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Evaluation of hydromethanolic extract and aglycone fraction of *Actiniopteris semiflabellata* for antioxidant activity

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Abstract
Actiniopteris semiflabellata Pic-Ser grows in Yemen and is used by the local population for wounds and burns. The goal of the current study was to assess the *Actiniopteris semiflabellata's* chemical composition and antioxidant activity. Phenols, flavonoids, triterpenes and sterols were identified in the hydromethanolic extract and in the aglycone fraction, while carbohydrates were only found in the hydromethanolic extract. In the hydromethanolic extract and the aglycone fraction, the total phenolic content was evaluated as 17.73 mg/g and 7.22 mg/g respectively, while the total flavonoid content was 11.21 mg/g and 4.13 respectively. Studied extract and fraction exhibited antioxidant activity by TLC bioautography and DPPH radical scavenging activity assay. The IC50 values for the hydromethanolic extract and the aglycone fraction were 79.64 and 148.60 µg/ml respectively. These results confirm the traditional use and are useful for further study of this plant, in addition, it may be a good source of natural antioxidants.

Keywords: Yemen, *Actiniopteris semiflabellata*, hydromethanolic**,** aglycone, antioxidant

Introduction
The significance of medicinal plants comes from the presence of chemical constituents that have physiological effects on the human body [1, 2]. The use of plant-derived medicines as indigenous treatments in the traditional medical system has been connected to their introduction into modern medicine [3], and has attracted intense investigation, both in terms of preservation and to determine whether its traditional uses are backed by real pharmacological effects [4]. The natural resources used to create new drugs are medicinal plants [5, 6, 7]. In recent years, several plants, especially medicinal plants, have undergone extensive testing for their antioxidant capacities [8]. Foods and medicinal plants are the major sources of antioxidants [9, 10]. Natural antioxidants, especially carotenoids and polyphenols, provide a range of biological advantages, including anti-inflammatory, anti-bacterial, antiviral, anti-aging, and anti-cancer activities [9, 10, 11]. There is no doubt that 70% of current medications have their roots in or are derived from plants or other natural materials [12]. It makes sense to investigate the chemical components of plants in order to screen them for the creation of innovative medicines and to determine the scientific basis for their traditional usage.
Yemenis have long used plants to treat a variety of medical, physiological, mental and social illnesses, however, few scientific studies have been done on these plants [13, 14, 15]. The plant *Actiniopteris semiflabellata* Pic-Ser, (Actinopteridaceae) grows in many parts of Yemen such as Tihama foothills, Hagda, Ibb, al Baydha, Hajjah, and Socotra [16]. It is also found in Al-Dali (Al-Saib) and is employed by locals to cure burns and wounds. The hydromethanolic extract and aglycone fraction of the plant were prepared, and then the phytochemical analysis and antioxidant activity were carried out. When necessary, statistical tests and the right references were used to assess the study's data and set up as parameters for further reference of the plant.

Materials and Methods
Collection and identification
The aerial parts of *Actiniopteris semiflabellata* (Pteridaceae) were collected in June 2021 from Al-Dali (Ashaib), dried in the shade, manually crushed, then kept at room temperature for investigation. Professor Othman S. Alhawshibi, a taxonomist from the University of Aden, Faculty of Science, identified the plant sample.

Preparation of the extract
The dried powder of the investigated plant's aerial part (100 gm) was defatted with petroleum ether in a Soxhlet extractor (boiling point 60-80 °C). The remaining marc was completely dried in a hot air oven below 50 °C, packed in a Soxhlet extractor, and extracted with 80% methanol (80-90 °C) until the extraction was complete. The hydromethanolic extract was filtered and evaporated to dryness by a rotary evaporator. The percentage yield of hydromethanolic extract was calculated and then kept at 8 °C in a sealed bottle until experiments were carried out [17, 18].

Acid hydrolysis of hydromethanolic extract
Seven grams of hydromethanolic extract were dissolved in 100 ml of 10% (v/v) sulfuric acid (H2SO4), heated for an hour in a water bath, and the hydrolysate (aglycone fraction) was then extracted with ethyl acetate. For further examination, the obtained fraction was kept in a sealed bottle at 8 °C [19].

Qualitative phytochemical analysis
The hydromethanolic extract and its aglycone fraction of *Actiniopteris semiflabellata* aerial part were subjected to phytochemical screening according to standard methods [17, 18, 20, 21].

Quantitative estimation of total phenolic content
Estimation of the total phenolic content (TPC) of the hydromethanolic extract and the aglycone fraction of the aerial section of *Actiniopteris semiflabellata* was calculated using a modified Folin-Ciocalteu method [22, 23]. Results were expressed as milligrams of gallic acid equivalent (GAE) for each gram of sample. Gallic acid and samples were prepared in methanol at various concentrations (40–200 g/ml). Each test sample and the standard solution (1 mg/ml in methanol) were mixed in a volume of 0.5 ml with 2 ml of the Folin-Ciocalteu reagent and 4 ml of a saturated sodium carbonate solution (7.5% w/v). Silver foils were used to cover the tubes, which were then incubated for 30 minutes at room temperature with intermittent shaking. At 765 nm, the absorbance was measured using a spectrophotometer. The analysis of all the samples was carried out three times. The total phenolic content was determined using a standard curve produced from gallic acid (standard solution).

Quantitative estimation of total flavonoid content
Estimation of the total flavonoid content (TFC) of the hydromethanolic extract and aglycone fraction of the *Actiniopteris semiflabellata* aerial part was performed by the aluminum chloride colorimetric technique [23, 24]. Different concentrations (40-200 µg/mL) of standard quercetin and samples were prepared in methanol. Each test sample and the standard solution (1 mg/ml) in a volume of 0.5 ml were mixed with 2 ml of distilled water, then 0.15 ml of sodium nitrite solution (5% NaNO2, w/v) was added. After 6 minutes, 0.15 ml of a 10% AlCl3 (w/v) solution was added. Next, after another 6 minutes of standing, 2 ml of a 4% NaOH (w/v) solution was added to the mixture. The final volume was then adjusted to 5 mL by adding distilled water, mixing well, and then allowed to stand for an additional 15 minutes. At 510 nm, the absorbance of each mixture was measured against a blank sample of the same mixture but without extract. The total flavonoid content was determined as mg of quercetin equivalent per gram of sample using the quercetin calibration curve. Three replications of all the samples' analyses were performed.

Thin layer chromatography
Prepared hydromethanolic extract and aglycone fraction were subjected to thin layer chromatography on silica gel G 60 F254 with a layer thickness of 0.2 mm (Allugram-Germany) to separate spots and determine their Rf values [25, 26].

Evaluation of antioxidant activity
TLC bioautography assay
The TLC-bioautography technique was used to screen for antioxidant chemicals in the investigated plant [27]. Approximately 2 µg of each extract/fraction was loaded onto a TLC plate (20 cm × 20 cm). The TLC plate was developed using hexane-ethyl acetate-formic acid (4:1:0.1) before being sprayed with 0.05% DPPH reagent to detect antioxidant chemicals.

DPPH radical scavenging assay
The hydromethanolic extract and the aglycone fraction from the aerial part of *Actiniopteris semiflabellata* were tested for their ability to scavenge free radicals according to the method described by Chan et al. with modification [28]. Various dilutions of the extract, fraction and quercetin (50, 100, 150, and 200 µg/ml) were prepared. Then, 1 mL of each dilution was added into the test tube containing 2 mL of DPPH solution (6.0 mg in 100 ml methanol). The solution was rapidly mixed and incubated in a dark place for 30 minutes. The absorbance of each solution was measured at 517 nm using UV/Vis spectrophotometer A mixture of 2 mL of DPPH solution with 1 mL of methanol was used as a blank, while quercetin was taken as a positive control.
The percentage of the DPPH scavenging effect was calculated using the following equation:

Inhibition % = [(A blank –A sample) /A blank] x 100

IC50 value
Hydromethanolic extract and aglycone fraction antioxidative activity was represented as IC50 values and compared to the standard. The 50% inhibition (IC50) of antioxidant activity was calculated using sample concentrations that inhibited 50% of the DPPH radicals [29].

Statistical analysis
The level of statistical significance between groups was examined using the Student's t-test. $p<0.05$ was considered statistically significant.

Result and Discussion
Qualitative phytochemical analysis
Medicinal plants, including phytochemicals such as alkaloids, tannins, flavonoids, and phenolic acids, have a variety of biological activities including anti-ulcer, anti-inflammatory, antioxidant, cytotoxic, antispasmodic, angioprotective, and antineoplastic, illustrating their therapeutic value [30, 31, 32, 33, 34]. The percentage yield of the hydromethanolic extract in the current study was determined to be 16.29%. Acid hydrolysis of hydromethanolic extract with 10% (v/v) sulfuric acid gives a black precipitate (2 gm), which collected with ethyl acetate (Figure 1). Qualitative phytochemical tests revealed the presence of phenols, flavonoids, triterpenes and sterols in the hydromethanolic extract and in the aglycone fraction, while carbohydrates were only found in the hydromethanolic extract.

Fig 1: Separated funnels with black precipitate, which collected with ethyl acetate after hydrolysis of hydromethanolic extract.

Total phenolic and flavonoid content

Many biological activities of flavonoids and other phenolic constituents have been reported, such as antioxidant, anticancer, antibacterial, cardioprotective, anti-inflammatory, etc [35, 36, 37]. The regression equation of the calibration curve (y=0.0063x + 0.2696, R2 = 0.9939) was used to calculate the content of phenolic compounds, expressed in mg of gallic acid equivalent (GAE) per gram of dry extract. The regression equation for the calibration curve of quercetin (y=0.0031x + 0.3461, R2 = 0.9713) was used to determine the flavonoid content in the quercetin equivalent. The results are shown in Table 1 and Figures 2 and 3.

Table 1: Total phenolic and flavonoid content of *Actiniopteris semiflabellata* aerial part.

Sample	Total phenolic content mg/g	Total flavonoid content mg/g
Hydromethanolic extract	17.73±3.30	11.21±4.32
Aglycone fraction	7.22±4.43	4.13±6.11

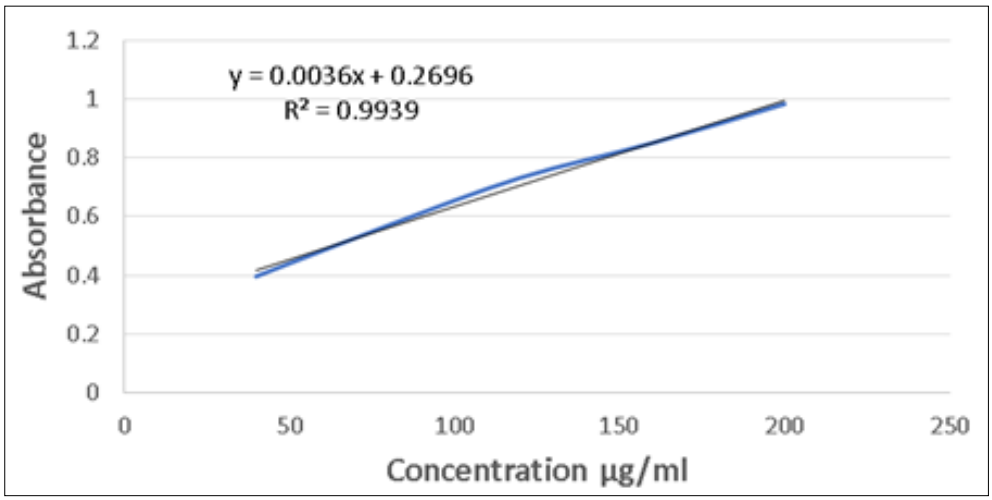

Fig 2: Gallic acid calibration curve.

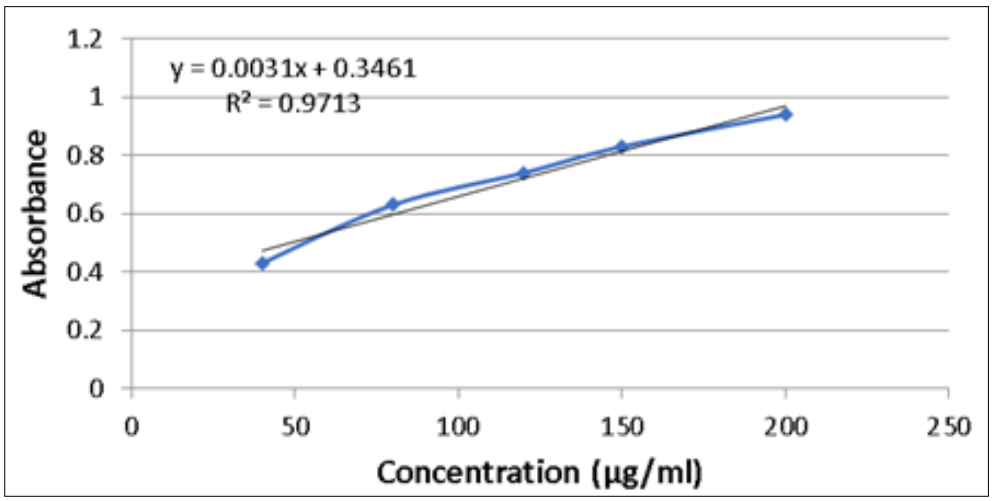

Fig 3: Quercetin calibration curve.

Thin layer chromatography

Thin layer chromatography was used to separate the spots and determine their Rf values in the prepared extract and aglycone fraction. The best solvent mixture was hexane-ethyl acetate-formic acid (4:1:0.1). Pictures of the plates were taken in daylight and in the UV chamber, and the Rf values of the developed spots were calculated (Table 2 and Figure 4).

Table 2: Observations of TLC of aglycone fraction and hydromethanolic extract of *Actiniopteris semiflabellata* in system hexane-ethyl acetate-formic acid (4:1:0.1)

Fraction/ Extract	No. of spots	Rf values	Spot color in daily light	Spot color at 254 nm	Spot color at 366 nm
Aglycone fraction	10	0.80	Yellow	quenching	Light pink
		0.66	Blue	quenching	Red
		0.57	Greenish yellow	quenching	Red
		0.52	Light yellow	quenching	Pink
		0.46	Blue	quenching	Blue
		0.42	Greenish yellow	quenching	Pink
		0.35	Greenish yellow	quenching	Pink
		0.32	Yellow	quenching	Reddish pink
		0.28	Greenish yellow	quenching	Sky blue
		0.22	Light blue	quenching	Purple
Hydromethanolic	7	0.55	Invisible	Invisible	Light pink
		0.53	Invisible	Invisible	Light pink
		0.41	Invisible	Invisible	Light pink
		0.37	Invisible	Invisible	Light pink
		0.32	Invisible	Invisible	Light pink
		0.28	Invisible	Invisible	Light pink
		0.22	Invisible	Invisible	Light pink

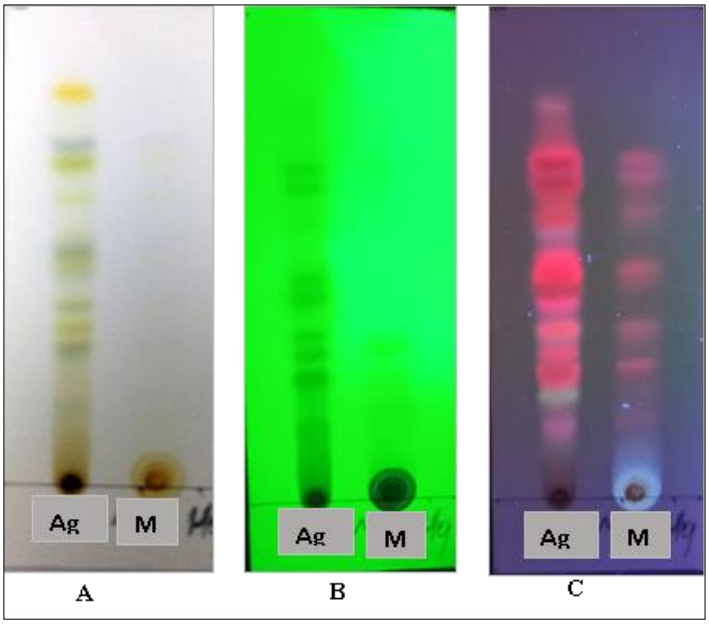

Fig 4: TLC plates of aglycone fraction (Ag) and hydro-methanolic extract (M), obtained in hexane-ethyl acetate-formic acid (4:1:0.1), A- in daylight, B-under UV 254 nm and C- under UV 365 nm.

Evaluation of antioxidant activity

TLC bioautography assay

For the qualitative and quantitative measurement of antioxidants, several TLC methods have been developed and effectively employed [38, 39], and as a derivatization reagent for this purpose, the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was commonly used [40]. Hydromethanolic extract and aglycone fraction were subjected to TLC-bioautography assay. In hexane-ethyl acetate-formic acid (4:1:0.1) the TLC plate was developed, and then 0.05% DPPH reagent was sprayed onto it to detect the antioxidant constituents. Three spots (Rf, 0.22, 0.28 and 0.31) were detected in hydromethanolic extract and five spots (Rf, 0.22, 0.28, 0.30, 0.46 and 0.64) in aglycone fraction. The spots that appeared in a yellow color indicate antioxidant behavior (Figures 5).

Fig 5: TLC plate of aglycone fraction (Ag) and hydro-methanolic extract (M), obtained in hexane-ethyl acetate-formic acid (4:1:0.1) in daylight after derivatization with DPPH reagent

DPPH radical scavenging activity assay

As compared to standard quercetin, the hydromethanolic extract and aglycone fraction of the investigated plant showed significant antioxidant activity at a concentration of 200 µg/mL (Table 3). The lower IC50 value in the DPPH assay indicates more antioxidant activity. It has been suggested that samples with IC50 less than 50 g/mL are extremely powerful antioxidants, samples with IC50 between 50 and 100 g/mL are strong, samples with IC50 between 101 and 150 g/mL are moderate, and samples with IC50 beyond 150 g/mL are weak antioxidants [41]. The quantity of antioxidants needed to lower the initial concentration of DPPH by 50% (IC50) is a commonly used parameter to assess antioxidant activity [42]. The IC50 values for quercetin, hydromethanolic extract, and aglycone fraction were determined to be 23.02, 79.64 and 148.60 g/ml, respectively, and from this result, it is evident that the hydromethanolic extract has higher antioxidant activity than the aglycone fraction (Table 3 and Figure 6).

Table 3: Inhibition (%) of DPPH free radical by hydromethanolic extract, aglycone fraction and quercetin.

Concentration µg/ml	Radical scavenging effect (%)		
	Quercetin	Hydromethanolic extract	Aglycone fraction
40	53.1	41.7	27.4
80	66.22	50.87	40.2
120	80.6	56.92	47.5
160	93.2	67.49	52.1
200	97.7	77.2	57.2

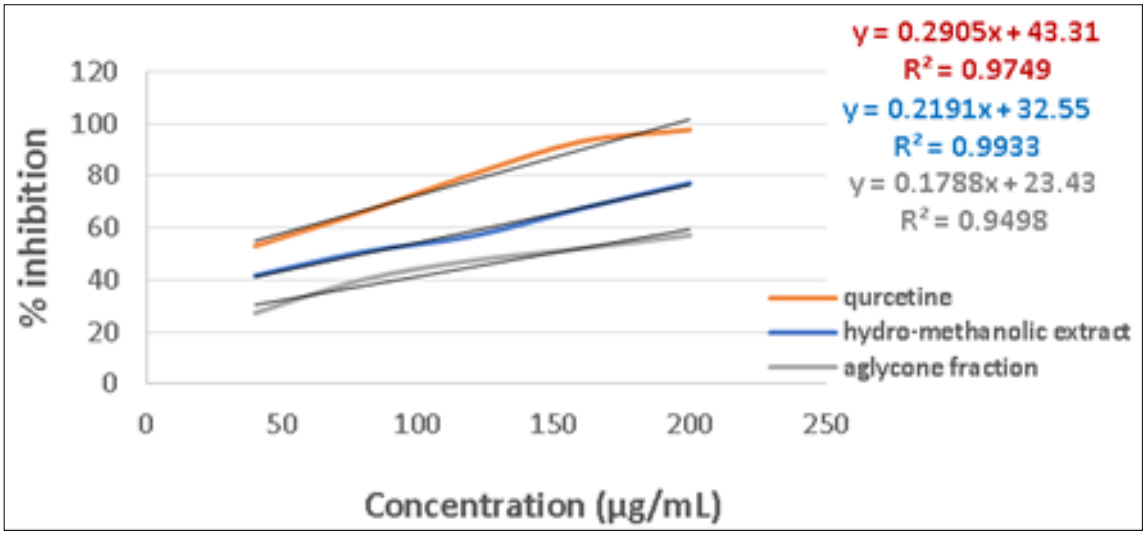

Fig 6: Inhibition (%) of DPPH free radical by hydromethanolic extract, aglycone fraction and quercetin.

Conclusion

The aerial part of *Actiniopteris semiflabellata* was used to prepare the hydromethanolic extract and the aglycone fraction, which were then chemically analyzed and tested for antioxidant activity. The hydromethanolic extract and the aglycone fraction show antioxidant activity by TLC bioautography and DPPH radical scavenging activity assay. The phenols, flavonoids, triterpenes, and sterols found in the plant under study are responsible for the antioxidant activity. The scientific data produced by this study will be important for further study on the plant and will support its traditional use. In addition, the plant may also be a good source of natural antioxidants. However, a deep phytochemical and pharmacological study is necessary.

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