



Densitometric HPTLC analysis of the *Acacia catechu* wild fractions for phenolics

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Abstract: Traditional "Ayurvedic" medicine from India has traditionally used *Acacia catechu*. The herbal extract is the primary component, although there have been no attempts to standardize it as an active agent or marker. A chromatographic fingerprint represents the chemical components of herbal remedies that are therapeutically advantageous. This study suggests utilizing an HPTLC approach to assess phenols like protocatechuic acid and gallic acid in *Acacia catechu* extract fractions made of water, ethyl acetate, and butanol. According to the study, protocatechuic acid (11.85%) in the water fraction of the *Acacia catechu* is more concentrated than gallic acid (6.92%). In contrast, the ethyl acetate fraction contains more gallic acid (11%) and less protocatechuic acid (4.10%). However, the butanol fraction only has 6.62% gallic acid. By the Folin-Coicalteu method, total phenolic content was determined, and antioxidant activity of all fractions was resolute using the DPPH method. It was discovered that ethyl acetate fraction had higher phenolic content (211 mg/g) than aqueous fraction (129 mg/g) and butanol fraction (101.2 mg/g). Ethyl acetate fraction has more potent antioxidant activity than water and Butanol fractions. The research emphasizes the potential of this methodology for efficient and economical phenolic profiling, which may aid in the assessment and use of *A. catechu* in the nutraceutical and pharmaceutical sectors.

Introduction

The medicinal qualities of *Acacia catechu* make it valuable. The medicinal potential of various components of the tree, such as its heartwood, bark, and leaves, is worth exploring (Chatterjee and Pakrashi, 1992). Due to its beneficial nutritional qualities, this plant has a long history of usage in Eastern medicine, particularly in Asia. *Acacia catechu* formulations have a diverse range of pharmacological activities, encompassing antibacterial, hepatoprotective, anti-inflammatory, anti-diarrheal, anti-pyretic, and anti-inflammatory effects (Singh and Lal, 2006; Singh et al., 1976; Naik et al., 2003). Traditionally used to treat cancer, bronchitis, asthma, chest wound healing discomfort, sore throats, diarrhoea, ulceration, vitiligo, and eczema, the *Acacia catechu* plant can produce therapeutic foods and medications due to its

antifungal, antiviral, spasmolytic, and hypoglycemic properties. Numerous references in Ayurveda support the idea that *A. catechu* is a helpful herb with many therapeutic benefits, including those for skin conditions (Adhikari et al., 2021). The bark and heartwood of *A. catechu* are used to make the well-known ayurvedic skin tonic known as Khadira. Aside from that, heartwood extract has been used in various therapeutic applications. Alkaloids provide strong anti-microbial and hypoglycemic properties (Bhattarai et al., 2020). Traditional and folk remedies are the only ones that utilize this tree for therapeutic purposes, providing more investigation into novel medicinal compounds with known activities. It has traditionally been used as a treatment for dermatological conditions, sore throats, and fuel and feed (Rout et al., 2021). Its anti-diabetic, anti-



hypertensive, antibacterial, antifungal, antiplaque, antioxidant, antiviral, anti-inflammatory, anti-cancer, and wound healing capabilities have all been demonstrated in experimental research. As a result, new and more modern approaches must be developed for various purposes. Further investigation is necessary in order to ascertain the active constituents. The primary aim of the present work was to measure the concentrations of phenolic indicators, namely protocatechuic acid and gallic acid, as well as determine the overall phenolic content in different extracts of *Acacia catechu* (Sharma and Lingha, 2021).

Materials and methods

Plant materials

Heartwood of *Acacia catechu* was purchased at Akluj, Taluka-Malshiras, and Dist-Solapur in February 2022, and the authenticity was confirmed by the botany department of Solapur's DBF Dayanand College of Arts and Sciences.

Physicochemical parameters

Physicochemical criteria are used to verify the drug's quality and purity. Water-soluble, acid-soluble, and total ash were measured. Extractive values for substances soluble in ethanol and water were calculated (Table 2) (Evans, 2009).

Preparation of plant extract

The powder of *Acacia catechu* was subjected to Soxhlet extraction with hydro-alcoholic solvent (10%) to obtain hydro-alcoholic extract with a 27% yield. After that, the dried extract dissolved in water to obtain water extract. After drying the water extract, we got a water fraction with a 17% yield. The dry hydro-alcoholic extract was again dissolved into ethyl acetate, and then ethyl acetate was evaporated to obtain an ethyl acetate fraction with an 11.5% yield. Then, the dry hydro-alcoholic extract was again dissolved in butanol and evaporated to obtain a Butanol fraction with an 8% yield.

Phytochemical screening of extract

To find out presence of different phytoconstituents, the phytochemical test was done on all the fractions, including the ethyl acetate, water and butanol fractions (Table 3), using standard techniques (Khandelwal, 2006).

Total phenolic content (Folin-Ciocalteu method)

The extract was diluted to 1 mg/ml, and samples were taken from the water, ethyl acetate, and butanol fractions. In a 25 ml volumetric flask, combine 1 ml extract with 9 ml of distilled water to make a reaction mixture. Folin-Ciocalteu reagent (1.0 ml) was added after being thoroughly combined. After waiting 5 minutes, 10 ml of NaCO₃, 7% was added to the mixture. Twenty-five millilitres more were added. Standard solutions of gallic

acid at concentrations of 200, 400, 600, 800, and 1000 µg/ml were prepared following the same protocol. After 90 minutes, we compared the absorbance of the test solution, the standard solution, and the reagent blank using a spectrophotometer set to 550 nm. Triplicate measurements of absorbance were taken. (Bhardwaj et al., 2021).

Antioxidant activity by DPPH method

Using DPPH free radicals, the antioxidant activity of WF, EAF, and BF was estimated. We measured the WF, EAF, and BF concentrations in the microtiter plate at 200, 400, 600, 800, and 1000 (g/ml). After incubating samples at room temperature for 30 minutes in the dark, 100 µL of 0.1% DPPH was added. Colour variations in the samples (from purple to yellow and pale pink were considered strong and weak positive) were analysed using an Elisa plate reader at 490nm (Fuso et al 2023).

The following formula was used to determine the radical scavenging activity:

$$\text{DPPH radical scavenging activity (\%)} = \frac{[(\text{Absorbance of control} - \text{Absorbance of test sample}) / (\text{Absorbance of control})] \times 100}$$

HPTLC analysis of *Acacia catechu*

Chemicals, reagents and Method

YUCCA Enterprises from Mumbai has provided Protocatechuic acid and gallic acid as markers; all chemicals were analytical grade E-Merck reagents. Apparatus CAMAG TLC chamber with Linomat IV automatic sample spotter with a Hamilton Syringe of 3 µL sample and 5 µL Standard. The Plate: 10 x 10 cm, 0.2 mm silica gel 60F254 precoated. Densitometer: TLC scanner 3 with installed SPI software 1.21

Standard solvent

- Protocatechuic acid 5.175mg of protocatechuic acid was weighed, and then methanol was added. Sonicate the sample and mix 10 ml of methanol into it.
- Mobile Phase: Ethyl acetate: Toluene: Methanol: Formic Acid: (3:3:0.2:0.8)
- Gallic Acid: 5.519 mg of Gallic acid was Weighed, and methanol was added. The sample was sonicated, 10 ml of methanol was added, and 5 ml of the resulting solution was diluted to 50 ml. Mobile Phase: Ethyl acetate: Toluene: Methanol: Formic Acid (3:3:0.2:0.8)

Preparation of sample solution

- BF: BF sample was weighed at 5.469 mg and dissolved in 1 ml of methanol after being sonicated.
- EAF: 5 mg of EAF sample was weighed, and methanol was added to dissolve the sample. Next, sonicate and dilute 1 ml of the methanol solution with water.

Table 1. Physicochemical study of *Acacia catechu*

Parameters	Mean %	Standard deviation
Total Ash	13.7	0.09
Acid in-soluble ash	1.325	0.003
water soluble ash	5.413	0.03
Loss on drying	6.104	0.02

Table 2. Extractive value of *Acacia catechu*

Sr. No	Parameters	Mean %	Standard deviation
1.	Alcohol soluble extractive value	3.92	0.02
2.	Water soluble extractive value	15.86	0.05

Table 3. Preliminary phytochemical examination of the *Acacia catechu* extract's water, ethyl acetate and butanol fractions

Constituents	Test	Hydro-alcoholic Fraction	Ethyl acetate Fraction	Butanol Fraction
Triterpenes	Liebermann burchard	++	+++	+
Flavonoids	Lead acetate, Alkaline reagent	+	+++	++
Tannins	Gelatin	++	++	++
Saponins	Froth formation	-	-	-
Glycosides	Modified borntagers	+	++	+
Alkaloids	Mayers and wagner reagent	-	-	-
Steroids	Salkowski	+	+	+

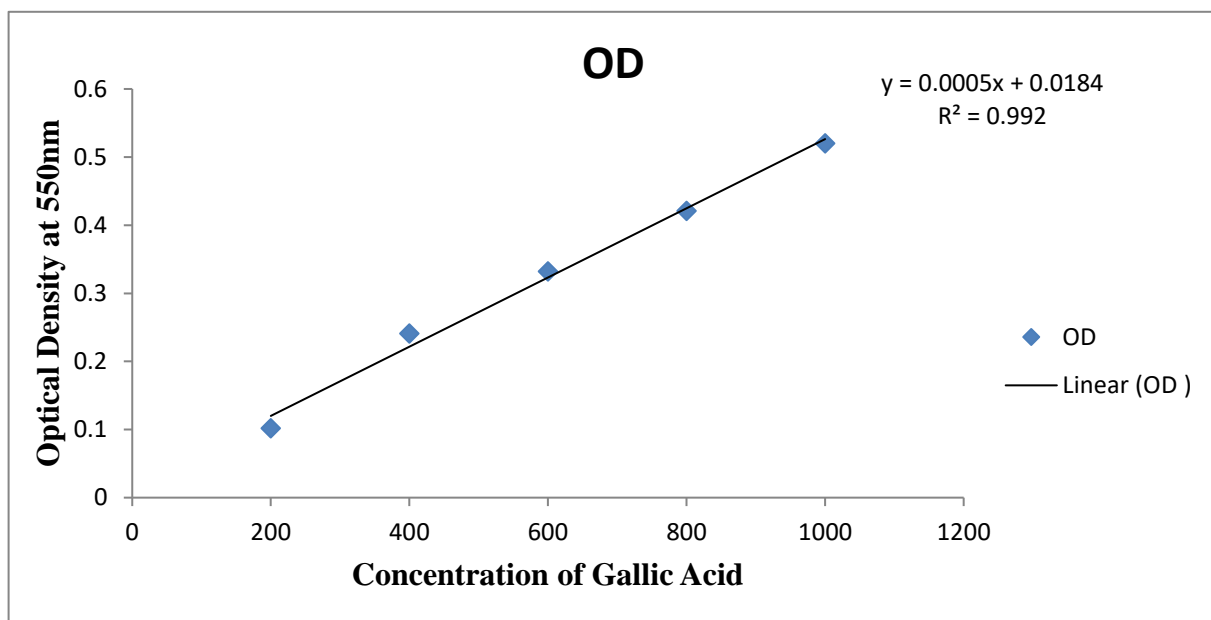
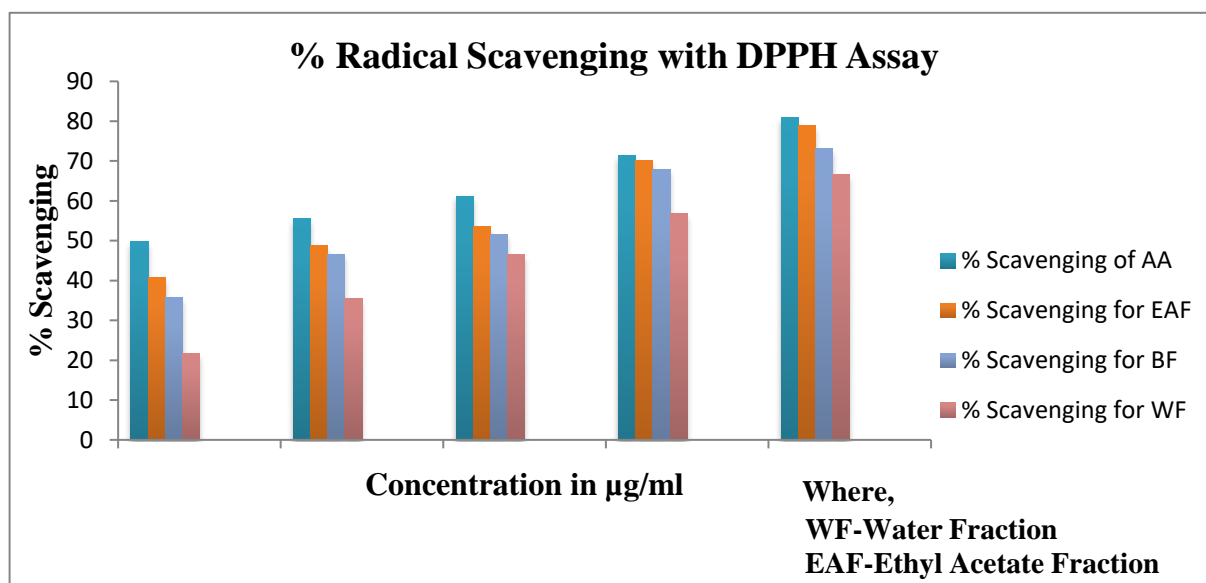
**Figure 1. Total Phenolic concentration of WF, EAF and BF of *Acacia catechu***

Table 4. Total Phenolic content of WF, EAF and BF of *Acacia catechu*

Extract	Total Phenolic Concentration (mg Gallic acid /gm of Phenols in dry extract)
WF	129 mg/gm
EAF	211 mg/gm
BF	101.2 mg/gm

Table 5. Antioxidant activity of EAF, BF & WF by DPPH method

Concentration ($\mu\text{g/ml}$)	% Scavenging of AA	% Scavenging for EAF	% Scavenging for BF	% Scavenging for WF
200	49.89	40.85	35.62	21.63
400	55.65	48.7	46.66	35.52
600	61.19	53.65	51.66	46.6
800	71.42	70.23	67.76	56.88
1000	80.84	78.95	73.19	66.57

**Figure 2. Antioxidant activity of EAE, BE & WE by DPPH method****Table 6. Quantitative estimation of the marker in the butanol, ethyl acetate and water fractions**

Sr. No	Marker	Concentration		
		WF	EAF	BF
1.	Protocatechuic Acid	11.85%	4.10%	-
2.	Gallic Acid	6.92%	11.00%	6.62%

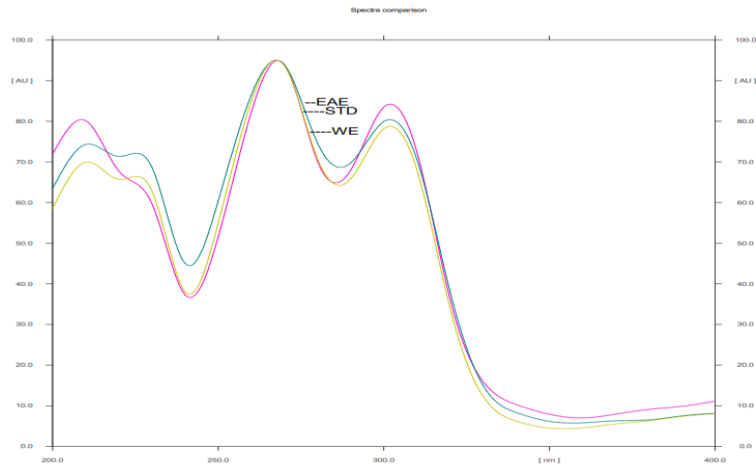


Figure 3. protocatechuic acid spectrum demonstrating concentration in WF, EAF and BF

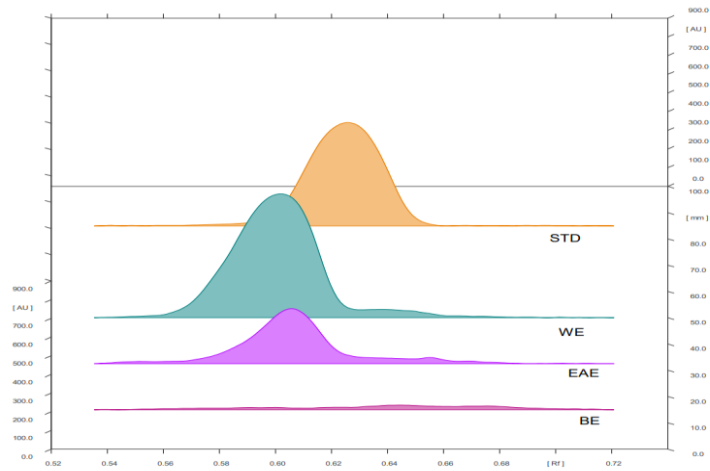


Figure 4. Protocatechuic acid concentration in WF, EAF and BF in a 3D view

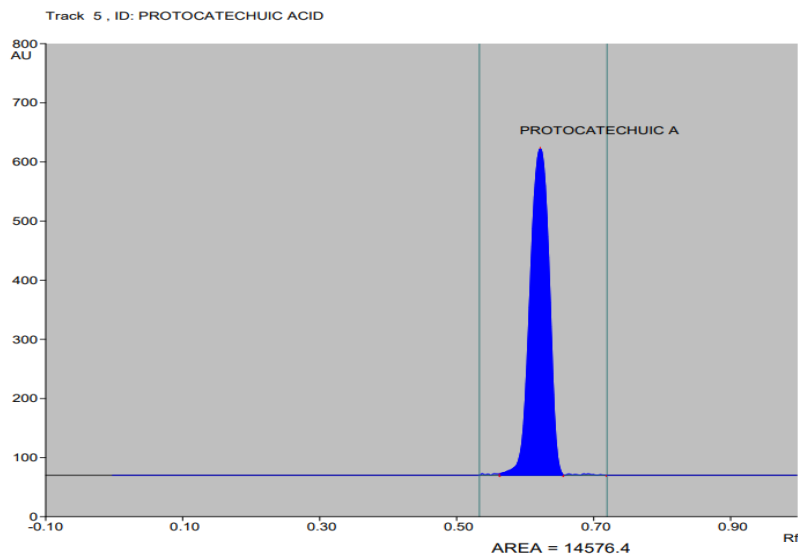


Figure 5. Standard protocatechuic acid

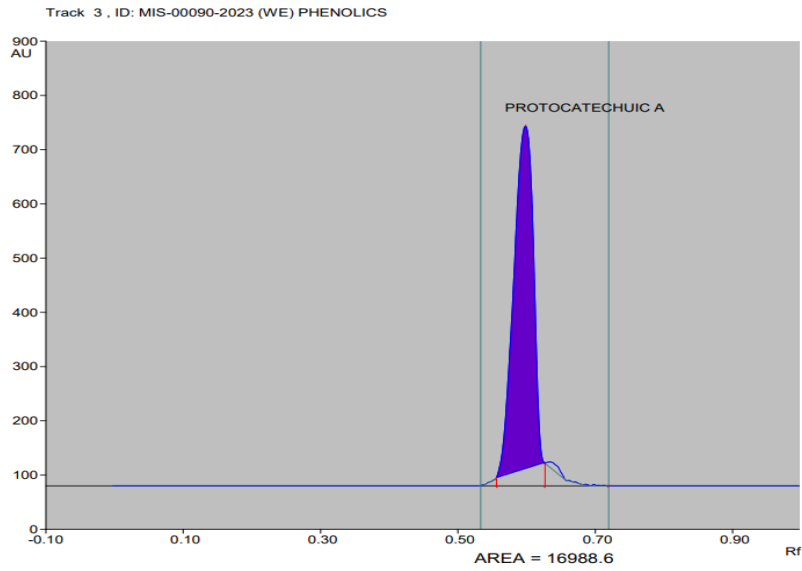


Figure 4. Protocatechuic acid in Water Fraction (WF)

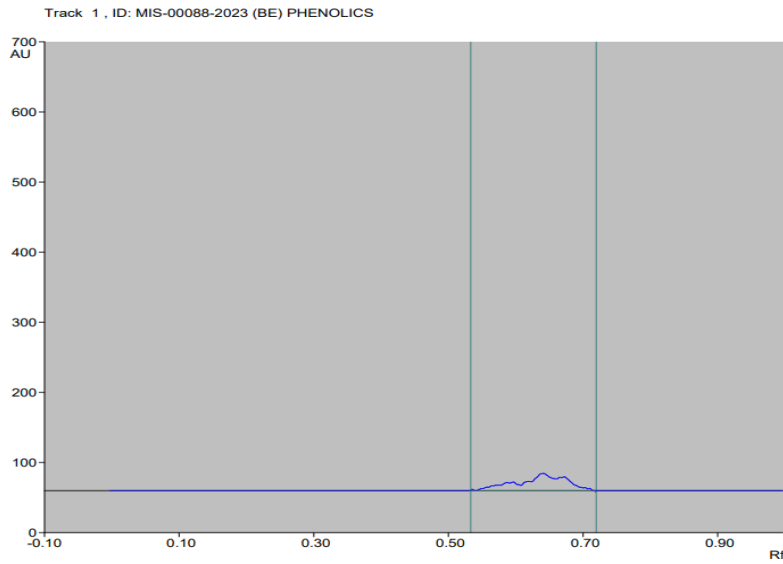


Figure 5. Protocatechuic acid in Butanol Fraction (BF)

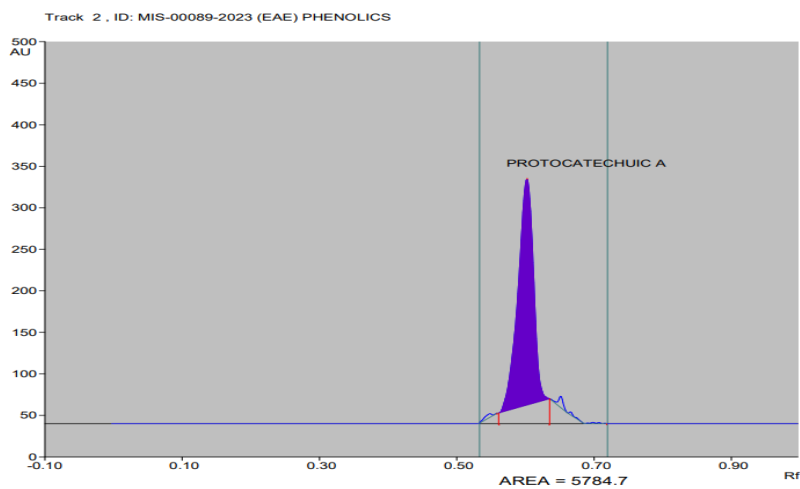


Figure 8. Protocatechuic acid in Ethyl Acetate Fraction (EAF)

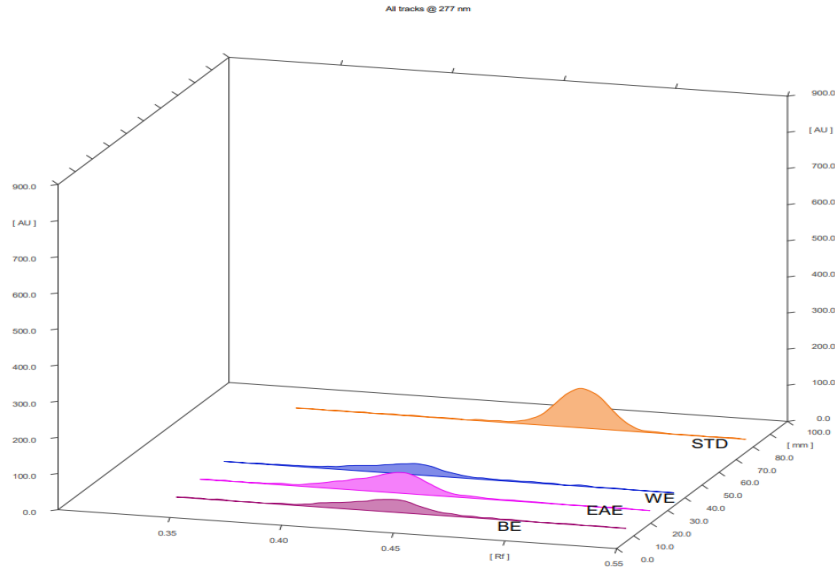


Figure 9. Gallic acid concentration in WF, EAF and BF in a 3D view

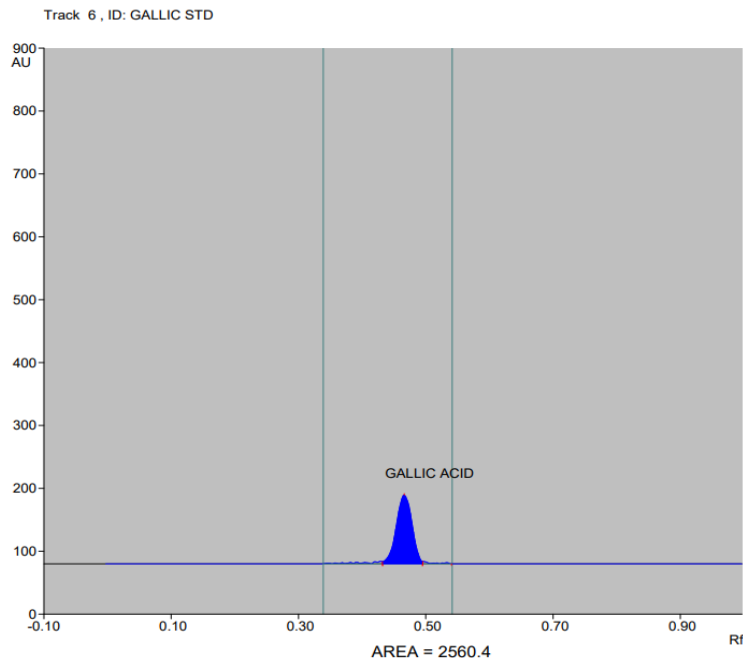


Figure 10. Standard Gallic acid

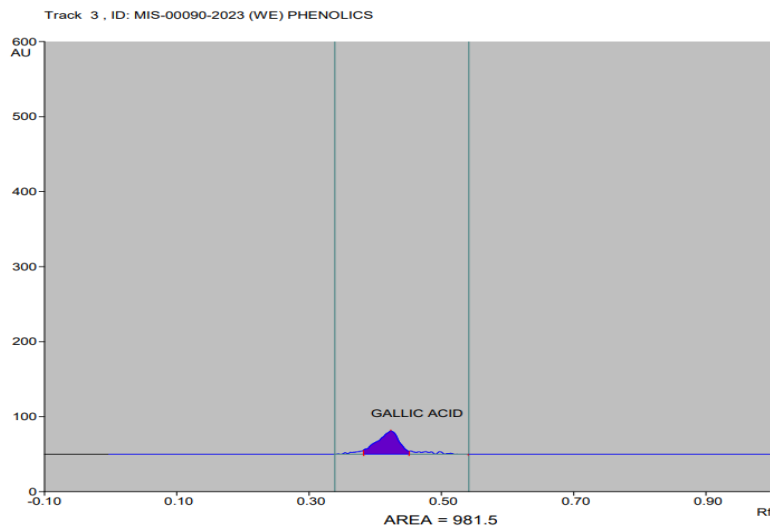


Figure 11. Gallic acid in Water Fraction (WF)

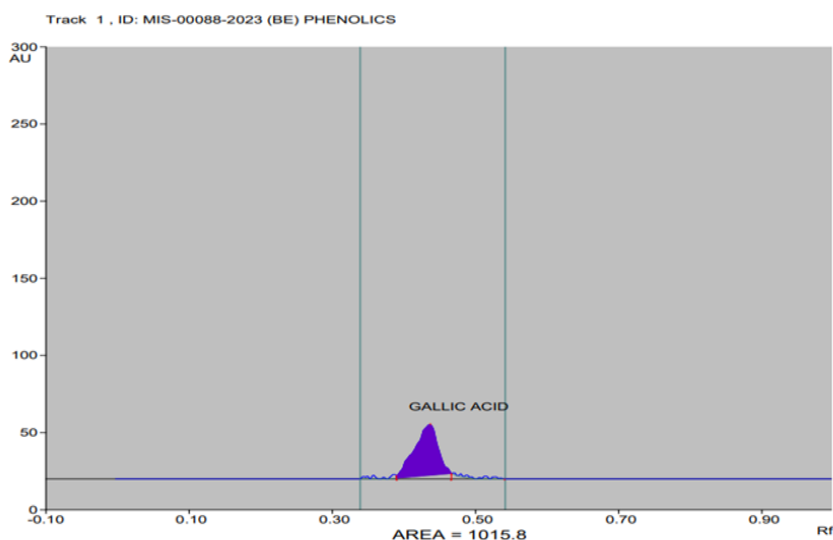


Figure 12. Gallic acid in Butanol Fraction (BF)

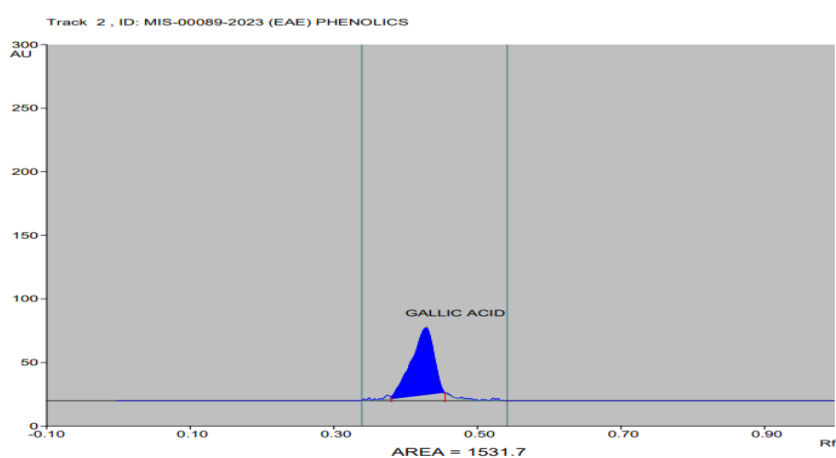


Figure 13. Gallic acid in Ethyl Acetate Fraction (EAF)

- WF: 5 mg of the WF sample weighed and then added methanol to dissolve it, sonicate and dilution to 1 ml with methanol

Spot development

The sample plate was placed in a developing chamber, which was saturated with solvent vapour for 20 minutes, and then the plate was developed up to 90mm using the appropriate mobile phase.

Scanning

The plate was screened with Camag TLC Scanner 3 at 288nm, 210nm, and 277nm wavelengths. Then, peak densitograms were observed.

Results and discussion

Physicochemical parameters

Hydro-alcoholic extract was tested for physicochemical characteristics, and the results are described in Tables 1 and 2.

Phytochemical screening

Acacia catechu was screened for several basic phytochemicals in water, ethyl acetate, and butanol, and

those compounds were found to be present, along with flavonoids, phenolics, tannins, triterpenes, glycosides, and steroids. As shown in Table 3, the ethyl acetate fraction of *Acacia catechu* has higher levels triterpenes flavonoids than the water and butanol fraction.

Total Phenolic content

Due to their redox characteristics, phenolic substances can function as antioxidants (Soobrattee et al., 2005). Considering their hydroxyl groups render it simpler to scavenge free radicals, total phenolic concentration could serve as a baseline for rapid antioxidant activity screening. The relative amount of 550 nm maximum wavelength-screened sample fractions, a coloured blue phosphor-tungstate phosphomolybdate complex, can be observed by spectrophotometry as it forms using sodium carbonate in an alkaline environment. A linear Gallic acid calibration curve was used as a standard and obtained with a coefficient of determination (R^2) value of 0.992, as shown in Figure 1. Phenolic content in butanol fractions, water fractions, ethyl acetate fractions listed in Table 4. The ethyl acetate fraction of *Acacia catechu*

contains phenolics (211 mg/gm) than the water (129 mg/gm) or butanol (101.2 mg/gm) fractions.

Antioxidant activity

The butanol, ethyl acetate, and water fractions are screened for antioxidant activity. It was discovered that ethyl acetate fraction had a higher percentage of scavenging activity (78.95%) than water fraction (66.57%) and butanol fraction (73.19%)

HPTLC profile of *Acacia catechu* fractions

Several phenolic phytoconstituents are found in *Acacia catechu*, two of these were measured using the HPTLC densitometric method. The phenolic content in ethyl acetate, water and butanol fraction were screened at wavelengths 288nm, 277nm and 210 nm. Protocatechuic acid and gallic acid were used as markers. Protocatechuic acid and gallic acid contents were measured in *Acacia catechu* Extract water, ethyl acetate, and butanol fractions. Protocatechuic acid was found in the water fraction and the ethyl acetate fraction; however, it was absent from the ethyl acetate fraction. Its concentration is higher in the water fraction (11.85%) with an RF value of 0.56 than in the ethyl acetate fraction (4.10%) with an RF value of 0.57 and absent from the butanol fraction as shown in table 6 and graphs 4-graph 8. The Gallic acid is present in each of the three fractions; however, it is concentrated more in the ethyl acetate fraction (11%) with an RF value of 0.38 than in the water fraction (6.92%) with an RF value of 0.38 or butanol fraction (6.62%) with RF value 0.38 as shown in table 6 and graphs 9 to graph 13 (Halder and Jha, 2023).

Conclusion

According to this study, ethyl acetate is a potential solvent for phenolic extraction. The *Acacia catechu* extract contained sizable levels of phenolic components. To get the most phenolic compounds out of the extract, butanol, water, and ethyl acetate were used in a fractionation process. For the purpose of measuring certain phenolics like protocatechuic acid and gallic acid, each fraction is subjected to an HPTLC analysis. The results showed that butanol was devoid of protocatechuic acid, although the water fraction had a higher concentration (11.85%) than the ethyl acetate fraction (4.10%). The concentration of gallic acid, which is present in all three fractions, is higher in the ethyl acetate extract (11%) than in the water fraction (69.2%) and butanol fraction (6.62%). Total phenolic content ethyl acetate fraction has a greater total phenol

concentration (211 mg/gm) than water fraction (129 mg/gm), which is followed by butanol fraction (101.2 mg/gm). As a result, the phenolic content of ethyl acetate and water fractions is high. Ethyl acetate has more excellent antioxidant activity than water fraction. Therefore, ethyl acetate fraction is better suited for employment in biological activities.

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Conflict of Interest

None

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