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Standardization, Antidaibetics And Antioxidant Assessment Of Hydroalcoholic Extract Of Abies Webbiana Fruit With Isolation Of Ferulic Acid

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Abstract
The present study aimed to evaluate the pharmacognostical, phytochemical, and antioxidant properties of Abies webbiana *fruit, with an emphasis on its hydroalcoholic extract. Pharmacognostical analysis confirmed the quality of the crude drug through parameters such as ash values and loss on drying. Extraction yield and solubility studies indicated that the hydroalcoholic extract was richer in polar phytoconstituents compared to the petroleum ether extract. Preliminary phytochemical screening revealed the presence of flavonoids, alkaloids, glycosides, tannins, phenolics, and terpenoids in the hydroalcoholic extract. Quantitative assays showed a high content of total phenolics (154.44 mg GAE/g) and flavonoids (98 mg RE/g), which correlated with strong antioxidant activity in DPPH, reducing power, and superoxide scavenging assays. TLC and HPTLC confirmed the presence of ferulic acid,. The study concludes that the hydroalcoholic extract of* Abies webbiana *fruit, rich in phenolic compounds like ferulic acid, exhibits significant antidiabetic and antioxidant potential, supporting its traditional use and potential pharmaceutical applications.*
Keywords:Abies webbiana, *hydroalcoholic extract, ferulic acid, antioxidant activity, phytochemical screening, HPTLC, DPPH, pharmacognostical evaluation.*

INTRODUCTION

Medicinal plants have served as the foundation for traditional systems of medicine for centuries, and their importance in modern pharmacological research continues to grow due to their therapeutic potential and relatively lower side effects compared to synthetic drugs. Among these, *Abies webbiana* Lindl. , commonly known as Talispatra, is a coniferous tree belonging to the family Pinaceae and is indigenous to the Himalayan region. Traditionally, its leaves and fruits have been used in Ayurvedic medicine for the treatment of respiratory disorders, inflammation, and nervous system-related ailments [1, 2].

The fruit of *Abies webbiana* is reported to be rich in bioactive phytochemicals such as flavonoids, phenolics, terpenoids, and glycosides, which are known to contribute to its antioxidant and therapeutic properties [3]. Antioxidants are critical in mitigating oxidative stress, which is implicated in the pathogenesis of several chronic diseases, including cancer, cardiovascular disorders, neurodegenerative conditions, and diabetes [4]. Therefore, evaluating the antioxidant potential of herbal extracts is a vital step in their standardization and validation for pharmaceutical use [5].

Ferulic acid, a phenolic compound found in many plant species, is widely recognized for its potent antioxidant, anti-inflammatory, and antimicrobial activities [6]. Isolating ferulic acid from *Abies webbiana* and confirming its presence using advanced spectroscopic techniques not only supports the plant's traditional use but also provides a scientific basis for its therapeutic potential.

The present study aims to evaluate the pharmacognostical and phytochemical properties of *Abies webbiana* fruit, determine its antioxidant capacity, and isolate and characterize ferulic acid from the hydroalcoholic extract. This investigation will contribute to the standardization of the plant and promote its utilization in evidence-based herbal formulations.

MATERIAL AND METHODS

Material

The materials used for this study included the dried fruit of *Abies webbiana*, which was authenticated and coarsely powdered for extraction. Petroleum ether and hydroalcoholic solvents (ethanol: water, 70:30) were employed for successive extraction using a Soxhlet apparatus. Standard laboratory reagents and chemicals such as methanol, ethyl acetate, DMSO, and reagents for phytochemical tests (Molisch's, Fehling's, Mayer's, etc.) were used, all of analytical grade. Ferulic acid standard was used for TLC and HPTLC comparison. Instruments used included a HPTLC system for qualitative and quantitative phytochemical and spectral analysis.

Methods

Pharmacognostical evaluation

Total ash value

About 5 g of the powdered plant material was accurately weighed and transferred into a previously ignited and weighed silica crucible. The powder was evenly spread to form a thin layer at the bottom of the crucible. It was then gradually incinerated by increasing the temperature until it became dull red hot, ensuring complete removal of carbon. After incineration, the crucible was allowed to cool and weighed. This process was repeated until a constant weight was obtained. The percentage of total ash was then calculated based on the air-dried weight of the powder [7].

$$\% \textbf{ Ash Content} = \frac{\textbf{Weight of crucible + ash − Weight of crucible}}{\textbf{Weight of crucible + sample − Weight of crucible}}$$

Moisture content

Place 2 to 6 g of the sample into a weighing bottle which has been accurately weighed, and weigh it accurately. Then, dry it at 105 °C for 5 - 6 hours and cool it in desiccators with silica gel. When the material is dried to a constant weight, the percent of Moisture content is determined [8].

$$\textbf{LOD } \% = \frac{\textbf{Wt. of petridish + crude drug − After drying Wt. of petridish + sample}}{\textbf{Weight of crude drug}} \textbf{x100}$$

Alcoholic extractive value

5g of powdered material was weighed into 250mL stopper conical flask containing 100mL of 90% ethanol and the stopper replaced. The flask and content was placed in a mechanical shaker for 6hrs and then allowed to stand for 18hrs. The mixture was filtered and 20mL of the filtrate was measured into an evaporating dish with a known weight, and evaporated to dryness. The constant weight of the residue was gotten after drying in the oven at 105 °C for about 3 minutes. The extractive value was calculated [9].

$$\textbf{Alcohol soluble extractive value} = \frac{\textbf{Weight of reside}}{\textbf{Weight of the drug}} \textbf{x100}$$

Water extractive value

The procedure was the same as above except that water used in place of 90% ethanol [10].

$$\textbf{Water soluble extractive value } = \frac{\textbf{Weight of reside}}{\textbf{Weight of the drug}} \textbf{x 100}$$

Extraction

Powdered sample were placed in a thimble of Soxhlet apparatus. The extraction was carried out using different organic solvents; petroleum ether and Hydroalcholic for 8-10 hours and 40-60°C temperature of the heating mantle were adjusted. After the extraction process, the extract of sample were filtered and concentrated to dryness. Extracts were collected in air tight container [11]. Extraction yield of all extracts were calculated using the following equation below:

$$\textbf{Formula of Percentage yield} = \frac{\textbf{Actual yield}}{\textbf{Theoretical yield}}$$

Qualitative phytochemical analysis of plant extract

Extracts obtained was subjected to the preliminary phytochemical analysis. The extracts were screened to spot the presence or absence of many active constituents like carbohydrates, glycosides, phenolic compounds, alkaloids, flavonoids, saponins, fats or fixed oils, protein, amino acid and tannins [12].

Quantitative Tests

Spectrophotometric Quantification of Total Phenolic Content

The total phenolic content of plant extract was determined using the Folin-Ciocalteu Assay. The *Abies webbiana* extract (0.2 mL from stock solution) were mixed with 2.5 mL of Folin-Ciocalteu Reagent and 2mL of 7.5% sodium carbonate. This mixture was diluted up to 7 mL with distilled water. Then the resulting solutions were allowed to stand at room temperature for 2 hrs before the absorbance was measured spectrophotometrically at 760 nm. Calibration curves were composed using standard solutions of Gallic Acid Equivalent (GAE) mg/gm. Concentration of 20, 40, 60, 80, and 100 µg/mL of Gallic acid was prepared. The Folin-ciocalteu reagent is sensitive to reducing compounds including polyphenols. They produce a blue colour upon reaction. This blue colour was measured spectrophotometrically [13].

Spectrophotometric Quantification of Total Flavonoid Content

The flavonoid content was determined using Aluminium chloride method. 0.5 ml of extract solution of *Abies webbiana* was mixed with 2 ml of distilled water. Then, 0.15 ml of sodium nitrite (5%) was added and mixed properly. After that, wait for 6 minutes before adding 0.15 ml Aluminium chloride (10 %) and allowed to stand for 6 minutes. Then, 2 ml of 4 % sodium hydroxide was added. The mixture was shaken and mixed thoroughly. Absorbance of mixture was estimated at 510 nm using UV spectrophotometer. Calibration curves were composed using standard solutions of Rutin Equivalent (GAE) mg/gm. Concentration of 20, 40, 60, 80, and 100 µg/mL of Rutin was prepared. Total flavonoid content was determined from the calibration curve and results were indicated as mg Rutin equivalent per gram dry extract weight [14].

In vitro anti-oxidant activity of plant extract

DPPH assay

Free radical scavenging activity of the extract of *Abies webbiana*, based on the scavenging activity of the stable free radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was determined. Different volume of extracts/standard (20 – 100µg/ml) was taken from stock solution in a set of test tubes and methanol was added to make the volume to 1 ml. To this, 2 ml of 0.1mM DPPH reagent was added and mixed thoroughly. Absorbance at 517 nm was determined after 30 min and the percentage inhibition activity was calculated by using the equation: % scavenging activity [(A0-A1)/A0]×100. Where A0 is the absorbance of the control and A1 is the absorbance of the extract. Lower the absorbance, the higher is the free radical scavenging activity. The curves were prepared and the IC50 value was calculated using linear regression analysis [15].

$$(\%)\ \textbf{Scavenging Efect} = 1 - \frac{\textbf{Absorbance of Sample}}{\textbf{Absorbance of Control}} * 100$$

Superoxide anion radical scavenging activity

1 ml of nitroblue tetrazolium (NBT) (100 µl of NBT in 100 mM phosphate buffer, pH 7.4), 1 ml of NADH (468 µl in 100 mM phosphate buffer, pH 7.4), solution as well as varying volumes of extract of *Abies webbiana* (20, 40, 60, 80 and 100 µg/ml), were mixed well with methanol. The reaction was started by the addition of 1 ml of phenazine methosulfate (PMS) (60 µl/100 mM phosphate buffer, pH 7.4). The reaction mixture was incubated at 30°C for 15 min. The absorbance was measured at 560 nm in a spectrophotometer. Incubation without the sample (extract) was used as a blank sample. Ascorbic acid was used as the standard in comparing the different sample. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity [15]. The percentage scavenging was calculated by using the formula shown below:

$$\%\ \textbf{Inhibition} = \frac{\textbf{Ab of control} - \textbf{Ab of sample}}{\textbf{Ab of control}} \textbf{x100}$$

Reducing power assay

Preparation of standard solution

3 mg of ascorbic acid was dissolved in 3 ml of distilled water/solvent. Dilutions of this solution with distilled water were prepared to give the concentrations of 20, 40, 60, 80 and 100 µg/ml.

Preparation of extracts

Stock solutions of extract of *Abies webbiana* were prepared by dissolving 10 mg of dried extracts in 10 ml of methanol to give a concentration of 1mg/ml. Then sample concentrations of 20, 40, 60, 80 and 100 µg/ml were prepared.

Protocol for reducing power

According to this method, the aliquots of various concentrations of the standard and extract of *Abies webbiana* (20 to 100µg/ml) in 1.0 ml of deionized water were mixed with 2.5 ml of (pH 6.6) phosphate buffer and 2.5 ml of (1%) potassium ferricyanide. The mixture was incubated at 50°C in water bath for 20 min after cooling. Aliquots of 2.5 ml of (10%) tri-chloroacetic acid were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution 2.5 ml was mixed with 2.5 ml distilled water and a freshly prepared 0.5 ml of (0.1%) ferric chloride solution. The absorbance was measured at 700 nm in UV spectrometer (Shimadzu UV-1700). A blank was prepared without adding extract. Ascorbic acid at various concentrations (20 to 100µg/ml) was used as standard [16].

Preliminary Thin layer chromatography:-

Thin-layer chromatography is a "solid-liquid adsorption" chromatography. In this method stationary phase was TLC plates of silica gel 60 F_{254} pre coated with layer thickness of 0.2 mm using different solvent system. In this method, the mobile phase travels upward through the stationary phase. Spots were applied manually using capillary tube, plates were air dried using and TLC chamber were developed at room temperature with respective solvent system. The solvent travels up the thin plate soaked with the solvent by means of capillary action. During this procedure, it also drives the mixture priorly dropped on the lower parts of the plate with a pipette upwards with different flow rates. Thus the separation of analytes was achieved. This upward travelling rate depends on the polarity of the material, solid phase, and of the solvent [17].

$$\textbf{Rf Value} = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

Solvent system developed in preliminary TLC for *Abies webbiana* Hydroalcoholic extract in which the maximum spots were visible in Toluene: Ethyl acetate: Acetic acid (4:2:0.2) mobile phase with std. Phenol. So that Toluene: Ethyl acetate: Acetic acid (4:2:0.2) solvent was taken as mobile phase for column chromatography.

Column chromatography

Hydroalcoholic extract was subjected to silica gel column chromatography for isolation of Phenol from *Abies webbiana* Hydroalcoholic extract. A vertical glass column made of borosilicate material was used for chromatography. The column was rinsed with the acetone and was completely dried before packing. Column was packed using wet packing technique using silica gel (60-120) as the adsorbent. Slurry was prepared using toluene and was poured in to the column. 1gm of extract was added over the top of the column. Gradient elusion technique was followed for column chromatography. The column was eluted with Toluene: Ethyl Acetate: Acetic acid (4:2:02) number of elutes were collected. The fractions/elutes collected were concentrated and TLC was performed to identify the presence of single compound [18].

Chromatography condition for *Abies webbiana* (Fruit) sample

Determination of the phytochemical profile of *Abies webbiana* (Fruit) sample was performed by using advanced chromatographic technique like High performance thin layer chromatography (HPTLC). TLC co-chromatography was performed on AW sample and standard Ferulic acid where 2 & 6 µl of AW and Ferulic acid were spotted on the TLC plate along with the standard solution of Ferulic acid for AW sample. Stationary phase consisted of TLC aluminium sheets pre-coated with silica gel 60 F 254 (E. MERCK KGaA), Mobile phase consisted of Toluene: Ethyl acetate: Acetic acid (4: 2: 0.2) for AW sample. Aliquots of AW sample was separately applied from Ferulic acid (Samples and standard) to the plate as 6 mm wide Band length, Application position Y - 8 mm with an automatic TLC applicator Linomat-V (CAMAG). Densitometric scanning was performed on CAMAG scanner IV at 254 and 366 nm. The plates were prewashed by methanol and activated at 60 °C for 5 min prior to chromatography [19].

Detection and Qualitative analysis by HPTLC

After sample application, plates were developed in a CAMAG through a glass tank pre-saturated with the mobile phase Toluene: Ethyl acetate: Acetic acid (4: 2: 0.2) for 20 min. The plate was developed in Camag horizontal developing chamber at the room temperature up to 7 cm. Ascending mode was used for the development of Thin Layer chromatography.

After the development, the plates were dried and then derivatization of the chromatogram was performed. The plate was observed after 30 min under Camag UV cabinet at 254 and 366nm. Qualitative analysis was performed on scanning the plates at 254 nm using Camag scanner IV.

RESULTS AND DISCUSSION

The total ash (5.91 %), acid-insoluble ash (0.7 %) and water-soluble ash (1.42 %) values reported in Table 1 indicate that *Abies webbiana* fruit contains only modest inorganic matter and very little siliceous contamination, while the 7.62 % loss on drying confirms acceptable moisture for crude-drug stability. The markedly higher water-extractive value (11.98 %) than the alcoholic extractive value (8.77 %) in the same table foreshadows a predominance of polar constituents. Yield data in Table 2 support this expectation: the hydroalcoholic extract furnished a 2.745 % yield compared with just 0.682 % from petroleum ether, underscoring the greater solubility of the fruit's metabolites in a polar medium. Solubility behaviour (Table 3) corroborates those findings. The hydroalcoholic extract is freely soluble in water and methanol, whereas the petroleum-ether extract prefers non-polar solvents evidence that the two extracts capture chemically distinct fractions of the fruit. Qualitative screening (Table 4) confirms this dichotomy. The hydroalcoholic extract responds positively to tests for carbohydrates, alkaloids, flavonoids, tannins, phenolics, terpenoids and glycosides, while the petroleum-ether fraction is largely limited to terpenoids and saponins. High total phenolic (154.44 mg GAE g^{-1}) and flavonoid (98 mg RE g^{-1}) contents in the hydroalcoholic extract (Table 5) translate into notable antioxidant performance. Its DPPH IC_{50} of 30.585 µg mL^{-1} is about one-third that of ascorbic acid (10.313 µg mL^{-1}) (Table 6), yet still denotes a strong free-radical-scavenging capacity. Reducing-power results (Table 7) and superoxide-scavenging data (Table 8) follow the same concentration-dependent trend, again placing the extract slightly behind but close to the reference standard. Chromatographic separation of the hydroalcoholic extract yielded nine TLC fractions (Table 9). Fraction F, with an Rf of 0.52, matched the standard phenolic marker. Spectroscopic interrogation of this fraction (Table 10) UV λmax 330 nm, the diagnostic FT-IR bands, characteristic ^{1}H-NMR signals and an ESI-MS molecular ion at m/z 194.0142 identified the compound as ferulic acid [IUPAC: 3-(4-hydroxy-3-methoxyphenyl)prop-2-enoic acid]. HPTLC images (Figures 1 and 2) and the overlaid chromatograms of the reference and sample (Figures 3 and 4) visually confirm this assignment, while the peak table (Table 11) shows a single dominant spot at Rf ≈ 0.59 accounting for virtually 100 % of the area on every track clear evidence that ferulic acid is the major antioxidant principle in *A. webbiana* fruit.

Table 1: Pharmacognostical evaluation of *Abies webbiana* fruit

S. No.	**Parameters**	**Value in percentage** (%)
1	Total ash value	5.94
2	Acid insoluble ash	0.7
3	Water soluble ash	1.42
4	Loss on Drying	7.62
5	Water extractive value	11.98
6	Alcoholic extractive value	8.77

Table 2: Percentage yield

S. No.	Plant name	Solvent	Theoretical yield (in gm)	Actual Yield (in gm)	Percentage Yield (%)
1.	*Abies webbiana (fruit)*	Petroleum ether	350	2.39	0.682
2.	*Abies webbiana (fruit)*	Hydroalcoholic	326.41	8.96	2.745

Table 3: Solubility Determination of Extracts

S. No.	Solvent	*Abies webbiana Pet. ether*	*Abies webbiana Hydroalcoholic*
1.	Water	Insoluble	Soluble
2.	Methanol	Insoluble	Soluble
3.	Ethyl Acetate	Slightly Soluble	Slightly Soluble
4.	DMSO	Soluble	Soluble
5.	Petroleum Ether	Soluble	Insoluble

Table 4: Qualitative Phytochemical analysis of extracts

S. No.	Experiment	Result	
		Abies webbiana Pet. ether	*Abies webbiana Hydroalcoholic*
Test for Carbohydrates			
1.	Molisch's Test	-	+
2.	Fehling's Test	-	+
3.	Benedict's Test	-	+
4.	Bareford's Test	-	+
Test for Alkaloids			
1.	Mayer's Test	-	+
2.	Hager's Test	-	+
3.	Wagner's Test	-	+
4.	Dragendroff's Test	-	+
Test for Terpenoids			
1.	Salkowski Test	+	+
2.	Libermann-Burchard's Test	+	+
Test for Flavonoids			
1.	Lead Acetate Test	-	+
2.	Alkaline Reagent Test	-	+
3.	Shinoda Test	-	+
Test for Tannins and Phenolic Compounds			
1.	$FeCl_3$ Test	-	+
2.	Lead Acetate Test	-	+
3.	Gelatine Test	-	+
4.	Dilute Iodine Solution Test	-	+
Test for Saponins			
1.	Froth Test	-	-
Test for Protein and Amino acids			
1.	Ninhydrin Test	-	-
2.	Biuret's Test	-	-
3.	Million's Test	-	-

Test for Glycosides			
1.	Legal's Test	-	+
2.	Keller Killani Test	-	+
3.	Borntrager's Test	-	+

Table 5: Total Phenolic and flavonoid Content in *Abies webbiana* Hydroalcoholic extract

Extract	**Total phenolic content (mg/gm GAE)**	**Total flavonoid content (mg/gm RE)**
Absorbance Mean ± SD	0.599±0.006	0.255±0.002
TPC	154.44	98

Table 6: DPPH radical scavenging activity of Ascorbic acid and *Abies webbiana* hydroalcoholic extract

Concentration	**% Inhibition**	
	Ascorbic acid	**Hydroalcoholic extract**
20	54.065	44.410
40	60.670	53.150
60	67.378	65.243
80	74.186	71.443
100	84.654	77.337
IC50	**10.313**	**30.585**

Table 7: Reducing power assay of Ascorbic acid and *Abies webbiana* hydroalcoholic extract

Concentration	**% Inhibition**	
	Ascorbic acid	**Hydroalcoholic extract**
20	0.142	0.081
40	0.198	0.143
60	0.254	0.199
80	0.311	0.259
100	0.359	0.324

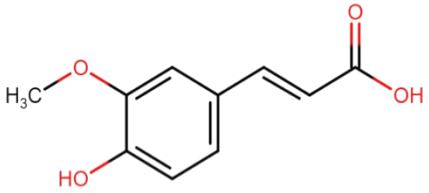
Table 8: SOS activity of Ascorbic acid and *Abies webbiana* hydroalcoholic extract

Concentration	**% Inhibition**	
	Ascorbic acid	**Hydroalcoholic extract**
20	52.192	43.969
40	60.855	51.096
60	66.995	59.210
80	73.355	65.789
100	82.236	73.574
IC50	**11.84**	**35.44**

Table 9: TLC of isolated fractions (A, B, C, D, E, F, G, H & I) of AW Hydroalcoholic extract

Sr. No.	Fraction	Solvent system	No. of spots	Colour of spots at Wavelenth (254 & 365nm)	Rf value (Extract)	Rf value (Std. Phenol)
1.	A	Toluene: Ethyl Acetate: Acetic acid (4:2:02)	-	-	-	0.51
2.	B		01	Fluorescence	0.81	
3.	C		02	Blue (365nm) Light Green (254nm)	0.42 0.37	
4.	D		-	-	-	
5.	E		01	Blue (365nm) Light Green (254nm)	0.44 0.47	
6.	F		01	Fluorescence (365nm) Light Green (254nm)	0.52 0.52	
7.	G1		01	Fluorescence (365nm)	0.31	
8.	G2		-	-	-	
9.	H		01	Light Green (254nm)	0.23	
10.	I		01	Light Green (254nm)	0.24	

Table 10: Spectral interpretation of isolated fraction F

Method	Spectral interpretation
UV	330 nm
FT-IR	The FTIR spectrum of fraction F showed a strong, broad peak at 3432.34 cm^{-1} indicating O-H stretching of hydroxyl groups, suggesting the presence of phenols or alcohols. Peaks at 2969.44 and 2920.61 cm^{-1} correspond to C-H stretching of alkanes, while 2365.64 cm^{-1} further supported alkane characteristics. Aromatic features were evident from C-H bending (1677.02 cm^{-1}) and C=C stretching (1431.15 cm^{-1}). The band at 1604.40 cm^{-1} indicated C-O stretching of a carbonyl group, and 1508.99 cm^{-1} was due to C-H bending of methyl groups. Phenolic O-H bending appeared at 1324.45 cm^{-1}, while C-O stretching bands at 1275.16 and 1165.45 cm^{-1} suggested esters and aromatic esters. Additional peaks at 1112.75, 972.43, 803.46, and 751.63 cm^{-1} confirmed the presence of alkanes, alkenes, and monosubstituted aromatic rings, indicating a chemically diverse composition.
^{1}HNMR (ppm)	The ^{1}H-NMR spectrum of isolated fraction F of AW revealed distinct proton signals: a singlet at 3.29 ppm (1H) and 3.76 ppm (3H) corresponding to aliphatic protons, while aromatic protons appeared at 6.33 ppm (d, 1H), 6.75 ppm (dd, 1H), and 7.24 ppm (dd, 1H). Two additional aromatic protons were observed as doublets at 7.42 ppm and 7.46 ppm. A strong singlet at 12.08 ppm (1H) indicated the presence of a hydroxyl proton, likely from a phenolic or carboxylic group. These signals suggest the presence of both aromatic and aliphatic moieties in the compound.
ESI-MS (m/z)	193.0142
Structure	
IUPAC Name	3-(4-hydroxy-3-methoxyphenyl) propenoic acid

HPTLC of *Abies webbiana* (Fruit) sample

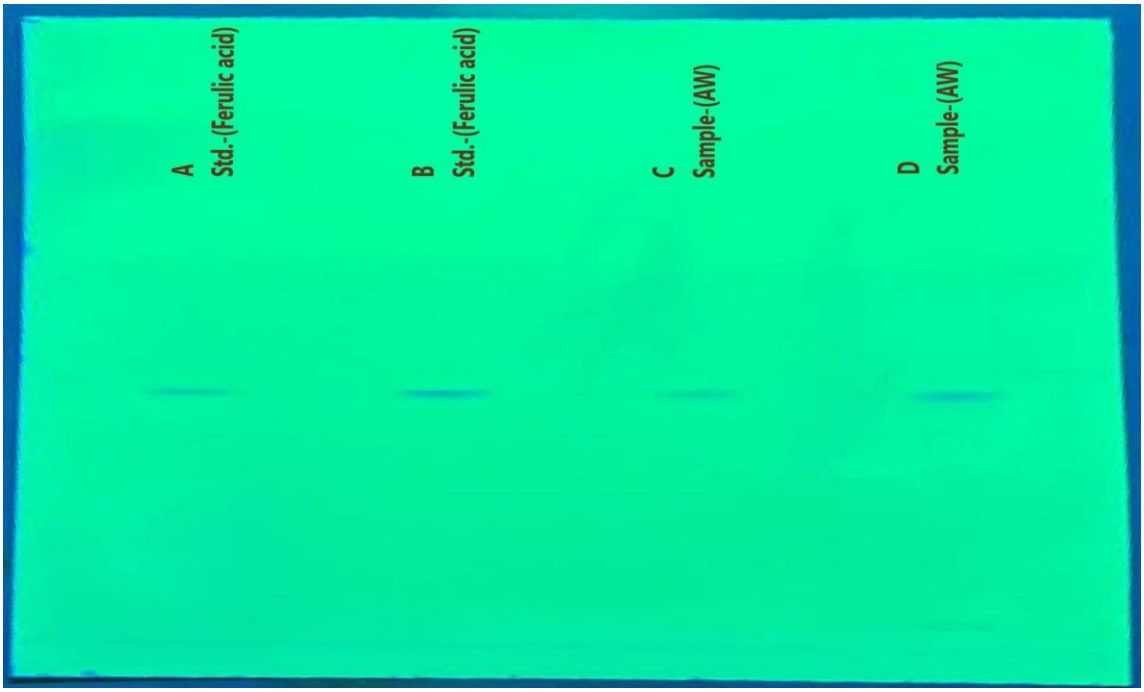


Figure 1: Chromatogram obtained from separation of AW Sample and visualized under UV light of wavelength 254 nm

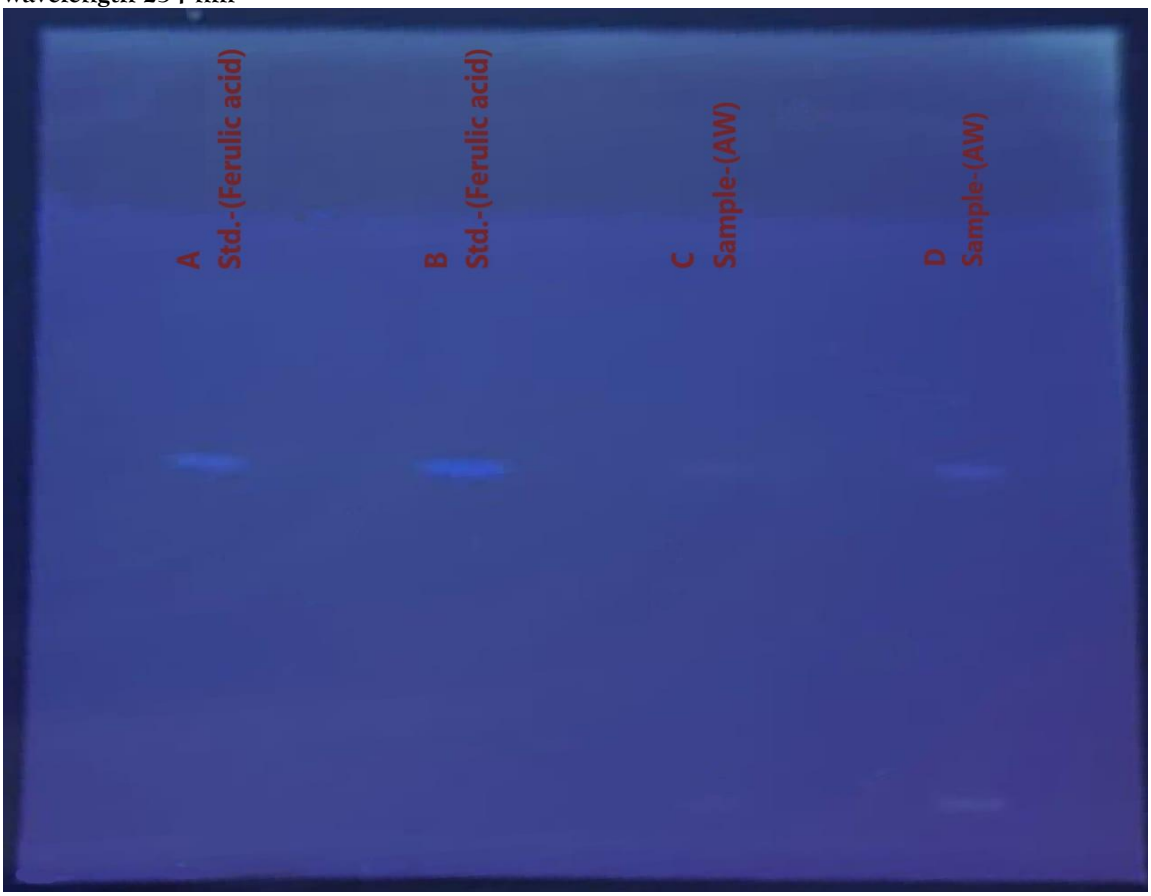


Figure 2: Chromatogram obtained from separation of AW Sample and visualized under UV light of wavelength 366 nm

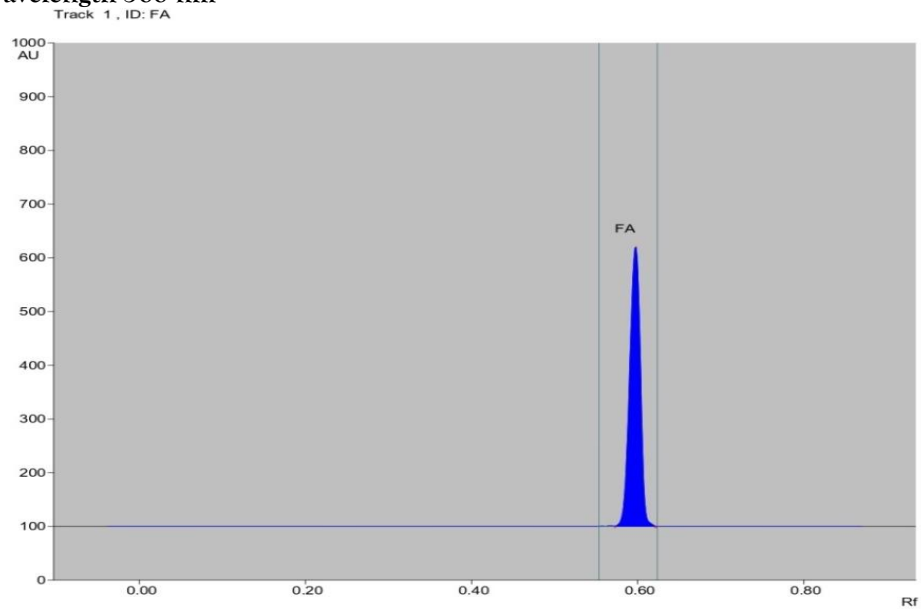


Figure 3: HPTLC chromatogram (Peak of Ferulic acid) scanned at 254 nm for Standard

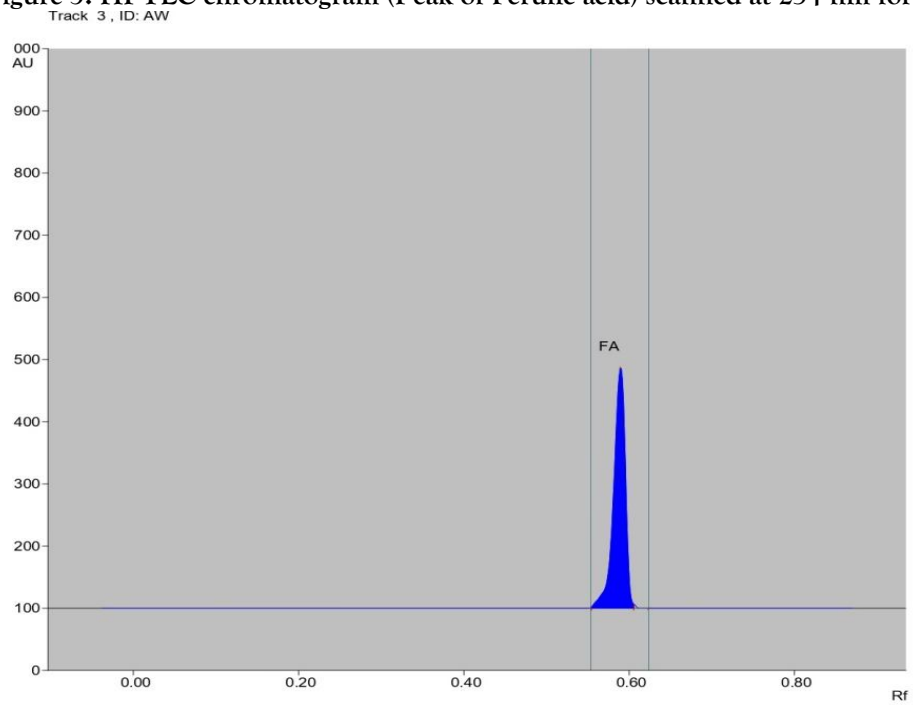


Figure 4: HPTLC chromatogram (Peak of Ferulic acid) scanned at 254 nm in *Abies webbiana* (Fruit)

Table 11: HPTLC peak table of sample at 254 nm of *Abies webbiana* (Fruit)

Track	Peak	Start R_f	Start height	Max R_f	Max height	End R_f	End height	Area	Area %	Assigned substance
1	1	0.57	0.8	0.60	519.2	0.62	0.4	5972.0	100.00	Ferulic acid
2	1	0.56	2.4	0.59	729.2	0.62	2.1	9734.2	100.00	Ferulic acid

3	1	0.55	1.4	0.59	386.1	0.61	6.5	4942.3	100.00	Ferulic acid
4	1	0.55	4.9	0.59	600.0	0.61	0.4	9350.9	100.00	Ferulic acid

CONCLUSION

The study highlighted the phytochemical, pharmacognostical, and antioxidant potential of *Abies webbiana* fruit, particularly its hydroalcoholic extract. The extract showed higher yield and solubility in polar solvents, indicating rich polar constituents. Phytochemical screening confirmed the presence of flavonoids, alkaloids, tannins, and phenolics, with strong antioxidant activity demonstrated in DPPH, reducing power, and superoxide assays. TLC, HPTLC, and spectral analyses identified ferulic acid as the major active compound. The extract shows promise for pharmaceutical applications due to its potent antioxidant properties.

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