Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v30i17.2718

PHYTOCHEMICAL PROFILE, ANTIMICROBIAL, ANTIOXIDANT, ANTHELMINTIC AND ANTIPROLIFERATIVE ACTIVITIES OF *ASPLENIUM TRICHOMANES* L. OF MALAM JABBA, SWAT VALLEY KP, PAKISTAN

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Ethnopharmacological potential of *Asplenium trichomanes* L.

Abstract: In this study the crude extracts of *Asplenium trichomanes* L. collected from Malam Jabba, Swat valley KP, Pakistan obtained in non-polar (n-Hexane, chloroform) to polar (ethanol and distilled water) solvents. These crude extracts were evaluated for their phytochemical composition, antibacterial, antifungal, antioxidant, anthelmintic, and anticancer effects. Qualitative analysis of these crude extracts showed presence of saponins, cardiac glycosides, tannins, reducing sugars, flavonoids, alkaloids, anthraquinones, and terpenoids in the rhizome, fronds, and sori extracts of this fern. Using the well diffusion method, the antifungal activity was evaluated against *Aspergillus niger* and *Aspergillus oryzae* and the antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* in triplicates. Zone of inhibition was measured in (mm) and was compared to standard discs. Chloroform extract showed notable inhibition, particularly in fronds that showed a zone of inhibition measuring 16.17±0.52 mm against *Bacillus subtilis* as opposed to the typical disc Gentamycin measuring 18±0.11 mm. Antioxidant activity was carried out to assess ABTS radical scavenging, metal chelating, DPPH assay, Total phenolic content and Total Flavonoid content. Results were compared with standard curve. For anthelmintic activity intestinal parasite of sheep and goat (*Haemonchus contortus*) which resides in fouth part of stomach (abomasum) and intestine was selected. Ethanolic extracts were evaluated for anthelmintic activity dueto its remarkable response in antimicrobial activity. Ethanol extracts of fronds, rhizome and sori were prepared in concentration of 12.5 mg/ml, 25 mg/ml, 50 mg/ml, 100 mg/ml and 200 mg/ml. 2 ml of each concentration was poured on ten adult *Haemonchus contortus* worms in a petriplate. Experiment was carried out in triplicates. Paralysis and death time of worms was noted every hour for about 7 hours. Results were compared with albendazole taken as standard. For anticancerous activity, crude extracts were screened for their cytotoxicity on aforementioned cell lines using MTT assay. The obtained result demonstrated selective anticancerous activity. It has been determined that *A. trichomanes* L. has a long ethnopharmacological history and is a potent medicinal herb.

Keywords: *Asplenium trichomanes* L., Antibacterial, Antioxidant, Anticancer, Anthelmintic.

1. INTRODUCTION:

Since ancient times, medicinal plants are considered as an important source and therapeutic agent to cure human ailments. Discovery of new drugs is still important because of ever changing resistance in microbes. Natural products works best for human body as compared to the synthetic drugs. Herbal sources are remarkable for discovery of new products having therapeutic value and drug development. The remarkable potential of antibacterial, antifungal, anthelmintic, antioxidant and antipoliferative abilities of herbal sources are due to variety of secondary metabolites found in them. From ancient times the natural flora has served as source of medicinal drugs which are being isolated for synthesizing new formulations to treat modern human ailments [1]. The secondary metabolites of crude extracts that effects the functions of human body describes the medicinal value of plants [2].

A lot of research has been done on such plants and multiple herbal products are recommended for synthesis of new medicines, but due to high resistance in pathogens, there is need of new resources to extract chemical substances. Mycotic infections are rising day by day and are considered the root of vascular infections involving mucosa especially in persons with weak immune system [3]. Cancer patients are also increasing day by day. It is important to investigate new resources to cure such serious ailments. Based on this lime light the current research is aimed to cover the phytotherapeutic applications of phytochemicals. In this study the crude extracts of the targeted fern (*Asplenium trichomanes* L.) obtained in polar and non-polar solvents is evaluated for its phytochemical composition, anthelmintic, antioxidant, antibacterial, antifungal and anticancer effects. It is demand of current scenario to investigate new plants having folklore reputation in a more elaborative way. *Asplenium trichomanes* belongs to family Aspleniaceae and is 20 to 30 cm tall. It has a scaly rhizome and evergreen fronds. There are 4-8 sori per pinna and the chromosomal count is 72.

2. MATERIAL AND METHODS:

2.1 Sample Collection And Material Preparation:

The targeted fern was collected from Malam Jabba, Swat valley, KP, Pakistan and was identified by expert taxonomist of Department of Botany, GCU Lahore. Well preserved samples were mounted on sheets and were deposited in Dr. Sultan Ahmad Ch. Herbarium GCU Lahore as voucher specimens. The plant was separated into specific parts (fronds, rhizome and sori), dried in shady conditions and powder of respective parts were prepared. Each part's (500 g) powder was immersed for 7 days in a different solvent (1 L), such as n-hexane (0.1), chloroform (4.1), ethanol (5.1), and distilled water (10.2). To concentrate the extracts at 40 °C, the surplus solvent was evaporated using a water bath and an evaporator.

2.2 Phytochemical Analysis:

Qualitative tests for presence of secondary metabolites were performed. These tests were performed to detect the presence of cardiac glycosides, tannins, alkaloids, reducing sugars, saponins, flavonoids, terpenoids, anthraquinones and saponins following protocols by Ayoola *et al.*, 2008 [4].

2.3 Antibacterial and antifungal activity:

Four bacterial strains, including two Gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) and two Gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) germs, were examined for antibacterial activity. Chugtai Laboratories Shahdara, Lahore, provided the bacterial strains. Both *Aspergillus niger* and *Aspergillus oryzae* were used as test subjects for antimycotic activities. Samples for testing antimycotic activity were obtained from GCU Lahore's biotechnology department. According to procedures by Jorgenson et al., 2007, Cruick-shank et al., 1975, and Johansen, 1940 employing Nutrient Broth medium for bacteria and potato dextrose agar for fungal strains [5][6][7], the activity was carried out using the agar well diffusion method. The techniques from Jorgenson et al., 2007 [5] were used to calculate MIC (Minimum Inhibitory Concentration)

using the agar microdilution method. Extracts were prepared in concentration of 0.625, 1.25, 2.5, 5and 10 mg/ml concentration. 2 ml of respective extract concentration was added to 18 ml of medium and was allowed to set into semi-solid medium. Bacterial and fungal samples were streaked on respective concentration plates. The minimum concentration on which no growth appeared was reported as minimum inhibitory concentration.

2.4 Antioxidant Assay:

The crude extracts were tested for antioxidant potential by determining DPPH scavenging following Lee and Shibamoto [8], Total phenolic contents (TPC) and metal chelating activity following protocols of Dinis *et al.*, 1994 [9], Total flavonoid contents (TFC) following Dewanto *et al.*, 2002 [10] and ABTS assay following Re *et.al.*, 1999 [11]. Results were compared with standard i.e., with Gallic Acid for TPC, Quercetin for TFC,and Trolox for ABTS activity.

2.5 Anthelmintic activity:

Fronds, rhizomes, and sori (singular: sorus) ethanolic crude extracts were made at concentrations of 12.5 mg/ml, 25 mg/ml, 50 mg/ml, 100 mg/ml, and 200 mg/ml. As a reference drug, albendazole was used, and preparations with similar amounts of this medication were also made. *Haemonchus contortus* which is an intestinal parasite of goat and sheep was collected from the slaughter house located in Sharakpur sharif, Sheikhupura, Pakistan. Anthelmintic activity was performed following Ghosh et al., 2005 [12] technique. Ten adult worms weretaken in a watch glass and 2 ml of respective extract concentration was poured to dip the worms. The reference medication Albendazole was used, while the control was distilled water. Following an incubation period of 37 °C, the worms' motility was noticed. To see if there was any movement, worms were washed in a washbasin. By tapping the worms' tips with the index finger, the death of the worms was verified. Those showing no motility were considered dead. The worms showing motility were further placed in the same extract concentration to determine the time of death. Experiment was run in triplicates. After every hour the number of worms paralyzed or died in each petriplate wasnoted for total 7 hours duration and was recorded in form of table. The death of worms was confirmed by shaking them vigorously. Moreover, dipping in warm water was also considered to confirm the results.

2.6 Anticancer activity:

Two cell lines MCF-7 and HCT-116 cells were considered for assessment of antipoliferative activity. The humified incubators with 5% CO_2 passage flow were used to maintain cell lines for less than 6 months in DMEM or RPMI medium supplemented with 1 A antibiotic and 10% FBS at 37˚C . Cell lines were observed for cytotoxicity following [3-(4,5 dimethylthiazol-2-yl)-2,5,-diphenyl, tetrazolium bromide] (MTT) assay. The IC_{50} values of these extracts was determined. Stock solution of extracts was prepared in DMSO at 100 mg/ml concentration. 3000 cells/well of MCF-7 and 3000 cells/well of HCF-116 were seeded in 96 well microplate with total volume of 100 µL cell culture per well. After 24 hrs 1µL of stock plant extract and 99µL of culture medium (for control 1µLDMSO and 99µL culture medium) was added in each well. Concentration ofextractwas500µL/ml in eachwell. After 72 hours, 20µL MTT (5mg/mlin phosphate buffer solution) was added and incubated for 4 hrs. The culture medium was replaced by 100µL DMSO and the microplate was shaken for10 minutes to dissolve the crystals thus formed. O.D. was measured at490nm and each experiment was run in triplicates. Growth was calculated using formula GI%=100-100x T/C where T is O.D of cell line treated with extract and C is O.D of cell line treated with DMSO. Cell lines with 80% growth inhibition were put for dose-dependent cytotoxic effect. Tested doses were 4µg/ml, 8µg/ml, 16µg/ml, 32µg/ml, 64µg/ml, 128µg/ml and 256µg/ml. Results were compared to standard Gemcitabine hydrochloride at 1.0116 µg/ml 0.116 µg/ml, 1.16 µg/ml, 11.6 µg/ml and 116 µg/ml and dose effect was expressed as IC_{50}.

2.7 Statistical Evaluation of the Data:

Co-stat (version 3.03) was used for the statistical analysis of the data using analysis of variance (ANOVA) with Duncan's multiple range 5%. Standard errors were included with the mean values. The parameters were tested three times.

3. RESULTS:

When *A. trichomanes*'s fronds, rhizome, and Sori crude extracts were screened for phytochemical compounds, alkaloids, tannins, reducing sugars, flavonoids, terpenoids, cardiac glycosides, saponins, and anthraquinones were found in all of the extracts in varying amounts (Figure 1).

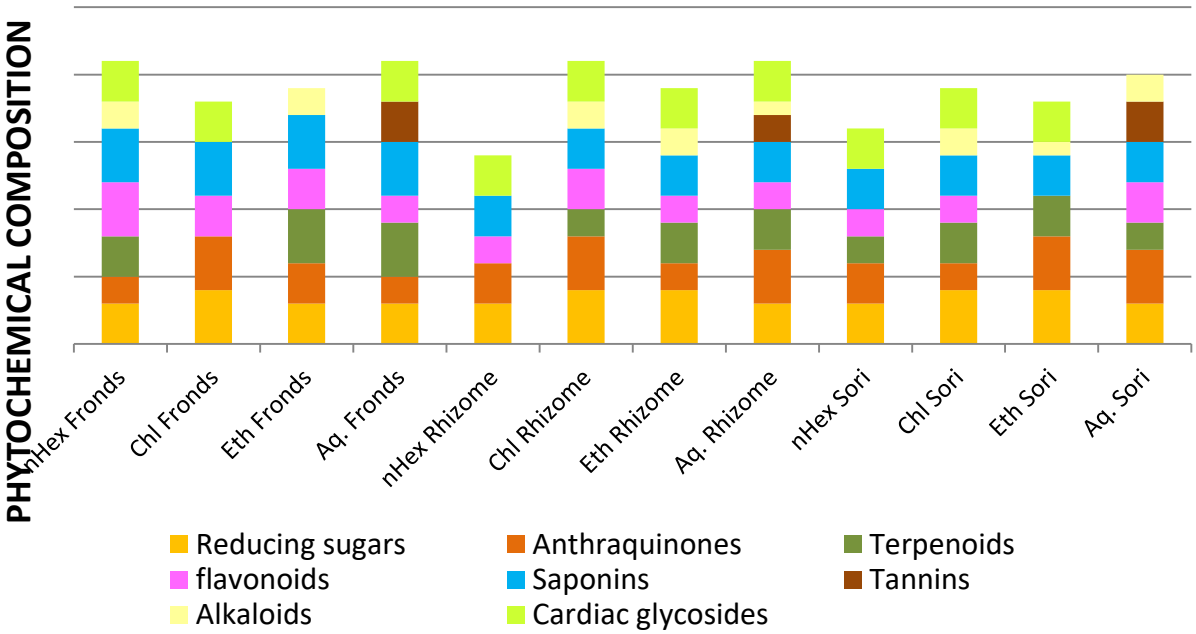

Fig. 1: Phytochemical profile of fronds, rhizomeand sori of *Asplenium trichomanes* L. Two mycotic strains, *A. oryzae* and *A. niger,* were chosen for their anti-mycotic action while *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Bacillus subtilis* were taken into consideration for their antimicrobial activity. The standard discs Amikacin showed 17±0.58 mm zone against *Bacillus subtilis* and 18.7±1.04 mm zone against *E.coli*. Cephalaxin (standard disc) showed 18±0.76 mm zone against *Pseudomonas aeruginosa* and Erythromycin showed 15±0.98mm zone against *Staphylococcus aureus*. Griseofulvin and Terbinafine were used as antifungal standard discs and showed a zone of 18±2.54mm and 15±2.32 mm for *Aspergillus niger* while 17±1.52 mm and 15±2.01 mm zone against *Aspergillus oryzae.* To demonstrate that only plant extracts were shown to have antibacterial properties, solvents without extracts were utilised as a negative control. The strongest antibacterial effect against *Bacillus subtilis* was shown by chloroform extract of fronds i.e., 16.17±0.5 mm while minimum by n-hexane extract of rhizome i.e., 10±0.2 mm. Maximum inhibition against *Staphylococcus aureus* was shown by ethanol extract of fronds i.e., 13.3±0.4 mm while minimum was shown by ethanol extract of rhizomes. For *Pseudomonas aeruginosa,* maximum zone was shown by ethanol extract of fronds i.e., 14±0.5 mm while minimum zone was demonstrated by aqueous extract of rhizome. For *Escherichia coli* maximum zone was shown by chloroform extract of rhizome while minimum zone was given by aqueous extracts of fronds and rhizome i.e., 10.01±0.2 and 10.02±0.1 mm respectively. The antibacterial and antifungal properties of several *A. trichomanes* sections against bacterial and fungal strains are listed in Table 1. Significant antifungal activity was executed by fronds, rhizome and sori of *A. trichomanes*. The largest zone of inhibition against *Aspergillus niger* i.e., 14.02±0.2 mm was obtained by chloroform extract of fronds while minimum zone was executed by aqueous extract of sori. For *Aspergillus oryzae* maximum zone was given by chloroform and ethanol extracts of

rhizome i.e., 15.06±0.2 mm and 15.01±0.1 mm respectively, while minimum was given by aqueous extracts of fronds and sori i.e., 10.04±0.2 mm and 10.03±0.1 mm respectively.

Table 1: Antibacterial and antifungal activities of different parts of *A. trichomanes* against bacterial and fungal strains.

	Zone of inhibition against *Bacillus subtilis* (mm)			
	n-hexane	**Chloroform**	**Ethanol**	**D. Water**
Fronds	8.02±0.2f	16.17±0.5a	14±0.4b	11.00±0.5de
Rhizome	10±0.2e	15.4±0.1ab	14.05±0.2b	12.00±0.2d
Sori	12.02±0.3d	13.04±0.6bc	12.11±0.3cd	13.1±0.4bc
Amikacin	17±0.5			
	Zone of inhibition against *Staphylococcus aureus* (mm)			
	n-hexane	**Chloroform**	**Ethanol**	**D. Water**
Fronds	11.0±0.3c	12.02±0.32b	13.3±0.4a	12.2±0.2b
Rhizome	13.02±0.2ab	11.01±0.12bc	10.07±0.3d	11.02±0.3bc
Sori	12.01±0.2b	12.04±0.1b	13.01±0.2ab	11.07±0.3bc
Erythromycin	15±0.58			
	Zone of inhibition against *Pseudomonas aeruginosa* (mm)			
	n-hexane	**Chloroform**	**Ethanol**	**D. Water**
Fronds	12.01±0.2bc	13.02±0.1ab	14±0.5a	12.01±0.3bc
Rhizome	11.03±0.2c	12.04±0.5bc	13.05±0.1ab	10.02±0.1d
Sori	11.07±0.3c	12.01±0.2bc	12.05±0.3bc	13.01±0.3ab
Cephalaxin	18±0.76			
	Zone of inhibition against *Escherichia coli* (mm)			
	n-hexane	**Chloroform**	**Ethanol**	**D. Water**
Fronds	12.05±0.2b	12.01±0.1b	13.02±0.3ab	10.01±0.2d
Rhizome	13.01±0.3ab	15.01±0.2a	13.01±0.3ab	10.02±0.1d
Sori	10.15±0.2c	11.02±0.3bc	11.01±0.1bc	12.02±0.2b
Amikacin	18±0.76			
	Zone of inhibition against *Aspergillus niger* (mm)			
	n-hexane	**Chloroform**	**Ethanol**	**D. Water**
Fronds	13.01±0.1ab	14.02±0.2a	13.03±0.1ab	11.01±0.3c
Rhizome	12.02±0.2bc	12.05±0.3bc	11.01±0.3c	11.05±0.4c
Sori	12.06±0.7bc	12.36±0.2b	12.34±0.5b	10.05±0.5d
Griseofulvin	18±2.54			
Terbinafine	15±2.32			
	Zone of inhibition against *Aspergillus oryzae* (mm)			
	n-hexane	**Chloroform**	**Ethanol**	**D. Water**
Fronds	12.07±0.3bc	13.02±0.3b	14.06±0.7ab	10.04±0.2d
Rhizome	13.05±0.7b	15.06±0.2a	15.01±0.1a	11.01±0.2c
Sori	13.02±0.2b	12.02±0.1bc	12.01±0.2bc	10.03±0.1d
Griseofulvin	17±1.52			
Terbinafine	15±2.01			

Chloroform extracts were chosen for MIC (Minimum inhibitory concentration) testing due to their promising antibacterial and antifungal activity, which demonstrated MIC ranges from 5 to 1.25 mg/ml with maximum MIC 1.25 mg/ml exhibited by chloroform extract of fronds against *B. subtilis* and *E. coli* and minimum MIC 5 mg/ml by chloroform extracts of rhizome and sori against *S. aureus* and *E. coli* (Table 2).

Table 2: Minimum inhibitory concentration of extracts against tested pathogens

Tested pathogen	Fern macerate conc. (mg/ml)	fern macerates		
		Chloroform Fronds	Chloroform Rhizome	Chloroform sori
Bacillus subtilis	10	-	-	-
	5	-	-	-
	2.5	-	-	-
	1.25	-	+	+
	0.625	+	+	+
Staphylococcus aureus	10	-	-	-
	5	-	-	-
	2.5	-	+	+
	1.25	-	+	+
	0.625	+	+	+
Pseudomonas aeruginosa	10	-	-	-
	5	-	-	-
	2.5	-	-	-
	1.25	+	+	+
	0.625	+	+	+
Escherichia coli	10	-	-	-
	5	-	-	-
	2.5	-	+	+
	1.25	-	+	+
	0.625	+	+	+
Aspergillus niger	10	-	-	-
	5	-	-	-
	2.5	-	-	+
	1.25	+	+	+
	0.625	+	+	+
Aspergillus oryzae	10	-	-	-
	5	-	-	-
	2.5	-	-	-
	1.25	-	+	+
	0.625	+	+	+

*Key: - = Absence of growth, + = Presence of growth.

A. trichomanes' antioxidant potential was assessed using a variety of techniques. For the ABTS+ assay, the sori chloroform extract demonstrated the lowest percentage inhibition at a concentration of 100 mg/ml, 22.64±1.68, and the frond aqueous extract demonstrated the highest percentage inhibition, 91.31±1.69. The values were compared with trolox curve to find TEAC values that lies between 1.941±0.24 to 7.94±0.04 mM trolox. Total flavonoid concentrations were determined using a colorimetric method, and ranged from 175.49±0.70 to 1185.74±0.01 µg/ml of quercetin, with n-hexane rhizome having the highest activity and aqueous sori extract having the lowest. To calculate the total phenolic content of *A. trichomanes*, the Folin-Ciocalteu reagent was used. The n-hexane rhizome extract, which had 40.33±0.23 µg/ml of GAE, had the highest reported activity, while the chloroform extract of the fronds, which contained 5.48±0.08 µg/ml of GAE, had the lowest (Tables 2,3,4).

For A. trichomanes' metal chelating activity, the proportion of bound iron falls between 0.066±0.63 and 60.30±0.93%. The ethanolic extract of the rhizome had the highest DPPH radical scavenging

activity, at 92.92±0.08%, whereas the aqueous extract of the rhizome had the lowest, at 32.72±0.56 (Table 3,4,5).

Table 3: Antioxidant activity of *A. trichomanes* fronds crude extracts

		Fronds			
		n-hex	Chl.	Ethanol	D. water
ABTS	Absorbance at 734 nm	0.914±1.21	0.472±1.30	0.127±1.03	0.065±0.50
	% inhibition	63.476±0.49	31.50±0.72	81.83±0.80	91.31±1.69
	TEAC (mM of Trolox)	5.71±0.86	2.65±1.04	7.06±0.26	7.94±0.04
Total Flavonoid contents	Absorbance at 510 nm	0.73±0.65	1.55±1.43	2.33±0.99	0.54±0.03
	QUE (µg/ml of Quercetin)	266±0.09	544±0.39	826±0.03	191±1.35
Total phenolic contents	Absorbance at 765 nm	0.39±0.19	0.29±1.10	0.55±0.84	0.84±1.06
	GAE (µg/ml of Gallic acid)	6.94±0.85	5.48±0.08	27.62±1.50	14.54±0.06
Metal chelating activity	Absorbance at 520 nm	1.49±0.09	1.67±0.93	1.35±0.03	1.64±0.91
	% bound iron	33.14±0.01	8.07±0.63	24.14±0.54	9.43±0.04
DPPH radical scavenging	% DPPH remaining	76.75±0.09	89.59±0.66	77.70±0.72	74.92±0.32

Table 4: Antioxidant activity of *A. trichomanes* rhizome crude extracts

		Rhizome			
		n-hex	Chl.	Ethanol	D. water
ABTS	Absorbance at 734 nm	0.346±0.50	0.370±0.99	0.135±0.72	0.063±0.85
	% inhibition	53.53±1.35	47.74±1.20	84.64±0.51	91.06±0.68
	TEAC (mM of Trolox)	4.65±0.49	4.133±1.35	7.33±0.23	7.93±0.27
Total Flavonoid contents	Absorbance at 510 nm	3.46±0.70	2.44±0.34	2.52±0.32	0.55±0.08
	QUE (µg/ml of Quercetin)	1185±0.01	845±0.48	716±0.06	194±0.32
Total phenolic contents	Absorbance at 765 nm	2.28±0.31	0.98±0.50	1.22±0.97	0.54±0.82
	GAE (µg/ml of Gallic acid)	40.33±0.23	17.49±0.19	22.59±0.90	10.55±0.05
Metal chelating activity	Absorbance at 520 nm	1.13±0.01	1.77±0.02	1.58±0.71	1.63±0.02
	% bound iron	35.83±0.03	0.34±0.78	12.08±0.32	9.43±0.03
DPPH radical scavenging	% DPPH remaining	83.13±0.35	84.96±0.33	32.72±0.56	92.92±0.08

Table 5: Antioxidant activity of *A. trichomanes* sori crude extracts

		Sori			
		n-hex	Chl.	Ethanol	D. water
ABTS	Absorbance at 734 nm	0.349±1.70	0.56±0.80	0.08±1.20	0.06±1.68
	% inhibition	53.37±0.99	22.64±1.68	87.57±1.04	90.76±0.99
	TEAC (mM of Trolox)	4.58±1.69	1.94±0.24	7.55±0.51	7.86±0.68
Total Flavonoid contents	Absorbance at 510 nm	3.23±1.02	2.53±0.48	2.52±1.35	0.55±0.03
	QUE (µg/ml of Quercetin)	1109±0.06	868.3±0.57	729.5±0.99	175.4±0.70
Total phenolic contents	Absorbance at 765 nm	1.83±0.97	0.31±1.20	0.73±1.50	0.73±0.69
	GAE (µg/ml of Gallic acid)	32.38±0.64	5.62±1.20	13.50±0.09	12.57±0.01
Metal chelating activity	Absorbance at 520 nm	0.73±0.68	1.77±0.05	1.35±0.63	1.59±0.01
	% bound iron	60.30±0.93	0.06±0.63	24.17±0.31	10.62±0.05
DPPH radical scavenging	% DPPH remaining	90.89±0.31	76.63±0.13	42.88±0.23	35.27±0.56

Anthelmintic assay was performed using adult *Haemonchus contortus* which is an interstinal parasite of sheep and goats. A concentration of albendazole (standard drug) was prepared as described earlier. Number of worms paralyzed and dead after every hour were noted in form of a table (Table 6).

Table 6: Anthelmintic activity of ethanolic crude extracts of *A. trichomanes*

Treatment	Conc. (mg/ml)	1 Hr	2 Hr	3 Hr	4 Hr	5 Hr	6 Hr	7 Hr
Ethanolic fronds	12.5	1.35±0.21	2.01±0.32	3.80±0.62	5.68±0.23	6.04±0.11	7.01±0.23	9.10±0.23
	25	2.11±0.22	3.01±0.66	4.12±0.56	6.11±0.44	6.80±0.13	8.11±0.32	9.80±0.32
	50	3.25±0.54	3.21±0.67	5.11±0.64	6.42±0.32	7.21±0.32	8.52±0.37	10.00±0.00
	100	3.20±0.43	4.25±0.66	5.23±0.88	7.21±0.66	7.66±0.66	9.87±0.33	10.00±0.00
	200	4.00±0.58	5.00±0.88	6.33±0.86	7.67±0.67	8.67±0.66	10.00±0.33	10.00±0.00
Ethanolic rhizome	12.5	2.35±0.24	2.83±0.22	3.90±0.92	5.66±0.23	6.11±0.17	7.11±0.23	9.14±0.25
	25	3.11±0.29	3.81±0.16	4.10±0.26	6.01±0.44	6.99±0.22	8.01±0.32	9.90±0.34
	50	3.85±0.24	4.21±0.67	5.29±0.64	6.42±0.31	7.60±0.32	8.52±0.37	10.00±0.00
	100	4.20±0.63	4.90±0.76	6.07±0.88	7.11±0.61	8.11±0.26	9.17±0.31	10.00±0.00
	200	4.34±0.18	5.40±0.68	6.99±0.86	7.57±0.62	8.99±0.16	9.66±0.33	10.00±0.00
Ethanolic Spores	12.5	1.30±0.24	2.63±0.22	3.90±0.12	4.66±0.23	4.11±0.17	5.11±0.23	6.14±0.25
	25	1.80±0.29	2.81±0.16	3.10±0.16	4.01±0.44	4.99±0.22	6.01±0.32	7.90±0.34
	50	2.85±0.24	3.21±0.67	3.80±0.14	4.42±0.31	5.60±0.32	6.52±0.37	8.67±0.54
	100	2.20±0.63	3.90±0.76	4.07±0.18	5.11±0.61	6.11±0.26	7.17±0.31	9.10±0.55
	200	2.34±0.18	4.01±0.68	4.99±0.16	6.57±0.62	6.99±0.16	7.66±0.33	9.20±0.63
Albendazole	12.5	4.25±0.21	5.23±0.21	6.13±0.43	6.66±0.23	7.11±0.12	8.61±0.28	10.00±0.00
	25	4.81±0.22	5.81±0.11	6.80±0.55	6.98±0.44	7.69±0.26	8.81±0.37	10.00±0.00
	50	5.15±0.23	6.01±0.17	7.21±0.32	7.42±0.31	8.10±0.12	9.52±0.36	10.00±0.00
	100	5.80±0.63	6.12±0.72	7.67±0.13	8.11±0.11	8.61±0.23	9.87±0.35	10.00±0.00
	200	6.34±0.28	6.94±0.63	7.99±0.81	8.57±0.66	8.99±0.18	9.96±0.34	10.00±0.00

For anticancerous activity, after 24 hours the inhibition effects of n-Hexane, chloroform, ethanolic and aqueous extracts was assayed for MCF-7 and HCT116 cell lines (Table-7) using 500µg/ml of extract. Ethanolic extracts showed more than 80% inhibition of growth thus dose dependent cytotoxic effects of ethanolic extracts were assayed and the IC_{50} values were acquired. IC_{50} value of ethanolic fronds against HCT116 was lowest i.e., 64 µg/ml while highest concentration for IC_{50} was 256 µg/ml of ethanolic sori extract against MCF-7 and HCT-116 cell lines (Table-8).

Table 7: Growth inhibition of MCF-7 and HCT-116 cells by crude extracts of A. trichomanes

Part	**Extract**	**MCF-7**	**HCT-116**
Fronds	n-Hexane	53.7	63.09
	Chloroform	62.8	29.49
	Ethanol	82.14	90.12
	Aqueous	4.14	8.30
Rhizome	n-Hexane	43.21	44.32
	Chloroform	68.13	54.21
	Ethanol	81.21	82.88
	Aqueous	3.12	8.13
Sori	n-Hexane	12.1	21.21
	Chloroform	28.1	22.18
	Ethanol	82.22	81.12
	Aqueous	1.42	4.11

Table 8: Cytotoxicity IC_{50} of crude extracts against MCF-7 andHCT-116 cell lines

Part	Extract	IC_{50} (µg/ml)	
		MCF-7	HCT-116
Fronds	Ethanol	128	64
Rhizome	Ethanol	128	128
Sori	Ethanol	256	256

4. DISCUSSIONS:

Asplenium trichomanes L. is traditionally used as an expectorant, anti-cough remedy, laxative, abortifacient and for treatment of irregular menstrual cycle. Despite their historic applications, the chemical components responsible for efficacy are little known. The fronds, rhizome, and sori phytochemical investigation revealed the presence of several compounds that are listed in table 1. These phytochemicals are in charge of a number of pharmacological processes, including diuretic, anticancer, antibacterial, antioxidant, and anti-inflammatory responses.

Similar glycosides, lignins, quinones, tannins, and terpenoids were found in crude extracts of *Asplenium scolopendrium* fronds, according to Ismail *et al.* in 2019 [14]. Cardiac glycosides are used to treat cardiac insufficiency and are crucial for heart health (Balch and Balch, 2000) [15]. Quinones and hydroquinones acts in electron transport chains of respiration and photosynthesis as mobile carriers (Fato *et. al.,* 1996) [16].

The most sensitive strain, according to the antibacterial activity of n-hexane, chloroform, and ethanol extracts of *A. trichomanes'* fronds, rhizome, and sori, was *Bacillus subtilis*, which was followed by *E. coli*, *S. aureus,* and *P. aeruginosa* (Table 2). Furthermore, frond extract demonstrated high antibacterial activity and was most effective against bacteria. The fronds' chloroform extracts were extremely antibacterial. Ahmad *et al.* 2022 [17] reported similar findings.
Reactive oxygen species (ROS) and free radicals impair the body's natural oxidation process by causing oxidative stress. Arteriosclerosis, Parkinson's disease, myocardial infarction, and Alzheimer's disease are the harmful impacts of oxidative stress (Selamoglu, 2018) [18]. The significance of plant extracts in preventing oxidative stress was emphasized in this particular study. Phytochemicals found in plant extracts aid in reducing the harm caused by the generation of free radicals (Barr *et al.,* 1992) [19].

Chloroform extracts of plant parts showed anthelmintic action at concentrations of 12.5 mg/ml, 25 mg/ml, 50 mg/ml, 100 mg/ml, and 200 mg/ml. The findings indicated that raising the dose or concentration of extracts increased their potency (Table 6). Prajanati *et al.,* 2022 [20] reported similar findings, leading them to draw the conclusion that plant extracts can be employed successfully for anthelmintic activity since they are convenient to collect, easy to extract, and reproducible in all aspects.

Results of anticancer activity revealed that ethanolic extracts of fronds, rhizomes, and sori were more effective at preventing the spread of MCF-7 andHCT-116 cell lines as shown by their growth inhibition values (GI> 80%) Table-7. According to results expressed in Table 8 the ethanolic extracts exhibited strong anticancerous activity with IC_{50} values between 64 µg/ml to 256 µg/ml. cell lines than chloroform and ethanol extracts. This suggests promising prospects of these plant extracts for developing anticancerous drugs.

5. CONCLUSION:

The findings suggest that the fern *Asplenium trichomanes* has medicinal value and could be used as a new drug source for ethnopharmaceutical companies looking for stable, pharmacologically active metabolites that healers can use in the field of medicine and the treatment of other disorders.

6. ACKNOWLEDGEMENTS

We are thankful to Miss Sidra Farid, (Department of Chemistry, GC University Lahore) for providing facilities to perform antioxidant activity in her lab, to Prof. Dr. Haroon Akbar (Department of Parisitology, UVAS, Lahore) for facilitating us to perform anthelmintic acitivity, and to Dr. Amir Fasil (Biological and Chemical Engineering department, LUMS, Lahore) for facilitating us to perform anticancerous activity.

7. CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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