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Phytochemical analysis and chemical fingerprinting of *Derris heyneana* (Wight and Arn.) Benth

Dr. Aparna Saraf and Pooja Shinde

Abstract
Origin of the most of the modern day pharmaceuticals can be traced to natural compounds. Herbal pharmaceutical industry suffers from inadequate knowledge of active constituents, their efficacy and dosage. The emerging recognition of herbals and recent development in analytical science, are gradually leading to the development standardized parameters for herbal evaluation. *Derris heyneana* (Wight and Arn.) Benth. is less known woody climber collected from western ghats region of India. Preliminary phytochemical analysis to detect the presence of various secondary metabolites was performed in six solvents of varying polarity. HPTLC fingerprint and FTIR spectra may be utilized for the purpose of authentication and identification of the crude plant drug by the pharmaceutical industry.

Keywords: *Derris heyneana* (Wight and Arn.) benth. HPTLC fingerprint, FTIR

Introduction
Various pharmacopoeia and WHO are coming up with global standards for evaluation of quality and standardization parameters for the evaluation of herbals [1-4]. There are different and newer techniques to standardize raw drug and finished products. The standardization of raw herbal drug can be done by using parameters prescribed by various pharmacopoeia and WHO [5].

HPTLC fingerprint is increasingly being utilized for the purpose of quality control, species identification and authentication [6]. It is an important tool to evaluate consistency of crude herbal drugs. HPTLC fingerprint is an accepted parameter in herbal drug assessment protocols in various pharmacopoeias. HPTLC fingerprint of botanically authenticated raw material is used as a primary reference for the purpose of identification and authentication of unknown herbal sample [7-8]. Popularity of HPTLC analytical method for analysis of herbal drugs due to economic, rapid, simultaneously screening of large number of herbal samples and less time consuming methods [9].

FTIR spectroscopy has developed quickly due to its low noise, rapid speed, high repeatability, easy operation, low expense, and so on. FTIR has become increasingly useful in the field of evaluating herbal qualities [10-11]. FTIR spectroscopy has been shown to be a valuable tool for differentiating and classifying closely related species of plants. It is reported that FTIR could be successfully exploited for determining phylogenetic relationships between flowering plants [12].

The different species of *Derris* has been worked upon and has proven to be good source of secondary metabolites. They have exhibited good pharmacological activities. Literature survey reveals *D. heyneana* has not been much worked upon for secondary metabolites and pharmacological activities. With this background current work was undertaken with the objective of phytochemical analysis and chemical fingerprinting of *Derris heyneana* (Wight and Arn.) Benth.

Material and Methods
The whole plant parts of *Derris heyneana* (Wight and Arn.) Benth. was collected from Amboli region of Western Ghats of Maharashtra. The herbarium of plant was authenticated by comparing specimen herbarium of ref. no. 15055 at Blatter herbarium, St. Xavier's college, Mumbai. Plant part was then washed to get rid of dust load and shade dried. It was then grounded using mechanical grinder and fine powder of plant material was used for further studies.

Preliminary Phytochemical screening

Preparation of extracts

For the present work 10gm powdered material of leaves, root and stem of *Derris heyneana* (Wight and Arn.) Benth. was extracted in 100 ml of petroleum ether, ethyl acetate, chloroform and methanol, successively for 24 hrs. Extract was then filtered. Filtered extract was used for qualitative analysis of secondary metabolites.

Phytochemical screening

Phytochemical screening for presence of various secondary metabolites such as Alkaloids, flavonoids, glycosides, phenols, saponins sterol and tannins was carried out using standard tests [13].

HPTLC fingerprinting

Sonicated extracts of 500 mg of root, stem and leaf powder in 10 ml of methanol was used for the fingerprinting studies. 2, 4, 6, 8, 10 µl of each extract was loaded on the TLC plate using automated TLC sampler IV by Hamilton microsyringe (Switzerland), with the nitrogen flow providing a delivery speed of 150 nl/s. Spotting was performed at 25±2ºC. Plate was then developed using Toluene: Methanol: Diethyl amine (8:1:1) as mobile phase. Plate was developed upto 8 cm, it was then dried and images were captured using Camag visualizer. Plate was then derivatized using dipping chamber. Anisaldehyde sulphuric acid was used as derivatizing agent. After derivatization plate was dried using Plate heater. Plate was then scanned using TLC scanner. WIN CATS software is used for programming [14].

FTIR fingerprinting

The root, stem and leaf powder was grounded with KBr salt to remove scattering effect from large crystals. Translucent pellet of this mixture was then formed through which beam of light was passed. The analysis was carried out using 3000 Hyperion microscope with vertex 80 FTIR System. The transmittance spectra was analyzed between 400 to 4000/cm wave number.

Results and Discussion

Preliminary phytochemical screening

Preliminary Phytochemical analysis performed in various solvents revealed an array of secondary metabolites in all the 3 extracts viz., root, stem and leaf of *Derris heyneana* (Wight and Arn.) Benth. Methanolic extract was found to be the best solvent for extraction of secondary metabolites for all plant parts of the plant under study. Ethyl acetate extract of root and petroleum ether extract of stem and leaf exhibited poor diversity of secondary metabolites (Table No. 1).

Table 1: Preliminary Phytochemical screening of root, stem and leaf of *Derris heyneana* (Wight and Arn.) Benth.

Secondary metabolites	Tests	Root Extracts				Stem Extracts				Leaf Extracts			
		PE	CH	EA	M	PE	CH	EA	M	PE	CH	EA	M
Flavonoid	Pews	-	-	-	+	-	+	+	+	-	+	+	+
	Shinoda	-	-	-	+	-	+	+	+	-	+	+	+
	NaOH	-	-	-	+	-	+	+	+	-	-	+	+
Alkaloid	Draggendorf	-	-	-	+	-	-	-	+	-	-	-	+
	Mayer	-	-	-	+	-	-	-	-	-	-	-	+
	Wagner	-	-	-	+	-	-	-	-	-	-	-	+
Glycoside	Keller-Killani	+	+	-	+	+	+	-	+	+	-	-	+
	Glycoside	+	+	-	+	+	+	-	-	+	-		+
Phenol	Ellagic acid	-	-	-	+	+	+	+	+	+	-	+	+
	Phenol	-	-	-	+	-	-	+	+	-	-	+	+
Tannins	Gelatin	-	-	-	+	+	-	+	+	-	+	-	+
	Lead acetate	-	-	-	+	+	-	-	+	-	+	-	+
Sterol	Salkowaski	+	+	+	+	+	+	+	+	+	+	+	+
Saponins	Frothing test	-	-	-	-	-	-	-	-	-	-	-	-

(PE: Petroleum ether, CH: Chloroform, EA: Ethyl acetate, M: Methanol)
(**'+'** indicates presence; **'-'** indicates absence)

HPTLC fingerprinting studies

HPTLC fingerprint of methanolic leaf extract (Table no. 2) of *Derris heyneana* scanned at 540 nm after derivatization revealed presence of 14 components with Rf values 0.11, 0.16, 0.20, 0.27, 0.37, 0.42, 0.48, 0.55, 0.58, 0.65, 0.67, 0.79, 0.86, 0.89. Out of these 9 components with Rf 0.16, 0.37, 0.48, 0.55, 0.65, 0.67, 0.79, 0.89 were predominant with percentage areas 6.56, 7.37, 15.05, 16.08, 8.77, 0.78, 6.69, 6.91 respectively. Highest percentage area found to be 16.08 at Rf 0.55.

The fingerprint of methanolic root extract (Table no. 3) of *Derris heyneana* scanned at 540nm after derivatization revealed presence of 9 components with Rf values 0.10,0.15,0.19,0.37,0.46,0.54,0.58,0.84,0.88.Allthe components except, component with Rf 0.19 were predominant as their percentage area was found to be 5.15, 7.02, 2.86, 7.07, 7.50, 22.24, 24.08, 8.61 and 15.47 respectively. Among all these highest percentage area found was 24.08 at 0.58 Rf.

The results from HPTLC finger print scanned at wavelength 540 nm after derivatization with ASR for methanolic stem extract (Table no. 4) revealed the occurrence of 10 phytoconstituents with of R_f values 010, 0.16, 0.20, 0.31, 0.36, 0.40, 0.47, 0.56, 0.60, 0.90. Components with Rf 0.10, 0.16, 0.20, 0.36, 0.47, 0.56, 0.60 were predominant with percentage areas 7.56, 9.19, 5.19, 10.13, 9.43, 32.54, 12.56 respectively.

HPTLC fingerprint of different parts of *D. heyneana* was best observed in visible light after derivatization with ASR. The best solvent system was toluene: methanol: diethyl amine 8:1:1. The best loading volume was 2 µl for leaf, 2 µl for root and 10 µl for stem Out of total 33 components two components with Rf 0.16 and 0.20 were found in stem and leaf extract. One component with Rf0.10 was common to root and stem extract and two components with 0.37 and 0.58 were found in leaf and root extract. 7 components were found only in stem extract.6 components were unique to root extract. And 10 components were exclusively found in leaf extract. (Fig no. 6.4A) (Fig no. 6.4B) (Fig no. 6.4C)

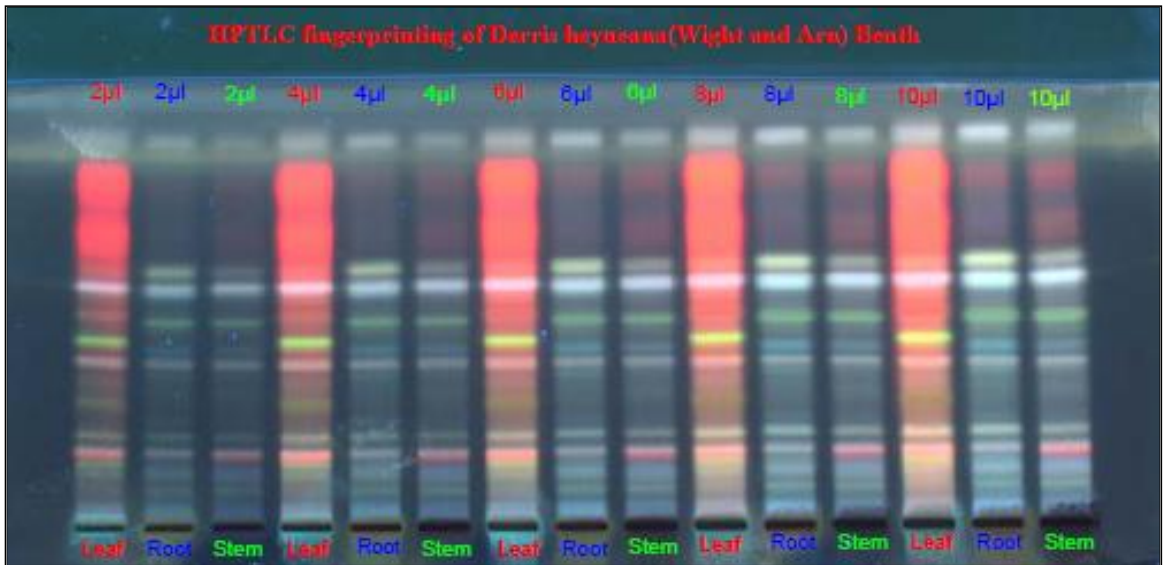

Plate 1: HPTLC fingerprint profile of Methanolic extract of leaf, root and stem of *Derris heyneana* (Wight and Arn) Benth. at 366nm after derivatization

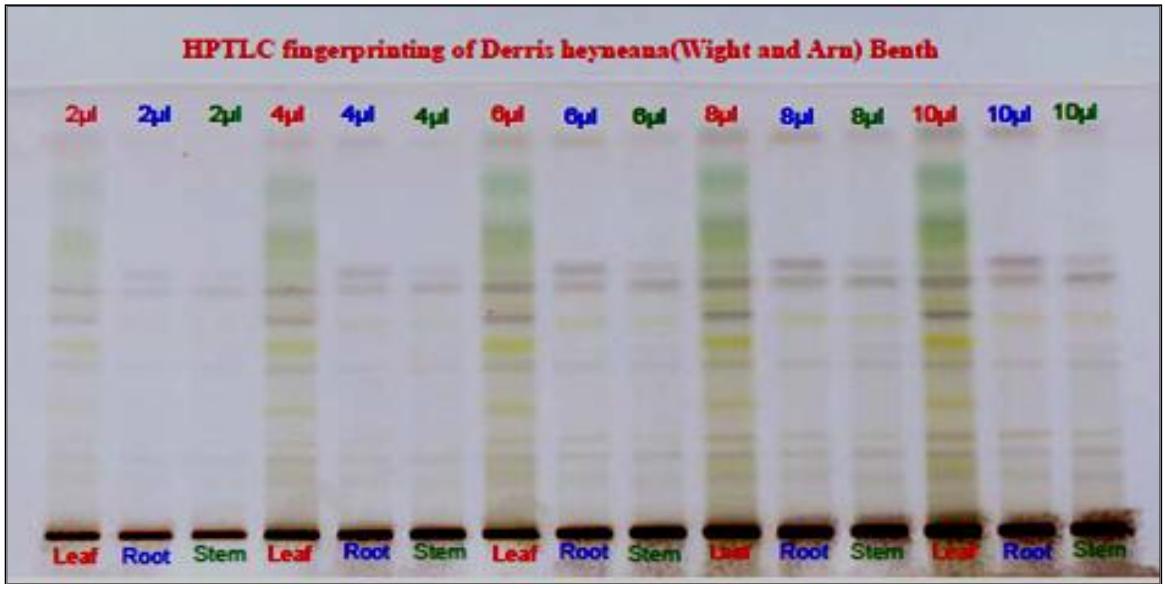

Plate 2: HPTLC fingerprint profile of Methanolic extract of leaf, root and stem of Derris heyneana (Wight and Arn) Benth. in visible light after derivatization

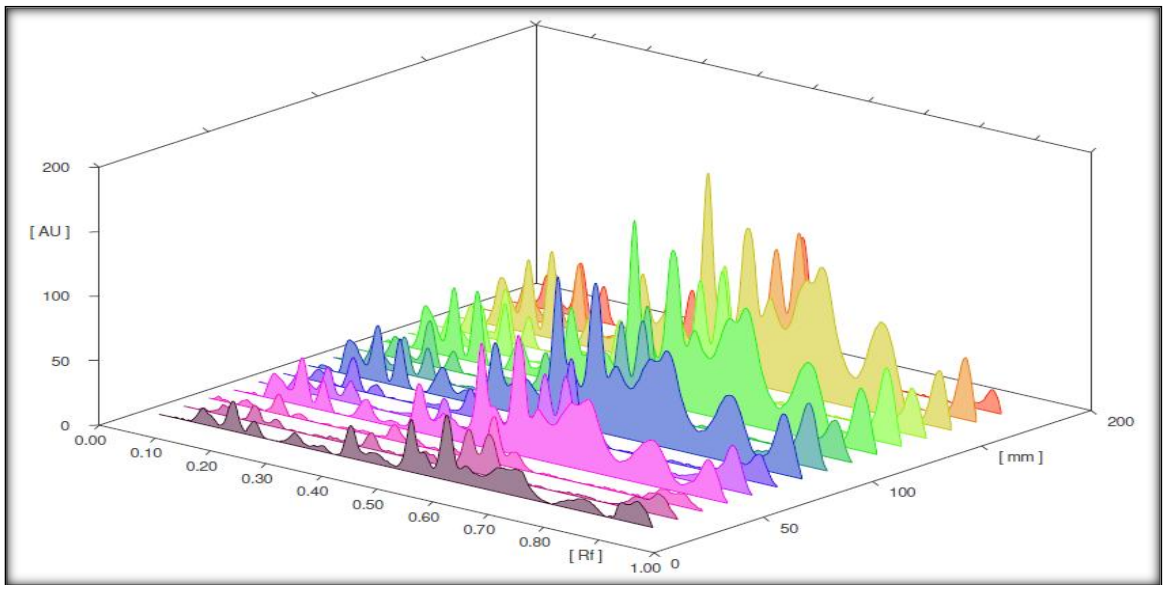

Fig 1: 3D- Densitogram of HPTLC fingerprints of Methanolic extract of leaf, root and stem of Derris heyneana (Wight and Arn) Benth. after derivatization in visible light

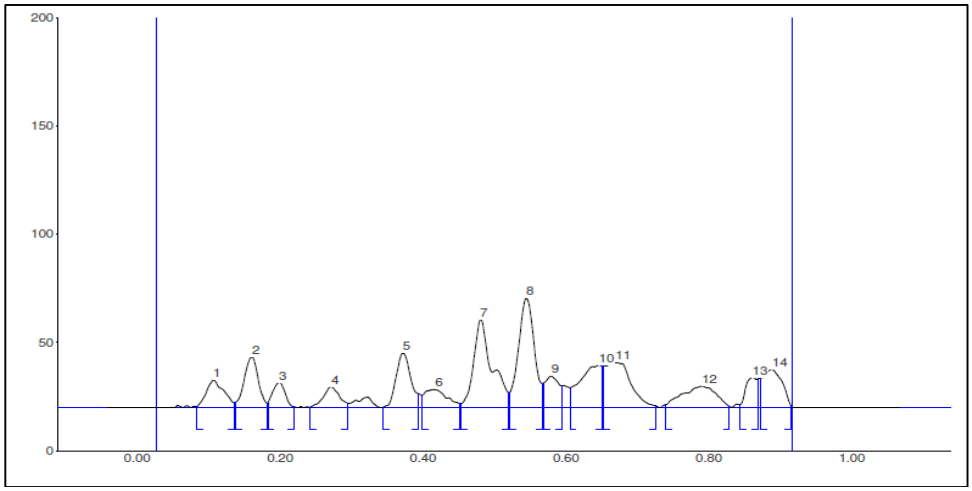

Fig 2: Densitogram of leaf methanolic extract (2µl) of *D. heyneana*(Wight and Arn) Benth.

Table 2: Rf values for fingerprint of leaf Methanolic extract of *D. heyneana*(Wight and Arn) Benth.

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.08	0.5	0.11	12.6	4.52	0.14	2.2	261.9	4.46	unknown *
2	0.14	2.3	0.16	23.3	8.40	0.18	2.0	384.8	6.56	unknown *
3	0.18	2.1	0.20	11.5	4.14	0.22	0.3	168.8	2.88	unknown *
4	0.24	0.1	0.27	9.5	3.42	0.30	2.0	179.8	3.06	unknown *
5	0.34	0.1	0.37	25.2	9.08	0.39	6.4	432.7	7.37	unknown *
6	0.40	5.7	0.42	8.4	3.02	0.45	1.9	227.2	3.87	unknown *
7	0.45	1.8	0.48	40.8	14.67	0.52	6.9	882.9	15.05	unknown *
8	0.52	6.9	0.55	50.5	18.15	0.57	11.1	943.2	16.08	unknown *
9	0.57	11.2	0.58	14.5	5.20	0.60	10.0	247.9	4.23	unknown *
10	0.61	9.3	0.65	19.6	7.04	0.65	19.0	514.3	8.77	unknown *
11	0.65	19.1	0.67	20.8	7.49	0.73	0.7	632.7	10.78	unknown *
12	0.74	1.5	0.79	9.9	3.57	0.83	0.5	392.4	6.69	unknown *
13	0.85	1.4	0.86	13.9	4.99	0.87	13.3	193.0	3.29	unknown *
14	0.87	13.6	0.89	17.6	6.32	0.92	0.8	405.6	6.91	unknown *

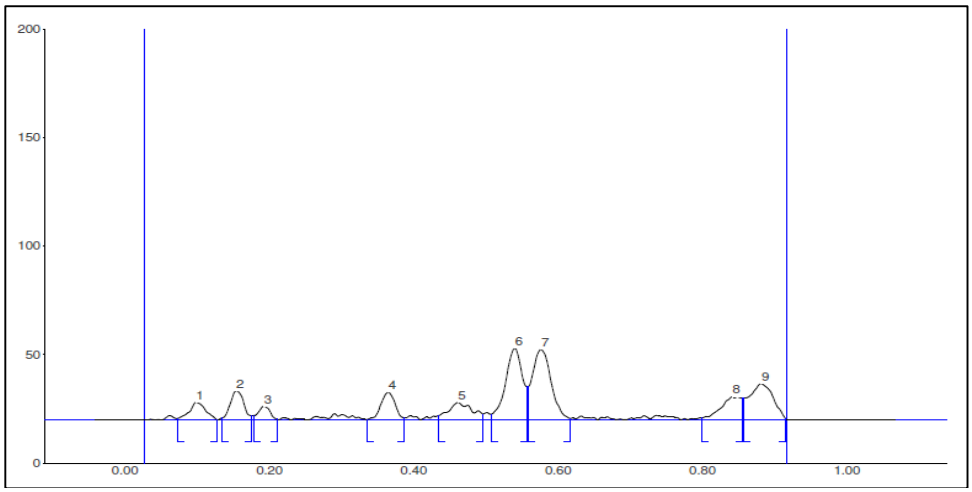

Fig 3: Densitogram of root methanolic extract (2µl) of *D. heyneana*(Wight and Arn) Benth.

Table 3: Rf values for fingerprint of root Methanolic extract of *D. heyneana*(Wight and Arn) Benth.

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.07	0.7	0.10	8.0	5.70	0.13	0.0	151.4	5.15	unknown *
2	0.13	0.9	0.15	13.2	9.40	0.17	1.9	206.4	7.02	unknown *
3	0.18	2.1	0.19	6.2	4.41	0.21	0.3	83.9	2.86	unknown *
4	0.34	0.2	0.37	12.8	9.06	0.39	1.1	207.8	7.07	unknown *
5	0.43	1.9	0.46	8.1	5.76	0.50	2.6	220.3	7.50	unknown *
6	0.51	2.6	0.54	32.8	23.29	0.56	15.2	653.8	22.24	unknown *
7	0.56	15.2	0.58	32.4	23.03	0.62	0.6	707.7	24.08	unknown *
8	0.80	0.9	0.84	10.6	7.51	0.86	9.9	253.0	8.61	unknown *
9	0.86	10.0	0.88	16.7	11.84	0.92	0.5	454.8	15.47	unknown *

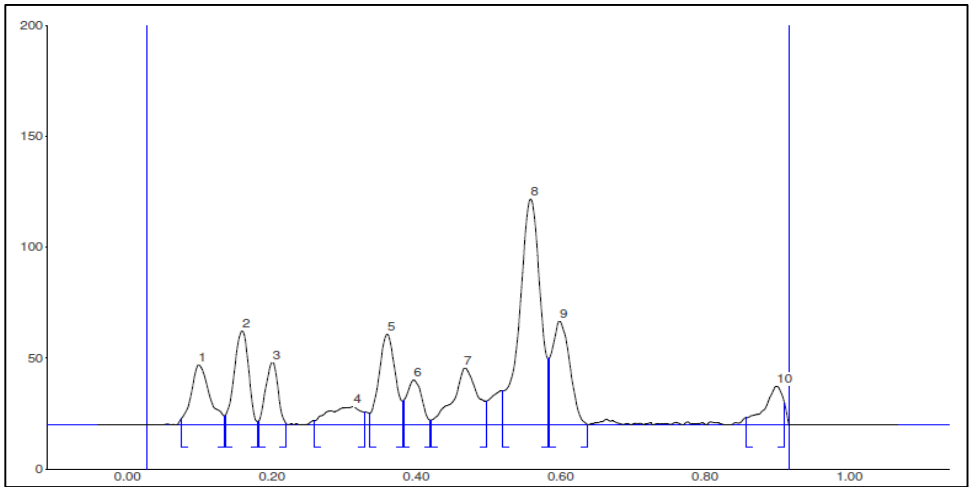

Fig 4: Densitogram of stem methanolic extract (10µl) of *D. heyneana* (Wight and Arn) Benth.

Table 4: Rf values for fingerprint of stem Methanolic extract of *D. heyneana* (Wight and Arn) Benth.

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.08	2.8	0.10	27.0	7.53	0.13	4.1	577.8	7.56	unknown *
2	0.14	4.3	0.16	42.2	11.78	0.18	1.4	702.4	9.19	unknown *
3	0.18	1.6	0.20	28.3	7.90	0.22	0.3	396.8	5.19	unknown *
4	0.26	1.8	0.31	8.2	2.29	0.33	5.6	309.0	4.04	unknown *
5	0.34	5.1	0.36	40.9	11.40	0.38	10.7	774.6	10.13	unknown *
6	0.38	10.8	0.40	20.2	5.63	0.42	2.0	356.5	4.66	unknown *
7	0.42	2.1	0.47	25.6	7.14	0.50	10.7	720.6	9.43	unknown *
8	0.52	15.2	0.56	101.8	28.39	0.58	29.7	2487.8	32.54	unknown *
9	0.59	30.3	0.60	46.9	13.08	0.64	0.1	960.2	12.56	unknown *
10	0.86	3.2	0.90	17.4	4.85	0.91	9.5	359.4	4.70	unknown *

FTIR fingerprinting

The FTIR analysis of stem exhibited peaks at 3422.86, 2921.91, 1740.66, 1637.85, 1514.24, 1439.01, 1375.24, 1321.51, 1246.21, 1033.85, 897.34, 827.88, 781.18, 666.61, 617.15 and 534.65 (Fig. 4.4). Peak observed at 3422.86 indicates presence of phenols and alcohols. Second peak was observed at 2921.91. This indicates C- H asymmetrical stretching of methylene group. The C-H asymmetric methylene group represents aliphatic compounds. Carboxylic group also absorbs in this region. No absorbance was observed in the region of 2220-2260 which indicates absence of cynide group. Thus, this result indicates that no toxic substances are present in the stem hence can be utilised for medicinal purpose. The third peak was observed at 1740.66. A peak was observes at1637.80. The area between 1600-1760cm^{-1} corresponds to bending vibrations of N-H (Amino acids), C=O stretching of Aldehyde and ketone esters as well as free fatty acids and glycerides. It indicates presence of proteins, coumarins and glycosides. C=O ranging from 1740-1736 is the characteristic feature of saponins. A peak was observed at 1514.24. 1555-1600cm^{-1} corresponds to aromatic domain and N-H bending vibrations.

Stem powder showed peak at 1439.01. The area between 1300-1450 cm^{-1}corresponds to stretching vibrations of C-O amide and C-C stretching from phenyl group. Peaks were observed at 1375.24 and 1321.15. C-O stretch at 1375.24 is also characteristic of anthraquinones. Peaks between 1150-1270 cm-1 corresponds to stretching vibrations of carbonyl C-O or OH bendings. One peak 1246.21was observed in this region. The area between 997-1130 cm^{-1} corresponds to stretching vibrations of C-O of mono and oligo carbohydrate. Single peak was observed in this area at 1033.85. The area <1000cm^{-1} corresponds to C-H bending from isoprenoids. Accordingly peaks were observed at 897.34, 827.88, 781.18, 666.61, 617.15 and 534.65.

FTIR spectra of leaf revealed the presence of different functional groups. The FTIR analysis of root give peaks at 3417.75, 2924.74, 2353.68, 2323.26,1737.63, 1638.29, 1518.17, 1439.12, 1370.29, 1238.11, 1155.07, 1079.34, 1020.63, 861.78, 773.43, 660.92, 617.32, 574.82, 529.82, 472.70, 442.62, 430.92,416.43 (Fig. 4.5).

Peak at 3417.75 shows presence of alcohol and phenol group. The C-H asymmetrical stretching of methylene group has been found near 2924.74. The C-H asymmetric stretching of methylene group represents aliphatic compounds. Peaks observed at 2924.74 is also an indicator of carboxylic acids since, carboxylic acid absorbs in the region between 2500-3300 cm^{-1}.It also indicates C-H stretch of lipids and C=C stretch of terpenes. There is no absorbance between 2220-2260 which indicates absence of cynidegroup. Peaksat 1735.84and 1638.29 corresponds to area between 1600-1760cm^{-1}which represents bending vibrations of N-H (Amino acids), C=O stretching of Aldehyde and ketone esters as well as free fatty acids and glycerides. It indicates presence of proteins, coumarins and glycosides. This result indicates the high protein content. Peaks at 1638.29 also indicates presence of C=O stretch in coumarin. A peak was observed at 1518.17 which representaromatic domain and N-H bending vibrations.

The area between 1300-1450 cm^{-1}corresponds to starching vibrations of C-O amide and C-C starching from phenyl group. Two peaks of 1439.12, 1370.29 were observed in this region. Readings between 1150-1270 cm-1 corresponds to stretching vibrations of carbonyl C-O or OH bendings [10]. Two absorbance peaks of 1238.11, 1155.07cm-1were found

in this region. The FTIR spectra show peaks at 1079.3 and 1020.63 this area indicates stretching vibrations of C-O of mono and oligo carbohydrates. Area < 1000cm^{-1}corresponds to C-H bending from isoprenoids. Accordingly peaks were observed at 861.78, 773.43, 660.92, 617.32, 574.82, 529.82, 472.70, 442.62, 430.92, 416.43, 997-1130cm^{-1} also corresponds to stretching vibrations of C-O of mono and oligo carbohydrates. Brominate compounds also shows absorbance ininfra red region 500-750.

The FTIR analysis of stem gives peaks 3420.73, 2920.74, 2852.08, 2375.85, 2345.45, 1735.84, 1636.93, 1518.59, 1444.11, 1379.75, 1317.84, 1245.56, 1160.14, 1061.39, 896.44, 827.58, 783.99, 662.12, 618.87, 592.81, 564.39, 536.98, 523.43, 483.00, 429.50. A peak at 3420.73 indicated N-H (amines) stretching. This lies in the range between 3350-3600 cm-1 corresponds to stretching vibrations of OH groups from water, alcohols, phenols, carbohydrates, peroxides as well as from amides [15]. Second peak was observed at 2920.74. This indicates C- H asymmetrical stretching of methylene group. The C-H asymmetric methylene group represents aliphatic compounds. Carboxylic group also absorbs in this region. C-H ranging from 2922-2929 is also a characteristic of oleane triterpenoid saponins.2800-2900 corresponds to C-H stretching vibrations specific to $CH_3$and CH_2 fromlipids metoxy derivatives, C-H (aldehydes), including cis double bonds. No absorbance was observed in the region of 2220-2260 which indicates absence of cynide group. Thus, this result indicates that no toxic substances are present in the stem hence can be utilised for medicinal purpose. Carboxylic acids are absorbed in the region 2500-3300. Two peaks are observed at 2852.08 and 2920.74. The next peak was observed at 1735.84and 1636.93. The area between 1600-1760cm^{-1} corresponds to bending vibrations of N-H (Amino acids), C=O stretching of Aldehyde and ketone esters as well as free fatty acids and glycerides. It indicates presence of proteins, coumarins and glycosides. C=O ranging from 1740-1736 is the characteristic feature of saponins [16]. Single peak was observed at1636.93.

Absorption band around 1600-1660 region indicates presence of amino acids. This result indicates high protein content.1500-1600 corresponds to aromatic domain and N-H bending vibrations. Peak was observed at 1518.59. The area between 1300-1450 cm^{-1} corresponds to stretching vibrations of C-O amide and C-C starching from phenyl group. Peaks were observed at 1444.11, 1379.75 and 1317.84. C-O-C ranging from 1034-1072is also a characteristic of saponins. A peak was observed at 1061.39. Peaks were observed at 1160.14and 1245.56. Readings between 1150-1270 cm-1 corresponds to stretching vibrations of carbonyl C-O or OH bendings. Two peaks were observed in this region, 1160.14 and1245.56. Area between 997-1130 corresponds to stretching vibrations C-O of mono oligo carbohydrates. Peak was observed at 1061.39.Area <1000 cm-1 corresponds to C-H bending from isoprenoids. Peaks were observed at 896.44, 827.58, 783.99, 662.12, 618.87, 592.81, 564.39, 536.98, 523.43, 483.00, and 429.50 [16]. Brominate compounds show infrared region 500-750. Peaks were observed at 662.12, 618.87, 592.81, 564.39,536.98, 523.43, 483.00and 429.50 [17].

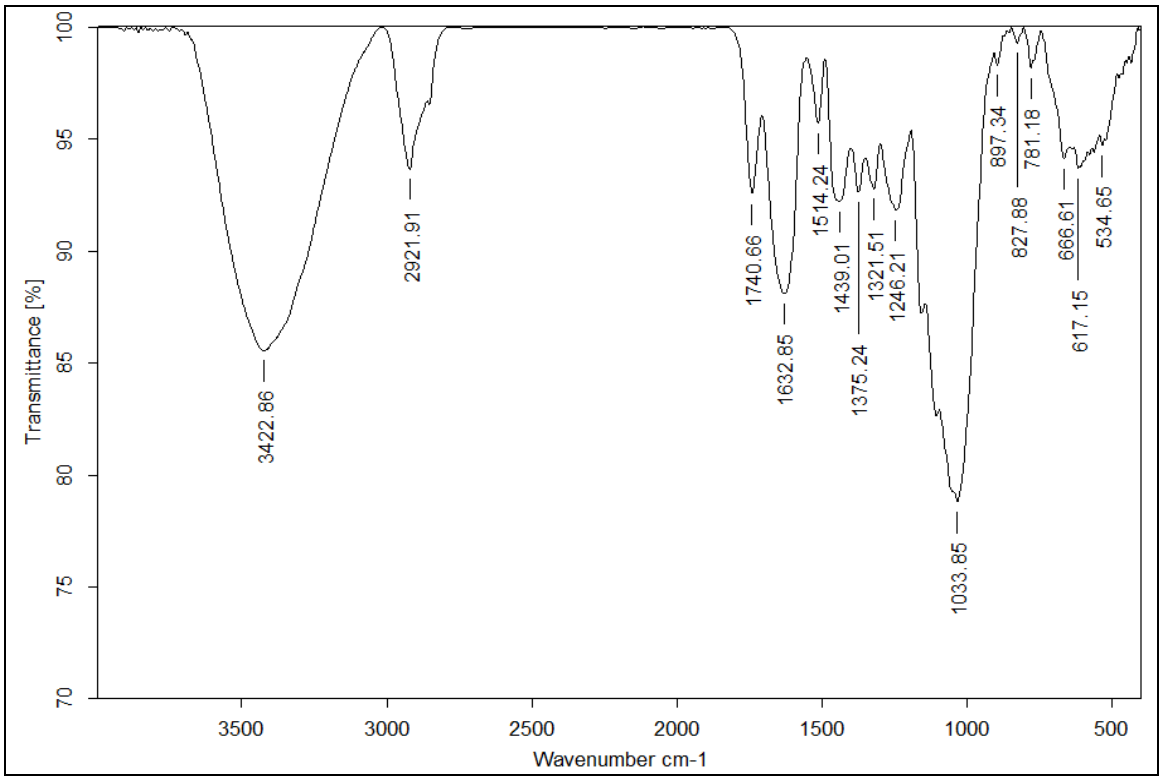

Fig 5: FTIR spectra of stem of *D. heyneana*

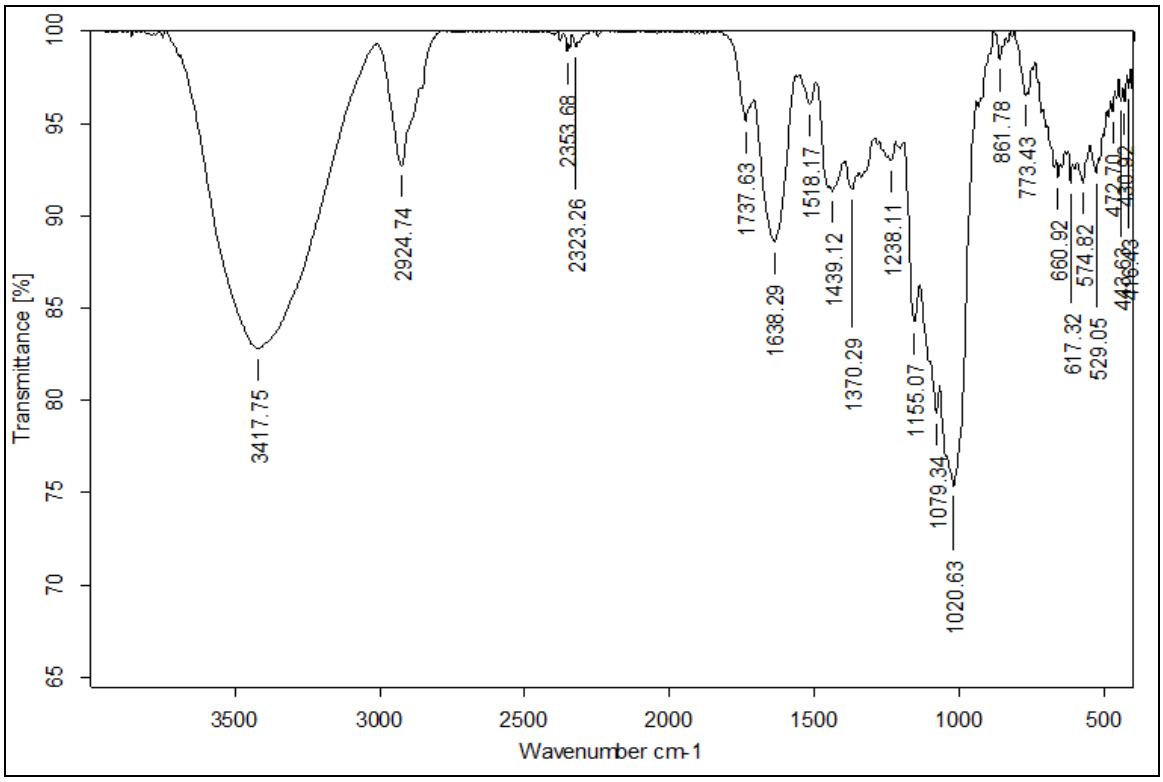

Fig 6: FTIR spectra of root of *D. heyneana*

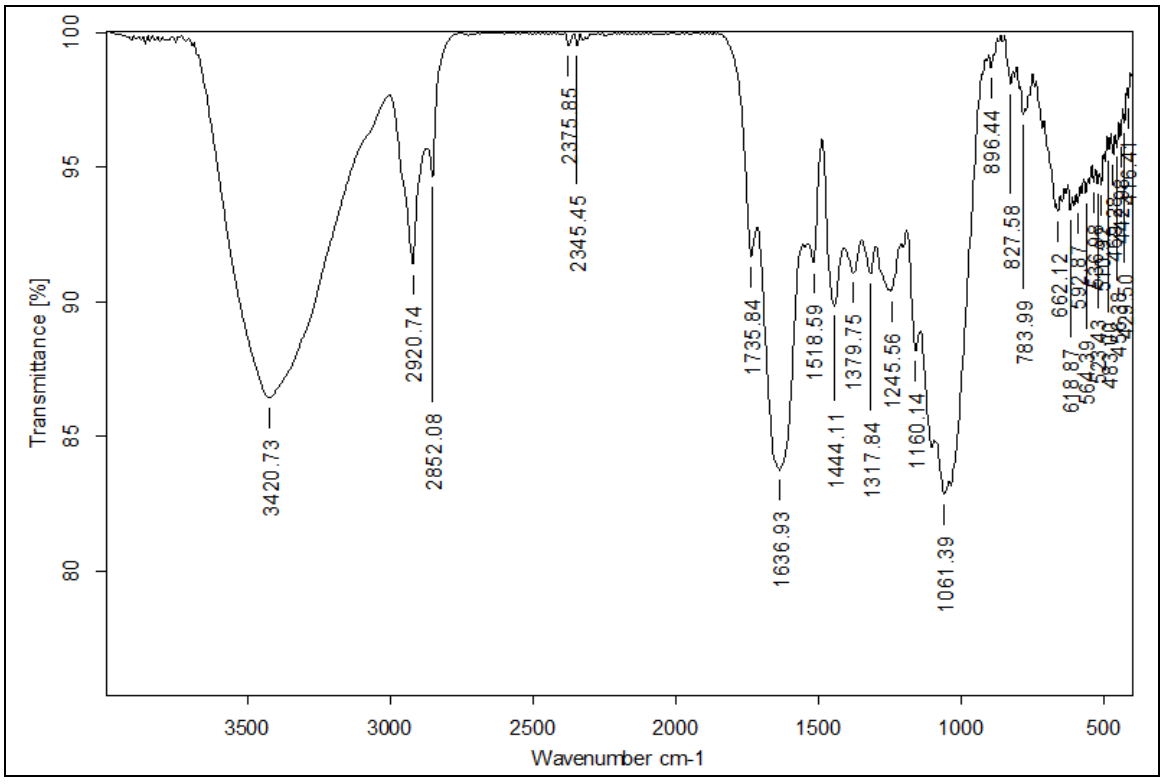

Fig 7: FTIR spectra of leaf of *D. heyneana*

Conclusion

The results indicated methanol can be used as extracting solvent for this plant. The medicinal properties exhibited by the plant can be attributed to the presence of various classes of secondary metabolites. Development of HPTLC fingerprint which will serve for standardization and authentication of the plant and also as a biomarker. The standardized FTIR spectrum may be employed as a molecular fingerprint of the plant.

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