Chapter 3

Results And Discussion

3. RESULTS AND DISCUSSION

In this chapter results obtained from various investigations carried out are compiled. An attempt has also been made to discuss these results in order to provide convincing reasons for the studies performed.

3.1 Pharmacognostic studies.

3.1.1 Macroscopic evaluation.

Dried leaf galls of *Pistacia integerrima* were hollow, horn like, thin walled, cylindrical and tapering at both ends. The galls were grayish brown internally and reddish brown externally. Size was 25-30cm or more. (Figure 1) Each gall contained numerous dead insects. Odor was terebinthine and taste strongly astringent and slightly bitter.

Dried rhizomes of *Hedychium spicatum* possess strong aromatic odor and bitter camphoraceous taste. They were available in form of slices 0.5inch or less in diameter and up to 0.25inch in thickness. (Figure 2) The rhizomes were white and starchy within covered by rough reddish brown bark with rootlets attached

3.1.2 Microscopic examination.

3.1.2.1 Transverse section (T. S.) of intact drugs.

T.S.of *P.integerrima* (Figure 3) showed collapsed epidermis on both the sides. Epidermal cells were thin walled and tangentially elongated. Ground tissues were thin walled oval / circular. Outer two layers were tangentially elongated while between the vascular bundles radially elongated. Outer few layers and some cells of ground tissue were filled with yellowish brown content. Vascular bundles were scattered throughout the ground tissue in two rows, consisting of phloem accompanied by a large tannin sac in each vascular bundle.

T.S. of *H. spicatum* (Figure 4) showed outermost thick layer of suberized dark brown cells of outer cork consisting of 10-15 or more layers of irregular parenchymatus cells. Inner cork consists of few layered brown, rectangular, radially arranged cells followed by wide zone of cortex which was 30-40 cells thick. Some cortical cells were filled with flattened and oval oblong starch grains. Numerous oleoresin cells were found in this region which has suberized walls. They showed presence of yellow green oil. A thin endodermal layer was present beneath the cortex. The central cylinder was distinguished by presence of peripheral plexus of irregular congested vascular bundles with poorly developed mechanical tissues. Vascular bundles were scattered irregularly throughout the ground tissue. Vascular bundles

were closed and collateral possessing group of two or more xylem elements. Ground tissue was composed of parenchymatous cells with abundant starch and oil.

Figure 1: Galls of *Pistacia integerrima*

Figure 2: Rhizomes of *Hedychium spicatum*

Figure 3:T. S. of *Pistacia integerrima*

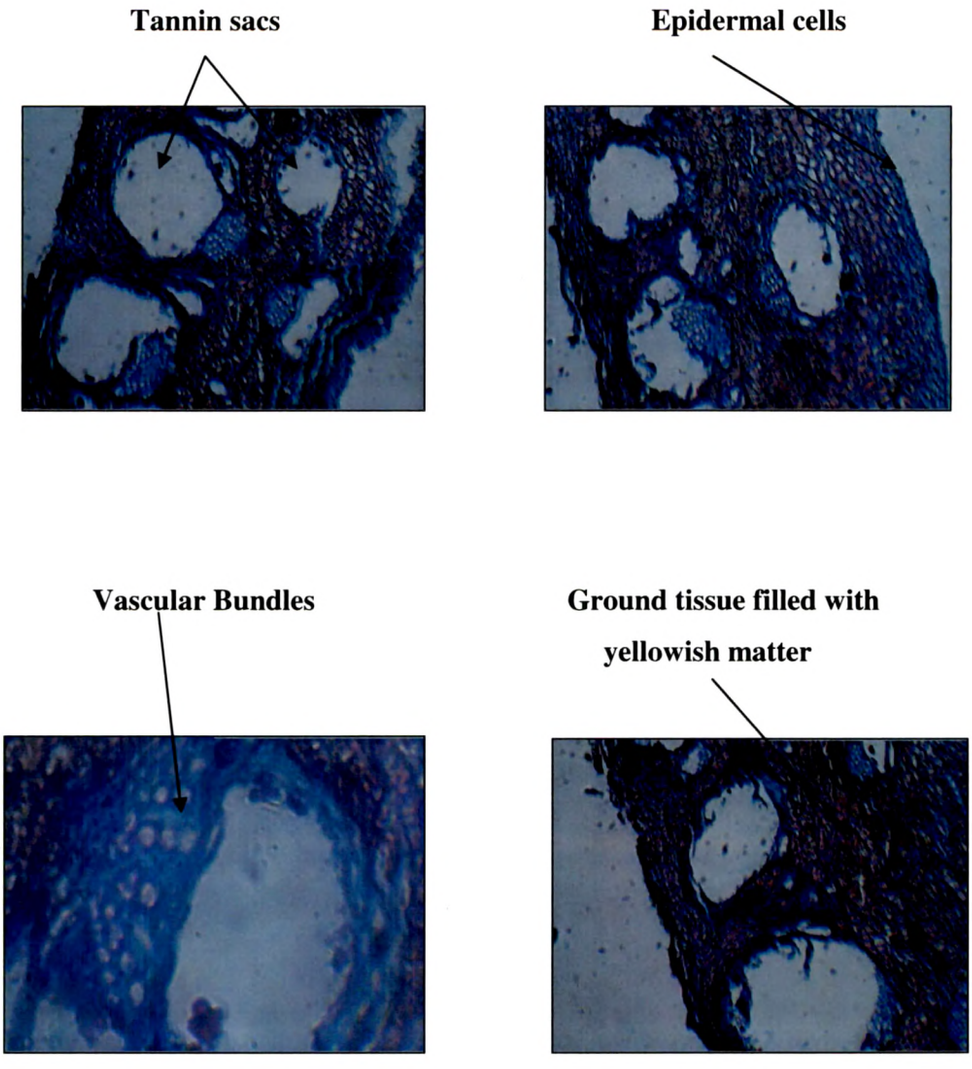
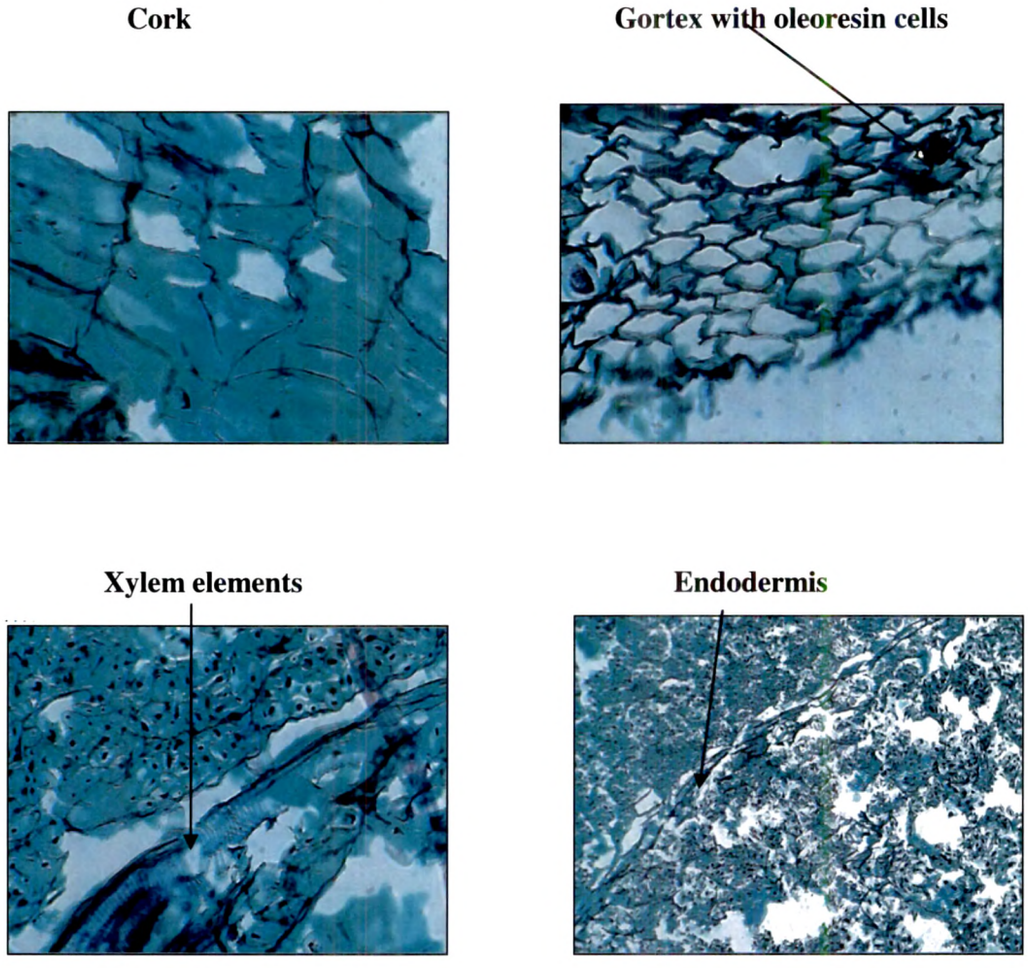


Figure 4: T.S. of *Hedychium spicatum*



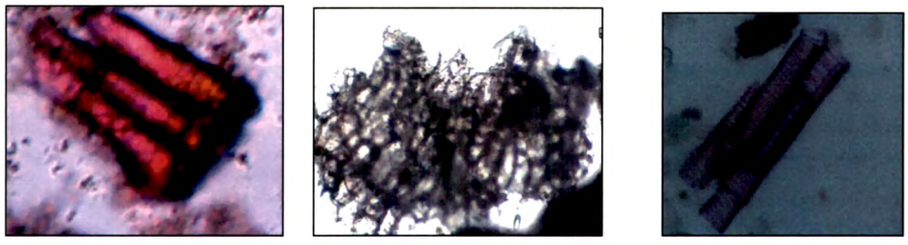
3.1.2.2 Microscopic features of powdered crude drugs

Powder of *P. integerrima* galls showed presence of lignified vascular bundles. Large fibres with tapering end (width 2-4 micron and length 15-20 microns) were observed when treated with phloroglucinol and conc. Hydrochloric acid. The powder showed presence of parenchymatous cells. Tannin sacs were observed. (Figure 5)

Powder of *H. spicatum* showed presence of cork cells (width 15-20 microns and length 30-35 microns) and parenchymatous cells. In the ground tissue oleoresin cells were observed. Large numbers of starch grains (10-15micron) were observed when the powder was treated with dilute iodine solution. (Figure 6)

Figure 5: Powder microscopy of *Pistacia integerrima*

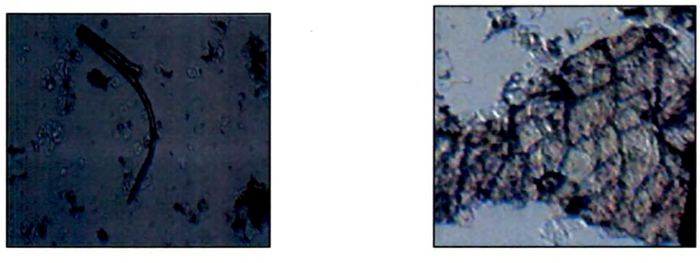
Lignified elements **Parenchyma** **Xylem Vessels**



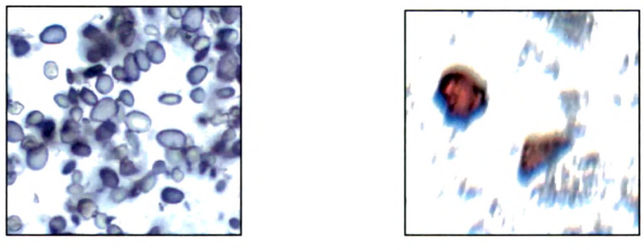
Tannin sacs **Fibre**

Figure 6: Powder microscopy of *Hedychium spicatum*

Xylem vessels **Cortex**



Starch grains **Oleoresin cells**



3.2 Proximate analysis

The results obtained from various determinations of proximate analysis are compiled in Table 2.The values represented are average of three readings taken.

(n= 3)

Table 2: Proximate analysis of selected plant drugs

Parameters	Average Values (% W/ W)	
	P. integerrima	***H. spicatum***
Ash Value		
Total Ash	3.33%	5.03%
Water Soluble ash	1.33%	8.21%
Acid Insoluble Ash	1.50%	6.99%
Extractive Value		
Water Soluble Extractive	4.52%	5.16%
Alcohol Soluble Extractive	5.62%	3.82%
Swelling Index	-	-
Foaming Index	Less than 100	Less than 100
Foreign Organic Matter	1.26%	1.90%
Water Content KF Method	4.12%	3.64%
Moisture Content RI Balance	3.33%	4.33%
Bitterness value	-	-
Haemolytic value	-	-
Heavy metal analysis		
Manganese	42.6 ppm	54.92 ppm
Zinc	86.21 ppm	49.75 ppm
Copper	6.54 ppm	10.79 ppm
Lead	19.51 ppm	17.26 ppm
Cadmium	Nil	Nil
Volatile Oil Content	0.56%	0.61%
Microbial content		
E.coli	4×10^3	7×10^3
Salmonella	AB	AB

The total ash value in case of *P. integerrima* was 3.33% and that of *H. spicatum* was 5.03%.A comparatively higher ash value in case of rhizomes of *H. spicatum* indicates presence of more inorganic matter being an underground drug. The values for foreign organic matter of *P. integerrima* was1.26% and that of *H. spicatum* was 1.90%. The moisture content of *P. integerrima* 3.33% whereas it was found to be 4.33% in *H. spiacatum.* Alcohol soluble extractive of *P. integerrima* was found to be 5.62 and that of *H. spicatum* was found to be 3.82%. Water soluble extractive value of *P. integerrima* was found to be 4.52% and that of *H. spicatum* was found to be 5.16%. Foaming index provides the data regarding presence of saponins in the crude dugs.

The foaming index less than 100 indicates absence of saponins. Therefore, there was no haemolysis observed. These values can be considered standard values and could therefore be used in standardization of these plant drugs in future.

3.3 Phytochemical studies

3.3.1 Successive solvent extraction:

Both the selected plants drugs are rich in content of volatile oil, apart from other phytoconstituents. In order to avoid the interference of volatile oils in different extracts these plant material were first made devoid of volatile oil and then were subjected to successive solvent extraction using solvents in the order of increasing polarity. The % yield and physical properties of the extracts so obtained are recorded in Table3. Results indicate maximum extractive values attained with polar solvents like Methanol, water in both the selected plants whereas extractive values with non polar solvents like Petroleum ether, Benzene, Acetone and Ethyl acetate were comparatively less. Methanol and Aqueous extract of *P. integerrima* was found to be12.25% 9.91% respectively. The Methanol extract of *H. spicatum* was found to be 7.23%. Aqueous extract of *H. spicatum* was 4.44%.

Table3: Preliminary phytochemical screening of selected plant drugs.

Sr. No.	**Solvents used**	**Plant drugs**			
		Pistacia integerrima		***Hedychium spicatum***	
		Color & consistency	Average extractive value (% W/W)	Color & consistency	Average extractive value (% W/W)
1	Petroleum ether	Yellow sticky	1.2	Light yellowish brown oily	2.28
2	benzene	Brown sticky	0.9	Light brown Nonsticky	0.16
3	Chloroform	Light brown sticky	1.3	Light brown Nonsticky	0.42
4	Ethyl acetate	Dark Brown sticky	5.41	Light brown Nonsticky	2.60
5	Acetone	Dark brown nonsticky	3.36	Light yellow Nonsticky	0.28
5	Methanol	Brown solid	12.25	Yellowish brown Nonsticky	7.23
6	Water	Brown solid	9.91	Cream Mucilagenous	4.44

3.3.2 Qualitative chemical tests.

The extracts obtained in the successive extraction were then subjected to various qualitative chemical tests to determine the presence of various phytoconstituents. *P.integerrima* showed presence of carbohydrates, phenolics, tannins, phytosterols and flavonoids. *H. spicatum* showed presence of carbohydrates, terpenoids, flavonoids and mucilage. Results are reported in Table 4.

Table 4: Qualitative chemical tests of different successive extracts of selected plant drugs.

Class of compounds	Plant drugs													
	Pistacia integerrima							*Hedychium spicatum*						
	P	B	C	E	A	ME	AQ	P	B	C	E	A	ME	AQ
Alkaloids	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Carbohydrates	-	-	-	-	-	+	+	-	-	-	-	-	+	+
Steroids/ Terpenoids	+	+	-	-	-	-	-	+	+	+	+	+	+	+
Proteins and Amino acids	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Saponins	-	-	-	-	--	-	-	-	-	-	-	-	-	-
Fl;avonoids	-	-	-	+	+	+	+	-	-	-	+	+	+	+
Phenolics/Tannins	-	-	+	+	+	+	+	-	-	-	-	-	-	
Volatile oil	Volatile oil was separated by hydrodistillation													

3.3.3 TLC profile of the extract obtained by successive solvent extraction.

The extracts obtained in the successive extraction process were subjected to determine the presence of various phytoconstituents by spraying different detecting agents. R_f of the compounds were then recorded. Results are summarized in Table 5 and Table 6 respectively.

Table 5: TLC profile of the extracts obtained by successive solvent extraction of *P. integerrima*

Class of compounds	Detection reagent	Solvent system	Extracts						
			P	B	C	E	A	M	W
Carbohydrate	AS reagent	B	-	-	-	-	-	0.85, 0.75 (Brown)	0.74,0.87, 0.35,0.25 (Brown)
Flavonoids	UV- 365 &NP-PEG	A	-	-	-	0.9 (Orange) 0.66(Orange) 0.5(Yellow) 0.35(Yellow)	0.60 (Orange) 0.41 (Yellow)	0.9 (Orange) 0.66(Orange) 0.4(Orange) 0.23(Yellow) 0.15(Orange)	0.66(Orange) 0.45(Orange) 0.25 (Yellow) 0.12(Yellow)
Saponins/ Terpenoids	VS reagent	A	0.68,0.9, 0.43, 0.35,0.26 (Voilet)	0.90,0.88,0. 79,0.25 (Violet)	0.75,0.66, 0.57 (Violet)	-	-	-	-
Phenolics	Ferric chloride	C	-	-		0.85,0.75,0.55,0.36 (Blue)	0.77,0.65,0.6 (Blue)	0.92,0.71,0.66 ,0.53,0.3 (Blue)	0.85,0.72,0.60, 0.48,0.29 (Blue)

A: Ethyl Acetate: Methanol: Water (10:1.35:1); B: n- Butanol: Glacial Acetic acid: Water (6:2:2) C: Chloroform: Methanol (1:1)

Table 6: TLC profile of the extracts obtained by successive solvent extraction of *H. spicatum*

Class of compounds	Detection reagent	Solvent system	Extracts					
			P	B	C	E	M	W
Carbohydrate	AS reagent	B	-	-	-	-	0.57,0.42 (Brown)	0.78,0.55,0.38 (Brown)
Flavonoids	UV- 365 &NP-PEG	A	-	-	-	0.84, 0.63 (Yellow)	0.78,0.62,(Yellow) 0.48,0.14(Yellowish green)	0.86,0.63,0.48 (Yellow)
Saponins/ Terpenoids	VS reagent	A	0.42,0.62,0.77 (Blue)	0.22,0.57,0.85 (Blue)	0.14,0.48,0.85 (Blue)	0.57,0.71,0.85 (Blue)		
Phenolics	Ferric chloride	C	-	-	-	-	-	-

A: Ethyl Acetate: Methanol: Water (10:1.35:1); B: n- Butanol: Glacial Acetic acid: Water (6:2:2) C: Chloroform: Methanol (1:1)

3.4 Preparation of selective extracts and their fractions.

The Aqueous and Methanol extracts of both the plant drugs were first subjected to preliminary screening for adaptogenic activity. The Aqueous and Methanol extract of *P. integerrima* and Methanol extract of *H. spicatum* were found considerably effective while the aqueous extract of *H. spicatum* was active in a very high dose. Solvents were selected based on the yield obtained and their qualitative chemical tests. Further the aqueous extract of *P. integerrima* was fractionated using n Butanol, Ethyl acetate and Methanol. The Methanol extract of *P. integerrima* was fractionated further using Chloroform, Acetone and Ethyl acetate.

Based on these observations, aqueous and methanol extracts of *P. integerrima* and methanol extract of *H. spicatum* were selected for fractionation. The fractions of Aqueous and Methanol extracts of *P. integerrima* and fractions of Methanol extract of *H. spicatum* were subjected to various qualitative chemical tests to determine the presence of various phytoconstituents. Results of fractionation of Aqueous and Methanol extracts of *P. integerrima* and fractionation of Methanol extract of *H. spicatum* are reported in Table 7 and Table 8 respectively. All the fractions of extracts of both the plant drugs were subjected to TLC. The results are recorded in Table 9 and Table 10.

Table 7: Qualitative chemical tests of different fractions of extracts galls of *P. integerrima.*

Class of compounds	Fractions of aqueous and methanol extract of *Pistacia integerrima*						
	BFAPI	EFAPI	MFAPI	CFMPI	AFMPI	EFMPI	RMFMPI
Alkaloids	-	-	-	-	-	-	-
Carbohydrates	-	-	+	-	-	-	+
Steroids/ Terpenoids	-	-	-	-	-	-	-
Proteins and Amino acids	-	-	-	-	-	-	-
Saponins	-	-	-	-	-	-	-
Fl;avonoids	+	+	+	-	+	+	+
Phenolics/ Tannins	+	+	+	+	+	+	+

Table 8:Qualitative chemical tests of different fractions of extracts of rhizomes of *H. spicatum.*

Class of compounds	Fractions of methanol extract of *Hedychium spicatum*						
	HFMHS	HBFMHS	PEFMHS	CFMHS	EFMEHS	AFMEHS	MFMHS
Alkaloids	-	-	-	-	-	-	-
Carbohydrates	-	-	-	-	-	-	-
Steroids/ Terpenoids	+	+	+	+	+	+	+
Proteins and Amino acids	-	-	-	-	-	-	-
Saponins	-	-	-	-	-	-	-
Flavonoids	-	-	-	-	-	-	-
Phenolics/Tannins	-	-	-	-	-	-	-

Table9: TLC profile of the fractions of Aqueous and Methanol extracts of galls of *P. integerrima*

Class of compounds	Detection reagent Solvent system		Extracts						
			BFAEPI	**EFAEPI**	**MFAEPI**	**CFMEPT**	**AFMEPI**	**EFMEPI**	**RMFMFPI**
Carbohydrate	AS reagent	B	-	-	0.74(Brown) 0.87Brown)	-	-	-	0.65 (Brown) 0.38(Brown)
Flavonoids	UV- 365 &NP-PEG	A	0.77(Orange) 0.86(Orange)	0.64(Yellow) 0.45(Orange)	0.77(Orange) 0.90 (Orange)	-	0.68 (Yellow)	0.82,0.69 (Yellow) 0.32(Yellow)	0.36(Yellow) 0.29(Yellow)
Phenolics	Ferric chloride	C	0.65,0.49 (Blue)	0.71 (Blue) 0.30(blue)	0.65 (Blue) 0.22(Blue)	0.9 (Blue)	0.36,0.32,0.19 (Blue)	0.60 (Blue) 0.66 (Blue)	0.56 (Blue) 0.66 (Blue)

A: Ethyl Acetate: Methanol: Water (10:1.35:1); B: n- butanol: Glacial Acetic acid; C: Hexane: ethyl acetate (1.7:0.3)

Table 10: TLC profile of the fractions of Methanol extracts *H. spicatum*

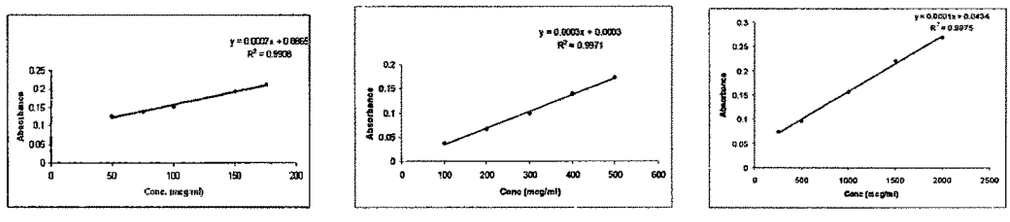
Class of compounds	Detection reagent	Solvent system	Extracts						
			HFMHS	**HBFMHS**	**PEFMHS**	**CFMHS**	**EFMHS**	**AFMHS**	**MFMHS**
Carbohydrate	AS reagent	B	-	-	-	-	-	-	0.55 Brown) 0.26(Brown)
Flavonoids	UV- 365 & NP-PEG	A	-	-	-	-	0.45,0.32 (Yellow)	0.28 (yellow)	0.81,058, 0.48(Yellow)
Terpenoids	10% H_2SO_4	C	0.34,0.6,0.74, 0.88 (Blue)	0.28,0.42,0.57 (Blue)	0.45,0.85,0.89 (Blue)	0.17,0.22 (Blue)	0.22,0.28,0.42 (Blue)	0.05,0.14 (Blue)	0.15(Blue)

A: Ethyl Acetate: Methanol: Water (10:1.35:1); B: n- butanol: Glacial Acetic acid; C: Hexane: ethyl acetate (1.7:0.3)

3.5 Estimation of total phenolic and flavonoid content in different extracts and fractions of *P. integerrima*

Aqueous and Methanol extracts of *P. integerrima* and their fractions were found to be rich in phenolics and flavonoids therefore they were subjected to determination of total phenolic and flavonoid content. The phenolic content of Aqueous extract and its fractions viz n Butanol, Ethyl acetate and Methanol fractions was determined. Similarly phenolic content of Methanol extract and its fractions viz Chloroform, Ethyl acetate, Acetone and remaining Methanol fraction was determined. Determination of phenolic content was done by Folin Ciocalteu methaod. All the above mentioned extracts and fractions were subjected to determination of flavonoid content by Aluminum chloride method and DNPH method.

Graph 1: Calibration curve for total phenolic and flavonoid content by Folin Ciocalteu Method (A), total flavonoids content by Aluminum chloride method (B) and DNPH method (C).



A B C

Folin Ciocalteu method was used to determine the total phenolic content of different extracts and fractions of *P. integerrima.* The total Aqueous extract showed presence of 12.96% phenolics and total Methanol extract showed presence of 14.29 % phenolics. Ethyl acetate fraction of both Aqueous and Methanol extract was found to content 5.38 and 6.82 % of phenolics. Methanol fraction of Aqueous extract showed 3.29%. Phenolic content of n Butanol fraction was 3.21%and that of acetone fractions was 4.72%.

The Aqueous extract showed 4.21% and Methanol extract showed 12.85% of total flavonoid when determined by aluminum chloride method. Ethyl acetate fraction of both aqueous and methanol extract was found to content 2.48 % and 3.15 % of total flavonoid respectively. The total flavonoid content in Butanol fraction of Aqueous extract was 1.65%, acetone fraction of Methanol extract was found to be 2.68% and in remaining Methanol fraction was 1.82%.

The Aqueous extract showed 5.66% and Methanol extract showed 7.82% of total flavonoid when determined by DNPH method. Ethyl acetate fraction of both Aqueous and Methanol extract was found to content 2.76% and 3.32 % respectively. The total flavonoid content in Butanol fraction of Aqueous extract was 1.05%, Acetone fraction of Methanol extract was found to be 2.68% and in remaining methanol fraction was 1.50%.

3.6 HPTLC fingerprint profile of active extracts and fractions:

Different active extracts and their fractions from selected plants were subjected to HPTLC fingerprint profiles. The extracts and fractions were analyzed by using different solvent systems of different polarity and at three different wavelengths. The solvent systems were selected on the basis of phytoconstituents present in the plants. The HPTLC studies confirm presence of different phytoconstituents present in the plant drug. The results are represented in the following section.

Figure 7: HPTLC profile for successive extracts of *P. integerrima* in solvent system 1(254 nm)

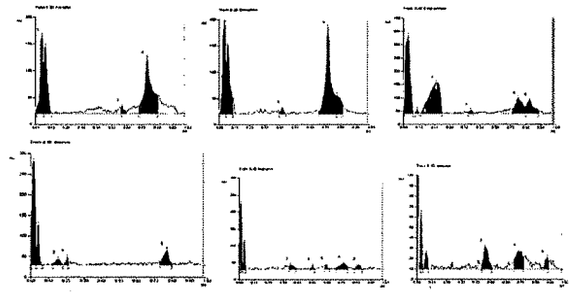


Table 11: HPTLC profile for successive extracts of *P. integerrima* in solvent system 1(254 nm)

Track	Extract	Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
1	Pet Ether	1	0.03	3.4	0.07	145.5	37.3	0.08	83.8	2392.7	26.95
		2	0.08	83.8	0.09	124.7	32.15	0.13	1.3	2118.2	23.86
		3	0.59	10.8	0.60	14.2	3.67	0.63	3.4	216.6	2.44
		4	0.71	14.4	0.76	103.3	26.65	0.83	33.5	4150.1	46.75
2	Chloroform	1	0.03	01.0	0.06	178.1	34.39	0.07	81.5	2565.1	23.85
		2	0.07	81.5	0.08	128.9	24.90	0.12	30.7	2426.3	22.56
		3	0.42	3.4	0.45	11.6	2.23	0.47	0.7	196.1	1.82
		4	0.68	4.6	0.75	163.7	31.61	0.80	33.6	4625.0	43.00
		5	0.80	33.6	0.81	35.6	6.87	0.85	16.1	944.4	8.78
3	Ethyl acetate	1	0.03	16.6	0.06	291.6	54.54	0.09	1.6	7064.6	39.32
		2	0.10	2.3	0.11	11.2	2.09	0.13	0.4	135.0	0.75
		3	0.14	0.1	0.24	115.9	21.68	0.28	0.3	6507.4	36.22
		4	0.45	3.9	0.47	11.7	2.18	0.49	2.7	176.6	0.98
		5	0.74	16.7	0.78	55.0	10.29	0.83	24.1	2332.6	12.98
		6	0.83	24.1	0.86	49.3	0.92	0.92	96	1748.7	9.73
4	Acetone	1	0.03	28.9	0.05	252.1	60.78	0.06	40.6	3558.6	61.59
		2	0.06	0.07	0.07	96.3	23.22	0.10	0.0	901.5	15.60
		3	0.15	0.19	0.19	13.5	3.26	0.21	0.4	315.6	5.46
		4	0.21	0.24	0.24	18.5	4.47	0.24	3.8	155.9	2.70
		5	0.76	6.7	0.80	34.3	8.27	0.83	3.2	846.6	14.65
5	Methanol	1	0.04	184.0	0.04	194.0	54.66	0.05	3.6	1182.6	34.14
		2	0.05	5.6	0.06	83.7	23.20	0.07	0.0	647.3	18.71
		3	0.37	8.9	0.38	15.7	4.42	0.42	2.6	345.7	9.99
		4	0.51	0.8	0.53	12.8	3.59	0.55	0.5	116.6	3.37
		5	0.61	6.3	0.62	15.2	4.30	0.65	1.4	86.8	5.40
		6	0.69	2.7	0.74	19.9	4.62	0.77	7.9	685.9	19.82
6	Aqueous	1	0.05	2.8	0.06	54.3	44.82	0.07	0.7	392.7	19.80
		2	0.07	0.0	0.09	15.9	13.16	0.10	0.3	203.5	10.25
		3	0.47	3.5	0.49	22.2	18.16	0.54	1.2	555.8	28.01
		4	0.68	7.4	0.72	17.6	14.56	0.75	9.2	669.5	33.44
		5	0.89	0.3	0.91	11.1	9.16	0.92	7.6	168.6	8.50

Figure 8: HPTLC profile for successive extracts of *P. integerrima* in solvent system 1(366 nm)

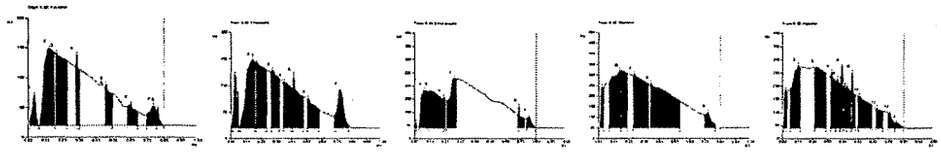


Table 12: HPTLC profile for successive extracts of *P. integerrima* in solvent system 1(366 nm)

Track	Extract	Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
1	Pet Ether	1	0.04	7.7	0.07	52.4	8.89	0.09	0.2	904.8	4.69
		2	0.09	0.2	0.15	129.3	21.96	0.18	121.3	6151.0	31.88
		3	0.19	121.3	0.19	122.1	20.74	0.26	108.1	5728.0	29.69
		4	0.31	97.9	0.31	117.9	20.01	0.33	93.1	1927.1	9.99
		5	0.48	64.0	0.49	67.1	11.39	0.52	52.0	1742.1	9.03
		6	0.61	31.9	0.63	36.4	5.19	0.67	23.4	1371.3	7.11
		7	0.72	17.9	0.76	32.6	5.54	0.78	21.8	1127.6	5.84
		8	0.78	21.8	0.79	31.0	5.27	0.82	1.0	344.0	7.78
2	Chloroform	1	0.04	17.3	0.06	99.2	12.13	0.08	0.4	1858.8	6.07
		2	0.09	1.3	0.17	18.3	16.46	0.19	12.37	6421.5	20.97
		3	0.19	123.7	0.20	126.2	16.20	0.26	108.6	6092.8	19.89
		4	0.29	104.2	0.30	109.5	14.05	0.35	92.2	3936.3	12.85
		5	0.38	86.1	0.38	87.6	11.20	0.43	74.8	3446.2	11.25
		6	0.43	74.1	0.44	99.1	12.72	0.51	58.1	3703.0	12.09
		7	0.53	53.8	0.54	56.8	7.29	0.61	35.0	2554.6	8.34
		8	0.71	23.8	0.75	72.6	4.32	0.81	5.0	2612.8	8.53
4	Acetone	1	0.03	28.9	0.05	252.1	60.78	0.06	40.6	3558.6	61.59
		2	0.06	0.07	0.07	96.3	23.22	0.10	0.0	901.5	15.60
		3	0.15	0.19	0.19	13.5	3.26	0.21	0.4	315.6	5.46
		4	0.21	0.24	0.24	18.5	4.47	0.24	3.8	155.9	2.70
		5	0.76	6.7	0.80	34.3	8.27	0.83	3.2	846.6	14.65
5	Methanol	1	0.04	184.0	0.04	194.0	54.66	0.05	3.6	1182.6	34.14
		2	0.05	5.6	0.06	83.7	23.20	0.07	0.0	647.3	18.71
		3	0.37	8.9	0.38	15.7	4.42	0.42	2.6	345.7	9.99
		4	0.51	0.8	0.53	12.8	3.59	0.55	0.5	116.6	3.37
		5	0.61	6.3	0.62	15.2	4.30	0.65	1.4	86.8	5.40
		6	0.69	2.7	0.74	19.9	4.62	0.77	7.9	685.9	19.82
		7	0.82	1.1	0.84	13.5	3.81	0.87	0.3	295.9	8.55
6	Aqueous	1	0.03	7.3	0.05	140.3	6.85	0.06	130.5	1782.9	3.26
		2	0.08	150.0	0.13	237.9	11.62	0.14	228.7	4334.8	17.01
		3	0.24	229.0	0.25	230.0	11.23	0.32	206.5	12326.1	22.57
		4	0.35	193.8	0.36	211.6	10.33	0.38	177.2	4761.2	8.72
		5	0.38	177.2	0.40	183.4	8.96	0.41	168.3	3868.6	7.08

HPTLC profile for successive extracts of *P. integerrima* in solvent system 1(366 nm) cont

Track	Extract	Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
		6	0.42	166.4	0.42	236.2	11.53	0.43	159.6	2842.6	5.21
		7	0.43	159.6	0.44	166.1	8.11	0.45	152.6	2222.8	4.07
		8	0.45	152.2	0.46	156.7	7.65	0.49	135.0	3527.7	6.46
		9	0.49	135.0	0.49	210.2	10.27	0.51	125.7	2989.7	5.47
		10	0.53	120.9	0.54	122.6	5.09	0.62	83.6	6931.6	12.69
		11	0.65	70.1	0.65	73.9	3.61	0.69	62.6	2306.6	4.22
		12	0.73	54.4	0.74	57.7	2.82	0.71	20.0	1205.9	2.21
		13	0.77	19.7	0.78	21.0	1.03	0.83	1.5	514.5	0.94

Figure 9: HPTLC fingerprint for successive extracts of *P. integerrima* in solvent system 1 (540 nm)

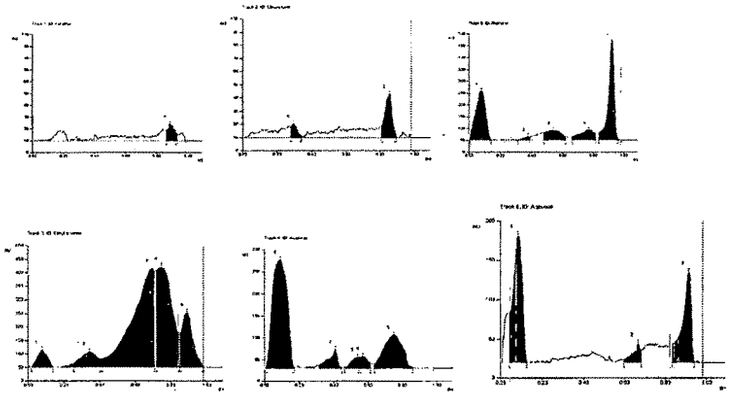


Table 13: HPTLC profile for successive extracts of *P. integerrima* in solvent system 1(540 nm)

Track	Extract	Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
1	Pet Ether	1	0.03	0.7	0.05	63.0	82.30	0.07	1.1	1000.6	94.27
		2	0.31	0.0	0.32	13.5	17.70	0.33	0.0	60.8	5.73
2	Chloroform	1	0.03	0.3	0.05	65.5	100	0.09	0.1	1228.3	100
3	Ethyl acetate	1	0.07	0.4	0.08	27.5	105.2	0.10	0.4	389.7	3.11
		2	0.11	0.4	0.22	218.1	83.53	0.25	0.1	11979.4	95.53
		3	0.26	0.0	0.29	15.5	5.93	0.30	0.0	170.9	1.36
4	Acetone	1	0.04	1.6	0.05	48.5	65.36	0.07	0.0	333.4	33.90
		2	0.12	0.04	0.16	25.7	34.64	0.18	0.4	450.2	66.10
5	Methanol	1	0.04	2.2	0.05	59.4	82.81	0.06	0.6	397.1	81.08
		2	0.25	2.7	0.26	12.3	17.19	0.27	0.0	92.6	18.92
6	Aqueous	1	0.04	0.0	0.05	29.7	57.82	0.06	0.07	210.3	40.65
		2	0.07	0.0	0.09	21.7	42.18	0.10	0.1	307.0	59.35

Figure 10: HPTLC fingerprint for successive extracts of *P. integerrima* in solvent system 2 (254 nm)

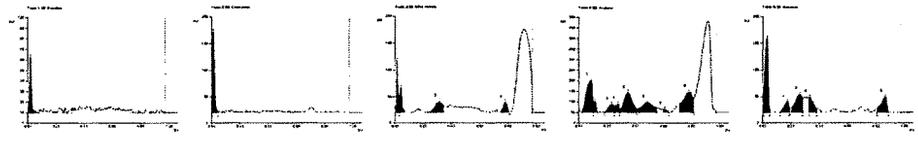


Table 14: HPTLC profile for successive extracts of *P. integerrima* in solvent system 2(254 nm)

Track	Extract	Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
1	Pet Ether	1	0.03	1.9	0.05	25.8	100	0.08	0.8	546.2	100
2	Chloroform	1	0.03	6.9	0.04	151.3	100	0.08	2.3	1643.8	100
3	Ethyl acetate	1	0.04	16.6	0.04	97.1	52.95	0.06	31.0	803.7	29.88
		2	0.06	31.0	0.07	47.3	25.77	0.10	0.4	677.9	25.20
		3	0.27	0.3	0.35	20.5	11.20	0.38	13.4	784.2	29.15
		4	0.78	0.5	0.81	18.5	10.07	0.84	0.0	424.2	15.77
4	Acetone	1	0.05	0.7	0.12	150.4	28.41	0.14	45.8	5214.4	26.04
		2	0.14	45.8	0.14	52.6	9.94	0.16	0.1	60.0	3.01
		3	0.21	0.2	0.26	38.1	7.19	0.29	31.1	1174.9	5.87
		4	0.29	31.1	0.31	40.4	7.62	0.32	14.5	797.1	3.98
		5	0.32	14.5	0.38	91.4	17.25	0.44	15.2	4102.6	20.50
		6	0.44	15.2	0.52	46.5	8.78	0.58	22.4	2991.0	14.94
		7	0.63	12.6	0.64	14.1	2.66	0.67	0.2	251.3	1.26
		8	0.75	42.8	0.81	96.0	18.14	0.85	68.7	4884.2	24.40
		2	0.16	3.0	0.20	16.1	3.69	0.22	7.0	445.1	3.06
5	Methanol	1	0.04	1.6	0.07	123.5	28.24	0.09	0.1	2179.8	15.01
		2	0.16	3.0	0.20	16.1	3.69	0.22	7.0	445.1	3.06
		3	0.23	4.0	0.29	42.1	9.63	0.30	34.4	1421.8	9.79
		4	0.39	4.7	0.40	42.7	9.76	0.51	1.5	1938.1	13.35
		5	0.68	7.1	0.72	19.8	4.52	0.76	2.5	6306.0	4.35
		6	0.80	8.9	0.91	193.2	44.17	0.96	0.2	7907.8	54.45
6	Aqueous	1	0.03	4.4	0.06	139.4	55.02	0.09	5.7	2680.2	43.08
		2	0.15	0.2	0.21	20.9	8.26	0.22	10.0	562.8	9.05
		3	0.24	8.2	0.29	34.3	13.54	0.32	27.1	1296.6	20.84
		4	0.36	26.0	0.36	29.5	11.66	0.41	5.0	721.1	11.59
		5	0.84	9.8	0.90	29.2	11.52	0.92	00	960.7	15.44

Figure 11: HPTLC fingerprint for successive extracts of *P. integerrima* in solvent system 2(366 nm)

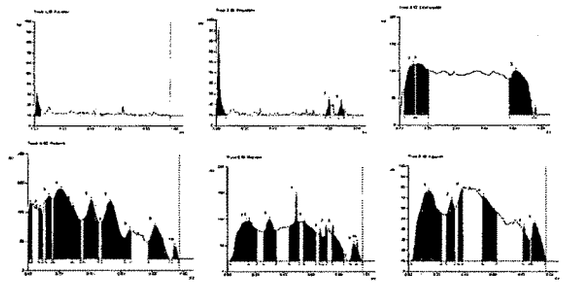


Table 15: HPTLC profile for successive extracts of *P. integerrima* in solvent system 2(366 nm)

Track	Extract	Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
1	Pet Ether	1	0.03	0.5	0.05	19.7	10	0.08	5.7	314.7	100
2	Chloroform	1	0.03	5.5	0.04	79.7	74.95	0.10	0.1	909.0	72.28
		2	0.81	0.2	0.83	15.0	14.14	0.84	8.2	167.1	13.28
		3	0.90	1.8	0.92	11.6	10.91	0.94	4.0	181.5	14.43
3	Ethyl acetate	1	0.06	46.1	0.13	93.1	32.97	0.14	92.0	4165.3	24.95
		2	0.15	92.4	0.17	94.1	33.34	0.24	81.9	5463.1	32.73
		3	0.81	65.6	0.86	81.4	28.82	0.98	0.3	6981.2	41.82
4	Acetone	1	0.04	83.7	0.05	94.6	11.39	0.05	94.2	1110.8	3.23
		2	0.10	85.4	0.11	87.8	10.56	0.12	75.0	1518.5	4.42
		3	0.14	100.8	0.17	108.1	13.01	0.18	104.5	2834.8	8.25
		4	0.19	104.4	0.25	119.5	14.38	0.31	87.9	8897.0	25.89
		5	0.31	87.9	0.32	92.8	11.16	0.37	65.9	2865.8	8.34
		6	0.39	67.7	0.44	100.6	12.11	0.48	62.9	5347.6	15.56
		7	0.50	63.2	0.56	99.3	11.95	0.65	35.8	7090.4	20.63
		8	0.65	35.8	0.69	48.8	5.87	0.69	47.6	1207.8	3.51
		9	0.80	29.0	0.85	58.0	6.97	0.94	0.2	3182.7	9.26
5	Methanol	1	0.05	0.1	0.16	76.9	10.97	0.18	75.7	4441.0	17.94
		2	0.18	75.7	0.19	78.8	11.25	0.24	61.1	2858.2	11.55
		3	0.29	56.8	0.33	80.7	11.52	0.38	59.7	4214.7	17.03
		4	0.47	66.2	0.52	129.2	18.43	0.55	75.0	3921.5	15.85
		5	0.58	74.8	0.58	77.8	11.10	0.67	55.3	3946.3	15.95
		6	0.69	51.1	0.70	56.3	8.04	0.72	44.4	892.9	3.61
		7	0.72	44.4	0.74	66.1	9.43	0.75	55.6	1113.0	4.50
		8	0.78	52.1	0.79	66.2	9.44	0.87	13.6	2271.2	9.148
		9	0.91	0.0	0.94	32.2	4.60	0.96	26.5	659.4	4.50
6	Aqueous	1	0.05	0.0	0.18	67.2	20.72	0.27	44.9	6836.4	41.3
		2	0.30	44.1	0.34	58.1	17.91	0.36	47.8	2179.0	3.19
		3	0.38	56.4	0.41	67.8	20.90	0.41	65.4	1390.6	8.42
		4	0.55	61.3	0.56	61.8	19.20	0.66	46.7	3681.9	22.29
		5	0.84	29.2	0.85	32.8	10.12	0.89	19.5	825.6	5.00

Figure 12: HPTLC fingerprint for successive extracts of *P. integerrima* in solvent system 2 (540 nm)

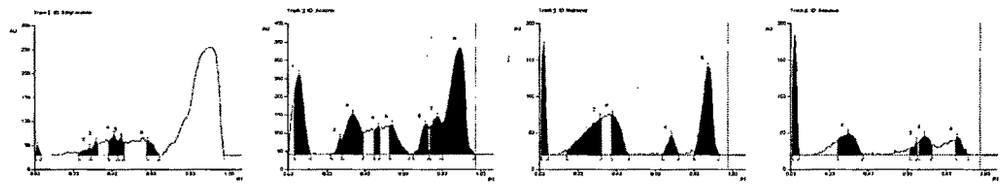


Table 16: HPTLC profile for successive extracts of *P. integerrima* in solvent system 2(540 nm)

Track	Extract	Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
1	Ethyl acetate	1	0.03	1.8	0.04	19.8	11.50	0.06	0.2	228.6	6.03
		2	0.26	4.9	0.31	15.6	9.05	0.32	13.6	430.0	11.35
		3	0.32	13.6	0.35	28.6	16.63	0.36	23.2	507.3	13.39
		4	0.41	29.5	0.44	40.8	23.74	0.46	29.4	1135.5	29.96
		5	0.46	29.4	0.48	37.3	21.68	0.49	24.4	667.2	17.61
		6	0.61	28.0	0.61	29.9	17.40	0.67	0.1	821.1	21.67
2	Acetone	1	0.06	157.3	0.09	218.8	21.62	0.15	0.1	7418.3	17.37
		2	0.25	0.7	0.30	44.7	4.42	0.31	40.4	782.5	1.83
		3	0.31	40.4	0.37	112.2	11.08	0.42	6.6	6150.6	14.40
		4	0.48	63.8	0.50	77.5	7.66	0.51	72.5	1555.9	3.64
		5	0.56	76.6	0.57	80.9	8.00	0.66	0.4	2765.5	6.47
		6	0.68	1.0	0.75	82.5	8.15	0.77	75.6	2504.4	5.86
		7	0.77	75.6	0.81	102.3	10.11	0.83	87.1	4027.8	9.43
3	Methanol	1	0.04	70.0	0.05	150.2	36.97	0.07	1.1	2284.0	17.04
		2	0.17	5.6	0.34	51.0	12.54	0.35	49.9	3489.4	26.03
		3	0.40	54.1	0.41	56.4	13.87	0.50	0.0	2026.0	15.11
		4	0.66	1.0	0.71	27.1	6.67	0.75	2.2	795.9	5.94
		5	0.83	4.6	0.91	121.7	29.96	0.96	1.0	4809.9	35.88
4	Aqueous	1	0.04	84.3	0.05	188.3	61.53	0.07	0.1	2385.4	38.65
		2	0.27	20.5	0.33	29.8	11.59	0.40	0.0	1806.2	29.27
		3	0.64	8.2	0.68	18.3	7.12	0.68	16.3	351.3	5.69
		4	0.69	17.6	0.72	26.5	10.29	0.76	13.3	985.1	15.96

Figure 13: HPTLC fingerprint for successive extracts of *P. integerrima* in solvent system 3 (254 nm)

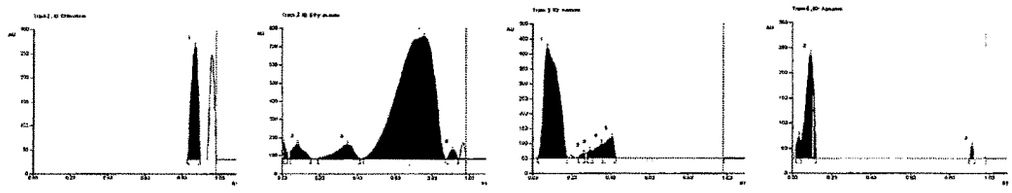


Table 17: HPTLC profile for successive extracts of *P. integerrima* in solvent system 3(254 nm)

Track	Extract	Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
1	Chloroform	1	0.86	2.2	0.90	235.5	100	0.92	9.4	7255.7	100
2	Ethyl acetate	1	0.03	87.4	0.04	87.4	9.04	0.06	28.5	934.8	0.75
		2	0.07	42.4	0.11	80.4	8.30	0.18	1.6	3907.9	3.12
		3	0.22	1.4	0.37	76.9	7.94	0.44	1.8	5916.2	4.12
		4	0.46	1.7	0.78	673.4	69.59	0.90	0.2	113391.0	90.41
		5	0.91	0.2	0.94	49.6	5.13	0.97	0.1	1266.9	1.01
3	Acetone	1	0.05	0.5	0.10	367.3	70.16	0.21	0.4	23428.8	83.07
		2	0.26	0.2	0.29	14.5	2.77	0.29	12.5	242.3	0.86
		3	0.31	16.1	0.32	24.9	4.76	0.34	24.1	517.4	1.83
		4	0.34	24.1	0.38	46.2	8.82	0.39	43.9	1931.2	4.72
		5	0.39	43.9	0.43	70.6	13.48	0.46	0.9	2682.9	9.51
4	Methanol	1	0.04	4.9	0.11	315.0	40.21	0.17	3.8	17531.0	56.75
		2	0.91	3.5	0.95	468.3	59.79	0.98	4.2	13359.5	43.25
5	Aqueous	1	0.05	27.0	0.06	43.4	16.75	0.07	33.3	691.7	8.73
		2	0.07	33.3	0.12	206.6	79.76	0.15	2.7	7049.2	89.0
		3	0.92	0.0	0.94	8.79	3.39	0.95	0.0	179.4	2.27

Figure 14: HPTLC fingerprint for successive extracts of *P. integerrima* in solvent system 3 (366 nm)

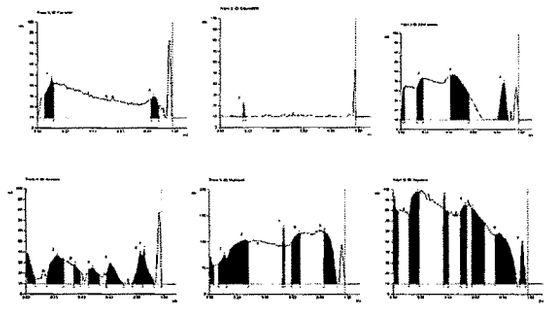


Table 18: HPTLC profile for successive extracts of *P. integerrima* in solvent system 3(366 nm)

Track	Extract	Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
1	Pet Ether	1	0.08	21.3	0.13	35.7	63.79	0.14	33.1	1454.3	67.35
		2	0.85	16.5	0.87	20.3	36.21	0.91	8.5	705.1	32.65
2	Chloroform	1	0.19	1.1	0.28	11.8	100	0.21	1.0	76.6	100
3	Ethyl acetate	1	0.03	16.6	0.05	33.2	20.53	0.07	0.5	456.1	5.60
		2	0.16	32.1	0.20	43.1	26.60	0.21	42.7	1660.1	20.40
		3	0.44	46.1	0.47	46.8	28.90	0.55	26.0	45960.0	56.48
		4	0.83	0.1	0.88	38.8	23.97	0.9	0.1	1425.2	17.51
4	Acetone	1	0.03	28.7	0.04	29.4	17.36	0.10	4.7	777.6	11.51
		2	0.18	4.2	0.25	26.9	15.90	0.30	2.3	1969.3	29.14
		3	0.37	16.7	0.38	18.1	10.71	0.42	11.3	536.2	7.93
		4	0.48	10.8	0.51	14.7	8.71	0.5	8.6	748.8	1.08
		5	0.61	4.6	0.64	19.7	11.55	0.73	0.1	950.3	14.06
		6	0.80	0.4	0.86	28.0	16.51	0.87	25.7	906.6	13.42
		7	0.87	25.7	0.88	32.6	19.26	0.96	0.0	868.7	12.39
5	Methanol	1	0.03	50.9	0.04	50.9	10.27	0.06	36.1	810.8	3.62
		2	0.09	37.7	0.14	56.2	11.34	0.15	49.4	2300.8	10.28
		3	0.15	49.4	0.29	83.5	16.86	0.31	79.2	8634.7	38.58
		4	0.56	71.3	0.56	108.7	21.94	0.57	71.9	1114.7	4.98
		5	0.62	77.9	0.68	96.4	19.46	0.68	96.2	4490.9	20.06
		6	0.85	97.9	0.86	99.7	20.13	0.95	0.1	5030.9	22.48
6	Aqueous	1	0.03	81.7	0.04	81.7	16.68	0.06	68.6	1664.6	8.13
		2	0.14	66.6	0.21	88.1	17.99	0.22	86.7	4544.5	22.10
		3	0.39	72.1	0.40	85.4	17.43	0.43	68.7	2177.5	10.64
		4	0.52	67.8	0.56	76.1	15.54	0.57	75.1	2626.6	12.83
		5	0.60	69.8	0.61	72.4	14.79	0.70	57.8	4574.4	22.35
		6	0.77	46.7	0.80	48.1	9.82	0.93	0.2	4215.3	20.59

Figure 15: HPTLC fingerprint for successive extracts of *P. integerrima* in solvent system 3 (540 nm)

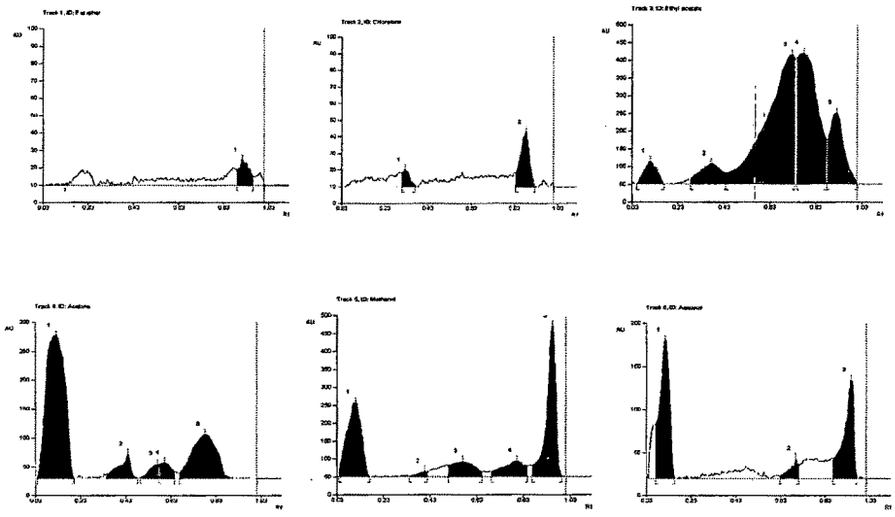


Table 19: HPTLC profile for successive extracts of *P. integerrima* in solvent system 3(540 nm)

Track	Extract	Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
1	Pet Ether	1	0.89	8.7	0.91	14.8	100	0.96	5.4	561.2	100
2	Chloroform	1	0.31	8.6	0.23	10.6	24.37	0.37	0.8	311.5	20.88
		2	0.83	8.6	0.88	33.0	75.63	0.92	0.4	1180.1	79.12
3	Ethyl acetate	1	0.05	0.1	0.11	62.8	5.95	0.17	0.2	2971.9	3.59
		2	0.28	14.0	0.37	56.5	5.35	0.44	30.6	4554.6	5.51
		3	0.44	30.6	0.73	365.3	34.58	0.74	353.7	37428.5	5.26
		4	0.75	354.5	0.78	370.9	35.11	0.88	125.9	2697.1	32.62
4	Acetone	1	0.03	0.5	0.12	248.1	58.89	0.20	0.7	18299.1	62.02
		2	0.34	6.2	0.44	43.3	10.27	0.48	0.0	1874.4	6.35
		3	0.49	2.2	0.57	25.5	6.06	0.57	24.3	918.8	3.11
		4	0.57	24.3	0.60	27.4	6.52	0.64	12.1	1178.5	3.99
5	Methanol	1	0.03	3.5	0.11	207.6	28.54	0.17	0.1	11248.7	34.59
		2	0.34	101	0.41	13.1	1.81	0.42	10.5	447.5	1.38
		3	0.50	31.7	0.57	41.9	5.76	0.65	12.7	3600.5	11.07
		4	0.69	14.1	0.80	44.0	6.05	0.84	28.9	3456.7	10.63
		5	0.6	31.8	0.95	420.7	57.58	0.99	1.3	13764.7	42.33
6	Aqueous	2	0.63	3.8	0.70	23.7	7.90	0.71	16.1	741.5	6.66
		3	0.86	23.8	0.94	114.9	38.32	0.97	0.3	4234.9	38.02

Figure 16: HPTLC fingerprint for successive extracts of *H. spicatum* in solvent system 1(254 nm)

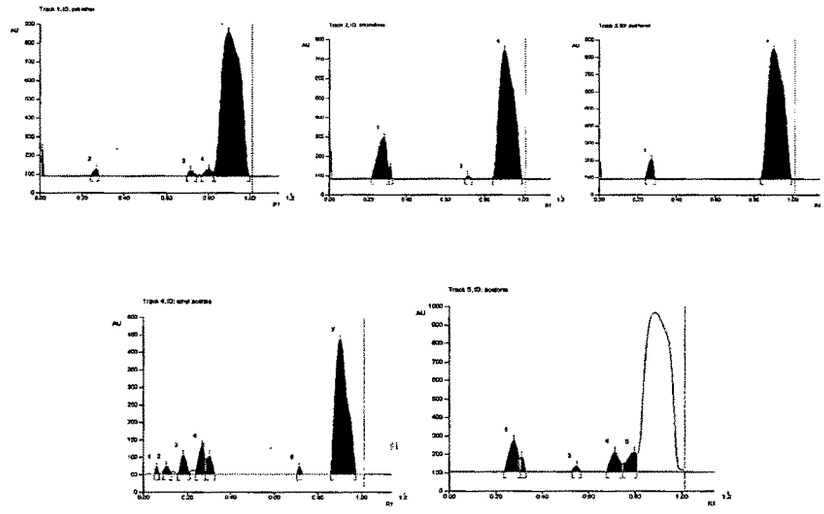


Table 20: HPTLC profile for successive extracts of *H. spicatum* in solvent system 1(254 nm)

Track	Extract	Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
1	Pet Ether	1	0.01	128.4	0.01	144.0	14.40	0.02	0.0	675.4	1.15
		2	0.24	0.0	0.27	33.5	3.35	0.28	2.6	545.3	0.93
		3	0.70	0.5	0.71	26.3	2.63	0.74	4.3	542.5	0.92
		4	0.77	0.1	0.80	35.6	3.56	0.82	20.8	885.1	1.51
2	Chloroform	1	0.24	1.2	0.28	215.3	22.54	0.31	54.8	8305.6	16.87
		2	0.31	54.8	0.31	62.3	6.52	0.33	2.5	674.0	1.37
		3	0.70	0.0	0.71	17.2	1.81	0.74	0.8	189.1	0.38
3	Methanol	1	0.24	0.27	0.27	112.1	12.99	0.29	1.1	2554.9	4.89
4	Ethyl acetate	1	0.05	0.6	0.06	20.6	3.24	0.07	0.8	202.2	0.89
		2	0.09	7.7	0.10	21.8	3.44	0.13	4.8	424.6	1.87
		3	0.15	1.7	0.18	58.7	8.62	0.21	7.1	1202.2	5.30
		4	0.24	10.9	0.27	81.0	12.76	0.29	43.5	1894.4	8.35
		5	0.29	43.5	0.30	52.7	8.30	0.33	1.5	1117.2	4.93
		6	0.70	0.1	0.72	20.8	3.27	0.73	1.2	229.2	1.01
		7	0.86	5.4	0.90	383.6	6.38	0.98	4.1	17607.4	77.61
5	Acetone	1	0.24	1.5	0.28	165.8	35.12	0.30	71.0	5072.1	38.90
		2	0.30	71.0	0.31	74.4	15.75	0.33	2.8	1072.2	8.10
		3	0.53	0.7	0.55	25.4	5.38	0.57	0.0	447.5	3.40
		4	0.68	1.0	0.71	104.5	22.15	0.75	41.8	3153.4	24.18
		5	0.75	41.8	0.80	101.9	21.59	0.81	95.5	3311.2	25.39

Figure 17: HPTLC fingerprint for successive extracts of *H. spicatum* in solvent system 1(540 nm)

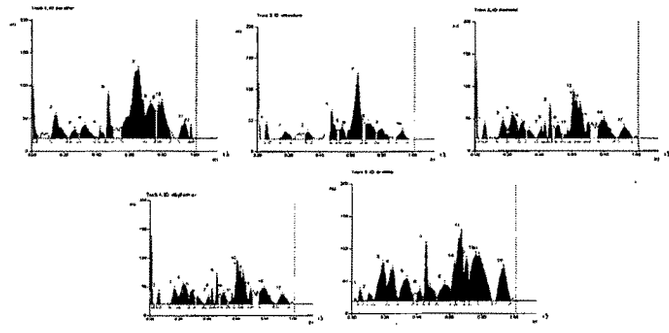


Table 21: HPTLC profile for successive extracts of *H. spicatum* in solvent system 1(540 nm)

Track	Extract	Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
1	Pet Ether	1	0.01	80.2	0.01	80.2	15.20	0.03	4.7	501.0	3.40
		2	0.12	0.2	0.15	35.8	6.78	0.22	1.4	1158.5	7.86
		3	0.24	2.5	0.27	12.4	2.34	0.29	5.9	331.1	2.25
		4	0.30	6.1	0.33	20.0	3.79	0.39	1.4	742.6	5.04
		5	0.41	0.5	0.42	12.7	2.41	0.46	0.1	227.6	1.54
		6	0.46	0.4	0.47	67.0	12.70	0.50	12.6	908.7	6.17
		7	0.55	15.4	0.66	103.9	19.69	0.70	32.2	5949.2	40.30
		8	0.70	32.2	0.73	52.1	9.89	0.76	42.0	1978.1	13.43
		9	0.77	39.8	0.75	50.9	9.65	0.79	48.4	697.4	4.73
		10	0.79	48.4	0.80	55.6	10.52	0.85	8.9	643.0	10.27
		11	0.90	1.7	0.94	21.4	4.05	0.97	0.5	643.0	4.36
		12	0.97	0.6	0.98	16.0	3.02	0.99	0.2	83.1	0.56
2	Chloroform	1	0.05	0.0	0.06	24.2	8.56	0.08	0.1	266.2	3.66
		2	0.14	2.0	0.18	11.4	4.04	0.22	0.4	360.7	4.95
		3	0.31	8.4	0.32	11.0	3.89	0.35	3.8	250.1	3.44
		4	0.41	1.1	0.48	45.5	16.08	0.51	14.0	775.0	10.64
		5	0.53	13.6	0.55	18.8	6.66	0.57	1.3	423.5	5.82
		6	0.57	1.3	0.59	15.5	5.48	0.60	12.6	167.1	2.29
		7	0.60	12.6	0.65	101.1	35.70	0.68	33.1	3017.8	41.45
		8	0.70	22.8	0.72	25.9	9.16	0.77	10.9	1060.9	14.57
		9	0.77	10.3	0.80	15.9	5.61	0.85	2.6	604.7	8.30
		10	0.89	0.7	0.94	13.6	4.82	0.97	0.5	355.5	4.88
3	Methanol	1	0.01	118.1	0.01	118.1	19.57	0.03	0.0	526.7	5.11
		2	0.04	0.1	0.06	21.3	3.53	0.08	0.1	240.5	2.33
		3	0.13	0.0	0.17	27.2	4.51	0.20	13.5	670.9	6.51
		4	0.20	13.5	0.23	34.9	5.77	0.29	14.2	1297.7	12.58
		5	0.27	14.2	0.29	23.6	3.91	0.31	11.1	544.2	5.28
		6	0.33	11.2	0.34	12.3	2.03	0.38	0.2	272.7	2.64
		7	0.39	1.3	0.41	13.5	2.23	0.42	8.0	229.4	2.22

		8	0.42	8.0	0.43	21.3	3.54	0.45	0.7	220.1	2.13
		9	0.45	0.4	0.47	47.7	7.90	0.48	0.9	443.8	4.30
		10	0.50	14.2	0.51	20.6	3.41	0.54	6.3	203.6	4.30
		11	0.55	2.5	0.57	13.1	2.117	0.59	9.8	1138.7	1.97
		12	0.59	9.8	0.61	69.5	11.52	0.62	56.0	783.0	11.04
		13	0.62	56.0	0.63	58.1	9.63	0.64	44.8	1045.0	7.59
		14	0.64	44.8	0.65	53.2	8.81	0.69	7.7	336.3	10.13
		15	0.69	7.7	0.70	24.8	4.10	0.71	18.7	1335.2	3.26
		16	0.76	48.0	0.80	27.8	4.61	0.87	1.8	579.9	12.95
		17	0.89	0.7	0.93	16.6	2.75	0.97	1.3	355.5	5.62
4	Ethyl acetate	1	0.02	0.0	0.03	12.3	2.07	0.04	0.9	114.0	0.86
		2	0.05	0.6	0.06	26.6	4.47	0.09	0.1	337.1	2.56
		3	0.13	0.0	0.18	29.6	4.97	0.21	1.5	787.3	5.97
		4	0.21	1.5	0.25	49.0	8.22	0.29	12.7	1378.6	10.45
		5	0.29	12.7	0.32	36.2	6.08	0.37	11.0	1454.2	11.02
		6	0.31	11.0	0.41	21.5	3.60	0.43	10.8	567.2	4.30
		7	0.37	10.8	0.43	19.1	3.21	0.44	1.2	174.5	1.32
		8	0.43	2.1	0.46	96.9	16.66	0.50	25.7	1786.2	13.54
		9	0.45	25.7	0.51	30.4	5.10	0.55	4.7	658.7	4.99
		10	0.50	4.7	0.60	50.2	9.93	0.62	26.3	1148.30	8.70
		11	0.55	26.3	0.64	44.1	7.39	0.64	41.9	674.3	5.11
		12	0.62	41.9	0.65	46.5	7.79	0.66	29.3	648.0	4.91
		13	0.64	29.3	0.67	41.7	6.99	0.69	15.1	510.9	3.87
		14	0.66	15.1	0.71	39.0	6.54	0.74	23.0	1129.0	8.57
		15	0.69	23.0	0.77	33.6	5.64	0.85	1.1	281.1	11.69
		16	0.74	2.00	0.92	10.3	1.73	0.95	4.7	281.1	2.13
5	Acetone	1	0.04	0.1	0.05	17.1	2.33	0.07	0.0	188.8	0.96
		2	0.09	0.3	0.11	10.5	1.43	0.13	0.8	184.9	0.94
		3	0.13	0.8	0.19	56.7	7.73	0.22	23.3	1842.7	9.37
		4	0.22	23.3	0.25	49.1	6.69	0.28	3.7	1483.8	7.55
		5	0.28	3.7	0.34	34.2	4.666	0.38	9.6	1414.1	7.19
		6	0.40	11.5	0.42	17.7	2.41	0.44	0.3	324.6	1.65
		7	0.44	1.9	0.46	87.2	11.88	0.48	19.6	997.0	5.07
		8	0.48	19.6	0.48	20.2	.82	0.52	5.2	516.6	2.63
		9	0.52	5.2	0.57	25.5	3.48	0.61	18.5	1106.9	5.63
		10	0.61	18.5	0.63	57.6	7.86	0.64	55.6	1058.5	5.39
		11	0.64	55.6	0.67	104.8	14.28	0.69	43.3	2740.8	13.94
		12	0.9	43.3	0.71	62.9	8.57	0.72	32.1	1250.5	6.36
		13	0.72	32.1	0.76	70.4	9.60	0.78	67.0	2327.2	11.84
		14	0.78	67.0	0.79	69.1	9.42	0.87	1.2	2404.4	12.23
		15	0.89	0.2	0.94	50.2	6.85	0.98	0.3	1817.5	9.25

Figure 18: HPTLC fingerprint for successive extracts of *H. spicatum* in solvent system 2(254nm)

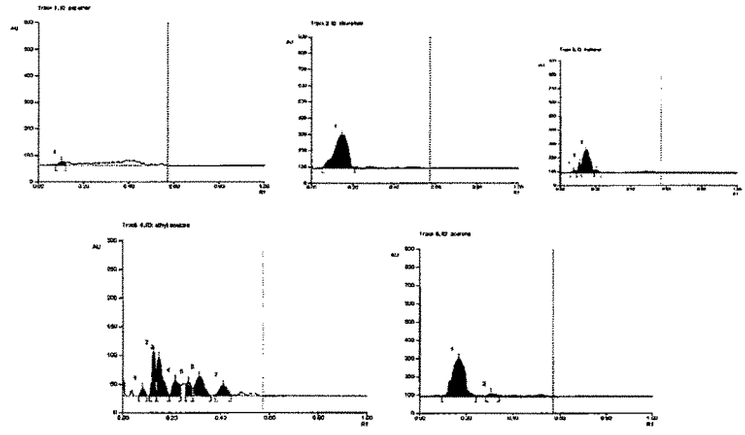


Table 22: HPTLC profile for successive extracts of *H. spicatum* in solvent system 2(254nm)

Track	Extract	Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
1	Pet Ether	1	0.07	0.7	0.10	15.1	100	0.12	8.6	323.0	100
2	Chloroform	1	0.05	2.3	0.15	204.3	100	0.21	3.6	10744.0	100
3	Methanol	1	0.06	0.1	0.08	16.0	5.91	0.09	10.5	256.8	3.78
		2	0.09	10.5	0.11	71.2	26.25	0.12	47.9	762.6	11.24
		3	0.12	47.9	0.15	165.7	61.12	0.19	16.4	5443.5	80.23
		4	0.19	16.4	0.20	18.2	6.72	0.23	0.1	322.1	4.75
4	Ethyl acetate	1	0.06	0.2	0.8	13.1	5.02	0.10	0.5	184.7	3.72
		2	0.11	0.5	0.13	76.4	29.20	0.14	47.0	902.9	18.21
		3	0.14	47.0	0.15	67.3	25.72	0.19	0.8	1354.7	27.32
		4	0.19	0.8	0.21	27.5	10.49	0.24	19.3	600.9	12.12
		5	0.26	20.5	0.27	24.4	7.33	0.29	14.0	452.2	9.12
		6	0.29	14.0	0.31	33.8	12.91	0.36	0.1	965.9	19.48
		7	0.38	1.3	0.41	19.2	7.33	0.44	0.4	497.5	10.03
5	Acetone	1	0.10	3.2	0.17	20.51	93.58	0.24	4.2	9122.1	96.08
		2	0.28	0.4	0.31	14.1	6.42	0.34	2.4	317.8	3.92

Figure 19: HPTLC fingerprint for successive extracts of *H. spicatum* in solvent system 2(366nm)

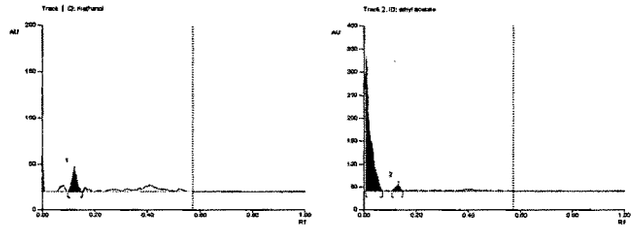


Table 23: HPTLC profile for successive extracts of *H. spicatum* in solvent system 2(366nm)

Track	Extract	Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
1	Methanol	1	0.10	0.1	0.12	22.7	100	0.0	350.7	246.6	100
2	Ethyl acetate	1	0.01	289.9	0.01	287.9	95.92	0.07	0.3	3877.9	95.10
		2	0.11	0.2	0.13	12.2	14.08	0.15	0.0	199.6	4.90

Figure 20: HPTLC fingerprint for successive extracts of *H. spicatum* in solvent system 2(540nm)

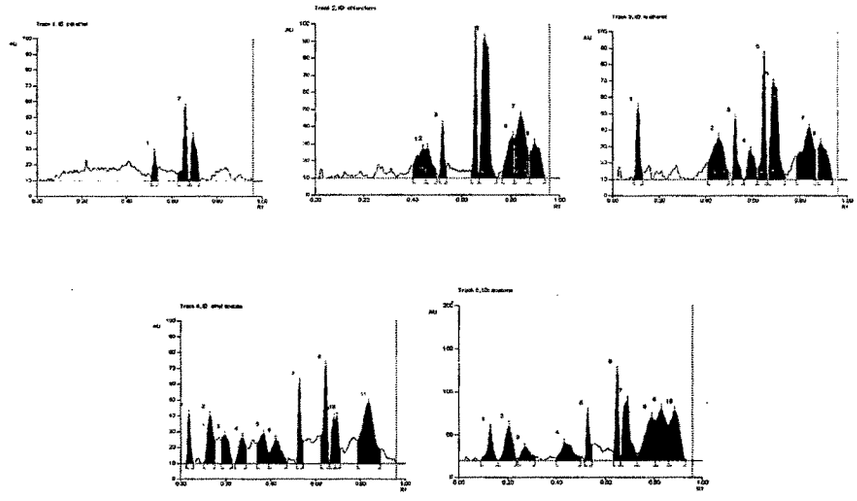


Table 24: HPTLC profile for successive extracts of *H. spicatum* in solvent system 2(540nm)

Track	Extract	Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
1	Pet Ether	1	0.51	2.8	0.52	17.9	19.46	0.54	8.3	225.9	17.51
		2	0.63	4.8	0.66	46.6	50.58	0.68	0.0	54834	42.50
		3	0.68	0.7	0.69	27.6	29.96	0.72	0.5	516.1	39.99
2	Chloroform	1	0.40	7.7	0.44	17.0	5.41	0.45	16.4	501.1	7.92
		2	0.45	16.4	0.46	17.3	5.50	0.50	2.6	359.6	5.69
		3	0.51	0.3	0.52	31.4	9.98	.54	6.8	322.8	5.11
		4	0.64	3.7	0.66	86.1	27.41	0.67	1.5	766.3	12.44
		5	0.67	1.5	0.69	81.6	25.97	0.74	0.3	1935.7	30.62
		6	0.77	3.8	0.81	24.5	7.79	0.82	22.9	599.0	9.47
		7	0.82	22.9	0.84	36.1	11.48	0.88	14.2	1168.7	18.48
		8	0.88	14.2	0.90	20.2	6.44	0.94	0.1	649.5	10.27
3	Methanol	1	0.09	0.1	0.11	43.2	13.86	0.13	3.9	496.3	7.35
		2	0.41	10.2	0.46	25.5	8.19	0.50	4.4	1072.2	15.88
		3	0.51	0.1	0.53	36.7	11.79	0.56	0.0	435.2	6.45
		4	0.56	0.1	0.59	17.9	5.76	0.62	7.1	430.2	6.37
		5	0.62	7.1	0.65	75.4	24.23	0.66	0.5	825.1	12.22
		6	0.67	0.8	0.69	58.9	18.91	0.74	2.9	1517.8	22.4
		7	0.79	14.1	0.84	31.5	10.13	0.87	17.9	1278.3	18.93
		8	0.88	18.7	0.89	22.2	7.14	0.94	0.2	497.0	10.32
4	Ethyl acetate	1	0.02	0.8	0.04	31.5	9.26	0.05	1.3	309.9	4.76
		2	0.10	0.1	0.13	30.3	8.91	0.15	14.1	612.9	9.42
		3	0.18	14.8	0.19	17.5	5.14	0.23	0.4	448.5	6.89
		4	0.24	0.2	0.28	16.4	4.83	0.29	10.6	376.8	5.79
		5	0.34	12.8	0.37	18.9	5.57	0.39	7.2	546.7	8.40
		6	0.39	7.2	0.42	15.2	4.48	0.47	0.7	517.0	7.95
		7	0.51	0.6	0.53	51.1	15.03	0.54	14.6	536.4	8.24
		8	0.62	17.0	0.65	61.7	18.15	0.66	0.2	772.7	11.87
		9	0.66	0.3	0.68	29.0	8.54	0.69	27.2	364.8	5.61
		10	0.69	27.2	0.70	30.0	8.84	0.71	8.8	344.6	5.30
		11	0.79	14.3	0.84	38.2	11.24	0.89	8.9	1676.9	25.77
5	Acetone	1	0.09	0.1	0.13	36.2	7.10	0.6	0.1	589.5	5.07
		2	0.16	0.1	0.20	40.0	7.86	0.23	2.6	947.2	8.15
		3	0.24	2.6	0.27	15.3	2.99	0.31	4.8	487.3	3.93
		4	0.40	3.5	0.43	21.3	4.18	0.50	3.5	823.4	7.08
		5	0.52	0.7	0.53	55.4	10.87	0.54	18.1	579.0	4.98
		6	0.63	10.2	0.65	103.7	20.35	0.66	2.9	1002.5	8.62
		7	0.66	05	0.69	70.0	13.72	0.73	8.6	1657.3	14.25
		8	0.73	8.6	0.79	50.8	9.96	0.81	46.0	1732.7	14.90
		9	0.81	46.0	0.83	59.7	11.71	0.86	40.7	1965.3	16.90

Figure 21: HPTLC fingerprint for successive extracts of *H. spicatum* in solvent system 3(254nm)

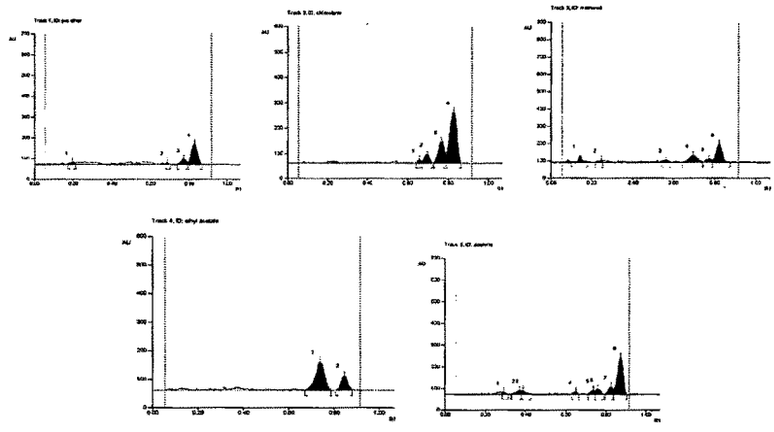


Table 25: HPTLC profile for successive extracts of *H. spicatum* in solvent system 3(254nm)

Track	Extract	Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
1	Pet Ether	1	0.17	2.2	0.20	11.4	7.71	0.21	7.6	205.6	6.32
		2	0.68	0.7	0.69	12.9	8.70	0.70	2.4	96.6	2.97
		3	0.74	0.4	0.77	26.1	17.60	0.79	10.2	570.8	17.56
		4	0.79	10.2	0.83	97.8	65.99	0.87	0.7	2377.6	73.14
2	Chloroform	1	0.64	0.2	0.66	12.1	3.61	0.67	8.5	134.2	1.70
		2	0.67	8.5	0.70	35.8	10.70	0.72	0.0	735.9	8.72
		3	0.73	0.0	0.77	85.6	25.59	0.79	39.9	2025.1	23.99
		4	0.79	39.9	0.83	201.0	60.10	0.87	0.6	5535.7	65.59
3	Methanol	1	0.10	0.0	0.14	35.8	16.67	0.18	2.9	483.4	10.70
		2	0.22	0.1	0.25	12.5	5.83	0.25	7.5	133.3	2.95
		3	0.54	5.4	0.57	11.5	5.35	0.59	0.1	228.4	5.05
		4	0.65	0.1	0.70	37.6	17.51	0.75	0.1	1123.7	24.87
		5	0.75	0.2	0.77	17.8	8.31	0.79	11.2	351.9	7.79
		6	0.79	11.2	0.82	99.5	46.33	0.88	0.0	2197.5	48.64
4	Ethyl acetate	1	0.67	0.2	0.74	97.5	66.03	0.79	0.0	3048.4	72.88
		2	0.81	0.1	0.85	50.1	33.97	0.88	0.6	1134.2	27.12
5	Acetone	1	0.28	7.6	0.29	10.2	3.24	0.31	0.0	117.1	1.87
		2	0.33	0.9	0.37	18.9	6.01	0.38	15.5	368.3	5.87
		3	.38	15.5	0.39	18.3	5.82	0.43	3.6	320.0	5.10
		4	0.63	0.8	0.65	13.0	4.14	0.67	1.6	184.3	2.94
		5	0.71	1.7	0.74	19.7	6.29	0.75	17.5	333.9	5.33
		6	0.75	17.5	0.76	26.2	8.34	0.79	0.0	449.1	7.16
		7	0.80	0.4	0.83	36.8	11.72	0.84	27.7	64.1	10.22
		8	0.84	27.7	0.86	170.9	54.44	0.91	0.5	3856.7	61.51

Figure 22: HPTLC fingerprint for successive extracts of *H. spicatum* in solvent system 3(366nm)

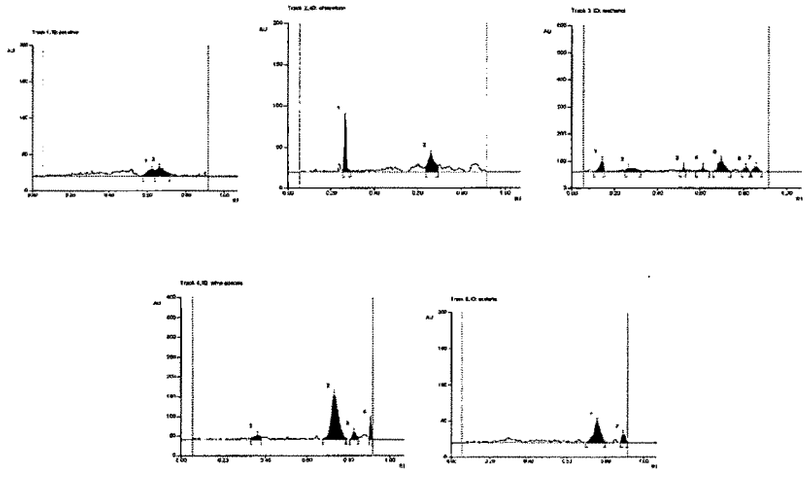


Table 26: HPTLC profile for successive extracts of *H. spicatum* in solvent system 3(366nm)

Track	Extract	Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
1	Pet Ether	1	0.58	0.6	0.63	10.1	45.48	0.54	8.3	280.7	39.36
		2	0.64	8.3	0.66	12.1	54.52	0.72	3.6	432.6	60.64
2	Chloroform	1	0.25	0.25	1.1	0.27	65.3	75.50	0.29	0.8	452.2
		2	0.64	5.0	0.66	21.2	24.50	0.70	6.9	473.7	51.16
3	Methanol	1	0.10	0.0	014	39.6	26.55	0.15	0.3	508.10	19.19
		2	0.25	1.0	0.26	11.4	7.64	0.32	2.1	388.8	14.69
		3	0.50	3.0	0.52	14.4	9.68	0.53	3.3	126.5	4.78
		4	0.58	1.8	.61	15.7	10.55	0.65	0.2	203.3	7.48
		5	0.66	0.5	0.70	38.1	25.54	0.74	0.1	924.2	34.92
		6	0.79	0.2	0.81	13.3	8.91	0.83	0.4	206.1	7.78
		7	0.84	0.4	0.86	16.6	11.14	0.89	0.2	290.0	10.95
4	Ethyl acetate	1	0.33	3.9	0.36	10.7	0.71	0.38	6.5	258.0	6.87
		2	0.68	0.1	0.73	112.7	59.96	0.79	3.0	3018.8	80.41
		3	0.81	0.2	0.83	17.8	9.47	0.85	3.6	254.3	6.77
		4	0.90	1.8	0.91	46.7	24.84	0.92	1.0	223.0	5.94
5	Acetone	1	0.70	0.0	0.76	28.1	71.31	0.80	0.9	776.3	83.55
		2	0.88	0.1	0.89	11.3	28.69	0.92	0.4	152.8	16.45

Figure 23: HPTLC fingerprint for successive extracts of *H. spicatum* in solvent system 3(540nm)

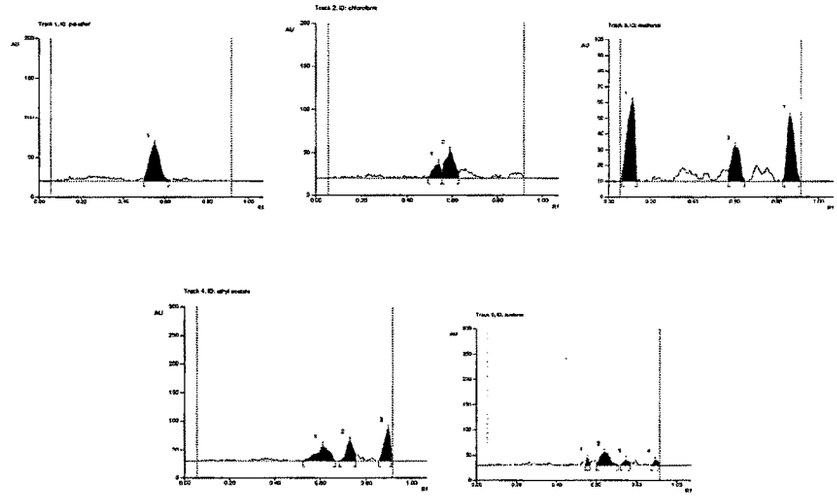


Table 27: HPTLC profile for successive extracts of *H. spicatum* in solvent system 3(540nm)

Track	Extract	Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
1	Pet Ether	1	0.50	4.7	0.55	45.7	100	0.62	1.1	1742.2	100
2	Chloroform	1	0.49	1.6	0.54	16.6	35.21	0.55	10.8	440.7	31.17
		2	0.55	10.8	0.59	30.5	64.79	0.63	7.0	973.1	68.83
3	Methanol	1	0.06	4.6	0.11	50.3	44.08	0.14	0.5	1403.7	43.79
		2	0.57	6.8	0.60	22.2	19.40	0.65	0.0	709.0	22.12
		3	0.83	0.0	0.87	41.8	36.56	0.92	0.1	1092.6	34.09
4	Ethyl acetate	1	0.53	0.1	0.61	25.7	22.17	0.67	0.0	1134.3	36.99
		2	0.68	0.6	0.73	34.7	30.00	0.76	12.1	810.3	26.43
		3	0.86	0.1	0.90	55.4	47.83	0.91	20.9	1121.8	36.58
5	Acetone	1	0.55	2.2	0.56	15.2	24.38	0.57	5.2	133.8	9.23
		2	0.60	4.7	0.64	26.1	41.82	0.71	0.1	938.0	64.69
		3	0.72	0.0	0.75	10.5	16.83	0.77	4.8	218.3	15.05
		4	0.87	0.1	0.90	10.6	16.96	0.91	6.1	159.9	11.03

Figure 24: HPTLC fingerprint for different fractions of aqueous and methanol extracts of *P. integerrima* in solvent system 1(254nm)

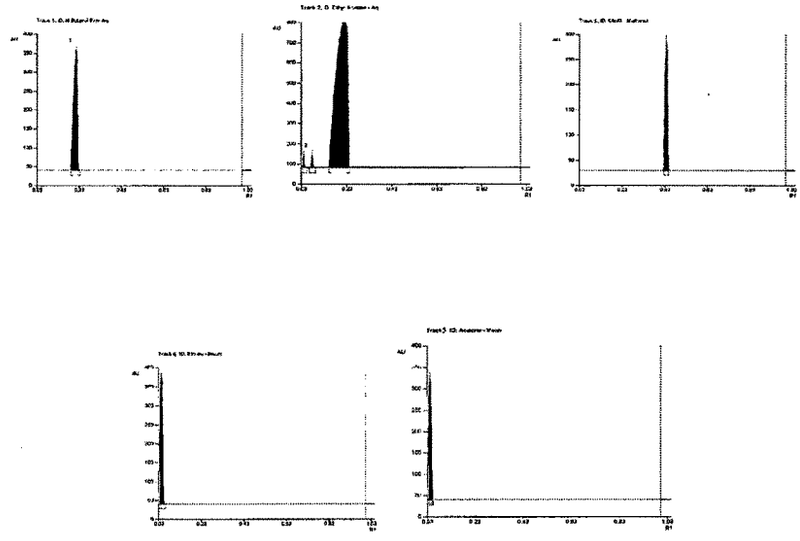


Table 28: HPTLC profile for different fractions of aqueous and methanol extracts of *P. integerrima* in solvent system 1(254nm)

Track	Fraction	Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
1	BFAPI	1	0.19	6.8	0.22	318.4	100	0.23	0.0	490.7	100
2	EAFAPI	1	0.04	36.5	0.04	56.2	6.70	0.05	0.0	209.1	0.68
		2	0.06	0.0	0.8	60.5	7.20	0.09	0.0	306.5	1.00
		3	0.15	4.9	0.22	722.6	86.10	0.24	1.1	30212.0	98.32
3	CFMPI	1	0.42	10.2	0.44	258.9	100	0.45	12.0	2674.2	100
4	EAFMPI	1	0.04	254.8	0.04	333.8	100	0.06	0.0	2568.2	100
5	AFMPI	1	0.04	226.4	0.04	286./8	100	0.05	5.2	2321.2	100

Figure 25: HPTLC fingerprint for different fractions of aqueous and methanol extracts of *P. integerrima* in solvent system 1(366nm)

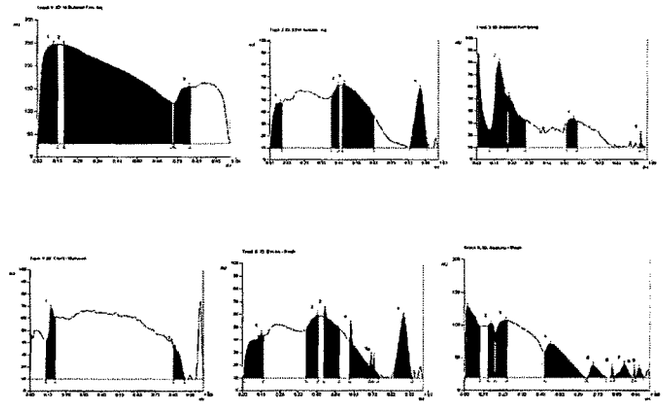


Table 29: HPTLC profile for different fractions of aqueous and methanol extracts of *P. integerrima* in solvent system 1(366nm)

Track	Fraction	Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
1	BFAPI	1	0.04	15.0	0.09	179.3	19.17	0.23	94.5	16360.4	25.6
		2	0.23	94.5	0.26	133.6	14.28	0.27	133.1	3433.8	5.39
		3	0.27	133.2	0.34	167.0	17.85	0.36	163.3	9753.2	15.31
		4	0.37	163.0	0.38	164.1	17.54	0.48	24.1	8916.1	13.99
		5	0.48	24.1	0.56	151.5	16.19	0.74	78.9	19661.3	30.85
		6	0.77	71.6	0.77	99.2	10.61	0.86	9.3	3858.2	6.05
		7	0.86	39.3	0.87	40.8	4.36	0.99	2.1	740.5	2.73
2	EAFAPI	1	0.05	0.1	0.07	15.2	2.30	0.08	6.5	257.0	0.63
		2	0.08	6.5	0.13	38.8	5.87	0.14	37.1	1109.8	2.72
		3	0.17	37.1	0.13	39.8	6.03	0.22	0.2	996.0	2.44
		4	0.27	92.3	0.33	151.8	22.98	0.40	0.4	9855.8	24.17
		5	0.41	0.5	0.47	125.4	18.99	0.48	119.0	4008.5	9.83
		6	0.48	119.0	0.51	167.6	25.37	0.65	101.1	15448.1	37.89
		7	0.68	92.9	0.69	100.6	15.23	0.78	37	8418.0	20.65
3	RMFAPI	1	0.04	31.7	0.07	30.2	22.82	0.13	4.3	3809.8	21.36
		2	0.17	0.3	0.19	56.9	9.97	0.20	55.1	1161.1	6.51
		3	0.31	62.2	0.34	73.7	12.92	0.35	77.0	1783.2	10.00
		4	0.40	68.1	0.43	78.3	13.72	0.44	72.1	2301.8	12.91
		5	0.44	72.5	0.47	85.0	14.89	0.50	72.5	3029.2	16.99
		6	0.50	72.1	0.52	80.3	14.07	0.60	34.4	3694.8	20.72
		7	0.67	29.2	0.68	30.7	5.38	0.78	16.3	1795.9	10.07
4	CFMPI	1	0.04	30.7	0.05	100.3	5.52	0.11	42.3	3395.2	5.78
		2	0.12	42.3	0.17	81.8	4.50	0.18	81.1	2851.8	4.86

		3	0.22	80.0	0.23	93.8	5.16	0.24	77.1	1014.6	1.73
		4	0.26	87.9	0.29	96.4	5.31	0.32	91.9	3504.2	5.97
		5	0.32	91.9	0.32	93.4	5.14	0.43	0.2	5091.0	8.67
		6	0.44	0.0	0.48	276.0	15.19	0.50	244.6	6784.5	11.56
		7	0.50	244.6	0.52	329.0	18.11	0.60	137.5	15105.8	25.73
		8	0.60	133.9	0.61	217.0	11.95	0.66	113.0	4877.5	8.31
		9	0.66	113.0	0.69	188.3	10.37	0.78	0.0	3725.6	13.40
		10	0.82	17.6	0.87	115.1	6.33	0.91	0.3	4485.4	6.35
		11	0.91	0.3	0.95	225.6	12.42	0.97	2.7	4675.6	7.64
5	EAFMPI	1	0.04	1.7	0.06	30.7	2.81	0.08	20.3	573.4	1019
		2	0.08	20.3	0.12	80.7	7.38	0.14	24.9	1972.9	4.08
		3	0.14	24.9	0.22	141.8	12.98	0.22	133.3	5365.1	11.10
		4	0.23	133.4	0.25	138.3	12.65	0.35	99.6	11074.8	22.92
		5	0.35	99.6	0.40	162.2	14.84	0.41	159.5	5320.1	11.01
		6	0.41	159.5	0.42	162.7	14.89	0.46	139.8	5662.1	11.72
		7	0.46	139.8	0.49	309.5	28.32	0.62	80.8	14930.1	30.90
		8	0.69	63.9	0.69	66.9	6.12	0.77	45.9	3421.5	7.08
6	AFMPI	1	0.04	0.9	0.06	30.1	3.01	0.07	24.4	441.0	0.92
		2	0.07	23.4	0.12	84.1	8.40	0.13	38.2	2282.8	4.78
		3	0.13	38.2	0.20	144.8	14.46	0.21	144.1	5444.4	11.40
		4	0.23	144.3	0.25	150.1	14.99	0.34	120.5	11288.5	23.63
		5	0.36	116.8	0.39	159.6	15.93	0.40	157.8	4358.8	9.13
		6	0.42	150.3	0.43	152.3	15.21	0.47	130.2	8111.2	10.70
		7	0.47	130.2	0.51	243.3	24.29	0.70	62.9	17272.8	36.16
		8	0.83	33.9	0.84	37.3	3.72	0.93	12.1	1564.9	328

Figure 26: HPTLC fingerprint for different fractions of aqueous and methanol extracts of *P. integerrima* in solvent system 1(540 nm)

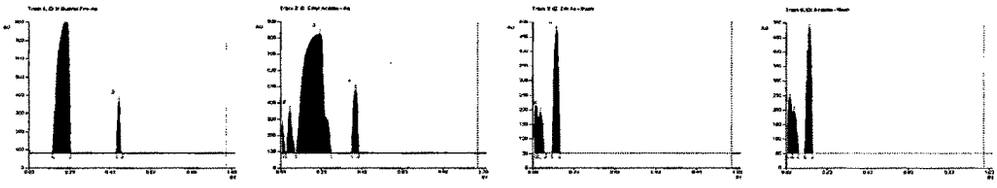


Table 30: HPTLC profile for different fractions of aqueous and methanol extracts of *P. integerrima* in solvent system 1(540nm)

Track	Fraction	Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
1	BFAPI	1	0.14	6.5	0.21	727.1	71.60	0.23	39.3	31366.6	92.59
		2	0.46	11.0	0.47	288.4	28.40	0.49	0.0	2511.4	7.41
2	EAFAPI	1	0.04	171.9	0.04	171.9	0.005	11.10	2.8	1093.2	1.60
		2	0.06	0.4	0.07	256.8	0.10	16.59	1.8	4072.8	5.96
		3	0.10	2.6	0.22	731.4	0.28	47.23	9.0	57041.4	83.25
		4	0.38	23.5	0.40	388.30	0.41	25.08	26.3	6306.9	9.21
3	RMFAPI	1	0.41	23.4	0.44	661.0	100.0	0.46	26.5	15093.5	100
4	EAFMPI	1	0.04	125.9	0.04	165	22.74	0.06	117.2	20146.6	17.6
		2	0.06	117.2	0.01	140.6	19.38	0.09	0.0	2304.6	20.21
		3	0.12	7.1	0.14	419.9	57.88	0.16	12.6	7081.3	62.11

Figure 27: HPTLC fingerprint for different fractions of aqueous and methanol extracts of *P. integerrima* in solvent system2(254 nm)

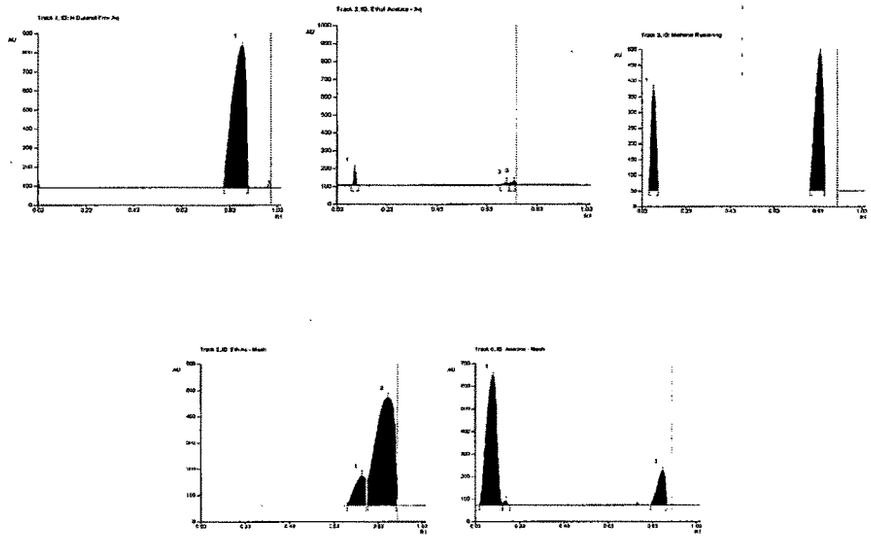


Table 31: HPTLC profile for different fractions of aqueous and methanol extracts of *P. integerrima* in solvent system 2(254nm)

Track	Fraction	Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
1	BFAPI	1	0.81	7.6	0.89	741.0	100	0.91	23.9	33605.7	100
2	EAFAPI	1	0.08	0.0	0.10	81.3	15.12	0.11	0.0	539.3	2.16
		2	0.70	21.5	0.74	116.4	21.65	0.77	0.5	3594.7	14.39
		3	0.78	0.2	0.87	339.9	63.23	0.91	137.6	20852.3	83.39
3	RMFAPI	1	0.06	7.1	0.08	321.7	42.38	0.10	14.1	5971.3	31.77
		2	0.80	3.6	0.84	437.5	57.62	0.86	16.2	12823.7	68.23
4	EAFAPI	1	0.69	7.9	0.76	113.7	21.70	0.77	99.9	4357.0	13.55
		2	0.78	98.2	0.88	410.3	78.30	0.91	141.3	27786.7	86.45
5	AFAPI	1	0.05	3.5	0.11	575.6	77.03	0.15	21351.6	21351.6	82.19
		2	0.15	10.9	0.17	18.4	2.47	0.18	211.1	221.1	0.85
		3	0.82	0.9	0.88	153.2	20.50	0.90	4407.0	4407.0	16.96

Figure 28: HPTLC fingerprint for different fractions of aqueous and methanol extracts of *P. integerrima* in solvent system 2(366nm)

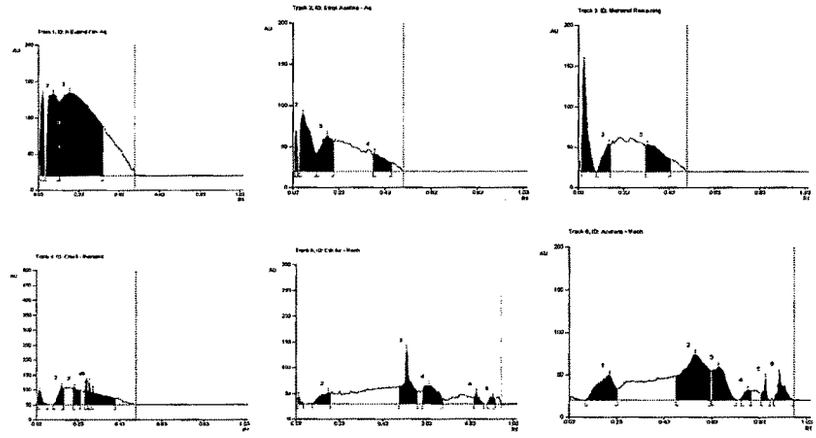


Table 32: HPTLC profile for different fractions of aqueous and methanol extracts of *P. integerrima* in solvent system 2(366nm)

Track	Fraction	Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
1	BFAPI	1	0.04	50.2	0.05	111.9	19.54	0.06	6.5	1320.9	2.99
		2	0.07	3.2	0.11	127.5	22.26	0.13	123.9	4779.6	10.88
		3	0.13	123.9	0.24	164.7	28.76	0.26	164.1	13486.6	30.48
		4	0.49	150.1	0.50	154.8	27.03	0.92	0.1	24499.4	65.37
		5	0.94	0.1	0.96	13.8	2.40	0.97	1.6	159.8	0.36
2	EFAAPI	1	0.04	33.3	0.04	49.6	13.45	0.05	2.5	337.2	2.88
		2	0.06	1.5	0.07	71.7	19.45	0.13	31.4	2666.0	22.75
		3	0.13	31.4	0.18	58.5	15.86	0.19	56.2	20.0	17.36
		4	0.55	51.1	0.58	60.6	16.44	0.62	48.5	2738.6	23.54
		5	0.65	46.4	0.58	47.0	12.74	0.73	4.4	1728.6	14.95
		6	0.73	4.4	0.65	14.9	4.05	0.77	13.4	340.8	2.91
		7	0.78	13.5	0.76	31.3	8.44	0.80	24.0	1.1	2.65
		8	0.83	34.2	0.84	35.3	9.57	0.93	0.0	1542.7	13.17
3	RMFAPI	1	0.04	1.0	0.05	137.8	137.8	0.08	9.0	2336.4	15.46
		2	0.11	9.0	0.2	69.8	69.8	0.17	68.4	4358.2	28.85
		3	0.54	75.6	0.57	87.9	87.9	0.6	51.6	5962.4	39.46
		4	0.74	34.3	0.75	35.6	35.6	0.78	0.8	1477.3	9.78
		5	0.88	0.4	0.90	28.7	28.7	0.91	1.7	973.9	6.45
4	CFMPI	1	0.04	31.6	0.04	45.7	5.60	0.08	1.0	620.6	3.64
		2	0.11	0.4	0.15	65.4	8.01	0.16	61.2	1099.3	6.45
		3	0.26	65.6	0.27	95.9	11.75	0.28	70.4	1022.0	6.00
		4	0.28	70.4	0.28	99.9	12.24	0.29	64.7	964.8	5.66
		5	0.52	57.6	0.55	65.5	8.02	0.58	53.6	2634.5	15.59
		6	0.59	53.4	0.65	66.3	8.12	0.65	60.1	2855.8	16.77
		7	0.76	52.0	0.78	75.6	9.26	0.79	73.0	1600.3	9.40
		8	0.79	73.0	0.80	76.7	9.40	0.82	63.8	1581.2	9.28

		9	0.82	63.8	0.84	80.2	9.83	0.86	65.4	2088.9	12.26
		10	0.87	54.8	0.88	83.8	10.27	0.90	43.4	1143.2	6.71
		11	0.90	43.5	0.92	61.3	7.51	0.96	0.9	1430.6	8.22
5	EAFMPI	1	0.04	13.3	0.04	13.6	6.46	0.07	0.2	141.1	2.43
		2	0.11	0.4	0.18	22.3	10.61	0.19	20.6	814.47	14.05
		3	0.50	33.7	0.54	106.0	50.37	0.59	23.6	2535.3	43.71
		4	0.61	25.7	0.64	35.5	16.87	0.71	9.2	1843.6	31.78
		5	0.85	11.8	0.86	20.3	9.67	0.89	0.8	322.9	5.56
		6	0.91	0.1	0.94	12.7	6.04	0.95	10.3	142.7	2.46
6	AFMPI	1	0.10	0.0	0.0	29.6	15.48	0.23	13.3	1675.7	19.22
		2	0.48	28.8	0.56	63.5	27.99	0.63	5.1	4206.6	48.24
		3	0.63	35	0.66	39.1	20.46	0.73	0.2	1713.5	19.65
		4	0.75	2.7	0.78	12.0	6.29	0.80	10.6	277.7	3.18
		5	0.84	7.8	0.86	25.8	13.49	0.88	0.1	229.8	2.64
		6	0.89	0.4	0.91	31.1	16.29	0.97	0.0	616.7	7.07

Figure 29: HPTLC fingerprint for different fractions of aqueous and methanol extracts of *P. integerrima* in solvent system 2(540nm)

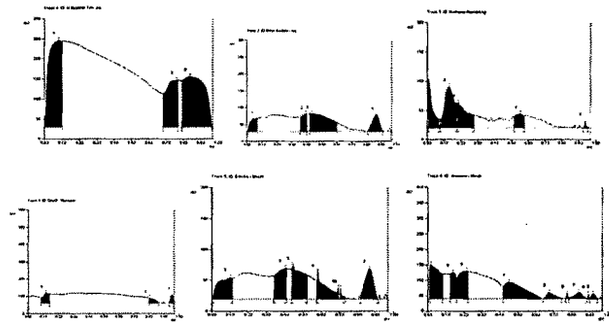


Table 33: HPTLC profile for different fractions of aqueous and methanol extracts of *P. integerrima* in solvent system 2(540nm)

Track	Fraction	Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
1	BFAPI	1	0.03	4.8	0.11	216.0	46.98	0.13	214.8	13289.3	39.62
		2	0.71	83.4	0.79	117.9	25.65	0.80	116.8	6699.7	19.97
		3	0.82	117.1	0.87	125.9	27.38	0.10	3.3	13553.7	40.41
2	EAFAPI	1	0.03	0.9	0.09	38.2	19.43	0.10	38.0	1522.9	14.0
		2	0.38	43.3	0.42	53.1	27.06	0.43	52.4	1606.2	14.77
		3	0.45	52.8	0.46	54.1	27.83	0.63	27.2	5716.0	52.66
		4	0.83	0.1	0.90	50.4	25.69	0.94	2.4	2029.3	18.65
3	RMFAPI	1	0.03	77.0	0.04	78.2	34.29	0.1	15.2	1615.9	19.6
		2	0.10	15.2	0.16	70.7	31.0	0.2	4.2	3523.2	41.42
		3	0.21	41.9	0.21	43.4	19.01	0.31	22.2	2261.8	26.6
		4	0.56	19.3	0.58	24.0	10.54	0.6	21.3	1021.1	12.01
		5	0.95	0.6	0.91	11.8	5.15	0.98	1.03	82.3	0.97
4	CFMPI	1	0.11	31.5	0.14	58.5	43.22	0.17	50.8	1927.0	54.59
		2	0.83	26.3	0.84	28.8	21.26	0.9	1.8	884.2	25.05
5	EAFMPI	1	0.03	1.1	0.13	36.0	13.52	0.14	33.8	2205.5	19.54
		2	0.37	37.5	0.43	50.3	18.89	0.44	48.4	2351.9	20.84
		3	0.47	48.0	0.47	54.3	20.42	0.55	32.2	2821.1	25.00
		4	0.60	29.5	0.61	42.7	16.04	0.71	9.1	1623.0	14.38
		5	0.71	9.1	0.72	17.6	6.60	0.73	7.2	145.7	1.28
		6	0.73	7.2	0.74	16.5	6.19	0.76	3.6	168.1	1.48
		7	0.84	3.5	0.90	48.8	18.33	0.94	0.6	1963.1	17.40
6	AFMPI	1	0.03	89.8	0.04	108.2	26.20	0.11	78.9	5205.7	28.98
		2	0.15	79.1	0.17	83.6	20.24	0.19	69.8	2211.8	12.31
		3	0.19	69.8	0.24	88.0	21.31	0.25	87.2	3883.2	21.62
		4	0.345	39.4	0.48	51.6	12.51	0.61	0.2	4866.3	27.01
		5	0.67	0.2	0.70	18.6	4.50	0.77	0.3	684.3	3.81
		6	0.80	0.0	0.81	15.4	3.73	0.81	1.6	95.8	0.53
		7	0.81	1.6	0.91	19.2	4.65	0.91	0.4	692.6	3.86
		8	0.91	0.4	0.93	13.7	3.31	0.93	1.3	64.5	0.36

Figure 30: HPTLC profile for fractions of methanol extracts of *H. spicatum* in solvent system 1 (254 nm)

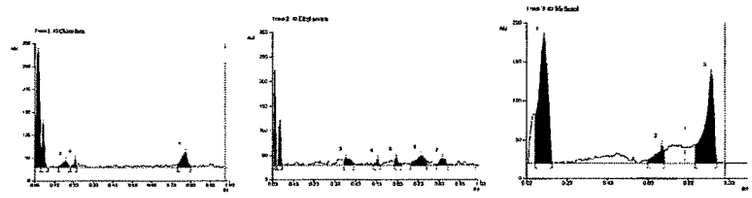


Table 34: HPTLC profile for fractions of methanol extracts of *H. spicatum* in solvent system1 (254nm)

Track	Extract	Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
1	CFMHS	1	0.03	28.9	0.05	252.1	60.78	0.06	40.6	3558.6	61.59
		2	0.06	0.07	0.07	96.3	23.22	0.10	0.0	901.5	15.60
		3	0.15	0.19	0.19	13.5	3.26	0.21	0.4	315.6	5.46
		4	0.21	0.24	0.24	18.5	4.47	0.24	3.8	155.9	2.70
		5	0.76	6.7	0.80	34.3	8.27	0.83	3.2	846.6	14.65
2	EAFMHS	1	0.04	184.0	0.04	194.0	54.66	0.05	3.6	1182.6	34.14
		2	0.05	5.6	0.06	83.7	23.20	0.07	0.0	647.3	18.71
		3	0.37	8.9	0.38	15.7	4.42	0.42	2.6	345.7	9.99
		4	0.51	0.8	0.53	12.8	3.59	0.55	0.5	116.6	3.37
		5	0.61	6.3	0.62	15.2	4.30	0.65	1.4	86.8	5.40
		6	0.69	2.7	0.74	19.9	4.62	0.77	7.9	685.9	19.82
3	MFMHS	1	0.04	0.0	0.05	29.7	57.82	0.06	0.07	210.3	40.65
		2	0.07	0.0	0.09	21.7	42.18	0.10	0.1	307.0	59.35

Figure31: HPTLCprofilt for fractions of methanol extracts of *H. spicatum* in solvent system 1(366nm)

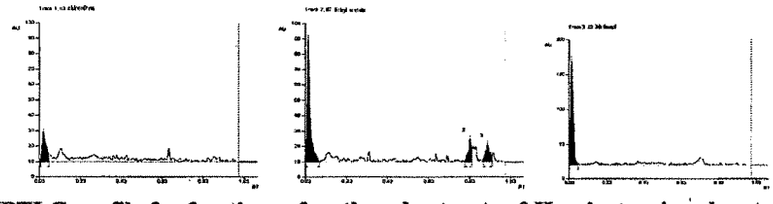


Table35: HPTLC profile for fractions of methanol extracts of *H. spicatum* in solvent system 1(366nm)

Track	Extract	Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
1	CFMHS	1	0.03	0.5	0.05	19.7	10	0.08	5.7	314.7	100
2	EAFMHS	1	0.03	5.5	0.04	79.7	74.95	0.10	0.1	909.0	72.28
		2	0.81	0.2	0.83	15.0	14.14	0.84	8.2	167.1	13.28
		3	0.90	1.8	0.92	11.6	10.91	0.94	4.0	181.5	14.43
3	MFMHS	1	0.03	1.9	0.05	25.8	100	0.08	0.8	546.2	100

Figure 32: HPTLC profile for fractions of methanol extracts of *H. spicatum* in solvent system 1 (540 nm)

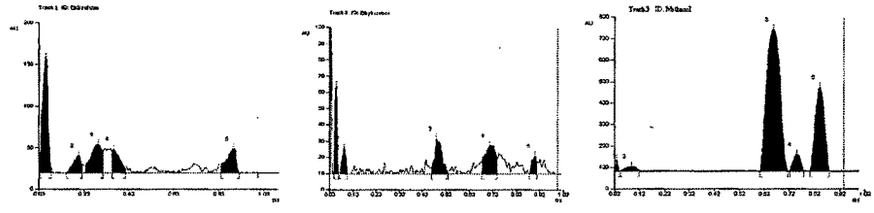


Table 36: HPTLC profile for fractions of methanol extracts of *H. spicatum* in solvent system1 (540 nm)

Track	Extract	Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
1	CFMHS	1	0.03	4.4	0.06	139.4	55.02	0.09	5.7	2680.2	43.08
		2	0.15	0.2	0.21	20.9	8.26	0.22	10.0	562.8	9.05
		3	0.24	8.2	0.29	34.3	13.54	0.32	27.1	1296.6	20.84
		4	0.36	26.0	0.36	29.5	11.66	0.41	5.0	721.1	11.59
		5	0.84	9.8	0.90	29.2	11.52	0.92	00	960.7	15.44
2	EAFMHS	1	0.05	2.8	0.06	54.3	44.82	0.07	0.7	392.7	19.80
		2	0.07	0.0	0.09	15.9	13.19	0.10	0.3	203.5	10.25
		3	0.47	3.5	0.49	22.2	18.16	0.54	1.2	555.8	28.01
		4	0.68	7.4	0.72	17.6	14.56	0.75	9.2	669.5	33.44
		5	0.89	0.3	0.91	11.1	9.16	0.92	7.6	168.6	8.50
3	MFMHS	1	0.51	4.0	0.56	346.1	73.62	0.60	1.4	13112.0	58.42
		2	0.61	0.5	0.6	25.9	5.50	0.66	0.9	1334.6	5.95
		3	0.72	0.9	0.77	98.2	20.88	0.79	1.6	7999.4	35.64

Figure 33: HPTLC profile for fractions of methanol extracts of *H. spicatum* in solvent system 2 (254 nm)

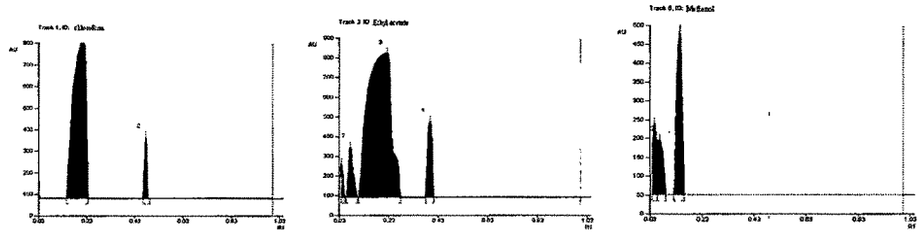


Table 37: HPTLC profile for fractions of methanol extracts of *H. spicatum* in solvent system 2 (254 nm)

Track	Fraction	Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
1	CFMHS	1	0.14	6.5	0.21	727.1	71.60	0.23	39.3	31366.6	92.59
		2	0.46	11.0	0.47	288.4	28.40	0.49	0.0	2511.4	7.41
2	EAFMHS	1	0.04	171.9	0.04	171.9	0.005	11.10	2.8	1093.2	1.60
		2	0.06	0.4	0.07	256.8	0.10	16.59	1.8	4072.8	5.96
		3	0.10	2.6	0.22	731.4	0.28	47.23	9.0	57041.4	83.25
		4	0.38	23.5	0.40	388.30	0.41	25.08	26.3	6306.9	9.21
4	MFMHS	1	0.04	125.9	0.04	165	22.74	0.06	117.2	20146.6	17.6
		2	0.06	117.2	0.01	140.6	19.38	0.09	0.0	2304.6	20.21
		3	0.12	7.1	0.14	419.9	57.88	0.16	12.6	7081.3	62.11

Figure 34: HPTLC profile for fractions of methanol extracts of *H. spicatum* in solvent system 2 (366 nm)

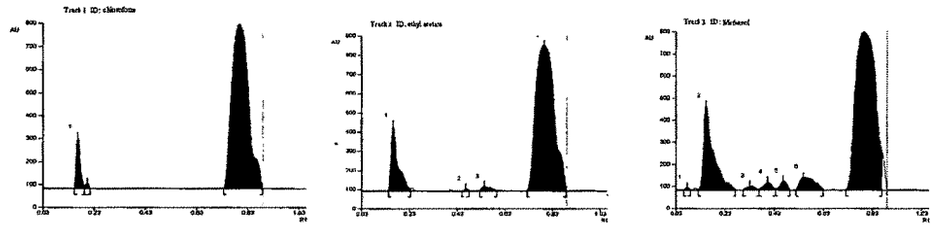


Table 38: HPTLC profile for fractions of methanol extracts of *H. spicatum* in solvent system 2 (366 nm)

Track	Fraction	Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
1	CFMHS	1	0.15	2.6	0.17	220.4	23.08	0.19	13.4	2750.5	6.86
		2	0.19	13.4	0.20	20.3	2.13	0.21	0.8	235.4	0.59
		3	0.74	1.0	0.80	114.2	74.79	0.89	0.69	37108.1	92.55
2	EAFMHS	1	0.14	2.4	0.16	341.3	30.12	0.23	11.4	7882.0	14.12
		2	0.45	0.0	0.46	11.7	1.03	0.48	1.0	127.6	0.23
		3	0.53	0.8	0.54	22.5	1.98	0.60	0.4	623.2	1.12
		4	0.72	1.5	0.79	757.8	66.87	0.89	9.5	47179.5	84.53
4	MFMHS	1	0.06	0.0	0.07	11.9	0.94	0.09	0.4	129.5	0.20
		2	0.12	1.8	0.15	380.8	30.18	0.27	0.6	12131.2	19.15
		3	0.30	2.9	0.33	17.4	1.38	0.31	4.8	497.6	0.79
		4	0.37	4.8	0.40	35.9	2.85	0.44	4.7	939.3	1.48
		5	0.44	4.7	0.47	41.5	3.29	.49	1.1	926.3	1.46
		6	0.52	0.8	0.55	59.5	4.72	0.63	6.4	2845.2	4.49
		7	0.72	0.3	0.80	714.7	56.64	0.87	178.9	45872.8	72.42

Figure 35: HPTLC profile for fractions of methanol extracts of *H. spicatum* in solvent system 2 (540 nm)

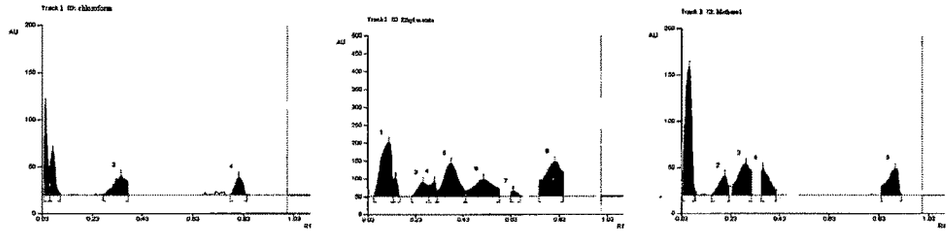


Table 39: HPTLC profile for fractions of methanol extracts of *H. spicatum* in solvent system 2 (540 nm)

Track	Extract	Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
1	CFMHS	1	0.04	16.6	0.04	97.1	52.95	0.06	31.0	803.7	29.88
		2	0.06	31.0	0.07	47.3	25.77	0.10	0.4	677.9	25.20
		3	0.27	0.3	0.35	20.5	11.20	0.38	13.4	784.2	29.15
		4	0.78	0.5	0.81	18.5	10.07	0.84	0.0	424.2	15.77
2	EAFMHS	1	0.05	0.7	0.12	150.4	28.41	0.14	45.8	5214.4	26.04
		2	0.14	45.8	0.14	52.6	9.94	0.16	0.1	60.0	3.01
		3	0.21	0.2	0.26	38.1	7.19	0.29	31.1	1174.9	5.87
		4	0.29	31.1	0.31	40.4	7.62	0.32	14.5	797.1	3.98
		5	0.32	14.5	0.38	91.4	17.25	0.44	15.2	4102.6	20.50
		6	0.44	15.2	0.52	46.5	8.78	0.58	22.4	2991.0	14.94
		7	0.63	12.6	0.64	14.1	2.66	0.67	0.2	251.3	1.26
		8	0.75	42.8	0.81	96.0	18.14	0.85	68.7	4884.2	24.40
		2	0.16	3.0	0.20	16.1	3.69	0.22	7.0	445.1	3.06
3	MFMHS	1	0.04	1.6	0.07	123.5	28.24	0.09	0.1	2179.8	15.01
		2	0.16	3.0	0.20	16.1	3.69	0.22	7.0	445.1	3.06
		3	0.23	4.0	0.29	42.1	9.63	0.30	34.4	1421.8	9.79
		4	0.39	4.7	0.40	42.7	9.76	0.51	1.5	1938.1	13.35
		5	0.68	7.1	0.72	19.8	4.52	0.76	2.5	6306.0	4.35
		6	0.80	8.9	0.91	193.2	44.17	0.96	0.2	7907.8	54.45

Figure 36: HPTLC fingerprint of methanol extract of *H. spicatum* by column chromatography.

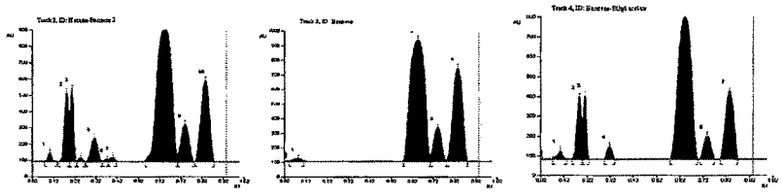


Table 40: HPTLC profile of methanol extract of *H. spicatum* by column chromatography.

Track	Fraction	Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	Area %
1	HB	1	0.08	3.4	0.10	75.9	2.41	0.14	4.2	1433.2	1.11
		2	0.14	4.2	0.18	481.0	15.30	0.20	371.4	10723.1	8.34
		3	0.20	371.4	0.21	521.8	16.59	0.23	11.6	8766.2	6.82
		4	0.23	1.6	0.25	35.2	1,12	0.27	0.0	747.3	0.58
		5	0.27	0.1	0.31	195.0	6.20	0.35	4.0	5991.0	4.66
		6	0.35	4.0	0.38	25.2	0.80	0.38	25.0	472.2	0.37
		7	0.39	24.7	0.41	50.0	1.59	0.43	0.9	1248.4	0.97
		8	0.56	0.7	0.66	585.8	27.31	0.72	97.2	63410.1	49.30
		9	0.72	97.2	0.75	305.1	9.70	0.80	15.9	11347.9	8.82
		10	0.80	15.9	0.84	597.0	18.98	0.89	2.1	24489.1	19.04
2	B	1	0.51	17.2	0.55	346.1	73.62	0.60	1.4	11390.0	75.60
		2	0.61	0.4	0.64	25.9	5.50	0.66	0.9	579.4	3.85
		3	0.72	0.8	0.76	98.2	20.88	0.79	1.6	3097.8	20.56
3	BEA	1	0.08	10.4	0.11	40.4	2.11	0.14	0.8	934.6	1.38
		2	0.16	0.2	0.19	313.5	16.34	0.20	199.0	6023.9	8.90
		3	0.20	199.0	0.21	324.0	16.89	0.23	1.3	4929.3	7.28
		4	0.30	0.9	0.32	62.9	3.28	0.34	1.2	1376.1	2.03
		5	0.58	1.3	0.65	718.1	37.43	0.70	2.5	39089.2	57.74
		6	0.71	0.9	0.74	115.8	6.03	0.77	1.8	11884.4	5.11
		7	0.80	2.0	0.84	343.8	17.92	0.88	1.9	1536.2	17.56

3.7 Isolation and characterization of compounds from Ethyl acetate fraction of Methanol extract of *P. integerrima* and Hexane Benzene fraction of *H. spicatum*

Ethyl acetate fraction of Methanol extract of *P. integerrima* was found to be rich in phenolic and flavonoid content; it was subjected to column chromatography for isolation of flavonoids and phenolic compounds. The fractions obtained after elution with different solvents were collected separately and subjected to HPTLC for identification of compounds present. The fractions so obtained were further subjected to preparative TLC for separation of compounds. Methanol extract of *H. spicatum* which was found to be rich in terpenoids was subjected to column chromatography and hexane benzene fraction so obtained was further separated by preparative TLC.

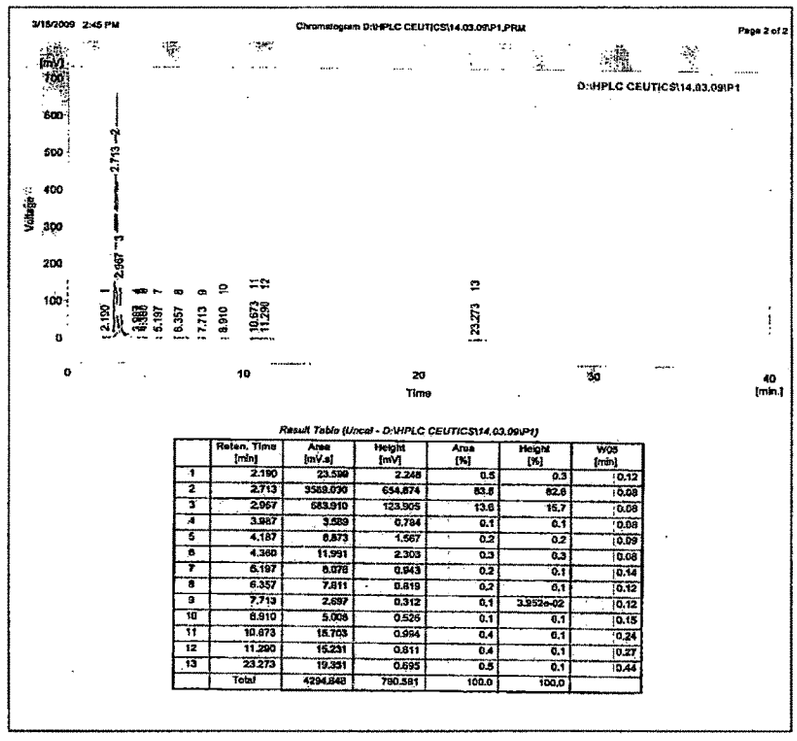
3.7.1Characterization of isolated compounds

The isolated compounds from *P. integerrima* and *H. spicatum* were identified using physicochemical and spectral data.

3.7.1.1 Characterization of PI 1

Description: Yellow colored powder, m. p. 212-213° C and λ max 256 nm in methanol. The compound was found to be 83.65% pure by HPLC (Figure 37)

Figure 37: Assessment of % purity of PI 1 by HPLC



	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.190	23.599	2.248	0.5	0.3	0.12
2	2.713	3589.030	654.874	83.6	82.6	0.08
3	2.957	683.910	123.905	13.6	15.7	0.08
4	3.987	3.589	0.784	0.1	0.1	0.08
5	4.187	8.873	1.567	0.2	0.2	0.09
6	4.380	11.991	2.303	0.3	0.3	0.08
7	5.197	8.076	0.943	0.2	0.1	0.14
8	6.357	7.811	0.819	0.2	0.1	0.12
9	7.713	2.697	0.312	0.1	3.952e-02	0.12
10	8.910	5.008	0.526	0.1	0.1	0.15
11	10.573	15.703	0.994	0.4	0.1	0.24
12	11.290	15.231	0.811	0.4	0.1	0.27
13	23.273	19.351	0.695	0.5	0.1	0.44
	Total	4294.848	790.581	100.0	100.0	

Solubility: Freely soluble in Ethyl acetate and Methanol, insoluble in Chloroform and Benzne.

Chromatographic studies: Thin layer chromatography of the compound using Ethyl acetate: Formic acid: Acetic acid: Water(100:11:11:27) as mobile phase, silica gel 60 F_{254} as stationary phase and NP- PEG as a detecting agent revealed single spot at R_f 0.45 indicating flavonoid nature of the compound.

FTIR spectra

The IR spectra of the sample show the peaks from3410.10-370.34 cm^{-1}.The peaks in the IR spectra from 3600-3300 cm^{-1} represent alcoholic O-H, amine or amide N-H and alkyne C-H. The spectra show broad peak in the range 3410.26-3336.96 cm^{-1}. The alcoholic stretch is broad and strong near 3400 cm^{-1}. It indicates presence of alcoholic O-H. Peaks at 3000-2800 cm^{-1} indicate presence of alkyl C-H. The spectra show presence of peak at the range 2983-2908.82 cm^{-1}.The spectra show peaks at 1654.98 cm^{-1} indicating presence of alkene, C=C and C=O unsaturation. Peak at 1863.30 cm^{-1} indicates presence of C=O as this group is present in the range 1870-1540. Peaks over 3000 cm^{-1} confirm presence of aromatic compounds. Peaks at 1300-1000 cm^{-1} indicated the presence of C-O. The peaks at 1294.28, 1205.55, 1163.11 and 1064.74 confirm presence of C-O. The peaks at 1000-675 cm^{-1} confirm presence of C-H bending vibrations. Peaks at 870-625 cm^{-1} indicate phenyl rings substitution bands. The FT-IR spectrum is shown in the figure 38.

Mass spectra

Mass spectra indicate molecular weight of the compound. In case of PI 1, M. Wt was found to be 312. (Figure 39)

^{1}H NMR spectra

The NMR data showed following results

PNMR(DMSO):1.03(3H,d); 2.52(4H,s); 3.165(4H,d); 3.74(2H,d); 4.42(4H,s); 5.22(2H,s); 5.24(2H,d); 6.14(1H,s); 6.32(1H,s); 6.82(1H,m); 7.32(2H,d); 7.65(2H,s); 8.05(1H,s) (Figure 40)

The spectra were matched with that of standard quercetin to confirm the identity of the compound.

Figure 38: IR spectra of PI 1

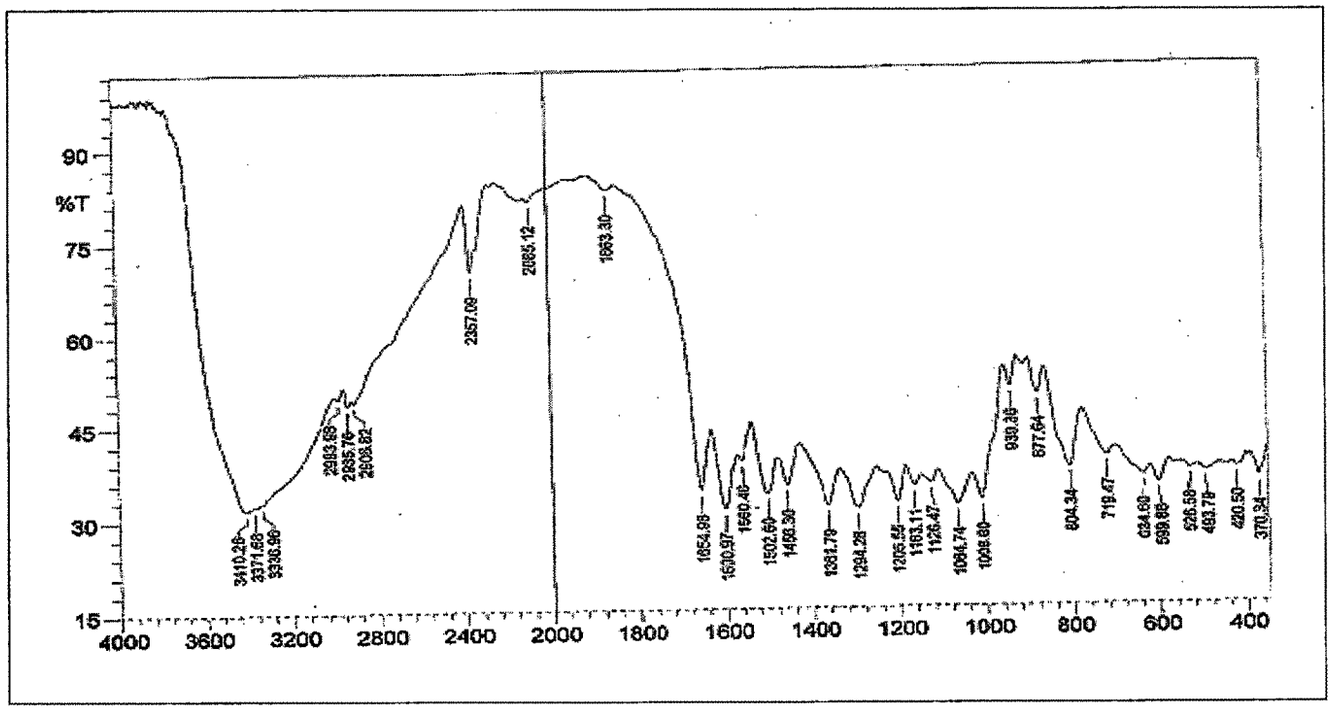


Figure 39: Mass spectra of PI 1

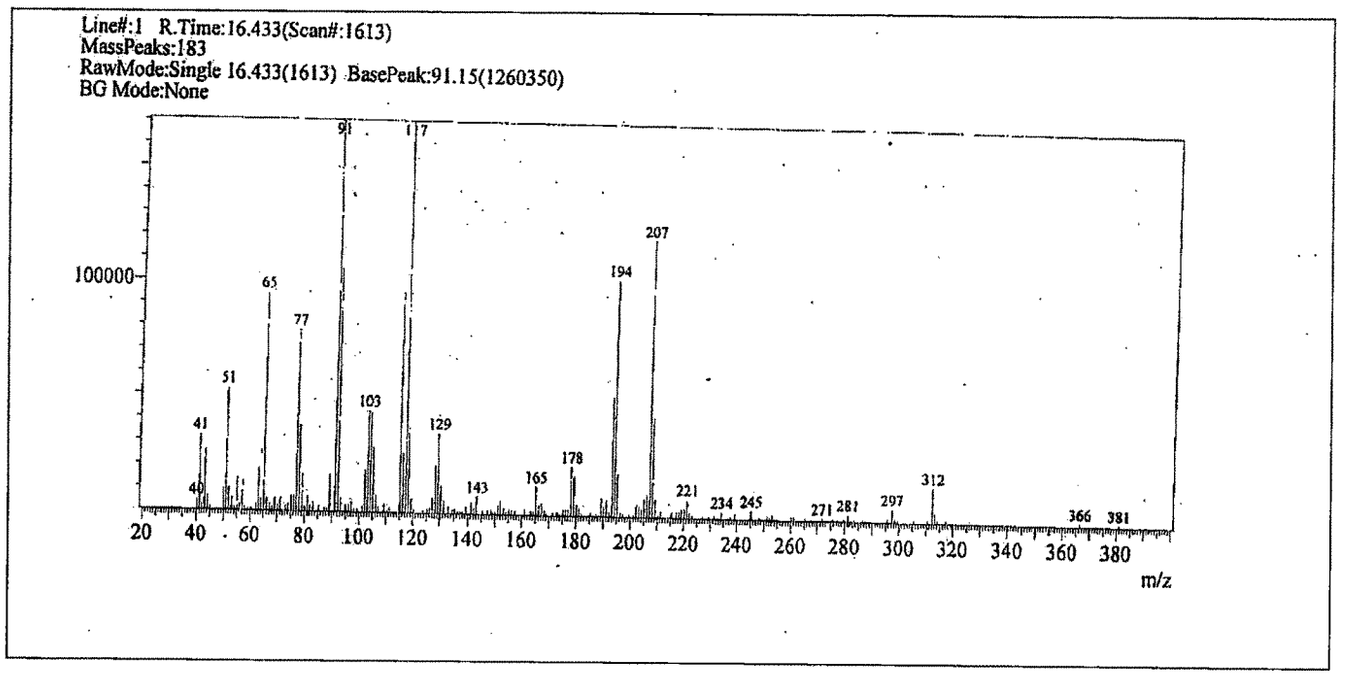


Figure 40: NMR spectra of PI 1

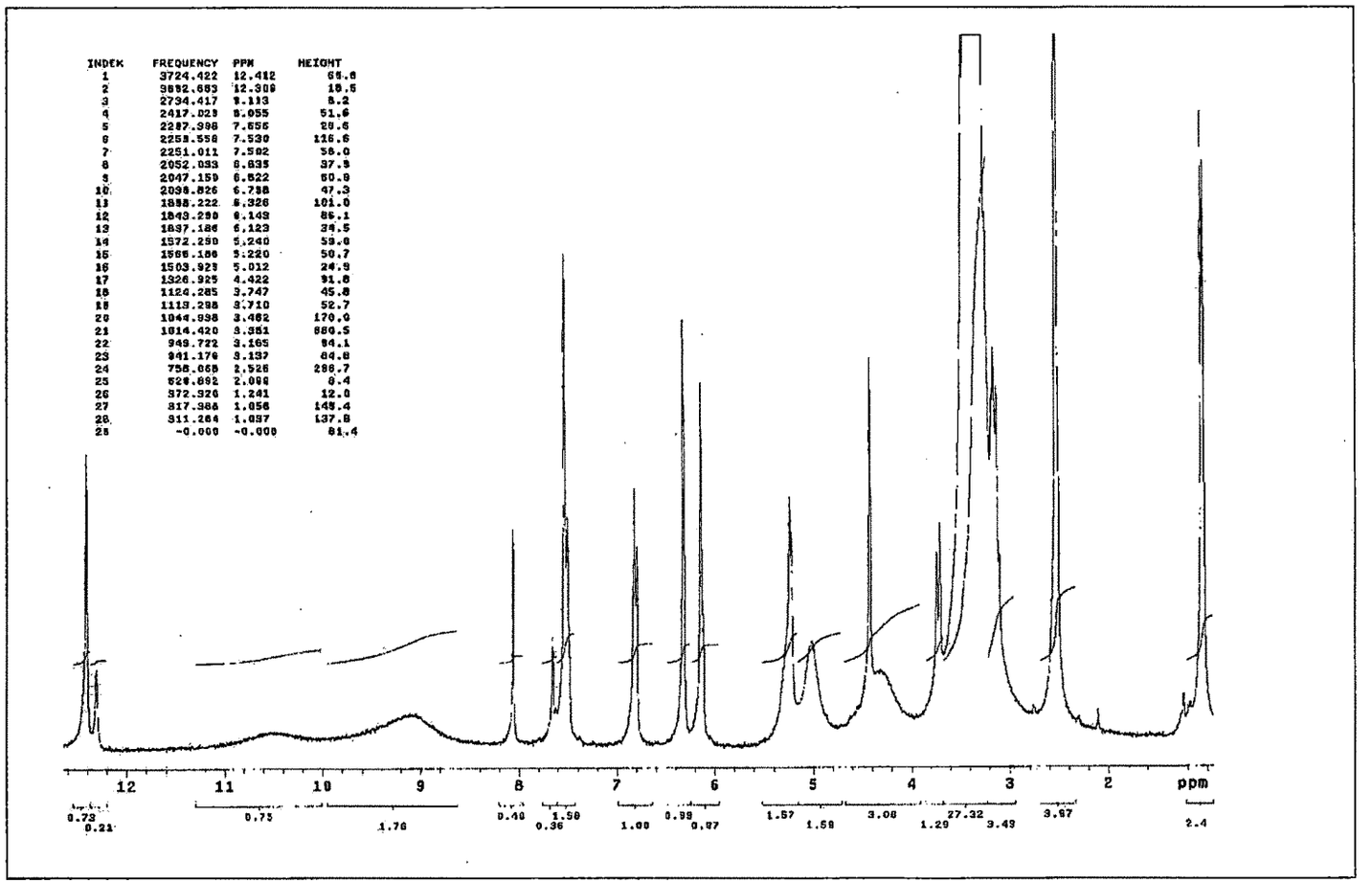
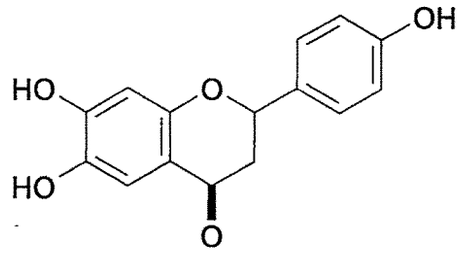


Figure 41: Probable structure of PI 1

Molecular formula $C_{15}H_{10}O_4$

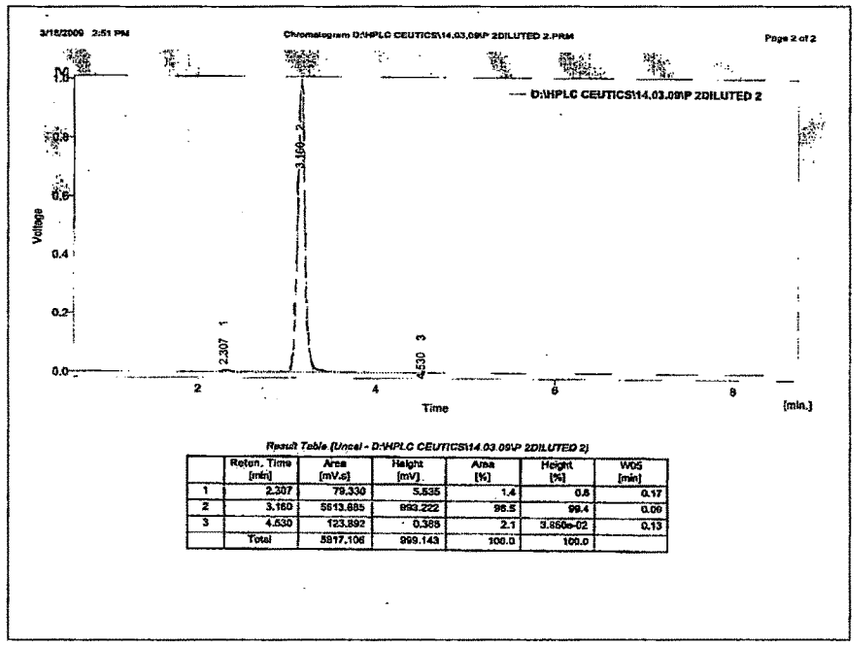


Quercetin

3.7.1.2 Characterization of PI 2

Description: White powder, m. p. 205-206° C and λ max 272 nm in methanol. The compound was found to be 96.7% pure. (Figure 42)

Figure 42: Assessment of % purity of PI 2 by HPLC



	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.307	79.330	5.535	1.4	0.6	0.17
2	3.160	5613.685	993.222	98.5	99.4	0.09
3	4.530	123.892	0.386	2.1	3.850e-02	0.13
	Total	5817.106	999.143	100.0	100.0	

Solubility: Freely soluble in Ethyl acetate and Methanol, insoluble in Chloroform.

Chromatographic studies: Thin layer chromatography of the compound using Ethyl acetate: Formic acid: Acetic acid: Water(100:11:11:27) as mobile phase, silica gel 60 F_{254} as stationary phase and NP- PEG as a detecting agent revealed single spot at R_f 0.6 indicating flavonoid nature of the compound.

FTIR spectra

The IR spectra of the sample show the peaks from3491.27-441.71 cm^{-1}.The peaks in the IR spectra from 3600-3300 cm^{-1} represent alcoholic O-H, amine or amide N-H and alkyne C-H. The spectra show broad peak in the range 3491.27-3362.04 cm^{-1}. The alcoholic stretch is broad and strong near 3400 cm^{-1}. It indicates presence of alcoholic O-H. Peaks at 3064-2802 cm^{-1} indicate presence of alkyl C-H. The spectra show presence of peak 3064.99 and3014.84 cm^{-1} indicating presence of aromatic C-H stretching. The spectra show peaks at 1654.98 cm^{-1} indicating presence of alkene, C=C and C=O unsaturation. Peaks at 2802.66, 2659.93 and 2592.41 cm^{-1} confirm presence of carboxylic acid O-H stretch. Also the peaks in the range 3300-2500^{cm-1} represent carboxylic group. Peaks over 3000 $^{cm-1}$ indicate presence of aromatic compounds. The peaks at 1000-675 $^{cm-1}$ confirm presence of C-H bending vibrations. Peaks at 870-625 $^{cm-1}$ indicate phenyl ring substitution bands. The FT-IR spectrum is shown in the figure 43.

Figure 43: IR spectra of compound PI 2

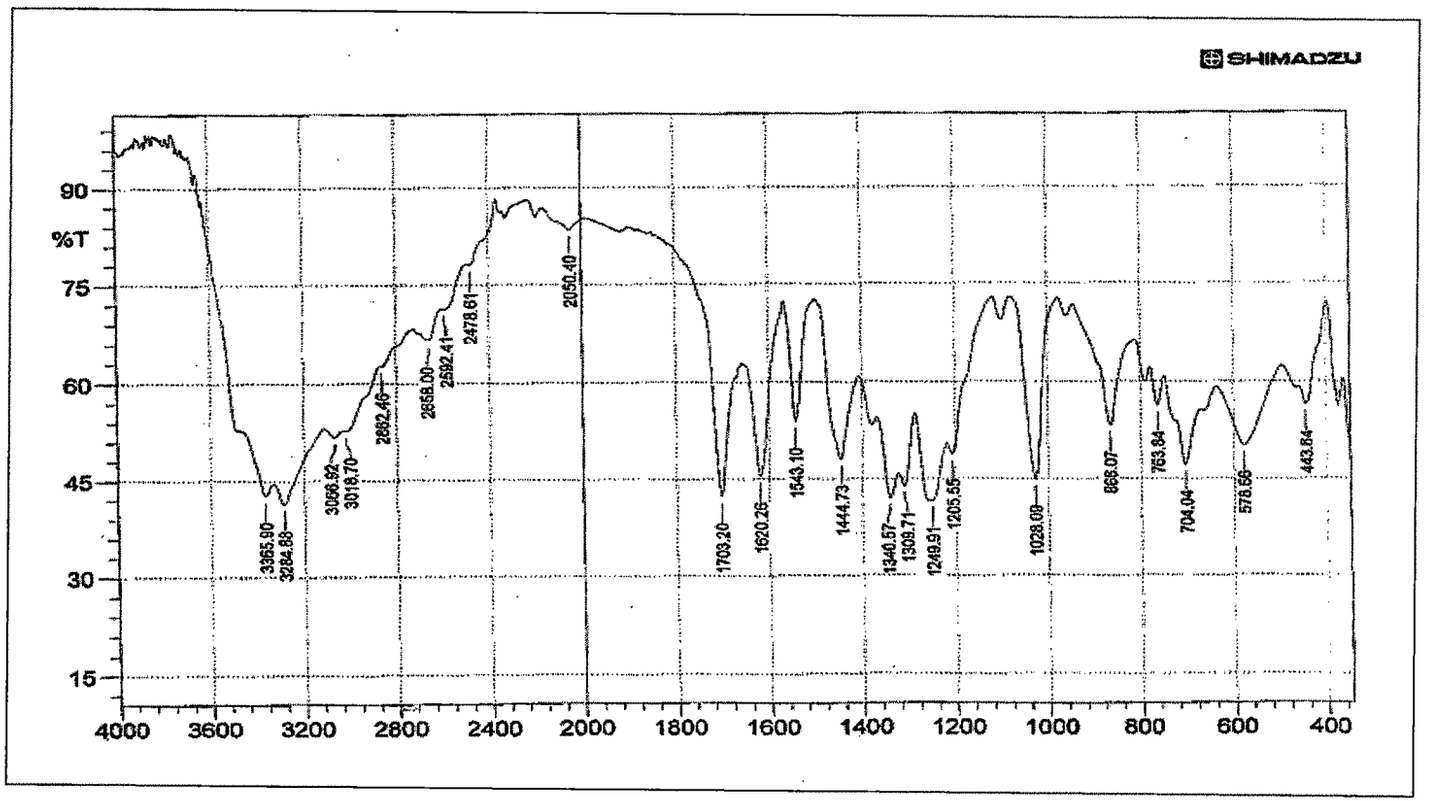


Figure 44: Mass spectra of PI 2

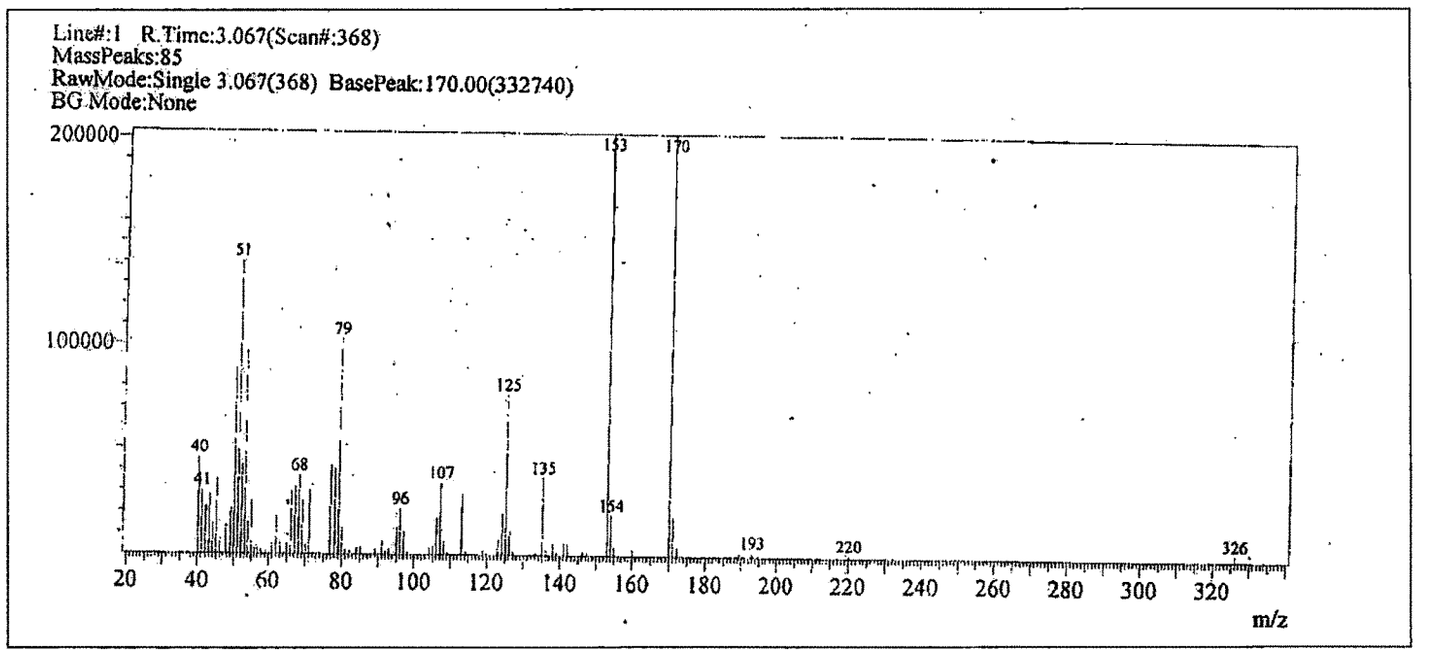
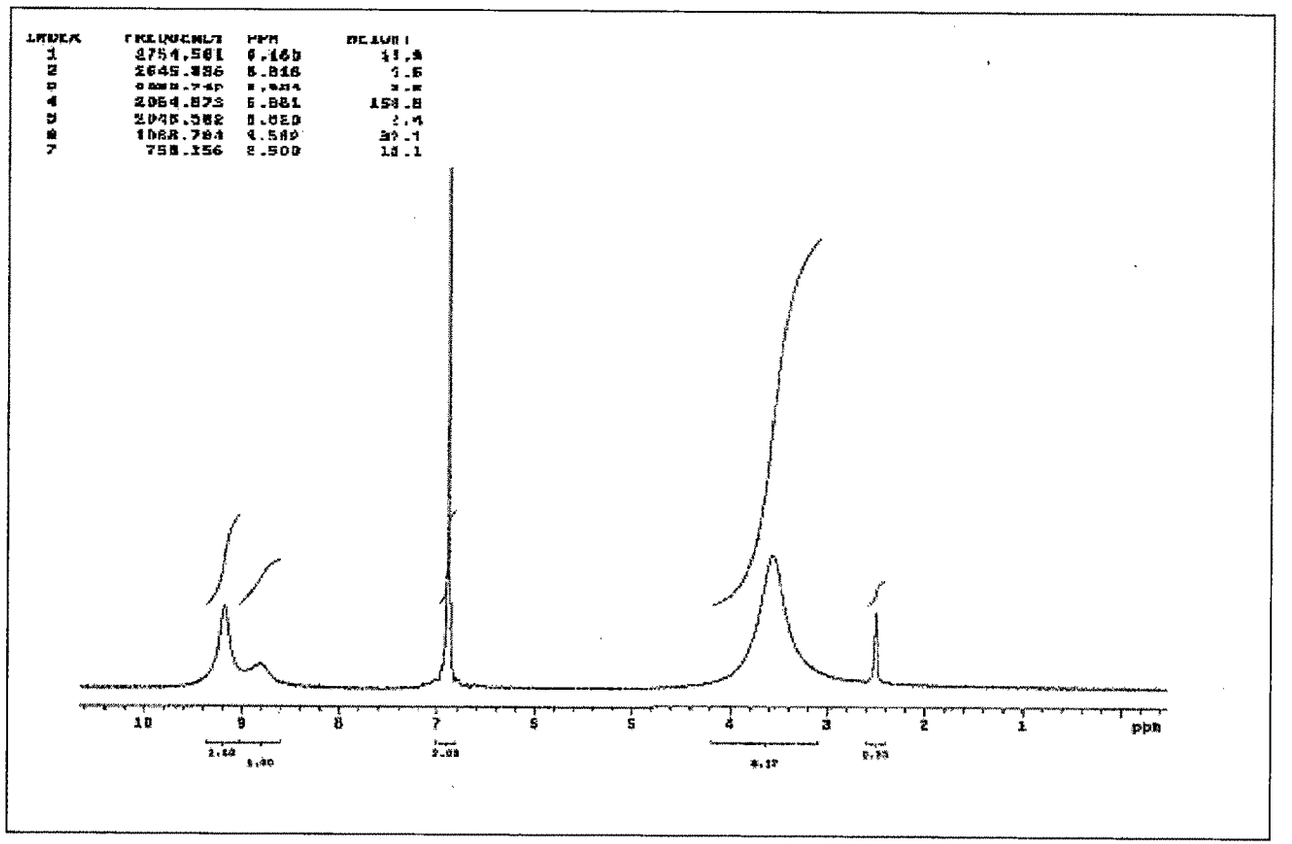


Figure 45: NMR of compound PI 2



Mass spectra

Molecular weight of PI 2 was found to be 253. (Figure 44)

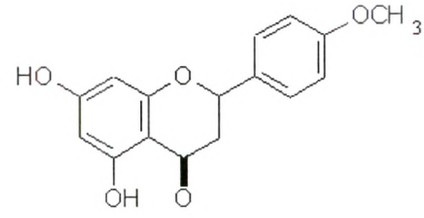
^{1}H NMR spectra

The NMR data showed following results which are represented in Figure 45.

PNMR(DMSO):2.5(1H,s); 3.45(8H,s); 3.165(4H,d); 6.89(2H,d); 6.9(1H,m); 9.2(2H,s)

Figure 46: Probable structure of PI 2

Molecular formula $C_{16}H_{12}O_4$



Leutiolin

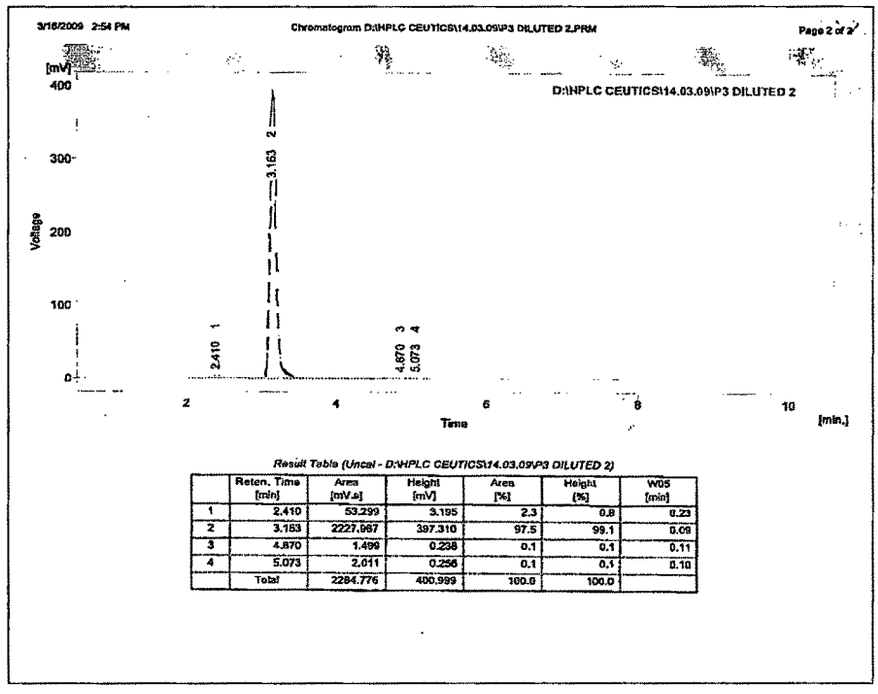
3.7.1.3 Characterization of PI 3

Description: Buff colored powder, m. p. 210° C and λ max 217 nm in methanol. The compound was 97.6% pure. (Figure 47)

Solubility: Freely soluble in Ethyl acetate and Methanol, insoluble in Chloroform.

Chromatographic studies: Thin layer chromatography of the compound using chloroform: ethyl acetate (7:3)as mobile phase, silica gel 60 F_{254} as stationary phase and Ferric chloride as a detecting agent revealed single spot at R_f 0.7 indicating phenolic nature of the compound.

Figure 47: Assessment of % purity of PI 3 by HPLC



Result Table (Uncal - D:\HPLC CEUTICS\14.03.09\P3 DILUTED 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.410	53.299	3.195	2.3	0.8	0.23
2	3.163	2227.867	397.310	97.5	99.1	0.09
3	4.870	1.499	0.238	0.1	0.1	0.11
4	5.073	2.011	0.256	0.1	0.1	0.10
	Total	2284.776	400.999	100.0	100.0	

FTIR spectra

The IR spectra of the sample show the peaks from3365.90-443.64 cm^{-1}.The peaks in the IR spectra from 3365.90-2592.41cm^{-1} represent O-H stretch in carboxylic acid region. The spectra show peaks at 3365.90 - 3018.70 cm^{-1}. This indicates C-H stretch in alkenes. The peak at 3066.92 and 3018.70 cm^{-1} confirms aromatic C-H stretch. The peak at 2050.40 cm^{-1} indicates presence of alkene. The peaks in the range 1620.26-1309.71 cm^{-1} indicate C-C stretch within the ring indicating presence of aromatic group. The spectra show peaks at 1241.91, 1205.55 and 1028.09 cm^{-1} indicating C-O stretch of alcohol and phenol. Aromatic bending of C-H is represented by peaks 866.07, 763.84 and 704.04. The FT-IR spectrum is shown in the figure 48.

Mass spectra

Molecular Weight of PI 3was found to be 170. (Figure 49)

^{1}H NMR spectra

The NMR data showed following results (Figure 50)

PNMR(DMSO):2.50(1H,s); 3.5(9H,s); 6.8(3H,s); 8.8(1H,s); 9.18(2H,s)

Figure 48: IR spectra for PI 3

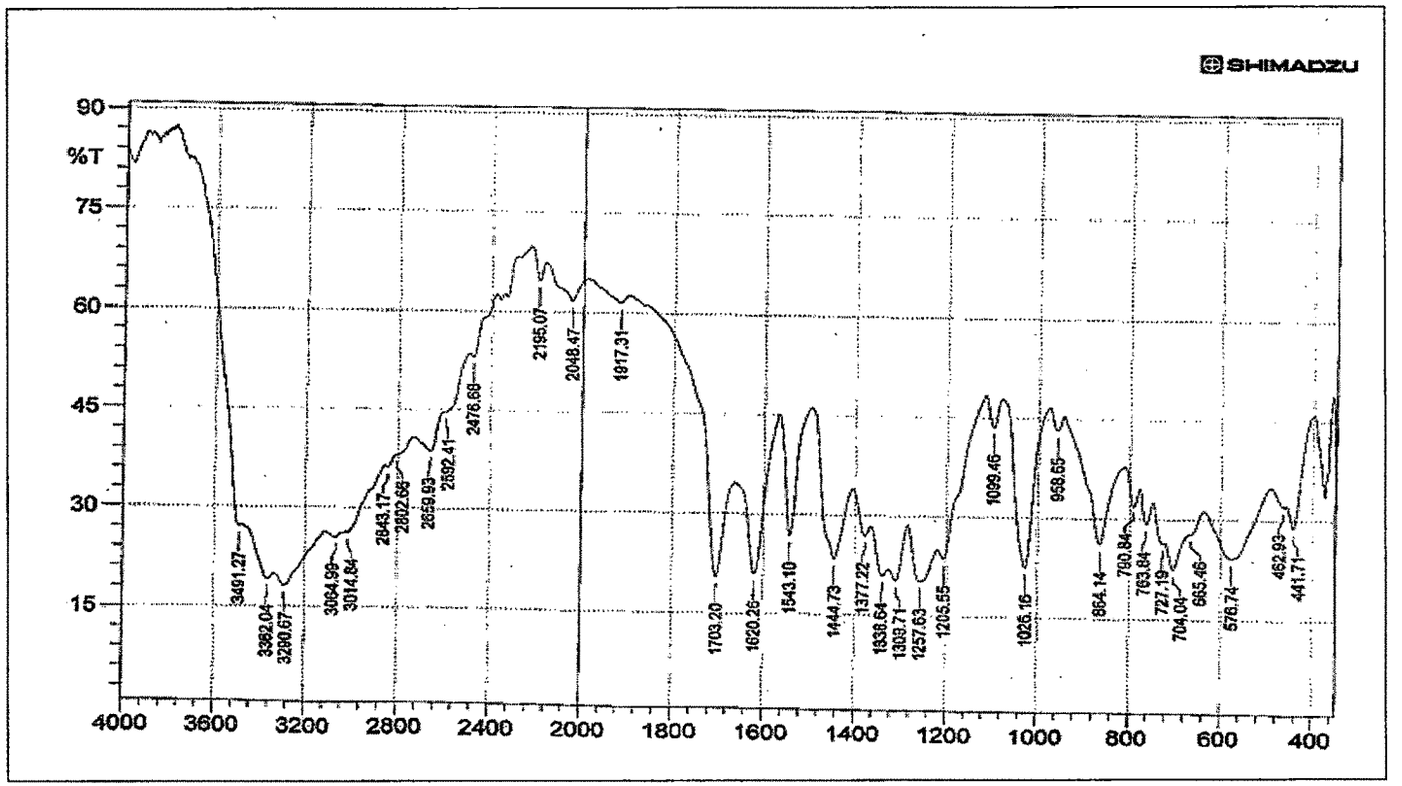


Figure 49: Mass spectra for PI 3

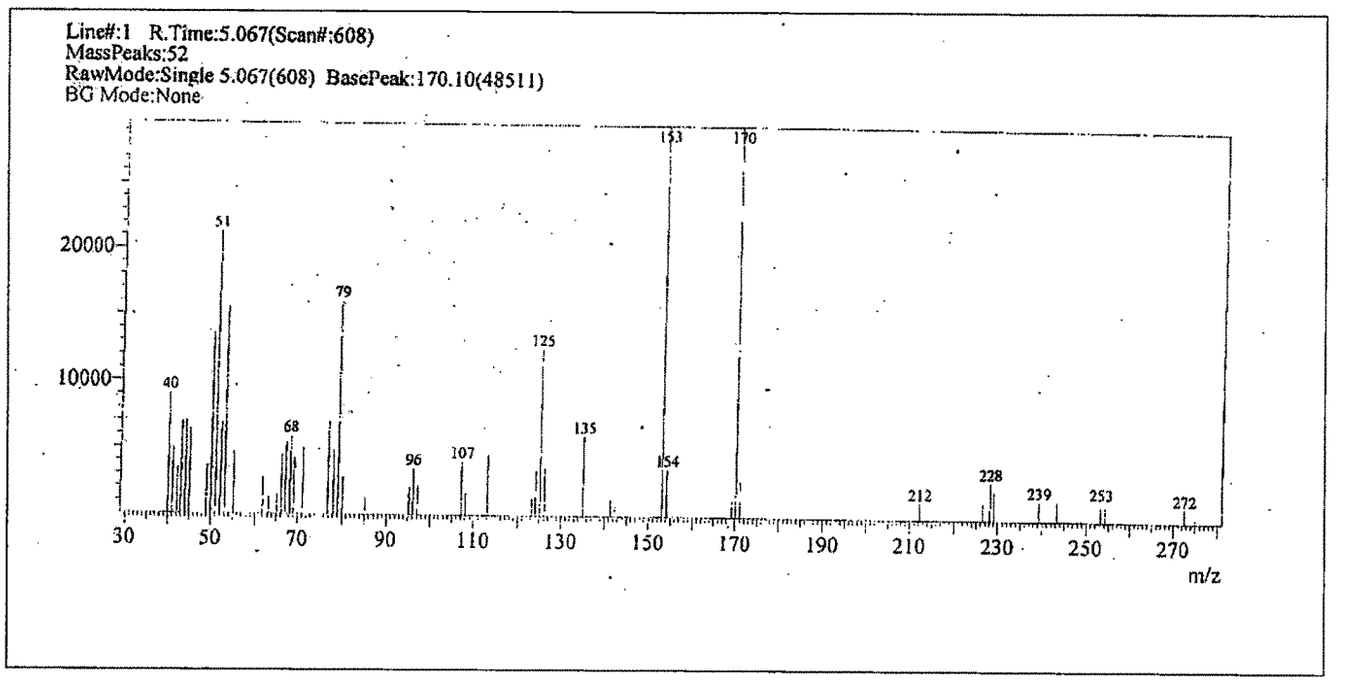


Figure 50: NMR spectra for PI 3

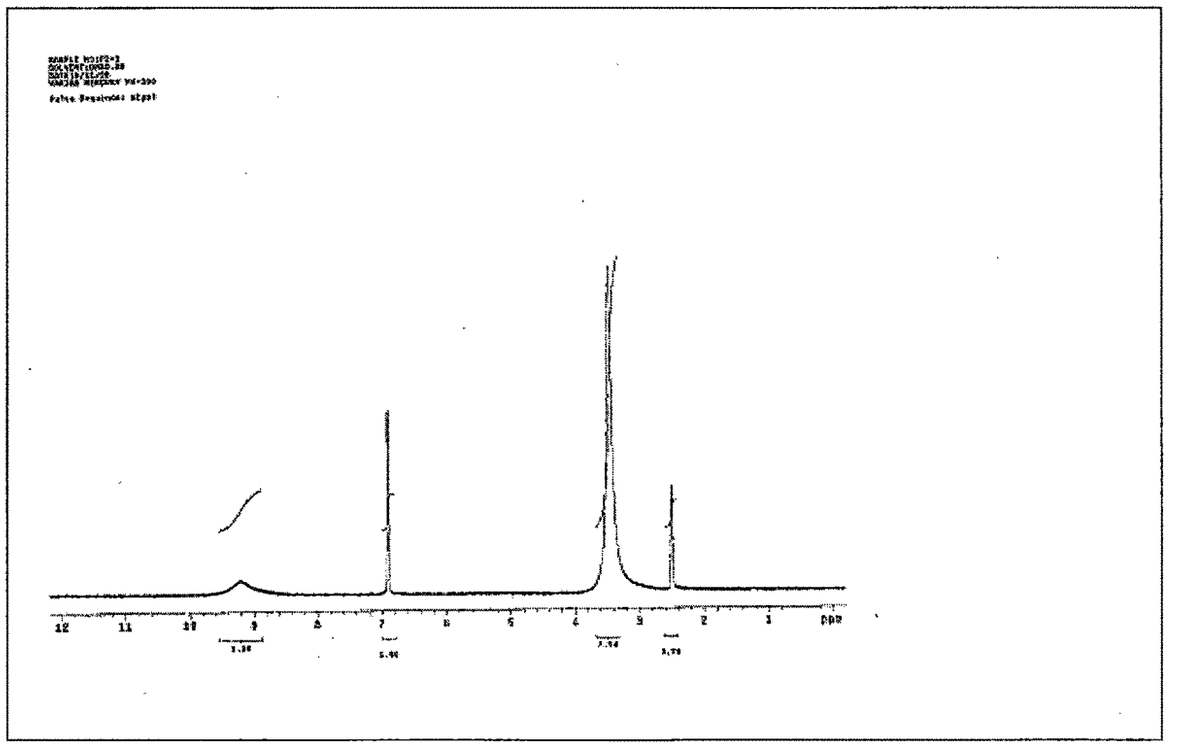
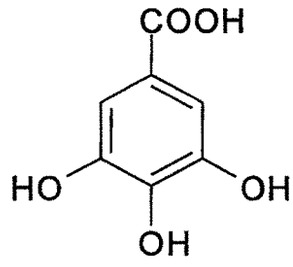


Figure 51: Probable structure of PI 3

Molecular formula $C_7H_{10}O_5$



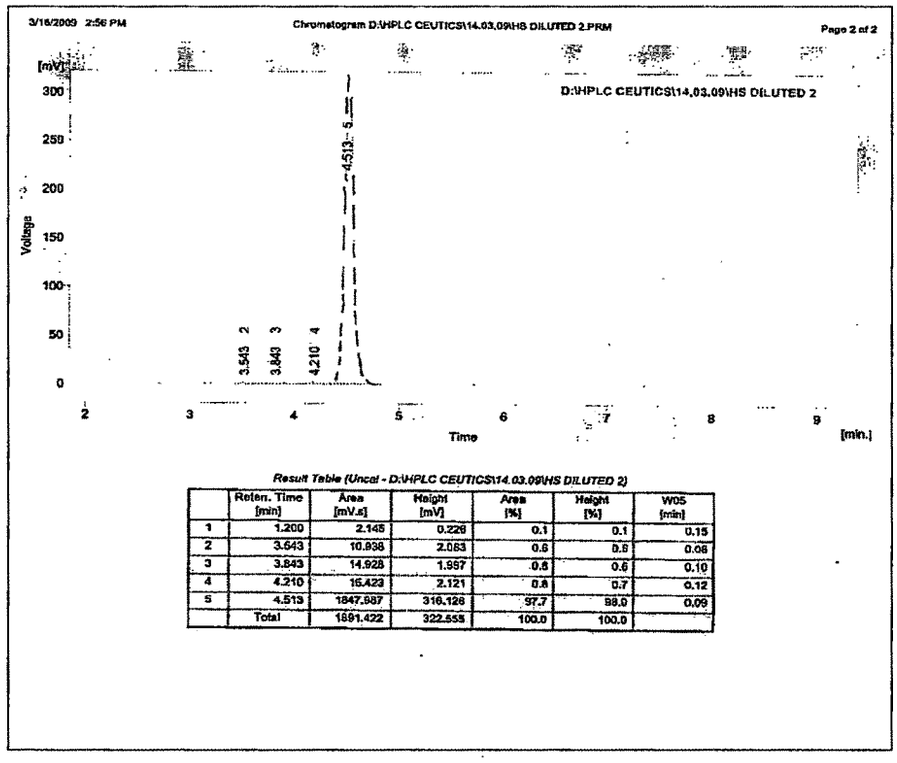
Gallic acid

The spectra were matched with that of standard gallic acid to conform the identity of the compound.

3.1.7.4 Characterization of HS

Description: White crystalline needles, m. p. 107-108° C and λ max 217 nm in methanol. Purity of the compound was found to be 97.8%. (Figure 52)

Figure 52: Assessment of % purity of HS by HPLC



Result Table (Uncal - D:\HPLC CEUTICS\14.03.09\HS DILUTED 2)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	1.200	2.145	0.226	0.1	0.1	0.15
2	3.543	10.938	2.083	0.6	0.6	0.08
3	3.843	14.928	1.957	0.8	0.6	0.10
4	4.210	16.423	2.121	0.8	0.7	0.12
5	4.513	1847.587	316.126	97.7	98.0	0.09
	Total	1891.422	322.555	100.0	100.0	

Solubility: Freely soluble in Benzene, Hexane and Chloroform, insoluble in Methanol and Ethyl acetate.

Chromatographic studies: Thin layer chromatography of the compound using Toluene: Chloroform: Ethanol (4:4:1) as mobile phase, silica gel 60 F_{254} as stationary phase and AS reagent as a detecting agent revealed single spot at R_f 0.17 indicating diterpene nature of the compound

FTIR spectra

The IR spectra of the sample show the peaks from3387.11-385.78 cm^{-1}.The peaks in the IR spectra from 3387.11-3230.87cm^{-1} represent C-H stretch in alkenes. Peaks at 1388, 1367 and 1174 cm^{-1} indicate presence of gem dimethyl. The spectra show peaks between 3039.91-2931.90 cm^{-1}. This indicates C-H stretch. The peaks at 1512, 885, 7 77 cm^{-1} confirms presence of furan ring. Presence of alkanes is represented by peaks 1292.35, 1253.77, 1209.41 and 1174.69. The peaks at 1250-1111 cm^{-1} confirms the stretch in lactones and 1650-1500 cm^{-1} indicates presence of C=O. The FT-IR spectrum is shown in the figure 53.

Mass spectra

Molecular Weight was found to be 206. (Figure 54)

^{1}H NMR spectra

The results of NMR (Figure 55) are as follows:

PNMR($CDCl_3$):0.82(1H,s);0.878(1H,s);1.25(3H,s);2.5(1H,s);2.27(2H,s);1.9(2H,d); 2.41(2H,d); 2.461(2H,d); 2.76(1H,s); 3.82(6H,d); 4.22(4H,d); 4.59(2H,s); 5.32(1H,s); 5.38(1H,s); 5.82(1H,s); 6.26(1H,d); 6.31(1H,s); 6.89(4H,d); 7.24(1H,s); 7.35(1H,s);7.46(1H,s); 7.67(2H,d)

The formula was derived on the basis of elemental analysis and the structure was predicted on the basis of IR and NMR spectra. The physicochemical properties and the spectra of the predicted compound were matched with that of standard to confirm the nature of the compound.

Figure 53: IR spectra of HS

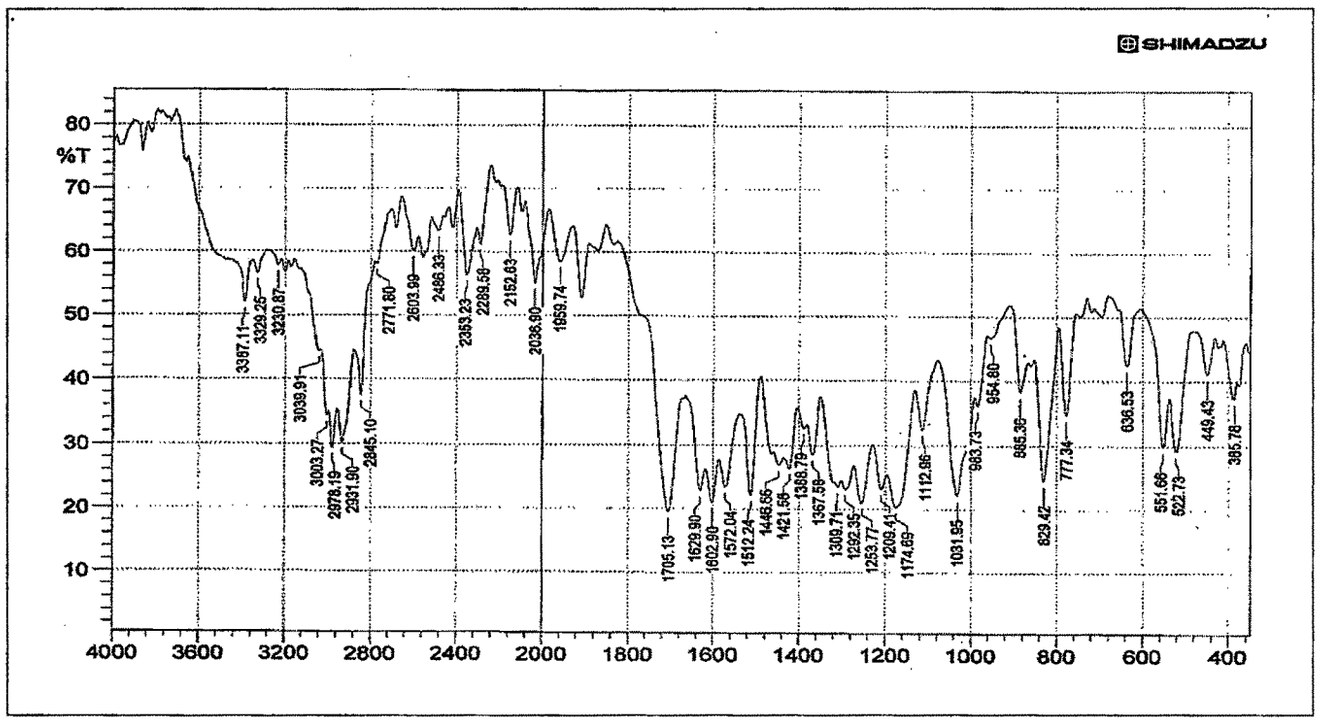


Figure 54: Mass spectra of HS

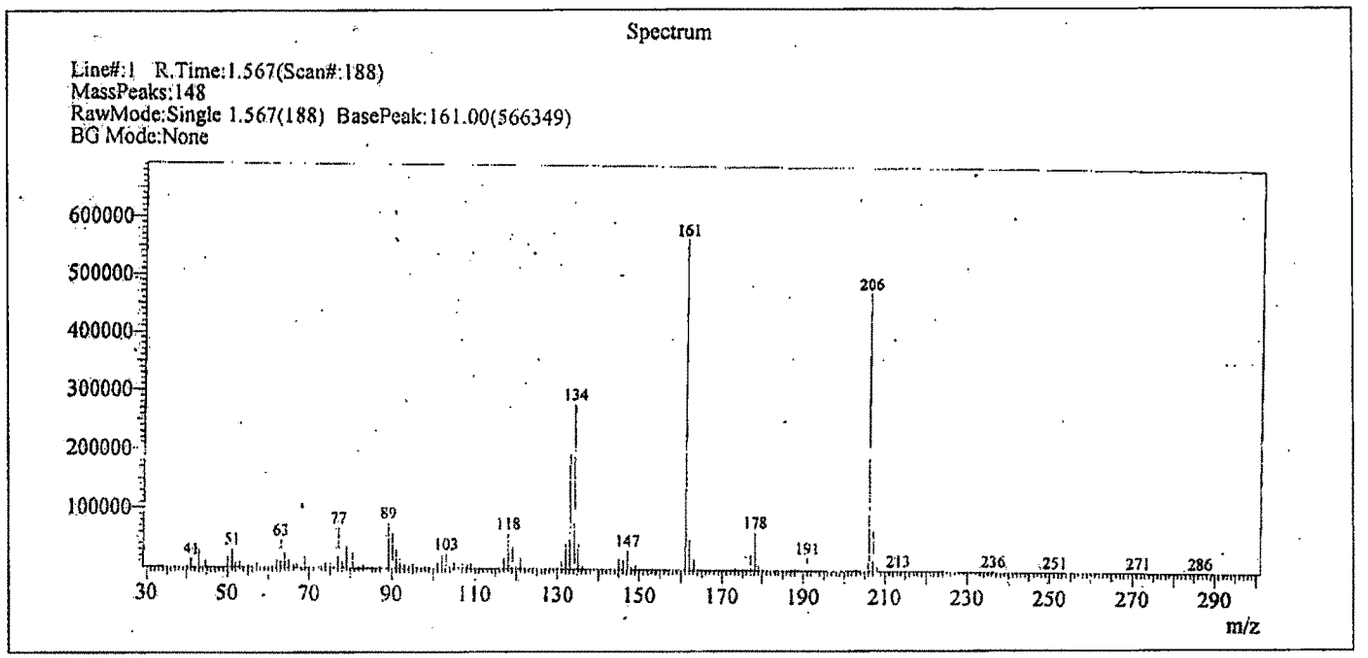
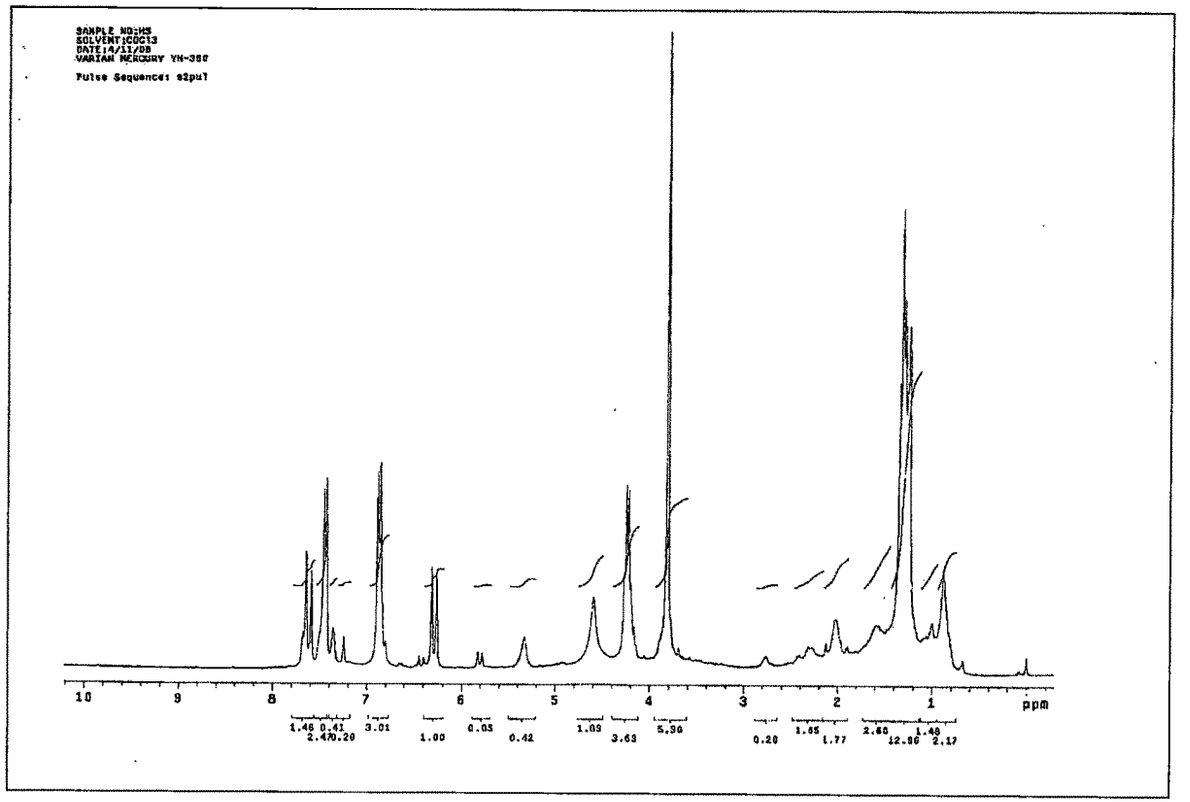


Figure 55: NMR spectra of HS

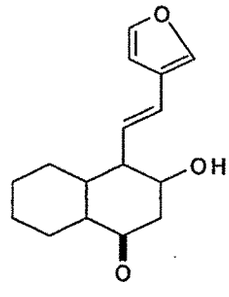


Frequencies of NMR of HS

INDEX	FREQUENCY	PPM	HEIGHT	INDEX	FREQUENCY	PPM	HEIGHT
1	2304.293	7.678	8.0	40	328.163	1.094	7.4
2	2294.666	7.647	28.5	41	319.086	1.063	7.8
3	2278.712	7.594	23.7	42	300.656	1.002	11.1
4	2240.201	7.466	44.6	43	283.521	0.878	23.9
5	2231.949	7.438	47.4	44	247.566	0.825	8.3
6	2208.293	7.359	9.0				
7	2175.008	7.249	6.9				
8	2068.280	6.893	49.1				
9	2060.028	6.865	51.8				
10	2041.323	6.803	6.0				
11	1895.259	6.316	24.8				
12	1879.304	6.263	22.7				
13	1748.644	5.828	9.2				
14	1735.991	5.785	2.9				
15	1598.729	5.328	7.0				
16	1378.770	4.598	17.3				
17	1275.242	4.250	45.6				
18	1268.365	4.227	44.7				
19	1261.489	4.204	20.7				
20	1249.985	4.166	9.9				
21	1243.059	4.143	5.2				
22	1146.283	3.820	182.0				
23	1107.722	3.692	4.5				
24	828.797	2.762	2.7				
25	730.596	2.435	2.6				
26	723.719	2.412	2.8				
27	692.361	2.307	4.8				
28	684.384	2.281	4.8				
29	638.171	2.127	5.7				
30	608.738	2.029	12.0				
31	603.237	2.010	11.7				
32	570.228	1.900	5.1				
33	544.096	1.813	3.9				
34	477.803	1.592	10.5				
35	405.184	1.350	72.2				
36	398.307	1.327	116.8				
37	391.155	1.304	93.6				
38	382.078	1.273	70.8				
39	375.476	1.251	87.0				

Figure 56: Probable structure of HS

Molecular formula: $C_{20}H_{26}O_3$.



Furanoditerpenoid

3.8 Quantification of identified compounds in *P. integerrima* and *H. spicatum* by HPTLC

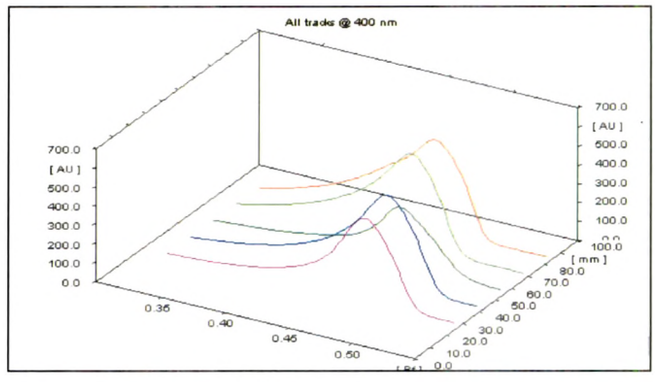
3.8.1 Quantification of Kaempferol in *H.spicatum* by HPTLC

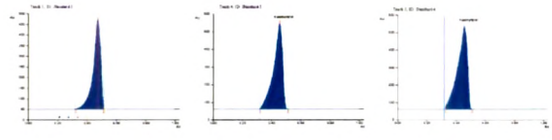
The TLC profile showed presence of flavonoids at different R_f viz 0.14, 0.48, 0.62 and 0.77. The test extract and standard marker were run simultaneously by TLC and found similar R_f, for test extract it was 0.71 and for standard Kaempferol 0.78. Hence, it was further subjected to HPTLC for quantitative determination Results are given in Table 41. The 5 of Kaempferol was found to be 0.8% in methanol extract of *H. spicatum.*

3.8.2 Quantification of Quercetin and gallic acid in methanol extract of *P. integerrima* by HPTLC

The TLC profile showed presence of quercetin and gallic acid in Methanol extract of *P. integerrima.* Their presence was confirmed by co-TLC with the standard. Therefore, it was further subjected to quantification by reported method. Results are reported in Table 43 and 45. The methanol extract was found to be rich in quercetin and gallic acid and the % was 4.94 and 5.63 respectively.

Figure 57: Quantification of Kaempferol in methanol extract *H. spicatum*





Standard Extrcat Standard

Graph 2: Calibration curve for Kaempferol

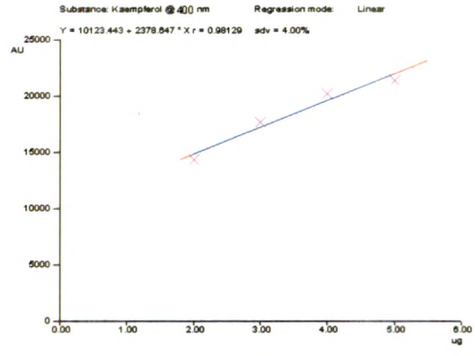
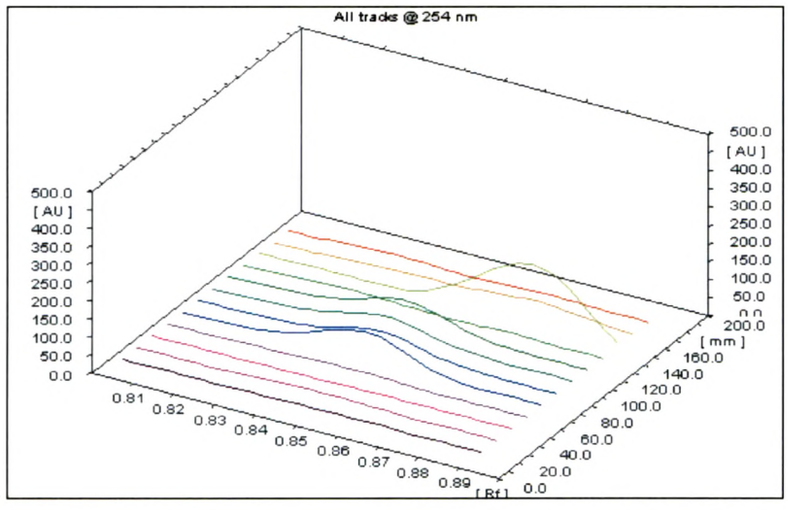
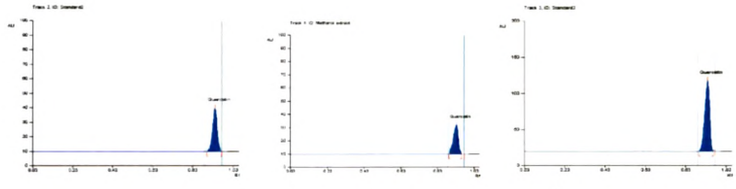


Table 41: Quantification of Kaempferol in methanol extract of *H.spicatum*

Track	Start R_f	Start Height	Max R_f	Max Height	End R_f	End Height	Area	Amt of Kaempferol
1	0.33	2.1	0.47	418.5	0.51	8.1	14395.9	1.0µg
2	0.32	0.4	0.47	451.0	0.51	9.5	17680.2	2.0µg
3	0.32	1.1	0.47	289.0	0.51	5.6	10270.6	3.0µg
4	0.32	2.2	0.46	486.2	0.51	5.6	20250.9	0.08µg
5	0.32	17.1	0.46	475.9	0.51	17.1	21467.8	4.0µg

Figure 58: HPTLC fingerprint for quantification of Quercetin in Methanol extract *P.integerrima*





Standard Extract Standard

Table 42: HPTLC fingerprint of Quercetine in methanol extract *P.integerrima*

Track	Start R_f	Start Height	Max R_f	Max Height	End R_f	End Height	Area
1	0.90	0.2	0.94	26.9	0.97	0.2	574.7
2	0.89	0.2	0.94	97.7	0.97	1.6	2003.8
3	0.89	0.0	0.93	22.6	0.96	0.0	510.8
4	0.89	15.3	0.94	258.2	0.96	0.8	6374.8
5	0.89	5.6	0.94	288.9	0.96	0.3	7420.6
6	0.89	26.7	0.94	289.9	0.97	0.9	7824.1
7	0.90	5.6	0.94	318.1	0.97	1.7	9141.3

Graph 3: Calibration curve for Quercetin

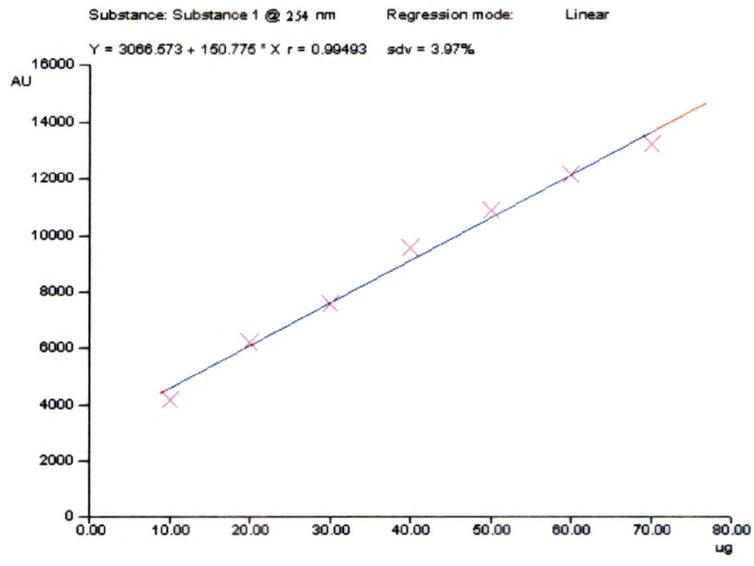
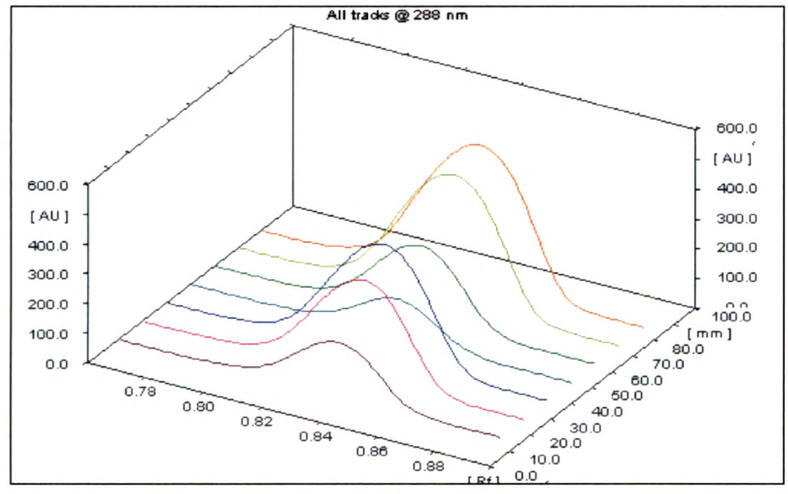
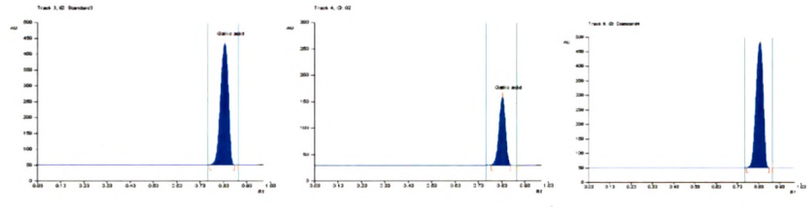


Table 43: Quantification of Quercetine in methanol extract *P.integerrima*

Track	R_f	Amount (ng)	Height	Max %	X(Cal) (ng)	Area	X(Cal) (ng)
1	0.94	400	29.89	100		574.12	
2	0.94	600	97.89	100		2003.80	
3	0.93		22.59	100	360.0	510.82	360.0
4	0.93		88.25	100	471.0	2003.20	494.5
5	0.93	800	258.23	100		6374.83	
6	0.94	1000	288.91	100		7420.63	
7	0.94	1200	289.64	100		7824.09	
8	0.94	1400	318.11	100		9141.30	

Figure 59: HPTLC fingerprint for quantification of Gallic acid in Methanol extract *P.integerrima*





Standard Methanol extract Standard

Table 44: HPTLC fingerprint of Gallic acid in methanol extract *P.integerrima*

Track	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area
1	0.78	0.8	0.84	177.7	100	0.87	0.6	4393.7
2	0.78	1.2	0.84	325.9	100	0.87	0.4	8843.5
3	0.77	0.8	0.83	383.2	100	0.88	0.0	10897.1
4	0.78	0.9	0.83	129.1	100	0.87	0.1	3040.5
5	0.78	2.3	0.83	245.2	100	0.87	0.0	6262.1
6	0.77	0.0	0.83	432.0	100	0.88	0.1	12640.3
7	0.77	0.4	0.84	470.6	100	0.89	0.7	14406.9

Graph 4: Calibration curve for Gallic acid

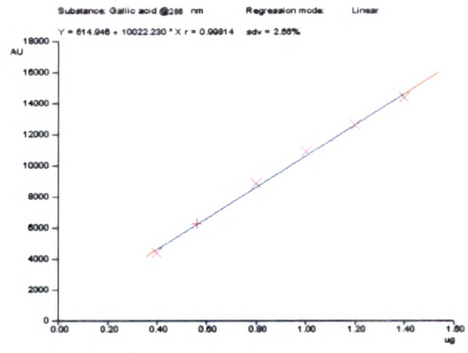


Table 45: Quantification of Gallic acid in methanol extract *P.integerrima*

Track	R_f	Amount (ng)	Height	Max %	X(Cal) (ng)	Area	X(Cal) (ng)
1	0.84	400	177.67	100		4393.70	
2	0.84	800	325.92	100		8843.48	
3	0.83	1000	383.23	100		10897.05	
4	0.83		129.09	100	360.0	3040.46	360.0
5	0.83		245.22	100	577.61	6262.12	563.47
6	0.83	1200	432.05	100		12640.29	
7	0.83	1400	470.56	100		14406.91	

3.9 In vitro antioxidant activity of different extracts and factions of *P. integerrima*

Free radicals are generated in the body due to several reasons one of which is stress. The free radicals are implicated for several disorders like cancer, hepatitis, arthritis etc. in treatment of these diseases antioxidant therapy gained utmost importance. *P. integerrima* was found to be rich in phenolics and flavonoids therefore all the extracts and fractions were subjected to in vitro antioxidant activity. The results are discussed in this section.

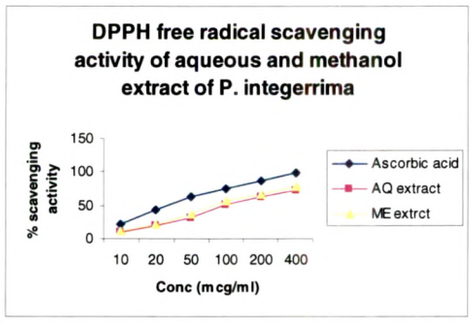
Table 46:DPPH free radical scavenging activity of extracts, fractions and isolated compounds of *P. integerrima.*

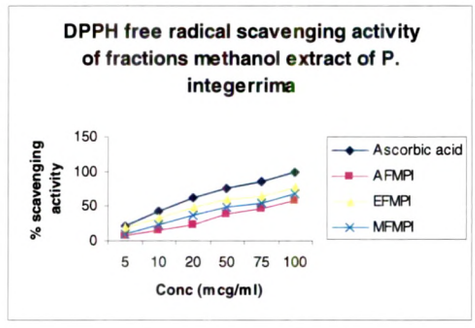
Conc (mcg/ml)	% scavenging activity											
	Ascorbic acid	Aqueous extract	Methanol extract	AFMPI	EFMPI	MFMPI	BFAPI	MFAPI	EFAPI	PI1	PI 2	PI 3
10	22.36	10.25	12.36	8.25	18.64	10.25	10.21	14.21	18.26	15.23	14.21	18.26
20	42.63	19.36	21.05	16.02	32.98	24.31	25.21	25.66	27.58	28.32	25.65	35.65
50	62.35	32.36	36.94	24.15	48.21	36.94	31.36	34.65	39.56	39.45	34.65	48.35
100	75.42	52.03	56.25	39.21	59.78	68.20	46.89	49.28	49.32	52.69	49.28	62.36
200	86.25	62.54	66.58	46.32	71.06	78.32	58.54	62.15	62.54	67.45	62.15	75.42
400	98.56	73.25	79.54	59.32	82.13	91.03	64.21	68.45	74.54	78.25	68.45	82.61

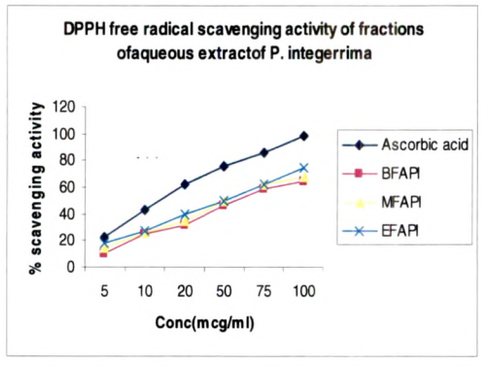
Table 47: Reducing power asssay of extracts, fractions and isolated compounds of *P. integerrima.*

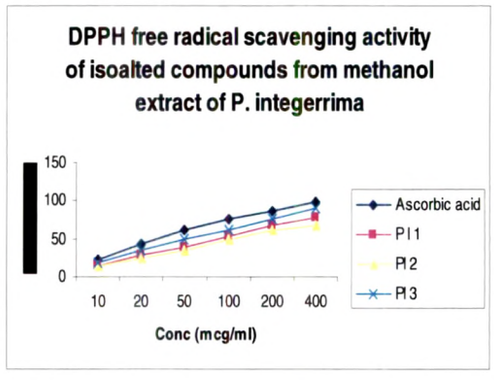
Conc (mcg/ml)	Absorbance											
	Ascorbic acid	Aqueous extract	Methanol extract	AFMPI	EFMPI	MFMPI	BFAPI	MFAPI	EFAPI	PI1	PI 2	PI 3
5	0.152	0.075	0.096	0.075	0.102	0.095	0.065	0.080	0.105	0.094	0.098	0.109
10	0.239	0.098	0.115	0.098	0.156	0.196	0.098	0.106	0.196	0.154	0.122	0.199
20	0.345	0.153	0.201	0.152	0.231	0.231	0.152	0.198	0.235	0.249	0.228	0.312
50	0.569	0.240	0.348	0.265	0.386	0.302	0.296	0.236	0.356	0.387	0.314	0.428
75	0.721	0.463	0.513	0.359	0.439	0.369	0.354	0.378	0.478	0.459	0.396	0.519
100	0.89	0.526	0.598	0.541	0.653	0.554	0.425	0.512	0.612	0.547	0.489	0.648

Figure 60: DPPH free radical scavenging activity of extracts, fractions and isolated compounds of *P. integerrima*



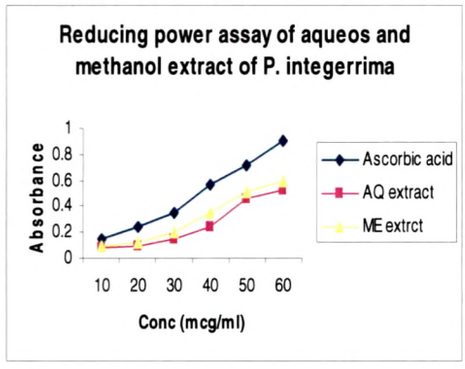


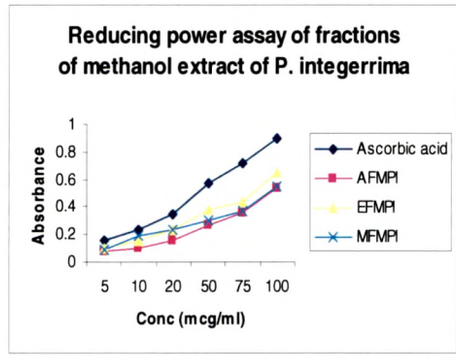


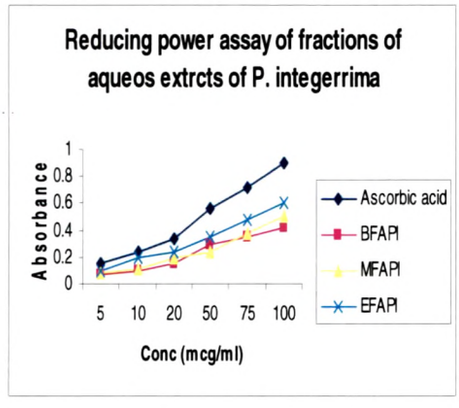


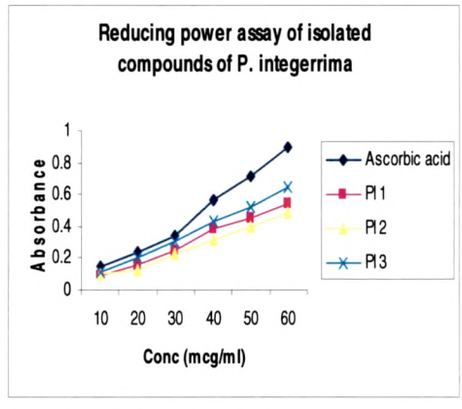
DPPH activity is a quick and reliable parameter to assess in vitro antioxidant activity. DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares, 1997). The absorption maximum of a stable DPPH radical in methanol was at 512 nm. The decrease in the absorbance of DPPH radical caused by antioxidant because the reaction between antioxidant molecule and free radical progresses which results in the scavenging of radical by hydrogen donation (Chang, 2002). The results of our studies indicate that the extracts and the fraction showed dose dependant activity. IC_{50} was found at the concentration100mcg/ml. (Table 46)

Figure 61: Reducing power assay of extracts, fractions and isolated compounds of *P. integerrima.*









The antioxidant activity has been attributed to various mechanisms. Among which are the prevention of chain initiation, the binding of transition, the prevention of continued hydrogen abstraction, the reductive capacity and radical scavenging. The reducing capacity of extract or compound may serve as a significant indicator of its potential antioxidant activity (Meir, 1995). For measurement of the reductive ability, ferric to ferrous transformation was assessed. The extracts and fractions showed comparable activity to that of the reference standard. The results of antioxidant activity of extracts, fractions and isolated compounds are reported in Table 47.

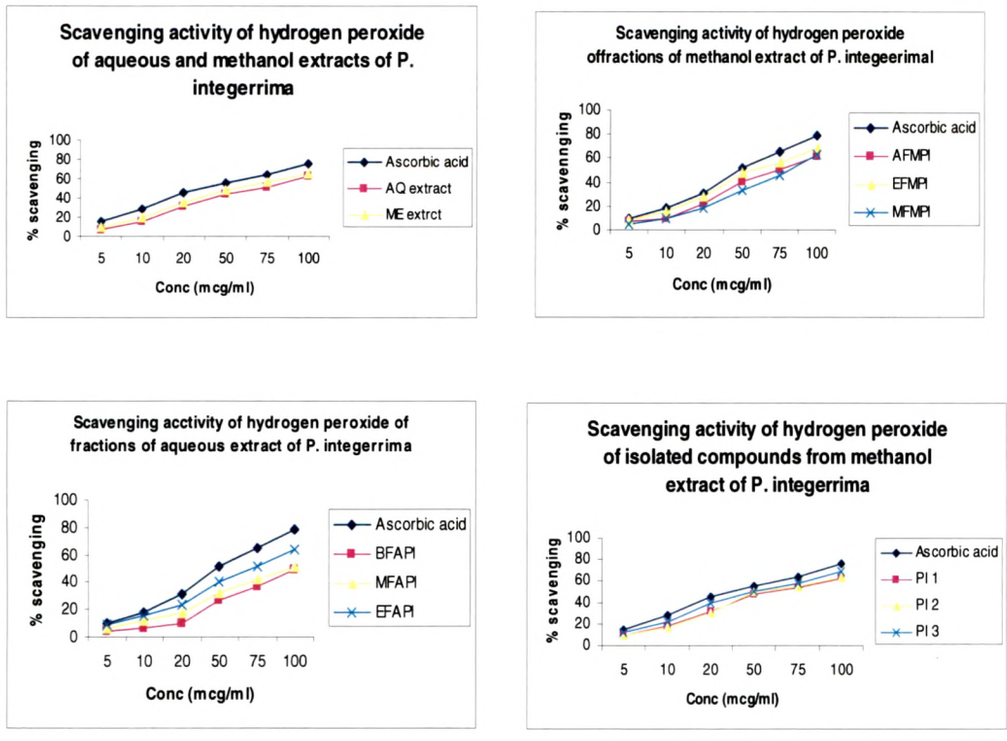
Table 48: Scavenging of hydrogen peroxide of extracts, fractions and isolated compounds of *P. integerrima.*

Conc (mcg/ml)	% scavenging activity											
	Ascorbic acid	Aqueous extract	Methanol extract	AFMPI	EFMPI	MFMPI	BFAPI	MFAPI	EFAPI	PI1	PI 2	PI 3
5	15.36	7.21	10.25	7.12	8.95	5.32	4.32	6.54	9.45	9.26	10.15	12.48
10	28.65	16.32	20.45	10.25	15.65	9.32	6.38	12.36	15.69	18.45	16.84	22.65
20	45.21	31.25	35.65	22.65	28.98	18.21	10.5	18.31	24.01	32.65	31.26	39.35
50	55.63	44.29	49.24	40.29	48.15	33.65	26.82	32.08	40.09	48.21	50.32	50.21
75	64.69	51.06	56.89	50.06	56.32	46.25	37.21	42.69	51.29	54.25	55.16	58.45
100	76.32	62.39	65.21	62.15	69.06	63.21	4.97	52.09	52.09	62.36	64.28	69.03

Table 49: Hydroxyl radical scavenging activity of extracts, fractions and isolated compounds of *P. integerrima.*

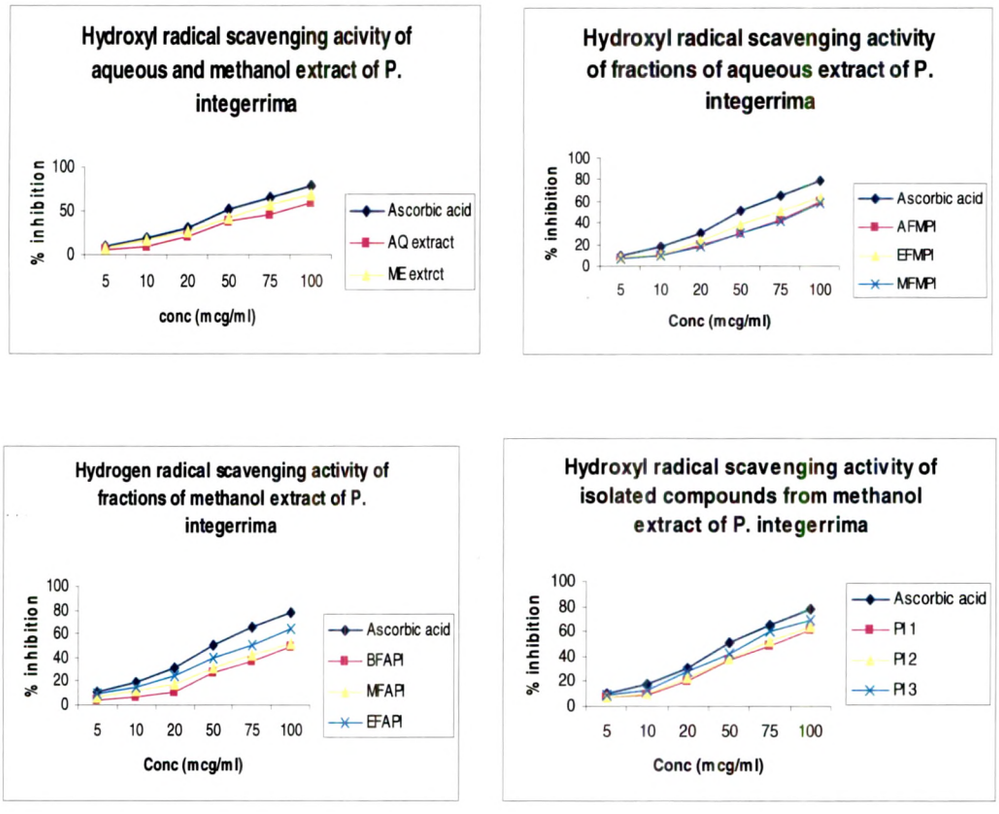
Conc (mcg/ml)	% scavenging activity											
	Ascorbic acid	Aqueous extract	Methanol extract	AFMPI	EFMPI	MFMPI	BFAPI	MFAPI	EFAPI	PI1	PI 2	PI 3
5	10.36	5.21	8.26	6.54	8.56	6.27	4.32	6.54	9.45	7.54	8.21	9.08
10	18.51	9.54	16.54	9.45	11.02	9.05	6.38	12.36	15.69	9.56	10.06	12.36
20	31.05	20.65	26.47	19.21	23.65	17.54	10.5	18.31	24.01	20.15	23.65	28.64
50	51.25	38.19	42.69	31.25	39.54	30.26	26.82	32.08	40.09	36.89	38.41	42.36
75	65.69	45.56	57.45	42.69	51.26	42.15	37.21	42.69	51.29	48.94	52.64	60.25
100	78.54	59.32	68.54	59.21	64.25	58.45	48.97	52.09	64.02	61.64	64.21	68.74

Figure 62: Scavenging of hydrogen peroxide of extracts, fractions and isolated compounds of *P. integerrima*.



Although hydrogen peroxide is not very reactive, it can sometimes cause cytotoxicity giving rise to hydroxyl radicals in the cell. Thus removing hydrogen peroxide is very important throughout the food system. Scavenging of hydrogen peroxide may be due to donation of electrons to hydrogen peroxide thus neutralizing it to water (Elmasta, 2006). Extracts, fractions as well as isolated compounds were found to possess good antioxidant activity by scavenging hydrogen peroxide. The results are given in Table 48.

Figure 63: Hydroxyl radical scavenging activity of extracts, fractions and isolated compounds of *P. integerrima.*



Hydroxyl radical is very reactive and can be generated in the cells through Fenton reaction. The potential scavenging ability of phenolic substances may be due to the active hydrogen donor ability of hydrogen substitution. Similarly, high molecular weight and proximity of many aromatic rings and hydroxyl groups are more important for free radical scavenging by specific functional groups (Korycka, 1978). The results mentioned in Table 49 indicate the extracts, fractions and isolated compounds possess activity comparable to that of standard.

3.9 Biological screening of extracts and fractions

3.9.1 Acute toxicity studies

All the selected extracts and fractions were subjected to acute toxicity studies as per OECD guidelines. None of the extracts and fractions showed mortality even at the dose of 2000mg/kg and therefore considered to be safe.

All the extract and fractions were investigated for adaptogenic activity using different models.

3.9.2 *E.coli* induced abdominal sepsis in mice

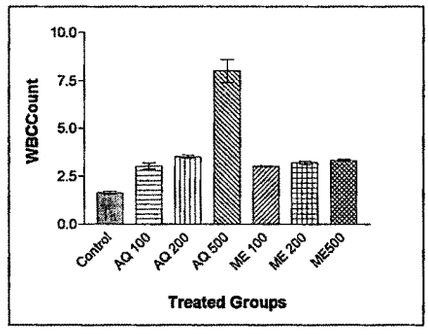
Different extracts and their fractions were subjected to evaluate the increase in WBC and % neutrophils in *E.coli* induced abdominal sepsis.

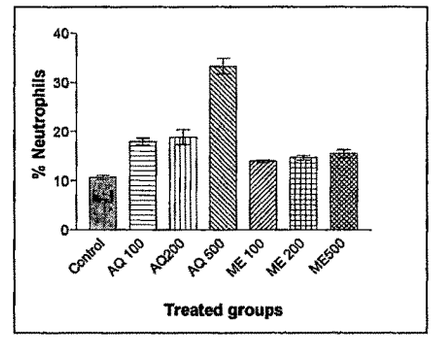
Table 50: Effect of Aqueous and Methanol extracts of *P. integerrima* on WBC and neutrophils count in *E. coli* induced abdominal sepsis in mice.

No	Groups	Dose (mg/Kg)	WBC (10^3 / mm^3)	Neutrophils (%)
1	Group I	Control (0.1% Sod CMC)	1.800 ±0.17	9.33 ±1.16
2	Group III	Aqueous 100	2.337±0.07	13.83 ±1.7
3	Group IV	Aqueous 200	2.40 ± 0.18**	14.00± 1.2*
4	Group V	Aqueous 500	3.65_±0.28**	23.67± 1.22**
5	Group VI	Methanol 100	2.11_±0.14	11.17 ±1.6
6	Group VII	Methanol 200	1.95 ±0.14	12.83 _±2.04
7	Group VIII	Methanol 500	2.350 ±0.17**	13.50 ± 2.07*

Six animals were used. *p< 0.5, ** p<0 .01, *** p< 0.001 Statistical analysis is done by applying One way ANOVA followed by Bonferronis test.

Graph 5: Effect of Aqueous and Methanol extracts of *P. integerrima* on WBC and neutrophils in *E. coli* induced abdominal sepsis in mice.





Aqueous and Methanol extracts of *P. integerrima* and their fractions in different solvent were subjected to peritonitis by *E.coli.* The effect of extracts and fractions on WBC and % Neutrophils were evaluated. The mortality due to peritonitis was assessed in control and treatment groups. It was observed that there was 100% mortality in control group. In the treatment groups in 100 and 200mg/kg body wt dose in aqueous and Methanol extract 50% mortality was observed. The WBC count was found to be 2.337±0.07 and 2.11±0.14 respectively. In the group treated with 500mg/kg, 33 % mortality was observed. The WBC count was 3.65±0.28 and 2.350 ±0.17 ($p<0.01$). The results are recorded in Table 50. Both the extracts, at 500 mg/kg were found to be more significant i.e. $p<0.01$. There was significant increase in WBC and % of Neutrophils. The % Neutrophils in Aqueous 500mg/kg dose was 23.67± 1.22 ($p<0.01$) and Methanol extract 500mg/kg 13.50 ± 2.07($p<0.05$).

Table 51: Effect of fractions of Methanol extract of *P. integerrima* on WBC and Neutrophils in *E. coli* induced abdominal sepsis in mice

No	Groups	Dose (mg/Kg)	*E.coli* induced abdominal sepsis in mice	
			WBC (10^3 / mm^3)	Neutrophils (%)
1	Group I	Control (0.1% Sod. CMC)	1.667± 0.16	9.500±1.64
2	Group III	CFMPI 50	4.033±0.64	10.67±1.21
3	Group IV	CFMPI 100	6.700±1.58	10.00±1.41
4	Group V	CFMPI 150	8.617±1.34*	11.33±0.81
5	Group VI	AFMPI 50	4.050±1.83	10.00±1.09
6	Group VII	AFMPI 100	7.683±1.70	10.00±1.41
7	Group VIII	AFMPI 150	8.717±2.46**	18.50±1.87**
8	Group IX	EFMPI 50	3.083±0.45***	17.17±5.26***
9	Group X	EFMPI 100	9.600±0.57***	14.83±2.78***
10	Group XI	EFMPI 150	9.217±2.64***	16.17±2.56***
11	Group XII	MFMPI 50	5.683±1.30	17.50±2.88***
12	Group XIII	MFMPI 100	8.800±2.34***	18.50 ±4.32***
13	Group XIV	MFMPI 150	8.050±2.12*	16.00±4.050*

Six animals were used. *$p< 0.5$, ** $p<0.01$, *** $p< 0.001$ Statistical analysis is done by applying One way ANOVA followed by Bonferronis test.

Graph 6: Effect of fractions of Methanol extract of *P. integerrima* in on WBC and Neutrophils *E. coli* induced abdominal sepsis in mice.

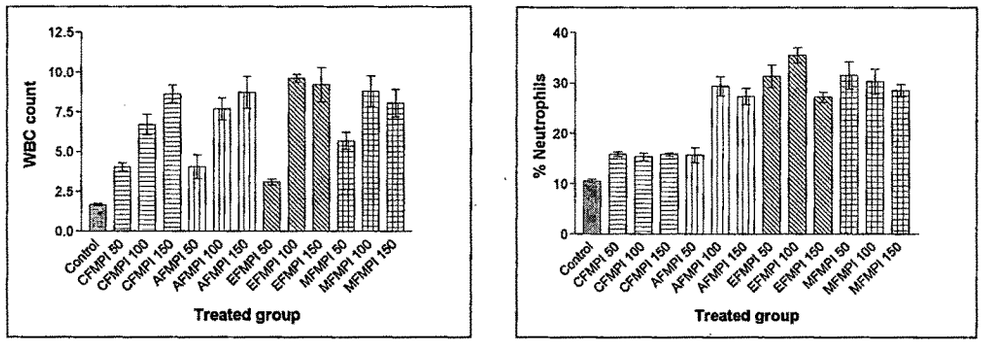
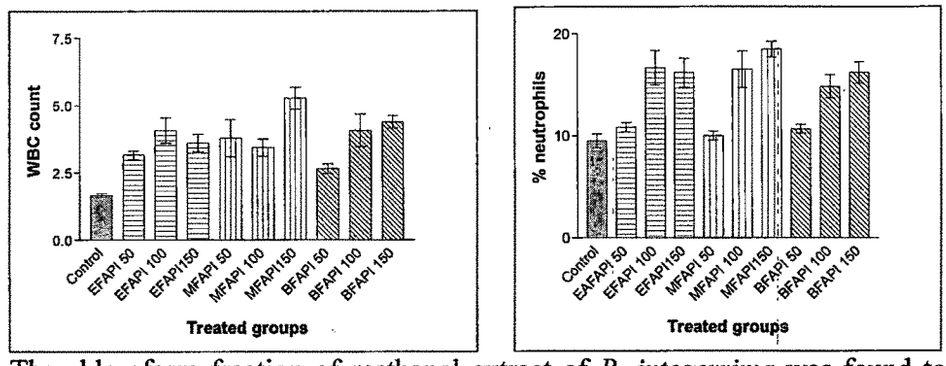


Table 52: Effect of fractions of Aqueous extract of *P. integerrima* on WBC and Neutrophils in *E. coli* induced abdominal sepsis in mice

No	Groups	Dose (mg/Kg)	*E.coli* induced abdominal sepsis in mice	
			WBC (10^3 / mm^3)	Neutrophils (%)
1	Group I	Control (0.1% Sod. CMC)	1.667± 0.16	9.500±1.64
2	Group II	EFAPI 50	3.150±0.39	10.83±1.16
3	Group III	EFAPI 100	4.067±1.16*	16.67±4.13**
4	Group IV	EFAPI 150	3.600±0.79	16.17±3.48***
5	Group V	MFAPI 50	3.783±1.68*	10.00±1.09
6	Group VI	MFAPI 100	3.433±0.78	16.50±4.37**
7	Group VII	MFAPI 150	5.267±0.99**	18.50±1.87**
8	Group VIII	BFAPI 50	2.650±0.43	10.67±1.03
9	Group IX	BFAPI 120	4.067±1.50**	14.83±2.78
10	Group X	BFAPI 150	4.383±0.57***	16.17±2.56***

Six animals were used. *p< 0.5, ** p<0 .01, *** p< 0.001 Statistical analysis is done by applying One way ANOVA followed by Bonferronis test.

Graph 7: Effect of fractions of Aqueous extract of *P. integerrima* on WBC and Neutrophils in *E. coli* induced abdominal sepsis in mice



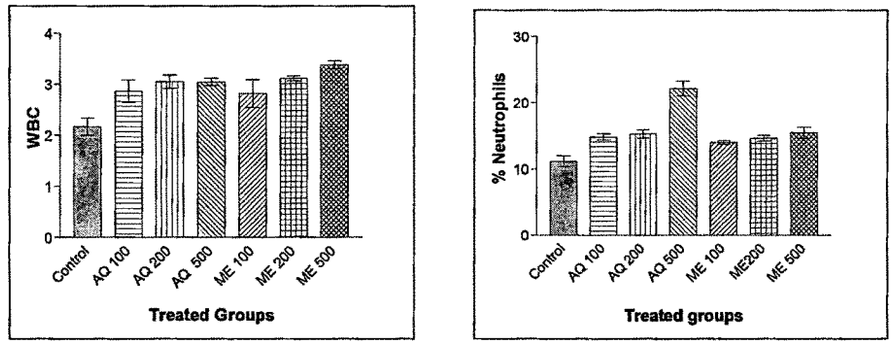
The chloroform fraction of methanol extract of *P. integerrima* was found to be toxic as there was 100% mortality in all the groups. Acetone fraction of Methanol extract of *P .integerrima* at the dose level 150mg/kg was found to be significant i.e. p<0 .01 with WBC 8.717±2.46 (p<0 .01) and Neutrophils 18.50±1.87 (p<0 .01) .Mortality in case of 50 and 100mg/kg was found to be 66% and there was no significant increase in WBC and % of Neutrophils. Ethyl acetate fraction of Methanol extract and remaining Methanol fraction of Methanol extract of *P. integerrima* was found to be most significant with p< 0.001.The mortality in this group was 33%. The WBC count and % neutrophils were considerably increased. The results are given in Table 52.

Table53: Effect of Aqueous and Methanol extracts of *H. spicatum* on WBC and neutrophils in *E. coli* induced abdominal sepsis in mice.

No	Groups	Dose (mg/Kg)	WBC (10^3 / mm^3)	Neutrophils (%)
1	Group I	Control(0.1% Sod CMC)	2.167 ±0.40	11.17±2.04
2	Group II	Aqueous 100	2.867±0.53	12.83±1.16
3	Group III	Aqueous 200	3.050± 0.32*	15.33±1.50**
4	Group IV	Aqueous 500	3.050±0.17*	22.17±2.78***
5	Group VII	Methanol 100	2.817±0.67	14.00±0.63
6	Group VIII	Methanol 200	3.117±0.11**	14.67±1.03*
7	Group IX	Methanol 500	3.383±0.19***	15.50 ±2.07**

Six animals were used. *p< 0.5, ** p<0 .01, *** p< 0.001 Statistical analysis is done by applying One way ANOVA followed by Bonferronis test.

Graph 8: Effect of Aqueous and Methanol extracts of *H. spicatum* in WBC and Neutrophils *E. coli* induced abdominal sepsis in mice



In case of *E. coli* induced peritonitis in mice, there was 50% mortality in control group. The groups treated with Aqueous extract of *H. spicatum* showed 66% mortality whereas in the groups treated with Methanol extract of *H. spicatum* showed 33.3% mortality. There was significant increase in WBC and % neutrophils with Methanol extract and it showed dose dependant response. Aqueous and Methanol extract of *H. spicatum* at the dose 100mg/kg was not found to be effective as there was no significant increase in WBC and Neutrophils. WBC count in groups treated with methanol extract at 200 and 500mg/kg was3.117±0.11 and 3.383±0.19 ($p<0.01$, $p<0.001$). % neutrophils was found to be 14.67±1.03 and 15.50 ±2.07 ($p< 0.5$, $p<0.01$).Though aqueous extract showed significance $p< 0.001$ at 500 mg/kg in % neutrophils, the extract was not found to be active. The results are represented in Table 53.

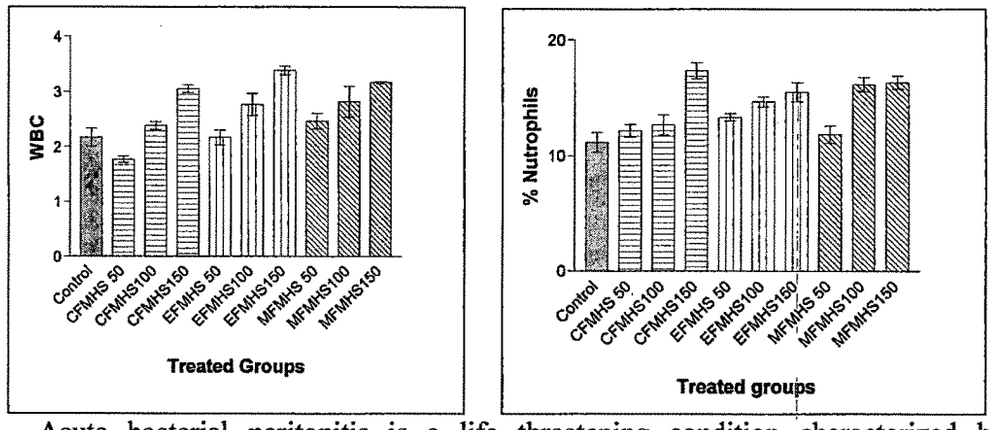
Chloroform fraction, Ethyl acetate fraction and remaining Methanol fraction of Methanol extract were found to be effective in groups treated with 150mg/kg. WBC count in these groups was found to be 3.050±0.17, 3.383±0.19 and 3.167±0.51 respectively ($p< 0.001$). The same groups showed increase in % neutrophils i.e. 17.33±1.75 ($p< 0.001$), 15.50± 2.07 and 16.33 ±1.36 respectively ($p<0.01$). Table 54 represents results of fractions of Methanol extract of *H. spicatum*.

Table 54: Effect of fractions of Methanol extracts of *H spicatum* in *E. coli* induced abdominal sepsis in mice on WBC and neutrophils count

No	Groups	Dose (mg/Kg)	WBC (10^3 / mm^3)	Neutrophils (%)
1	Group I	Control (0.1%Sodium CMC)	2.167 ±0.40	11.17±2.04
2	Group II	CFMHS 50	1.767±0.15	12.17±1.32
3	Group III	CFMHS 100	2.383±0.17*	12.67± 2.16
4	Group IV	CFMHS 150	3.050±0.17***	17.33±1.75***
5	Group V	EFMHS 50	2.167±0.33	13.33±0.81
6	Group VI	EFMHS 100	2.767±0.49*	14.67±1.03*
7	Group VII	EFMHS 150	3.383±0.19***	15.50± 2.07**
8	Group VIII	MFMHS 50	2.467±0.34	11.83±1.83
9	Group IX	MFMHS 100	2.817±0.67*	16.17±1.47**
10	Group X	MFMHS 150	3.167±0.51***	16.33 ±1.36**

Six animals were used. *p< 0.5, ** p<0 .01, *** p< 0.001 Statistical analysis is done by applying One way ANOVA followed by Bonferronis test.

Graph 9: Effect of fractions of Methanol extracts of *H spicatum* in *E. coli* induced abdominal sepsis in mice on WBC and neutrophils count



Acute bacterial peritonitis is a life threatening condition characterized by presence of bacteria in the germfree peritoneal cavity (Martin et al, 1992). Host defense is a classical domain of the innate immune system as a rapid response to pathogens is essential for the host to survive. Treatment of this condition has focused

on surgery, antibiotics and nutritional support. But in spite of this, fatal complications have been reported. A factor which influences the recovery is host defense mechanism (Bigoniya and Rana, 2008). In *E. coli* induced abdominal sepsis, protection offered by the extracts could be attributed to secretion of IL-1 and GM-CSF from activated macrophages. Activated macrophages secrete number of cytokines like IL-1 and GM-CSF which in turn stimulate other immunocytes like neutrophils (Rao and Patel, 1978). Intra abdominal sepsis is a major cause of morbidity and mortality following trauma. The positive immune prophylactic efficacy of the extracts can be assessed by measuring mortality rate against *E.coli* induced abdominal sepsis (Rao et al 1994). Neutrophils and macrophages are active in phagocytosis. They ingest and destroy bacteria with lysozyme defensins and strong antioxidants like superoxide anion, hydrogen peroxide and hypochlorite anion (Tortora, 1996). In case of *H. Spicatum* whole Methanol extract found to possess significant protection against peritonitis. Similar observation was reported by Puri etal (1993).Ethanol extract and purified diterpene andrographaloid of *A. paniculata* have been shown to induce significant stimulation of macrophages in peritonitis. The stimulation was lower with purified andrographaloid indicating contribution of other phytoconstituents towards immunostimulation. Extracts also showed significant increase in WBC and neutrophils thus it may have humoral immune response potentiating effect. *T. cordifolia* was found to be effective against *E. coli* induced abdominal sepsis. The mortality in the treatment group was reduced as compared to the immunosuppressed group. Immunotherapeutic modification of *E. coli* peritonitis was investigated by Thatte (1992). Peritonitis with *T. cordifolia* was reduced from 100% in control to 17.8% in the treated groups. The extracts of *P. integerrima and H. spicatum* and their fractions significantly protected the animals against peritonitis. The results of our findings are comparable to the earlier reports.

3.9.3 Carbon clearance test in mice

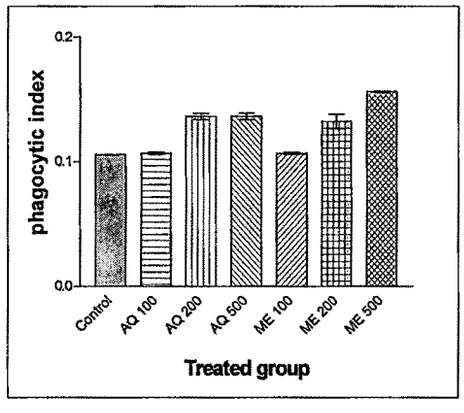
Different extracts and the fractions were subjected to carbon clearance test in mice to assess the increase in the phagocytic index. The results are summarized below

Table 55: Effect of Aqueous and Methanol extracts of *P. integerrima* on Phagocytic index in mice.

No	Groups	Dose (mg/Kg)	Phagocytic index
1	Group I	Control (0.1% Sod CMC)	0.1056 ± 0.03
2	Group II	Aqueous 100	0.1066 ± 0.02
3	Group III	Aqueous 200	0.1362 ± 0.05***
4	Group IV	Aqueous 500	0.1362 ± 0.05***
5	Group V	Methanol 100	0.1056 ± 0.02
6	Group VI	Methanol 200	0.1322 ± 0.01***
7	Group VII	Methanol 500	0.1558 ± 0.01***

Six animals were used. *p< 0.5, ** p<0 .01, *** p< 0.001 Statistical analysis is done by applying One way ANOVA followed by Bonferronis test.

Graph 10: Effect of Aqueous and Methanol extracts of *P. integerrima* on Phagocytic index in mice



Aqueous and methanol extracts at dose level 200 and 500mg/kg possess macrophage stimulatory activity as evidence by increased phagocytic index in carbon clearance test. The phagocytic index for control was found to be 0.1056 ± 0.03 and that of standard was found to be0.1533±0.02573. Aqueous and Methanol extract at 200mg/kg body wt was 0.1362 ± 0.05 and 0.1322 ± 0.01 respectively (p< 0.001). Aqueous and methanol extract at 500mg/kg body wt was 0.1362 ± 0.05 and 0.1558 ± 0.01 respectively (p< 0.001). (Table 55)

In case of different fractions of Methanol extract, Chloroform extract of Methanol extract of *H. spicatum* was ineffective at 50,100,150mg/kg body wt. Acetone fraction

and remaining Methanol fraction of Methanol extract were found to be effective at dose level 100 and 150mg/kg body wt. Ethyl acetate fraction of Methanol extract at all three dose levels were found to be effective i.e. 0.1564 ±0.010 ($p<0.01$), 0.1613±0.02and 0.1662±0.005 ($p< 0.001$) The results are reported in Table 56.

Aqueous extract and Methanol extract of *H. spicatum* at 500mg/kg were found to be effective with the evidence in increase in the phagocytic index. The phagocytic index was 0.1362± 0.0059 and 0.1558±0.0012 for Aqueous extract and Methanol extract at 500mg/kg ($p< 0.001$) respectively. The increase in the phagocytic index was comparable to standard.

Table 56: Effect of fractions of Methanol extract of *P. integerrima* on Phagocytic index in mice

No	Groups	Dose (mg/Kg)	Phagocytic index
1	Group I	Control (0.1% Sod. CMC)	0.1056 ± 0.03
2	Group II	CFMPI 50	0.1533±0.010
3	Group III	CFMPI 100	0.09222±0.002
4	Group IV	CFMPI 150	0.1217±0.033
5	Group V	AFMPI 50	0.1089±0.028
6	Group VI	AFMPI 100	0.1590±0.017***
7	Group VII	AFMPI 150	0.1645±0.015***
8	Group VIII	EFMPI 50	0.1564 ±0.010**
9	Group IX	EFMPI 100	0.1613±0.02***
10	Group X	EFMPI 150	0.1662±0.005***
11	Group XI	MFMPI 50	0.1311±0.01
12	Group XII	MFMPI 100	0.1721±0.0060***
13	Group XIII	MFMPI 150	0.1690±0.0078**

Six animals were used. *$p< 0.5$, ** $p<0.01$, *** $p< 0.001$ Statistical analysis is done by applying One way ANOVA followed by Bonferronis test.

Graph 11: Effect of fractions of Methanol extract of *P. integerrima* on Phagocytic index in mice

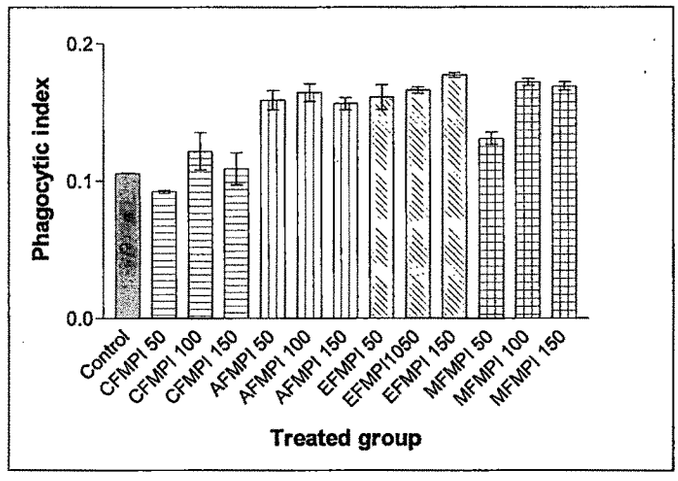
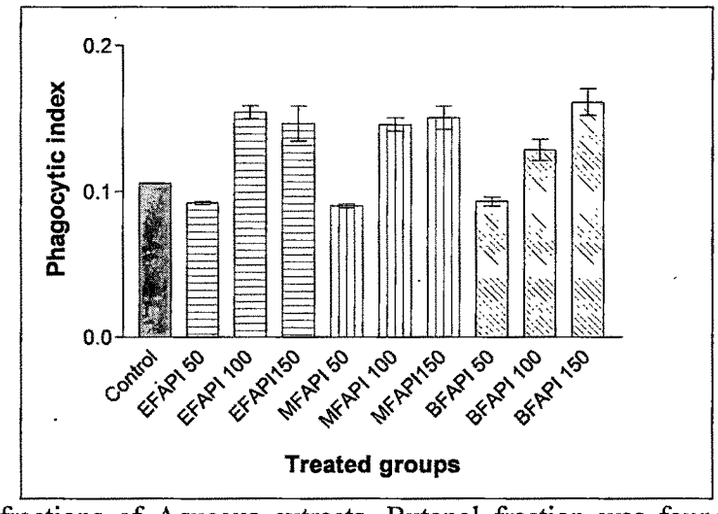


Table 57: Effect of fractions of Aqueous extract of *P. integerrima* on Phagocytic index in mice

No	Groups	Dose (mg/Kg)	Phagocytic index
1	Group I	Control (0.1% Sod. CMC)	0.1056 ± 0.03
2	Group II	EFAPI 50	0.09222±0.002
3	Group III	EFAPI 100	0.1544±0.010***
4	Group IV	EFAPI 150	0.1467±0.02***
5	Group V	MFAPI 50	0.09007±0.002
6	Group VI	MFAPI 100	0.1460±0.01**
7	Group VII	MFAPI 150	0.1505±0.01**
8	Group VII	BFAPI 50	0.09327±0.07
9	Group IX	BFAPI 120	0.1287±0.01
10	Group X	BFAPI 150	0.1613±0.02*

Six animals were used. *p< 0.5, ** p<0 .01, *** p< 0.001 Statistical analysis is done by applying One way ANOVA followed by Bonferronis test.

Graph 12: Effect of fractions of Aqueous extract of *P. integerrima* on Phagocytic index in mice



In case of fractions of Aqueous extracts, Butanol fraction was found to be least effective. At dose level 150mg/kg, the phagocytic index was 0.1613±0.02 ($p< 0.5$) Ethyl acetate fraction at dose level 100 and 150mg/kg body wt was 0.1544±0.010and 0.1467±0.02 respectively ($p< 0.001$). Methanol fraction at dose level 100 and 150mg/kg body wt was 0.1460±0.01and 0.1505±0.01 respectively ($p< 0.01$) (Table 57)

Table 58: Effect of Aqueous and Methanol extracts of *H. spicatum* on Phagocytic index in mice.

No	Groups	Dose (mg/Kg)	Phagocytic index
1	Group I	Control (0.1% Sod CMC)	0.1056±0.0003
2	Group II	Aqueous 100	0.1106 ±0.010
3	Group III	Aqueous 200	0.1206±0.014
4	Group IV	Aqueous 500	0.1362± 0.0059***
5	Group V	Methanol 100	0.1170±0.015
6	Group VI	Methanol 200	0.1322±0.014**
7	Group VII	Methanol 500	0.1558±0.0012***

Six animals were used. *$p< 0.5$, ** $p<0.01$, *** $p< 0.001$ Statistical analysis is done by applying One way ANOVA followed by Bonferronis test.

Graph 13: Effect of Aqueous and Methanol extracts of *H. spicatum* on Phagocytic index in mice

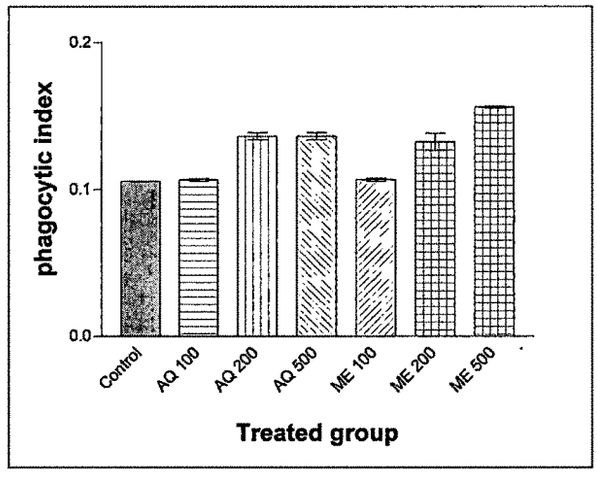
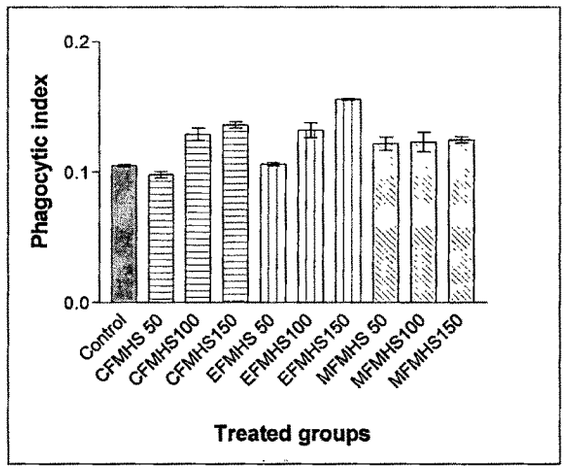


Table 59: Effect of fractions of Methanol extracts of *H. spicatum* on Phagocytic index in mice.

No	Groups	Dose (mg/Kg)	Phagocytic index
1	Group I	Control (0.1% Sod CMC)	0.1056±0.0003
2	Group II	CFMHS 50	0.09793±0.0060
3	Group III	CFMHS 100	0.1290±0.011**
4	Group IV	CFMHS 150	0.1362±0.0059***
5	Group V	EFMHS 50	0.1060±0.0027
6	Group VI	EFMHS 100	0.1322±0.014***
7	Group VII	EFMHS 150	0.1558±0.0012***
8	Group VIII	MFMHS 50	0.1220±0.012
9	Group IX	MFMHS 100	0.1230±0.018
10	Group X	MFMHS 150	0.1246±0.0059*

Six animals were used. *p< 0.5, ** p<0 .01, *** p< 0.001 Statistical analysis is done by applying One way ANOVA followed by Bonferronis test.

Graph 14: Effect of fractions of methanol extracts of *H. spicatum* on Phagocytic index in mice.



Chloroform fraction and Ethyl acetate fraction of Methanol extract of *H. spicatum* at dose level 100 and 150mg/kg body weight showed dose dependent response. Phagocytic index of Chloroform fraction at dose level 100 and 150mg/kg body weight was found to be 0.1290±0.011 and 0.1362±0.0059 ($p<0.01$, $p< 0.001$). Phagocytic index in Ethyl acetate fraction of methanol fraction of *H. spicatum* was 0.1558±0.0012 and 0.1322±0.014 at 100 and 150mg/kg body weight respectively ($p<0.01$, $p< 0.001$). Remaining Methanol fraction was not found to be effective as there was no increase in the phagocytic index. (Table 59)

Phagocytosis provides the first line defense to the host against infectious microorganisms. Polymorphnuclear leukocytes (Neutrophils and Eosinophils) and mononuclear phagocytes (monocytes and macrophages) are most commonly recognized 'professional' phagocytes. The primary target of most of the adaptogens is believed to be macrophages which play a major role by engulfing pathogens or foreign substances and initiating innate immune response. Macrophages are known to secrete number of cytokines which in turn stimulate other immunocytes. This may enhance the defense ability to counter the infection stress. It suggests enhancement in phagocytic function of macrophages and thus nonspecific immunity. Phagocytosis by macrophages is important against the smaller parasites and its effectiveness is markedly enhanced by opsonization of the parasites with the antibody and complement C3b, leading to more rapid clearance of parasites from the blood. *P.*

integerrima and H. spicatum showed significantly high phagocytic index which indicates marked increase in the rate of carbon clearance (Sagle, 2004). Similar results were observed in case of *T. cordifolia.* There was an increase in neutrophil count and peritoneal macrophages which was associated with increased phagocytic activity. It has been suggested that *T. cordifolia* activates macrophages to release GM-CSF activity. The results of our study are comparable with the previous reports.

3.9.4 Cyclophosphamide induced myelosuppression

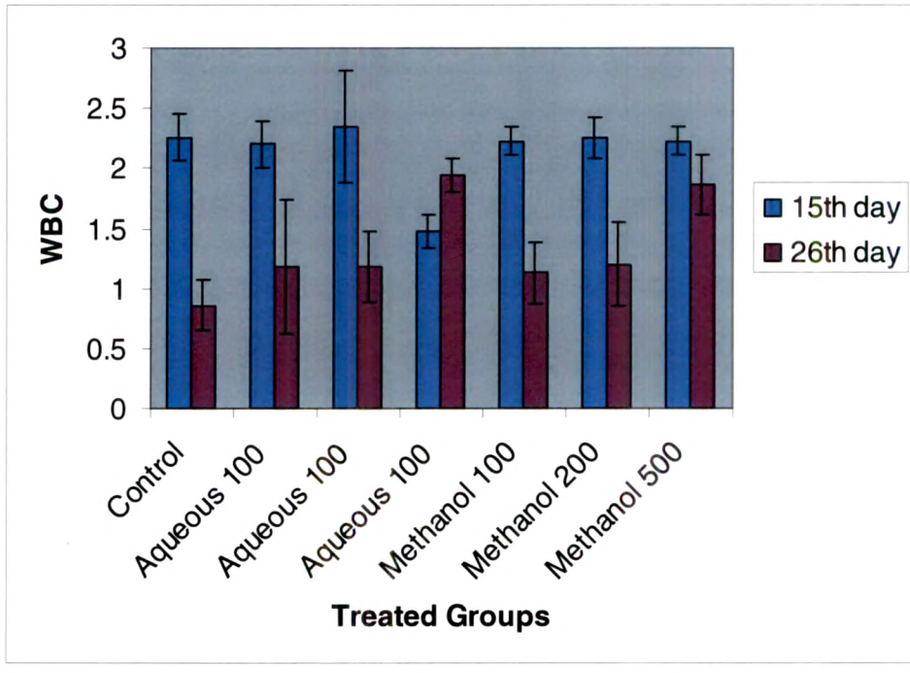
In cyclophosphamide induced myelosuppression, the extracts of the plant drugs were evaluated for different haematological parameters like Hemoglobin content (HB), Haematocrit value (HCT), Leukocytes count, Erythrocyte count, and Mean corpuscles volume (MCV) to determine the efficacy of the extracts. The results are described as follows.

Table 60: Effect of Aqueous and Methanol extracts of *P. integerrima* on haematological parameters after 15 days of treatment with extract and on 26th day in cyclophosphamide induced myelosuppression in mice.

No	Groups	Dose (mg/Kg)	RBC (10^6/ mm^3)		WBC (10^3/ mm^3)		HB		MCV		HCT	
			15th day	26th day	15th day	26th day	15th day	26th day	15th day	26th day	15th day	26th day
1	Group I	Control (0.1%Sod CMC)	7.95±0.15	5.05± 0.22	2.26±0.19	0.86± 0.21	10.0±1.63	8.53± 0.36	49.70±1.33	49.34± 1.2	34.72±1.33	33.31±0.21
2	Group II	Aqueous 100	7.32±0.41	7.10 ± 0.28	2.20±0.20	1.18± 0.65*	9.22±0.14	9.38± 0.29	52.38±1.00	52.00± .23	37.80±0.64	35.21± .40
3	Group III	Aqueous 200	6.27±0.25	6.14± 0.20	2.35±0.47	1.18± 0.30*	9.26±0.18	9.80± 0.38	47.12±0.83	46.85± 0.3	36.54±0.66	36.84± .02
4	Group IV	Aqueous 500	8.88±0.25	8.85± 0.25	1.48±0.14	1.95± 1.96***	10.18±0.15	9.75± 0.22	51.65±1.9	52.00± 0.9	34.53±0.57	35.11±0 .9
5	Group V	Methanol 100	7.62±0.13	6.76± 0.36	2.23±0.12	1.13± 1.56	10.12±0.20	9.66± 0.20	51.00±1.44	51.98± .87	40.53±1.62	39.15± .14
6	Group VI	Methanol 200	7.78±0.25	6.24± 0.22	2.26±0.17	1.20± 1.00**	10.11±0.27	9.66± 0.57	52.43±1.10	50.62± 0.9	39.30±0.83	40.02± 0.9
7	Group VII	Methanol 500	6.00±0.25	5.87± 0.20	2.23±0.12	1.86± 0.81***	9.02±0.18	9.06± 0.22	44.23±1.07	44.18± 0.8	34.53±0.58	35.98± 0.5

Six animals were used. *p< 0.5, ** p<0 .01, *** p< 0.001 Statistical analysis is done by applying One way ANOVA followed by Bonferronis test.

Graph 15: Effect of Aqueous and Methanol extracts of *P. integerrima* on WBC count after 15 days and on 26th day of treatment with extracts in cyclophosphamide induced myelosuppression in mice



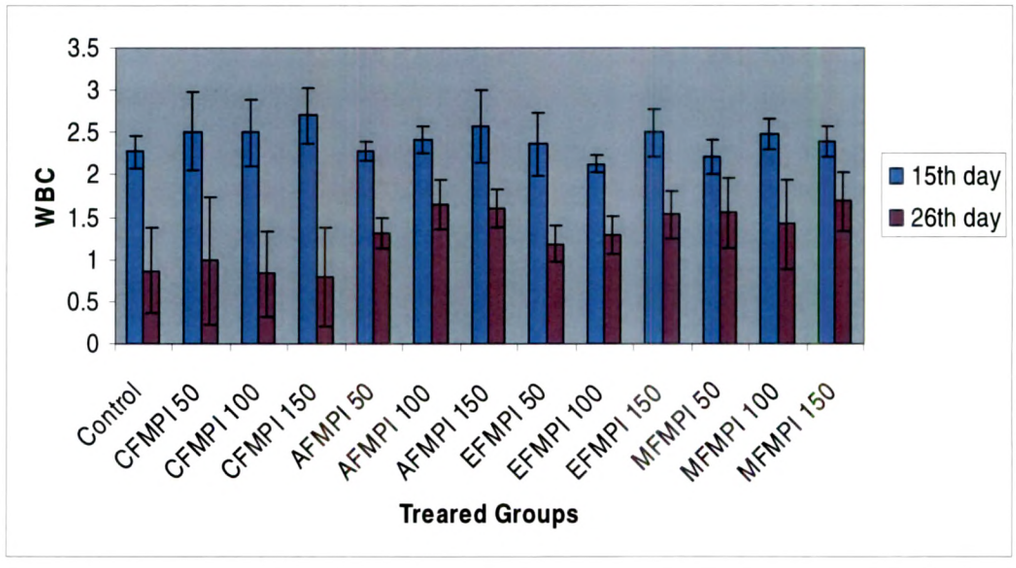
In cyclophosphamide induced myelosuppression, effect of Aqueous and Methanol extracts and their fractions was evaluated in different haematological parameters like WBC, RBC, HB, HCT and MCV on 15th day and 26th day. No significant changes were found in RBC, HB, HCT and MCV when compared to control on 15th day and 26th day. There was significant change in WBC when the values on 15th day and 26th day were compared. WBC count in case of control 1on 15th day was 2.26±0.19 and on 26th day 0.86± 0.21. Aqueous and methanol extracts at dose 500mg/kg were found to be significant with the values 1.95± 1.96 and 1.86± 0.81($p< 0.001$).Aqueous and methanol extracts at dose levels 100 and 200 mg/kg were not found to be effective. The results are reported in Table 60.

Table 61: Effect of fraction of Methanol extracts of *P. integerrima* on haematological parameters after 15 days of treatment with extracts and on 26th day in cyclophosphamide induced myelosuppression in mice

No	Groups	Dose (mg/Kg)	RBC (10^6/ mm^3)		WBC (10^6/ mm^3)		HB		MCV		HCT	
			15th day	26th day	15th day	26th day	15th day	26th day	15th day	26th day	15th day	26th day
1	Group I	Control (0.1% Sod. CMC)	7.950±0.38	7.067±1.347	2.270±0.19	0.8667±0.51	9.852±0.67	8.102±0.85	49.65±4.19	39.38±1.6	34.48±1.42	28.40±2.68
2	Group II	CFMPI 50	6.937±1.72	5.918±0.8351	2.517±0.47	0.9833±0.75	10.10±1.32	8.868±0.58	46.90±1.37	44.50±2.12	37.27±0.8	30.82±1.56
3	Group III	CFMPI 100	7.218±1.40	5.963±1.398	2.500±0.4	0.8333±0.51	10.15±1.31	8.450±0.93	47.93±2.78	45.10±2.76	34.19±0.5	32.90±3.617
4	Group IV	CFMPI 150	7.318±1.94	7.138±1.381	2.700±0.32	0.7833±0.98	7.735±3.28	6.400±2.72	49.68±2.46	45.09±2.01	35.56±2.19	35.70±4.116
5	Group V	AFMPI 50	7.723±1.18	6.518±0.4867	2.283±0.11	1.317±0.18	10.10±0.28	8.617±1.04	49.45±2.40	42.83±1.94	41.25±4.01	36.17±2.847
6	Group VI	AFMPI 100	8.077±1.00	6.783±0.4665	2.417±0.16	1.650±0.30**	9.833±0.97	7.517±0.5	44.78±2.38	41.82±2.12	38.40±2.49	33.83±3.251
7	Group VII	AFMPI 150	7.415±1.14	6.645±0.4599	2.567±0.43	1.600±0.22**	9.227±1.40	7.760±0.77	50.11±3.04	42.17±2.48	37.91±2.06	32.93±2.113
8	Group VIII	EFMPI 50	8.255±1.25	6.562±1.024	2.367±0.37	1.183±0.22	10.17±1.24	8.232±1.27	47.59±3.18	40.17±1.16	34.61±2.49	34.60±1.905
9	Group IX	EFMPI 100	6.708±1.14	6.448±0.8172	2.133±0.10	1.283±0.23	9.833±0.32	7.798±2.08	44.95±4.25	40.83±2.48	36.23±1.92	36.17±3.125
10	Group X	EFMPI 150	6.956±2.65	6.308±2.151	2.500±0.28	1.533±0.28***	10.09±0.65	8.458±1.47	47.60±2.60	40.67±1.51	41.05±4.14	33.25±3.65
11	Group XI	MFMPI 50	6.868±1.05	7.525±0.9796	2.217±0.21	1.550±0.41**	9.913±0.73	7.133±1.28	49.59±2.12	43.67±2.87	40.14±1.55	35.67±2.503
12	Group XII	MFMPI 100	6.390±2.46	5.167±1.178	2.483±0.18	1.417±0.53*	9.352±0.52	8.885±0.37	47.46±3.091	42.17±3.54	35.48±2.02	33.21±2.500
13	Group XIII	MFMPI 150	6.323±2.46	5.400± 1.888	2.400±0.18	1.683±0.35***	10.83±0.75	8.717±0.47	47.31±3.81	43.33±4.32	34.63±1.61	31.17±0.9832

Six animals were used. *p< 0.5, ** p<0 .01, *** p< 0.001 Statistical analysis is done by applying One way ANOVA followed by Bonferronis test

Graph 16: Effect of fraction of Methanol extracts of *P. integerrima* on WBC count after 15 days and 26 days of treatment in cyclophosphamide induced myelosuppression in mice



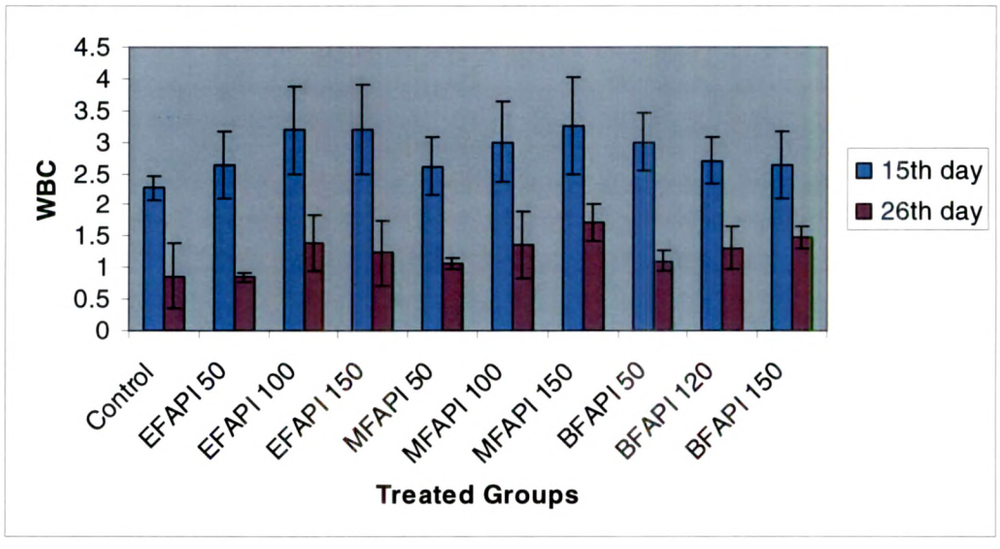
Chloroform fraction of methanol extract was not found to be effective. Acetone extract showed significant activity $p< 0.01$ at 100 and 150mg/kg dose. Ethyl acetate fraction at 150mg/kg was found to be effective with the value 1.533±0.28 ($p< 0.001$) WBC count in remaining methanol fraction of methanol extract was 1.550±0.41 ($p< 0.001$), 1.417±0.53 ($p< 0.001$) and 1.683±0.35($p< 0.001$). (Table 61)

Table 62: Effect of fractions of Aqueous extract of *P. integerrima* on haemayological parameters on 15th day of treatment and 26th day of cyclophosphamide induced myelosuppression in mice.

No	Groups	Dose (mg/Kg)	RBC (10^6/ mm^3)		WBC (10^6/ mm^3)		HB		MCV		HCT	
			15th day	26th day	15th day	26th day	15th day	26th day	15th day	26th day	15th day	26th day
1	Gr I	Control (0.1% Sod CMC)	7.950±0.38	7.067±1.347	2.270±0.19	0.8667±0.51	9.852±0.67	8.102±0.85	49.65±4.19	39.38±1.6	34.48±1.42	28.40±2.68
2	Gr II	EFAPI 50	6.390±2.46	6.758±1.53	2.633±0.52	0.8500±0.08	8.183±0.37	6.950±0.60	47.28±2.21	40.6±2.69	34.53±0.57	30.75±1.30
3	Gr III	EFAPI 100	6.937±1.72	6.292±1.04	3.183±0.71	1.400±0.44*	7.900±0.41	6.967±0.85	47.67±2.43	41.83±3.97	36.54±0.66	29.53±1.61
4	Gr IV	EFAPI 150	7.218±1.40	6.830±1.15	3.200±0.71	1.233±0.52	8.800±0.80	6.400±0.80	51.36±2.08	45.00±3.46	33.21±1.76	30.17±1.65
5	Gr V	MFAPI 50	7.318±1.94	6.777±1.44	2.617±0.47	1.067±0.10	8.378±0.46	6.250±0.55	49.69±3.19	44.50±4.31	33.69±2.85	30.32±1.52
6	Gr VI	MFAPI 100	7.723±1.18	7.143±0.88	3.000±0.64	1.367±0.53*	8.967±0.86	7.333±1.23	50.11±2.70	43.79±3.53	41.41±3.9	31.67±2.16
7	Gr VII	MFAPI 150	8.077±1.03	7.237±0.44	3.267±0.77	1.717±0.31**	8.300±0.8	7.300±1.28	46.22±5.48	42.90±3.67	38.88±1.35	30.83±1.83
8	Gr VII	BFAPI 50	7.415±1.14	7.007±0.94	3.000±0.46	1.107±0.16	8.780±1.30	6.667±0.76	53.49±1.81	48.67±1.96	37.79±1.25	32.93±2.92
9	Gr IX	BFAPI 120	8.255±1.25	7.512±1.08	2.700±0.37	1.317±0.34*	8.315±0.79	6.667±0.70	48.29±1.58	42.67±2.25	36.54±0.66	33.01±2.78
10	Gr X	BFAPI 150	6.708±1.14	6.035±0.61	2.633±0.53	1.483±0.18**	8.900±0.68	7.112±0.43	50.53±3.08	43.17±5.15	37.95±2.3	31.73±2.69

Six animals were used. *p< 0.5, ** p<0 .01, *** p< 0.001 Statistical analysis is done by applying One way ANOVA followed by Bonferronis test

Graph 17: Effect of fractions of aqueous extracts of *P.integerrima* on WBC count after 15 days of treatment with extracts and on 26th day in cyclophosphamide induced myelosuppression in mice



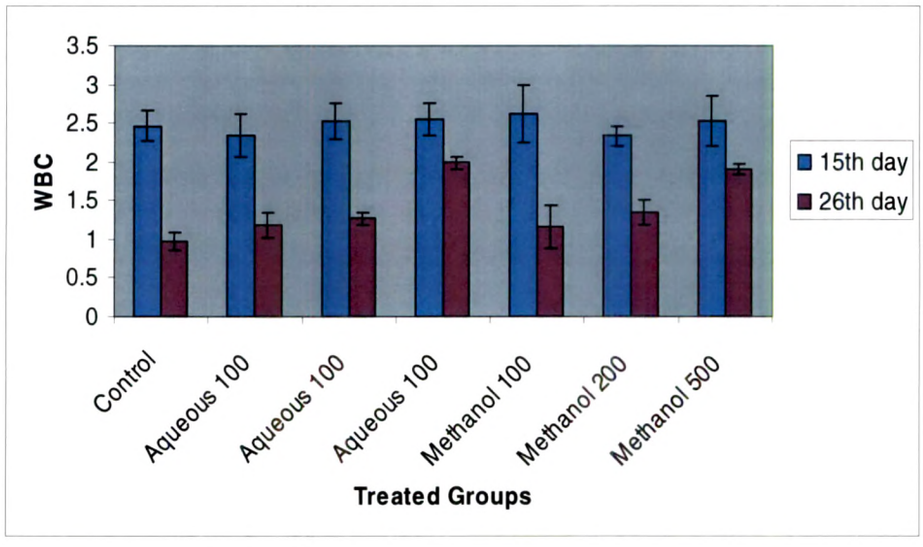
Methanol fraction of Aqueous extract was found to be significant at dose level 100 and 150mg/kg. At these dose levels, WBC count were 1.367±0.53 ($p< 0.05$) and 1.717±0.31 ($p< 0.01$) respectively. The results are recorded in Table 62.

Table 63: Effect of Aqueous and Methanol extracts of *H. spicatum* on haematological parameters after 15 days of treatment with extracts and on 26th day in cyclophosphamide induced myelosuppression in mice.

No	Groups	Dose (mg/Kg)	RBC (10^6/ mm^3)		WBC (10^3/ mm^3)		HB		MCV		HCT	
			15th day	26th day	15th day	26th day	15th day	26th day	15th day	26th day	15th day	26th day
1	Group I	Control	7.95±0.15	5.05± 0.22	2.26±0.19	0.86± 0.21	10.0±1.63	8.53± 0.36	49.70±1.33	49.34± 1.2	34.72±1.33	33.31±0.21
2	Group III	Aqueous 100	7.32±0.41	7.10 ± 0.28	2.20±0.20	1.18± 0.65	9.22±0.14	9.38± 0.29	52.38±1.00	52.00± .23	37.80±0.64	35.21± .40
3	Group IV	Aqueous 200	6.27±0.25	6.14± 0.20	2.35±0.47	1.18± 0.30	9.26±0.18	9.80± 0.38	47.12±0.83	46.85± 0.3	36.54±0.66	36.84± .02
4	Group V	Aqueous 500	8.88±0.25	8.85± 0.25	1.48±0.14	1.95± 1.96***	10.18±0.15	9.75± 0.22	51.65±1.9	52.00± 0.9	34.53±0.57	35.11±0 .9
5	Group VI	Methanol 100	7.62±0.13	6.76± 0.36	2.23±0.12	1.13± 1.56	10.12±0.20	9.66± 0.20	51.00±1.44	51.98± .87	40.53±1.62	39.15± .14
6	Group VII	Methanol 200	7.78±0.25	6.24± 0.22	2.26±0.17	1.20± 1.00**	10.11±0.27	9.66± 0.57	52.43±1.10	50.62± 0.9	39.30±0.83	40.02± 0.9
7	Group VIII	Methanol 500	6.00±0.25	5.87± 0.20	2.23±0.12	1.86± 0.81***	9.02±0.18	9.06± 0.22	44.23±1.07	44.18± 0.8	34.53±0.58	35.98± 0.5

Six animals were used. *p< 0.5, ** p<0 .01, *** p< 0.001 Statistical analysis is done by applying One way ANOVA followed by Bonferronis test.

Graph 18: Effect of aqueous and methanol extracts of *H. spicatum* on WBC count after 15 days and on 26th day of treatment with extracts in cyclophosphamide induced myelosuppression in mice



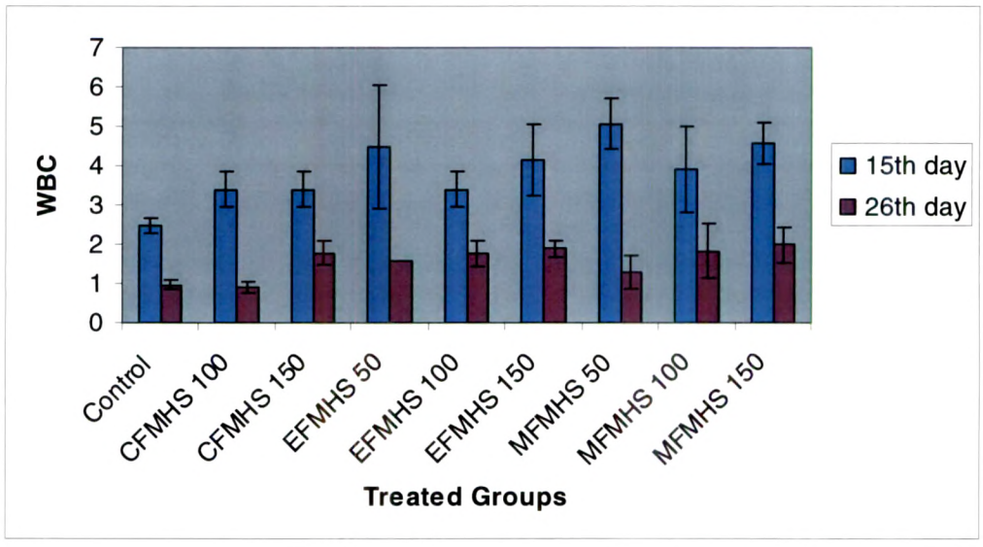
WBC count in control group on 15th day was 2.26± 0.19 and on 26th day after treatment with cyclophosphamide it was decreased to 0.86 ±0.21. Aqueous extract of *H. spicatum* at dose level 100 and 200mg/kg and methanol extract of *H. spicatum* at dose level 200mg/kg was not found to be effective as there was no significant increase in the WBC on 26th day on treatment with cyclophosphamide. Aqueous extract of *H. spicatum* at 500mg/kg dose showed significant increase in WBC 1.96±1.96 ($p< 0.001$). Methanol extract of *H. spicatum* at 200 and 500mg/kg dose showed dose dependent response. The WBC count was increased to 1.20 ±1.00 and 1.86± 0.86 respectively ($p< 0.01$, $p< 0.001$).reduction in RBC was found in the control group but there was no reduction in treated groups. There was no change in the values of HB, HCT and MCV in the treated groups. (Table 63)

Table 64: Effect of fractions of Methanol extracts of *H. Spicatum* on haematological parameters after 15 days of treatment with extracts and on 26th day in cyclophosphamide induced myelosuppression in mice

No	Groups	Dose (mg/Kg)	RBC (10^6/ mm^3)		WBC (10^6/ mm^3)		HB		MCV		HCT	
			15th day	26th day	15th day	26th day	15th day	26th day	15th day	26th day	15th day	26th day
1	Group I	Control(0.1%Sod CMC)	8..7095±0.35	6.05± 0.38	2.467±0.19	0.9667±0.12	11.0±158	8.53± 0.36	50.750±2.65	49.34± 1.2	33.85±2.24	31.25±3.25
2	Group II	CFMHS 50	7.848±1.03	6.567±0.46	3.400±0.47	0.9150±0.14	8.033±3.36	7.317±0.81	49.86±2.92	41.77±2.75	38.39±0.96	34.38±1.69
3	Group III	CFMHS 100	7.303±1.30	6.373±0.66	4.483±1.58	1.333±0.42	9.705±0.36	7.833±0.5	49.50±2.40	41.93±3.43	38.18±1.57	34.58±3.27
4	Group IV	CFMHS 150	9.253±0.71	6.683±1.10	3.400±0.47	1.383±0.29	9.327±1.149	7.250±0.9	51.45±1.08	47.92±5.07	37.09±2.60	32.23±3.42
5	Group V	EFMHS 50	8.972±0.82	5.867±0.93	4.483±1.58	1.583±0.40*	9.765±0.746	0.9460±1.22	50.87±1.39	45.65±5.47	38.47±3.03	33.95±3.13
6	Group VI	EFMHS 100	8.612±0.96	6.500±0.43	3.400±0.47	1.750±0.34*	9.992±0.674	6.883±0.89	49.37±1.45	40.78±1.30	37.21±2.66	34.10±2.91
7	Group VII	EFMHS 150	7.768±1.23	6.550±1.03	4.150±0.91	1.883±0.23**	9.183±0.43	6.783±0.63	50.84±4.48	43.53±4.33	36.60±1.94	32.96±1.87
8	Group VIII	MFMHS 50	8.473±0.78	6.533±0.46	5.067±0.65	1.267±0.43	8.957±0.77	6.567±0.94	51.68±2.33	42.85±3.25	37.98±1.45	34.50±1.30
9	Group IX	MFMHS 100	7.627±0.89	6.133±1.12	3.900±1.108	1.33±0.68	9.202 ±1.30	6.617±1.0	50.51±2.37	40.65±1.73	37.62±1.88	34.37±2.00
10	Group X	MFMHS 150	9.140±0.81	7.483±0.40	4.567±0.53	1.3±0.46	9.638±0.67	6.600±0.46	51.39±3.70	43.55±2.06	36.77±1.32	32.98±2.60

Six animals were used. *p< 0.5, ** p<0 .01, *** p< 0.001 Statistical analysis is done by applying One way ANOVA followed by Bonferronis test.

Graph 19: Effect of fractions of Methanol extracts of *H. Spicatum* on WBC count after 15th day and 26th day of treatment with extracts in cyclophosphamide induced myelosuppression in mice



Chloroform fraction of methanol extract of *H. spicatum* showed significant increase in WBC on 26th day. There was a dose dependent increase in WBC i.e. 1.733±0.42 and 1.883±0.29 at 100 and 150 mg/kg dose level. Ethyl acetate fraction was found to be statistically significant at 100 and 150mg/kg ($p< 0.01$).There was reduction in RBC in control group. There were no alteration in other parameters like HB, MCV and HCT. (Table 64)

Cyclophosphamide is an alkylating agent resulting in cross linking of DNA and causes inhibition of DNA synthesis. Major drawback of this drug is myelosuppression. An attempt to overcome this problem has been made by pro-host therapy (Davis, 1998). In case of cyclophosphamide induced myelosuppression Aqueous and Methanol extracts of *P. integerrima* and their fractions and Methanol extract of *H. spicatum* and its fractions were able to bring back the levels of WBC to normal while aqueous extract of *H. spicatum* and Chloroform fraction of Methanol extract of *P. integerrima* were not found to be effective. The results were comparable to those obtained for *Asparagus racemosus* and *Withania somnifera* (Thatte and Dahanukar, 1988). There was no significant change in other haematological parameters suggesting these drugs can be used for long term treatment. The preventive effect of *A. racemosus* against myelosuppression induced by single dose of cyclophosphamide has been reported by Thatte and Dahanukar (1988). The results of our studies are comparable to these findings. Similar findings have been reported for

T. cordifolia against myelosuppression induced by cyclophosphamide and compared with glucan. A significant modulation of immune reactivity by Ashwagandha was observed in an animal model of myelosuppression induced by cyclophosphamide, azathioprin and prednisolone. Ashwagandha prevented myelosuppression in mice treated with all three immunosuppressive drugs.

3.9.5 Anoxia stress tolerance test

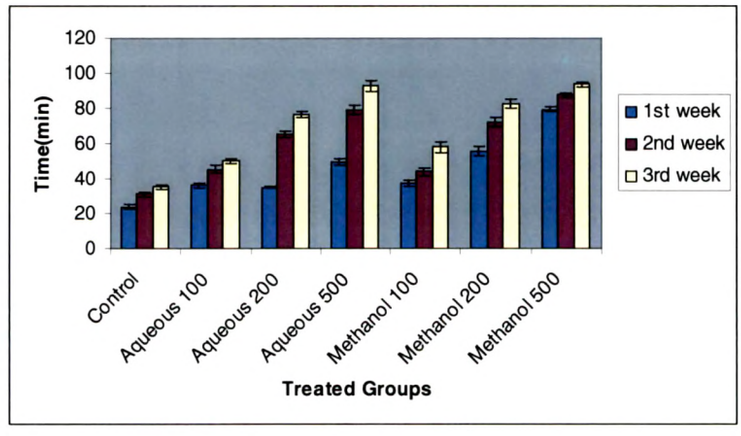
Anoxia stress tolerance test was performed using albino mice. The time for first convulsion was noted for different extracts and fractions of the selected plant drugs. The results are described in the following tables.

Table 65: Anoxia stress tolerance test in Aqueous and Methanol extracts of *P. integerrima*

No	Groups	Dose(mg/kg)	Mean duration of tolerance(min)		
			1st week	2nd week	3rd week
1	Group I	Control (0.1% Sod CMC)	39.66±1.25	46.89±1.65	55.32±0.98
2	Group II	Aqueous 100	36.24±1.25	45.56±1.89	50.21±1.48
3	Group III	Aqueous 200	35.21±0.54	65.23±1.32*	76.32±1.59**
4	Group IV	Aqueous 500	49.78±1.57*	78.98±2.53**	92.65±2.87***
5	Group V	Methanol 100	37.45±1.64	43.98±1.87	57.89±3.02
6	Group VI	Methanol 200	55.79±2.54**	72.35±2.64**	82.65±2.58**
7	Group VII	Methanol 500	79.54±1.49***	87.54±1.56***	93.56±1.34***

Six animals were used. *p< 0.5, ** p<0 .01, *** p< 0.001 Statistical analysis is done by applying One way ANOVA followed by Bonferronis test.

Graph 20: Anoxia stress tolerance test in Aqueous and Methanol extracts of *P. integerrima.*



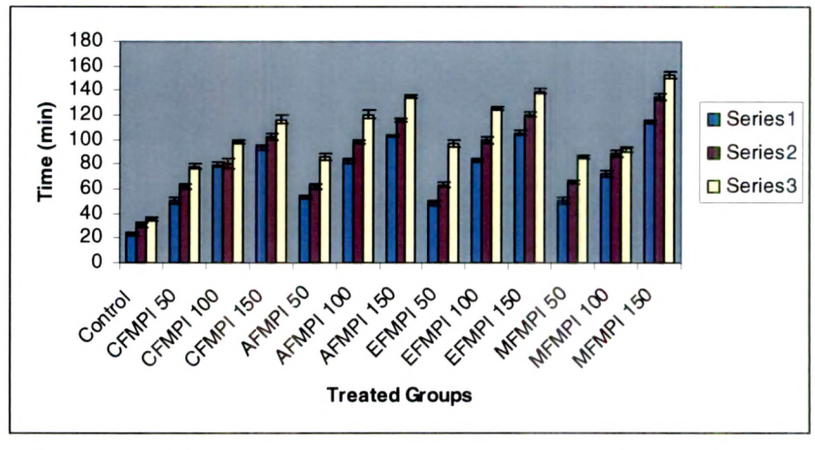
It was observed in the treatment group there was delayed time for convulsion. In the control group, at the end of 1st week, anoxic stress time was 39.66±1.25 min, at the end of 2nd week 46.89±1.65 min and at the end of 3rd week it was 55.32±0.98 min. There was increase in the anoxic stress time at the end of each week. Methanol extract at 200 and 500 mg/kg body weight was found to be effective. There was no significant change at the end of the 1st week but with increase in duration the anoxic stress tolerance. (Table 65)

Table 66: Anoxia stress tolerance test in fractions of methanol extracts of *P. integerrima.*

No	Groups	Dose(mg/kg)	Mean duration of tolerance(min)		
			1st week	2nd week	3rd week
1	Group I	Control (0.1% Sod MC)	39.66±1.25	46.89±1.65	55.32±0.98
2	Group II	CFMPI 50	50.32±2.34	62.12±1.89	78.21±1.68
3	Group III	CFMPI 100	79.65±1.65*	80.54±3.78**	98.45±1.03*
4	Group IV	CFMPI 150	93.45±1.54**	102.03±2.87***	116.54±2.98**
5	Group V	AFMPI 50	53.12±1.25	62.54±1.86	85.54±2.94
6	Group VI	AFMPI 100	82.54±1.87***	98.06±1.23**	120.54±3.84**
7	Group VII	AFMPI 150	102.52±0.64***	115.56±1.54***	135.21±1.29***
8	Group VIII	EFMPI 50	48.54±1.97	63.25±1.87	96.65±2.85
9	Group IX	EFMPI 100	83.21±1.54**	99.61±2.54**	125.65±1.05
10	Group X	EFMPI 150	105.63±1.98***	120.54±1.95***	139.65±2.54***
11	Group XI	MFMPI 50	49.87±2.68	65.28±1.26**	86.54±1.4
12	Group XII	MFMPI 100	72.32±2.59**	88.54±2.54**	92.21±1.79*
13	Group XIII	MFMPI 150	115.02±1.53***	135.20±2.57***	152.89±2.69***

Six animals were used. *p< 0.5, ** p<0 .01, *** p< 0.001 Statistical analysis is done by applying One way ANOVA followed by Bonferronis test.

Graph 21: Anoxia stress tolerance test in fractions of methanol extracts of *P. integerrima.*



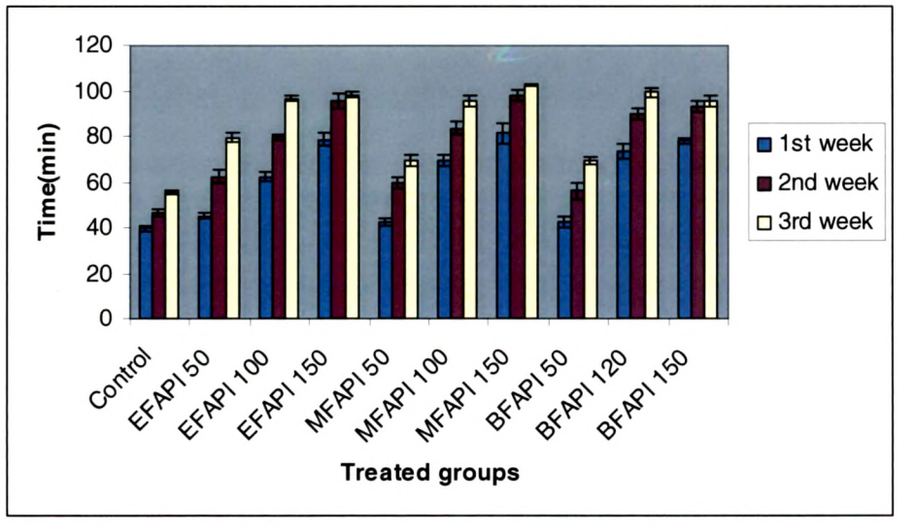
Acetone and Ethyl acetate fractions of Methanol extract of *P. integerrima* were found to possess significant activity at 100 and 150mg/kg body weight dose. In case of fractions of aqueous extract, all the extracts showed significant activity at the end of 3rd week. (Table 66)

Table 67: Anoxia stress tolerance test in fractions of Aqueous extracts of *P. integerrima*

No	Groups	Dose(mg/kg)	Mean duration of tolerance(min)		
			1st week	2nd week	3rd week
1	Group I	Control (0.1%Sod CMC)	39.66±1.25	46.89±1.65	55.32±0.98
2	Group II	EFAPI 50	45.21±1.23	62.29±2.97	79.58±2.36*
3	Group III	EFAPI 100	62.32±2.25	79.60±1.36*	96.65±1.39**
4	Group IV	EFAPI 150	78.54±2.69*	95.64±3.25**	98.32±1.25**
5	Group V	MFAPI 50	42.32±1.58	59.78±2.58	69.33±2.39
6	Group VI	MFAPI 100	69.45±2.62	83.65±2.94*	95.60±2.69**
7	Group VII	MFAPI 150	81.39±4.32*	98.14±2.48**	102.6±0.59***
8	Group VIII	BFAPI 50	42.63±2.59	56.20±3.58	69.31±1329
9	Group IX	BFAPI 100	73.21±3.26	89.89±2.58*	99.26±2.06**
10	Group X	BFAPI 150	78.32±1.26	93.26±2.26**	95.63±2.65**

Six animals were used. *p< 0.5, ** p<0 .01, *** p< 0.001 Statistical analysis is done by applying One way ANOVA followed by Bonferronis test.

Graph 22: Anoxia stress tolerance test in fractions of aqueous extracts of *P. integerrima*



Ethyl acetate fraction of Aqueous extract at all three dose levels was found to be effective at the end of the 3rd week. Ethyl acetate fraction of Aqueous extract at the

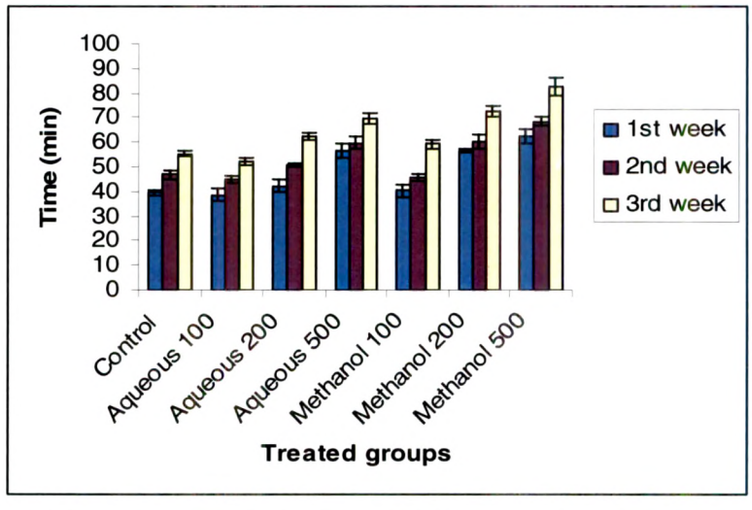
dose level 100 and 150 mg/kg was effective at the end of 1st and 2nd week too. Methanol and Butanol extracts were found to be effective at 100 and 150 mg/kg dose levels. (Table 67)

Table 68: Anoxia stress tolerance test in Aqueous and Methanol extracts of *H. spicatum*

No	Groups	Dose(mg/kg)	Mean duration of tolerance(min)		
			1st week	2nd week	3rd week
1	Group I	Control (0.1% Sod CMC)	39.66±1.25	46.89±1.65	55.32±0.98
2	Group II	Aqueous 100	38.65±2.39	45.21±1.39	52.26±1.32
3	Group III	Aqueous 200	42.21±2.66	50.69±0.63	62.36±1.64*
4	Group IV	Aqueous 500	56.50±3.15*	59.54±2.45*	69.68±2.22*
5	Group V	Methanol 100	40.26±2.56	45.63±1.28	59.32±1.87
6	Group VI	Methanol 200	56.54±0.95*	60.15±2.59*	72.69±2.15*
7	Group VII	Methanol 500	62.28±3.02*	68.32±1.69*	82.39±3.65**

Six animals were used. *p< 0.5, ** p<0 .01, *** p< 0.001 Statistical analysis is done by applying One way ANOVA followed by Bonferronis test.

Graph 23: Anoxia stress tolerance test in Aqueous and Methanol extracts of *H. spicatum*



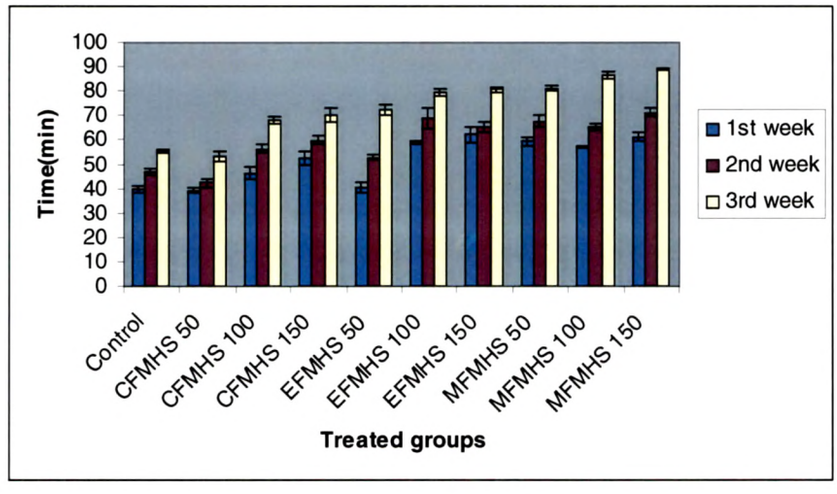
In case of H. spicatum, Methanol extract 500 mg/kg was found to be significant with p<0.01 at the end of 3rd week. Aqueous extract at 500 mg/kg showed delayed response to anoxic stress at the end of 1st, 2nd and 3rd week. Methanol extract was more effective at 200 and 500 mg/kg and there was a delayed response at to anoxic stress at the end of 1st, 2nd and 3rd week.

Table 69: Anoxia stress tolerance test in fractions of Methanol extracts of *H. spicatum*

No	Groups	Dose(mg/kg)	Mean duration of tolerance(min)		
			1st week	2nd week	3rd week
1	Group I	Control (0.1% Sod CMC)	39.66±1.25	46.89±1.65	55.32±0.98
2	Group II	CFMHS 50	39.21±1.21	42.26±1.62	53.25±2.36
3	Group III	CFMHS 100	46.33±2.59	56.21±1.63	68.25±1.36*
4	Group IV	CFMHS 150	52.36±3.01	59.87±1.64	70.35±2.98*
5	Group V	EFMHS 50	40.23±2.26	52.64±109	72.26±2.28*
6	Group VI	EFMHS 100	58.78±0.93*	68.54±4.21*	79.62±1.37**
7	Group VII	EFMHS 150	62.26±3.08*	65.34±1.98*	80.64±0.98**
8	Group VIII	MFMHS 50	59.23±1.98	67.58±2.56*	81.15±0.84**
9	Group IX	MFMHS 100	56.89±0.23	65.21±1.59*	86.56±1.64**
10	Group X	MFMHS 150	61.25±1.73*	71.21±1.64*	88.36±1.25**

Six animals were used. *p< 0.5, ** p<0 .01, *** p< 0.001 Statistical analysis is done by applying One way ANOVA followed by Bonferronis test.

Graph 24: Anoxia stress tolerance test in fractions of methanol extracts of *H. spicatum*



Chloroform fraction of Methanol extract was found to be effective at 500 mg/kg at the end of 3rd week. Ethyl acetate fraction of Methanol extract showed delayed response to anoxic stress at the dose level 100 and 150 mg/kg at the end of 1st, 2nd and 3rd week. Remaining Methanol fraction was effective at the end of 2nd and 3rd week. (Table 69)

3.9.6 Forced swim model

In forced swim model, evaluation was done for different biochemical parameters like serum glucose, cholesterol, triglycerides and blood urea nitrogen. Blood counts like RBC, WBC and differential counts were measured. Changes in the organ weight like spleen, liver and adrenals were observed. In case of animals treated with the extracts and fractions, no change was observed in the organ weight. Hyperglycemic effect was observed in stress control group whereas in the treatment groups there was reduction in glucose level. Statistically significant decrease was observed in groups treated with Aqueous extract 100 mg/kg and Methanol 100,200,500mg/kg ($p< 0.001$). It was observed that there was increase in the cholesterol level in the stress control group which was found to be decreased in groups treated with methanol extract at dose levels 100,200 and 500mg/kg. The values were 47.88±3.4, 27.59±1.88 and 31.58±2.44 respectively. Triglycerides value was increased in case of stress control and found to be significantly decreased in case of aqueous extract 500mg/kg 37.26±0.65 ($p< 0.001$). The reduction in triglyceride level was found in groups treated with methanol extract 100,200and 500mg/kgi.e 49.19±3.46, 46.30±1.38 and 46.30±1.38 ($p< 0.01$).In case of fraction of Methanol extract, Ethyl acetate and remaining Methanol fraction was found to be statistically more significant with $p< 0.01$ and $p< 0.001$. Chloroform fraction of methanol extract was not found to be effective in any of the parameters. In case of fraction of Aqueous extract, Ethyl acetate fraction and Methanol fractions were found to be more effective than Butanol fraction.

There was increase in RBC, WBC and differential count in stress control group. Group treated with aqueous extract 500mg/kg was found to be significant with RBC 7.37±0.68 ($p< 0.001$) Methanol extract have shown dose dependent response in 200and 500mg/kg dose level. The RBC was found to be 8.667±0.65 and 7.40±1.14 at200and 500mg/kg dose respectively. In case of WBC, significant decrease was found in group treated with aqueous extract 500mg/kg and dose dependent response was observed in 200 and 500mg/kg in methanol extract. Significant results were obtained with methanol 200 and 500 mg/kg dose in differential count.

Chloroform fraction of Methanol extract did not show any change in RBC, WBC and differential count and was not found to be effective. Acetone fraction of Methanol extract was found to be significant at 100mg/kg dose ($p< 0.05$). Ethyl acetate fraction

and remaining Methanol fraction were found to be statistically significant at the dose level 100 and 150mg/kg. Butanol fraction of Aqueous extract did not show significant change on blood count and differential count. Ethyl acetate fraction and Methanol fractions were found to be effective. In case of Ethyl acetate fraction RBC count was 9.070±0.96 and8.847±0.77 at 100 and 150mg/kg respectively and WBC count was 8.900±1.12and 7.662±1.20 respectively. Methanol fraction showed decrease in RBC count 8.863±1.00 and 7.909±1.16 at 100 and 150 mg/kg dose level respectively and WBC 8.016±1.15 and 7.443±1.03 respectively. Ethyl acetate and Methanol fraction have shown significant effect on differential count. (Table 74) When the animals were treated with extracts and different fractions of Methanol extract of *H. spicatum* there were alteration in biochemical parameters and blood count. There was no change on the organ weight. The glucose level in stress control group was increased which was found to be reduced in the treatment groups. Aqueous extract of *H. spicatum* at 500 mg/kg was found to be effective. Methanol extract of *H. spicatum* at 200 and 500mg/kg showed a dose dependent response. Concentration of glucose at dose level 500mg/kg of aqueous extract was 107.8±4.04 ($p<0$ .01). Methanol extract showed dose dependent response with amount of glucose 121.3± 3.67 and 103.2± 2.70 at dose 200 and 500mg/kg ($p<0$ .01 and $p<0$ .001). Cholesterol level in stress control group was 115.16±2.04. It was found to be significantly lowered in Aqueous extract 500mg/kg ($p<0$ .001) and Methanol extract 200 and 500mg/kg ($p<0$ .01 and $p<0$ .001). In case of triglyceride and BUN, there was increase in stress control groups which was found to be decreased in the treatment groups. The RBC and WBC count was increased in stress control group which was found to be significantly decreased in the treatment groups at the dose level 500mg/kg ($p<0$ .01) of Aqueous extract and 200 and 500 mg/kg of Methanol extract ($p<0$ .01 and $p<0$ .001).In differential count similar results were seen indicating that Methanol extract was more effective.(Table 76 and 77) When treated with different fractions of methanol extract, it was observed that remaining methanol extract was not found to be effective. Chloroform fraction and Ethyl acetate fraction were found to be more effective. The glucose level was found to be significantly reduced in Chloroform fraction of *H. spicatum* at 150mg/kg ($p<0$ .01) and Ethyl acetate fraction at 100 and 150 mg/kg ($p<0$ .01 and $p<0$ .001). Cholesterol and triglyceride levels were decreased in chloroform fraction at 150mg/kg ($p<0$ .01) and ethyl acetate fraction at 100 and 150 mg/kg ($p<0$ .01 and $p<0$ .001). There was alteration in blood count in all the fractions at dose level 150mg/kg.

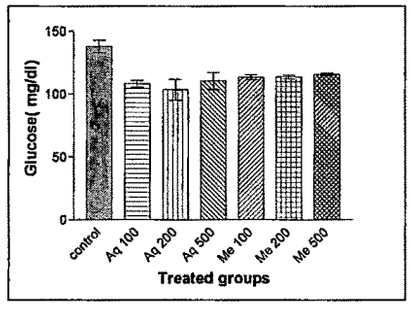
Table 70: Effect of Aqueous and Methanol extracts of *P. integerrima* on stress mediated changes

No	Groups	Dose (mg/Kg)	Swimming survival time(min)	Biochemical Parameters				Organ weight		
				Glucose (mg/ dl)	Cholesterol (mg/dl)	Triglyceride (mg/dl)	BUN (mg/ dl)	Liver (g)	Spleen (mg)	Adrenals (mg)
1	Group I	Control (0.1% Sod CMC)	87.25 ±2.32	137.7 ±4.96	42.29 ±4.96*	56.65 ±0.25	35.40 ±3.866	4.983 ±0.18	934.0± 0.41	0.044 ±0.004
2	Group II	Aqueous 100	105.65 ±1.65	108.0 ±2.7*	47.05 ±0.86**	44.77 ±1.97*	21.15 ±1.509***	4.900 ±0.089	930.5± 0.4	0.038 ±0.01
3	Group III	Aqueous 200	120.20 ±52*	103.4 ± 4.33**	96.34 ±0.58	44.26 ±1.56*	22.08 ±0.9843**	4.583 ±0.36	911.3± 0.14	0.034 ±0.01
4	Group IV	Aqueous 500	130.69 ±1.87**	110.3 ±6.71***	83.87 ±0.65	37.26 ±0.65 ***	23.65 ±1.867**	4.233 ±0.29**	1.061± 0.19	0.029 ±0.007
5	Group V	Methanol 100	102.67 ±3.15	113.2 ±1.78***	47.88 ±3.4**	46.19 ±3.46*	45.04 ±1.620	4.733 ±0.33	856.7± 0.6	0.031 ±0.011
6	Group VI	Methanol 200	126.65 ±1.38*	113.5 ±1.31***	27.59 ±1.88***	46.30 ±1.38*	47.15 ±1.507	4.650 ±0.16	861.2± 0.97	0.028 ±0.01
7	Group VII	Methanol 500	135.98** ±0.98	115.4 ±1.04***	31.58 ±2.44***	46.30 ±1.38*	45.96 ±2.155	4.333 ±0.43*	971.2± 0.13	0.029 ±0.011

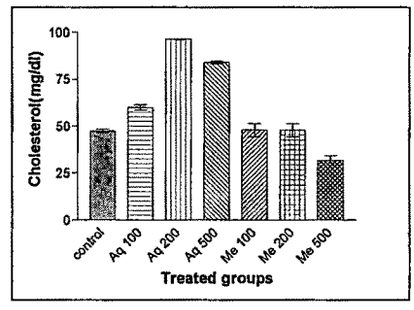
Six animals were used. $p< 0.5$, ** $p<0.01$, *** $p< 0.001$ Statistical analysis is done by applying One way ANOVA followed by Bonferronis test

Graph 25: Effect of Aqueous and Methanol extracts of *P.integerrima* on stress mediated changes in biochemical parameters in rats

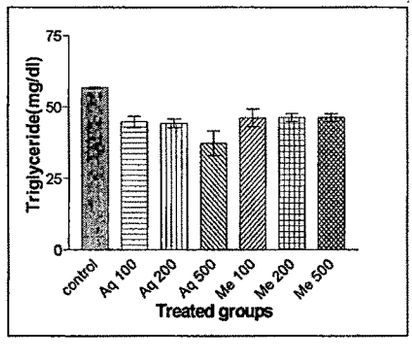
Glucose



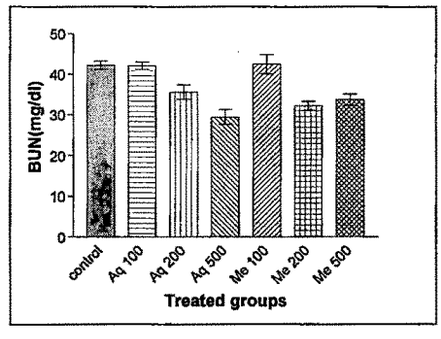
Cholesterol



Triglyceride

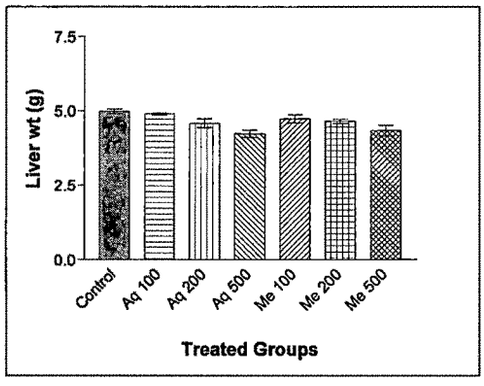


BUN

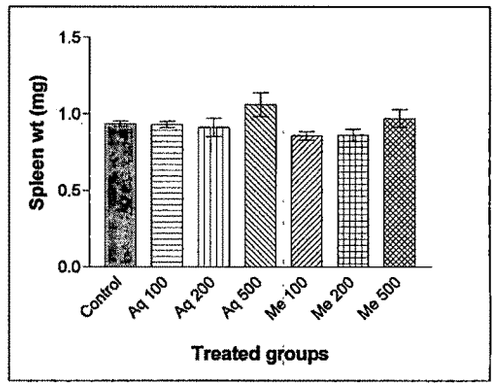


Graph 26: Effect of Aqueous and Methanol extracts of *P. integerrima* on stress mediated changes on organ weight

Liver



Spleen



Adrenals

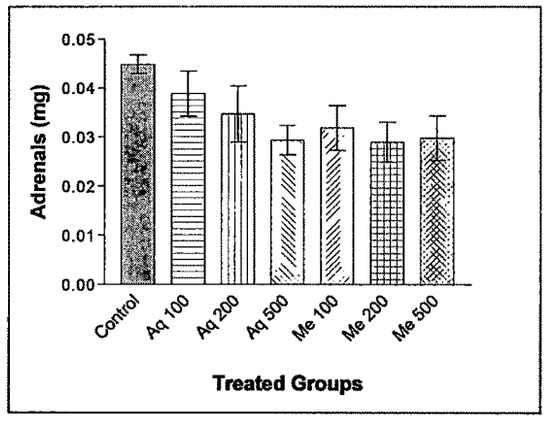
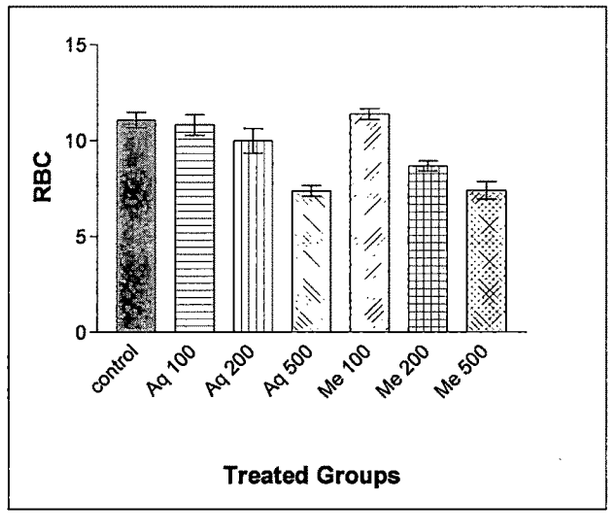


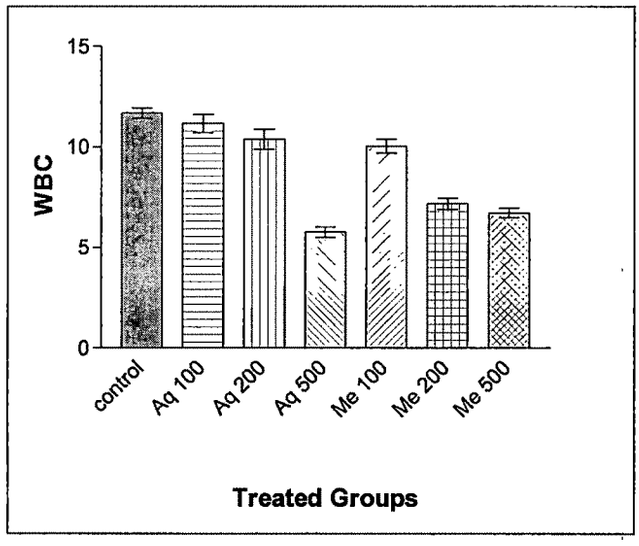
Table71: Effect of Aqueous and Methanol extracts of *P. integerrima* on stress mediated changes on blood count

No	Groups	Dose (mg/Kg)	RBC (10^6/ mm^3)	WBC (10^3/ mm^3)	DLC			
					L	N	E	M
1	Group I	Control (0.1% Sod CMC)	11.07 ±0.97	11.69 ±0.64	7478 ±265.2	3037 ±29.83	256.0 ±7.72	12.50± 0.8367
2	Group II	Aqueous 100	10.82 ±1.32	11.17 ±1.10	6704 ±569.9	2943 ±39.32**	261.6 ±9.93	13.33± 1.506
3	Group III	Aqueous 200	9.983 ±1.55	10.39 ±1.23	6700 ±171.1	2872 ±30.60***	248.0 ±6.87**	13.17± 1.169
4	Group IV	Aqueous 500	7.370 ±0.67**	5.763 ±0.62***	5371 ±115.3***	2193 ±8.058	200.2 ±2.53	10.50± 0.5477
5	Group V	Methanol 100	11.38 ±0.66	10.04 ±0.84	7338 ±132.2	3021 ±25.59***	229.4 ±8.49	12.00± 1.789
6	Group VI	Methanol 200	8.667 ±0.65**	7.178 ±0.68**	5983 ±157.2*	2667 ±41.00***	189.8 ±2.65***	9.833± 0.7528
7	Group VII	Methanol 500	7.400 ±1.14***	6.715 ±0.59***	5120 ±78.89***	2108 ±25.19	149.0 ±2.40***	9.667± 0.5164

Six animals were used. $p< 0.5$, ** $p<0.01$, *** $p< 0.001$ Statistical analysis is done by applying One way ANOVA followed by Bonferronis test.

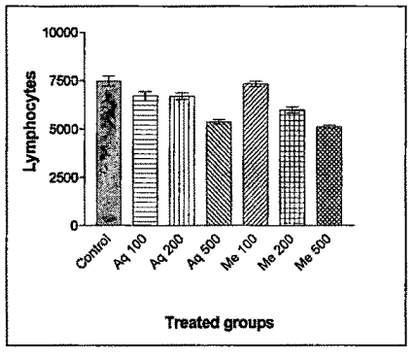
Graph 27: Effect of Aqueous and Methanol extracts of *P. integerrima* on stress mediated changes on RBC and WBC



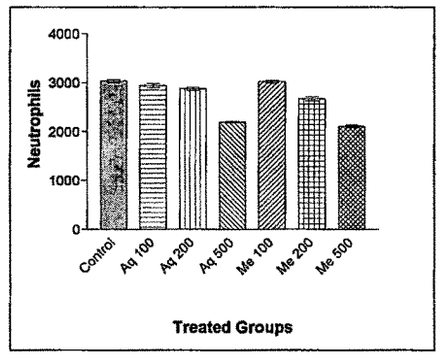


Graph 28: Effect of Aqueous and Methanol extracts of *P. integerrima* on stress mediated changes on DLC

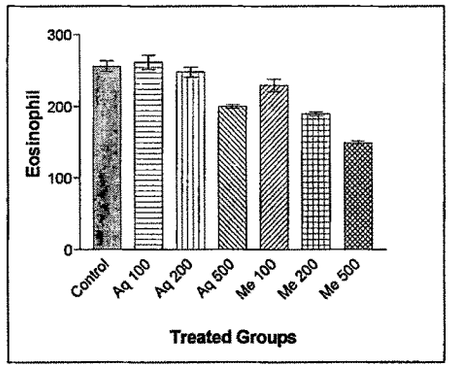
Lymphocytes



Neutrophils



Eosinophils



Monocytes

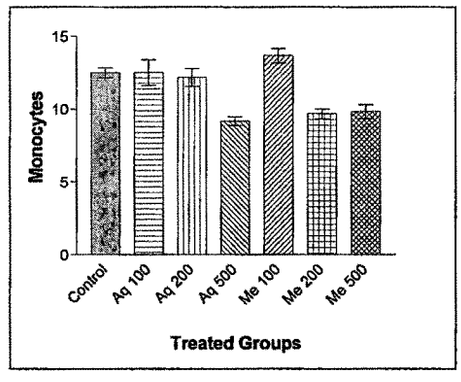


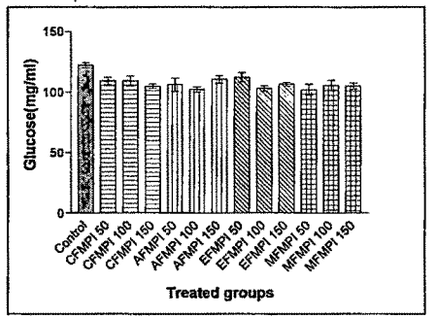
Table 72: Effect of fractions of Methanol extracts of *P. integerrima* on stress mediated changes.

No	Groups	Dose (mg/Kg)	Swimming survival time(min)	Biochemical Parameters				Organ Weight		
				Glucose (mg/ dl)	Cholesterol (mg/dl)	Triglyceride (mg/dl)	BUN (mg/ dl)	Liver (g)	Spleen (mg)	Adrenals (mg)
1	Group I	Control	87.25 ±2.32	122.3±5.29	65.38±2.16	57.37±1.46	53.81±4.75	4.983±0.18	934.3 ±0.041	0.04483 ±0.004622
2	Group II	CFMPI 50	88.21±3.21	109.2±4.23	58.47±4.20	48.92±3.75	40.50±2.58	4.917±0.13	1.001±0.09	0.0385±0.007
3	Group III	CFMPI 100	88.54±0.25	104.8±4.25	59.32±3.16	49.04±5.07	41.58±4.66	4.733±0.17	939.0±0.13	0.03383±0.01
4	GroupI V	CFMPI 150	102.32±1.68*	106.2±5.56***	54.85±1.60*	46.04±3.41*	41.63±4.30	4.633±0.32	1.016±0.21	0.0415±0.01
5	Group V	AFMPI 50	110.45±1.78**	102.3±2.29**	57.52±3.35*	52.81±2.95*	39.96±4.69**	4.700±0.30	963.8±0.14	0.03467±0.01
6	Group VI	AFMPI 100	125.32±1.10**	110.5±3.34***	57.60±2.26*	48.43±2.41*	36.82±1.62*	4.800±0.1	976.0±0.28	0.03598±0.01
7	Group VII	AFMPI 150	134.21±4.21**	112.2±3.83	57.47±4.00*	45.92±3.47	37.33±2.25**	4.800±0.17	1.036±0.18	0.04465±0.008
8	Group VIII	EFMPI 50	126.79±1.25**	102.9± 2.13	54.75±3.26**	46.58±4.31	36.27±2.24**	4.900±0.14	1.000±0.25	0.04517±0.01
9	Group IX	EFMPI 100	134.69±3.02**	106.4± 3.21***	58.40±4.68	46.76±2.75**	40.66±2.96	4.583±0.27	869.5±0.081	0.0460±0.01
10	Group X	EFMPI 150	133.45±1.14**	102.2±4.42*	52.88±4.3***	45.21±3.83***	27.87±3.35***	4.450±0.21	966.7±0.1	0.03517±0.003
11	Group XI	MFMPI 50	115.36±0.38*	102.2±4.42***	59.37±4.04	46.19±3.00***	27.10±5.98***	4.683±0.11	1.034±0.28	0.0435±0.01
12	Group XII	MFMPI 100	129.34±2.54**	105.5±4.17***	52.06±2.76***	46.30±3.39***	38.58±4.31*	5.100±0.81	926.3±0.06	0.03665±0.01
13	Group XIII	MFMPI 150	136.78±3.21**	104.9±2.64***	50.23±2.71***	42.41±2.08**	33.30±5.54***	4.633±0.18	831.3±0.08	0.03967±0.006

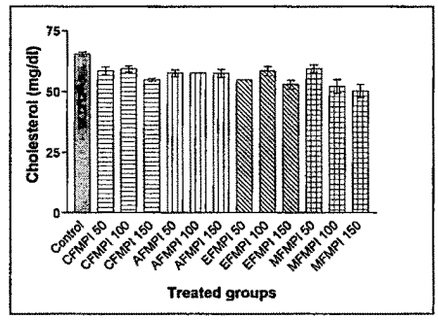
Six animals were used. p< 0.5, ** p<0 .01, *** p< 0.001 Statistical analysis is done by applying One way ANOVA followed by Bonferronis test

Graph 29: Effect of fractions of Methanol extracts of *P. integerrima* on stress mediated changes in biochemical parameters in rats.

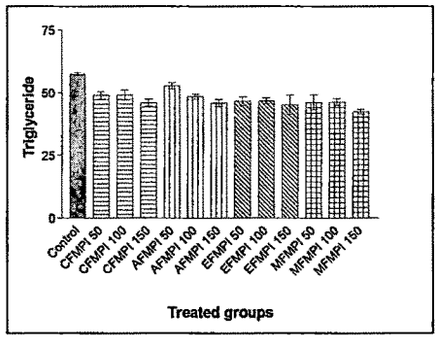
Glucose



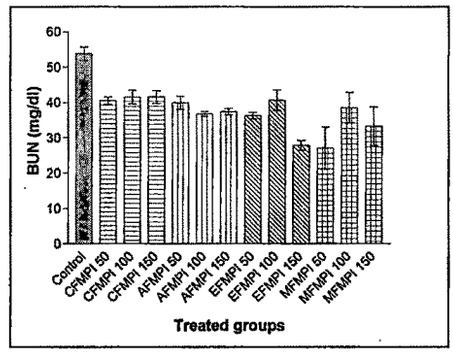
Cholesterol



Triglycerides

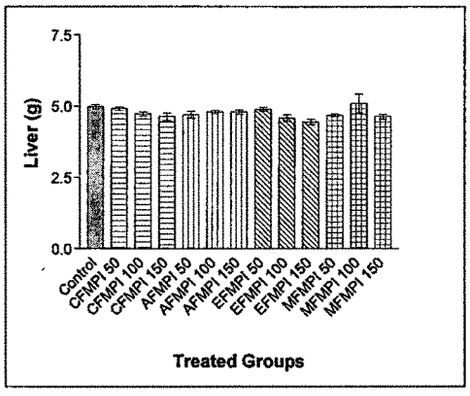


BUN

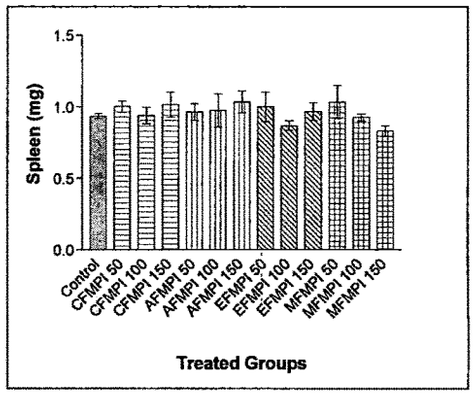


Graph 30: Effect of fractions of Methanol extracts of *P. integerrima* on stress mediated changes on organ weight

Liver



Spleen



Adrenals

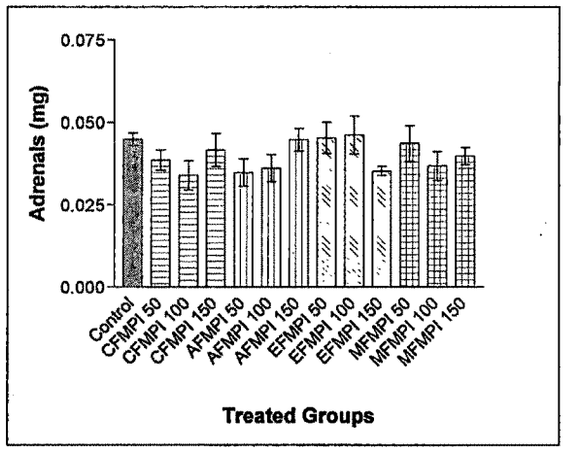
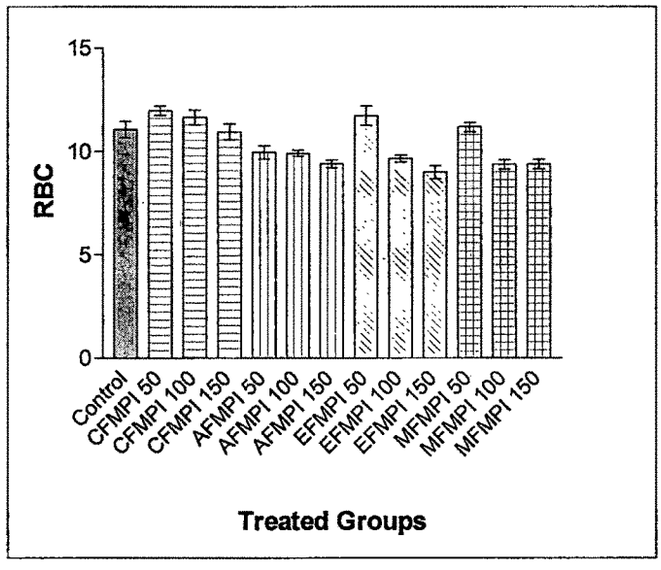


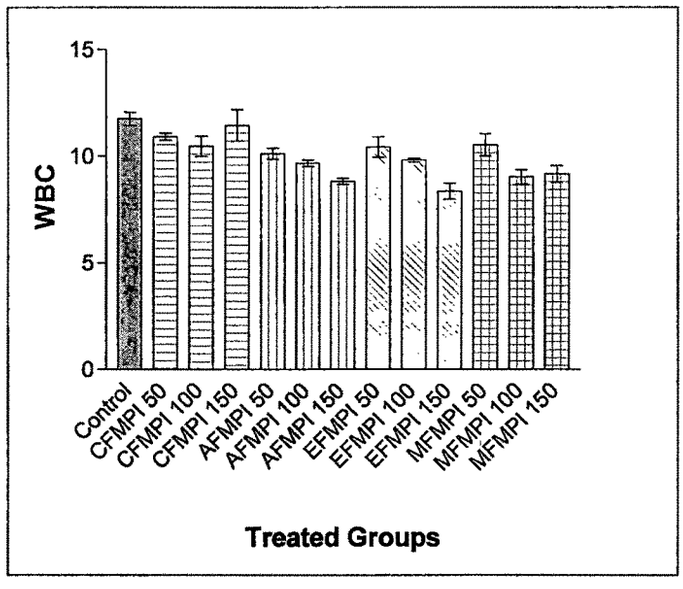
Table 73: Effect of fractions of Methanol extracts of *P. integerrima* on stress mediated changes on blood cell count

No	Groups	Dose (mg/Kg)	RBC	WBC	DLC			
					L	N	E	M
1	Group I	Control	11.07±0.97	11.74±0.69	7478±265.2	3037±29.83	256.0±5.15	12.50±0.83
2	Group II	CFMPI 50	11.98±0.57	10.91±0.37	7469±40.05	2970±29.24	251.3±2.00	12.00±0.63
3	Group III	CFMPI 100	11.65±0.89	10.46±1.04	7395±25.67	2947±20.16	250.1±3.44	11.83±0.75
4	GroupI V	CFMPI 150	10.95±0.96	11.44±1.63	7156±39.57	2849±35.51	228.5±3.20	10.67±0.81
5	Group V	AFMPI 50	9.953±0.78	10.12±0.56	7003±14.96**	2933±29.45	236.8±7.23	11.00±0.89
6	Group VI	AFMPI 100	9.828±0.37	9.674±0.35*	7012±19.78**	2760±65.81***	219.7±2.24**	10.33±0.51
7	Group VII	AFMPI 150	9.685±0.35*	8.818±0.29	5900±20.12***	2500±36.11***	208.9±5.55***	9.833±0.75**
8	Group VIII	EFMPI 50	11.73±1.138	10.43±1.05	6990±49.89**	2956±44.93	232.0±7.51	11.83±1.72
9	Group IX	EFMPI 100	9.652±0.45***	9.816±0.17*	7059±27.93*	2811±30.06*	220.9±14.9**	10.00±0.89*
10	Group X	EFMPI 150	8.990±0.78	8.353±0.8***	5947±19.50***	2272±36.38***	14.93±4.79*	9.333±0.51**
11	Group XI	MFMPI 50	11.18±0.52	10.52±1.13	6996±28.14**	2977±32.93	231.0±1.7	11.67±1.63
12	Group XII	MFMPI 100	9.363±0.53**	9.022±0.76***	7001±23.01***	2806±73.97*	188.4±7.92***	10.50±1.37**
13	Group XIII	MFMPI 150	9.380±0.55***	9.167±0.87***	5265±15.39***	2338±46.25***	145.4±2.73***	9.667±1.36**

Six animals were used. p< 0.5, ** p<0 .01, *** p< 0.001 Statistical analysis is done by applying One way ANOVA followed by Bonferronis test

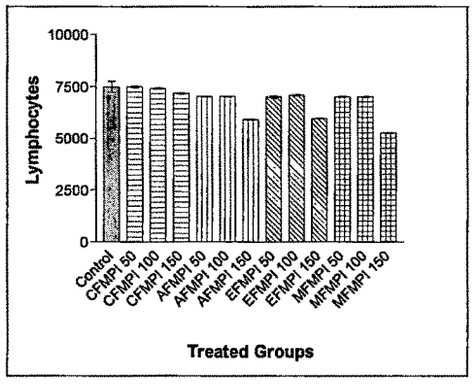
Graph 31: Effect of fractions of Methanol extracts of *P. integerrima* on blood count



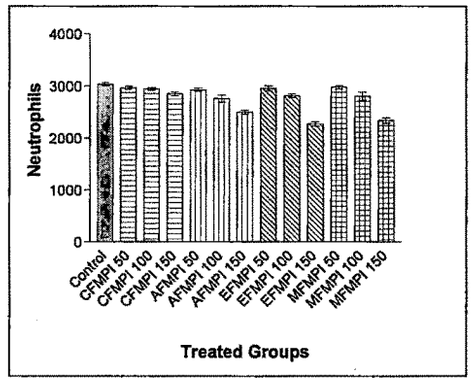


Graph 32: Effect of fractions of Methanol extracts of *P. integerrima* on DLC

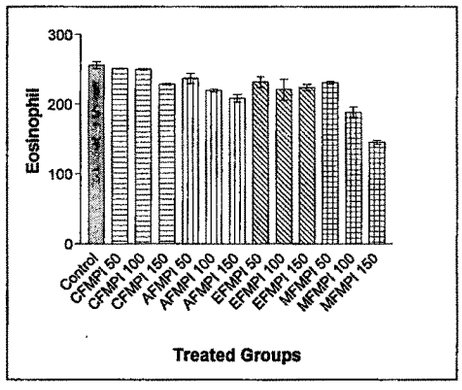
Lymphocytes



Neutrophils



Eosinophils



Monocytes

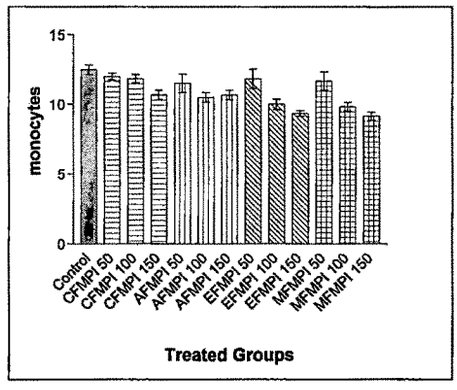


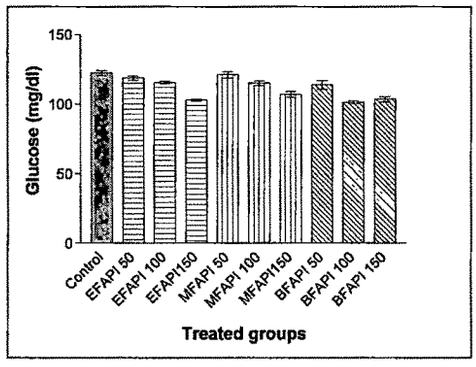
Table 74: Effect of fractions of Aqueous extract of *P. integerrima* on stress mediated changes.

No	Groups	Dose (mg/Kg)	Swimming survival time(min)	Biochemical parameters				Organ weight		
				Glucose (mg/ dl)	Cholesterol (mg/dl)	Triglyceride (mg/dl)	BUN (mg/ dl)	Liver (g)	Spleen (mg)	Adrenals (mg)
1	Group I	Control (0.1%Sod CMC)	87.25±2.32	122.3±5.29	65.38±2.16	57.37±1.46	53.81±4.75	4.983± 0.18	0.9343±0.04	263.3±0.2
2	Group II	EFAPI 50	104.32±1.65	118.7±3.36	63.89±4.481	52.39±3.39	48.10±2.72	4.617±0.4	1.053±0.11	420.0±0.8
3	Group III	EFAPI 100	120.36±1.25**	115.5±2.31	57.46±1.782***	50.67±2.92*	45.47±3.73*	4.783±0.21	0.9718±0.14	471.7±0.01
4	Group IV	EFAPI 150	135.89±0.98**	102.7±1.78***	55.79±3.419***	44.49±4.0***	44.58±2.58***	4.417±0.31	0.9852±0.14	451.7±0.16
5	Group V	MFAPI 50	95.89±1.29	121.0±2.27	66.30±2.740	52.93±2.59	46.77±2.62	4.567±0.37	0.9014±0.03	454.8±0.01
6	Group VI	MFAPI 100	115.21±1.29	111.5±2.67	56.32±3.118***	48.38±2.09	47.17±2.63***	4.250±0.18	0.9160±0.07	503.3±0.5
7	Group VII	MFAPI 150	132.89±1.89**	106.9±2.12***	60.32±3.064*	46.13±2.11***	41.85±1.73*	4.633±0.42	0.9091±0.06	541.7±0.12
8	Group VIII	BFAPI 50	98.65±0.87	113.8±3.02	76.67±2.989	53.28±3.28	46.45±3.01	4.783±0.17	0.9297±0.04	586.7±0.7
9	Group IX	BFAPI 100	115.19±1.67*	101.3±2.53***	64.67±4.629	51.06±4.08*	40.66±2.96***	4.333±0.36	0.8343±0.03	425.0±0.9
10	Group X	BFAPI 150	140.67±2.59**	103.5±4.69***	59.46±2.387*	50.52±4.90***	47.57±1.35	4.583±0.34	0.9857±0.13	588.3±0.6

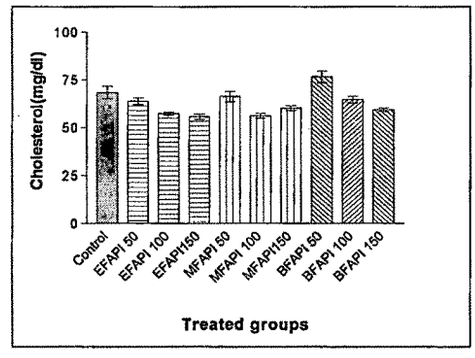
Six animals were used. $p< 0.5$, ** $p<0.01$, *** $p< 0.001$ Statistical analysis is done by applying One way ANOVA followed by Bonferronis test

Graph 33: Effect of fractions of Aqueous extract of *P. integerrima* on stress mediated changes in biochemical parameters in rats.

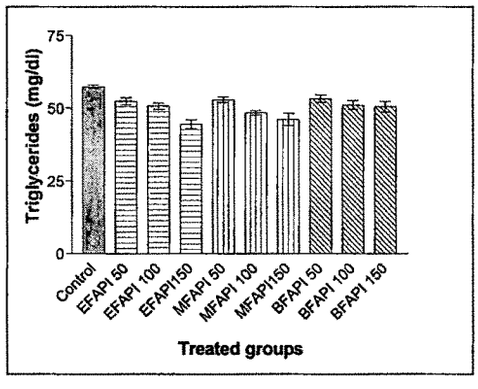
Glucose



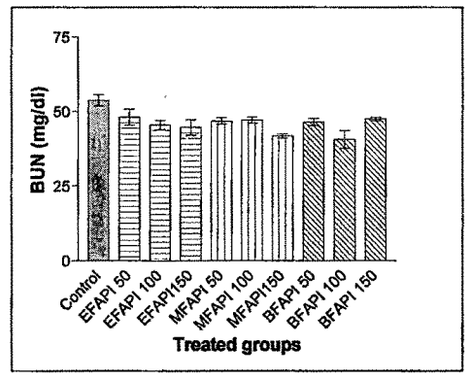
Cholesterol



Triglycerides

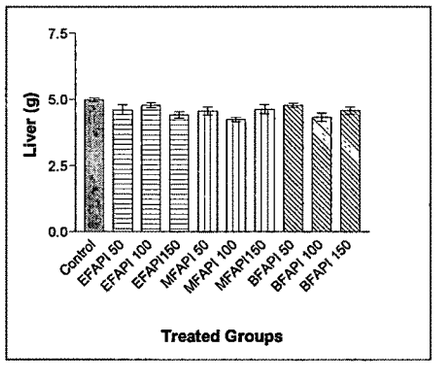


BUN

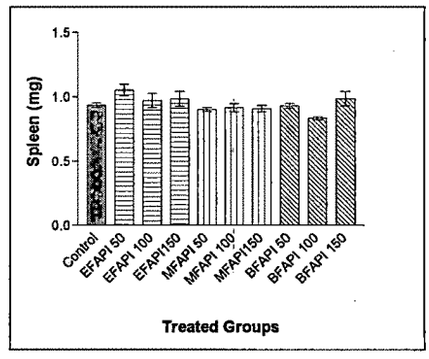


Graph 34: Effect of fractions of Aqueous extract of *P. integerrima* on stress mediated changes in organs weight

Liver



Spleen



Adrenals

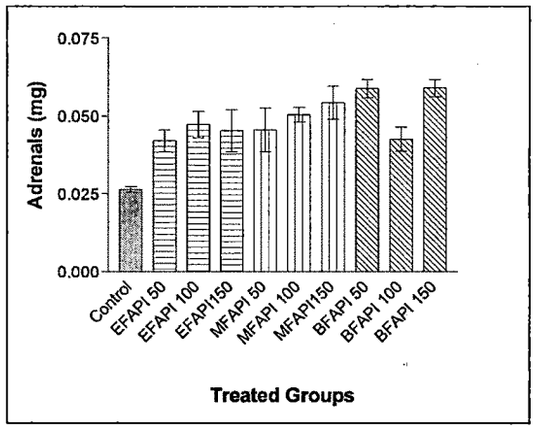
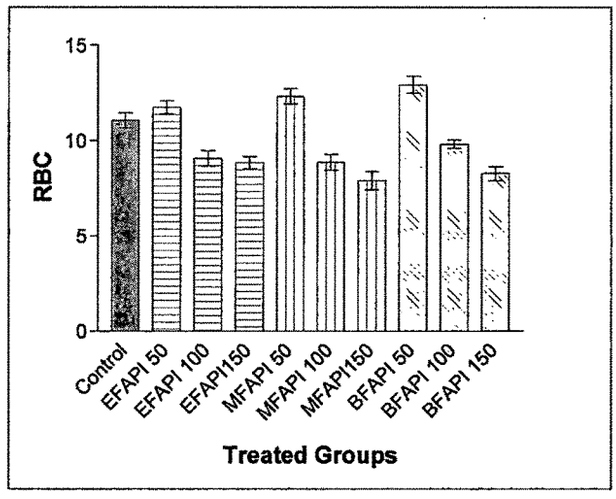


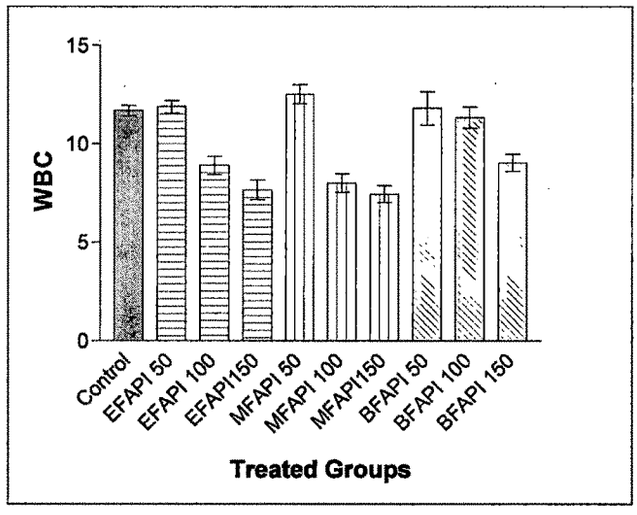
Table 75: Effect of fractions of Aqueous extract of *P. integerrima* on stress mediated changes on blood count

No	Groups	Dose (mg/Kg)	RBC	WBC	DLC			
					L	N	E	M
1	Group I	Control (0.1% Sod. CMC)	11.07±0.97	11.69±0.64	7478±26.52	3037±29.83	256.0±17.28	12.50±0.83
2	Group II	EFAPI 50	11.74±0.83	11.88±0.77	7275±19.51	2931±10.90	250.2±1.78	13.00±0.89
3	Group III	EFAPI 100	9.070±0.96*	8.900±1.12**	6955±17.16**	2901±33.20	250.4±2.01	13.17±0.75
4	Group IV	EFAPI 150	8.847±0.77**	7.662±1.20***	5252±7.17***	2670±21.63**	226.0±1.41**	10.17±0.98**
5	Group V	MFAPI 50	12.32±0.99	12.51±1.19	7259±6.38	3087±39.70	256.8±2.68	14.33±0.81
6	Group VI	MFAPI 100	8.863±1.00***	8.016±1.15***	5969±10.45***	2814±69.85**	224.4±2.88**	11.50±1.04
7	Group VII	MFAPI 150	7.909±1.16***	7.443±1.03***	5467±2.48***	2937±44.23	225.0±3.93**	10.17±0.752**
8	Group VIII	BFAPI 50	12.91±1.09	11.80±2.07	7308±38.86	3058±37.02	253.2±5.21	13.33±0.81
9	Group IX	BFAPI 120	9.815±0.52	11.33±1.31	7323±10.26	2897±29.62	262.8±11.02	12.33±1.03
10	Group X	BFAPI 150	8.275±0.86***	9.037±1.06*	6959±11.73**	2915±34.55	231.6±8.42	10.33±1.03**

Six animals were used. $p< 0.5$, ** $p<0.01$, *** $p< 0.001$ Statistical analysis is done by applying One way ANOVA followed by Bonferronis test.

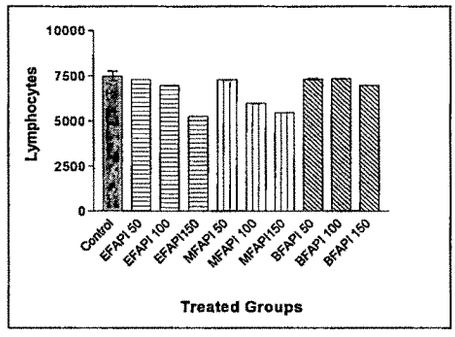
Graph 35: Effect of fractions of Aqueous extract of *P. integerrima* on stress mediated changes on blood count (RBC and WBC)



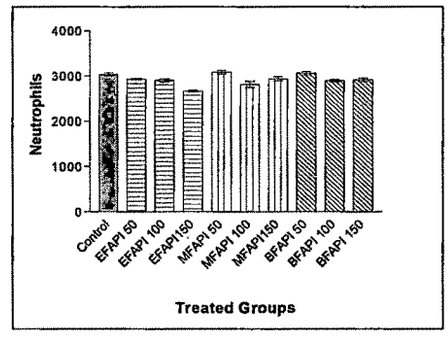


Graph 36: Effect of fractions of Aqueous extract of *P. integerrima* on stress mediated changes on DLC

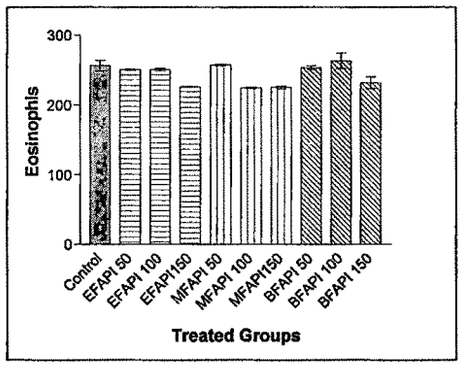
Lymphocytes



Neutrophils



Eosinophils



Monocytes

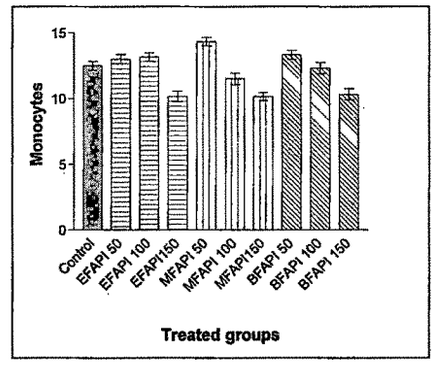


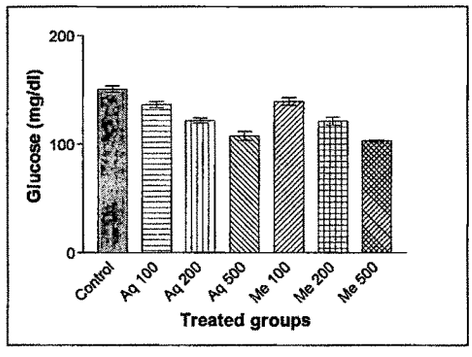
Table76: Effect of Aqueous and Methanol extracts of *H. spicatum* on stress mediated changes in rats

No	Groups	Dose (mg/Kg)	Swimming survival time(min)	Biochemical parameters				Organ Weight		
				Glucose (mg/ dl)	Cholesterol (mg/dl)	Triglyceride (mg/dl)	BUN (mg/ dl)	Liver (g)	Spleen (mg)	Adrenals (mg)
	Group I	Control (0.1%Sod CMC)	87.25 ±2.32	150.7 ±2.81	115.16 ±2.04	55.22 ±2.35	52.19 ±2.48	4.983 ±0.18	934.0 ±0.041	0.044 ±0.0046
2	Group II	Aqueous 100	90.32 ±2.36	136.7 ±2.87*	84.27 ±5.53	44.84 ±1.81	42.06 ±2.23	4.750 ±0.45	915.7 ±0.03	0.0411 ±0.006
3	Group III	Aqueous 200	112.98 ±0.98*	131.8 ±2.22	76.54 ±3.70**	47.26 ±2.56	35.60 ±4.39*	4.750 ±0.25	868.7 ±0.72	0.032 ±0.006
4	Group IV	Aqueous 500	125.98 ±2.14**	107.8 ±4.04**	46.77 ±2.85***	35.87 ±4.52***	29.45 ±4.43***	4.350 ±0.43*	1156.0 ±0.11	0.0321 ±0.004**
5	Group V	Methanol 100	102.98 ±1.87*	139.5 ±3.39	82.38 ±6.38	43.29 ±3.41*	42.46 ±2.35	4.867 ±0.13	884.0 ±0.39	0.036 ±0.007
6	Group VI	Methanol 200	129.87 ±1.50**	121.3 ±3.67**	61.41 ±3.59**	41.77 ±1.23**	32.22 ±1.07**	4.750 ±0.22	838.2 ±0.30	0.043 ±0.0046
7	Group VII	Methanol 500	138.65 ±1.73**	103.2 ±2.70***	51.66 ±2.35***	43.27 ±2.39**	33.74 ±1.39**	4.300 ±0.23*	1237.0 ±0.75*	0.039 ±0.0059

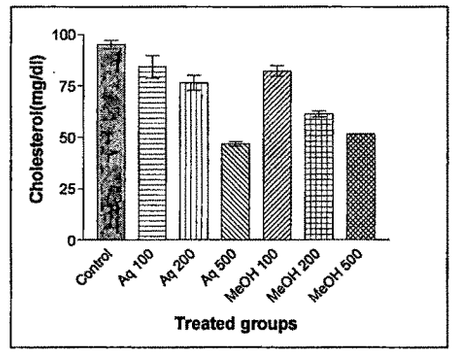
Six animals were used. $p< 0.5$, ** $p<0.01$, *** $p< 0.001$ Statistical analysis is done by applying One way ANOVA followed by Bonferronis test

Graph 37: Effect of Aqueous and Methanol extracts of *H. spicatum* on stress mediated changes in rats

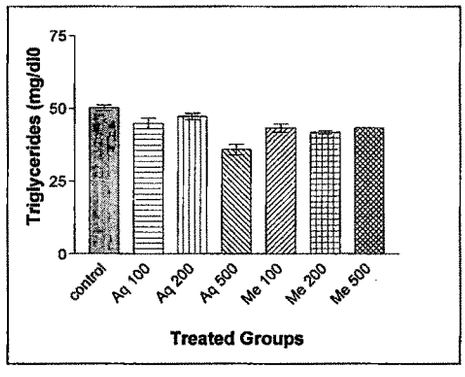
Glucose



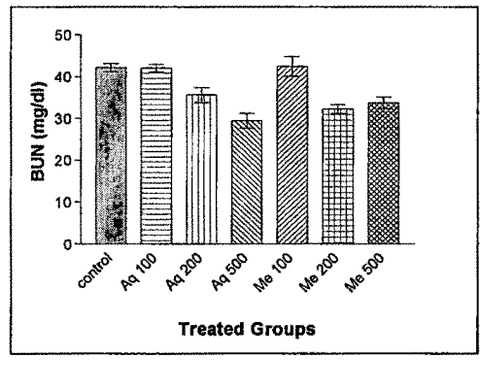
Cholesterol



Triglycerides

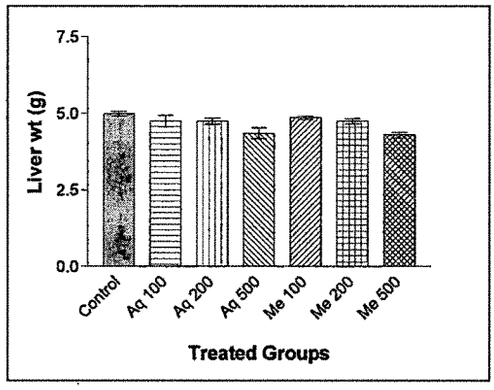


BUN

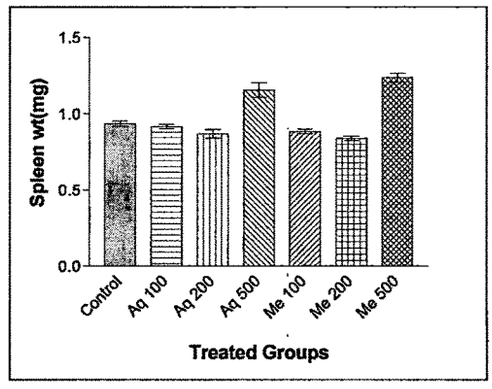


Graph 38: Effect of Aqueous and Methanol extracts of *H.spicatum* on stress mediated changes on organ weight

Liver



Spleen



Adrenals

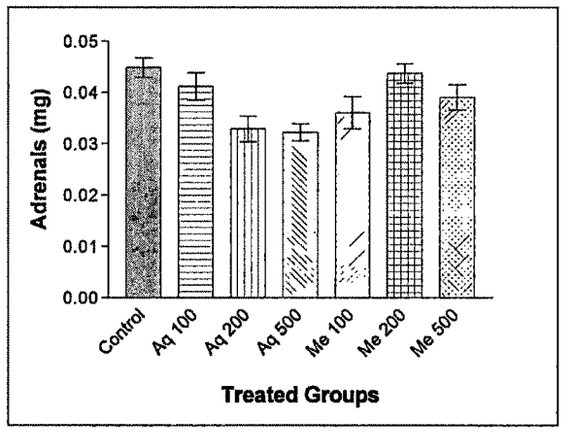
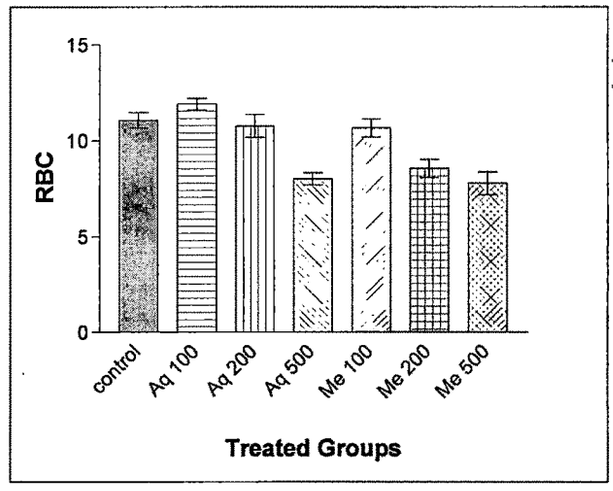


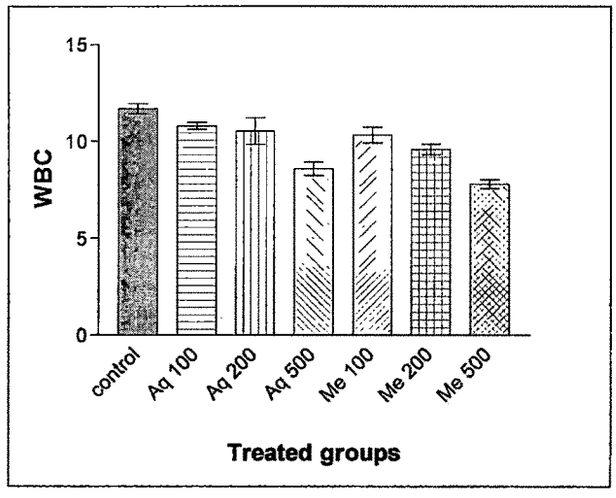
Table77: Effect of Aqueous and Methanol extracts of *H. spicatum* on stress mediated changes blood cell count.

No	Groups	Dose (mg/Kg)	RBC	WBC	DLC			
					L	N	E	M
1	Group I	Control	11.07 ±0.9771	11.69 ±0.6411	7478 ±26.52	3037 ±29.83	12.50 ±0.83	12.50 ±0.83
2	Group II	Aqueous 100	11.92 ±0.7333	10.80 ±0.4401	7095 ±19.74	2943 ±39.32	12.50 ±0.83	13.33 ±1.50
3	Group II	Aqueous 200	10.77 ±1.475	10.53 ±1.710	6868 ±20.19*	2872 ±30.60*	11.50 ±1.04	13.17 ±1.16
4	Group III	Aqueous 500	8.017 ±0.7782**	8.583 ±0.8658***	5453 ±19.34**	2193 ±8.058**	9.667 ±0.51***	10.50 ±0.54
5	Group IV	Methanol 100	10.67 ± 1.148	10.33 ±1.017	7055 ±37.49	3021 ±25.59	14.00 ±1.09	12.00 ±1.78
6	Group V	Methanol 200	8.550 ±1.143**	9.580 ±0.6705**	6938 ±23.16***	2667 ±41.00***	10.83 ±0.98***	9.833 ±0.75**
7	Group VI	Methanol 500	7.783 ±1.477***	7.789 ±0.5670***	6281 ±7.024***	2108 ±25.19***	9.333 ±1.03***	9.667 ±0.51**

Six animals were used. p< 0.5, ** p<0 .01, *** p< 0.001 Statistical analysis is done by applying One way ANOVA followed by Bonferronis test

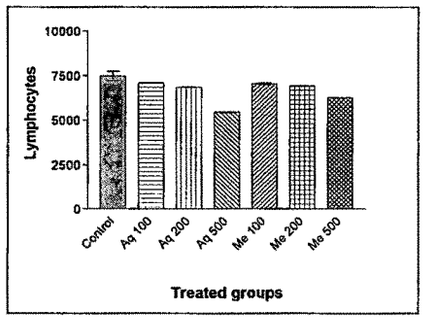
Graph 39: Effect of Aqueous and Methanol extracts of *H. spicatum* on stress mediated changes on blood cell count (RBC and WBC)



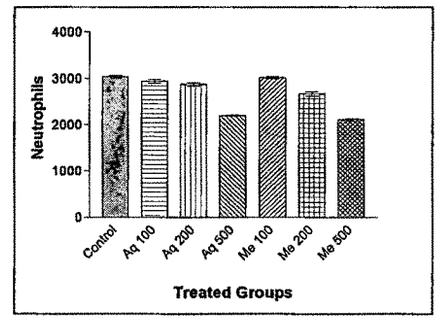


Graph 40: Effect of Aqueous and Methanol extracts of *H. spicatum* on stress mediated changes on DLC

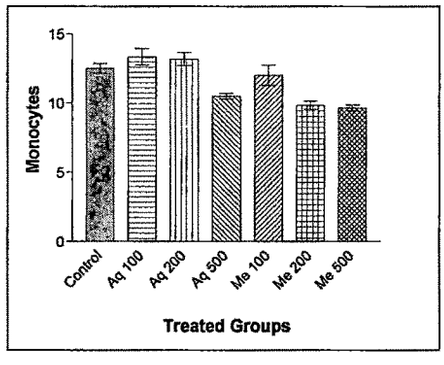
Lymphocytes



Neutrophils



Eosinophils



Monocytes

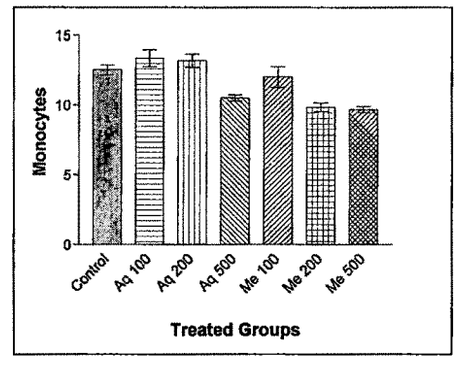


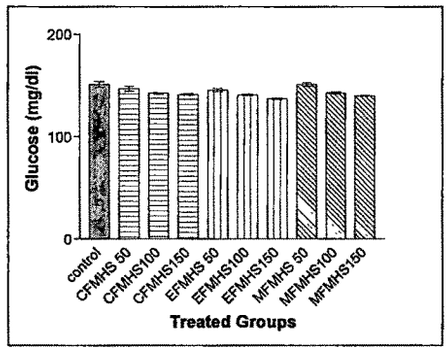
Table 78: Effect of fractions of Methanol extracts of *H. spicatum* on stress mediated changes in rats.

No	Groups	Dose (mg/Kg)	Swimming survival time (min)	Biochemical Parameters				Organ Weight		
				Glucose (mg/ dl)	Cholesterol (mg/dl)	Triglyceride (mg/dl)	BUN (mg/ dl)	Liver (g)	Spleen (mg)	Adrenals (mg)
1	Group I	Control (0.1%Sod CMC)	87.25±2.32	150.7± 2.81	115.16±2.04	55.22±2.35	52.19±2.48	4.983±0.18	934.3±0.041	0.04483±0.04
2	Group II	CFMHS 50	92.32±1.64	146.6±2.37	94.82±2.24	53.42±2.85	48.10±2.72	4.933±0.16	923.0±0.03	0.03683±0.09
3	Group III	CFMHS 100	109.26±2.05*	142.3±2.09**	95.70±2.58	52.00±1.71	45.47±3.73	4.867±0.17	880.0±0.03	0.04283±0.08
4	Group IV	CFMHS 150	128.56±1.94**	141.3±1.72**	84.08±2.56**	49.75±1.40*	44.58±2.58	4.817±0.36	0.8965±0.07	0.03967±0.05
5	Group V	EFMHS 50	102.6±1.26	145.3± 3.37	94.24±2.85	54.45±1.89	42.22±2.20	4.883±0.07	903.2±0.05	0.04333±0.07
6	Group VI	EFMHS 100	125.32±0.98**	140.6±1.13***	82.98±3.73***	50.30±2.18*	39.23±1.30**	4.933±0.12	857.3±0.04	0.0465±0.1
7	Group VII	EFMHS 150	138.98±1.26**	136.9±1.72***	78.88±3.38***	49.28±1.51*	39.28±2.41**	4.933±0.10	960.0±0.11	0.04517±0.09
8	Group VIII	MFMHS 50	98.65±2.98	150.9±3.61	88.73±1.61	52.37±0.916	40.97±2.46	4.950±0.10	943.3±0.05	0.0455±0.7
9	Group IX	MFMHS 100	115.23±1.76	145.6±2.06	85.08±3.14**	52.37±0.916	40.43±2.45*	4.833±0.19	890.8±0.05	0.04583±0.01
10	Group X	MFMHS 150	134.25±1.72	142.8±1.10	79.88±1.29***	53.03±2.04	38.58±2.20**	4.433±0.23	843.3±0.08	0.0430±0.01

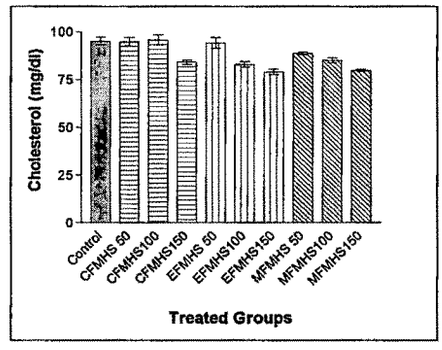
Six animals were used. p< 0.5, ** p<0 .01, *** p< 0.001 Statistical analysis is done by applying One way ANOVA followed by Bonferronis test

Graph 41: Effect of fractions of Methanol extracts of *H. spicatum* on stress mediated changes in biochemical parameters in rats

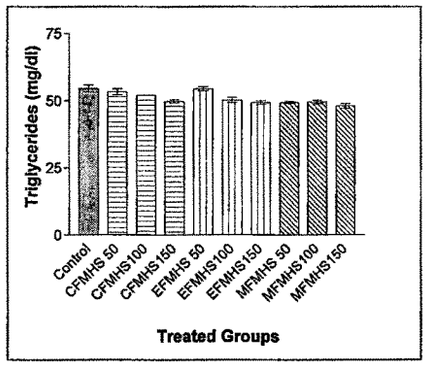
Glucose



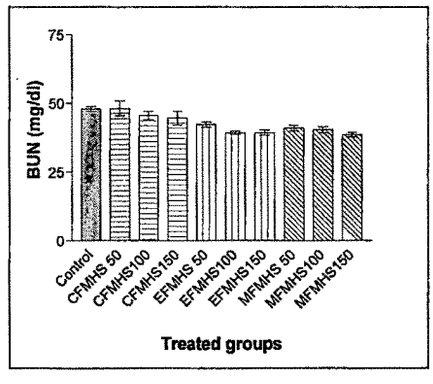
Cholesterol



Triglycerides

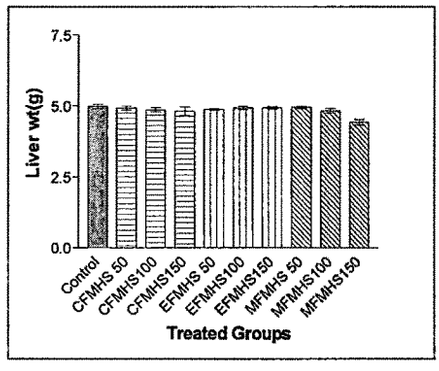


BUN

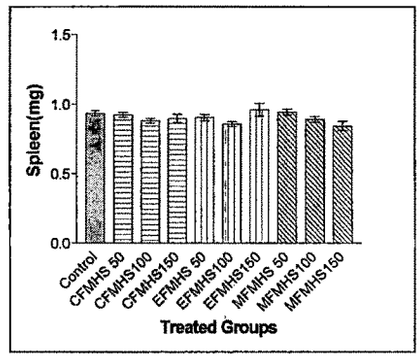


Graph 42: Effect of fractions of Methanol extracts of *H. spicatum* on stress mediated changes on organ weight

Liver



Spleen



Adrenals

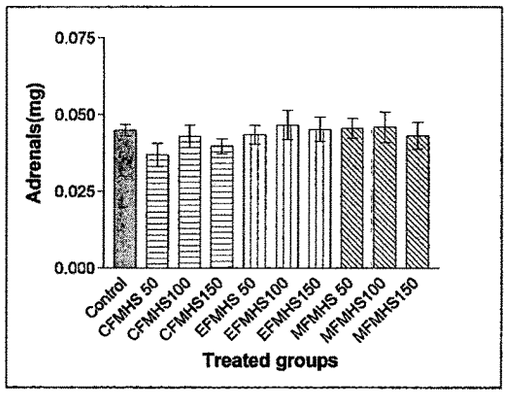
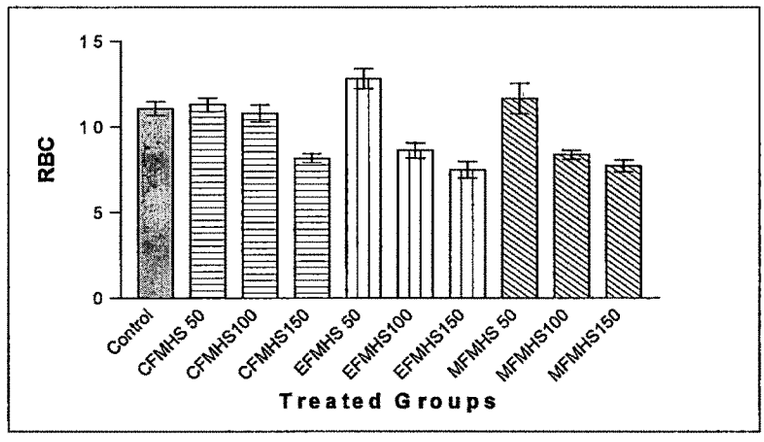


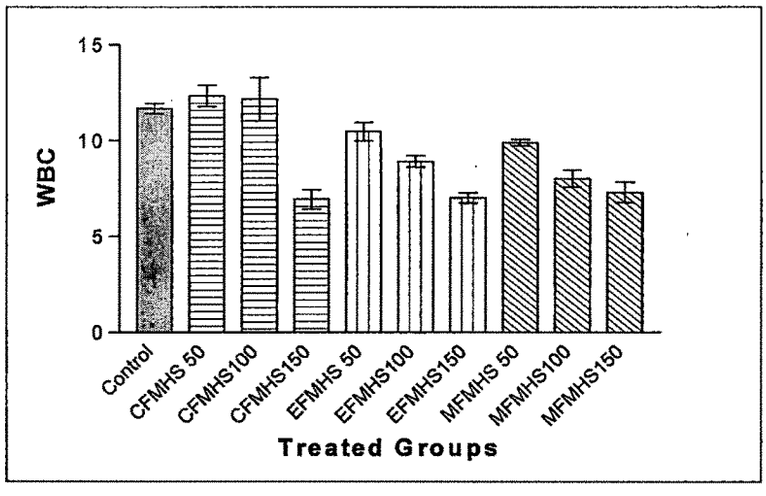
Table 79: Effect of fractions of Methanol extracts of *H. spicatum* on stress mediated changes on blood count

No	Groups	Dose (mg/Kg)	RBC	WBC	DLC			
					L	N	E	M
1	Group I	Control (0.1%Sod CMC)	11.07±0.97	11.69±0.64	7478±26.52	3037±29.83	12.50±0.83	12.50±0.83
	Group II	CFMHS 50	11.29±0.97	12.331.36	7523±10.33	3083±47.65	13.17±0.75	13.50±1.37
2	Group II	CFMHS 100	10.79±1.20	12.17±2.78	6998±6.756*	2981±5.457	12.00±0.63	12.67±1.21
3	Group III	CFMHS 150	8.158±0.63**	6.938±1.21	5436±20.83**	2650±17.21***	9.667±0.51***	9.667±0.51***
4	Group IV	EFMHS 50	12.81±1.38	10.47±1.18	7009±22.21	2970±15.85	14.17±0.75	12.33±1.50
5	Group V	EFMHS 100	8.617±1.06*	8.920±0.743	6398±26.23**	2769±24.00	10.17±0.98***	9.500±0.54***
6	Group VI	EFMHS 150	7.483±1.17***	7.000±0.66	5935±14.00***	2315±8.288***	9.667±0.81***	9.500±0.54***
7	Group VII	MFMHS 50	11.64±2.18	9.897±0.38	6944±32.45*	2956±16.87	12.17±0.75	13.00±0.63
8	Group VIII	MFMHS 100	9.367±0.62	8.023±1.07	6921±15.78	3027±21.41	11.17±1.16	9.667±0.51***
9	Group IX	MFMHS 150	9.717±0.85	7.300±1.33	5476±48.41**	2141±32.85*	9.167±0.75***	10.00±1.41**

Six animals were used. p< 0.5, ** p<0 .01, *** p< 0.001 Statistical analysis is done by applying One way ANOVA followed by Bonferronis test

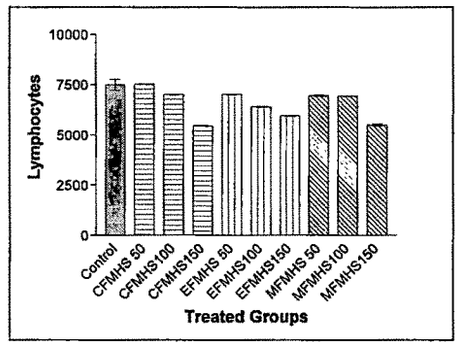
Graph 43: Effect of fractions of Methanol extracts of *H. spicatum* on stress mediated changes on blood count (RBC and WBC)



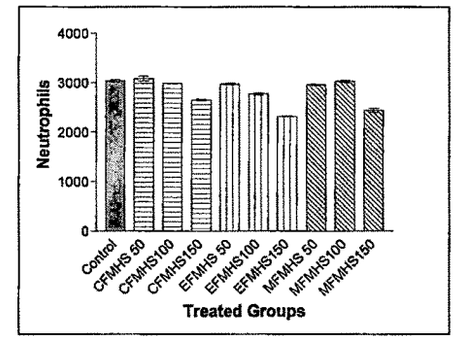


Graph 44: Effect of fractions of Methanol extract of *H. spicatum* on stress mediated changes on DLC

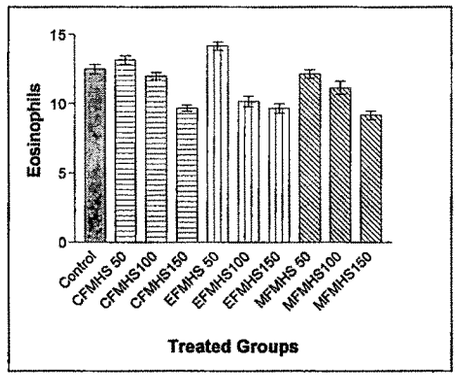
Lymphocytes



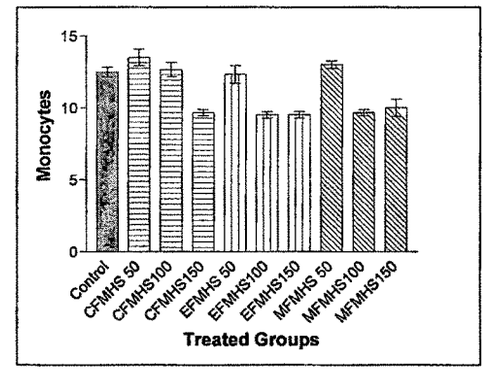
Neutrophils



Eosinophils



Monocytes



Chronic fatigue syndrome is a heterogeneous disorder of unknown etiology characterized by neuropsychiatry symptoms and various other somatic complaints (Kaur and kulkarni, 1998). Lazarev in 1947 described a concept about existence of new substances causing a state of nonspecifically increased resistance. Stress may lead to improvement or deterioration of mental physical performance depending on its magnitude but certainly it leads to disease (Brekhman, 1969 and Patil, 1997). Forced swim involves physical exercise and physiological stress which leads to increased

serum cholesterol and protein levels. (Krupavaram et al, 2007). Blood sugar levels in response to stress in rats shows fluctuation in blood sugar level ranging slight decrease, relative increase or no change. In the present study, hyperglycemia was observed. Under stressful conditions adrenal cortex secretes cortisol in man and corticosterone in rats. Hyper secretion of cortisol helps maintenance of internal homeostasis through the process of gluconeogenesis and lipogenesis (Dadkar, 1987). The stress raises serum cholesterol level through enhanced activity of hypothalamus hypophyseal axis resulting in increased liberation of catecholamines and chorticosteroids (Bijlani, 1985) The effect of triglycerides is probably due to mobilization of lipids from adipose tissues by catecholamine (Tagar, 1973). There was increase in BUN level in stress control group as these are the end products of protein metabolism and in excess adrenocorticoid activity, urea excretion increases. The decreased BUN level in the treated groups indicates diminished amount of protein catabolism in extract treated groups. Stress induced adrenomedullary response in man leading to increased production of corticotropic hormone that leads to increase in weight adrenal gland and liver and reduction in spleen weight. In the study, there was no alteration in organ weight (Sardesai, 1993). Stress induces adrenomedullary response in man causing release of adrenalin which in turn stimulates β_2 receptors by pituitary gland causing greater release of ACTH. This stimulates the adrenal medulla and cortex leading to weight increased weight of adrenal gland. Cortisol increases m-RNA levels in the liver cells and facilitate metabolic anabolic effect. This leads to increase in weight of liver. Spleen contracts to release more blood cells during stress leading to decrease in weight of spleen. During stress, heart rate, blood pressure and flow rate increase and to meet the extra demands, RBC and WBC increase (Bapu, 2006).

3.6 Hepatoprotective activity in vitro

The flavonoid fraction, phenolic fraction of *P. integerrima* and terpenoid fraction of *H. spicatum* and isolated compounds from these fractions were subjected to in vitro hepatoprotective screening using paracetamol induced hepatotoxicity in rats.

Isolation and culturing of hepatocytes:

Hepatocytes were isolated from liver from according to the method of Sarkar and Sil with some modifications. The method adopted by Tingstrom and Obrink with slight modification was used for the purpose of primary culture of hepatocytes.

Table 80: Effect of fractions of *P. integerrima* and *H. spicatum* against Paracetamol induced toxicity in rats

Group	Viable cells (%)	GOT (IU/L)	GPT (IU/L)	TPTN (g/dl)
Control	97.03±0.26	20.35±0.53	24.63±0.27	4.16±0.12
Paracetamol (100µg/ml)	27.65±1.43	46.32±0.35	55.62±0.69	2.09±0.51
Sylimarine (100µg/ml)	84.23±1.26*	22.86±0.81*	29.06±0.48*	3.68±0.26*
PIFF 100	39.45±0.36	42.20±1.26	49.32±1.26	2.12±0.26
PIFF 500	49.74±1.19*	38.51±0.59*	43.26±0.87*	3.01±0.39*
PIFF 1000	56.34±1.22**	28.23±0.64**	34.25±0.54*	3.20±0.64**
PIPF 100	51.26±0.91*	45.26±1.31	43.02±1.09*	2.03±0.68
PIPF 500	60.21±2.72**	33.96±.036**	35.21±1.07*	2.69±0.94*
PIPF 1000	72.26±3.01***	29.51±0.83*	31.25±1.89**	3.28±0.94**
HSDF 1000	49.21±1.25*	44.21±0.23	45.26±0.39	2.08±0.24
HSDF 500	53.67±2.36*	37.59±0.37**	39.26±0.92*	3.10±0.37*
HSDF 1000	62.35±1.64**	31.26±0.56**	36.56±0.69**	3.18±0.58**

n=3. *p<0.5, ** p<0.01, ***p< 0.001. Statistical analysis was done by one way ANOVA followed by Bonferronis test.

Table 81: Effect of isolated compounds from *P. integerrima* and *H. spicatum* against Paracetamol induced toxicity in rats

Group	Viable Cells (%)	GOT (IU/L)	GPT (IU/L)	TPTN (g/dl)
Control	97.03±0.26	20.35±0.53	24.63±0.27	4.16±0.12
Paracetamol (100µg/ml)	27.65±1.43	46.32±0.35	55.62±0.69	2.09±0.51
Sylimarine (100µg/ml)	84.23±1.26*	22.86±0.81*	29.06±0.48*	3.68±0.26*
PI 1 10	32.45±0.53	48.16±0.26	48.16±0.16	2.08±0.89
PI 1 50	51.23±0.23*	35.69±1.06*	44.31±0.64	3.12±0.54
PI 1 100	58.26±0.26**	30.06±0.81**	36.14±1.28*	3.35±0.43*
PI 2 10	48.28±0.93*	46.29±0.35	47.12±0.26	2.12±0.20
PI 2 50	49.24±1.32*	38.89±.042*	40.02±0.95*	3.06±0.89*
PI 2 100	56.21±0.68**	33.26±0.46**	35.26±0.54**	3.45±0.16**
PI 3 10	36.21±0.26	45.26±0.59	49.09±0.66	2.11±1.02
PI 3 50	42.13±1.09	37.34±0.16	48.28±0.54	2.65±0.67
PI 3 100	51.22±0.64*	32.19±1.09**	38.40±0.68**	3.19±0.16*
HS 10	46.46±0.65	44.56±1.12	45.26±0.39	2.08±0.24
HS 50	49.21±1.09*	38.26±0.45	39.26±0.92*	3.10±0.37*
HS 100	56.29±0.95**	33.39±0.85*	36.56±0.69**	3.18±0.58*

n=3. *p<0.5, ** p<0.01, ***p< 0.001.

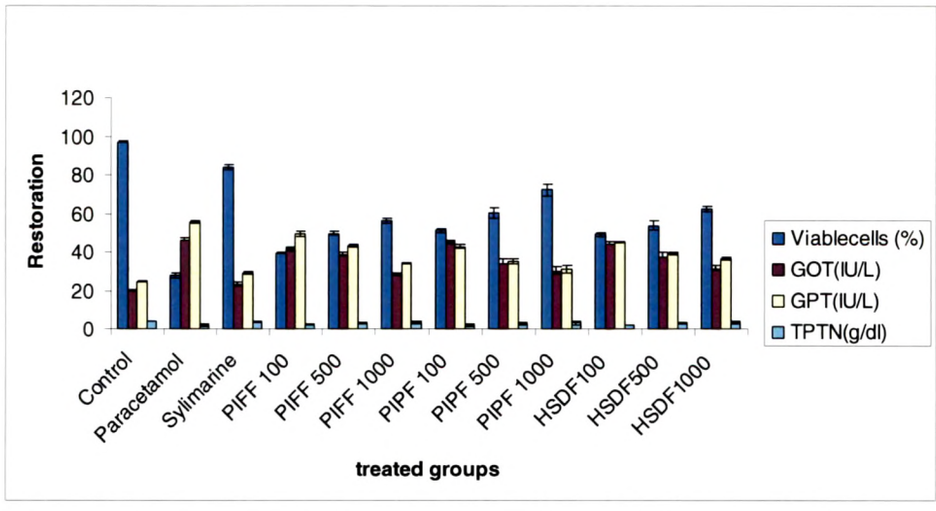
Statistical analysis was done by one way ANOVA followed by Bonferronis test.

PI 1, PI 2- compounds isolated from flavonoid fraction of *P. integerrima*

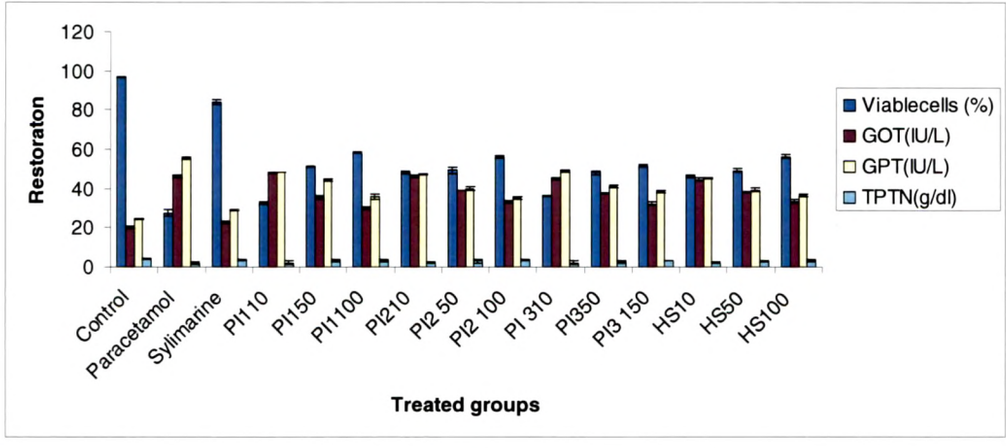
PI 3 - compound isolated from phenolic fraction of *P. integerrima*

HS- compound isolated from terpenoid fraction of *H. spicatum*

Graph 45: Effect of fractions of *P. integerrima* and *H. spicatum* against Paracetamol induced toxicity in rats



Graph 46: Effect of isolated compounds from *P. integerrima* and *H. spicatum* against Paracetamol induced toxicity in rats



Paracetamol an analgesic and antipyretic is assumed to be safe in recommended doses, overdoses however taken with suicidal intent produce hepatic necrosis. Small doses are eliminated by conjugation followed by excretion. When conjugation enzymes are saturated, the drug is diverted to an alternative metabolic pathway resulting in the formation of a hydroxylamine derivative by cytochrome P_{450}. The hydroxylamine derivative, a reactive electrophillic agent, reacts nonenzymatically with glutathione and detoxifies. When the hepatic reserves of glutathione depletes, the hydroxylamine reacts with macromolecules and disrupts their structure and function. Extensive liver damage by paracetamol decreases its rate of metabolism and other

substrates for microsomal enzymes (Savides et al, 1983).Paracetamol is metabolized by microsomal cytochrome P $_{450}$. The hepatotoxicity of paracetamol is due to formation of toxic and highly reactive metabolite N acetyl p benzoquinoneamine. This highly toxic substance starts a chain of free radicals which attack membrane lipids and proteins thereby causing destruction of microsomes and liver cells leading to cell lysis. Leakages of cytosolic enzymes out of the cells thus occur due to increase in cell permeability, membrane damage and cell necrosis. In the present study, it was observed that there was reduction in cell viability due to injury to plasma membrane. The enzyme level was increased due to leakage of cellular enzymes.

The fractions at dose level 100-1000 µg/ml were found to be effective as there was decrease in SGPT and SGOT levels. The cell viability was increased as compared to paracetamol treated group and was comparable to Sylimarine in all the treated groups. Cell viability in paracetamol treated group was reduced to 27.65±1.43 where as in the treatment groups it was found to be increased. The GOT level was 20.35±0.53 in the control group. When the cells were treated with toxicant, the level was increased to 46.32±0.35 which was brought back to 22.86±0.81 with Silymarin which was compared to control. All the treatment groups showed reduction in GOT level but at 1000 µg/ml dose the values were comparable to Sylimarin. Similarly, GPT levels were also raised on treatment with the toxicant i. e. 55.62±0.69 which was reduced to 29.06±0.48 on treatment with Silymarin. The treatment groups at dose level 1000 µg/ml could bring back GPT level comparable to Silymarin. Total protein level was found to be reduced in paracetamol treated group i. e. 2.09±0.51. In group treated with Silymarin, the total protein level was 3.68±0.26. The treatment groups at 500 and 1000 µg/ml dose level could show significant increase in the total protein level. (Table 80)

Similar results were obtained when restoration was observed with isolated compounds P1, P2, P3 and HS at 10, 50, and 100 µg/ml dose levels. The isolated compounds showed restoration of all the parameters at 50 and 100 µg/ml dose levels. The compounds isolated from ethyl acetate fraction of methanol extract of *P. integerrima* were of phenolic and flavonoid nature. It was confirmed by spectral studies and comparing the spectra with standard. These types of compounds may possess the hepatoprotective activity due to their antioxidant properties. The diterpenes are also reported to possess the hepatoprotective activity. *H. spicatum* was found to be rich in diterpenoids which was confirmed by preliminary studies, TLC and HPTLC analysis.

The compound isolated from *H. spicatum* was subjected to chemical tests, TLC, HPTLC and spectral studies. From the above observations it was concluded that the isolated compound may be the furanoditerpene which is responsible for hepatoprotective activity. The furanoditerpene which is present in *A. paniculata* also possess the hepatoprotective activity. Thus it can be concluded that hepatoprotective activity of *H. spicatum* may be due to the diterpene present. (Table 81)

The in vivo studies require a large number of animals (n=6) and needs up to 3-5 days of drug administration for significant effect to be produced. It needs large amount of drug. On the other hand the in vitro model is rapid and requires fewer amounts of test substances. Bioactive fractions obtained from the plant extracts are usually available in the small quantities. Therefore, in vitro models can be more useful in assessment of activity.

In the literature many authors have reported hepatoprotective activity of phenolic and flavonoidal compounds. Galisto et al (2006) reported the hepatoprotective activity of flavonoids of *Rosmarinus tomentosus*. The hepatoprotective effect of quercetin and rutin was reported by Janbaz (2004). Sylimarin from *Sylibum marianum* is a good hepatoprotective agent (1998). In accordance with these findings, it may be hypothesized that hepatoprotective activity of *P. integerrima* may be attributed to phenolics and flavonoids present in it. In conclusion the study confirms the therapeutic potential of *P. integerrima*. It hepatoprotective activity of *H. spicatum* is reported in the literature. The in vivo hepatoprotective activity was also reported earlies by Habbu etal (2002). The diterpens in the plant may be responsible for hepatoprotective activity. Saxena etal (2000) has reported presence of diterpene andrographaloid in *A. paniculata* which is responsible for hepatoprotective activity. Deng (1982) and Choudhary (1982) have reported the hepatoprotective activity of andrographaloid, a known diterpene isolated from hepatoprotective drug *A. paniculata*. It was observed that andrographaloide showed significant activity in paracetamol and galactosamine induced toxicity (Handa 1990; Sharma 1991). Results of our findings are comparable to that of andrographaloide.

In conclusion the study confirms the therapeutic potential of *H. spicatum* as a good hepatoprotective agent. The activity of *H. spicatum* may be due to diterpenes present. The findings of our study confirm the claims on *H. spicatum* as hepatoprotective agent.

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