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Phytochemical Profiling And Antioxidant Potential Of *Abies Webbiana* Fruit Extract: Isolation And Characterization Of Phenolic Compounds

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ABSTRACT
Abies webbiana (Wall ex D. Don) Lindl is called as Thalisapathiri in Tamil, Talisapathra or Patradhyam in Sanskrit, and Yew or Himalayan Silver in English. In Siddha system of medicine, it acts as a stomachic, carminative, expectorant, and tonic. The present study was undertaken to evaluate the pharmacognostical, phytochemical, and antioxidant properties of *Abies webbiana* fruit. We also identify the phenolic compound.Pharmacognostical evaluation revealed significant parameters, such as total ash value (5.61 ± 0.085%), acid-insoluble ash (0.59 ± 0.174%), water-soluble ash (1.23 ± 0.069%), and loss on drying (7.42 ± 0.341%). The water and alcoholic extractive values were 12.34 ± 0.418% and 8.57 ± 0.021%, respectively. Phytochemical screening of hydroalcoholic and petroleum ether extracts revealed the presence of alkaloids, terpenoids, steroids, carbohydrates, proteins, glycosides, and saponins. Quantitative analysis showed that the total phenolic content was 163.33 mg/g, and the total flavonoid content was 96 mg/g. The antioxidant activity of the hydroalcoholic extract was assessed using DPPH and superoxide radical scavenging assays. The IC50 values for DPPH and superoxide radicals were 30.785 µg/ml and 36.44 µg/ml, respectively, indicating moderate antioxidant potential. TLC, UV, FTIR, 1H-NMR, and mass spectroscopy further confirmed the presence of phenolic compounds, with isolated fraction F showing a molecular formula corresponding to 3-(4-hydroxy-3-methoxyphenyl) propenoic acid. These findings suggest that *Abies webbiana* fruit possesses significant phenolic and antioxidant properties, with potential for further exploration as a natural antioxidant source.However, *in vivo* studies to determine the antioxidant potential of the plant should be conducted before they can be recommended as nutritional substitutes.

1. INTRODUCTION

It is well known that free radicals, including reactive oxygen species (ROS) and reactive nitrogen species (RNS) produced in vivo, such as hydroxyl radicals, superoxide anions, and hydrogen peroxide, are extremely reactive and transient chemical species **(Apel, K., & Hirt, 2004).** They can damage proteins, DNA, and lipids. It has been reported that imbalances between ROS formation and scavenging systems contributes to the pathogenesis of different conditions, including aging, Alzheimer's disease, diabetes mellitus, atherosclerosis, and hypertension (**Valko et al., 2007**). Antioxidants can inhibit or delay oxidative chain reactions in lipids, proteins, carbohydrates, and DNA **(Nicolson and Ash, 2014).** Several studies have proposed that natural antioxidants may be less toxic than synthetic ones, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) **(Namiki, 1990).**

Abies webbiana (Wall ex D. Don) Lindl is called as Thalisapathiri in Tamil, Talisapathra or Patradhyam in Sanskrit, and Yew or Himalayan Silver in English. This lofty fir is widely distributed on higher ranges of Himalayas region from Kashmir to Assam states in India **(Ayurvedic Pharmacopoeia of India. Part I. Vol. IV).** In Siddha system of medicine, it acts as a stomachic, carminative, expectorant, and tonic. This plant powder is ground with vinegar and applied over the head for a head ache, head heaviness, and sinusitis. Gargling the leaf powder decoction for throat pain and mouth ulcer and it is also used as a tooth powder for tooth pain **(Mudhaliyar, 1988)**. It is used for a chronic cough, wheezing, fever, tuberculosis, vomiting, indigestion, gastritis, and bone fever **(Kuppusamy et al., 2009).**

A. webbiana leaf has been reported to exhibit antibacterial, antifungal, mast cell stabilizing, anxiolytic, antitumor, anti-inflammatory, antitussive, female antifertility, febrifuge, antispasmodic properties, central nervous system depressant actions and effective against hyperglycemia, conception, and rheumatism. Bronchodilator and antiplatelet activities of A. webbiana were investigated **(Rajalakshmi et al., 2016)**and evaluated the antioxidant and antimicrobial activity of *A. webbiana* extract. Effect of *A. webbiana* leaf extract on sleeping time and inflammation was analyzed **(Nayak et al., 2004).**

The present study aimed to evaluate the pharmacognostical, phytochemical, and antioxidant properties of *Abies webbiana* fruit.To deepen and fully characterize the phenol of *A. webbiana*, advanced techniques such as TLC, UV spectroscopy, FTIR, NMR, and mass spectrometry were performed.

2. MATERIAL AND METHODS

2.1 Plant material collection, identification, and authentication

Fresh fruits of *Abies webbiana* were collected in May from Nainital, which is located in the Kumaun area of Himalayas in India. Plant sample were submitted for identification and authentication to Department of Botany, Govt. College

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2.2 Physico-Chemical Analysis

The physicochemical parameters like loss on drying and ash value (total ash, water soluble ash, and acid insoluble ash) were determined by following the standard procedures **(Anonymous, 2008).** The cold maceration technique was used to determine the extractive values according to the procedure described with slight modification **(Ghosh et al., 2017).** About 5 g of the coarse powder of *Abies webbiana* was weighed accurately and placed in two conical flasks with a glass stopper separately in 100 mL of thefollowing solvents (alcohol, and water) macerated in a closed vessel for about 6 h with the help of a mechanical shaker and allowed to rest at normal room temperature (24 ± 2°C) for 18 hours. The extracts were filtered with Whatman No.1 filter paper and reduced using a rotary evaporator, and dried in an oven at 105°C. The weight of the residue obtained was used to calculate the extract value in % w/w for all the solvents.

2.3 Preparation of *Abies webbiana* fruit extract

The collected plant materials were washed with running tap water to avoid surface contaminations and shade dried for about 15 days. The dried leaves were cut into small dried powder was soaked with different organic solvents such as petroleum ether and 70% methanol and was subjected to solvent extraction using the Soxhlet apparatus. Then, the extracted sample was stored at 4°C for further analysis.

2.4 Preliminary phytochemical analysis

The extracts of *F. micrantha* leaves were then subjected to preliminary phytochemical analysis to assess the presence of various phytoconstituents **(Pardeshi et al., 2018).**

2.5 Determination of total phenolic content (TPC)

The TPC of the *Abies webbiana* extract was determined spectrophotometrically according to the Folin-Ciocalteu method with slight modifications. A standard solution of gallic acid was prepared using the distilled water in the concentration of 1 mg/ml. Different working standards were prepared to obtain the standard calibration curve, followed by diluting with distilled water with 3 ml and 2.5 ml of FC reagent (1:9) and incubated at room temperature for about 15 min, and then 2 ml of 7% sodium carbonate was added. Similar steps were followed for estimating phenolic content in the sample extract and the absorbance was measured at 765 nm against blank using spectrophotometer. All experiments were made in triplicates and the TPC was determined using the standard gallic acid calibration curve**(Palanisamy et al., 2020).**

2.6 Determination of total flavonoid content (TFC)

TFC of the *Abies webbiana* extract was determined spectrophotometrically according to the aluminum chloride method with slight modifications. A standard solution of rutin was prepared using methanol in the concentration of 1 mg/1 ml. Different working standards were prepared to obtain a standard calibration curve using 1ml with methanol and 0.15 ml of 10% $AlCl_3$, 0.15 ml of 5% sodium nitrite and incubated for 6 min. Then, 2 ml of 4 % sodium hydroxide was added. After 15 min, absorbance of mixture was estimated at 510 nm using UV spectrophotometer. Similar steps were followed for estimating flavonoid content in the sample extract and absorbance was measured at 510 nm against blank using spectrophotometer. All determinations were made in triplicates and the TFC was determined using the standard rutin calibration curve**(Palanisamy et al., 2020).**

2.7 Antioxidant assay

2.7.1Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (DPPH)

The free-radical scavenging activity was determined by DPPH method with slight modifications **(MA et al., 2008).**A standard solution of ascorbic acid was prepared in the concentration of 1 mg/ml in methanol. The standard calibration curve was obtained using different working standards and was made up of 1 ml with methanol. DPPH solution (2ml) was then added and mixed vigorously. Similar steps were followed for the sample extract. The reaction mixture was incubated for about 30 min in dark condition and absorbance was measured at 517 nm using a spectrophotometer. All determinations were made in triplicates and the standard curve was obtained using ascorbic acid. The % DPPH which was scavenged (% DPPH) was calculated using the formula:

$$\text{Scavenging effect (\%)} = 1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of Control}} \times 100$$

2.7.2 Scavenging activity of superoxide anion

The scavenging activity of superoxide anion was determined by the method of Yen and Chen **(Yen and Chen, 1995)**. The reaction mixture consists of different concentration of plant extract (1 mg/ml), 1 ml of phenazine methosulfate (PMS) (60 μM) prepared in phosphate buffer (0.1 M pH 7.4) and 1 ml of NADH (phosphate buffer) was incubated at 25°C for 5 min, the absorbance was read at 560 nm against blank samples.

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2.7.3 Determination of reducing power

The reducing power of the extract was evaluated according to the method of Oyaizu **(Oyaizu, 1986).** The mixture containing 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of $K_3Fe(CN)_6$ (1%w/v) was added to 1.0 ml of the extract dissolved in distilled water. The resulting mixture was incubated at 50°C for 20 min, followed by the addition of 2.5 ml of TCA (10% w/v). The mixture was centrifuged at 3000 rpm for 10 min to collect the upper layer of the solution (2.5 ml), mixed with distilled water (2.5 ml) and 0.5 ml of $FeCl_3$ (0.1%, w/v). The absorbance was then measured at 700 nm against blank sample **(Aiyegoro and Okoh, 2010).**

2.8 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 **(Ozlem *et al.*, 2016).**

$$\mathbf{R_f\ Value} = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

2.9 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 **(Srivastava *et al.*, 2021).**

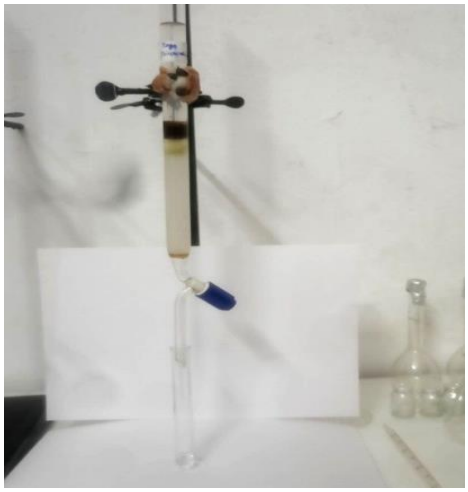

Figure 1 Isolation of components by column chromatography

2.10 Spectroscopic characterization

2.10.1 UV-visible Spectroscopy

The isolated fraction (F) of AWExtract was scanned from 200 to 800 nm wavelength using UV-Visible Spectrophotometer (Shimadzu UV-1700) and the characteristic peaks were detected and recorded **(Patel *et al.*, 2022)**.

2.10.2 FT-IR

To establish the presence of the functional groups in the isolated fraction (F) of AWExtract, FT-IR spectroscopy was performed using Perkin Spectrum BX spectrophotometer. The sample was dried and ground with KBr pellets and analyzed on Thermo Nicolet model 6700 spectrum instrument. A disk of 100 mg of KBr was prepared with a mixture of 2% finely dried sample and then examined under IR-spectrometer. Infrared spectra were recorded in the region of 400 - 4,000 cm-1 **(Lucien*et al.*, 2008).**

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2.10.3 NMR Spectroscopy

NMR spectroscopy was performed for the isolated fraction (F) of AWExtract to identify the structure of the compound present in the isolated fraction. JEOL RESONANCE NMR spectroscopy for this purpose was Fourier Transform Nuclear Magnetic Resonance spectroscopy **(Zia *et al.*, 2019).**

2.10.4 Mass Spectroscopy

Mass spectrometry converts molecules into ions and according to their mass and charge the ions can be separated and sorted. The mass spectrometer used for the identification of the molecular weight of isolated fraction (F) of AWextract was recorded on mass spectrometer instrumentmicrOTOF-Q 228888.10348 **(Wiley *et al.*, 1995).**

3. RESULTS AND DISCUSSION

3.1 Pharmacognostical evaluation

Table 1 Pharmacognostical evaluation of *Abies webbiana* fruit

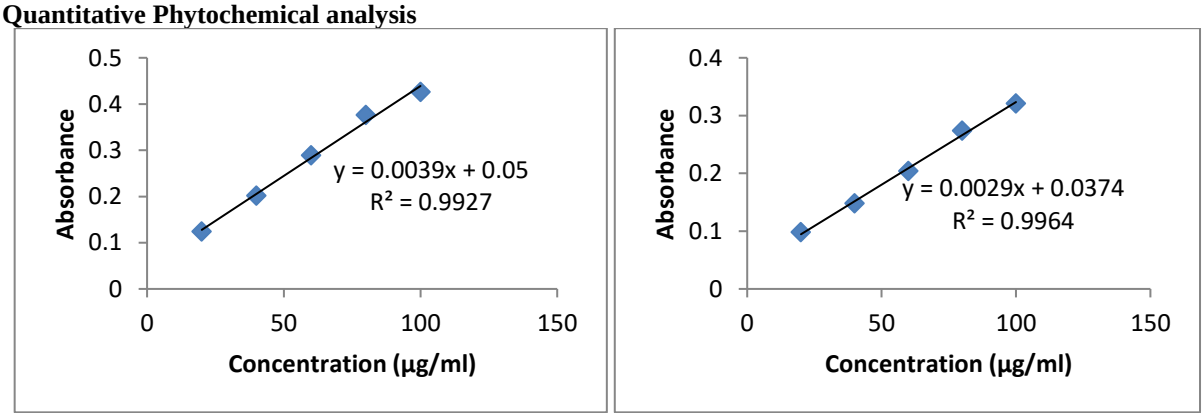
Parameters	Value in percentage (%)
Total ash value	5.61±0.085
Acid insoluble ash	0.59±0.174
Water soluble ash	1.23±0.069
Loss on Drying	7.42±0.341
Water extractive value	12.34±0.418
Alcoholic extractive value	8.57±0.021

Pharmacognostical and physicochemical studies, being reliable and inexpensive, play an important role in quality control issues of the crude drug samples. The physicochemical values of *Abies webbiana* fruit are displayed in Table 1. Loss on drying, total ash content, acid-insoluble ash and Water soluble ash content were determined to be 7.42±0.341, 5.61±0.085, 0.59±0.174 and 1.23±0.069% of the dry weight, respectively. These parameters were useful for detecting low-grade products as well as for determining the extractive values. Alcohol and waterextractive values were determined to be 8.57±0.021 and 12.34±0.418% of the dry weight, respectively.

3.2 Phytochemical Analysis

Successive Soxhlet extraction was performed using different solvents. The extract showed sticky nature for all solvents used and the color varied from yellowish brown to dark brown color. Respective extracts were subjected to preliminary phytochemical screening and the results showed the presence of alkaloids, terpenoids, steroids, carbohydrates, proteins and glycosides in hydroalcoholic extract, where terpenoids and saponin, were present in petroleum ether extract of *Abies webbiana* fruit.

3.3 Quantitative Phytochemical analysis



Graph 1 Graph represent standard curve of Gallic acid and Rutin

Table 2 Total Phenolic and Flavonoid Content in extract

Extract	Total phenolic content (mg/gm equivalent to Gallic acid) in *Abies webbiana Hydroalcoholic extract*	Total flavonoid content (mg/gm equivalent to rutin) in *Abies webbiana Hydroalcoholic extract*
Absorbance Mean±SD	0.540±0.008	0.229±0.012
Value (mg/gm)	163.33	96

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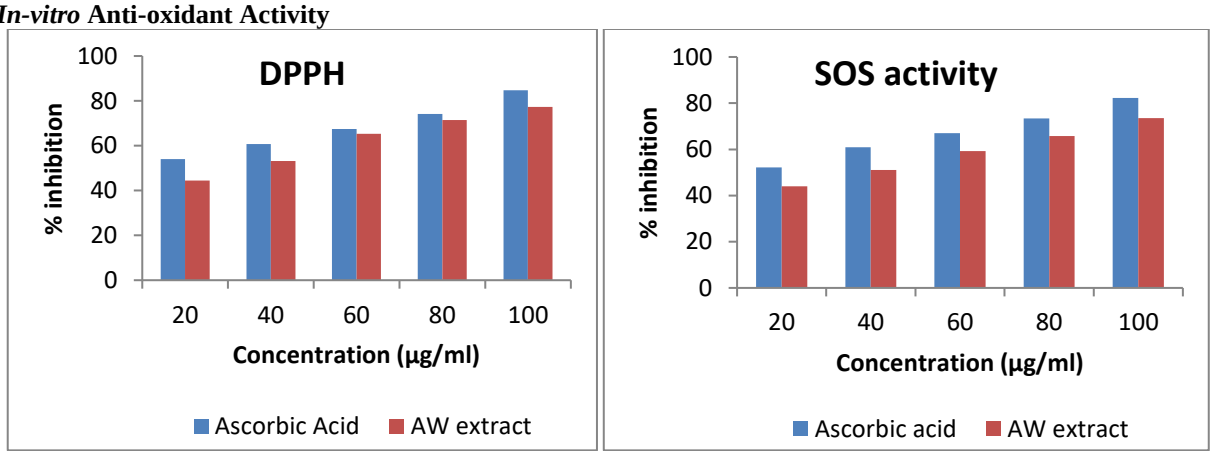
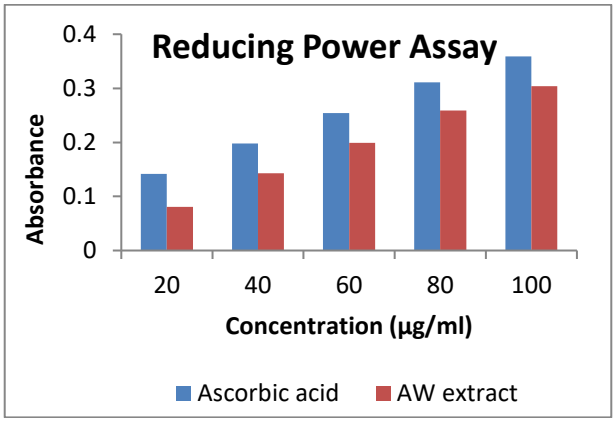
Phenolics are the most wide spread secondary metabolite in plant kingdom. These diverse groups of compounds have received much attention as potential natural antioxidant in terms of their ability to act as both efficient radical scavengers and metal chelator. It has been reported that the antioxidant activity of phenol is mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers**(Rice-evans et al., 1995).**In the present study, total phenolic content present in extract was estimated using modified Folin- ciocalteau method. In*Abies webbiana* hydroalcoholic extract, the phenolic content was found to be 163.33mg/gm.
It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of flavonoids are through scavenging or chelating process**(Kessler et al., 2003).** Therefore, in the present study, total flavonoid content present in extract was estimated using Aluminum chloride colorimetric method. In *Abies webbiana* hydroalcoholic extract, the flavonoid content was found to be 96mg/gm.

3.4*In-vitro* Anti-oxidant Activity



Graph 2 Graph represent DPPH and Superoxide activity of *A. webbiana extract*



Graph 3 Graph represent Reducing power activity of *A. webbiana extract*

The DPPH radical scavenging activity of the *Abies webbiana* extract, compared with ascorbic acid, as standard. IC50 values of extract and standard were 30.785µg/ml and 11.313µg/ml, respectively. The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability**(Shirwaikar et al., 2006).** Though the DPPH radical scavenging abilities of the extracts were less than those of ascorbic acid, the study showed that the extracts have the proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants.
It is well known that superoxide anions damage biomolecules directly or indirectly by forming H_2O_2, OH, peroxy nitrite or singlet oxygen during aging and pathological events such as ischemic reperfusion injury. Superoxide has also been observed to directly initiate lipid peroxidation**(Yen and Duh, 1994)**. The superoxide anion radical scavenging activity of *Abies webbiana* extract assayed by the PMS-NADH system is shown in **Graph 2.** The superoxide scavenging activity of *Abies webbiana* extract was increased markedly with the increase in concentrations. Thus, higher inhibitory effects of the rhizomes extracts on superoxide anion formation noted herein possibly renders them as a promising antioxidants. The half inhibition concentration (IC50) of *Abies webbiana* extract was 36.44 µg/ml while IC50 value for ascorbic acid was 12.84 µg/ml. These results suggested that *Abies webbiana* extract has a potent superoxide radical scavenging effects.
The reducing power of *Abies webbiana* extract was very potent and the power of the extract was increased with quality of sample. The plant extract could reduce the most Fe^{3+} ions, which had a lesser reductive activity than the standard of

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ascorbic acid. Increased absorbance of the reaction indicated increased reducing power. The reductive capability of the plant extract compared to ascorbic acid is shown in **Graph3.**

3.5 Preliminary TLC preparation for the estimation of active constituents

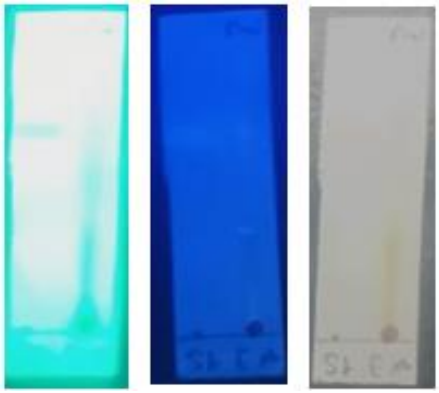

Figure 2 TLC estimation by UV lamp for AW extractwith Std. Phenol

In this thin-layer chromatography (TLC) analysis of the extract, a solvent system of toluene, ethyl acetate, and acetic acid (4:2:0.2) was employed to analyze phenolic compounds. The TLC plate was observed under UV light at two wavelengths: 254 nm and 365 nm. The phenolic standard used for comparison has an Rf value of 0.51. The Rf value of 0.51 in both the extract and the standard phenol indicates that the extract likely contains phenolic compounds, as the spot co-elutes with the phenolic standard. The multiple spots in both wavelengths reflect a diverse range of compounds present in the extract, with fluorescence indicating the presence of conjugated aromatic systems, possibly phenols or other related compounds. The variations in colors and Rf values suggest that the extract contains different flavonoid or phenolic fractions, supported by the characteristic fluorescence under UV light.

3.6 Column Chromatography

Nine fractions/elutes obtained from silica gel column chromatography of *Abies webbiana* hydro alcoholic extract was tested for the detection of various phyto compounds using TLC. The TLC analysis of the fractions (A, B, C, D, E, F, G, H, and I) of the hydroalcoholic extract (AW) was performed using a solvent system of toluene, ethyl acetate, and acetic acid (4:2:0.2). The Rf values of the extract and the phenolic standard were compared, and the observations at both 254 nm and 365 nm UV light were recorded.

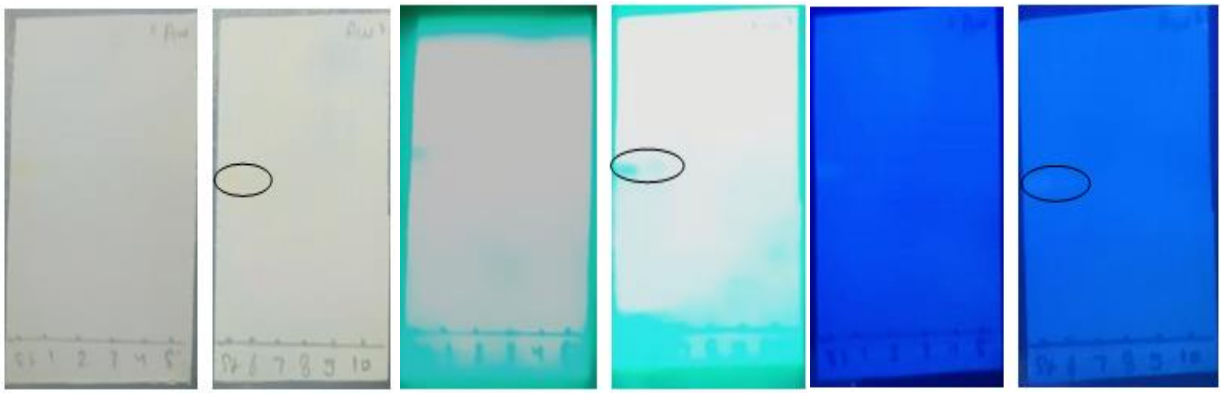

Figure 3TLC of AW fractions after column chromatography with Std. Phenol.a) visible light b) Short-UV (254 nm), c) Long-UV (365 nm)

Among the fractions analyzed, Fraction F show Rf values (0.52) that closely match the Rf of the phenolic standard, suggesting that these fractions contain phenolic compounds. Other fractions, such as B, C, G1, H, and I, display fluorescence and colors under UV light, indicating the presence of compounds like flavonoids or other aromatic molecules, but their Rf values suggest they are different from the phenolic standard.

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3.8Spectroscopic characterization

3.8.1 By UV-Spectroscopy

UV-Spectra of isolated fraction (F) of AW Hydroalcoholic extract was recorded with a Shimadzu 1700 double beam-UV-VIS spectrophotometer. UV spectra of the isolated fraction was recorded in solvent as Toluene: Ethyl acetate: Acetic acid (4:2:0.2) over a scanning range of 200-800 nm and λmax of isolated compound were determined. The Blank was Toluene: Ethyl acetate: Acetic acid (4:2:0.2). The wavelength of isolated fraction (F) of AW Hydroalcoholic extract was found to be 329 nm.

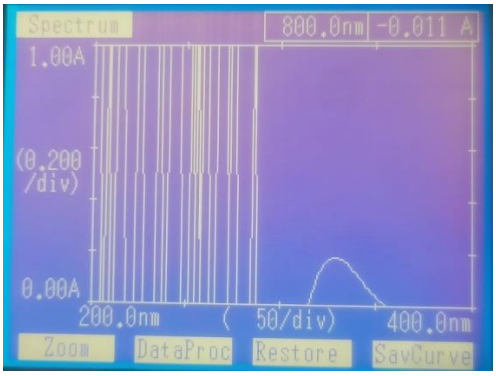

Figure 4 UV- Spectra of (F) fraction of AWHydroalcoholic extract after column chromatography

3.8.2 IR spectra of the isolated fraction (F) of AW Hydroalcoholic extract

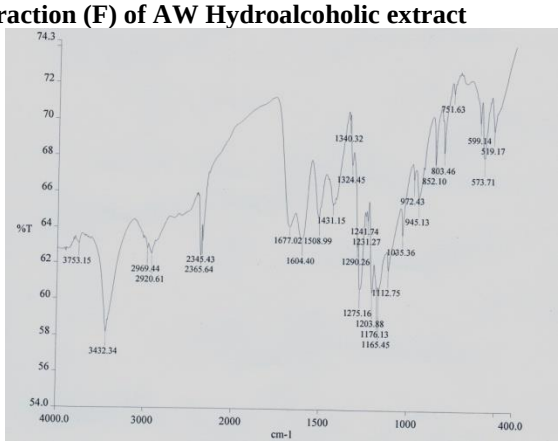

Figure 5IR spectra of the isolatedfraction (F) of AW Hydroalcoholic extract

The IR Spectra of isolated fraction (F) of AW Hydro alcoholic extract showed that -OH group Strong, Broad peak appeared at 3432.34 cm-1, C-H stretching peaks of Alkane at 2969.44, 2920.61 & 2920.61 cm-1. The C-H bending peak of Aromaticcompound at 1677.02 cm-1, Carbonyl group, C-O stretching peak at 1604.40 cm-1, C-H bending peak of Methyl group at 1508.99 cm-1, C=C Stretching peak of Benzene Ring at 1431.15 cm-1, O-Hbending peak of Phenol at 1324.45 cm-1, C-O stretching peak of Aromatic Ester at 1165.45 cm-1, C-O stretching peak of Ester at 1165.45 cm-1, C-C stretching peak of Alkane at 1112.75 cm-1, C=Cbending peak of Alkene at 972.43 & 803.46 cm-1 and C-H bending peak of Monosubstituted at 751.63 cm-1.

3.8.3^{1}H NMR spectra of the isolated Fraction (F) of AW

^{1}H NMR spectra of isolated fraction (F) of AW Hydroalcoholic extract was recorded on NMR Spectrometer. Tetramethylsilane used as an internal standard. The signals are denoted with the symbols s, d, t, and m for singlet, doublet, triplet, and multiplet, respectively.

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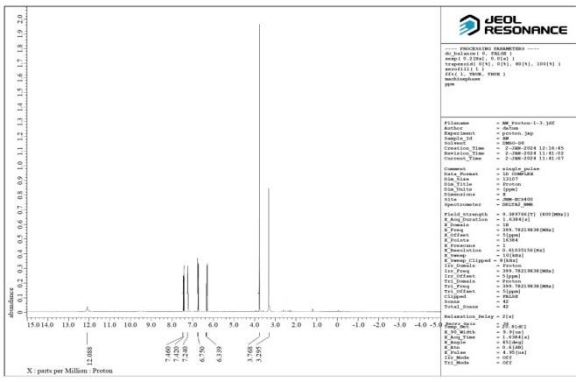

Figure 6[l]**H-NMR spectra of theisolated compound (Fraction (F)) of AW**

In [l]H-NMR spectra isolated Fraction (F) of AW showed that [l]H-1 proton appeared at 3.29 (s) ppm, [l]H-3 protons appeared at 3.76 (s) ppm, [l]H-1 proton appeared at 6.33 (d) ppm, [l]H-1 proton appeared at 6.75 (dd) ppm, [l]H-1 proton appeared at 7.24 (dd) ppm, [l]H-2 protons appeared at 7.40-7.50 (7.42 (dd) ppm, 7.46 (dd) ppm) and [l]H-1 proton appeared at 12.08 (s) ppm).

3.8.4 Mass spectra of theisolated Fraction (F) of AW

Mass spectra of isolated Fraction (F) of AW were recorded on Bruker micrOTOF-Q mass spectrometer.

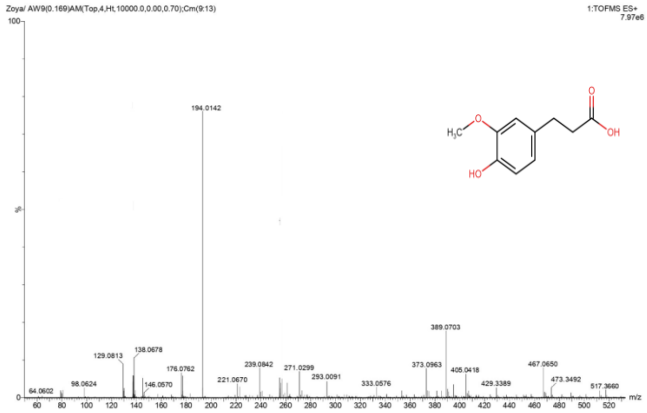

Figure 7: **Mass spectra of theisolated Fraction (F) of AW**

Mass spectra of isolated Fraction (F) of AW showed molecular ion [M^+] peaks at mlz 194.0142 The carbon (10), Hydrogen (10) and Oxygen (4) present in isolated Fraction (F) of AW which corresponds to the molecular formula $C_{10}H_{10}O_4$ of 3-(4-hydroxy-3-methoxyphenyl) propenoic acid according to their fragments.

4. CONCLUSION

The study provided comprehensive insights into the pharmacognostical and phytochemical profile of *Abies webbiana* fruit. The hydroalcoholic extract demonstrated significant antioxidant activity, with moderate DPPH and superoxide radical scavenging capabilities. Phenolic compounds, particularly 3-(4-hydroxy-3-methoxyphenyl) propenoic acid, were confirmed in the extract through advanced spectroscopic techniques. The total phenolic and flavonoid content further supported the plant's antioxidant potential, indicating its suitability as a natural source of antioxidants. These findings open avenues for further research into the medicinal applications of *Abies webbiana*, especially in oxidative stress-related conditions. However, further in vivo studies are needed to fully explore its therapeutic potential and validate its efficacy as a nutritional supplement.

5. REFERENCE

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