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Exploring the phyto- and physicochemical evaluation, fluorescence characteristics, and antioxidant activities of *Acacia ferruginea* Dc: an endangered medicinal plant

M. Jeevitha[1*], Pavithra V. Ravi[2], Vinodhini Subramaniyam[2], Moorthi Pichumani[2] and Shubashini K. Sripathi[1]

Abstract

Background: Herbal plants are potent in curing various ailments of ancient times as they have comparatively lesser side effects. The demands for natural drugs, mostly from plant sources, are increasing over the past few decades. Because of their potent antioxidant activity, Acacia species are used to treat a variety of diseases. One of the species *Acacia ferruginea*, an endangered medicinal plant, is widely used in the traditional medicine system, and it is considered that standardization would be beneficial. The present study investigates the physicochemical parameters, preliminary phytochemical screening, trace metals by SEM–EDS, and fluorescence properties of various extracts (non-polar to polar) of leaf and bark parts. Standard spectrophotometric methods (UV–Vis, FT-IR, fluorescence spectroscopy) are employed to analyze the functional groups, and the DPPH and total antioxidant methods are used to assess antioxidant potential.

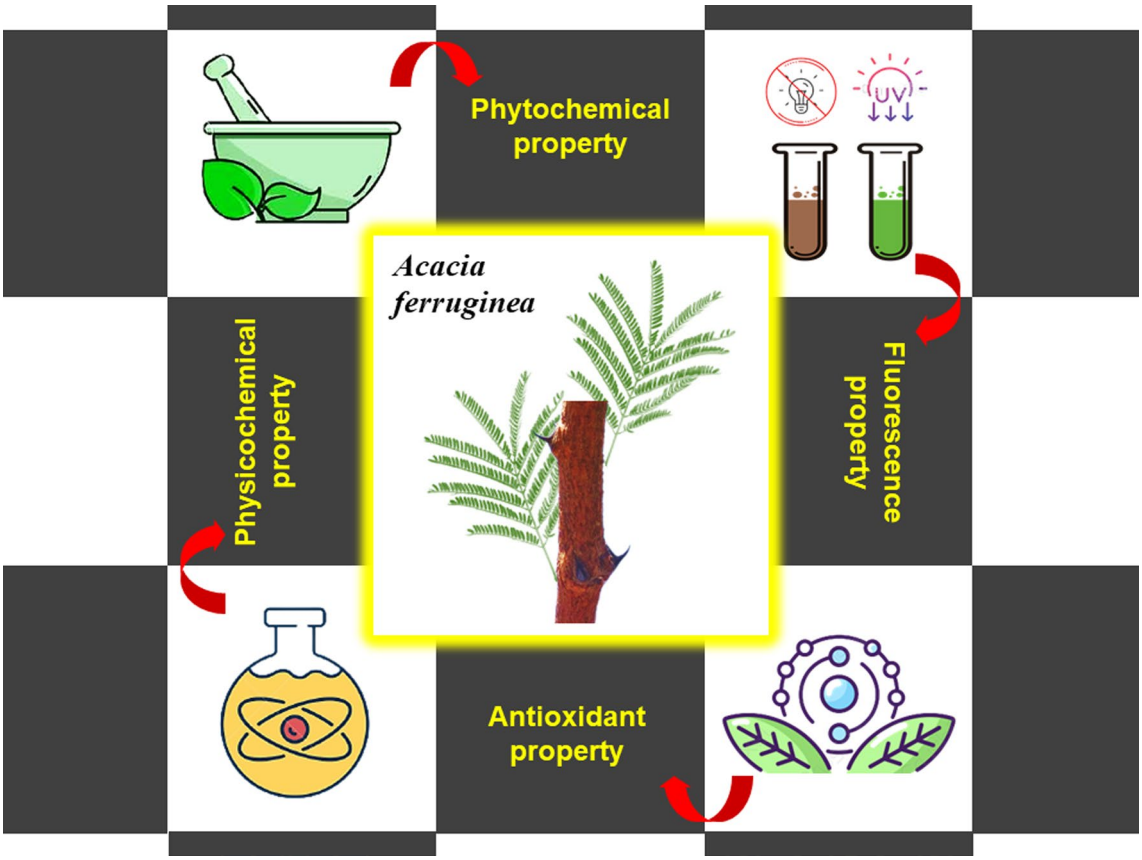
Results: The ethyl acetate extract of leaves and ethanol extract of the bark are found to be the highest in yield, 16.32% and 2.54%. Results reveal that the total ash percentage and moisture content are of bark and the water-soluble ash of leaves is higher (10.3 ± 0.85, 7.6 ± 0.34, 3.22 ± 0.24%). The bark polar extract contained more macro-elements such as Na, K, Mg, Ca, S, and Cl. Phytochemical analysis reveals the polar extracts of leaves and bark show saponins, flavonoids, steroids, phenolic compounds, and non-polar extracts show mild positive. The total alkaloids, phenolics, and terpenoids (1.58 ± 0.08%; 0.56 ± 0.11; 0.75 ± 0.15) are found to be higher in *A. ferruginea* leaves. The FT-IR result shows the presence of alkanes, alkenes, aromatic compounds, aldehydes, phenolics and does not contain any toxic substances since there is no peak observed in the region between 2220 and 2260 cm^{-1}. The in vitro antioxidant activity of the species demonstrated that both the leaf and bark parts have prominent antioxidant properties.

Conclusions: The results obtained from the preliminary standardization of *A. ferruginea* are very helpful in the determination of the quality and purity of the crude drug. The refurbished findings of *A. ferruginea* are promising, and further research is important to identify the bioactive compounds, thereby developing nutritional supplements and medications through therapeutic compound isolation.

Keywords: *Acacia ferruginea*, Medicinal plants, Phytochemicals, Physicochemical analysis, Fluorescence, Spectroscopy, FT-IR, Antioxidant, Endangered

*Correspondence: jeevichemistry@gmail.com
[1] Department of Chemistry, Avinashilingam Institute for Home Science and Higher Education for Women, Tamil Nadu, Coimbatore 641 043, India
Full list of author information is available at the end of the article

Graphical Abstract



Background

Medicinal plants are important sources and have been used for thousands of years for curing health conditions, flavoring, preserving food, and preventing diseases, including epidemics. The relationship between man and his quest for medications in nature dates back thousands of years, as evidenced by a variety of sources including written records, conserved locations, and even original plant medicines. Active compounds engendered during secondary metabolism are typically responsible for the biological properties of plant species [1].

In plants, approximately 50,000 metabolites have been discovered, with the total number expected to reach 200,000 [2]. Modern research has grasped its success and has employed numerous natural remedies known to ancient civilizations and utilized in modern pharmacotherapy for millennia. [3]. Traditional medication, primarily derived from plants, has played a critical role in keeping humans disease-free on our planet. It is difficult to pinpoint where these medicines emerged as a treatment option [4]. Despite the overwhelming impact of modern medicine and enormous breakthroughs in synthetic drug synthesis, traditional cures (now referred to as herbal pharmaceuticals/herbal treatments in many publications) are supported, suggested, and encouraged by the World Health Organization (WHO). Since these remedies are affordable, healthful, and trustworthy because they have no substantial adverse repercussions, they are not only popular but also a good way to cure the disease [4, 5]. Botanical medicines are gaining popularity around the globe, with consumer demand of more than $72 billion, especially in developed and developing nations over the last decade; traditional remedies are thought to enable individuals in Western countries to live healthier lives like their forefathers. People spend billions of dollars on home remedies because they believe herbal medicine is a balanced and proper formula for a healthy existence, which has become a hot commodity on the world market [6, 7]

Acacia species are multipurpose trees distributed worldwide which comprise nearly 1200 species of the *Fabaceae* family belonging to the tribe *Acaciacea.* Approximately 152 chemical components have been discovered from the *Acacia* species during the last seven

decades. The main factors for their possible biomedical values are the isolated key compounds (flavonoids, terpenoids, and phytosterols, phenolic acids, hydrocarbons, fatty acids, and other substances) accumulated primarily on leaves, stem barks, and pods. Protein and mineral content are abundant in numerous species, especially in the leaves, twigs, and pods [8, 9]. *Acacia* species have been extensively investigated because of their widespread ecological amplitude, dietary, and beneficial properties [10].

One of the species, *Acacia ferruginea*, belongs to *the Mimosaceae* family, a unique natural source widely used in the traditional systems of medicine to treat various ailments. Irritable bowel syndrome, leprosy, and hemorrhage can be traditionally treated by the bark of *A. ferruginea* as it has higher anti-ulcerogenic and antioxidant activities [11–13]. According to published literature, *A. ferruginea*, an effective Ayurvedic medicine, is used to cure "Vata" and "Kapha" illnesses. The leaf paste is applied externally to cure burns and scalds, while the pod decoction is used as an astringent and demulcent [14]. It has the potential to grow into a tree or a large shrub and can flourish in a variety of conditions, including tropical, subtropical, warm, and cool temperate zones [15]. *Acacia* leaves are either real leaves or phyllodes, and flowering (brilliant yellow to cream) occurs in late winter, early spring, or early summer [16]. Seeds are edible and are frequently crushed into flour, and gum flows from the stems and branches due to environmental conditions or stress [17, 18]. *A. ferruginea* leaves and bark possess anti-inflammatory, larvicidal, anticancer, anti-hemolytic, anti-diabetic, and anti-hemorrhoidal properties [19–23]. The quest for novel natural medications is now underway. The development of novel herbal medications is becoming more difficult owing to several challenges, including a lack of understanding of the most active family, genus, and species, required for a thorough investigation [9].

Adulteration and replacement constitute a significant issue in an enterprise that jeopardizes the reputation of the herbal medicine system, resulting from a lack of precise information, misidentification, and personal benefit [24]. To reform this unconstitutional act and enhance customer protection and poor medication handling, physicochemical parameters must be evaluated. From raw ingredients to final goods, the World Health Organization has established many criteria for the standardization of herbal products like quality, safety, and effectiveness of herbal medicines and quantitative norms (such as ash values, extractive values, and volatile matter) [4, 25]. The chromatographic and spectral fingerprints (UV–visible, FT-IR, fluorescence spectroscopy) of herbal medicines may be used to solve the issue of quality control of herbal medicines as well as offering detailed insights into the molecular properties and quantification of photo components from plant products. Chemical fingerprints collected by chromatographic procedures are highly recommended for establishing the authenticity of traditional medicinal [26, 27]. It necessitates a well-balanced blend of contemporary analytical methods and traditional knowledge. Stability testing studies are performed to assess the consistency of herbal drugs, which are impacted by various factors such as temperature, humidity, heat, oxygen, moisture, microbial contamination, and trace metal contamination. As a result, studies involving different forms of measurement such as chemical, physical, microbiological, spectral, chromatographic, and therapeutic studies may be useful to investigate opulence [28].

The South African Act on Alien and Invasive Species Regulations classifies *Acacia* species as an invasive plant (Category 2) [29], and *A. ferruginea* is red-listed under vulnerable category (updated red list-2015) by "The International Union for Conservation of Nature (IUCN)", a foremost expert on environmental issues that take steps to protect it via several significant international environmental agreements (IUCN conferences, etc.) [30].

The present investigation is beneficial to conserve *A. ferruginea* as it comes under endangered plant species due to overexploitation and environmental conditions. Since there has been no significant scientific evidence on the pharmacological potential and medicinal uses of this genus, the current study is conducted to resolve this lacuna by phytochemical and physicochemical (qualitative and quantitative) assessments.

Methods

Chemicals and reagents

In the present study, all the chemicals (hexane, ethanol, ethyl acetate, millipore water, conc. H_2SO_4, conc. HNO_3, conc. HCl, picric acid, Ferric chloride, α-naphthol, NaOH, sodium acetate, acetic acid, pet ether, chloroform, diethyl ether, acetone, methanol, lead acetate, ammonia, distilled water, DPPH, DMSO, sodium phosphate, ammonium molybdate, ascorbic acid, acetic anhydride, magnesium chips) are purchased from Ranbaxy/HI-MEDIA and are of analytical grade used without purifying further.

Sample collection and preparation of the samples

The leaves and bark of *Acacia ferruginea* are collected from the village Nalligoundanpalayam (11012′19.2″ N 77017′55.3″ E), Avinashi, Tamil Nadu state, India. The plant is authenticated by the Botanical Survey of India, Coimbatore, Tamil Nadu, India (BSI/SRC/5/23/2018/Tech/2080). The pre-cleaned leaves and bark are dried under shade and pulverized for extraction.

Extraction

The dried powder samples of *A. ferruginea* (170 g) are sequentially extracted with solvents with increasing polarities (hexane, ethyl acetate, ethanol, 90% ethanol, and distilled water (each 1200 mL)) using a Soxhlet extractor. Solvent traces are removed by drying and are concentrated by simple distillation. The crude extracts are further dried using a rotary evaporator, and aqueous extracts are concentrated by lyophilization (Christ Alpha 1-2 LD plus) and stored at 4^0 C for further analysis.

Physicochemical studies

The fresh plant (leaves and bark) powder is used to determine the physicochemical behavior according to methods described in Indian pharmacopeia.

The pH of different solutions of bark and leaves (1% w / v and 10% w / v) of *A. ferruginea* material is measured using a Hanna pH meter [31]. Ash values (total, water soluble, and acid insoluble) are determined by Thiex et al. [32]. Dry matter, moisture content (LOD) [33], foreign matter [34], solubility, water-soluble and alcohol-soluble extractive values [35], fluorescence studies [36] are also determined. All tests are carried out in triplicate, with the findings expressed as mean and standard deviation, and the details are given in Additional file 1: SI-M1.

Metal content analysis

The polar and non-polar extracts of *A. ferruginea* are subjected to elemental analysis by adhering the extract to adhesive carbon tape, which is subsequently placed on aluminum specimen stubs and sputtered with a gold sputter coater. The samples are then analyzed using a TESCAN MIRA 3 Scanning Electron Microscope with a magnification of 30 kV and an accelerating voltage of 200 V to 30 kV [25, 37].

Qualitative phytochemical studies

Qualitative phytochemical analyses for all of the extracts are done by the standard procedures [38]. The extracts are tested for alkaloids, tannins, phenols, flavonoids, steroids, saponins (Additional file 1: SI-M2).

Quantitative studies

The total terpenoids, alkaloids, and phenolics are quantified from *A. ferruginea* leaves and bark by standard methods [39].

Quantification of phenolic compounds

At room temperature, 10 g of the plant sample is extracted several times with 80% aq. methanol (100 mL). Whatman filter paper No. 42 is used to filter the entire solution (125 mm). Then, the filtrate is moved to a crucible, dried over a water bath, and weighed to a constant weight [39].

Quantification of alkaloids

To the plant sample (5 g) is added, and 10% acetic acid in ethanol (200 mL) is added and left to stand (4 h). Then, it is filtered and concentrated for a quarter of an hour in a water bath. Drop by drop ammonium hydroxide is applied until the precipitation is complete [39]. The precipitate is cleaned with ammonium hydroxide, dried, and weighed, and the following calculations are made:

$$\%\text{Alkaloid} = W_a/W_b \times 100$$

W_a—weight of alkaloid; W_s—weight of sample.

Quantification of terpenoids

The dried plant extract (100 mg (W_a)) is immersed in 9 mL ethanol for 24 h. Then, it is extracted with petroleum ether (10 mL) using a separating funnel. The ether extract is separated, into pre-weighed beakers (W_b) and allowed to dry completely [39]. After ether is evaporated, the yield (%) of total terpenoid content is estimated using the following formula

$$\%\text{Terpenoid} = W_a - W_b/W_a \times 100$$

Antioxidant activity

Total antioxidant activity (TAA)

Sample preparation The plant sample (10 mg) is dissolved in 1 mL DMSO solution and incubated (400^0 C) for 24 h in a shaking incubator (100–120 rpm).

Phatak and Hendre's [40] procedure, with minor modifications, is used to validate total antioxidant activity employing the phosphomolybdenum method. The sample (0.5 mL) is diluted with the reaction mixture (0.5 mL of 28 mM sodium phosphate, 0.6 M H_2SO_4, and 4 mM ammonium molybdate reagent solution) and incubated (500 °C; 90 min) with a blank tube (without sample). The tubes are sterilized after incubation, and the absorbance is measured at 695 nm using a UV–visible spectrophotometer (Shimadzu, 1601). TAA (mg/g) is measured using ascorbic acid as the standard.

DPPH radical scavenging assay

The DPPH radical scavenging activity of the different extracts is assessed based on the method given by Chaves et al. [41] The diluted test solutions (25–200 µg/mL) of each extract are added to 0.3 mM DPPH in methanol solution to reach the final volume of 2 mL. An equal volume of DPPH and methanol served as a blank. After 30 min of incubation at room temperature, the resultant

solutions appeared yellow rather than purple color. The colorimetric absorbance is fixed at 520 nm, and the percentage of inhibition is calculated using the formula below.

$$\text{Inhibition\%} = A_i - A_{ii}/A_i \times 100$$

A_i—standard absorbance control; A_{ii}—absorbance for extracts.

Spectral fingerprinting

UV

The extracts (2 mL/1000 ppm) are dissolved in their respective solvents, and the UV–Vis spectra are recorded in a double-beam UV spectrophotometer (Systronics U-2701) in the wavelength range of 200–800 nm. A shift reagent (NaOEt) is added to the polar extracts to observe the shift.

FT-IR

FT-IR spectral measurements are recorded in PerkinElmer FTIR00585 spectrophotometer in the range 4000–800 cm^{-1}.

Fluorescence measurements

The fluorescence (excitation/emission) spectra of *A. ferruginea* extracts are measured using a fluorescence spectrophotometer (Agilent technologies, G9800A) with a wavelength range of 190–1100 nm. Absorption and fluorescence measurements are taken for the extract solution (2 mL/1000 ppm) using quartz cuvettes (path length: 1 cm) at room temperature.

Results

Extractive values

In leaves and bark, significant differences in extraction yields are observed for methanol and other solvents. The ethyl acetate extract of leaves and ethanol extract of the bark are observed to be the highest in yield, 16.32% and 2.54%, followed by ethanol extract of leaves (8.26%), respectively, which may be because extractable bioactive components like carbohydrates and proteins are more soluble in ethanol than in the non-polar solvent hexane. The extractive values of *A. ferruginea* bark and leaves are given in Table 1.

Physicochemical evaluation of *A. ferruginea* bark and leaves

The results of total ash, acid-soluble and insoluble ash, water-soluble and insoluble ash, dry matter, and moisture content of bark and leaves of *A. ferruginea* are given in Tables 2 and 3, respectively. The results reveal that the total ash percentage and moisture content of bark are higher (10.3 ± 0.85, 7.6 ± 0.34). The total ash of bark (10.32%) is found to be more significant than that of leaves (8.84%), whereas the water-soluble ash of leaves is higher (3.22 ± 0.24%) than bark which may be due to the presence of a high level of biological residue. The pH of the bark is found to be higher compared to leaves.

The hexane extract of both bark and leaves is completely soluble in chloroform, and slight miscibility in ethanol is noted for aqueous extract. Ethyl acetate extract of leaves shows complete solubility in ethanol and less solubility in chloroform, acetone, and water. The solubility profile of bark and leaf extracts of *A. ferruginea* is given in Additional file 1: Table SI-T2.

The bark and leaf powder of the *A. ferruginea* extracted in various chemical reagents are observed under UV light (245 nm) and ordinary visible light and recorded in Additional file 1: Table SI-T3, T4**.** The bark powder of *A. ferruginea* showed olive green color under UV light and orange under visible light when treated with conc. HNO_3. The bark remained colorless under UV and visible light with many solvents. The fluorescent green color is observed

Table 1 Extractive values of *A. ferruginea* bark and leaf extracts

S. no	Extracts	Sample name	Solvent	Percentage (%)
1	*A. ferruginea* Bark Hexane	ACFE-B-H	Hexane	0.33
2	*A. ferruginea* Bark Ethyl acetate	ACFE-B-EA	Ethyl Acetate	1.59
3	*A. ferruginea* Bark Ethanol	ACFE-B-E	Ethanol	2.54
4	*A. ferruginea* Bark Hydroethanol	ACFE-B-HE	Hydroethanol (90%)	0.23
5	*A. ferruginea* Bark Aqueous	ACFE-B-Aq	Distilled water	1.67
6	*A. ferruginea* Leaf Hexane	ACFE-L-H	Hexane	4.32
7	*A. ferruginea* Leaf Ethyl acetate	ACFE-L-EA	Ethyl Acetate	16.32
8	*A. ferruginea* Leaf Ethanol	ACFE-L-E	Ethanol	8.26
9	*A. ferruginea* Leaf Hydroethanol	ACFE-L-HE	Hydroethanol (90%)	1.26
10	*A. ferruginea* Bark Aqueous	ACFE-L-Aq	Distilled water	2.35

Table 2 Physicochemical Analysis of *Acacia ferruginea* bark

S. no	Test	Weight of the sample taken (g)	Initial color	Final color	Temperature °C	% Value
1	pH 1% w/v solution	1.0	–	–	26.9 ± 0.08	7.2 ± 0.05
	pH 10% w/v solution	10.0	–	–	28.6 ± 0.33	6.4 ± 0.0
2	Total ash content	2.0	Brown	White	0–600, 16 h	10.3 ± 0.85
3	Acid-insoluble ash	2.0	White	Buff color	100, 5 min	1.36 ± 0.087
4	Water-soluble ash	2.0	White	Cloudy white	100, 5 min	1.74 ± 0.013
5	Dry content	2.0	Brown	Brown	105, 12 h	92.4 ± 0.35
6	Moisture content (Loss on drying)	2.0	Brown	Brown	105, 12 h	7.6 ± 0.34
7	Foreign matter	100	–	–	–	0.75 ± 0.21
8	Water-soluble extractive	5	–	–	–	9.45 ± 0.50
9	Alcohol-soluble extractive	5	–	–	–	12.26 ± 0.89

Values are expressed in Mean ± SD where $n = 3$

Table 3 Physicochemical analysis of *A. ferruginea* leaves

S. no	Test	Weight of the sample taken (g)	Initial color	Final color	Temperature °C	% Value
1	pH 1% w/v solution	1.0	–	–	27 ± 0.163	6.33 ± 0.047
	pH 10% w/v solution	10.0	–	–	29 ± 0.535	5.8 ± 0.00
2	Total ash	2.0	Green	White	0–500, 16 h	8.8 ± 0.30
3	Acid-insoluble ash	2.0	White	White	100, 5 min	1.22 ± 0.13
4	Water-soluble ash	2.0	White	White	100, 5 min	3.22 ± 0.24
5	Dry content	2.0	Green	Pale green	105, 12 h	95.8 ± 0.85
6	Moisture content (Loss on drying)	2.0	Green	Pale green	105, 12 h	4.2 ± 0.85
7	Foreign matter	100	–	–	–	0.35 ± 0.11
8	Water-soluble extractive value	5	–	–	–	18.11 ± 0.43
9	Alcohol-soluble extractive value	5	–	–	–	24.26 ± 0.66

Values are expressed in Mean ± SD where $n = 3$

under UV light when the leaf powder is treated with $FeCl_3$. On treatment with $FeCl_3$, leaf powder turned to a fluorescent green color under UV light which may be attributed to the presence of phenolic rings (-OH). Both the leaf and bark powder showed characteristic coloration, viz. pale green, pale brown, and dark in treatment with various chemical reagents under UV and visible light.

Metal content analysis

Identification of inorganic minerals of leaf and bark extracts of *A. ferruginea* by EDS indicated that the bark polar extract contained more macro-elements such as Na, K, Mg, Ca, S, and Cl. All extracts except bark hexane extract show the presence of Al, and there is no trace of toxic metals in any of the samples (Table 4). Figure 1 represents the elemental analysis of *A. ferruginea* extracts, and the corresponding values are given in Table 4.

Phytochemical screening

The polar extracts of leaves and bark show saponins, flavonoids, steroids, phenolic compounds, and non-polar extracts show mild positive. Ethyl acetate extract of the bark and leaves strongly shows the presence of alkaloids, flavonoids, steroids, terpenoids, and a trace amount of saponins (Table 5).

Quantification studies

The results given in Table 6 show that the total alkaloids, phenolics, and terpenoids of *A. ferruginea* leaves are found to be higher (1.58 ± 0.08%; 0.56 ± 0.11; 0.75 ± 0.15). Quantification results show the following order.

Table 4 Metal content analysis of hexane and hydroethanol extracts of *A. ferruginea* bark and leaves

Minerals	Weight %			
	ACFE-B-H	ACFE-B-HE	ACFE-L-H	ACFE-L-HE
Al	–	0.57	0.42	0.48
Au	–	1.47	1.05	0.78
C	80.16	45.14	77.40	61.03
Ca	–	1.74	–	–
Cl	0.39	2.20	–	–
K	–	2.99	–	–
Mg	–	1.12	–	–
Mo	1.81	–	–	–
Na	–	1.07	–	–
O	17.64	42.09	20.84	27.07
S	–	0.60	–	–
Si	0.39	1.01	0.29	–
Zn	–	–	–	10.64

A. ferruginea leaves: Total Alkaloids > Total Terpenoids > Total Phenolics.
A. ferruginea bark: Total Terpenoids > Total Phenolics > Total Alkaloids.

Spectral fingerprinting

UV

The UV profile shows the peaks at 230, 240, 235, 244, and 238 nm with the absorption of 1.87, 2.29, 1.87, 1.8, and 1.5 nm for bark extracts, respectively (Additional file 1: SI-T5, Figs. 2, 3).

FT-IR

The FT-IR peak values and functional groups of *A. ferruginea* leaves and bark extracts are represented in Additional file 1: SI-T6, SI-T7, and the corresponding FT-IR spectrum profile is shown in Figs. 4, 5. The FT-IR gave a broad peak around 3200 cm^{-1} for all polar extracts, indicating O–H stretching. The strong peak is observed at 1724.36 and 1249.87 cm^{-1}, indicating C=O stretching for leaf hexane extract. The stretching band at 1269.16 cm^{-1} attributes to C=O stretching (alkyl/aryl ether) bending vibrations, the peak around 1396.46 cm^{-1} and 1045.42 cm^{-1} indicates the presence of C-H bending and stretching, and C–C bending indicated the presence of alkenes and aromatic compounds, respectively.

Fluorescence spectroscopy

All fluorescence spectra are stimulated at different wavelengths based on the identification. The strongest

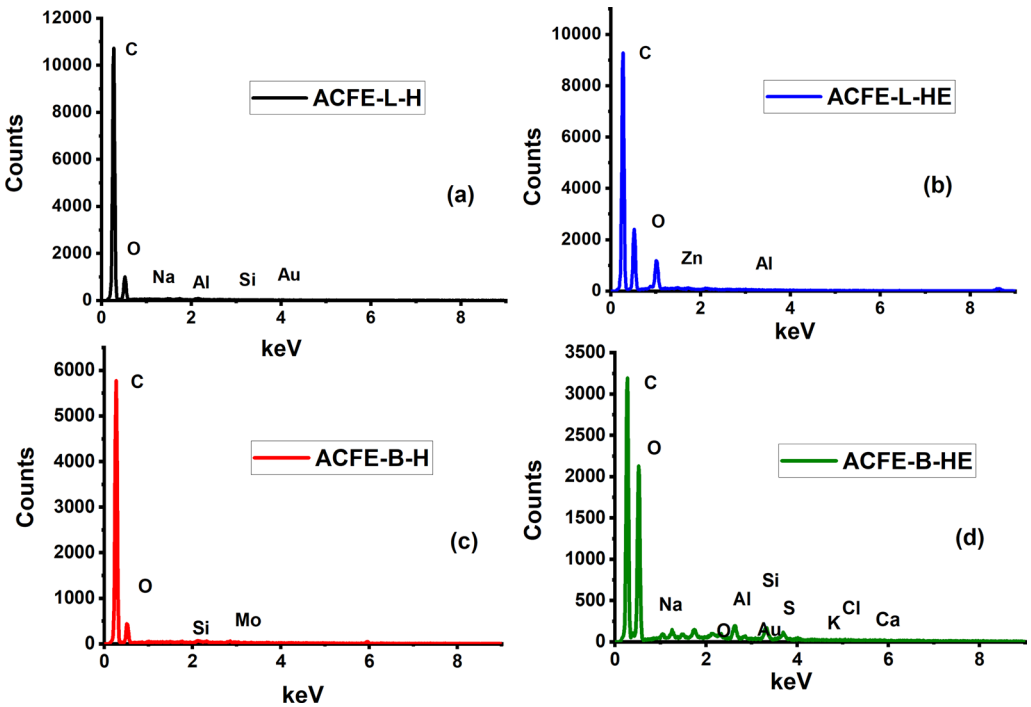


Fig. 1 Energy-dispersive X-ray spectroscopy (EDS) spectra of *A. ferruginea* leaf and bark extracts **a** ACFE-L-H, **b** ACFE-L-HE, **c** ACFE-B-H, **d** ACFE-B-HE. EDS Specifications: Magnification—30 kV; field of view—6.4 mm at WD analytical 10 mm, 20 mm at WD 30 mm; accelerating voltage 200 V to 30 kV; probe current—2 pA to 200 nA

Table 5 Results of phytochemical screening of leaf and bark extracts of *A. ferruginea*

Sample name	Hager's test	NaOH	Shinoda	Ferric chloride	Salkowski test	Molisch test	Liebermann's test	Foam test
ACFE-B-H	+	−	−	−	++	−	+	−
ACFE-B-EA	+++	+	++	++	++	−	++	−
ACFE-B-E	++	+	−	+++	++	−	−	−
ACFE-B-HE	−	−	−	++	−	−	−	+
ACFE-B-Aq	−	+	+	++	−	+	−	+
ACFE-L-H	−	−	−	−	+	−	−	−
ACFE-L-EA	++	+	+	+++	+	−	+	++
ACFE-L-E	+	+	+++	++	++	−	++	++
ACFE-L-HE	+	++	+	++	+++	−	++	++
ACFE-L-Aq	+	++	++	++	+	−	++	+++

+++ very strong, ++ trong, + mild, − nil

Table 6 Result of quantification studies—*A. ferruginea* leaves and bark

S. no	Test parameters	Results (ACFE)	
		Leaves (%)	Bark (%)
1	Total alkaloids	1.58 ± 0.08	0.11 ± 0.03
2	Total phenolics	0.56 ± 0.11	0.17 ± 0.06
3	Total terpenoids	0.75 ± 0.15	0.54 ± 0.16

emission intensity is detected for ACFE-B-H extract at the wavelength 791 nm when excited at 392 nm. No red-shift is observed in leaf extracts (ACFE-L-H, ACFE-L-E, ACFE-L-EA, ACFE-L-HE), and the emission wavelength remains the same at increasing emission intensity except for aqueous extract. Fluorescence spectra obtained from *Acacia* leaf and bark extracts are documented in Additional file 1: SI-T8.

Figures 6 and 7 show emission and excitation spectra of leaf and bark extracts in different solvents, respectively.

Total antioxidant activity (TAA) and DPPH assay

The propensity for phosphomolybdenum reduction in different sample extracts is in the range of: ACFE-B-Aq > ACFE-B-EA > ACFE-L-E > ACFE-L-EA > ACFE-L-Aq > ACFE-B-E > ACFE-B-H > ACFE-L-HE > ACFE-L–H > ACFE-B-HE, and the respective values are found to be 608 > 559 > 531 > 522 > 464 > 385 > 372 > 248 > 218 > 198 mg/g of total antioxidant activity.

The results reveal that both *A. ferruginea* leaves and polar bark extracts exhibit good potential for antioxidant activity at higher concentrations. Figures 8 and 9 show the TAA and DPPH radical scavenging potential of the extracts along with ascorbic acid as reference.

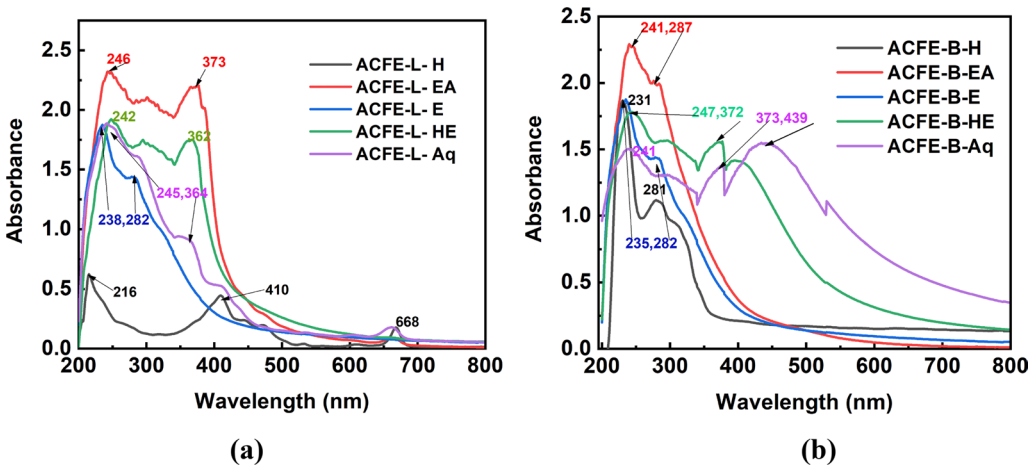


Fig. 2 **a** UV–Vis spectrum of *A. ferruginea* leaf extracts (ACFE-L-H, ACFE-L-EA, ACFE-L-E, ACFE-L-HE, ACFE-L-Aq), **b** UV–Vis spectrum of *A. ferruginea* bark extracts (ACFE-B-H, ACFE-B-EA, ACFE-B-E, ACFE-B-HE, ACFE-B-Aq)

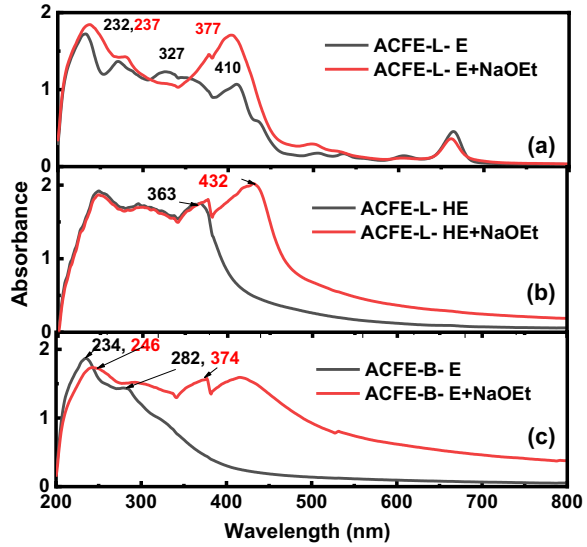


Fig. 3 UV–Vis spectrum of *A. ferruginea* extracts (concentration: 1 mg/mL) before and after adding shift reagent (NaOEt) **a** mild shift in ACFE-L-E, **b** redshift from 363 to 432 nm (absorption maxima) in ACFE-L-HE, **c** longer wavelength shift in ACFE-B-E

The percent DPPH scavenging activity of the leaf extracts ranged from 5 to 0.74% and bark from 14 to 78% at the concentration range of 25–200 µg/mL. A significant correlation exists between total antioxidant activity and DPPH• scavenging activity, and the results are comparable.

Discussion

Herbal plants were originally researched due to their broad medicinal values since the classical era especially Acacia species are utilized to cure a range of illnesses due to their high antioxidant activity. Water is an all-purpose solvent that is often used to extract plant materials. Many conventional healers extract plants mainly with water, although most plant extracts in organic solvents have been found to have more consistent therapeutic properties than water [42]. *A. ferruginea* bark ethanol extract (ACFE-B-E) has the highest solubility of phytochemicals when extracted with alcohol than the other extracts tested. Trishala and Lakshmi [43] report the same kind of study that *A. catechu* bark ethanol extract showed 2.436% extractive value which is similar to that of *A. ferruginea* (2.54%). The difference in extract yields is probably attributed to the different solvent polarities used, which also aids in improving phytochemical component solubility [44].

The term "organoleptic test" refers to observing properties of components that sense organs can use. It thus determines some of the material's unique features, which can be called a first step in determining the material's character and degree of purity. Results reveal that the hexane extract from bark appears as a buff-colored coarse powder with a fragrant odor, and the alcoholic extracts of leaves are of intense odor with a waxy/oily appearance which is listed in Additional file 1: SI-T1. One of the methods for determining the quantity and quality of minerals present in plants is ash content (i.e., total ash, acid-insoluble, and water-soluble ash) and moisture determination, in which the humidity levels in medicinal plants do not reach the recommended level (17%) [45, 46].

Dry matter is higher in leaves (95.8%) than in the bark. The moisture content of the bark sample is 7.61% and is under the recommended level. Low moisture indicates that the substance would be more resistant to degradation [28]. The results are in agreement with *A. ferruginea* bark extracts (acid-insoluble ash 1.13; water-soluble ash 2.59; water-soluble extractive 11.73; ethanol-soluble extractive 16.57; moisture content 10.2) [47]. The water-soluble and alcohol-soluble extractive values of *A. ferruginea* leaves are higher (91.6 and 97.9% increase, respectively) than those of the bark.

Solubility tests show the soluble efficacy of extracts in polar and non-polar solvents. Ethanol extract of the bark is soluble in polar solvents and hexane. Both the hexane extracts (bark and leaves) are completely soluble in chloroform, while the aqueous extract has a little miscibility in ethanol. This shift tends to be due to a variation in the polarity of components present in the extracts and the solvents used, which also plays a role in increasing phytochemical compound solubility. Therefore, this result confirms the richness of this plant in polar substances. The dielectric constant, chemical composition of organic solvents, and chemical properties of plant phytochemicals may all influence phytochemical recovery [44].

Fluorescence is a phenomenon that numerous chemical components in plant material exhibit. Many natural chemicals (such as alkaloids like berberine) do not glow in daylight, but under ultraviolet light they emit fluorescence. If the chemicals are not fluorescent by nature, other reagents can typically be used to transform them into fluorescent derivatives or breakdown products. As a result, several crude drugs are frequently evaluated qualitatively, which is an essential pharmacognostic indicator [48]. The bark powder remained colorless with several solvents, but the leaf powder turns a brilliant green color with $FeCl_3$, (under UV light) which might be due to the presence of phenolic rings (–OH). In treatment with various chemical reagents under UV and visible light, both the leaf and bark powder displayed distinctive coloration, notably pale green, pale brown, and black. A non-destructive technique, EDS, can be used to identify trace

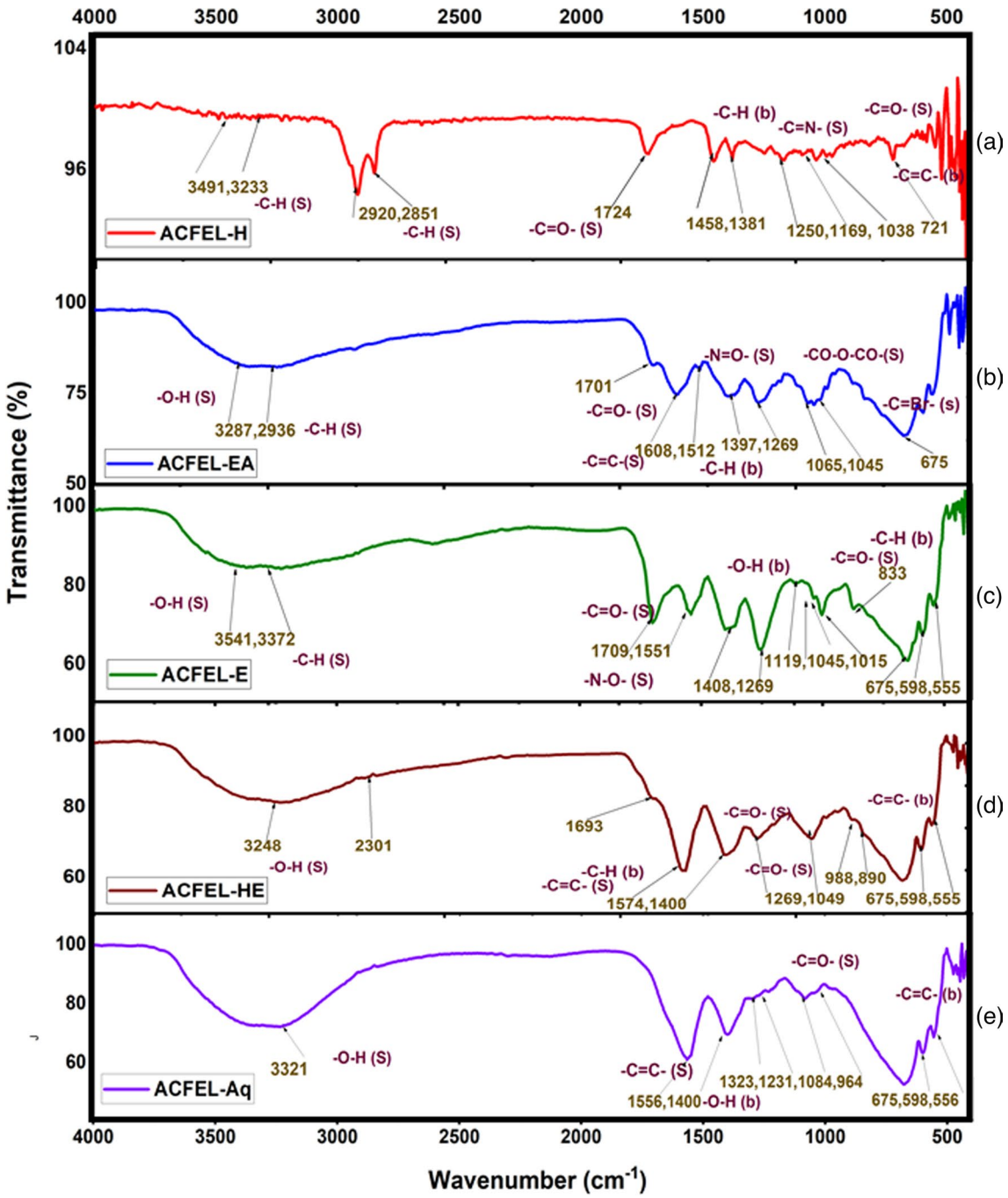


Fig. 4 **a** FT-IR spectrum of *A. ferruginea* Leaf Hexane extract (ACFE-L-H); **b** ethyl acetate extract (ACFE-L-EA); **c** ethanol extract (ACFE-L-E); **d** hydroethanol extract (ACFE-L-HE); and **e** aqueous extract (ACFE-L-Aq)

elements, crystals, and multiple sampling in different plant areas, both of which provide data from a nanometer-sized region. Zinc is an important micronutrient as it promotes certain metabolic reactions in plants [49]. A high percentage of zinc (10.64%) is shown in the leaf-polar extracts.

The discovery of phytochemicals may be used to predict the pharmacological operation of plants by calculating various methods, but qualitative assessments like preliminary phytochemical studies are still widely used. The most common chemicals isolated from the *Acacia* genus are flavonoids, terpenoids, and phenolic

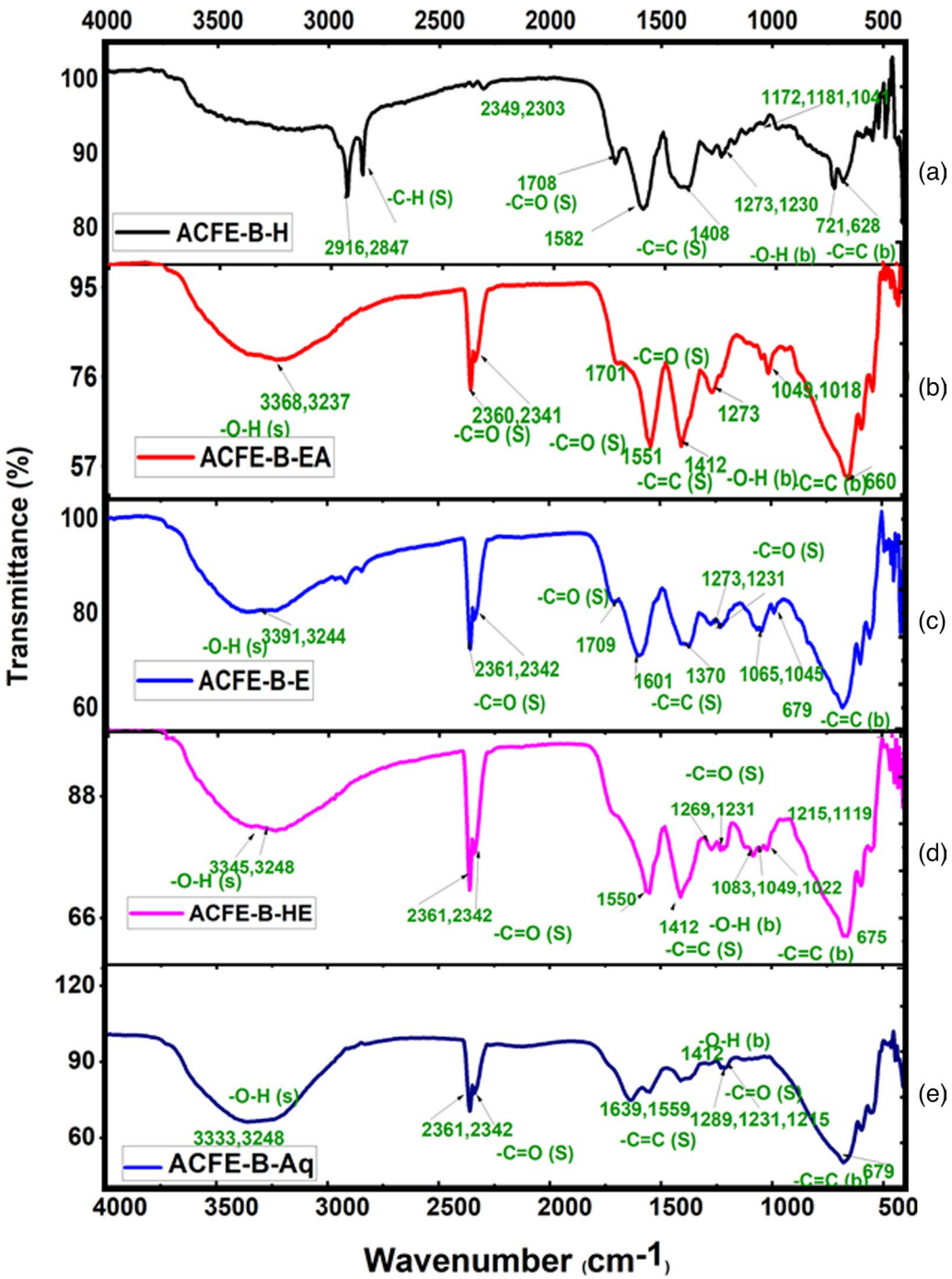


Fig. 5 **a** FT-IR spectrum of *A. ferruginea* Bark Hexane extract (ACFE-B-H); **b** ethyl acetate extract (ACFE-B-EA); **c** ethanol extract (ACFE-B-E); **d** hydroethanol extract (ACFE-B-HE); and **e** aqueous extract (ACFE-B-Aq)

acids [50, 51]. Previous studies have reported that *A. ferruginea* contains alkaloids, steroids, triterpenoids, saponins, flavonoids, tannins, phenolic compounds, carbohydrates, gums, mucilages, proteins, and amino acids [20–23, 52]. The UV–Vis spectra can offer information about compounds with conjugated double bonds of the active components [53]. When the phenolic structure/triterpenoids are present in molecules, the longer wavelength band experiences a bathochromic shift when an alkali is added [54, 55]. On adding shift reagent (NaOEt),

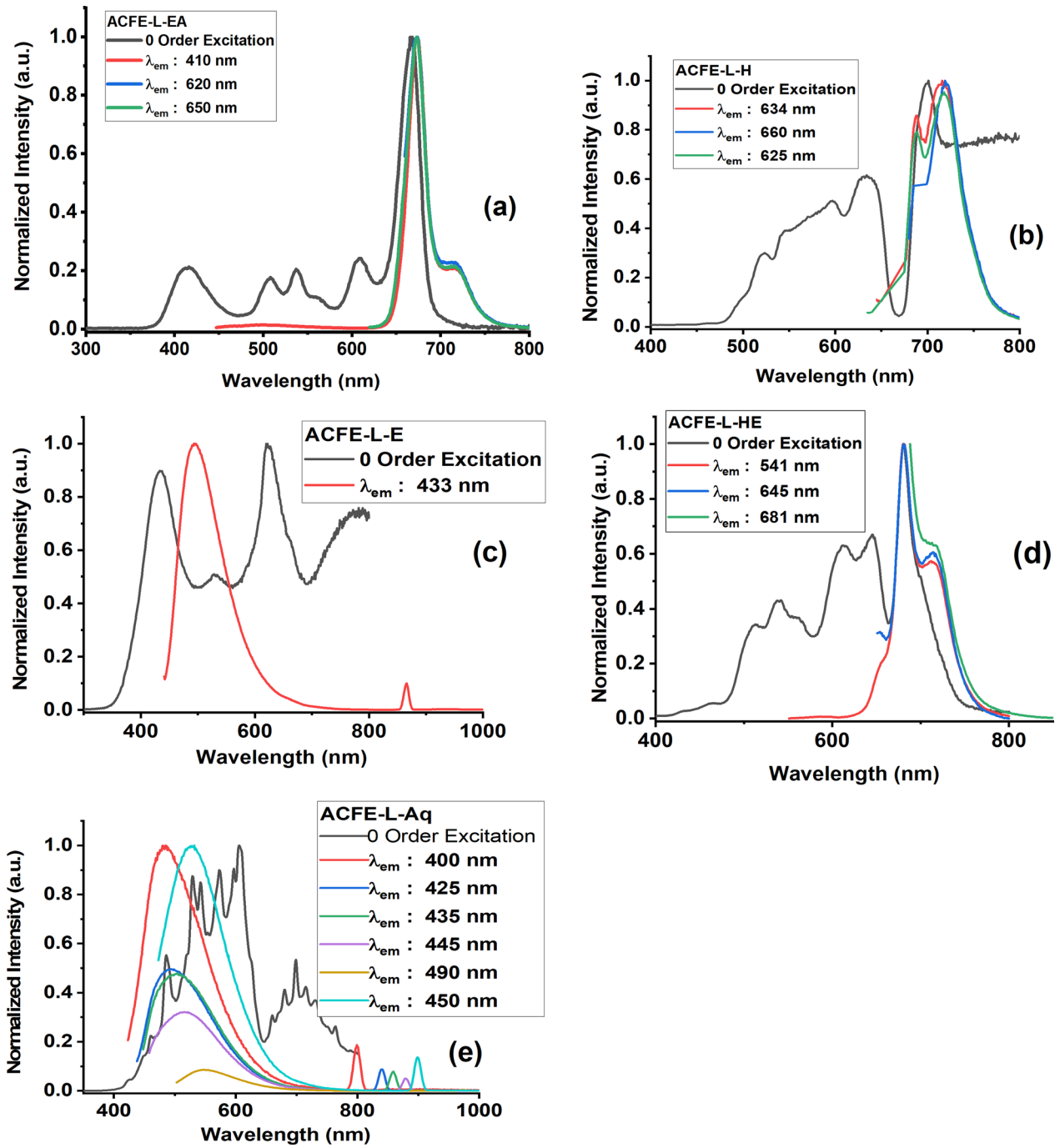


Fig. 6 **a** Fluorescence spectroscopic profile of *A. ferruginea* Leaf Hexane extract (ACFE-L–H); **b** ethyl acetate extract (ACFE-L-EA); **c** ethanol extract (ACFE-L-E); **d** hydroethanol extract (ACFE-L-HE); and **e** aqueous extract (ACFE-L-Aq)

wavelength shifts are observed for the polar extracts of both bark (235 to 246 nm) and leaves (232 nm to 237 nm). Surprisingly, the shift is greater (282 to 374 nm) in the bark, which may be attributed to the presence of phenolic/triterpenoid compounds.

Fourier-transform infrared spectroscopy (FT-IR) is a high-resolution analytical tool to identify the chemical constituents and elucidate the structural compounds, which offers a rapid and nondestructive investigation to fingerprint herbal extracts or powders [56]. There is no

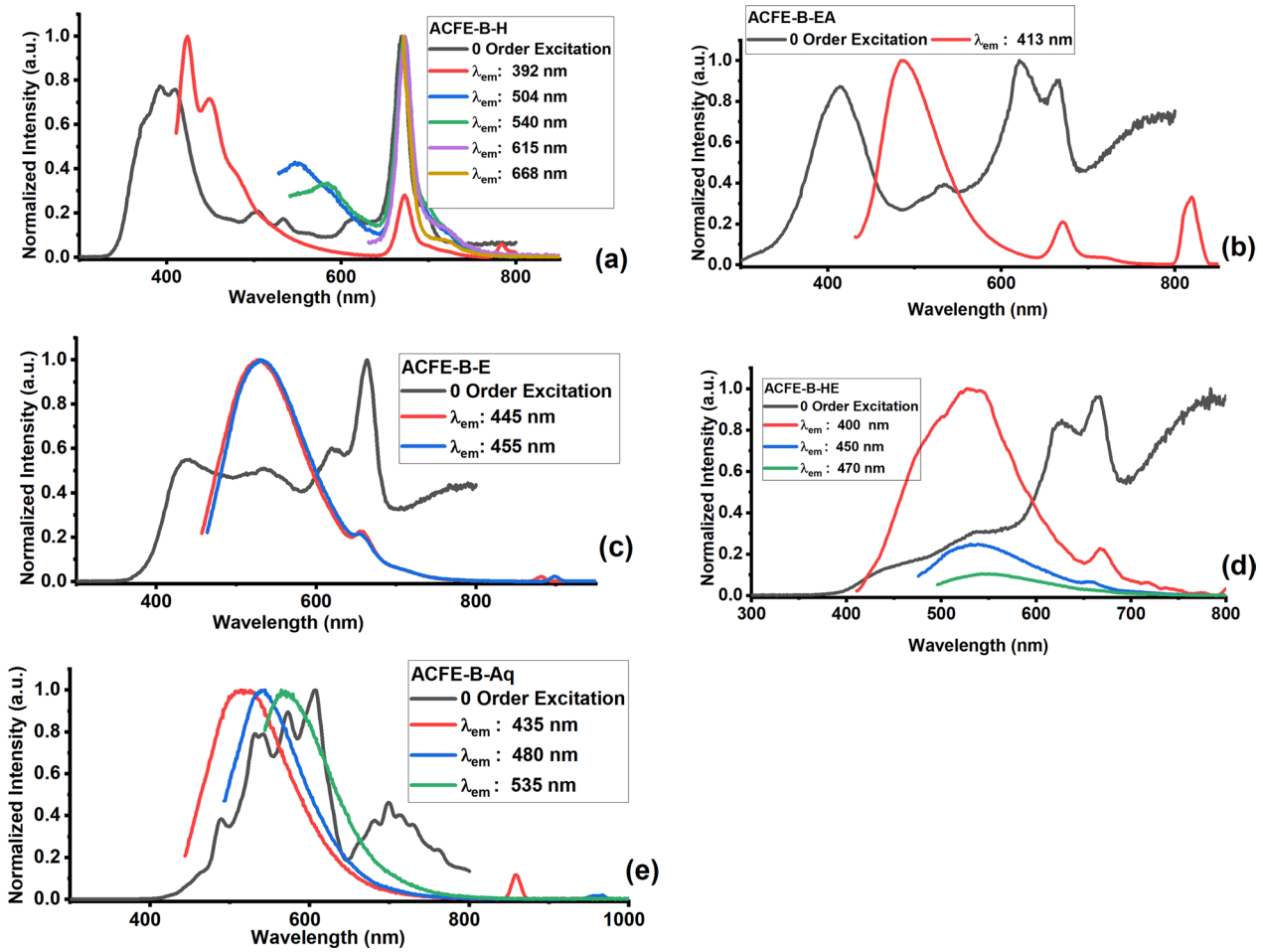


Fig. 7 **a** Fluorescence spectroscopic profile of *A. ferruginea* Bark Hexane extract (ACFE-B-H); **b** ethyl acetate extract (ACFE-B-EA); **c** ethanol extract (ACFE-B-E); **d** hydroethanol extract (ACFE-B-HE); and **e** aqueous extract (ACFE-B-Aq)

peak observed between the region 2220and 2260 cm^{-1}, which indicates the absence of the cyanide group in the extract. This result shows that *A. ferruginea* does not contain any toxic substances. There may be the presence of saponins in *A. ferruginea* extracts since it has characteristic triterpenoid saponin peaks of OH, C=O, C–H, and C=C [57]. The FT-IR spectrum confirmed the presence of alcohols, phenols, alkanes, alkynes, alkyl halides, aldehydes, aromatics, nitro compounds, and amines. The non-polar extract of ACFE-L-H and ACFE-B-H shows the predominant presence of aliphatic moieties with peaks at 2920, 2851 cm^{-1}, and 2916, 2847 cm^{-1}, respectively, which are absent in polar extract. This assumes the complete separation of non-polar constituents from polar constituents.

Fluorescence spectroscopy is a promising diagnostic method that can be used in plants to track physiological conditions, quality, phytochemicals, nutrient/environmental pressures, and diseases due to its higher sensitivity and specificity [58, 59]. In the fluorescence spectra, it can be seen that the fluorescence emission peak for ACFE-B-H and ACFE-B-EA is 489 and 424 nm when excited at 392 and 413 nm, respectively. Similar results are reported by Atta et al. [59], for p-coumaric acid (370–420 nm). When the samples (ACFE-L-Aq) are excited from 400 to 490 nm by an increment of 5–15 nm, the resultant fluorescence emission peak continuously redshifted from 482 to 549 nm. Beyond this excitation wavelength, the emission intensity gradually decreases [60]. All the bark extracts show a redshift with variation in intensities except hexane extract. Bands at 400–600 nm represent many leaf fluorophores and secondary metabolites; specifically, chlorogenic acid, ferulic acid, sinapic acid, tannins, carotenoids, flavonoid/flavin, chlorophyll, and anthocyanins (448 nm, 465 nm, 436 nm, 506 nm, 532 nm, 522 nm, 680–730 nm, 560 nm) [60]. The excitation wavelength for ACFE-L-H extract is 634 and

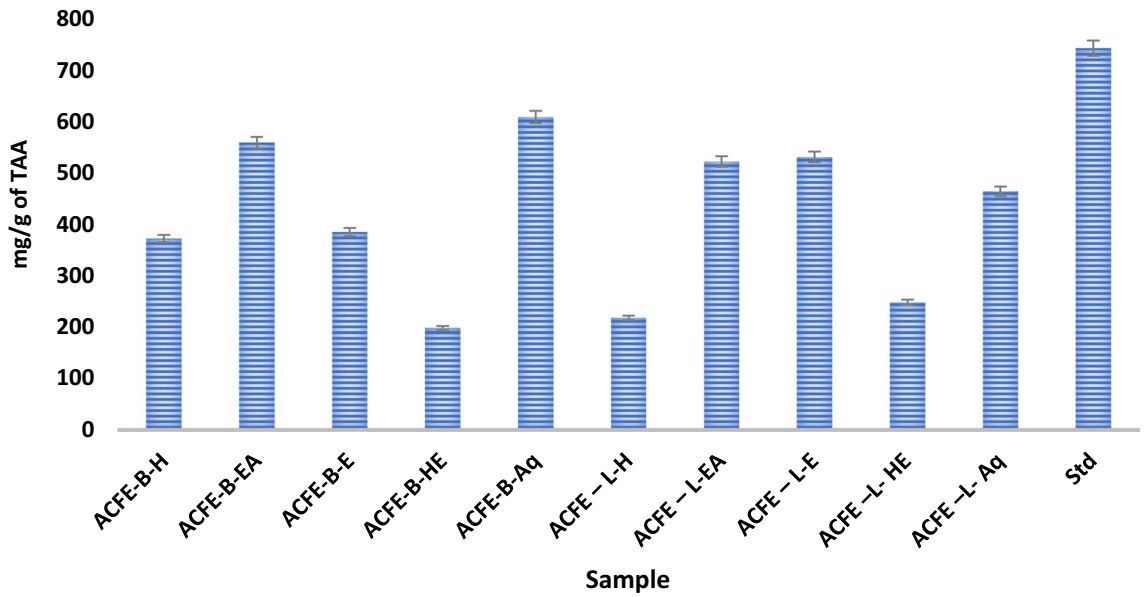


Fig. 8 Total antioxidant activity (TAA) of standard (ascorbic acid) and various extracts (5 mg/mL) of *A. ferruginea* leaves and bark is measured in relation to ascorbic acid. Error bars represent standard deviations of independent experiments conducted in triplicate. *A. ferruginea* Leaf Hexane extract (ACFE-L-H); Ethyl acetate extract (ACFE-L-EA); Ethanol extract (ACFE-L-E); Hydroethanol extract (ACFE-L-HE); Aqueous extract (ACFE-L-Aq); *A. ferruginea* Bark Hexane extract (ACFE-B-H); Ethyl acetate extract (ACFE-B-EA); Ethanol extract (ACFE-B-E); Hydroethanol extract (ACFE-B-HE); Aqueous extract (ACFE-B-Aq). Std-Standard ascorbic acid

660 nm. Two distinct maxima, one at 689 and 714 nm for excitation at 634 nm and 688, 722 nm, are observed for 660 nm excitation. Since the quantity of extractives present in hexane extract is very small, these two maximal observed may correspond to cellulose/lignin, respectively

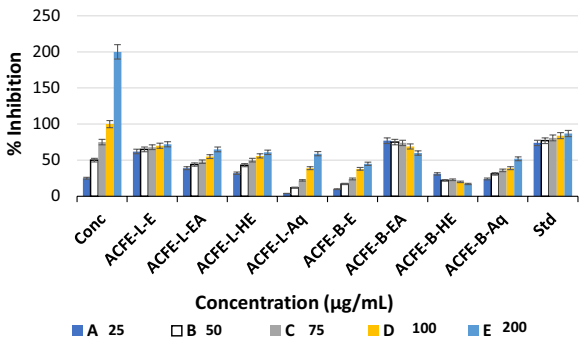


Fig. 9 DPPH free radical scavenging activity of standard and various extracts (25–200 µg/mL) of *A. ferruginea* leaves and bark is measured in relation to ascorbic acid. Error bars represent standard deviations of independent experiments conducted in triplicate. *A. ferruginea* Leaf Hexane extract (ACFE-L-H); Ethyl acetate extract (ACFE-L-EA); Ethanol extract (ACFE-L-E); Hydroethanol extract (ACFE-L-HE); Aqueous extract (ACFE-L-Aq); *A. ferruginea* Bark Hexane extract (ACFE-B-H); Ethyl acetate extract (ACFE-B-EA); Ethanol extract (ACFE-B-E); Hydroethanol extract (ACFE-B-HE); Aqueous extract (ACFE-B-Aq). Std-Standard ascorbic acid

[61]. Spectra measured for leaf extracts are identical to those obtained from the bark and are independent of excitation wavelength. A strong emission peak at 673 nm is observed for ACFE-L-EA extract when excited at 420, 620, 650 nm and is excitation independent. Both the bark and leaf aqueous extracts were dependent on the excitation wavelength, suggesting the existence of many fluorophores. A new emission peak for ACFE-B-H is observed at 424 and 448 nm when excited by 392 nm. Most of the extracts except ACFE-B-Aq and ACFE-L-Aq are independent of excitation wavelength, whereas the extracts that exhibit excitation wavelength dependence indicate the presence of many fluorescent chemicals. The need for the systematic study of the plant extracts in different polar and non-polar solvents would be useful for identifying *Acacia* species. Antioxidants work through various methods, including chain termination by scavenging active species, quenching metal ions, decreasing hydroperoxide and H_2O_2 generation, cell death, and cleansing [62]. The extracts of *A. ferruginea* are also used to determine their antioxidant capacities by forming a green phosphomolybdenum complex. The phosphomolybdenum method is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/Mo (V) complex with maximal absorption at 695 nm. The antioxidant property of acetone extracts from the tested *Acacia* species is higher

and comparable, which may be explained by the fact that the movement of electrons/hydrogen from antioxidants occurs at different redox potentials in different assay systems, and the transfer is also dependent on the composition of the antioxidants [11].

DPPH• radical scavenging activity has been widely used to evaluate the antioxidant activity of plant extracts and foods [63]. Sowndharrajan et al. [12] also reported that the acetone extract from the stem bark of *Acacia* species effectively inhibited H_2O_2-mediated oxidative stress and may be a useful source as a therapeutic agent to prevent oxidative stress-mediated disorders and diseases. The strong DPPH scavenging activity of *A. ferruginea* bark suggests that the leaves contain more phenolics that may be attributed to the antioxidant properties of *Acacia* species [11]. Total antioxidant ability and DPPH radical scavenging activity are all positively associated with saponins and flavonoids [10]. The reducing activity is correlated with aqueous/alcohol-soluble substances due to the presence of bioactive functional groups such as hydroxyl and carbonyl, which lead to reduced or inhibited oxidation [56].

Conclusions

The findings of the analysis suggest that the leaves and bark of *Acacia ferruginea* contain a wide variety of phytochemical compounds and higher phenolic content values. Physicochemical characterization studies may be a significant source of information to assess the purity and quality of this plant. Many studies including spectrometric studies and fluorescence studies are reported for the first time in the current work. Additionally, this is used as a diagnostic tool for the standardization of this medicinal plant and is helpful in the characterization of the crude drug. This study demonstrates in an unprecedented way that the crude extract of *A. ferruginea* leaves and bark is rich in pharmaceutically important phytochemicals like alkaloids, saponins, terpenoids, tannins, phenolic compounds, and flavonoids and can neutralize different sources of ROS and presents low toxicity. Thus, it is suggested that there is a synergism between the chemical composition of the extract, especially phenolic compounds, with the high antioxidant capacity demonstrated through different analysis techniques. To recapitulate, it is suggested that the *A. ferruginea* leaf and bark might be a potential source of useful bioactive molecules. It can be concluded that the species is effective in scavenging free radicals and has the potential to be a powerful antioxidant. Therefore, our results open the way for the possible development of natural antioxidants after further studies for the isolation of compounds and more specific investigations to elucidate the mechanisms of action of the extract. Further studies will focus on the isolation and characterization of the possible novel drugs present in this medicinal plant.

Abbreviations

ACFE-L-H: *A. ferruginea* Leaf Hexane Extract; ACFE-L-EA: *A. ferruginea* Leaf Ethyl acetate Extract; ACFE-L-E: *A. ferruginea* Leaf Ethanol Extract; ACFE-L-HE: *A. ferruginea* Leaf Hydroethanol Extract; ACFE-L-Aq: *A. ferruginea* Leaf Aqueous Extract; ACFE-B-H: *A. ferruginea* Bark Hexane Extract; ACFE-B-EA: *A. ferruginea* Bark Ethyl acetate Extract; ACFE-B-E: *A. ferruginea* Bark Ethanol Extract; ACFE-L-HE: *A. ferruginea* Bark Hydroethanol Extract; ACFE-L-Aq: *A. ferruginea* Bark Aqueous Extract.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43094-021-00375-4.

Additional file 1.. Materials and methodsSI-M1: 1. Organoleptic properties, 2. Solubility, 3. Determination of pH, 4. Total ash, 4.1. Determination of acid- insoluble ash, 4.2. Determination of water-soluble ash, 4.3. Determination of dry matter, 4.4. Moisture content by loss on drying (LOD) method, 4.5. Determination of foreign matter, 4.6. Water soluble and alcohol soluble extractive values4.7. Fluorescence analysis SI-M2: 1. Qualitative phytochemical studies, 1.1. Analysis of Alkaloids (Hager's Test), 1.2. Analysis of Flavonoids (Test with NaOH, Shinoda Test), 1.3. Analysis of Tannins, 1.4. Analysis of Carbohydrates (Molisch's Test), 1.5. Analysis of Terpenoid (Salkowski Test, Libermann- Buchard Test), 1.6. Analysis of Phenolic Compounds (Ferric Chloride Test, Lead Acetate Test), 1.7. Analysis of Steroids (Libermann-Buchard Test, Test with Concentrated Sulphuric acid), 1.8. Analysis of Saponins (Foam Test). SI- T1: Organoleptic Evaluation of A. ferruginea Bark and Leaf Extracts, SI-T2: Results of Solubility Test of Bark and Leaf Extracts of A. ferruginea, SI-T3: Fluorescence Analysis of A. ferruginea Bark, SI-T4: Fluorescence Analysis of A. ferruginea Leaves, SI-T5: UV Absorption Spectrum Values of A. ferruginea Bark and Leaf extracts, SI-T6: Table of Characteristic IR Absorptions of A. ferruginea Leaf Extracts, SI- T7: Table of Characteristic IR Absorptions of A. ferruginea Bark Extracts, SI-T8: Fluorescence Spectroscopic Profile of A. ferruginea Leaves and Bark Extracts.

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Studies involving plants

1. The leaves and bark parts of the plant material (BSI/SRC/5/23/2018/Tech/2080) were authenticated by the Botanical Survey of India, Coimbatore. 2. As per the local and national guidelines and legislation and the required or appropriate permissions and/or licenses for the study.

Authors' contributions

JM designed, analyzed the data, and wrote the manuscript. PVR and SV contributed to data interpretation, editing, formatting, and graphical work. SKS and MP were associated in supervising, advising, and structuring the study and the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article [and its Additional file 1] and can be accessed/shared with the public.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Department of Chemistry, Avinashilingam Institute for Home Science and Higher Education for Women, Tamil Nadu, Coimbatore 641 043, India. [2]Department of Nanoscience and Technology, Sri Ramakrishna Engineering College, Tamil Nadu, Coimbatore 641 022, India.

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