

The Antioxidant Potential of Leaf, Bark, and Flower Extracts of *Vateria indica* L.

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ABSTRACT

Background: *Vateria indica* L. is an extremely endangered tree belonging to Family Dipterocarpaceae. In almost all Unani, Ayurveda, and Siddha medicines, the resin obtained from this tree is used to treat various diseases. **Objectives:** The present study evaluates *Vateria indica* L's antioxidant potential and quantification of total phenol and flavonoid content in dry and fresh bark, leaf, and flower extracts. **Materials and Methods:** The analysis of phenol quantification has been determined utilizing Folin-Ciocalteu reagent, and gallic acid utilized as the standard, and flavonoid quantification has been computed by aluminium chloride colorimetric assay. DPPH radical scavenging assay is employed to analyze antioxidant activity. **Results:** Fresh bark extracts presented maximum phenol content (417.4mg GAE/g), whereas the fresh flower extract showed the least phenol (78.675 mg GAE/g). Highest flavonoid content has been found in fresh bark, afterward, dry bark, fresh leaf, dry leaf, dry flower, and fresh flower. Antioxidant activity is exhibited by all the extracts studied. Flower and leaf extracts showed comparable antioxidant activity. Maximum antioxidant activity has been exhibited by fresh bark extract, having (IC₅₀ value of 28.995 µg per mL. **Conclusion:** Results of current study recommend that bark, leaves, flower extracts, both fresh and dry, of *Vateria indica* L. contain phenols and flavonoids and exhibit good antioxidant properties, which could be exploited further as a potent source of natural antioxidants.

Keywords: *Vateria indica* L., Antioxidant activity, Total Phenol, Total Flavonoid, DPPH.

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INTRODUCTION

Medicinal plants improve the general public's health and safety in addition to complementing or replacing modern medical treatments. So, they have an important part in people's everyday lives in emerging nations. Existence of a broad variety of biologically active substances or phytochemicals makes these medicinal plants important. Plant foods contain a diverse range of non-nutritive chemical substances known as phytochemicals. A few examples of well-known phytochemicals include carotenoids, isoflavones, phenolic acids, flavonoids, and isothiocyanates. Additionally, plants have been a plentiful source of safe and effective medications since ancient times.^[1] Many nations today consider their traditional plant knowledge to be a cultural treasure trove. To maintain and retrieve information for the benefit of humanity, it is crucial to study and preserve traditional

knowledge and to create a database of traditional medicine. Since ancient times, India has gained international recognition for its abundant natural resources and diverse biological population, owing to the wide range of zones and natural environments, contributing to the floristic diversity.^[2] The Western Ghats, extending to six states, have a wide range of vegetation types and topographical features.^[3] But almost a third of the endemic plant species are endangered and in danger of extinction, *Vateria indica* being one of them, being an important flagship species.^[4]

Herbal medicine is reviving with huge demand, and there is good potential for new pharmacies. Medicinal plants are treasures for the use of mankind, and Tropical forests are repositories of terrestrial medicinal plant diversity.^[5] The two most significant groups of secondary metabolites, along with bioactive compounds in plants, are flavonoids or phenolic acids.^[6] They are also an antioxidant as well as natural product that can scavenge free superoxide radicals, prevent aging, alongside reduce risk of cancer. Many biological actions, including antioxidant, antiulcer, anti-inflammatory, cytotoxic and anticancer, antidepressant and antispasmodic properties, are possessed by phenols and flavonoids.^[7]



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The critically endangered *Vateria indica* L. tree is unique to India's South Western Ghats. Indian medicine uses the resin Kahruba (*Vateria indica* L.) as a tonic, expectorant, as well as carminative. Resin released by tree is also called White Dammar, Piney Resin, and Dhupa. The resin of *Vateria indica* L. is mentioned in almost all Unani literature as a treatment for diabetes mellitus, gonorrhea, syphilis, urinary discharges, amenorrhea, piles, chronic bronchitis, anemia, ear, skin, and pile disorders.^[8] Various research has been carried out to analyze phytochemical properties of plants, including research on chemical constituents in leaves, physicochemical evaluation of Sarja rasa, studies on the effect of stem bark of natural gum as a drug release modifier, and Herbo-Medicinal Formulation. The resin obtained from the plant possesses enormous pharmacological properties, including anti-ulcer, anti-tumor activity, anti-inflammatory, anthelmintic, anti-diabetic, antiepileptic, as well as neuroprotective.^[9] This study evaluates the total phenols, flavonoids, alongside antioxidant potential of leaf, bark, as well as flower extracts of *Vateria indica* L.

MATERIALS AND METHODS

Chemicals

This experiment utilizes only analytical-grade chemicals.

Collection and Extraction of Plant Materials

Bark, leaves, as well as flowers of *Vateria indica* L. have been gathered from botanical garden of Vimala College (Autonomous), Thrissur, Kerala, in February. Plant was authenticated at the Department of Botany, Vimala College (Autonomous), Thrissur. Herbarium of voucher specimens has been prepared as well as deposited at Department of Botany, Vimala College (Autonomous), Thrissur. Collected samples have been cleaned, shade-dried for two weeks, and powdered separately in a grinder. Samples were extracted with distilled water for 72 hr by the Soxhlet extraction method. After complete extraction, solvent has been evaporated in water bath at 40°C. Dried extracts had been sealed as well as kept for further analysis in a refrigerator between 2 and 8°C. Fresh samples of bark, leaves, and flowers of *Vateria indica* L. were collected and cleaned using water. A known weight of 0.6 g was macerated in a mortar and pestle with 60 mL distilled water as solvent. The aqueous extract was filtered using Whatman's filter paper, and the filtrate was taken for further analysis.

Quantitative Estimation of Phenol

TPC (Total phenolic content) of aqueous extract of *Vateria indica* L. has been determined using Folin-Ciocalteu reagent as an oxidizing agent, as well as gallic acid as the standard.^[10] 1 mL of several concentrations of extract (25, 50, 75, and 100 µg per mL) has been taken in a clean test tube. To each of these, 5 mL of 10% FCR (Folin-Ciocalteu reagent) alongside 4 mL of 7% Na₂CO₃

had been added, producing a final volume of 10 mL. After giving blue liquid a good shake, it was incubated in a water bath at 40°C for 30 min. At 760 nm, OD (absorbance) has been evaluated in comparison to blank. A UV-visible spectrophotometer measures dark blue color of phenols in plant extracts that have been oxidized by FCR reagent. Calibration curve has been plotted utilizing average absorbance readings at varying concentrations of gallic acid, and each experiment has been conducted in triplicate.

Quantitative Estimation of Flavonoid

The colorimetric assay for aluminium chloride has been utilized to assess total flavonoids. 4 mL of distilled water as well as 0.3 mL of 5% sodium nitrite solution had been added to every test tube containing 1 mL of aliquots, along with 1 mL of standard quercetin solution. 0.3 mL of 10% aluminium chloride has been added after 5 min. 2 mL of 1 M sodium hydroxide has been added at 6 min. Ultimately, distilled water has been added to get volume up to 10 mL and thoroughly blended. It gained an orange-yellowish color. At 510 nm, OD has been determined with UV-visible spectrophotometer. Distilled water has been utilized for blank. Total Flavonoid has been computed from a standard curve prepared using Quercetin.^[7]

Antioxidant Activity

DPPH free radical scavenging assay, which has been modified from Nithianantham *et al.*,^[11] has been utilized to assess extracts' *in vitro* antioxidant activities. Antioxidant activity of ascorbic acid is treated as a standard value.

Preparation of sample Solutions for DPPH assay

Each plant extract is dissolved in the necessary volume of methanol, and a stock solution containing 1 mg/mL of different extracts was created. From sample stock solution, concentrations of 25, 50, 75, 100 µg per mL solutions of every extract had been prepared. 1 mL DPPH solution has been administered to 1 mL of sample solutions, and they have then been incubated at ambient temperature for 30 min in dark. Similarly, 1 mL of various concentrations of ascorbic acid was added with 1 mL of DPPH, which served as the standard. A control has been made by combining 1 mL of methanol with 1 mL of DPPH solution. Lastly, a spectrophotometer set at 517 nm has been utilized to measure solutions (standard, plant samples, control) absorbance. The percentage of inhibition is used to express radical scavenging activity.

% of inhibition has been computed by utilizing below formula:

$$I \% = [AC - AO / AC] \times 100\%$$

Here, AC stands for absorbance of control (1 mL methanol + 1 mL DPPH solution),

AO stands for sample solution absorbance,

I % stands for percentage of inhibition.

Extracts' 50% inhibitory concentrations (IC_{50} values) had been computed. Data had been shown as mean $\pm$ standard deviation ($n=3$).

RESULTS

Quantitative Estimation of Phenol

TPC in dry and fresh extracts of bark, leaves, as well as flowers of *Vateria indica* L. has been established by Folin-Ciocalteu technique utilizing gallic acid as standard. Calibration curve's regression equation has been employed to calculate TPC, which has then been denoted as milligrams of gallic acid equivalents /g of dry weight sample (mgGAE per g). TPC of fresh bark extracts has been observed to be greater than dry bark extracts. Maximum TPC value has been noted for fresh bark, then dry flower, alongside lowest has been for the fresh flower extracts. TPC values for fresh and dry bark extracts were 417.4 and 236. (GAE/g) respectively (Table 1). For the dry leaves extracts (187 mg GAE per g), TPC value is higher than fresh leaves extract, 164.2 mg (GAE per g). Dried flower extract showed TPC value of 310.4 mg (GAE per g).

Quantitative Estimation of Flavonoid

Extracts' Total Flavonoid (TFC) content has been ascertained and stated as milligrams of QE (quercetin equivalents) /g of sample dry weight (mg/g). Total flavonoid content has been less than that of total phenol content in all extracts studied. Maximum flavonoid content has been seen in fresh bark extracts (151.245 mg QE /g). Least flavonoid content has been seen in fresh flower extracts (Table 2). Both fresh as well as dry leaf extracts showed almost same flavonoid content.

Antioxidant activity

The extracts' reducing power was calculated based on their concentration, yielding 50% inhibition (IC_{50}) values, and their antioxidant activity has been measured utilizing DPPH free radical scavenging assay. Mean % of DPPH free-radical scavenging activity at various extract concentrations as displayed in Table 3. There has been a dose-dependent rise in various extracts' capacity to scavenge radicals. The benchmark was ascorbic acid, alongside antioxidant qualities of several extracts varied. Ascorbic acid's IC_{50} value was 33.06 μ g/mL. Reduced antioxidant capability or

reduced radical scavenging activity is indicated by a higher IC_{50} value. In comparison to the bark and flower extracts, the fresh leaf extracts exhibited the highest antioxidant capacity. Fresh leaf extracts showed an IC_{50} value that was better than that of standard ascorbic acid. Bark extracts, both fresh and dry, have an IC_{50} value similar to that of Ascorbic acid. Here, all the extracts studied showed remarkable antioxidant properties. Bark fresh, also the IC_{50} value was less than that of the standard tried. Flower dry and fresh, leaves dry almost had comparable IC_{50} values. The bioactivity of these crude extracts is because of their high flavonoid or phenolic content.

DISCUSSION

This is a comparison with research of Gupta *et al.*, where TPC of aqueous extract of *Vateria indica* L. in the bark was 310 mg/g at 765 nm, and the ethanolic extract was 670 mg/g using Folin-Ciocalteu reagent.

Any plant's antioxidant properties are directly correlated with its phenol and flavonoid content.^[12] Phenolic compounds are efficient electron donors due to the direct antioxidant activity of their hydroxyl groups.^[13] Additionally, many of them stimulate cells' endogenous synthesis of antioxidant compounds.^[14] Numerous publications in literature claim that phenolic compounds minimize the burden of oxidative diseases by exhibiting free radical suppression, metal inactivation, peroxide breakdown, or oxygen scavenging in biological systems.^[15] An earlier study of flavonoid content of aqueous extracts and ethanolic extract by Gupta *et al.*, 2012^[16] has shown 62 mg QE/g in both samples using the Aluminium chloride method. This is far less than the current study's.

Most oxidizing chemicals, encompassing singlet oxygen along with other free radicals associated with a number of diseases, can be effectively scavenged by flavonoids.^[17] Flavonoids scavenge reactive species, chelate trace elements implicated in the creation of free radicals, inhibit synthesis of reactive oxygen, and upregulate as well as protect antioxidant defenses.^[18] Natural antioxidants have garnered particular attention due to their capacity to scavenge free radicals.^[19] One promising therapeutic strategy for hepatic damage is use of therapeutic herbs that contain greater levels of antioxidant components.^[20]

Table 1: Total phenolic contents in different extracts of *Vateria indica* L.

Sl. No.	Samples	75 mg/mL	100 mg/mL	Mean TPC value (mg GAE/g)
1	Bark (dry)	192.4	281.4	236.9
2	Bark (fresh)	357.8	477	417.4
3	Leaves (dry)	170.8	203.2	187
4	Leaves(fresh)	158.8	169.6	164.2
5	Flower (dry)	288.6	332.2	310.4
6	Flower(fresh)	75.7	81.65	78.675

Table 2: Total flavonoid contents in different extracts of *Vateria indica* L.

Sl. No.	Samples	0.75 mg/mL	1 mg/mL	Mean TPC value (mg QE/g)
1	Bark (dry)	74.464	103.75	89.107
2	Bark (fresh)	134.46	168.03	151.245
3	Leaves (dry)	84.64	85.17	84.905
4	Leaves(fresh)	62.5	99.46	80.98
5	Flower (dry)	56.60	82.32	69.46
6	Flower(fresh)	55.71	76.42	66.065

Table 3: Mean absorbance and IC₅₀ values of extract and ascorbic acid at different concentrations.

Concentration (µg/mL)	% Inhibition						
	Ascorbic Acid	Bark (dry)	Bark (fresh)	Leaves (dry)	Leaves (fresh)	Flower (dry)	Flower (fresh)
100	88.36	98.43	86.29	77.04	82.55	67.12	62.29
75	80.23	88.67	83.55	62.14	69.26	43.75	47.17
50	73.38	66.21	79.4	48.12	54.48	27.04	31.22
25	35.68	31.58	24.45	26.89	28.67	14.06	10.29
IC ₅₀ µg/mL	33.06	42.10	28.99	57.18	50.09	55.98	55.25

CONCLUSION

The findings of this research underscore the considerable phytochemical richness and antioxidant potential of *Vateria indica* L., particularly in its bark and leaves. The high levels of phenolic and flavonoid compounds contribute to its strong free radical scavenging activity, supporting its traditional therapeutic uses and indicating potential for modern pharmacological applications.

Given that *Vateria indica* is critically endangered, conservation efforts are urgently needed to preserve this valuable species and further explore its medicinal potential. Additionally, future research should aim to isolate and characterize individual bioactive compounds responsible for the observed antioxidant activity, assess their mechanisms of action, and evaluate their safety and efficacy through *in vivo* studies. Harnessing the therapeutic properties of *Vateria indica* may pave the way for the development of novel natural antioxidants and therapeutic agents.

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ABBREVIATIONS

DPPH: 2,2-Diphenyl-1-picrylhydrazyl; **IC₅₀:** Half Maximal Inhibitory Concentration; **mg GAE/g:** milligrams of Gallic Acid Equivalents per gram; **mg QE/g:** milligrams of Quercetin Equivalents per gram; **µg per mL:** Micrograms per milliliter; **°C:** degrees Celsius; **g:** Gram; **mL:** Milliliter; **FCR:** Folin-Ciocalteu

Reagent; **Na₂CO₃:** Sodium Carbonate; **nm:** Nanometer; **OD:** Optical Density; **UV:** Ultraviolet; **TPC:** Total Phenolic Content; **TFC:** Total Flavonoid Content; **AC:** Absorbance of Control; **AO:** Sample Solution Absorbance; **I %:** Percentage of Inhibition.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

The present study investigated the Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and antioxidant potential of the leaf, bark, and flower extracts of the critically endangered medicinal tree, *Vateria indica* L., endemic to the Western Ghats of India. Traditionally valued for its resin (White Dammar) in various medicinal systems, *Vateria indica* possesses significant bioactive properties due to its phytochemical constituents, notably phenols and flavonoids.

Aqueous extracts of fresh and dried plant parts were prepared and analyzed using standard spectrophotometric assays. TPC was assessed via the Folin-Ciocalteu method and expressed in Gallic Acid Equivalents (GAE), while TFC was estimated using the aluminum chloride colorimetric method and expressed in Quercetin Equivalents (QE). Antioxidant activity was evaluated through DPPH free radical scavenging assay, and IC₅₀ values were calculated.

The results revealed that fresh bark extracts exhibited the highest TPC (417.4 mg GAE/g) and TFC (151.245 mg QE/g), indicating substantial phenolic and flavonoid presence. Among the extracts tested, fresh leaf extracts demonstrated superior antioxidant potential, with an IC₅₀ value lower than that of the standard

ascorbic acid, suggesting potent radical-scavenging capacity. Overall, the study confirmed a positive correlation between phenolic/flavonoid content and antioxidant activity in *Vateria indica* extracts.

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