nm)	0.56 / 0.60
Ruggedness (Analyst 1 / Analyst 2)	0.78 / 0.90

3.7 Pharmacology of quercetin: A comment

One important dietary flavonoid belonging to the class of flavonols is quercetin. Plants contain it as glycosides, but many quercetin derivatives have also been identified⁹. Five hydroxyl groups are present in a quercetin particle, and their presence determines the pharmacological effect of the molecule and its many derivatives. Among polyphenols, it is one of the most potent agents for cell reinforcement^{10,11}. Additionally, quercetin has been demonstrated to have antiviral, antibacterial, anticarcinogenic, anti-inflammatory, and antidepressant properties¹². According to Joshi et al. (2011)¹³, quercetin has a potent anticarcinogenic impact due to its crucial role in increasing the death of altered cells, limiting DNA blending, impeding the growth of dangerous cells, and reducing and modifying cell signal transduction pathways. A thorough review of the literature revealed that quercetin is responsible for the antidepressant properties of a number of plants. Using FST, quercetin extracted from *Opuntia ficus-indica* shown significant antidepressant effects in rats at a dosage of 50 mg/kg¹⁴. According to Clarke and Ramsay (2011)¹⁵, *Hypericum perforatum*'s antidepressant properties are caused by quercetin at a dose of 100 mg/kg, p.o. At dosages of 10 or 50 mg/kg, p.o., ginkgo biloba leaf extract has been shown to exhibit significant antidepressant activity¹⁶. According to online scientific reports, quercetin exhibits its pharmacological actions through a number of related pathways. According to the observations, quercetin may be one of the beneficial compounds found in the bark of *Diospyros embryopteris*. In order to standardize *Diospyros embryopteris* bark, it is chosen as a marker.

SUMMARY AND CONCLUSION

The plant being studied, *Diospyros embryopteris*, is a member of the Ebenaceae family and is primarily known as the Gaub tree. The plant is widely distributed throughout India and neighboring countries, including Sri Lanka, Burma, Cambodia, Laos, Vietnam, Thailand, and Indonesia. Many illnesses, including dyspepsia, leprosy, diarrhea, dysentery, bleeding, skin burning, diabetes, spermatorrhea, vaginal infections, wounds, flatulence, prolepsis, and scabies, have historically been treated with the herbs. The plant has been used for a long time, but it hasn't been thoroughly studied for phytochemical research, such as determining the amount of total phenols, flavonoids, and antioxidants. According to the literature review, the plant has not yet been standardized based on the primary bioactive marker component. Therefore, the current research will be aimed to validate the suggested method in accordance with ICH criteria and standardize the plant based on quercetin (scientifically separated from plant) using a TLC densitometer. As a result, *D. embryopteris* will be tested for total phenol content, flavonoid content, antioxidant activity, quercetin content, and validation studies. Total phenols and total flavonoids were quantitatively determined using a standard curve for gallic acid and quercetin, respectively. With the exception of the methanol extract, none of the extracts displayed any total phenol or flavonoid content. It is clear that compared to all other examined extracts, the methanol extract of plant bark had a higher amount of total phenols and flavonoids. This finding implies that the majority of polyphenols are extracted from plant bark using the solvent methanol. The preliminary phytochemical screening and the outcomes are closely related. The DPPH assay was used to assess the in vitro antioxidant activity of the different plant bark crude extracts. The common antioxidant was rutin. Standard plots were created between sample concentrations and the percentage of DPPH inhibition. The IC₅₀ values of the standard and samples were calculated using regression equations of standard plots. Methanol extract had the highest antioxidant activity, with an IC₅₀ value of 24.23 µg/ml, whereas normal rutin had an IC₅₀ value of 4.68 µg/ml. The other extracts other than methanol extract did not shown any sign of antioxidant activity. In the current investigation, a TLC-densitometric method was developed to measure the amount of quercetin in plant bark. Additionally, the developed technique was accepted for the ICH rules' bounds. Plant bark was found to contain 0.780 ± 0.0003% w/w of quercetin. The developed method for evaluating quercetin in plant bark was approved in accordance with ICH guidelines. The percentage coefficient of variation found in the created method's validation parameters such as instrumental precision, inter- and intra-day precision, robustness, ruggedness, and repeatability complied with the specified limit. In accuracy studies, quercetin recovery exceeded 98%. Additionally, there was no difference between the sample and standard's thin layer chromatograms and ultraviolet spectra. These findings suggest that the established method for estimating

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