Evaluation of Phytchemicals and Fluorescent Analysis of Roots, Leaves and Stem of *Dryopteris chrysocoma* (Christ) C. Chr.

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Abstract: *Dryopteris chrysocoma* is male fern. It is widely distributed as ornamental plant. It is found in damp and moist areas and is common in high altitudes of Pakistan. The palnt has medicinal importance also as it has anthelmintic property. The present study is conducted to determine various phytochemicals present in roots, leaves and stem of *D. chrysocoma*. Various chemical tests as Lead acetate, Ferric chloride, Salwaski, Dragondroff's, Liebermans Burchart, Molisch's, Organic solvent, picric and tannic acids test were performed for the determination of chemical constituents. It showed presence of tannins, phenols and flavonoids, sterols, alkaloids, steroids and triterpenes, carbohydrates and proteins. For the standardization of plant material fluorescence analysis of powder of roots, leaves and stem was performed by treating powder with different chemicals and observing it at ordinary light and UV light at 254 and 366nm. That showed fluorescence and the presence of different chemicals. TLC analysis was performed that showed presence of certain constituents in the form of spots. HPLC and FTIR analysis was performed for identification of phytochemicals and their functional groups. These phytochemicals are responsible for medicinal activities as rhizome contains phloroglucinol derivatives of crude filicin that has vermifuge and abortifacient effects.

Keywords: *Dryopteris chrysocoma*, male fern, Powder analysis, Fluorescence analysis, TLC, HPLC, FTIR.

INTRODUCTION

Dryopteris chrysocoma belong to family Pteridaceae. Its English name is Filix-mas. That is commonly known as male fern. An evergreen Fern growing to 1.2 m by 1m high. The plant grows in moist and damp areas. The plant contains oleoresin, filicic acid, flavaspidic acid, filmaron, albaspidin [1]. The rhizome contains phloroglucinol derivatives of crude filicin consisted of albaspidin, filixic and flavaspidic acids [2]. Filmarone and aspidinol are the most important to have been identified. The secret of a reliable male fern preparation is that the drug must be fresh. After one year it becomes ineffective [3]. It is womifuge, taenifuge [4,5], insecticide, contraceptive, cytotoxic, astringent, antibacterial and laxative [6,7]. It has antirheumatic properties and is effective in tap worm infection [8,9]. The plant is widely utilized for ornamental purpose. The recent work is conducted on plant material collected from Karachi. For its standardization work conducted on different parts of *D. chrysocoma* as roots, stem and leaves. Presence of different chemical constituents varies in different parts which is responsible for its medicinal activity.

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MATERIALS AND METHODS

The plant material of *Dryopteris chrysocoma* collected from Karachi University, Karachi, Pakistan. The sample materials are deposited in the herbarium of the department. *Dryopteris chrysocoma* (root) (herbarium number, 001006-09), *Dryopteris chrysocoma* (leaves) (herbarium number, 001006-08), *Dryopteris chrysocoma* (stem) (herbarium number, 001006-07).

Extraction Procedure

Fresh plant material was copped in to pieces and percolated in methanol for 15 days. After 15 days it was filtered by filter paper and residue was again soaked in methanol for next 15 days. The process was repeated three times. Then filtrate was run on rotary evaporator to evaporate methanol. Thick residue collected at the end was used for testing [10].

Pharmacognostic Evaluation/Standardization of Drugs

1. Chemical Analysis

i. Lead Acetate Test for Tannins

Powder of dried plant material was used for identification of tannins. For this powder was boiled with water and then filtered it then lead acetate was

added in the filtrate. Presence of precipitates indicated tannins [11].

ii. Ferric Chloride Test

Phenols and hydroxamic acid flavonoids were determined by Ferric chloride test by the method of E. Merk, 1980 [12].

iii. Salwaski Test for Sterols

In methanolic solution of extract few drops of concentrated H_2SO_4 were added with the wall of test tube. Presence of purple ring indicates sterols [12].

iv. Dragendorff's Test

Alkaloids and nitrogen containing compounds were detected by using Dragendorff's test by the method of E. Merk, 1980. This reagent was use for spray on TLC chromatogram, which immediately gave dark orange colour with yellow background. It indicates presence of alkaloids [12].

v. Liebermann-Burchard Test

It is used for the detection of sterols, for number of steroid and triterpene glycosides [12].

vi. Molisch's Test for Carbohydrates

Small quantity of methanol extract was treated with α-naphthol and concentrated sulphuric acid, which gave purple color indicating the presence of carbohydrates [11].

vii. Test for Protein

Proteins are precipitated in organic solvents as ethanol. Proteins also precipitate in picric acid and tannic acid solution. Small amount of powder/extract mixed with these solvents separately. Precipitation indicates presence of proteins [11].

2. Fluorescence Analysis

Powder Drug Preparation

Plant parts leaves, stem and roots were separately ground in powder form. The powder of plant material was treated with different chemicals and fluorescence analysis was done in ordinary light, 254nm and 366nm [13].

3. Thin Layer Chromatographic Methods

The following solvent systems were applied for TLC:

1. Ethyl acetate-Methanol-Water (100:16.5:13.5)
2. Chloroform-Methanol-Water (80:20:2)

TLC chambers were used for performing chromatography. First mobile phase was used for lipophilic constituents and second mobile phase was used for hydrophilic constituents. Compounds were detected under UV light at 254 nm and under UV light at 366 nm [14,15]

4. HPLC Procedure

HPLC is based on Reversed phase chromatography. It is a separation technique in which column act as stationary phase or non-polar part and mobile phase act as liquid phase or polar part. Depending upon the type of stationary phase (column) used, separation is achieved by partitions, by adsorption, or ion exchange processes. HPLC was performed by the method of Christie, 1997 [16].

5. FTIR Procedure

FTIR performed by using the same method as given in User's guide [17].

RESULTS

The results of phytochemical analysis of roots, leaves and stem of Dryopteris chrysocoma are given in the Tables **1-3**. Test with different chemical reagents as Lead acetate, Ferric chloride, Salwaski, Dragondroff's, Liebermans Burchart, Molisch's, Organic solvent, picric and tannic acid tests showed the presence of chemical constituents. In the lead acetate test presence of precipitates in root and leaves extract showed presence of tannin while methanolic extract of stem give negative result for tannins. Ferric chloride test showed presence of phenols and flavonoids more in leaves and stem as compare to roots that was identified by the dark green and bluish black colors. In salwaski test purple to brown ring obtained. It showed more sterols in leaves and stem than roots. Results of Dragondroff's test showed alkaloids and nitrogen containing compounds more in leaves and stem than in roots. Liebermans Burchart test show positive result with red brown precipitates. It indicated presence of steroids and triterpene glycosides in roots, leaves and stem extracts. Molish's test for carbohydrates and Picric and tannic acids test for proteins give positive results for carbohydrate and proteins in all plant parts.

The powder of roots, leaves and stem of D. chrysocoma was treated with chemicals as NaOH,

Table 1: An Overview of Phytochemical Constituents Identified by Different Chemical Reagents

S. No.	Chemical Test/Reactions	Plant Name		
		***D. chrysocoma* (Root)**	***D. chrysocoma* (Leaves)**	***D. chrysocoma* (Stem)**
01	Lead acetate (Tannins)			
	a) Colour/ppt	ppt obtained	ppt obtained	No ppt obtained
	b) Result	++	++	-
02	Ferric chloride (Phenols, hydroxamic acid and flavonoids)			
	a) Colour/ppt b) Results	Dark green	Bluish black colour	Bluish Black colour
		++	+++	+++
03	Salwaski Reagent (Sterols)			
	a) Colour/ppt	Purple to brown ring obtained	Purple ring obtained	Purple ring obtained
	b) Results	++	+++	+++
04	Dragondroff's Test (Alkaloids and nitrogen containing compounds)			
	a) Colour/ppt	ppt obtained	Orange ppt obtained	Yellow ppt obtained
	b) Results	+	+++	+++
05	Liebermans Burchart Reaction (Steroid and triterpene glycosides)			
	a) Colour/ppt	Red-brown colour obtained	Green-brown colour obtained	Red-brown colour obtained
	b) Results	+++	+++	+++
06	Molisch's Test (Carbohydrates)			
	Colour/ppt	Purple colour	Purple colour	Purple colour
	Results	++	+++	+++
07	Organic solvent, picric and tannic acids test (Protein)			
	Colour/ppt	Insoluble in ethanol & ppt in picric and tannic acid	Insoluble in ethanol & ppt in picric and tannic acid	Insoluble in ethanol & ppt in picric and tannic acid
	Results	++	++	++

Table 2a: Fluorescence Analysis of Powder of *Dryopteris chrysocoma* (Root)

S. No.	Protocol	Observations Under		
		ORDINARY LIGHT	**UV LIGHT 254**	**UV LIGHT 366**
01	Dry powder as such	Blackish brown	Brown	Grey
02	Powder treated with 1.0 N NAOH in MEOH	Rust	Yellowish brown	Dark brown
03	Powder treated with 50% H_2SO_4 in water	Blackish brown	Dark brown	Dark brown
04	Powder treated with 1.0 N NAOH in H_2O	Rust brown	Black	Black
05	Powder treated with 50% HNO_3 in water	Light brown	Off white	Off white
06	Powder treated with chloroform	Brown	Brown	Dark brown
07	Powder treated with water	Rust brown	Yellowish brown	Brown
08	Powder treated with 66% H_2SO_4	Blackish brown	Dark brown	Dark brown
09	Powder treated with aniline	Dark brown	Yellowish black	Rust
10	Powder treated with tween 80	Dark brown	Yellowish brown	off White
11	Powder treated with aceticanhydride	Yellow	Pale yellow	Off white
12	Powder with conc. KOH	Rust brown	Brown	Brown

Table 2b: Fluorescence Analysis of Powder of *Dryopteris chrysocoma* (Leaves)

S. No.	Protocol	Observations Under		
		ORDINARY LIGHT	UV LIGHT 254	UV LIGHT 366
01	Dry powder as such	Greenish grey	Off white	Off white
02	Powder treated with 1.0 N NAOH in MEOH	Yellowish brown	Dark green	Dark brown
03	Powder treated with 50% H_2SO_4 in water	Greenish off white	Greenish off white	Dark brown
04	Powder treated with 1.0 N NAOH in H_2O	Greenish yellow	Dark green	Dark brown
05	Powder treated with 50% HNO_3 in water	Greenish off white	Greenish off white	Dark brown
06	Powder treated with chloroform	Greenish grey	Off white	Off white
07	Powder treated with water	Greenish grey	Dark green	Dark brown
08	Powder treated with 66% H_2SO_4	Off white	Off white	Dark brown
09	Powder treated with aniline	Greenish grey	Off white	Blackish brown
10	Powder treated with tween 80	Yellowish green	Dark green	Off white
11	Powder treated with aceticanhydride	Grayish green	Off white	Dark brown
12	Powder with conc. KOH	Yellowish brown	Dark green	Dark brown

Table 2c: Fluorescence Analysis of Powder of *Dryopteris chrysocoma* (Stem)

S. No.	Protocol	Observations Under		
		ORDINARY LIGHT	UV LIGHT 254	UV LIGHT 366
01	Dry powder as such	Off white	Brown	Dark brown
02	Powder treated with 1.0 N NAOH in MEOH	Brownish black	Black	Black
03	Powder treated with 50% H_2SO_4 in water	Light brown	Grey	Brownish black
04	Powder treated with 1.0 N NAOH in H_2O	Dark brown	Greenish black	Black
05	Powder treated with 50% HNO_3 in water	Light brown	Grey	Brownish black
06	Powder treated with chloroform	Light brown	Brown	Dark brown
07	Powder treated with water	Light brown	Light brown	Brownish black
08	Powder treated with 66% H_2SO_4	Light brown	Grey	Dark brown
09	Powder treated with aniline	Dark yellowish brown	Black	Brownish black
10	Powder treated with tween 80	Dark brown	Dark brown	Off white
11	Powder treated with aceticanhydride	Brown	Dark brown	Off white
12	Powder with conc. KOH	Brown	Dark brown	Light brown

H_2SO_4, HNO_3, chloroform, KOH and other chemicals and was observed for the color change in ordinary light as well as in UN light at 254 and 366nm. Results are given in Table **2a**, **2b** and **2c**. fluorescent analysis indicated presence of certain constituents in different parts of D. chrysocoma. Thin layer chromatography was performed in two solvent systems. One with Chloroform-Methanol-Water (80:20:2) and another with Ethylacetate-Methanol-Water (100:16.5:13.5). TLC showed presence of constituents in the form of spots. R_f values of all spots are given in Table **3**. Figure **1a** and **1b** show spots in 254 and 366nm UV light. The methanolic extract of roots showed 4 and 2 spots in chloroform and ethyl acetate solvent systems. Leaves extract showed 9 and 8 spots. Stem extract showed 11 and 4 spots in chloroform and ethyl acetate solvent systems. HPLC spectra show presence of different constituents in the form of peaks that is given in Figure **2a**, **2b** and **2c**. FTIR of roots, leaves and stem extracts showed presence of different functional groups and spectra are given as Figure **3a**, **3b** and **3c**.

Table 3: Thin Layer Chromatography of Crude Extracts

Extract	Solvent system	Spray	R_f value (254nm)	R_f value (366nm)
D. chrysocoma (root)	Chloroform-Methanol-Water (80:20:2)	Exposed to iodine vapour (Observed under UV light)	0.13 0.18 0.55 0.75	0.24
D. chrysocoma (root)	Ethylacetate-Methanol-Water (100:16.5:13.5)	Exposed to iodine vapour (Observed under UV light)	0.53 0.76	0.56 0.71
D. chrysocoma (leaf)	Chloroform-Methanol-Water (80:20:2)	Exposed to iodine vapour (Observed under UV light)	0.18, 0.35, 0.38, 0.49 0.6, 0.63 0.66, 0.69 0.89	0.33, 0.34 0.6, 0.69 0.85, 0.88 0.93
D. chrysocoma (leaf)	Ethylacetate-Methanol-Water (100:16.5:13.5)	Exposed to iodine vapour (Observed under UV light)	0.53, 0.54 0.55, 0.69 0.8, 0.81	0.35, 0.38 0.44, 0.48 0.65, 0.75 0.9, 0.94
D. chrysocoma (stem)	Chloroform-Methanol-Water (80:20:2)	Exposed to iodine vapour (Observed under UV light)	0.11, 0.16 0.19, 0.35 0.38, 0.5 0.54, 0.55 0.56, 0.63 0.69	0.14 0.18 0.29 0.54 0.56
D. chrysocoma (stem)	Ethylacetate-Methanol-Water (100:16.5:13.5)	Exposed to iodine vapour (Observed under UV light)	0.56 0.75 0.85 0.86	0.69 0.81 0.88

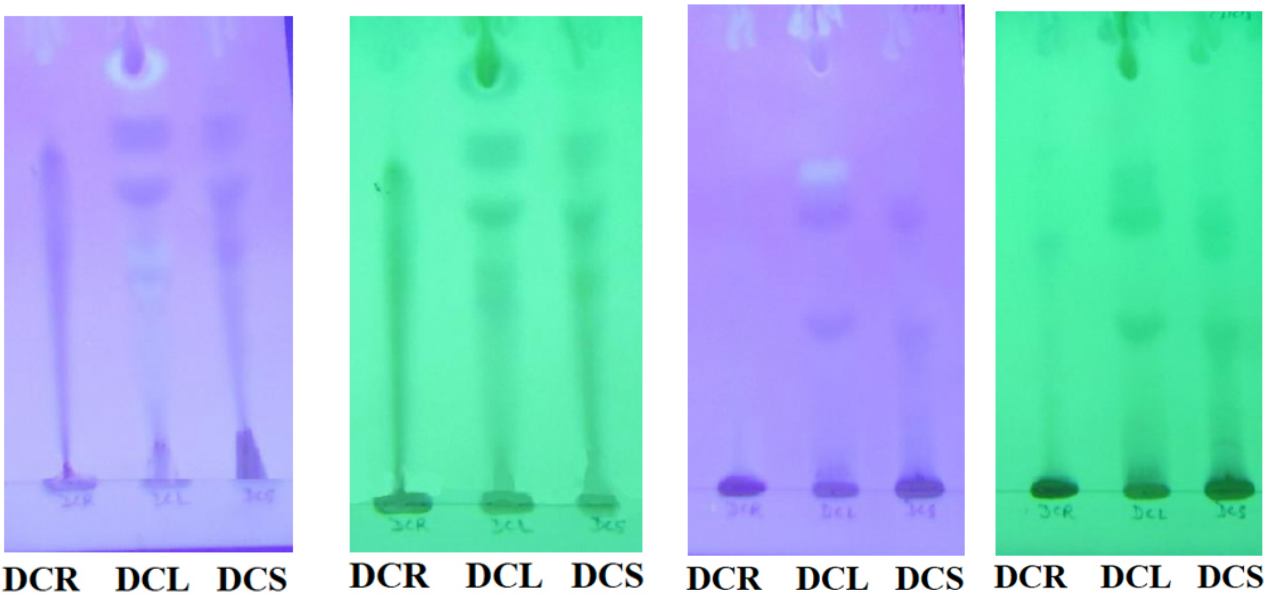
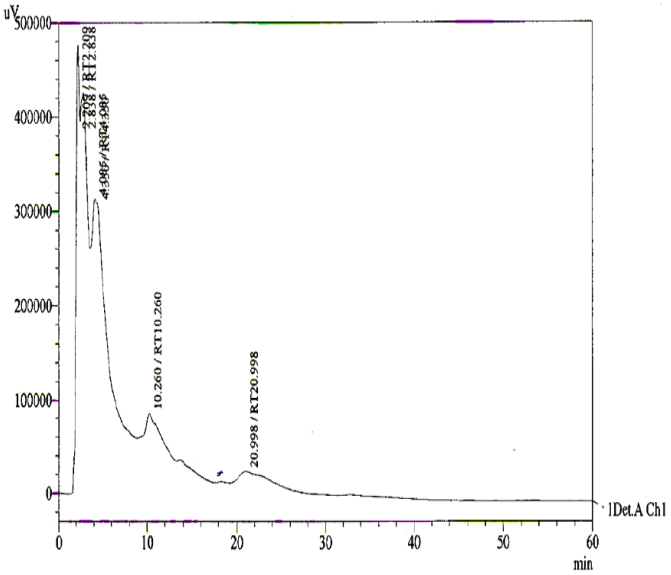


Figure 1: a: TLC of plant extracts in Ethyl acetate, methanol and water system at 254 and 366nm.

b: TLC of plant extracts in Chloroform, methanol and water system at 254 and 366nm: DCR=*D. chrysocoma* (root), DCL=*D. chrysocoma* (leaves), DCS=*D. chrysocoma* (stem).



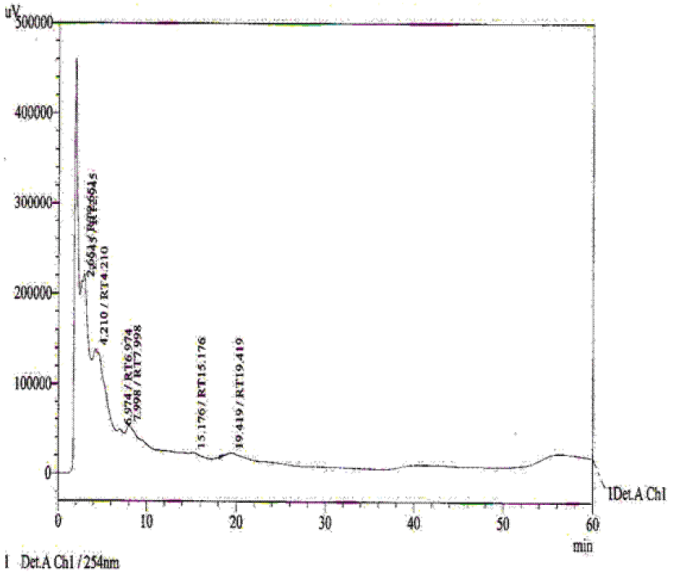
1 Det.A Ch1 / 254nm

PeakTable

Detector A Ch1 254nm

Name	Ret. Time	Area
RT2.209	2.209	12761243
RT2.838	2.838	24833094
RT4.085	4.085	11344619
RT4.350	4.350	23179512
RT10.260	10.260	3392981
RT20.998	20.998	3506216
		79017666

a



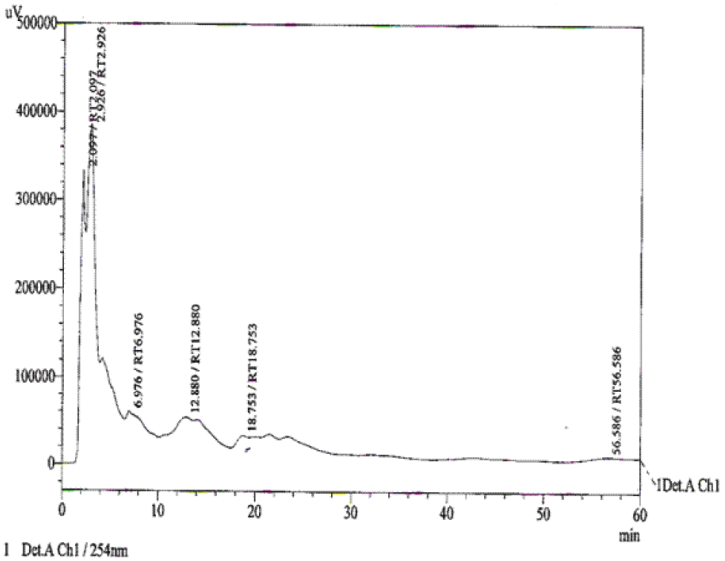
1 Det.A Ch1 / 254nm

PeakTable

Detector A Ch1 254nm

Name	Ret. Time	Area
RT2.651	2.651	353727
RT2.945	2.945	1390154
RT4.210	4.210	2143659
RT6.974	6.974	105091
RT7.998	7.998	983463
RT15.176	15.176	121000
RT19.419	19.419	1100445
		6197539

b



1 Det.A Ch1 / 254nm

PeakTable

Detector A Ch1 254nm

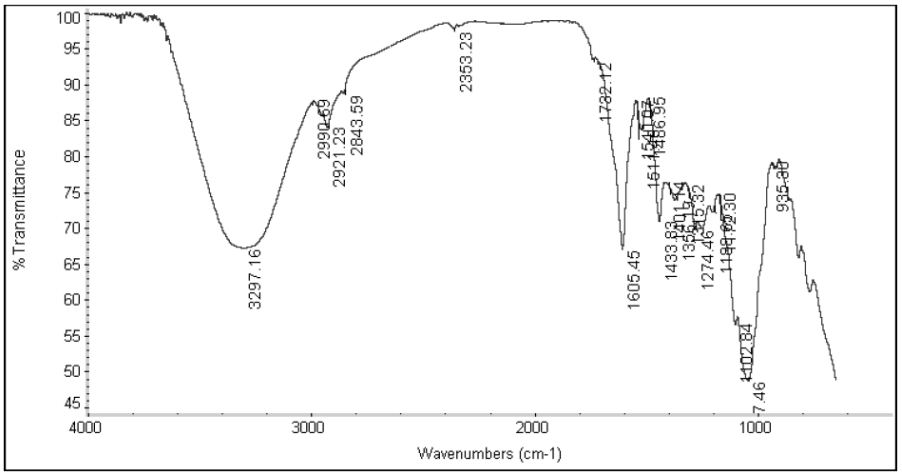
Name	Ret. Time	Area
RT2.097	2.097	3464554
RT2.926	2.926	11048759
RT6.976	6.976	1060620
RT12.880	12.880	715507
RT18.753	18.753	399243
RT56.586	56.586	621483
		17310165

c

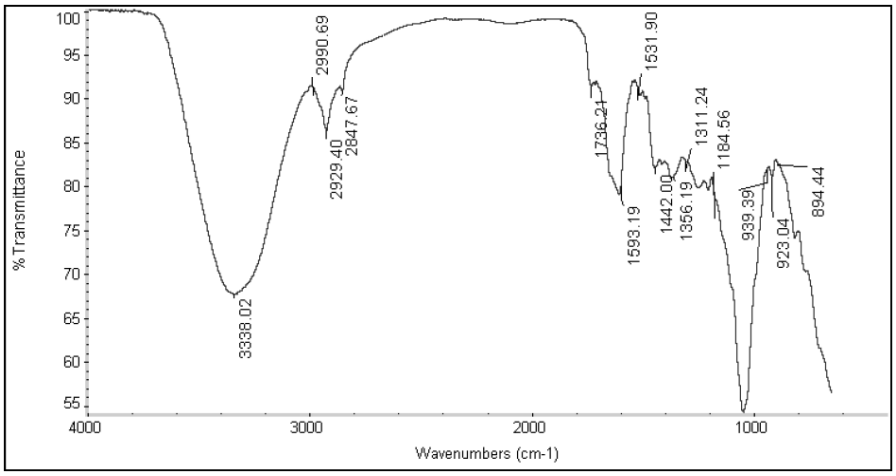
Figure 2: a: HPLC spectra of *Dryopteris chrysocoma* (Root).

b: HPLC spectra of *Dryopteris chrysocoma* (Leaf).

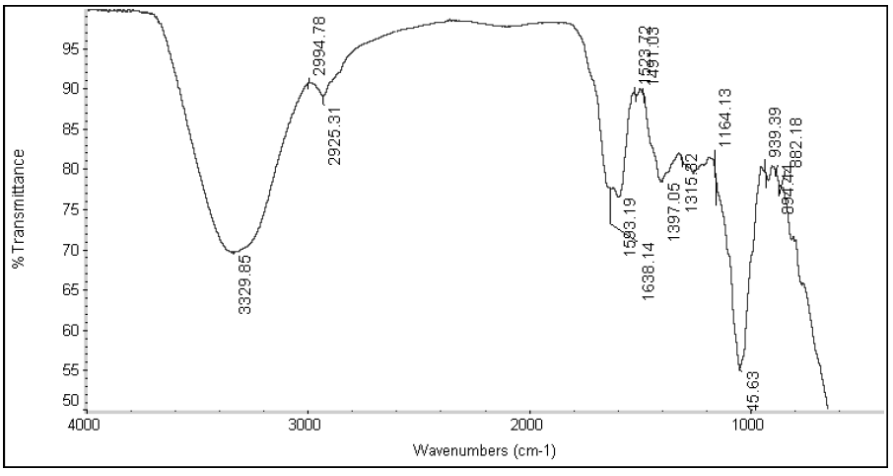
c: HPLC spectra of *Dryopteris chrysocoma* (Stem).



a



b



c

Figure 3: a: FTIR spectra of *D. chrysocoma* (root).

b: FTIR spectra of *D. chrysocoma* (leaves).

c: FTIR spectra of *D. chrysocoma* (stem).

DISCUSSION

The presence of different phytochemicals in roots leaves and stem of *D. chrysocoma* was identified by reaction with certain chemicals. The reactions showed positive results for the presence of triterpenes, tannins, flavonoids, alkaloids, carbohydrate, sterols and protein. Tannins are were absent in stem. Alkaloids were found in very less amount in roots as compare to the leaves

and stem. Tannins, flavonoids and sterols were also less in root extract. Dryopteris roots contain one ingredient that is oleoresin its active principal is filicin. Filicin has anthelmintic property that paralyse worms in intestine and other parasites, it also expel worms [5,18]. Filicin content in roots is about 1.5-2.5% f [19]. It is effective treatment for tapeworms that should followed with a non-oily purgative agent as magnesium sulphate. The plant has antibacterial and insecticidal activities due to presence of flavonoids and tannins [6]. It has anthelmintic and cytotoxic properties that is due to presence of alkaloids [8,9]. Tannins may also responsible to decrease the intestinal activity [20].

Fluorescent analysis of powder drug showed different colors that indicate different constituents in roots, leaves and stem. TLC analysis of crude methanolic extracts of roots, leaves and stem showed presence of different constituents and maximum number of spots found in leaves extract and than in stem extract. HPLC and FTIR spectra also showed different peaks that indicate presence of certain active constituents in roots, leaves and stem of *D. chrysocoma*.

CONCLUSION

Phytochemical analysis of crude extract and powder of roots, leaves and stem of *D. chrysocoma* showed presence of number of constituents in this plant. These constituents are responsible for biological activities of different plant parts. Some constituents are present in large amount in one part while less amount in another part that is also responsible for variation in potency or effectiveness in the treatment of certain medical conditions as the plant root is highly effective as wormifuge. Roots are widely utilized for medicinal purpose. TLC, HPLC and FTIR are used for purity and quality of drug. These shows presence of different constituents and functional groups and it can also utilized for proper standardization of Plant.

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