Chapter 5

RESULTS

5. RESULT

5.1 FREE RADICAL SCAVENGING ACTIVITY

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are beneficial for normal physiological functions when they occur at low or moderate concentrations. Overproduction of reactive ROS/RNS results in damage of cell structure, protein and DNA (Valko *et al*., 2006). In the present work free radical scavenging potential of eleven liverworts namely, *Pellia epiphylla, Conocephalum japonicum, Lunularia cruciata, Dumortiera hirsuta, Marchantia emarginata, M subintegra, M polymorpha, Plagiochasma cordatum, Asterella wallichiana*, and *Plagiochila nepalensis* and *Marchantia paleacea* were studied by their ability to scavenge Superoxide (SO), Nitric oxide (NO), $DPPH^{\cdot}$, $ABTS^{+}$ radicals and metal ion chelating Activity (MC). $DPPH^{\bullet}$ is mostly used for investing the free radical scavenging properties of natural compounds. Studied eleven liverworts showed DPPH radical scavenging activity ranging between 0.1 mg/ml and 34.7 mg/ml (IC_{50} value). Among the studied liverworts *Marchantia paleaea*, *Plagiochasma cordatum* and *Plagiochila nepalensis* showed the lowest IC_{50} value which signifies the highest $DPPH^{\cdot}$ scavenging activity of these liverworts (Fig 5.1.1). Another important finding of this assay was determination of suitable solvent for the extraction of phytochemicals with high $DPPH^{\cdot}$ scavenging potential. Table 5.1 lists the $DPPH^{\bullet}$ scavenging activity of studied liverworts. In all the studied liverworts, diethyl ether and ethyl acetate extracts have shown the highest $DPPH^{+}$ scavenging activity. $ABTS^{+}$ is another stable free radicals used to study the antioxidant activity of plants. In the present study, $ABTS^{+}$ scavenging activity ranged between 0.02 mg/ml and 10.19 mg/ml (IC_{50} value). *Marchantia polymorpha*, *Marchantia paleacea, Plagiochasma cordatum* and *Plagiochila nepalensis* showed highest $ABTS^{+}$ scavenging activity (Fig 5.1.2). $ABTS^{+}$ was scavenged with maximum strength by the diethyl ether and ethyl acetate extracts in studied liverworts except in case of *Dumortiera hirsuta* where acetone extract showed the highest $ABTS^{+}$ scavenging activity (Table 5.2).

In living organism superoxide generated by the auto-oxidation reaction is the most harmful free radical. It induces oxidation of lipid by singlet oxygen generation (Halliwell *et al*., 1978). Table 5.3 represents the SO scavenging activity of studied liverwort species. SO scavenging activity ranged between 0.11 mg/ ml and 15.57 mg/ml (IC_{50} value). Liverworts

like *Marchantia polymorpha*, *Marchantia paleacea*, *Marchantia subintegra*, *Marchantia emerginata* and *Asterella wallichiana* showed the highest superoxide scavenging activity (Fig 5.1.3). Nitric oxide is an important signalling molecule; however its sustained level in the body is very harmful. Liverworts showed large variation in the NO scavenging Activity (Fig 5.1.4). The highest NO scavenging activity was shown by heptane extract of *Dumortiera hirsuta*. Other liverworts like *Lunularia cruciata*, *Dumortiera hirsuta*, *Conocephalum japonicum* and *Marchantia paleacea* also showed significant NO scavenging activity. Lowest NO scavenging activity was shown by methanolic extract of *Marchantia subintegra* (Table 5.4). Suitable solvent for extracting active nitric oxide scavenging phytochemicals varied among studied liverworts. In *Marchantia paleacea*, *M. subintegra*, *Pellia epiphylla*, *Plagiochasma cordatum* and *Astetrella wallichiana* heptane extract showed highest NO scavenging activity. Whereas, in *Marchantia polymorpha* and *Dumortiera hirsuta*, butanol extract showed the NO scavenging activity. In *Marchantia emarginata* and *Lunularia cruciata* ethyl acetate extract and in *Conocephalum japonicum* and *Plagiochila nepalensis* acetone extract showed highest NO scavenging activity (Fig 5.1.4).

Metal chelating assay is another important antioxidant assay. Complexes of ferrozine and Fe^{2+} together with samples have metal chelating activity, and thus probability for the production of complexes to yield hydroxyl radical will be decreased. Studied liverworts have showed metal chelating activity ranging between 0.09 mg/ml and 25.48 mg/ml (IC_{50}value) (Table 5.5). Liverwort species like *Marchantia paleacea*, *M. subintegra*, *M. emarginata*, *Plagiochasma cordatum*, *Plagiochila nepalensis*, *Dumortiera hirsuta* and *Lunularia cruciata* displayed a strong metal chelating activity among studied liverworts.

Reducing power of bioactive compounds provides direct assessment to their antioxidative property. Figure 5.1.6 represents the ferrous ion reducing potential of studied eleven liverworts. Diethyl ether extract of *Plagiochasma cordatum* showed the highest metal ion reducing potential. Liverworts like *Marchantia paleacea*, *M. emerginta*, *Plagiochila nepalensis*, *Pellia epiphylla* also displayed an impressive potential to reduce ferrous ion. Result also revealed that in *Marchantia paleacea*, *M. subintegra*, *Conocephalum japonicum*, *Pellia epiphylla*, *Plagiochasma cordatum*, *Plagiochila nepalensis* and *Dumortiera hirsuta* diethyl ether showed highest ferrous ion reducing activity. Whereas in *Marchantia polymorpha*, *M. emarginata*, *Lunularia crucita* and *Asterella wallichiana* ethyl acetate extract showed highest ferrous ion reducing actcivity (Table 5.6

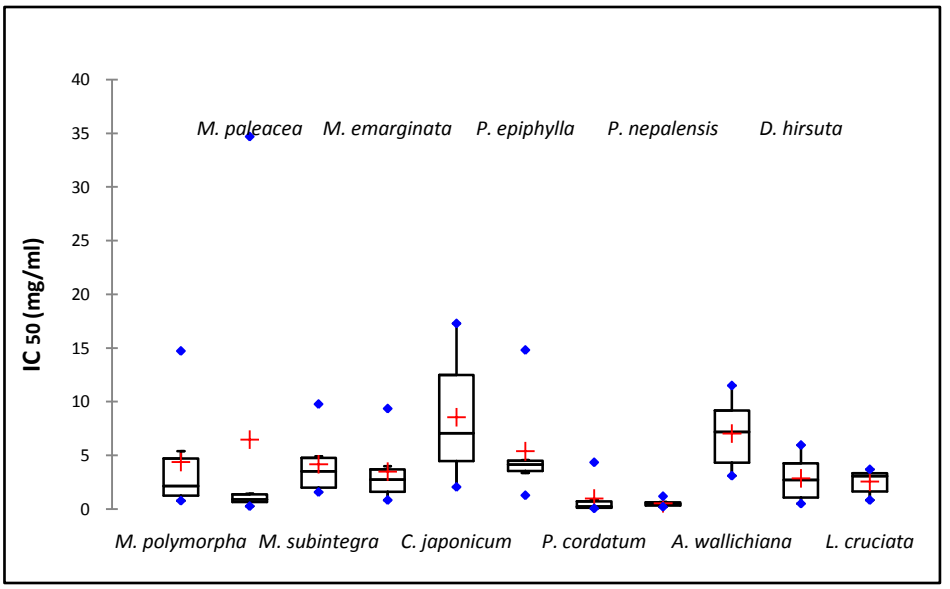


Fig 5.1.1: DPPH radical scavenging activity of studied eleven liverworts. For each sample, mean, standard deviation, maximum and minimum values, outliers are shown.

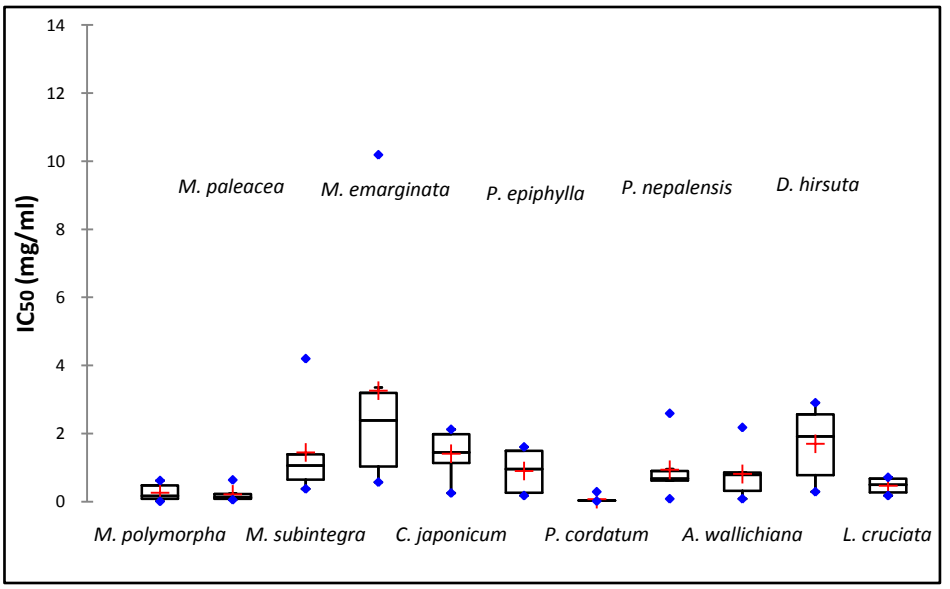


Fig 5.1.2 ABTS radical scavenging activity of studied eleven liverworts. For each sample, mean, standard deviation, maximum and minimum values, outliers are shown.

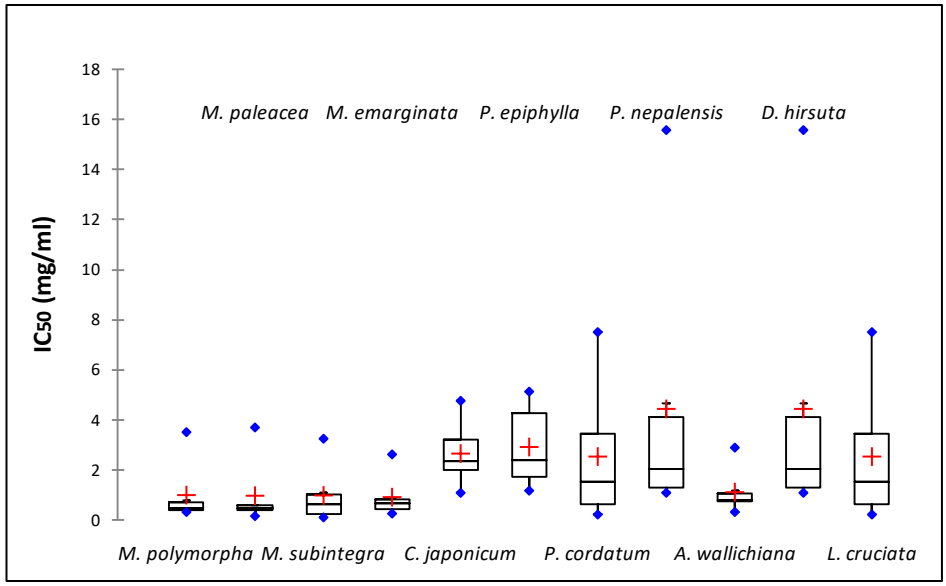


Fig 5.1.3: Super oxide scavenging activity of studied eleven liverworts. For each sample, mean, standard deviation, maximum and minimum values, outliers are shown.

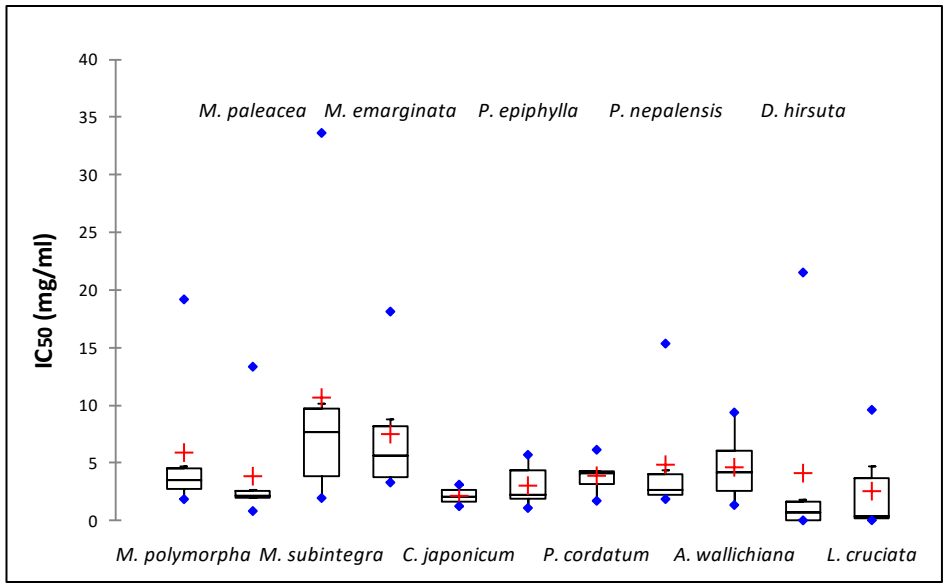


Fig 5.1.4: Nitric oxide scavenging activity of studied eleven liverworts. For each sample, mean, standard deviation, maximum and minimum values, outliers are shown.

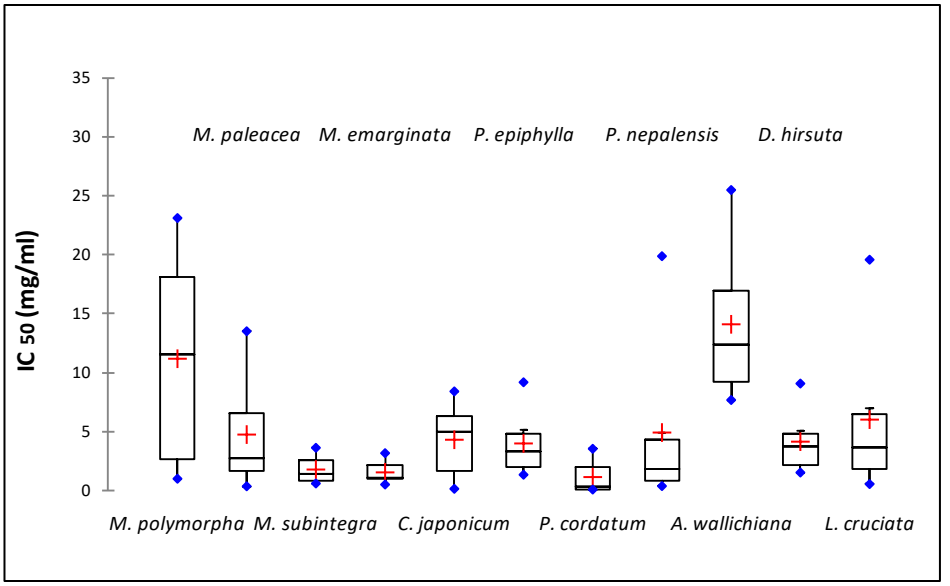


Fig 5.1.5: Metal chelating activity of studied eleven liverworts. For each sample, mean, standard deviation, maximum and minimum values, outliers are shown.

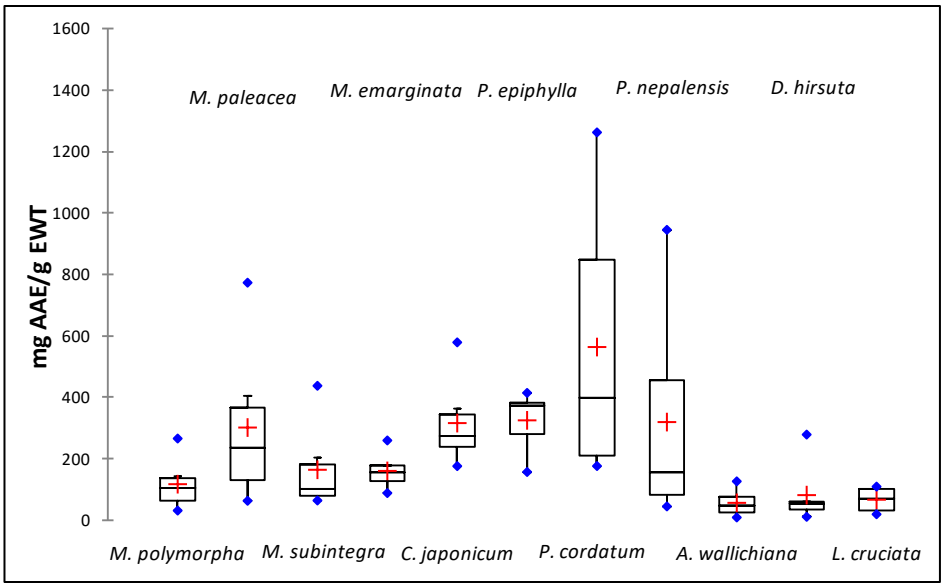


Fig 5.1.6: Reducing power of studied eleven liverworts. For each sample, mean, standard deviation, maximum and minimum values, outliers are shown.

Table 5.1.1: DPPH$^{\cdot}$ scavenging activity of studied liverworts (IC_{50} mg/ml)

Solvents used	*M. polymorpha*	*M. paleacea*	*M. subintegra*	*M. emarginata*	*C. japonicum*	*P. epiphylla*	*P. cordatum*	*P. nepalensis*	*A. wallichiana*	*D. hirsuta*	*L. cruciata*
Heptane	5.38±0.19	34.7±2.08	4.29±0.44	9.35±0.23	17.3±0.32	14.8±0.45	0.87±0.01	0.66±0.01	7.20±0.06	1.60±0.1	2.89±0.23
Diethyl ether	1.13±1.45	0.27±0.01	2.74±0.23	1.24±0.04	2.04±1.52	3.37±0.38	0.10±0.01	0.19±0.03	3.37±0.16	0.90±0.12	1.22±0.09
Ethyl acetate	0.76±0.67	0.62±0.37	1.58±0.04	0.84±0.07	4.30±0.58	1.27±0.07	0.18±0.02	0.27±0.01	3.10±0.36	3.78±0.59	0.84±0.38
acetone	1.62±0.21	1.43±0.07	9.77±0.32	3.98±0.13	9.19±0.88	4.55±0.11	0.06±0.05	0.41±0.13	11.5±0.67	4.41±0.38	3.25±0.36
Butanol	2.63±0.46	0.67±0.03	1.74±0.01	2.63±0.01	4.91±0.05	3.99±0.13	0.26±0.10	0.50±0.03	9.82±0.17	0.51±0.02	3.37±0.16
Methanol	14.73±3.66	1.11±0.05	4.92±0.44	2.82±0.10	13.6±0.7	4.29±0.59	4.34±0.10	1.18±0.11	7.16±0.25	5.96±1.15	3.70±0.58

Table 5. 1.2: $ABTS^{+}$ scavenging activity of studied liverworts (IC_{50} mg/ml)

Solvents used	*M. polymorpha*	*M. paleacea*	*M. subintegra*	*M. emarginata*	*C. japonicum*	*P. epiphylla*	*P. cordatum*	*P. nepalensis*	*A. wallichiana*	*D. hirsuta*	*L. cruciata*
Heptane	0.20±0.04	0.64±0.4	1.41±0.31	10.19±0.25	1.70±0.21	1.41±0.02	0.04±0.01	0.72±0.29	2.18± 0.04	1.40±0.10	0.70±0.08
Diethyl ether	0.57±0.10	0.06±0.01	0.37±0.14	0.69±0.02	0.26±0.03	0.19±0.01	0.02±0.01	0.08±0.02	0.19± 0.03	0.58±0.13	0.37±0.02
Ethyl acetate	0.07±0.04	0.16±0.01	0.59±0.07	0.57±0.03	2.12±0.39	1.61±0.26	0.03±0.01	0.96±0.27	0.09± 0.05	2.44±0.25	0.09±0.03
acetone	0.13±0.02	0.24±0.04	4.2±0.22	3.36±0.26	1.12±0.04	0.50±0.1	0.04±0.02	2.60±0.20	0.72± 0.01	2.61±0.29	0.71±0.01
Butanol	0.01±0.02	0.08±0.01	0.80±0.41	2.72±0.02	1.20±0.01	1.52±0.44	0.02±0.01	0.62±0.38	0.86± 0.04	0.29±0.09	0.24±0.1
Methanol	0.62±0.43	0.10±0.01	1.34±0.77	2.06±1.08	2.08±0.07	0.18±0.03	0.29±0.10	0.62±0.34	0.85± 0.03	2.90±0.21	0.62±0.16

Table5.1.3: Super oxide scavenging activity of studied liverworts (IC_{50} mg/ml)

Solvents used	*M. polymorpha*	*M. paleacea*	*M. subintegra*	*M. emarginata*	*C. japonicum*	*P. epiphylla*	*P. cordatum*	*P. nepalensis*	*A. wallichiana*	*D. hirsuta*	*L. cruciata*
Heptane	0.46±0.16	3.7±0.28	1.12±0.23	0.88±0.09	4.76±0.76	2.55±0.33	7.51±0.05	1.64±0.07	0.72±0.28	1.64±0.07	7.51±0.05
Diethyl ether	0.80±0.18	0.16±0.01	0.11±0.03	0.26±0.17	1.09±0.59	1.18±0.98	0.62±0.04	1.2±0.05	0.82±0.32	1.20±0.05	0.62±0.04
Ethyl acetate	0.32±0.03	0.39±0.26	0.15±0.07	0.38±0.17	1.91±1.09	1.57±0.92	0.64±0.09	2.47±0.02	0.32±0.07	2.47±0.02	0.64±0.09
acetone	0.51±0.33	0.61±0.05	0.81±0.12	0.71±0.09	2.26±0.31	2.24±0.55	0.22±0.06	4.67±0.07	1.16±0.40	4.67±0.07	0.22±0.06
Butanol	0.39±0.13	0.52±0.03	0.48±0.12	0.66±0.03	2.46±0.36	5.13±1.08	2.42±0.06	1.09±0.01	0.78±0.20	1.09±0.01	2.42±0.16
Methanol	3.51±0.17	0.47±0.01	3.25±0.53	2.63±0.33	3.46±0.7	4.86±0.91	3.82±0.28	15.5±0.12	2.90±0.66	15.6±0.12	3.82±0.28

Table 5.1.4: Nitric oxide scavenging activity of studied liverworts (IC_{50} mg/ml)

Solvents used	*M. polymorpha*	*M. paleacea*	*M. subintegra*	*M. emarginata*	*C. japonicum*	*P. epiphylla*	*P. cordatum*	*P. nepalensis*	*A. wallichiana*	*D. hirsuta*	*L. cruciata*
Heptane	1.85±0.6	13.35±1.4	8.62±2.33	6.42±0.3	2.08±0.69	1.89±0.46	3.97±0.73	1.86±0.64	6.09±2.7	0.02±0.01	0.03±0.02
Diethyl ether	4.73±1.6	0.82±0.3	1.95±3.52	4.92±0.1	1.25±0.42	1.09±075	2.87±0.72	2.21±0.21	1.34±0.4	0.03±0.02	0.15±0.69
Ethyl acetate	3.93±0.9	2.68±0.1	2.90±1.78	3.30±0.1	1.48±0.78	1.89±0.19	6.14±0.91	4.31±0.24	2.57±0.9	0.16±0.05	0.37±0.86
acetone	2.65±0.4	2.31±0.8	6.78±2.02	3.43±1.5	2.06±0.45	2.59±0.76	1.70±0.47	2.99±0.44	2.53±0.6	1.78±0.84	0.41±0.09
Butanol	3.03±0.2	1.98±0.6	10.1±0.42	8.79±0.3	3.11±0.16	5.7±0.95	4.31±0.50	2.33±0.12	5.80±1.8	1.18±0.19	9.6±083
Methanol	19.2±1.5	1.93±0.1	33.6±0.16	18.1±0.04	2.88±0.80	5±1.11	4.30±0.38	15.4±1.56	9.37±1.6	21.5±1.42	4.72±0.12

Table 5.1.5: Metal Chelating activity of studied eleven liverworts (IC_{50} mg/ml)

Solvents used	*M. Polymorpha*	*M. paleacea*	*M. subintegra*	*M. emarginata*	*C. japonicum*	*P. epiphylla*	*P. cordatum*	*P. nepalensis*	*A. wallichiana*	*D. hirsuta*	*L. cruciata*
Heptane	1.3±0.06	0.37±0.15	0.61±0.26	0.96±0.1	5.57±1.32	1.35±0.32	0.09±0.02	4.94±0.29	8.42±0.38	1.4±0.02	0.7±0.08
Diethyl ether	23.1±0.82	13.5±0.03	0.94±0.27	1.07±0.06	4.42±0.25	1.7±0.13	0.10±0.02	0.74±0.38	18.2±0.18	0.58±0.03	0.37±0.02
Ethyl acetate	18.6±0.64	2.95±0.44	0.78±0.01	0.52±0.04	8.41±0.11	2.85±0.36	0.16±0.09	1.09±0.45	11.7±0.52	2.44±0.16	0.18±0.04
acetone	16.5±0.65	7.74±0.11	2.83±0.43	2.48±0.06	0.15±0.31	9.19±0.53	0.44±0.01	0.38±0.37	25.5±0.55	2.61±0.18	0.71±0.01
Butanol	1.01±0.01	2.61±0.21	1.95±0.05	1.09±0.04	6.55±0.29	3.74±0.47	2.57±0.13	2.52±0.13	13.2±0.44	0.29±0.1	0.24±0.10
Methanol	6.59±1.3	1.34±0.24	3.63±1.16	3.18±0.30	0.79±0.43	5.18±0.64	3.55±0.43	19.8±0.45	7.68±0.03	2.9±0.02	0.62±0.16

Table 5.1.6: Reducing potential of studied eleven liverworts (mg ascorbic acid equivalent/ g extractive weight)

Solvents used	*M. polymorpha*	*M. paleacea*	*M. subintegra*	*M. emarginata*	*C. japonicum*	*P. epiphylla*	*P. cordatum*	*P. nepalensis*	*A. wallichiana*	*D. hirsuta*	*L. cruciata*
Heptane	98.9±17.1	63.2±17.2	92.3±3.03	175±30	176±18.6	383±22.7	215±0.08	169±0.87	54.2±15.9	61.3±9.2	88.9±14.5
Diethyl ether	144±16.5	773±8.3	437±93.1	134±29.9	578±65.6	414±20.5	1261±0.54	944±30.8	85.5±15.3	278±19.8	104±5.7
Ethyl acetate	265±23.3	242±27.1	203±7.13	259±10.3	233±27.7	251±40.1	210±0.07	550±47.1	126±25.3	55.6±14.5	109±16.4
acetone	113±0.48	226±7.45	64±15.8	125±15.7	363±15.7	382±33.4	936±0.02	44.9±15.6	42.5±21.0	28.9±10.1	53.5±5.8
Butanol	50.5±0.1	406±16.7	109±14.1	178±11.1	258±2.56	156±7.11	581±0.04	142±3.67	8.97±4.79	54.6±0.08	19.4±4.13
Methanol	31.6±7.9	98.6±7.57	77.4±10.3	88.4±7.5	287±21.4	360±39.3	176±0.07	64.9±12.5	21.7±16.2	10.9±0.82	24.8±3.13

5.2 ANTI-DIABETIC ACTIVITY

The α-glucosidase and α-amylase enzymes inhibitory activity was investigated and the results were presented in Figure 5.2.1 to 5.2.11. Studied liverworts inhibited the activity of α-glucosidase enzyme significantly; their IC_{50} value ranged between 0.09 mg/ml and 64.82 mg/ml and α- amylase inhibitory activity ranged between 0.058 mg/ml to 30.07 mg/ml (IC_{50} value). Diethyl ether extract of *Marchantia subintegra* showed the highest α-glucosidase enzyme inhibitory activity (IC_{50} value 0.09 mg/ml), the activity was almost similar to and α-amylase inhibitory activity of standard Metformin (0.17 mg/ml). Similarly, diethyl ether extract of *Marchantia subintegra* also showed highest α-amylase enzyme inhibitory activity (IC_{50} value 0.058 mg/mg). Variations in the α- amylase and α- glucosidase inhibitory activity was seen within different solvent extracts of a same species. Among six solvents used for extraction, diethyl ether and ethyl acetate extracts of *Marchantia polymorpha*, *M. paleacea*, *M. subintegra*, *M. Subintegra*, *Pellia epiphylla*, *Plagiochasma cordatum*, *Asteralla wallichiana* and *Dumortiera hirsuta* showed the highest α. -glucosidase and α- amylase inhibitory activities. In *Conocephalum japonicum* and *Lunularia cruciata*, acetone extract and in *Plagiochila nepalensis* heptane extract showed the highest α-glucosidase and α-amylase inhibitory activity.

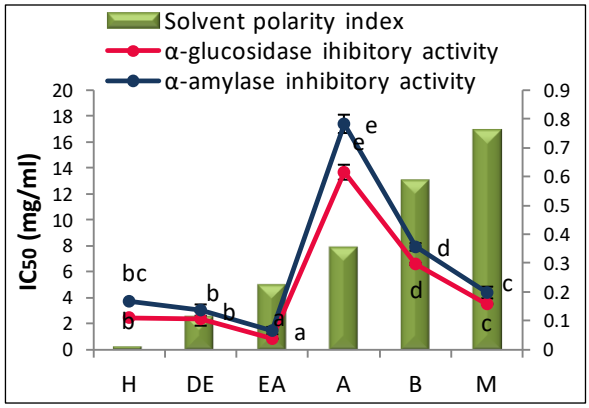


Fig 5.2.1: Anti-diabetic activity of *Asterella wallichiana*

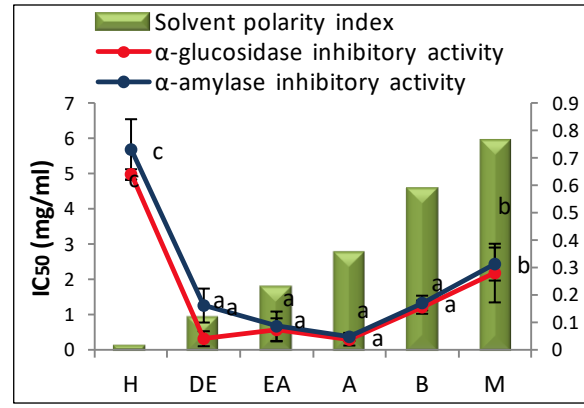


Fig 5.2.2: Anti-diabetic activity of *Conocephalum japonicum*

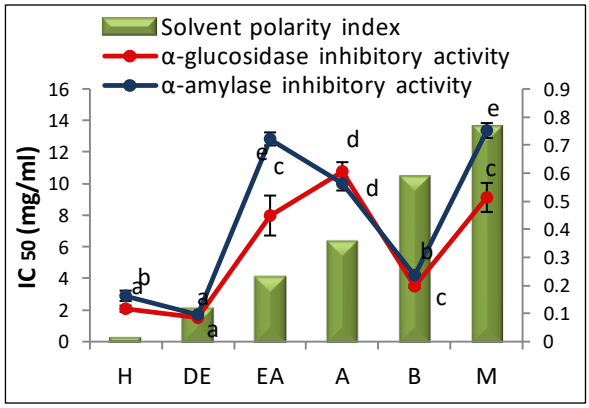


Fig 5.2.3: Anti-diabetic activity of *Dumortiera hirsuta*

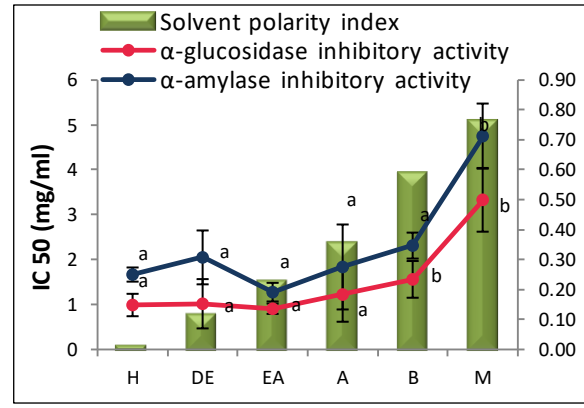


Fig 5.2.4: Anti-diabetic activity of *Pellia epiphylla*

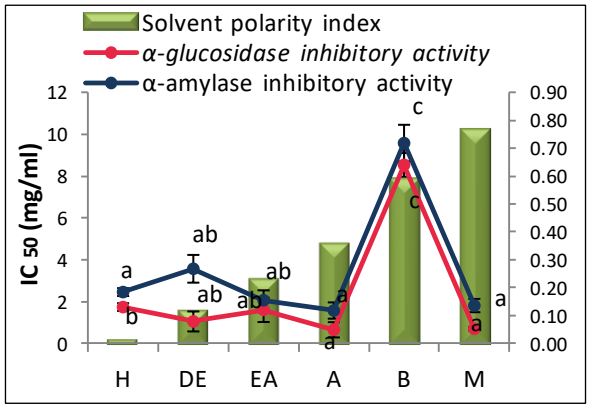


Fig 5.2.5: Anti-diabetic activity of *Lunularia cruciata*

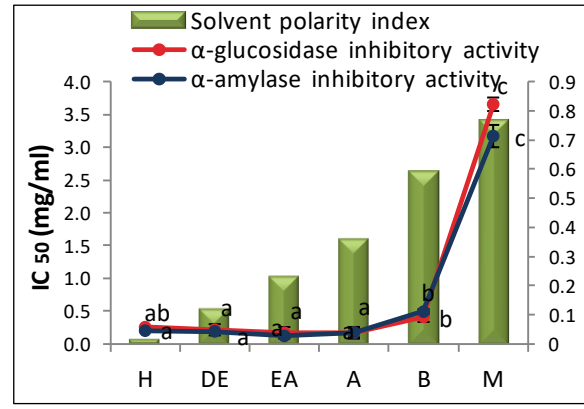


Fig 5.2.6: Anti-diabetic activity of *Marchantia emarginata*

NB: H= heptane, DE=diethyl ether, EA= ethyl acetate, A= acetone, B=butanol, M=methanol

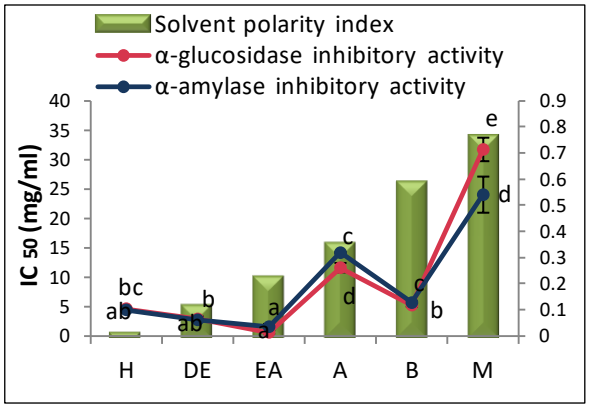


Fig 5.2.7: Anti-diabetic activity of *Marchantia polymorpha*

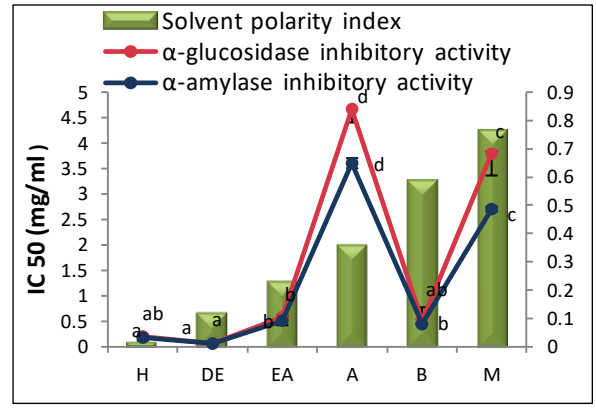


Fig 5.2.8: Anti-diabetic activity of *Marchantia subintegra*

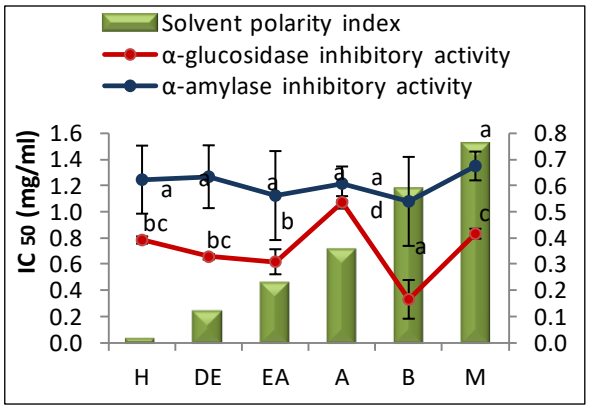


Fig 5.2.9: Anti-diabetic activity of *Plagiochasma cordatum*

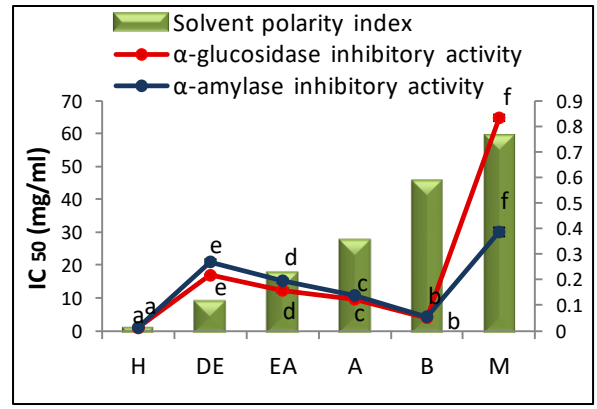


Fig 5.2.10: Anti-diabetic activity of *Plagiochila nepalensis*

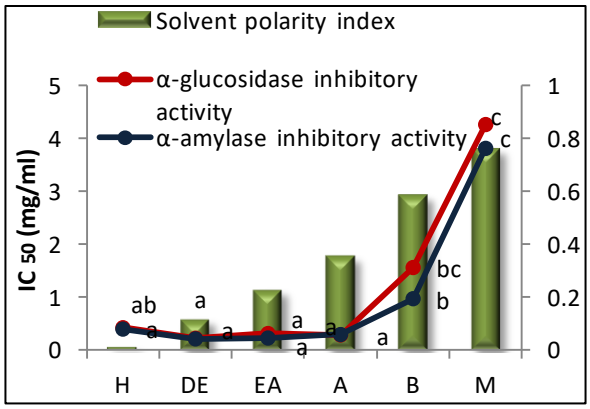


Fig 5.2.11: Anti-diabetic activity of *Marchantia paleacea*

NB: H= heptane, DE=diethyl ether, EA= ethyl acetate, A= acetone, B=butanol, M=methanol

5.3 *IN VITRO* CYTOTOXICITY SCREENING

In this study, we screened the extracts of five species of liverworts: *Plagiochasma cordatum, Asterella wallichiana, Lunularia cruciata Marchantia paleacea* and *Plagiochila nepalensis* against human kidney cancer cell line (ACHN). Perusal of literature revealed that liverwort under study has not been included previously in a bio prospecting program for anti-cancer activity. The cytotoxic effect of extracts on human kidney cancer cell line was studied by MTT assay. The effect of plant extract on viability of cell was studied by calculating the percentage of MTT reduction on incubation of ACHN cells with increasing concentration of extract. Extracts caused a dose dependent reduction in cell viability. Extract concentration range 100 µg/ ml to 500 µg/ ml were used. The calculation of cell viability percentage showed that in present work *Plagiochasma cordatum* had the highest potential to induce maximum cell death at a minimum concentration of 100 µg/ ml. Dose response studies of studied liverwort extracts are presented in figures 5.3.1 to 5.3.5. Studied liverworts showed up to 73 % cell death of human kidney cancer cell line. The IC_{50} value of anti-proliferative activity of liverwort under analysis ranged between 69.15 µg/ml to 308.98 µg/ml. Atjanasuppat *et al*. (2009) categorized the anti-proliferative activities of the extracts into four groups: ≤ 20 µg/mL, active; >20–100 µg/mL, moderately active; >100–1000 µg/mL, weakly active; and >1000 µg/mL, inactive. Based on the report, IC_{50} values of the studied liverworts falls under range of extracts having anti-proliferative activity. The lowest IC_{50} value was shown by *Plagiochasma cordatum* which indicated that this liverwort is most active in inducing death of human kidney cancer cell line than other studied liverworts (Fig 5.3.6).

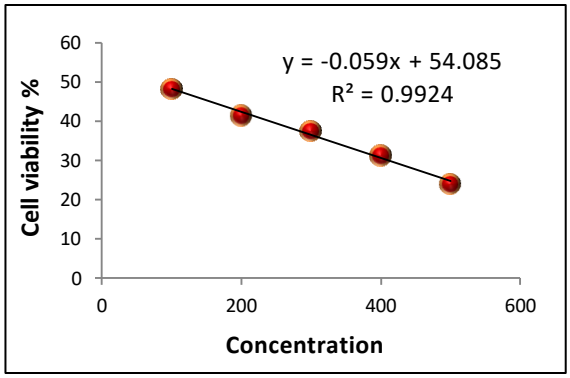


Fig 5.3.1: Inhibitory activity of *Plagiochasma cordatum* on ACHN cell proliferation.

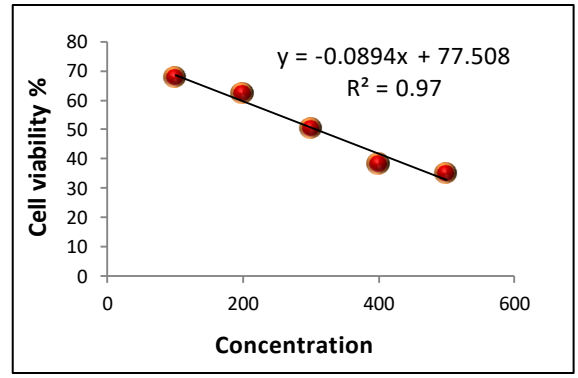


Fig 5.3.2: Inhibitory activity of *Asterella wallichiana* on ACHN cell proliferation.

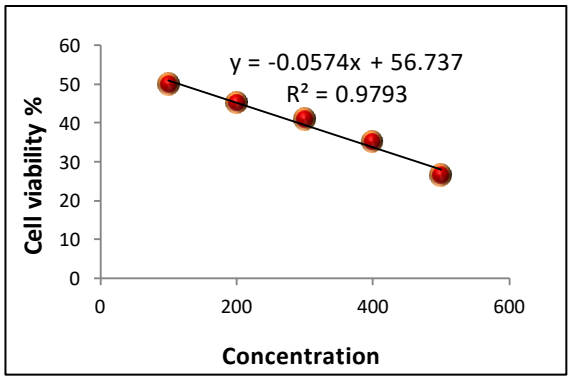


Fig 5.3.3: Inhibitory activity of *Plagiochila nepalensis* on ACHN cell proliferation.

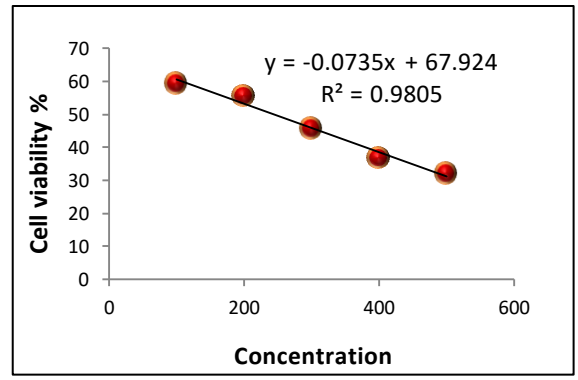


Fig 5.3.4: Inhibitory activity of *Lunularia cruciata* on ACHN cell proliferation.

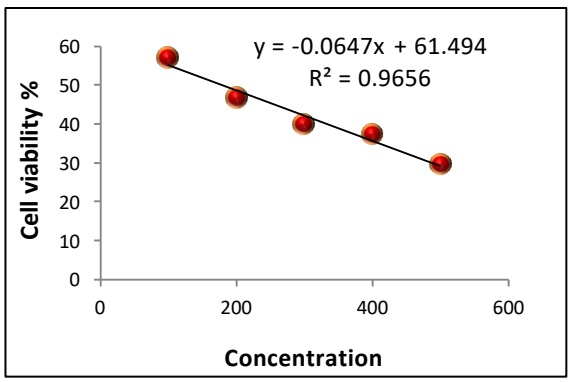


Fig 5.3.5: Inhibitory activity of *Marchantia paleacea* on ACHN cell proliferation.

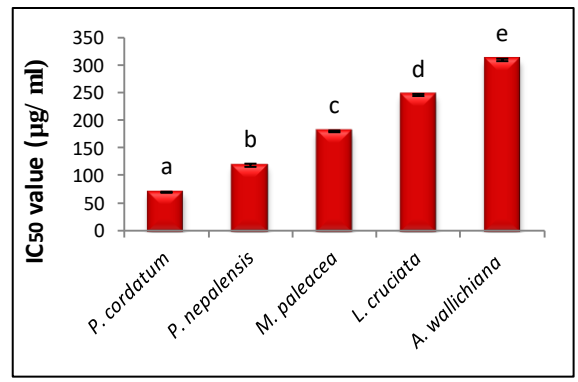


Fig 5.3.6: IC_{50} values of anti-proliferative activity studied liverworts against ACHN cells. Value with different letters (a, b, c, d etc.) differ significantly ($p < 0.05$) from each other by Duncan's Multiple Test (DMRT)

5.4 PHYTOCHEMICAL ANALYSIS

5.4.1 Quantitative estimation of phytochemicals

Quantitative estimation of phenols, flavonoids, orthodihydric phenol, steroid and tannin content was done. Studied liverworts showed large variation in the content of phenol which ranged between 4.8 mg Gallic acid equivalent / g extractive weight to 377.9 mg GAE/g EW (Fig 5.4.1). Highest phenol content was recorded in diethyl ether extract of *Marchantia emarginata* and lowest content of phenol was recorded in methanolic extract of *Dumortiera hirsuta*. Significant effects of solvent's polarity were observed on extraction of phenolic compounds. It was found that diethyl ether extract of all studied liverworts except *Marchantia polymorpha* and *Asterella wallichiana* contained the highest amount of phenols. Whereas in case of *Marchantia polymorpha* butanolic extract and in *Asterella wallichiana*, heptane extract showed highest phenol content. Flavonoid content of the studied plants ranged between 0.3 mg Quercetin equivalent/ g extractive weight and 94.2 mg Quercetin equivalent/ g extractive weight. *Plagiochasma cordatum* showed highest flavonoid content among studied liverworts (Fig 5.4.2).

Orthodihydric phenol content in the liverworts ranged between 0.34 mg catechol equivalent / g extractive weight and 47.20 mg catechol equivalent / g extractive weight (Fig 5.4.3). In *Dumortiera hirsuta* the presence of highest amount of orthdihydric phenol content was found. Presence of steroid was also detected in the studied liverworts which ranged between 0.002 mg solasodine equivalent/ g extractive weight and 1.64 mg solasodine equivalent/ g extractive weight (Fig 5.4.4). Tannin content of the plant ranged between 0.02 mg Tannic acid equivalent / g extractive weight and 2.56 mg Tannic acid equivalent / g extractive weight. Highest tannin content was recorded in methanolic extract of *Asterella wallichiana* (Fig 5.4.5).

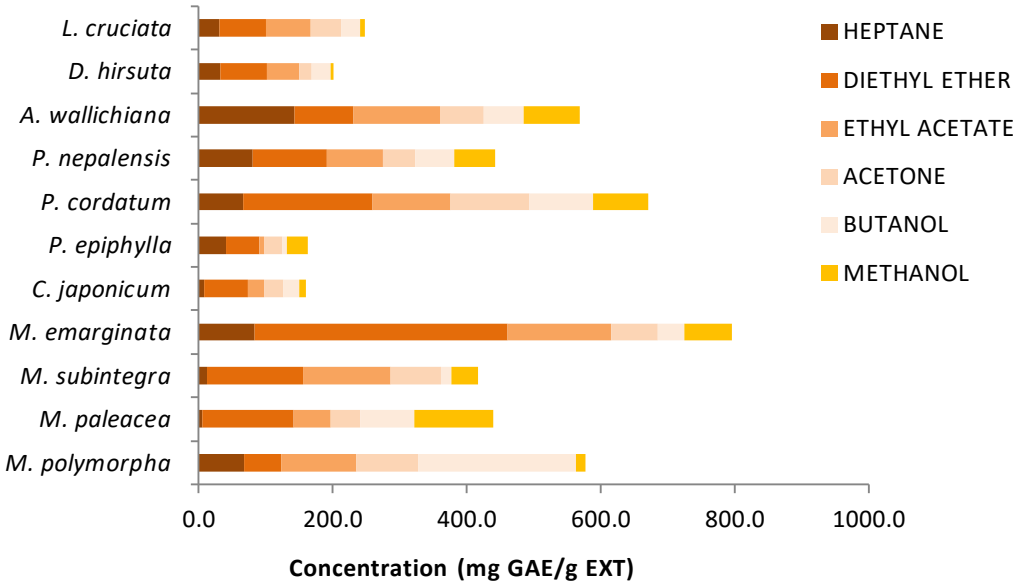


Fig 5.4.1: Phenol content of studied eleven liverwort species in different solvent extracts

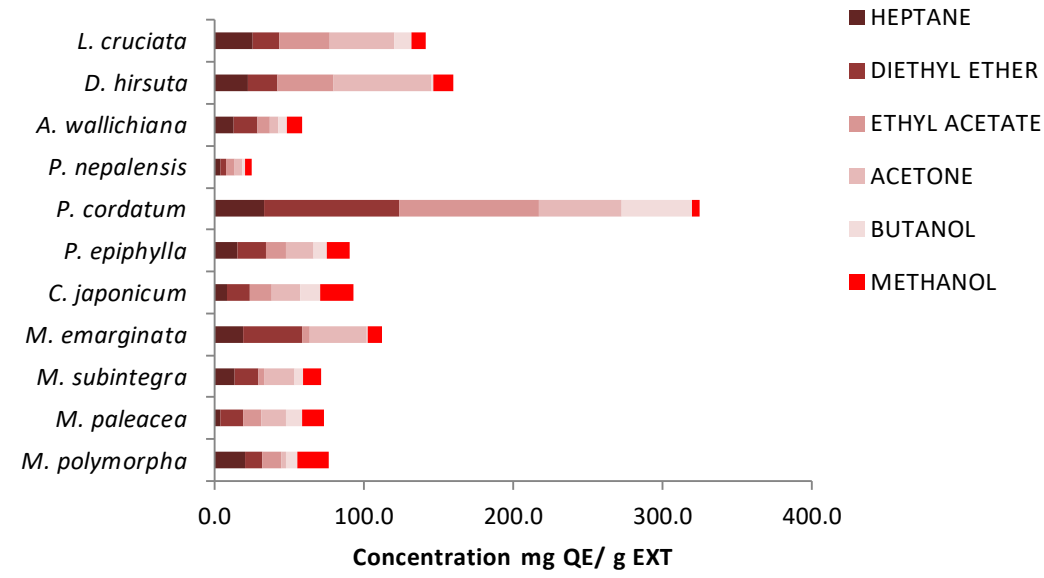


Fig 5.4.2: Flavonoid content of studied eleven liverwort species in different solvent extracts

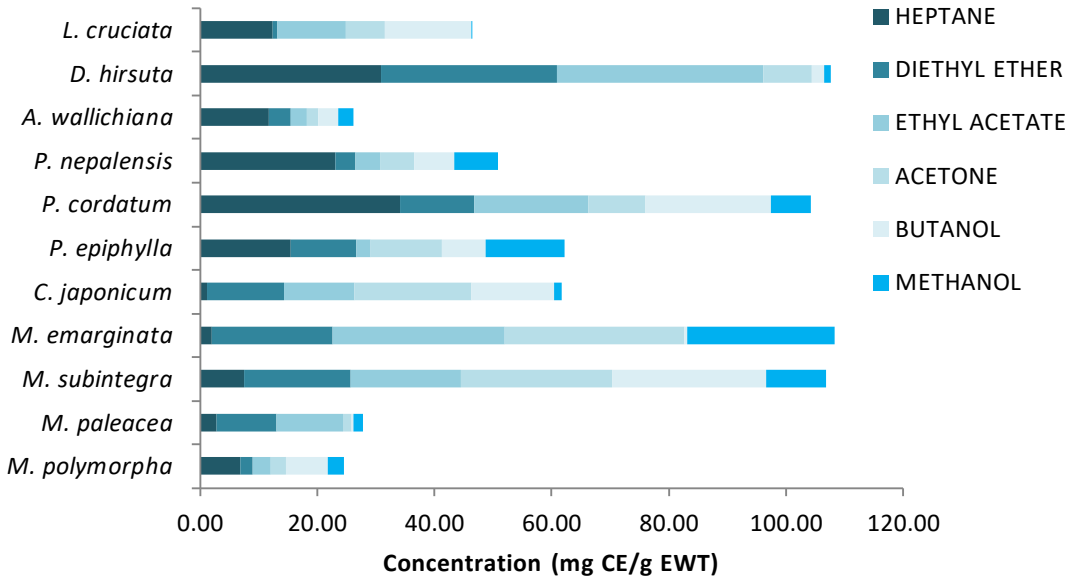


Fig 5.4.3: Ortho-dihydric phenol content of studied eleven liverwort species in different solvent extracts

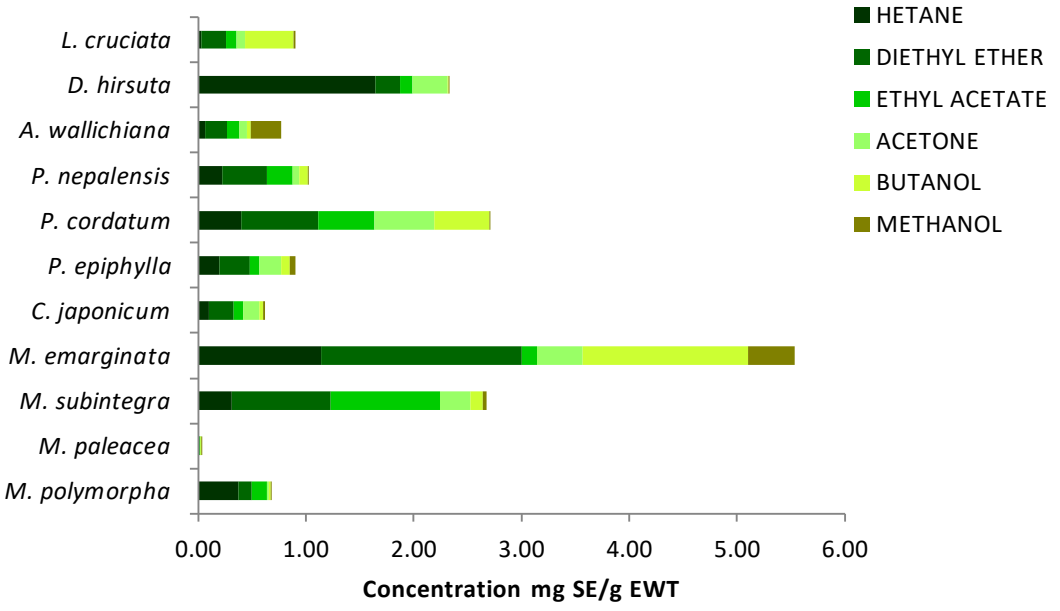


Fig 5.4.4: Steroid content of studied eleven liverwort species in different solvent extracts

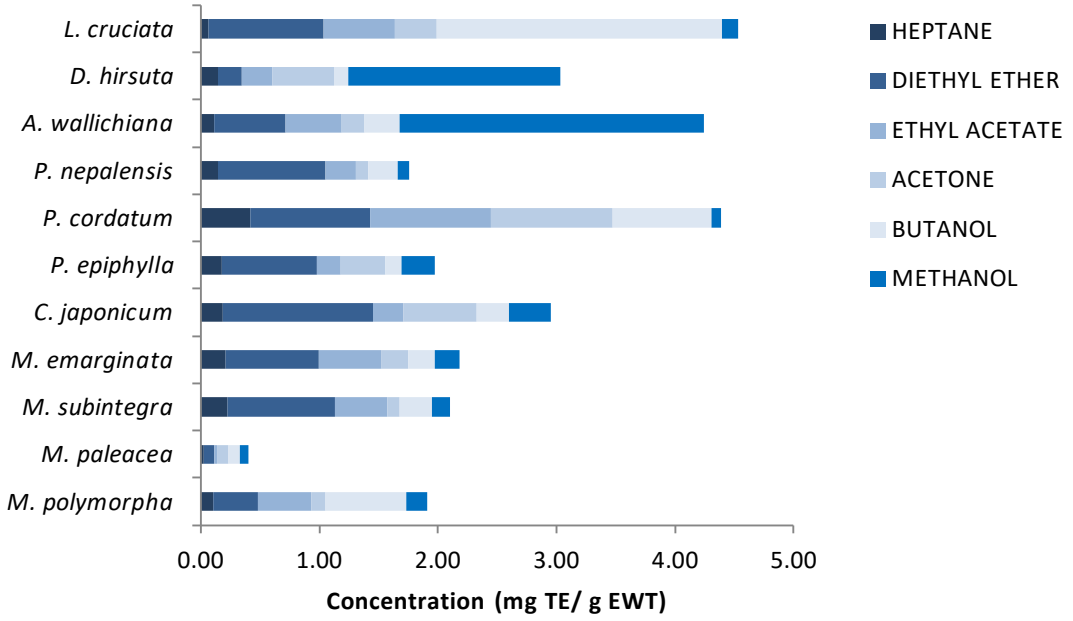


Fig 5.4.5: Tannin content of studied eleven liverwort species in different solvent extracts

5.4.2 Qualitative phytochemical analysis

Presence of phytochemicals like steroid (ST), tannin (TAN), triterpenoid (TT), amino acid (AA), resin (RES), cardiac glycoside (CG), alkaloid (ALK), flavonoid (FLA), reducing sugar (RS), anthraglycoside (ANT) and glycoside (GLY) were detected by qualitative phytochemical analysis (Table 5.2.1 -5.2.11).

5.4.3 Thin layer Chromatography (TLC)

Thin layer chromatography analysis has shown the presence of phytochemicals like steroid, tannin, triterpenoids, cardiac glycoside, flavonoids, resin, reducing sugar, amino acid, glycoside, anthraglycoside, arbutin, phenol, bitter principle and coumarin in studied liverworts.

5.5 CORRELATION BETWEEN PHYTOCHEMICAL CONTENT AND PHARMACOLOGICAL ACTIVITIES

5.5.1 Pearson Correlation Analysis

Pearson Correlation analysis was performed to evaluate how the pharmacological activities and phytochemical content of studied liverworts were correlated with each other (Fig 5.5.1). Result showed a negative correlation between total phenol content of the plant and the IC_{50} value of DPPH˙ and superoxide (SO) scavenging assay. This indicated that the phenolic compounds that are present in the plants actively participated in the reduction of DPPH radical and SO ions. Reducing power (RP) of the plant was positively correlated with total phenol and flavonoids content. Ferric reducing activity is considered equivalent to free radical scavenging potential of plant. Positive correlation between ferric reducing ability and phenolic content suggests that phenolic compounds are main free radical scavengers that are produced by plants as secondary metabolites. In Pearson Correlation analysis, another important class of phenolic compound, the orthodihydric phenol was found to be negatively correlated with IC_{50} value of metal chelating activity and the α-amylase (α-A) inhibitory activity. Thus, it can be interpreted that orthodihydric phenol was main phytochemical that are responsible for metal chelating activity displayed by liverworts. Data also suggested that anti-diabetic activity of the plants was mainly contributed by the orthodihydric phenol present in the liverworts.

- Absent + Low ++ High

Table 5.2.1: Phytochemical profile of *Plagiochasma cordatum*

	ST	TAN	TT	AA	RES	CG	ALK	FLA	RS	ANT	GLY
H	+ +	+	+ +	-	-	+ +	+	-	+ +	-	+
DE	+ +	+	+ +	-	+	+ +	+	-	+ +	-	+
EA	+ +	+	+ +	-	-	+ +	+	+	+ +	-	+
A	+	+	-	-	-	+ +	+	-	+ +	-	+
B	+ +	+	+ +	-	+	+ +	+	+	+ +	-	+
M	+ +	+ +	+	-	+	-	-	-	-	-	+ +

Table 5.2.2: Phytochemical profile of *Plagiochila nepalensis*

	ST	TAN	TT	AA	RES	CG	ALK	FLA	RS	ANT	GLY
H	+ +	+	+	-	+	+	-	-	-	-	+
DE	+ +	+	+	-	+	+	-	-	+	-	+
EA	+ +	+	-	-	-	+ +	-	-	+	-	+
A	+ +	+	+	-	-	+	-	-	+	-	+
B	+	+	+	-	-	-	-	-	+	-	+
M	+ +	+ +	+	+	+	-	-	-	+	-	+ +

Table 5.2.3: Phytochemical profile of *Marchantia polymorpha*

	ST	TAN	TT	AA	RES	CG	ALK	FLA	RS	ANT	GLY
H	+ +	+	+	-	-	+ +	-	-	+	-	+
DE	+ +	+ +	+ +	-	-	+ +	-	-	v+	-	+
EA	+ +	+ +	+ +	-	+	+ +	+	+	+	-	+
A	+	+ +	-	-	+	-	-	-	+	-	+
B	+	+ +	+ +	-	-	-	-	-	+	-	+
M	+ +	+ +	+	-	-	-	-	-	+	-	+ +

NB: ST= steroid, Tan= tannin, TT= triterpenoid, AA=amino acid, RES=resine, CG= cardiac glycoside, ALK=alkaloid, FLA= flavonoid, RS= reducing sugar, ANT=anthraglycoside, GLY= glycoside

\- Absent + Low ++ High

Table 5.2.4: Phytochemical profile of *Asterella wallichiana*

	ST	TAN	TT	AA	RES	CG	ALK	FLA	RS	ANT	GLY
H	+ +	+	+ +	-	-	+ +	-	-	+	-	+
DE	+ +	+	+	-	+	+ +	-	-	+	-	+
EA	+ +	+	+ +	+	+	+ +	+	-	+	-	+
A	+ +	+	+	+	-	+	-	-	+	+	+
B	+	+	+	-	-	-	-	-	+	-	+
M	+ +	+ +	+ +		-	-	-	-	+	-	+ +

Table 5.2.5: Phytochemical profile of *Pellia epiphylla*

	ST	TAN	TT	AA	RES	CG	ALK	FLA	RS	ANT	GLY
H	+ +	+	+	-	-	+ +	-	-	-	-	+
DE	+	+	+	-	+	+ +	-	+	+ +	-	+
EA	+ +	+	+	-	-	+	-	+	+	-	+
A	+	+	+	-	+	+	-	+	+	-	+
B	+ +	+	-	-	+	-	-	-	+	-	+
M	+ +	+ +	+	-	+	+	-	+	+	-	+ +

Table 5.2.6: Phytochemical profile of *Marchantia subintegra*

	ST	TAN	TT	AA	RES	CG	ALK	FLA	RS	ANT	GLY
H	+	+	+	-	-	+	-	-	+ +	-	+
DE	+	+	+	-	+	+	-	+	+	-	+
EA	+	+	+	+	-	-	-	-	+	-	+
A	+	+	-	+	-	-	-	+	+	-	+
B	+	+ +	+	-	+	-	-	-	+	-	+
M	+	+ +	+	+	+	+	-	+	+ +	-	+ +

NB: ST= steroid, Tan= tannin, TT= triterpenoid, AA=amino acid, RES=resine, CG= cardiac glycoside, ALK=alkaloid, FLA= flavonoid, RS= reducing sugar, ANT=anthraglycoside, GLY= glycoside

\- Absent + Low ++ High

Table 5.2.7: Phytochemical profile of *Dumortiera hirsuta*

	ST	TAN	TT	AA	RES	CG	ALK	FLA	RS	ANT	GLY
H	+ +	+	+	-	+	+ +	-	-	+	-	+ +
DE	+ +	+	+	-	+ +	+ +	-	+	+	-	+ +
EA	+ +	+	+	+	+ +	+ +	-	-	+	-	+
A	+ +	+	-	+	-	-	-	-	+	-	+
B	+	+	-	-	+	-	-	-	+	-	+
M	+ +	+ +	-	+	-	-	-	-	+	+	+ +

Table 5.2.8: Phytochemical profile of *Lunularia cruciata*

	ST	TAN	TT	AA	RES	CG	ALK	FLA	RS	ANT	GLY
H	+	+	-	-	-	+	-	-	+	-	+
DE	+	+	+	-	+	+ +	-	+	+	-	+
EA	+ +	+ +	+ +	+	+	+ +	+	+	+	-	+
A	+ +	+	+	-	-	+ +	-	+	+	-	+
B	+	+	-	-	+	+ +	+	-	+	-	+
M	+ +	+ +	-	-	-	-	-	-	+	-	+ +

Table 5.2.9: Phytochemical profile of *Conocephalum japonicum*

	ST	TAN	TT	AA	RES	CG	ALK	FLA	RS	ANT	GLY
H	+ +	+	+	-	-	+ +	-	-	-	-	+
DE	+ +	+	+	-	+	+ +	-	-	-	-	+
EA	+ +	+	+	-	+	+ +	-	-	-	-	+
A	+	+	-	-	-	-	-	-	-	-	+
B	+	+	-	-	-	-	-	-	-	-	+
M	+	+	-	-	-	-	-	-	-	-	+ +

NB: ST= steroid, Tan= tannin, TT= triterpenoid, AA=amino acid, RES=resine, CG= cardiac glycoside, ALK=alkaloid, FLA= flavonoid, RS= reducing sugar, ANT=anthraglycoside, GLY= glycoside

-	+	++
Absent	Low	High

Table 5.2.10: Phytochemical profile of *Marchantia emarginata*

	ST	TAN	+TT	AA	RES	CG	ALK	FLA	RS	ANT	GLY
H	+	+	+	-	-	+	-	-	-	-	+
DE	+	+	+	-	-	+	-	+	-	-	+
EA	+ +	+	+	-	-	+	-	-	-	-	+
A	+	+	-	-	-	-	-	-	-	-	+
B	+	+	-	-	-	-	-	-	-	-	+
M	+	+ +	-	-	+	-	+	-	-	-	+ +

Table 5.2.11: Phytochemical profile of *Marchantia paleacea*

	ST	TAN	TT	AA	RES	CG	ALK	FLA	RS	ANT	GLY
H	-	+	+ +	+	-	+ +	-	-	+ +	-	+
DE	-	+	+ +	+	+	+ +	-	+	+ +	-	+ +
EA	+	+ +	+ +	+	+ +	+ +	-	+ +	+ +	-	+ +
A	+ +	+ +	+ +	+	+	+ +	-	+ +	+ +	-	-
B	+ +	+ +	+	+	+ +	+ +	-	+ +	+ +	-	+ +
M	+ +	+ +	+	+	+ +	+ +	-	+ +	+ +	-	+ +

NB: ST= steroid, Tan= tannin, TT= triterpenoid, AA=amino acid, RES=resine, CG= cardiac glycoside, ALK=alkaloid, FLA= flavonoid, RS= reducing sugar, ANT=anthraglycoside, GLY= glycoside

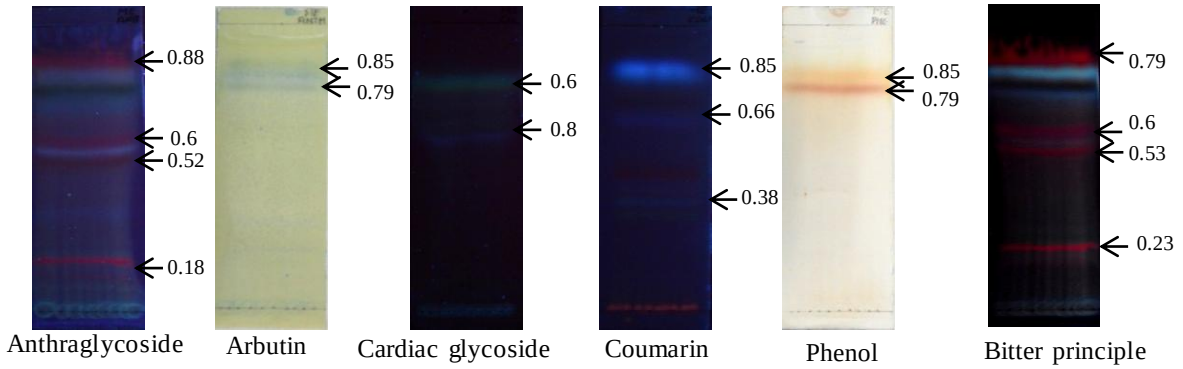


Fig 5.4.6.1: Detection of phytochemicals in *Marchantia emarginata* by TLC analysis

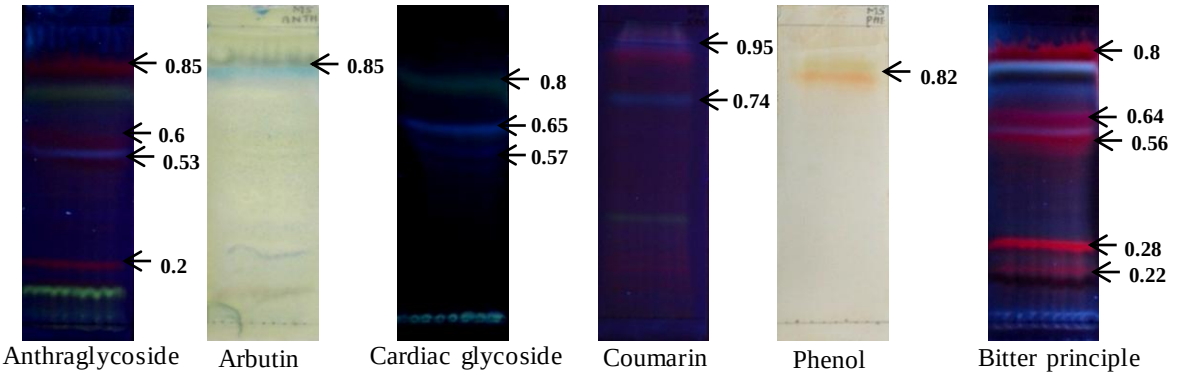


Fig 5.4.6.2 : Detection of phytochemicals in *Marchantia subintegra* by TLC analysis

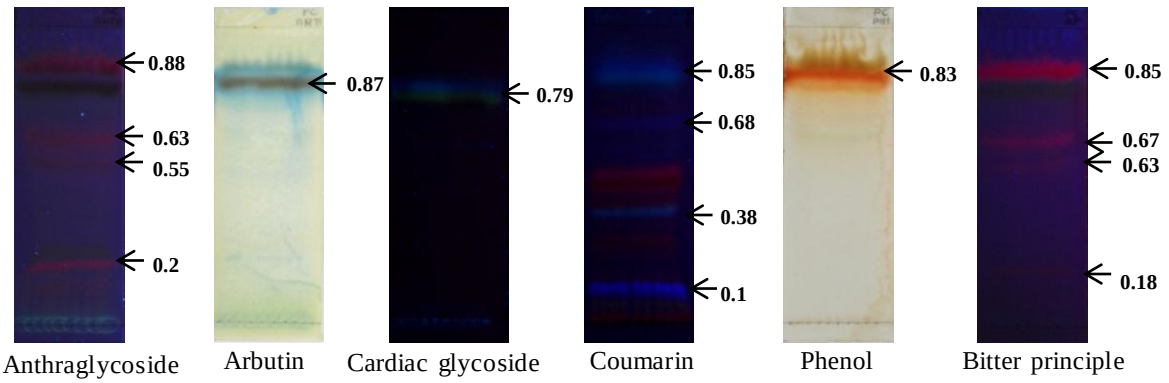
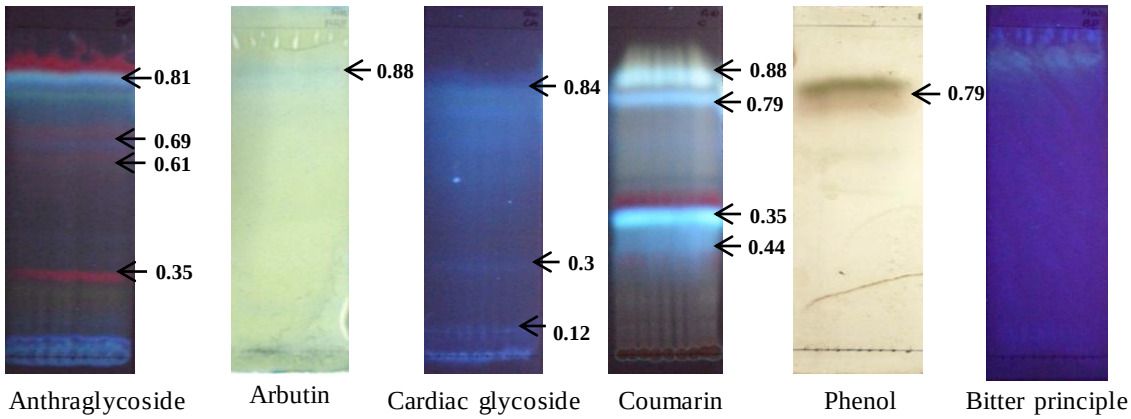
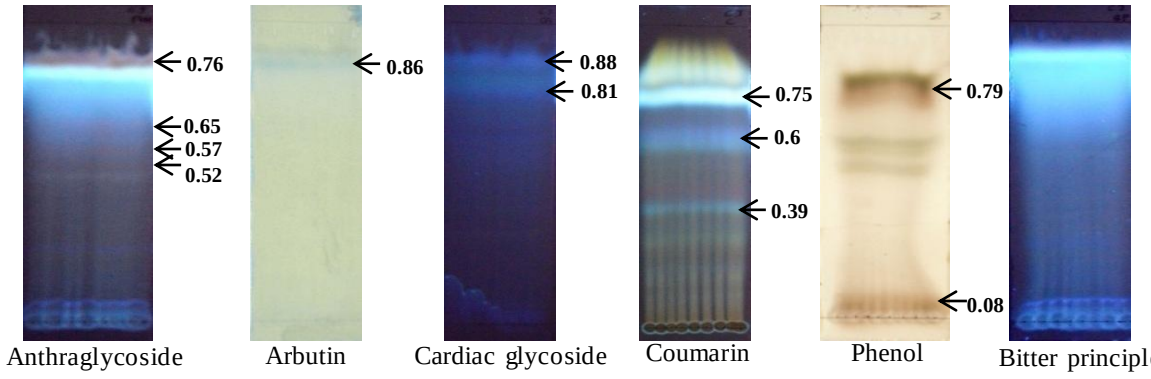


Fig 5.4.6.3 : Detection of phytochemicals in *Plagiochasma cordatum* by TLC analysis

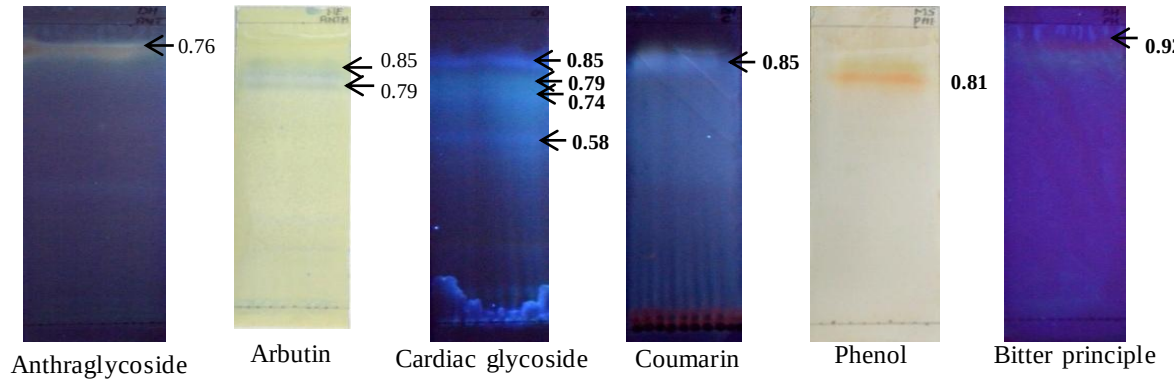
Phytochemical	***Identifying band colour***
Anthraglycoside	red band (UV-365)
Arbutin	blue band (Visible)
Cardiac glycoside	blue band(UV-365)
Coumarin	light blue band (UV-365)
Phenol	brown band (visible)
Bitter principle	red band (UV-365)



5.4.6.4 : Detection of phytochemicals in *Asterella wallichiana* by TLC analysis

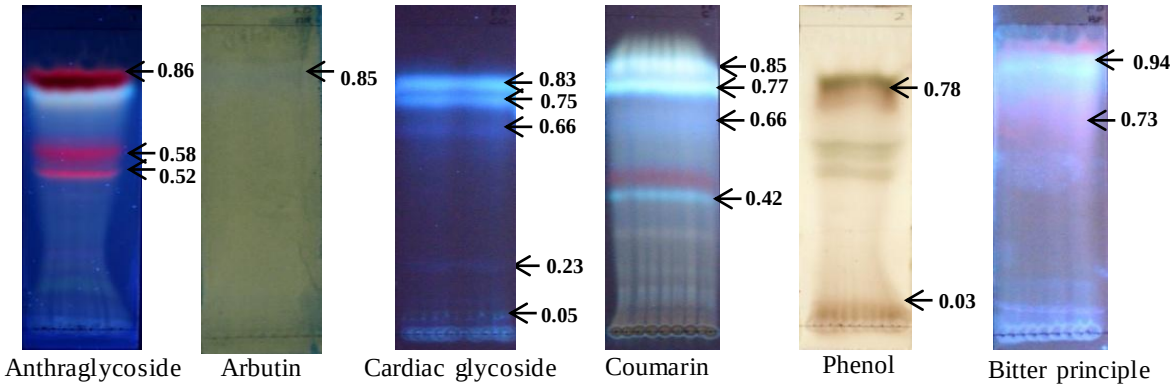


5.4.6.5 : Detection of phytochemicals in *Conocephalum japonicum* by TLC analysis

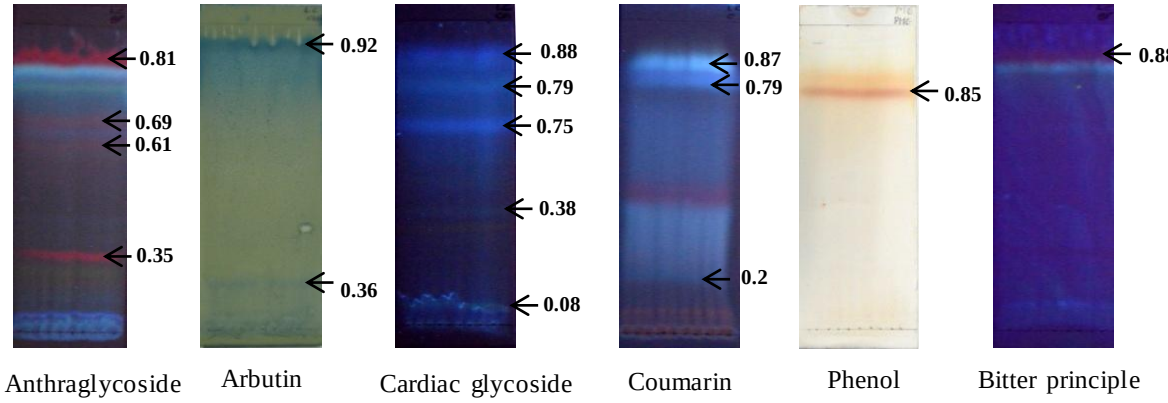


5.4.6.6 : Detection of phytochemicals in *Dumortiera hirsuta* by TLC analysis

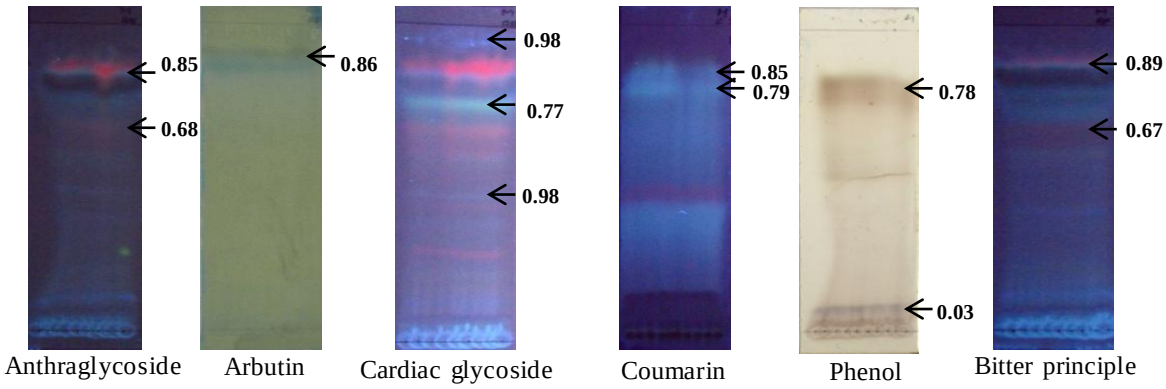
Phytochemical	***Identifying band colour***
Anthraglycoside	red band (UV-365)
Arbutin	blue band (Visible)
Cardiac glycoside	blue band(UV-365)
Coumarin	light blue band(UV-365)
Phenol	brown band(UV-365)
Bitter principle	red band(UV-365)



5.4.6.7: Detection of phytochemicals in *Pellia epiphylla* by TLC analysis

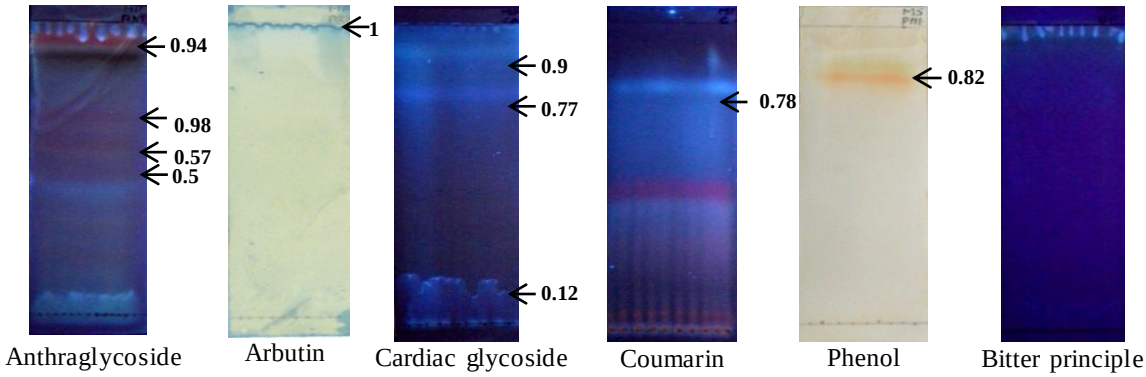


5.4.6.8: Detection of phytochemicals in *Lunularia cruciata* by TLC analysis

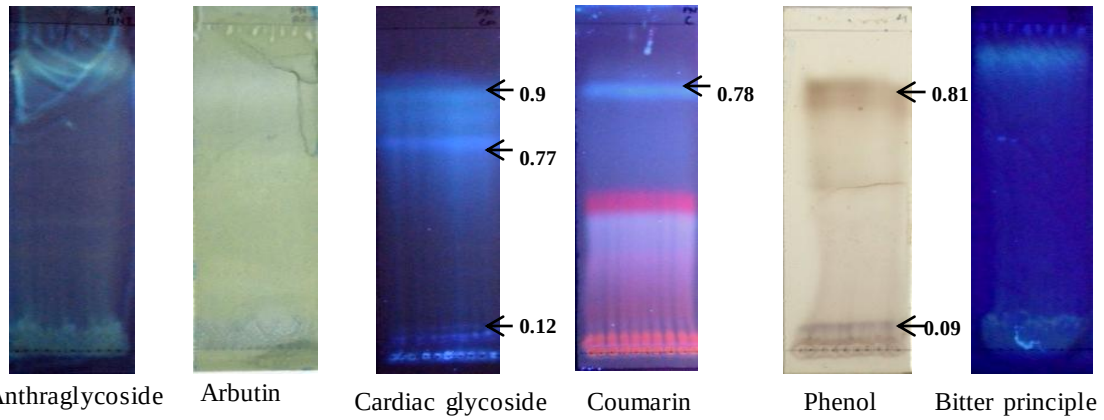


5.4.6.9: Detection of phytochemicals in *Marchantia polymorpha* by TLC analysis

Phytochemical	***Identifying band colour***
Anthraglycoside	red band (UV-365)
Arbutin	blue band (Visible)
Cardiac glycoside	blue band(UV-365)
Coumarin	light blue band(UV-365)
Phenol	brown band(UV-365)
Bitter principle	red band(UV-365)



5.4.6.10: Detection of phytochemicals in *Marchantia paleacea* by TLC analysis



5.4.6.11: Detection of phytochemicals in *Plagiochila nepalensis* by TLC analysis

Phytochemical	***Identifying band colour***
Anthraglycoside	red band (UV-365)
Arbutin	blue band (Visible)
Cardiac glycoside	blue band(UV-365)
Coumarin	light blue band(UV-365)
Phenol	brown band(UV-365)
Bitter principle	red band(UV-365)

Steroid content of the liverworts also found to be involved in metal chelating activity as suggested by the negative correlation between metal chelating activity and steroid content in Pearson Correlation analysis.

5.5.2 Principal Component Analysis

Multivariate analysis like principal component analysis helps to summarize the variability of complex set of data and translates it into most interpretable form. The PCA analysis of phytochemical content and pharmacological activities of the studied liverworts were carried out. The loading of the first principal component (PC1) accounted for 19.84% of the variance and second principal components (PC2) accounted for 13.59% of the variance (Fig 5.5.2). Analyzed parameters were grouped into three clusters (A, B and C). By the loadings of PC1, phytochemicals like phenol, flavonoids, alkaloid, cardiac glycoside were clustered together in group A. Ferric ion reducing activity was also clustered along with other phytochemicals in group A. By PC1, phytochemicals and reducing power (Group A) of the plants were separated from the antioxidant and anti-diabetic activities of plants (Group C). This indicated negative correlation between the phytochemicals of Group A and IC_{50} value of different antioxidant and anti-diabetic assays suggesting phytochemicals like phenols, flavonoids, alkaloid, cardiac glycoside present in the studied plants to be responsible for free radical scavenging and α- amylase, α- glucosidase enzyme inhibiting activities. Negative correlation between FRAP assay and biological activities suggested antioxidant and anti-diabetic activities increased with the increase of metal ion reducing potential of plant. On the basis of loading of PC2, phytochemicals such as tannin, resin and amino acid were included in group B and $ABTS^+$ scavenging activity in group D. PC2 separated the phytochemicals of group B from the IC_{50} value of $ABTS^+$ scavenging activity. This result suggested that tannin like compounds present in liverworts were involved in $ABTS^+$ scavenging activity.

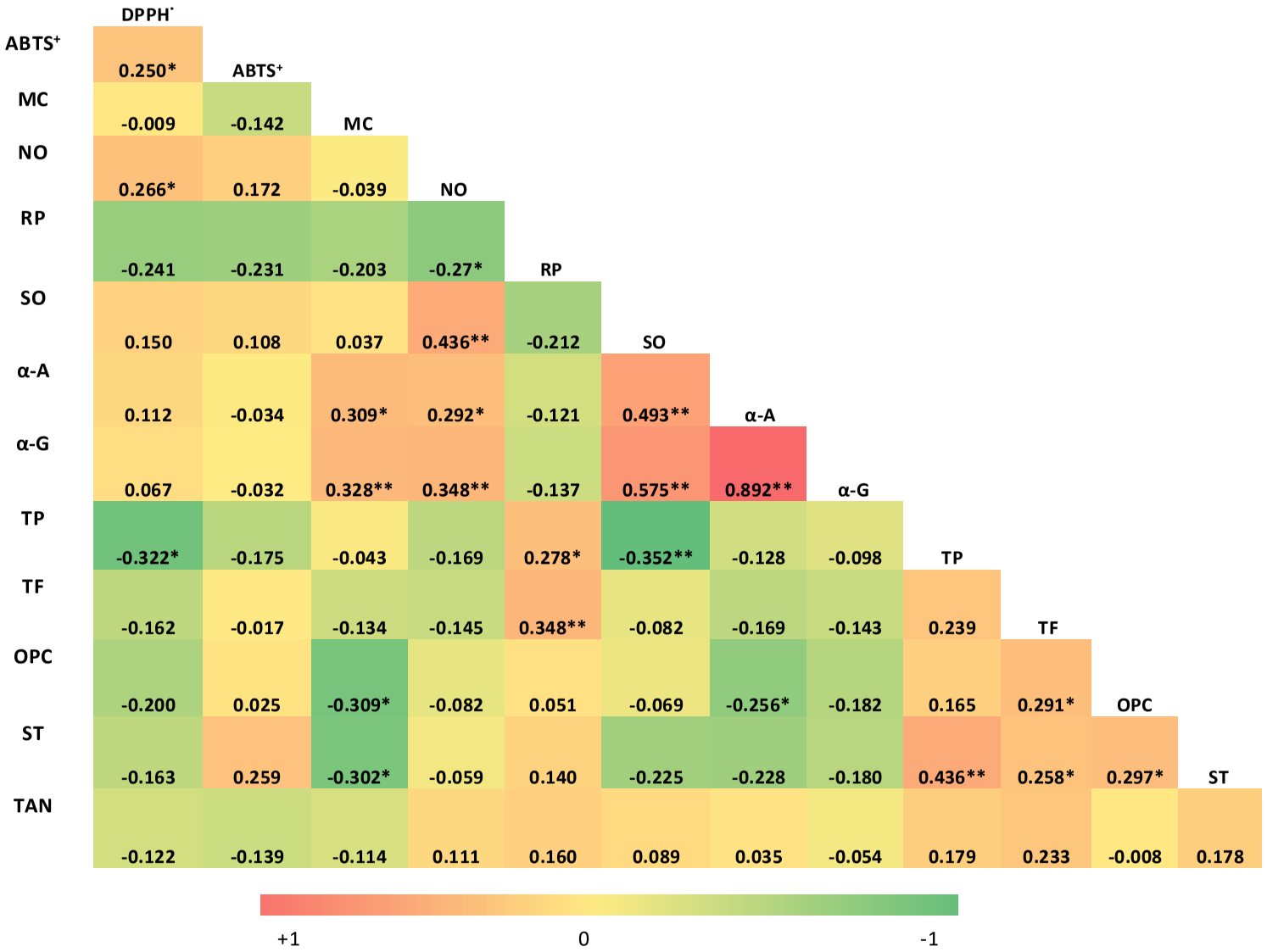


Fig 5.5.1: Correlation between the pharmacological activities and phytochemical content of the studied eleven liverwors. *. Correlation is significant at the 0.05 level (2 tailed) **. Correlation is significant at the 0.01 level (2 tailed)

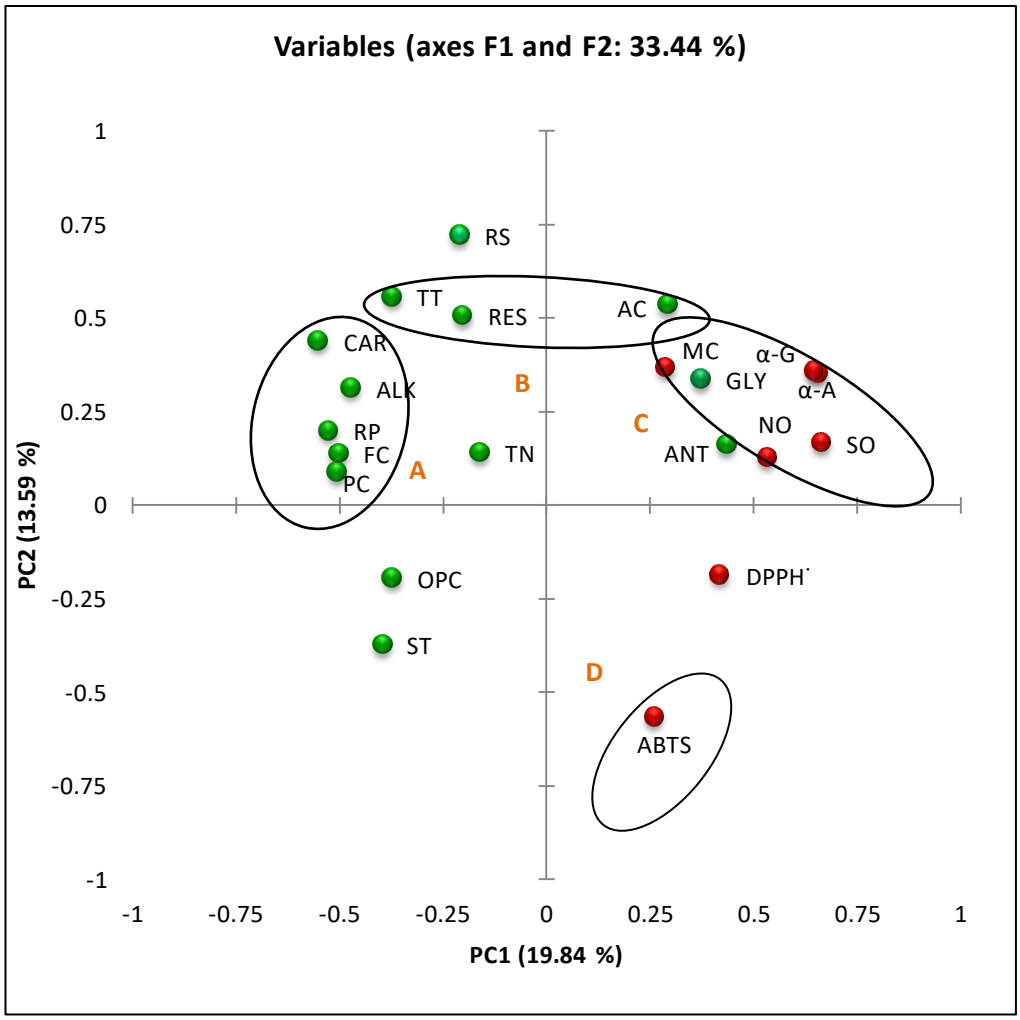


Fig 5.5.2: Principal component analysis of phytochemical content and antioxidant, anti-diabetic activities of studied liverworts

NB: CAR= cardiac glycoside, ALK= alkaloid, RP=reducing power, FC= flavonoid content, PC= phenol content, OPC= orthodihydric phenol content, ST= steroid, RS= reducing sugar, TT= triterpenoid, RES= resine, TN=tannin, AC= amino acid, ANT= anthraglycoside, GLY= glycoside, MC= metal chelating activity, NO= nitric oxide scavenging activity, SO= superoxide scavenging activity, DPPH˙= DPPH˙ scavenging activity, ABTS= ABTS scavenging activity, α-G= α-glucosidase inhibitory activity, α-A= α-amylase inhibitory activity

= antioxidant and anti-diabetic assays **= phytochemical content**

5.5.3 Heat map Analysis

Heat map analysis was performed specifically to find the effect of the polarity of solvents on the extraction of phytochemicals having high antioxidant and anti-diabetic activities. Solvents used for extraction were placed on the vertical axis and the phytochemical content, antioxidant and anti-diabetic activities of the studied plants were placed on the horizontal axis. In case of *Asterella wallichiana* (Fig 5.5.3.1) diethyl ether, ethyl acetate and heptane were clustered together (Group A) indicating that extracts of these solvents have similar pharmacological activities and phytochemical content. Colour key in graphical analysis signified the presence of high amount of plant phytochemicals having high free radical scavenging activity and anti-diabetic enzyme inhibition activity in diethyl ether, ethyl acetate and heptane extracts. Another cluster (Group B) on the vertical axis consisted of solvents acetone and butanol. These solvent extracts showed much lesser pharmacological activities and phytochemical content. Methanol was placed alone in group C. In methanolic extract tannin was found in higher concentration than other phytochemicals. Methanolic extract was noticed to be effective in scavenging selective free radicals only. Thus for *Asterella wallichiana*, non-polar (heptane, diethyl ether) to moderately polar (ethyl acetate) solvents were found to be most appropriate for extraction of phytochemicals having high pharmacological activities.

In case of *Conocephalum japonicum* butanol, ethyl acetate, acetone and diethyl ether were clustered in one group (A) while heptane and methanol extracts were clustered together in another group (Group B). Solvents falling under one group shows similar pattern of biological activities and phytochemical content. Solvents of group A displayed significant pharmacological activities but the content of phytochemicals under study was low. Graphical analysis interpreted that diethyl ether extract had the highest antioxidant, anti-diabetic activities and phytochemical contents. Heptane and methanol extracts showed low pharmacological activities and phytochemicals as well. Thus for *Conocephalum japonicum* moderately polar and non-polar solvents were better for extraction of plant metabolites with significant pharmacological activities (Fig 5.5.3.2).

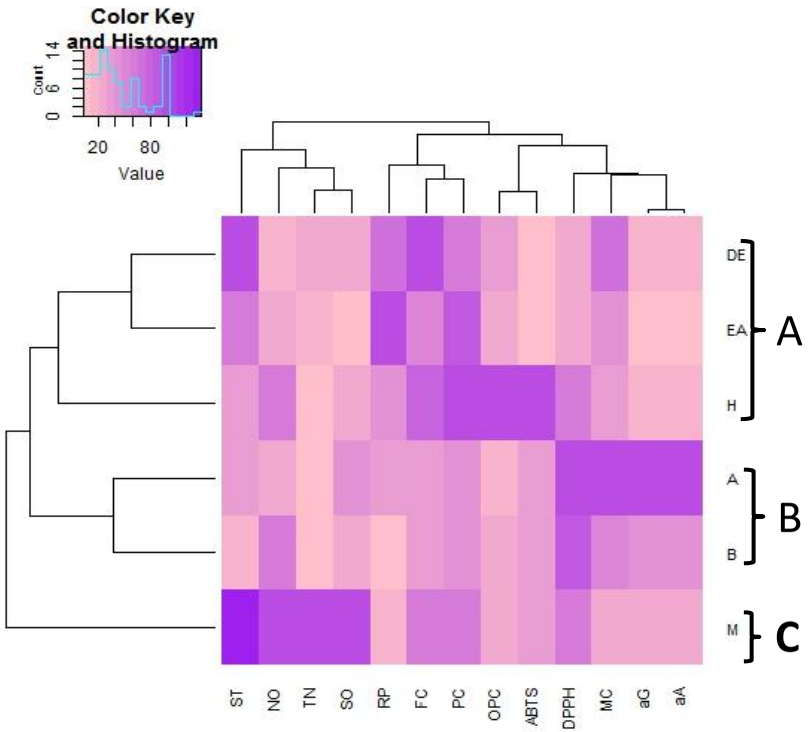


Fig 5.5.3.1: Heat map test for determining the role of different solvents on bioactive phytochemical extraction from *Asterella wallichiana*

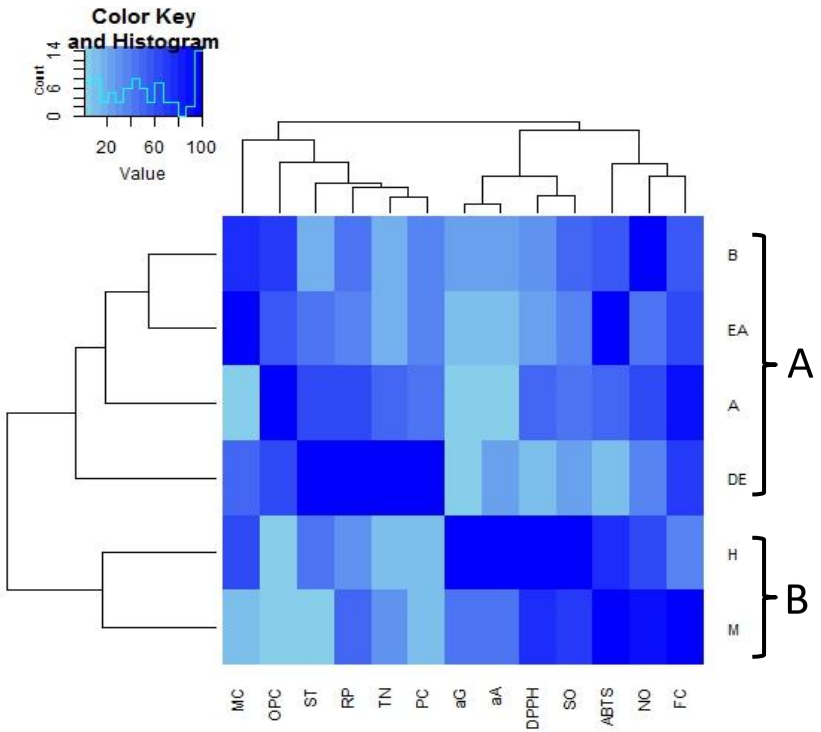


Fig 5.5.3..2 : Heat map test for determining the role of different solvents on bioactive phytochemical extraction from *Conocephalum japonicum*

Heat map of *Dumortiera hirsuta* clusters the solvent extracts into two major groups - A and B (Fig 5.5.3.3). In group A acetone, ethyl acetate and methanol were included and in group B diethyl ether, heptane and butanol were clustered. Graphical analysis signified that diethyl ether and heptane extracts had high antioxidant and anti-diabetic activities. Phytochemical content was also high in these solvent extracts. Apart from these, butanol extract had also showed high pharmacological activities but the content of phytochemicals was low indicating that plant metabolites other than those belonging to polyphenolic group are responsible of the pharmacological activities displayed by butanol extract. However, pharmacological activities and phytochemical content was insignificant in acetone, ethyl acetate and methanol extract.

Different solvents used for extraction of *Lunularia cruciata* were grouped into three major groups (Fig 5.5.3.4). Group A included ethyl acetate, diethyl ether and acetone. Group B included heptane and methanol, and group C included butanol. Colour key indicated that diethyl ether and ethyl acetate have extracted the phytochemicals that have high free radical scavenging potential. Acetone, heptane and methanol extracts were found to be active in scavenging only selective free radicals and also the content of polyphenols were low in these extracts. While in butanol both the pharmacological property and content of phytochemicals was found to be insignificant. Thus, it can be inferred that, moderately non-polar and moderately polar solvents like diethyl ether and ethyl acetate were most suitable for extraction of active phytochemical from *Lunularia cruciata*.

In case of *Pellia epiphylla*, acetone, diethyl ether, heptane and methanol extracts have showed high antioxidant, anti-diabetic activities and phytochemical content (Fig 5.5.3.5). While, butanol and ethyl acetate extracts (Group A) had high pharmacological activities but low phytochemicals content. Colour key in graphical analysis showed highest antioxidant and anti-diabetic activity in diethyl ether and ethyl acetate extracts. Thus in *Pellia epiphylla*, for extraction of biologically active substances non-polar to moderately polar solvents were found to be suitable.

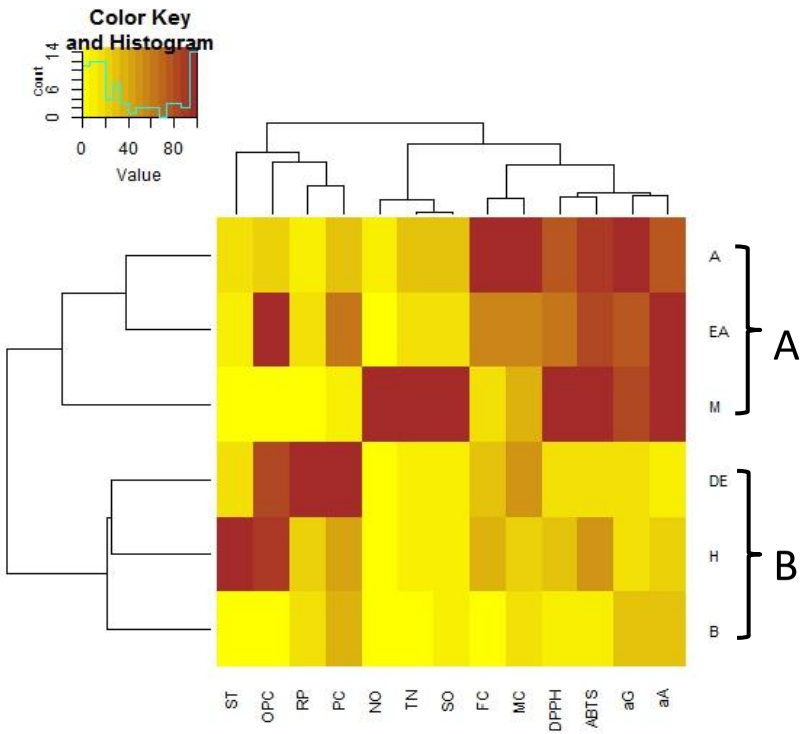


Fig 5.5.3.3: Heatmap test for determining the role of different solvents on bioactive phytochemical extraction from *Dumortiera hirsuta*

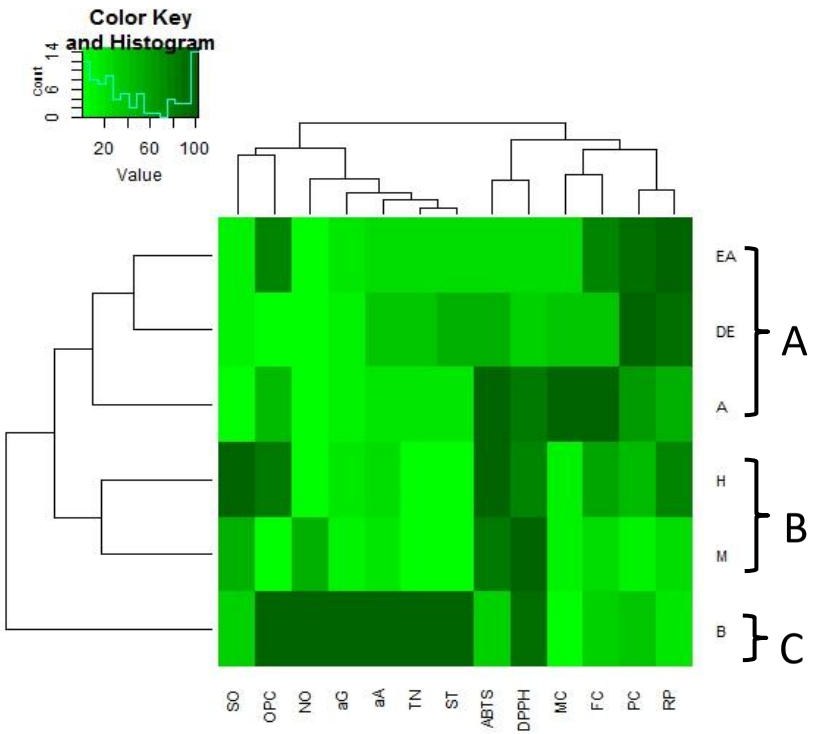


Fig 5.5.3.4: Heatmap test for determining the role of different solvents on bioactive phytochemical extraction from *Lunularia cruciata*

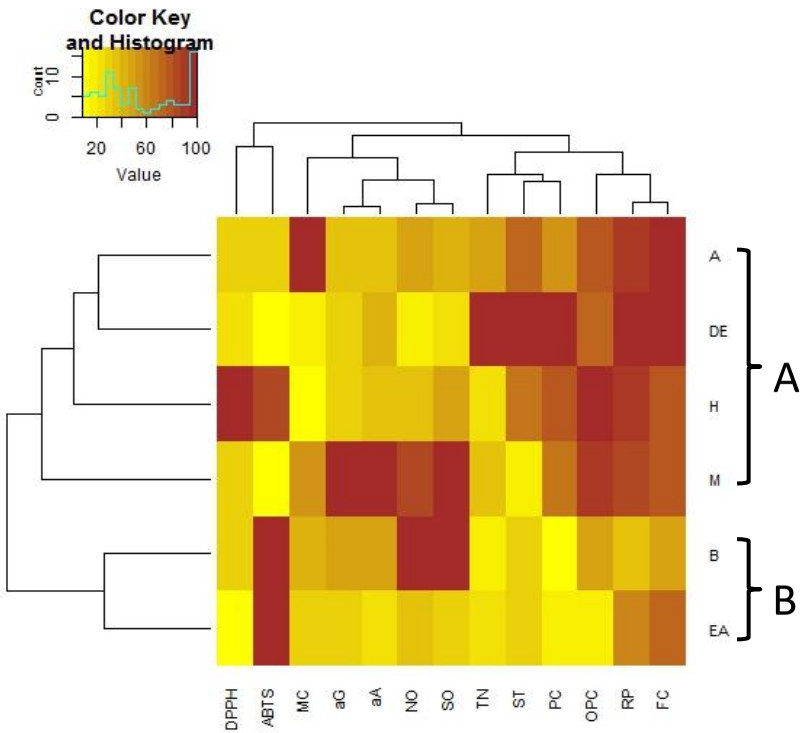


Fig 5.5.3.5: Heatmap test for determining the role of different solvents on bioactive phytochemical extraction from *Pellia epiphylla*

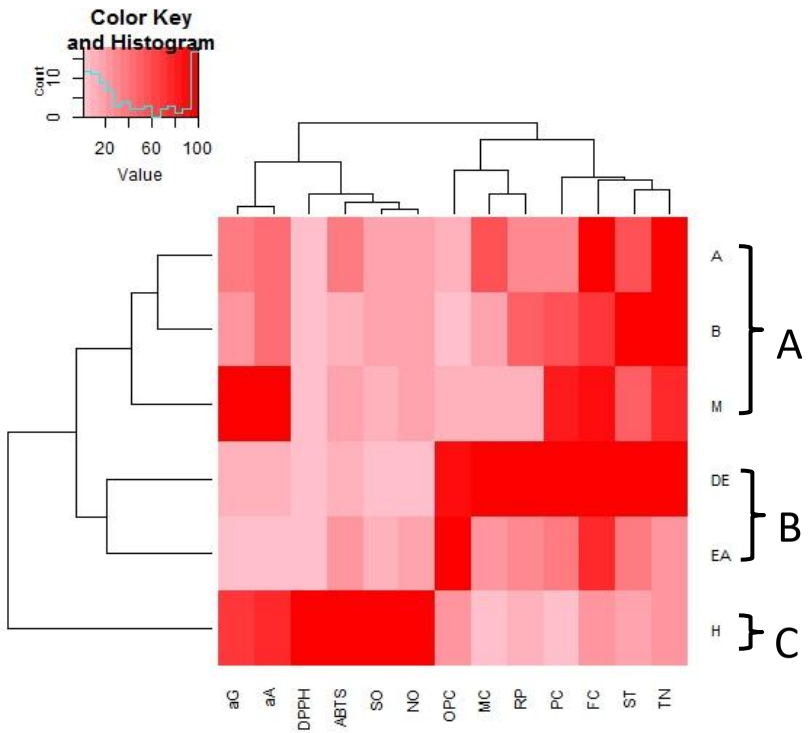


Fig 5.5.3.6: Heatmap test for determining the role of different solvents on bioactive phytochemical extraction from *Marchantia paleacea*

In *Marchantia paleacea*, hierarchical clustering placed the solvent extracts under study in three groups A, B and C (Fig 5.5.3.6). Graphical analysis suggested solvents included in group A (acetone, butanol and methanol) and B (diethyl ether and ethyl acetate) have extracted phytochemicals from *M. paleacea* that are active in scavenging free radicals. However, solvents of group A were less active in inhibiting the activity of α-amylase and α-glucosidase enzymes than solvents of group B. In heptane extract both phytochemicals and pharmacological activities were low. Thus, for *M. paleacea* too ethyl acetate and diethyl ether were best solvents for extracting phytochemicals having high pharmacological activities.

In *Marchantia polymorpha* except methanolic extract all the other extracts showed a significant potential to scavenge free radical and to inhibit α-amylase and α-glucosidase enzymes. Solvents of Group A (diethyl ether, ethyl acetate and acetone) extracted the studied phytochemicals in high amount and the extracted bio-active phytochemicals also displayed a strong antioxidant and anti-diabetic activities. In butanol and heptane extracts (Group B) steroid content was high whereas other phytochemicals like phenol, flavonoid, tannin etc were present in lower amount. These solvent extracts scavenged the free radicals and inhibited the activity of α-amylase and α-glucosidase efficiently. However methanol extract have shown low pharmacological activities and phytochemical content. Thus, for *M. polymorpha* non-polar and moderately polar solvents were found to be suitable for bio-active phytochemical extraction (Fig 5.5.3.7).

In *Marchantia emarginata*, six solvents used for extracting bioactive phytochemicals were grouped into three major groups by heatmap hierarchical clustering (Fig 5.5.3.8). Acetone, ethyl acetate and diethyl ether were clustered together in group A, heptane and butanol were clustered together in Group B and methanol was placed separately in group C on the vertical axis. Solvent extracts of group A showed good antioxidant, anti-diabetic activity and high phytochemical content. Butanol and heptane extracts showed good free radical scavenging and anti-diabetic activities suggesting that steroids present in these extracts are responsible for mentioned activities. In group B solvent extracts, amount of steroid and iron reducing potential were high whereas polyphenol content was low. Phytochemical content, antioxidant and anti-diabetic activities were insignificant in methanolic extract. Result indicated that, in *M. emarginata* too ethyl acetate and diethyl ether were best solvents for bioactive phytochemicals extraction. Acetone though included in

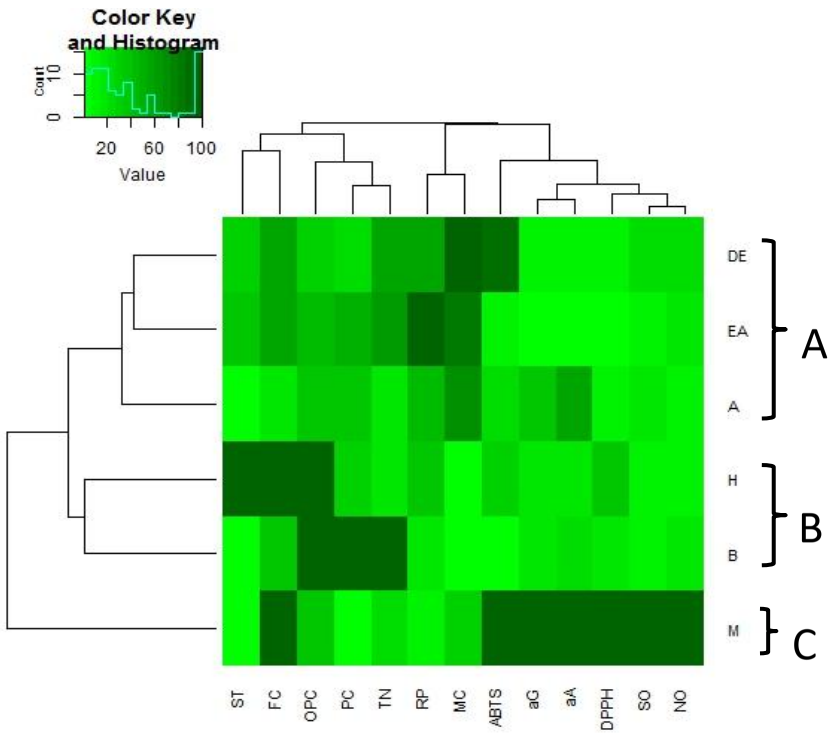


Fig 5.5.3.7. : Heat map test for determining the role of different solvents on bioactive phytochemical extraction from *Marchantia polymorpha*

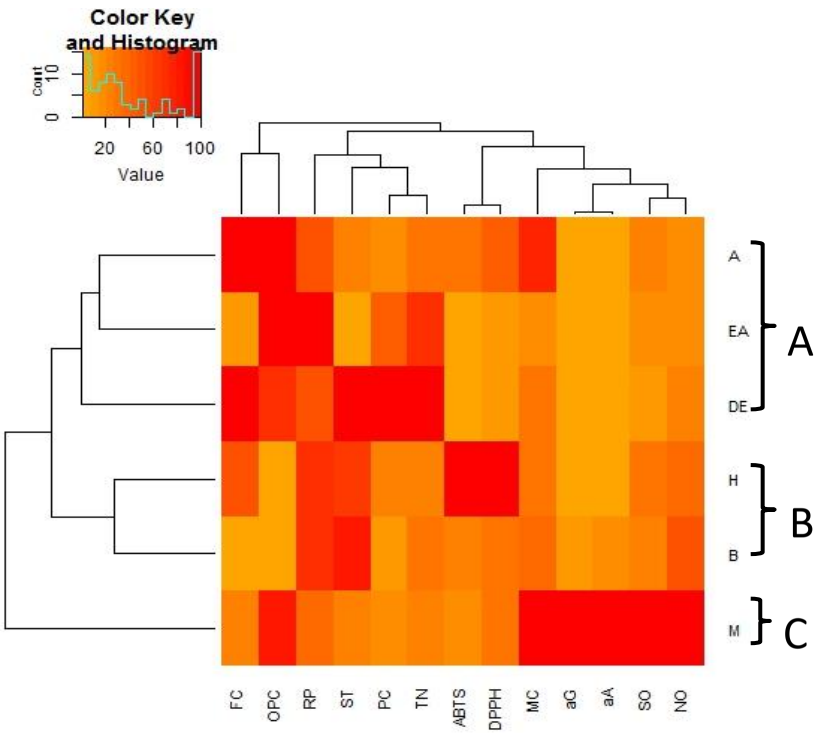


Fig 5.5.3.8 : Heat map test for determining the role of different solvents on bioactive phytochemical extraction from *Marchantia emarginata*

group A showed weaker metal chelating and DPPH˙ scavenging activities and thus was not considered as good solvent for active phytochemical extraction.

Heat map analysis for *Marchantia subintegra* placed the solvent extracts under study in three discrete groups namely A, B and C (Fig 5.5.3.9). The extracts of group C (diethyl ether and ethyl acetate extracts) showed high antioxidant, anti-daibetic activities as well as high bioactive phytochemical content. Butanol and heptane extracts placed under group B showed significant free radical scavenging and anti-diabetic activities but low polyphenol content. In methanol and acetone extracts (group A) both lower phytochemical content and pharmacological activities were noticed. Thus in *M. subintergra* non-polar and moderately polar solvents were found to be suitable for extracting bio-active phytochemicals while polar solvents were less effective in extraction of bioactive phytochemicals.

In *Plagiochasma cordatum*, hierarchical clustering clustered the studied solvents under three groups (Fig 5.5.3.10). Solvents included under group A (diethyl ether and acetone) and B (ethyl acetate and butanol) extracted phytochemicals having significant biological activities in high amount. While group C solvents extracts (methanol and heptane) showed low content of studied phytochemicals as well as low free radical scavenging activity. Thus for *Plagiochasma cordatum*, solvents with moderate polarity like diethyl ether, ethyl acetate, acetone and butanol were more suitable for bioactive phytochemical extraction.

In *Plagiochila nepalensis*, hierarchical clustering placed solvent extracts into four groups (Fig 5.5.3.11). In extracts of Group A (ethyl acetate and acetone) and B (heptane and butanol) significant antioxidant and anti-diabetic activities were recorded but the content of studied phytochemicals were low. While, diethyl ether extract showed high pharmacological activities and phytochemical content. However, methanolic extract showed very low antioxidant and anti-diabetic activities. Thus for *P. nepalensis* non polar and moderately polar solvents were found to be suitable for extraction of bioactive phytochemicals.

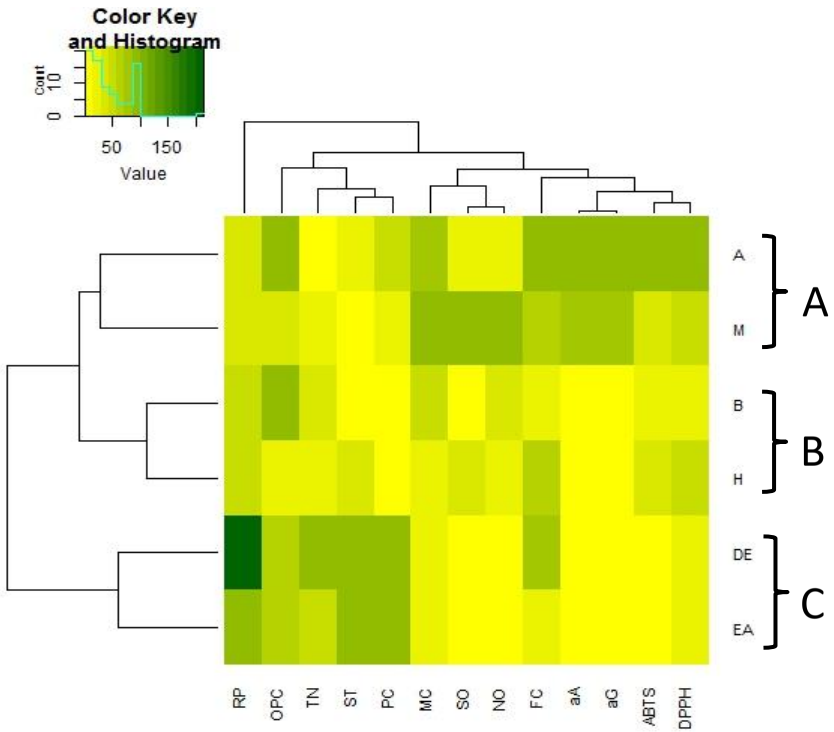


Fig 5.5.3.9 : Heat map test for determining the role of different solvents on bioactive phytochemical extraction from *M.archantia subintegra*

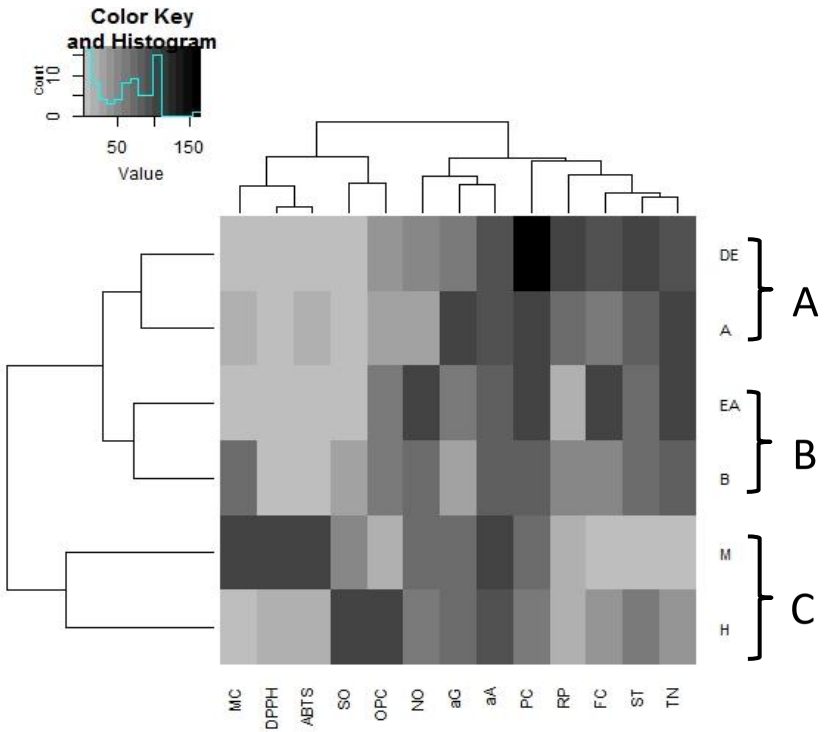


Fig 5.5.3.10 : Heat map test for determining the role of different solvents on bioactive phytochemical extraction from *Plagiochasma cordatum*

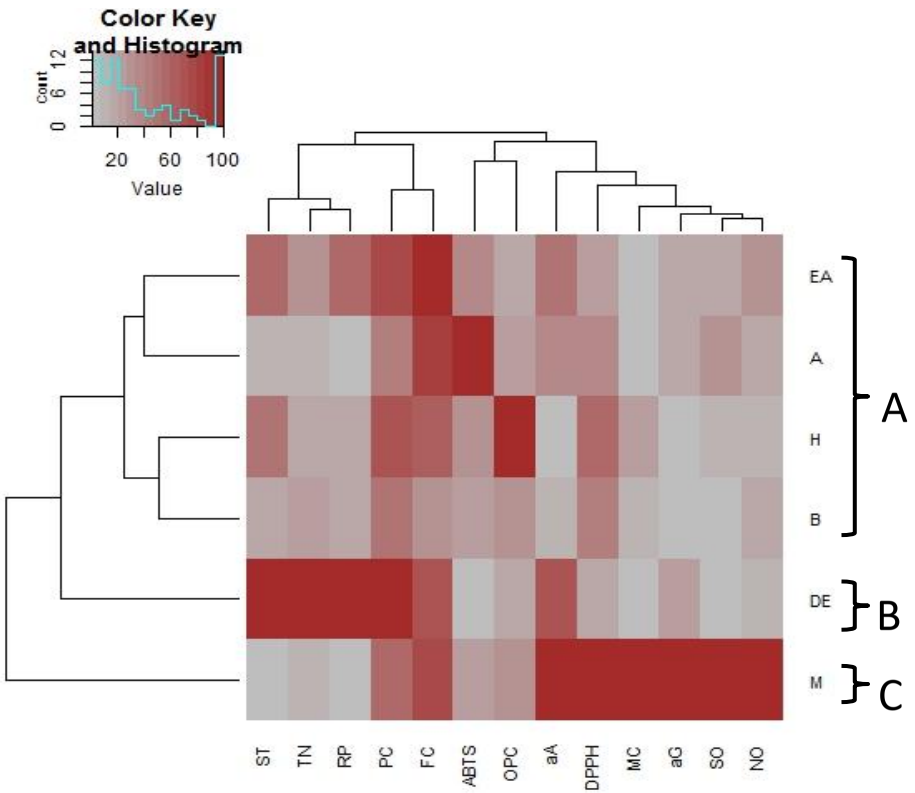


Fig 5.5.3.11: Heat map test for determining the role of different solvents on bioactive phytochemical extraction from *Plagiochila nepalensis*

5.6 SEASONAL CHANGES IN ANTIOXIDANT ACTIVITY AND PHENOLIC CONTENT IN LIVERWORTS

Seasonal changes in free radical scavenging activity and phenolic content were studied in liverwort *Marchantia paleacea* collected during two seasons *i.e.* rainy season prevailing from May till October and dry season prevailing from October till March of a year. Six different solvents, heptane, diethyl ether, ethyl acetate, acetone, butanol and methanol were used for the extraction of phytochemicals. Different kinds of free radicals are generated in the body that are responsible for inducing oxidative stress. We have selected $DPPH^{\cdot}$, $ABTS^{+}$, nitric oxide (NO), super oxide (SO) radicals to evaluate the variation in antioxidant activity of *M. paleacea* during rainy and dry season. $DPPH^{\cdot}$ scavenging activity of *M. paleacea* increased significantly during dry season. The $DPPH^{\cdot}$ scavenging activity (IC_{50}) ranged between 0.27 mg/ml and 34.75 mg/ml during rainy season and in dry season activity ranged between 0.22 mg/ml and 2.63 mg/ml in different solvent extracts. Significant increase in $DPPH^{\cdot}$ activity was observed in heptane, diethyl ether, ethyl acetate, acetone and butanol extracts during dry season while methanolic extract's potential to scavenge this free radical decreased during dry season as compared to rainy season (Fig 5.6.1).

$ABTS^{+}$ scavenging activity varied from 0.06 mg/ ml to 0.64 mg/ml (IC_{50} values) during rainy season in six solvent extracts. While in dry season, IC_{50} value ranged between 0.06 mg/ml and 0.40 mg/ml. $ABTS^{+}$ scavenging activity of heptane, ethyl acetate and acetone extracts increased during dry season. The activity decreased in methanol, butanol and very slightly in diethyl ether extract during dry season (Fig 5.6.2). In superoxide scavenging assay also similar result was obtained. Superoxide scavenging activity of *M. paleacea* increased significantly during dry season in heptane, ethyl acetate and acetone extracts, while in methanol, butanol and diethyl ether extracts, potential to scavenge SO was found to be better during rainy season. IC_{50} value of SO scavenging activity ranged from 0.16 mg/ml to 3.7 mg/ml during rainy season and from 0.19 mg/ml to 3.7 mg/ml during dry season (Fig 5.6.3). However when all other studied free radicals scavenging activity was increased during drought condition in selected solvent extracts, the result of nitric oxide scavenging assay were rather contradictory. *M. paleacea* showed increased potential to scavenge nitric oxide during rainy season than dry season (Fig 5.6.4). During rainy season the NO scavenging activity ranged between 0.82 mg/ml and 13.35 mg/ml (IC_{50}), while during dry season the said activity ranged between 2.56 mg/ml and 21.8 mg/ ml (IC_{50}).

Free ferrous ions are the most powerful pro-oxidants and thus its reduction is important for protection against oxidative damage and lipid peroxidation by Fenton reaction. Result of the present study showed that metal chelating potential of *M. paleacea* increased significantly during dry season in diethyl ether, ethyl acetate and acetone extract. While heptane, methanol and butanol extracts showed better ferrous iron chelating activity during rainy season. Metal chelating activity of *M. paleacea* varied from 0.37 mg/ml to 13.51 (IC_{50} value) during rainy season and 0.96 mg/ml to 4.72 mg/ml (IC_{50} value) during dry season (Fig 5.6.5).

As plant polyphenols are mainly reported of having a role as antioxidants, we have studied the change in pattern of polyphenolic compounds content during rainy and dry season to check if there exist any correlation between the phenolic content and the free radical scavenging activities. Content of phenols in studied extracts during two seasons explained the variation in the antioxidant activity to some extent. With the increase in antioxidant activity of heptane, diethyl ether, ethyl acetate and acetone extracts during dry season the increase in the phenolic compound was also recorded. Similarly, the increase in antioxidant activity of methanolic and butanolic extracts was accompanied with the increase in phenolic compound in these extracts. Phenol content ranged between 4.81 mg gallic acid equivalent/g extractive weight (mg GAE/g EW) and 113.9 mg GAE/g EW in rainy season and between 24.1 mg GAE/g EW and 117.4 mg GAE/g EW (Fig 5.6.7). Similarly, flavonoid content of the plant was also found to increase during dry season. Concentration of flavonoid increased significantly in butanol, ethyl acetate, diethyl ether and heptane extracts during dry season. Flavonoid content varied from 3.8 mg quercetin equivalent/ g extractive weight (mg QE/ g EW) to 16.3 mg QE/ g EW during rainy season and from 9.1 mg QE/ g EW to 32.1 mg QE/ g EW during dry season (Fig 5.6.8). However, orthodihydric phenol content of the plant was found to decrease during unfavorable condition where there is scarcity of water (Fig 5.6.7). During rainy season content of orthodihydric phenol ranged between 0.34 mg Catechol/ g extractive weight (mg CE/ g EW) to 11.45 mg CE/ g EW. In dry season 0.11 mg CE/ g EW to 7.11 mg CE/ g EW content of orthodihydric phenol was recorded in six solvent extracts.

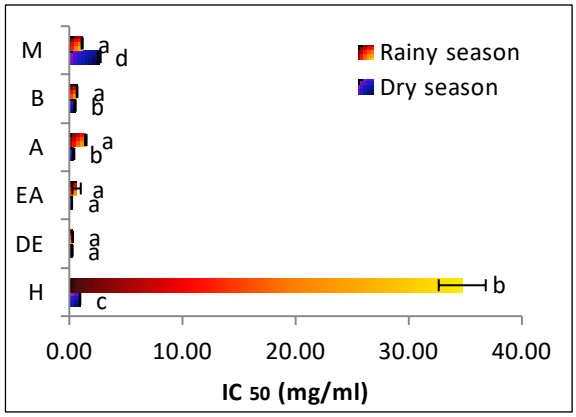


Fig 5.6.1: Comparison of DPPH˙ scavenging activity of *M. paleacea* during rainy and dry season

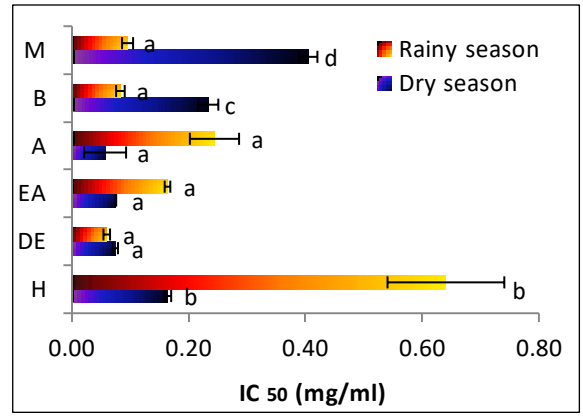


Fig 5.6.2: Comparison of ABTS˙ scavenging activity of *M. paleacea* during rainy and dry season

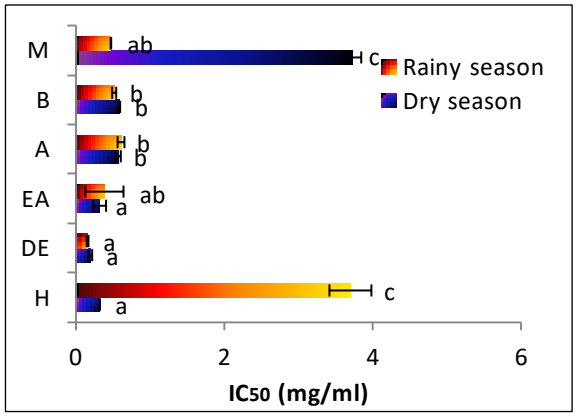


Fig 5.6.3: Comparison of SO scavenging activity of *M. paleacea* during rainy and dry season

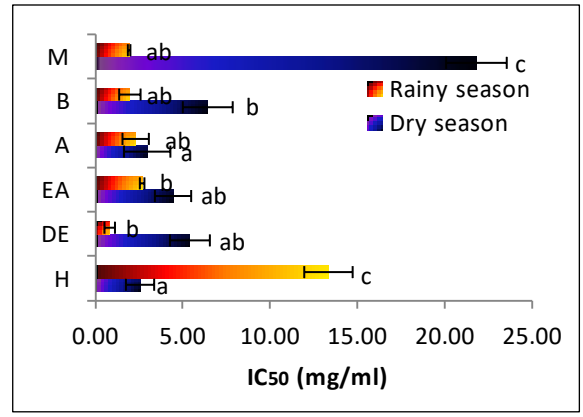


Fig 5.6.4: Comparison of NO scavenging potential of *M. paleacea* during rainy and dry season

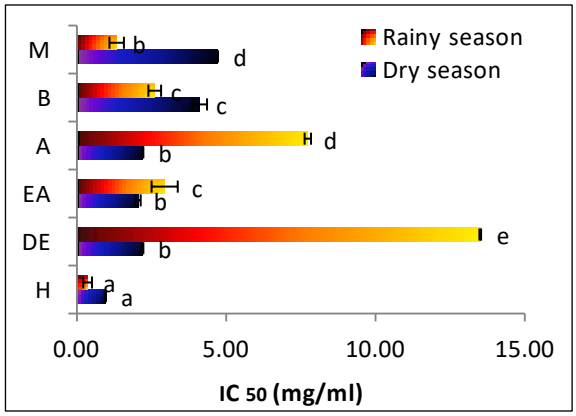


Fig 5.6.5: Comparison metal chelating activity of *M. paleacea* during rainy and dry season

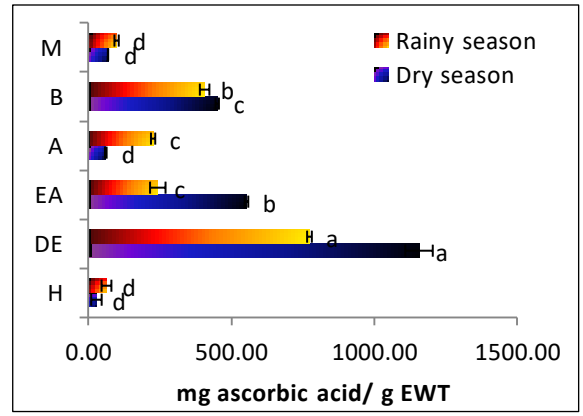


Fig 5.6.6: Comparison of reducing power of *M. paleacea* during rainy and dry season

NB: H= heptane, DE=diethyl ether, EA= ethyl acetate, A= acetone, B=butanol, M=methanol
Value with different letters (a, b, c, d etc.) differ significantly (p <0.05) from each other by Duncan's Multiple Test (DMRT).

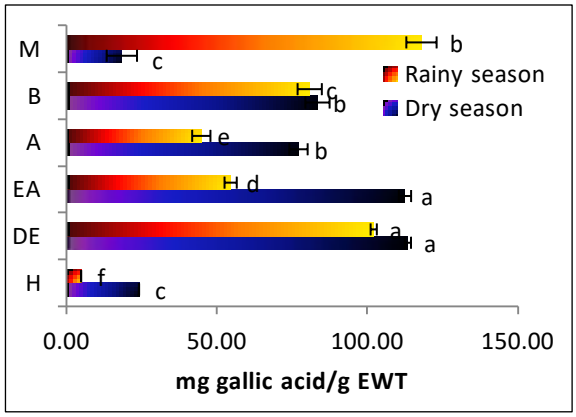


Fig 5.6.7: Comparison of phenolic content of *M. paleacea* during rainy and dry season

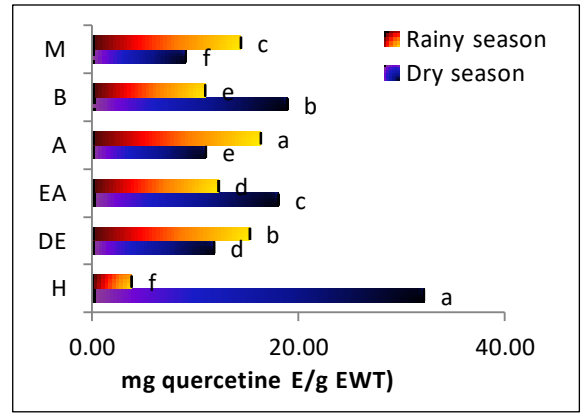


Fig 5.6.7: Comparison of flavonoid content of *M. paleacea* during rainy and dry season

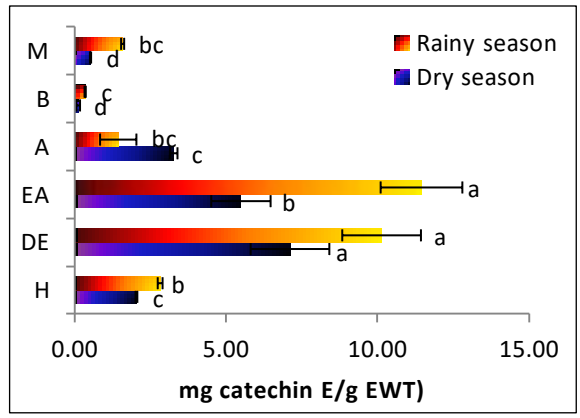


Fig 5.6.7: Comparison Orthodihydric phenol content of *M. paleacea* during rainy and dry season

NB: H= heptane, DE=diethyl ether, EA= ethyl acetate, A= acetone, B=butanol, M=methanol
Value with different letters (a, b, c, d etc.) differ significantly ($p < 0.05$) from each other by Duncan's Multiple Test (DMRT)

5.7 BIOASSAY GUIDED PARTIAL PURIFICATION OF LIVERWORT *MARCHANTIA PALEACEA*

Bryophytes were used as medicines in ancient Chinese, European and North American traditional medicine. One important class of bryophyte, the liverworts, has been used as ethnomedicine worldwide and few reports are also available recording their use in Indian culture. *Marchantia paleacea*, has been reported to be used in traditional medicines; it is used in skin tumefaction, hepatitis and also as an antipyretic (Chandra *et al*., 2017). Moreover, result of assays that were performed earlier in this work showed that *M. paleacea* has a significant free radical scavenging and anti-diabetic potential along with moderate anti-proliferation activity. In Darjeeling, *M. paleacea* grows abundantly, so, on the basis of medicinal properties and abundance, *M. paleacea* was selected for bioassay guided purification of phytochemicals. Phytochemicals present in *M. paleacea* was extracted successively with solvents of increasing polarity (hexane, toluene, diethyl ether, ethyl acetate, chloroform, acetone, acetonitrile, ethanol, methanol, water). All ten extracts obtained after successive extraction were screened for the phytochemical content and antioxidant activity. DPPH˙ assay is the most common assay for screening free radical scavenging potential (Brand-Williams *et al*., 2005).

On screening the preliminary extracts for its DPPH˙ scavenging activity, diethyl-ether extract was found to exhibit considerably stronger antioxidant property than other solvent extracts (Fig 5.7.1). Similarly Fig 5.7.2 represents the quantitative profile of flavonoid which shows that diethyl ether extract also contained higher amount of flavonoid compounds. On the basis of the antioxidant activity, anti-diabetic potential and content of flavonoid compounds, diethyl ether fraction was chromatographed on silica gel column. A total of 208 sub-fractions (20 ml each) were collected and screened for DPPH˙ scavenging activity (Fig 5.7.3). Sub-fractions with high antioxidant activity were then combined on the basis of their TLC profiles (Fig 5.7.4). Active sub-fractions were grouped into eleven main fractions (F1-A, F1-B, F1-C, F1-D, F1-E, F1-F, F1-G, F1-H, F1-I, F1-J, F1-K). These main fractions were further screened by antioxidant and anti-diabetic assays (Fig 5.7.5 – 5.7.7). Fraction F1-F showed best antioxidant and anti-diabetic activity. Therefore, F1-F was subjected to additional fractionation by second column chromatograph. In total, 546 sub fractions (10 ml each) were collected and screened for their antioxidant (DPPH˙ scavenging activity) property and flavonoid content (Fig 5.7.10). Less active fractions were discarded

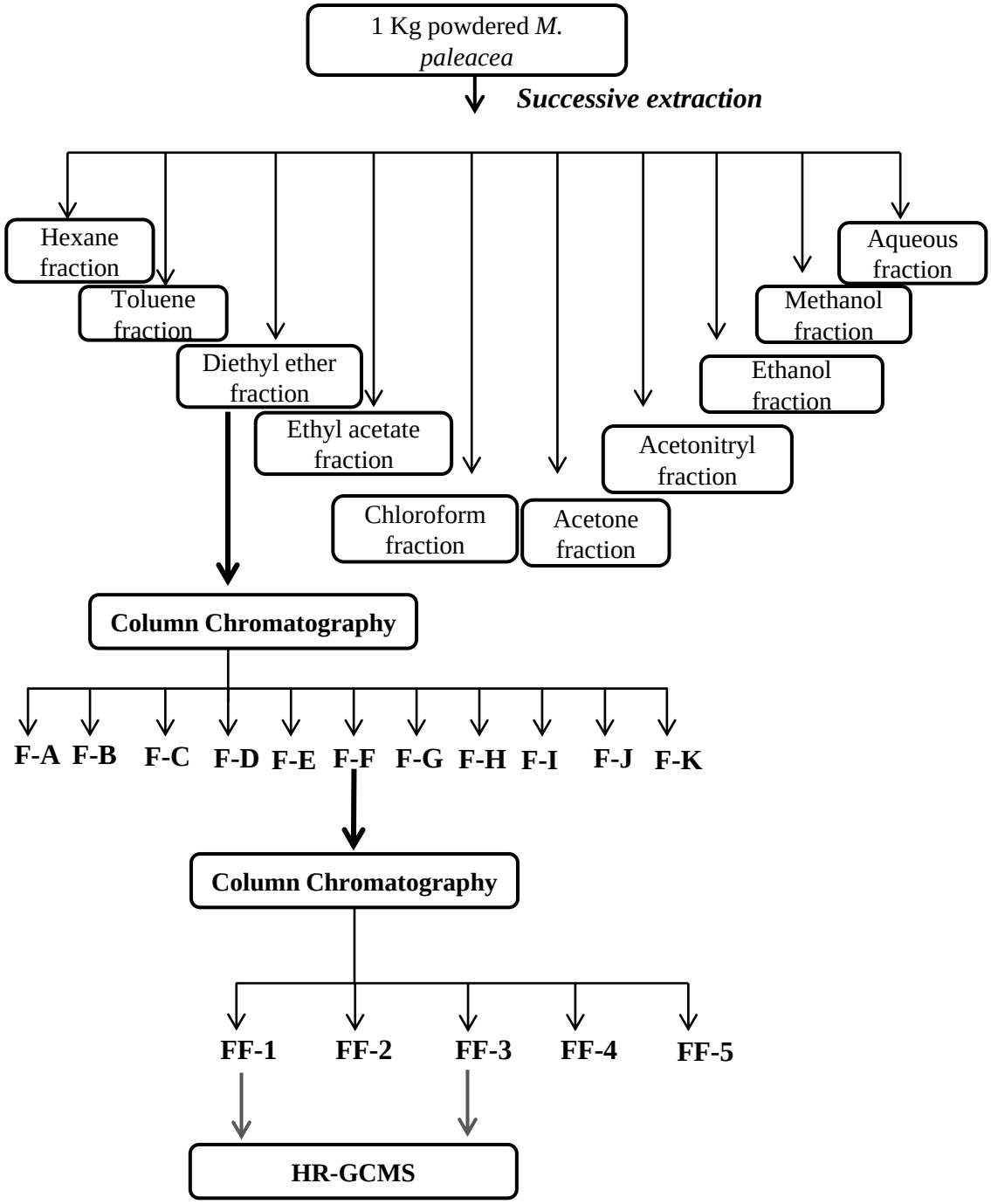


Fig 5.7: Schemes of fraction preparation of *Marchantia paleacea.* F= fractions obtained from first column chromatography, FF= fraction obtained from second column chromatography

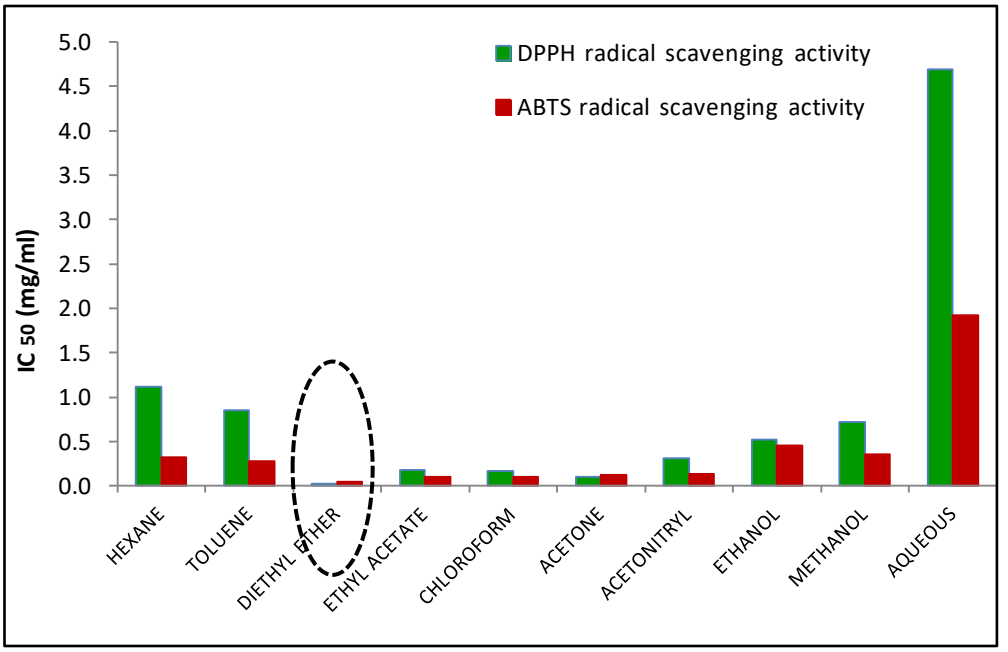


Fig 5.7.1: DPPH and ABTS radical scavenging activities of main fractions obtained from successive extraction

NB: Bar within the ellipse indicates highest DPPH$^{\cdot}$ and ABTS^{+} activity.

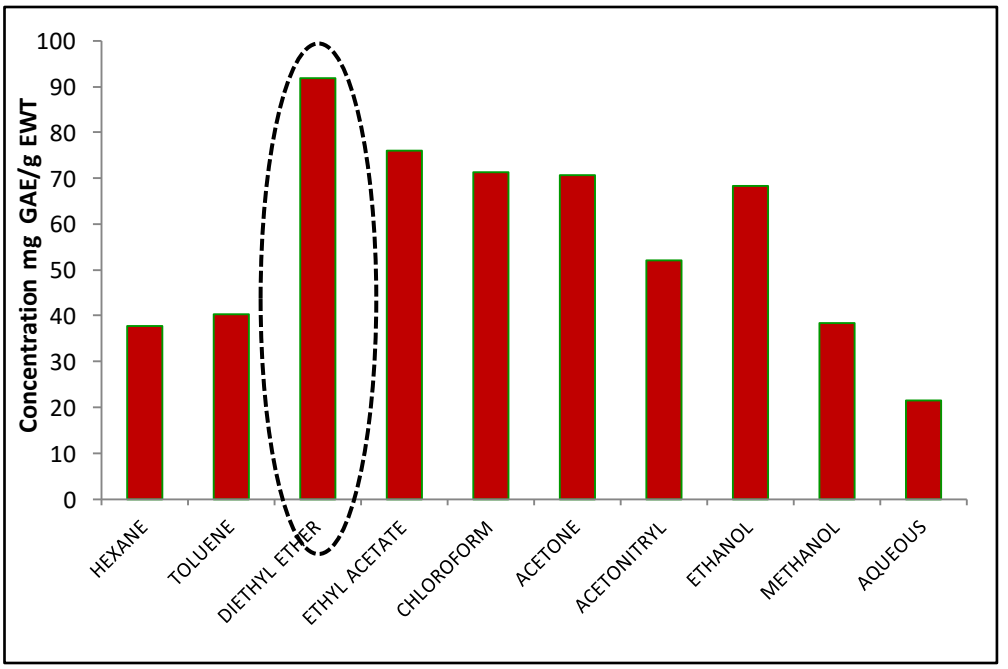


Fig 5.7.2: Flavonoid content of main fractions obtained from successive extraction

NB: Bar within the ellipse indicates highest flavonoid content.

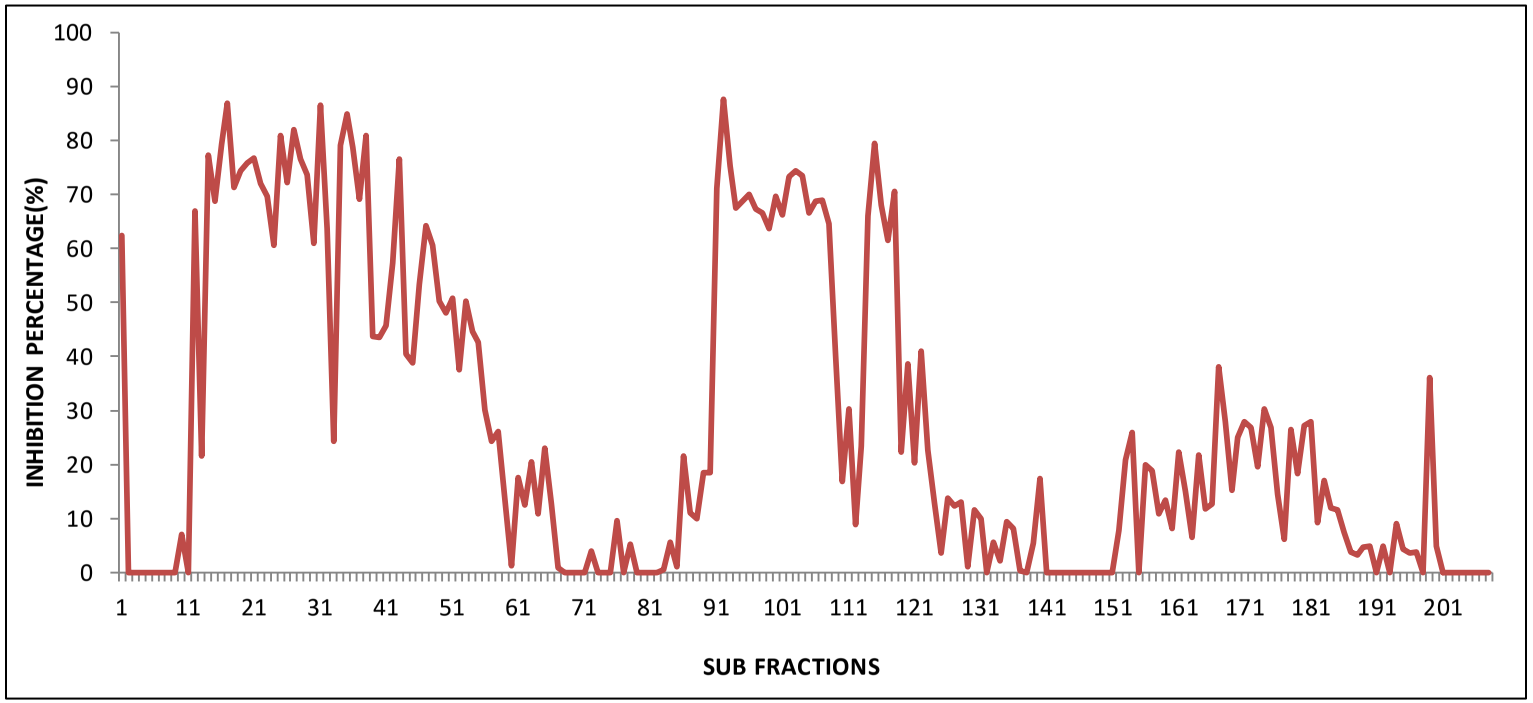


Fig 5.7.3: DPPH˙ scavenging activity of fractions of *M. paleacea* obtained from first column chromatography

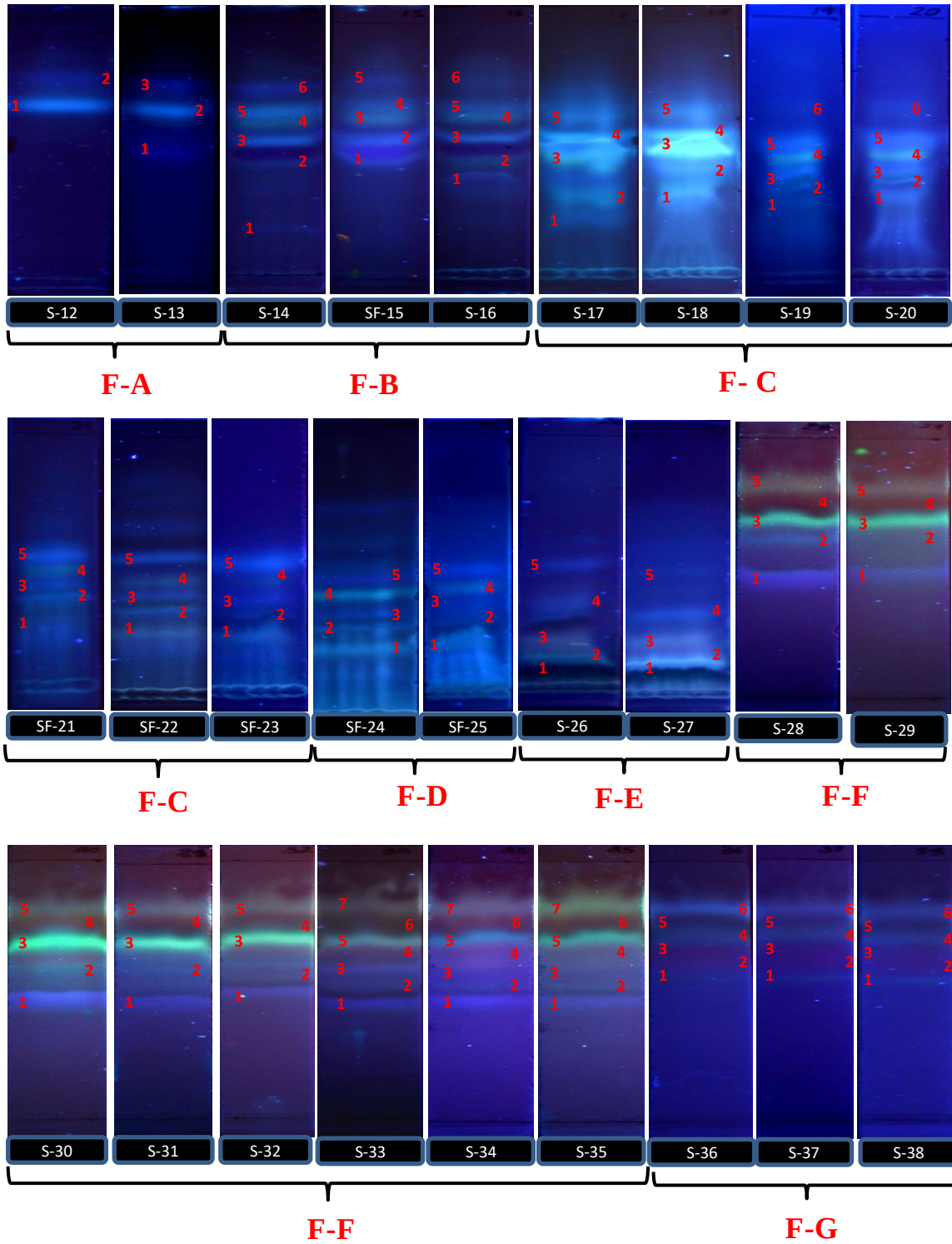


Fig 5.7.4 : TLC profiling of bioactive sub-fractions of *M. paleacea* obtained from first column chromatograph

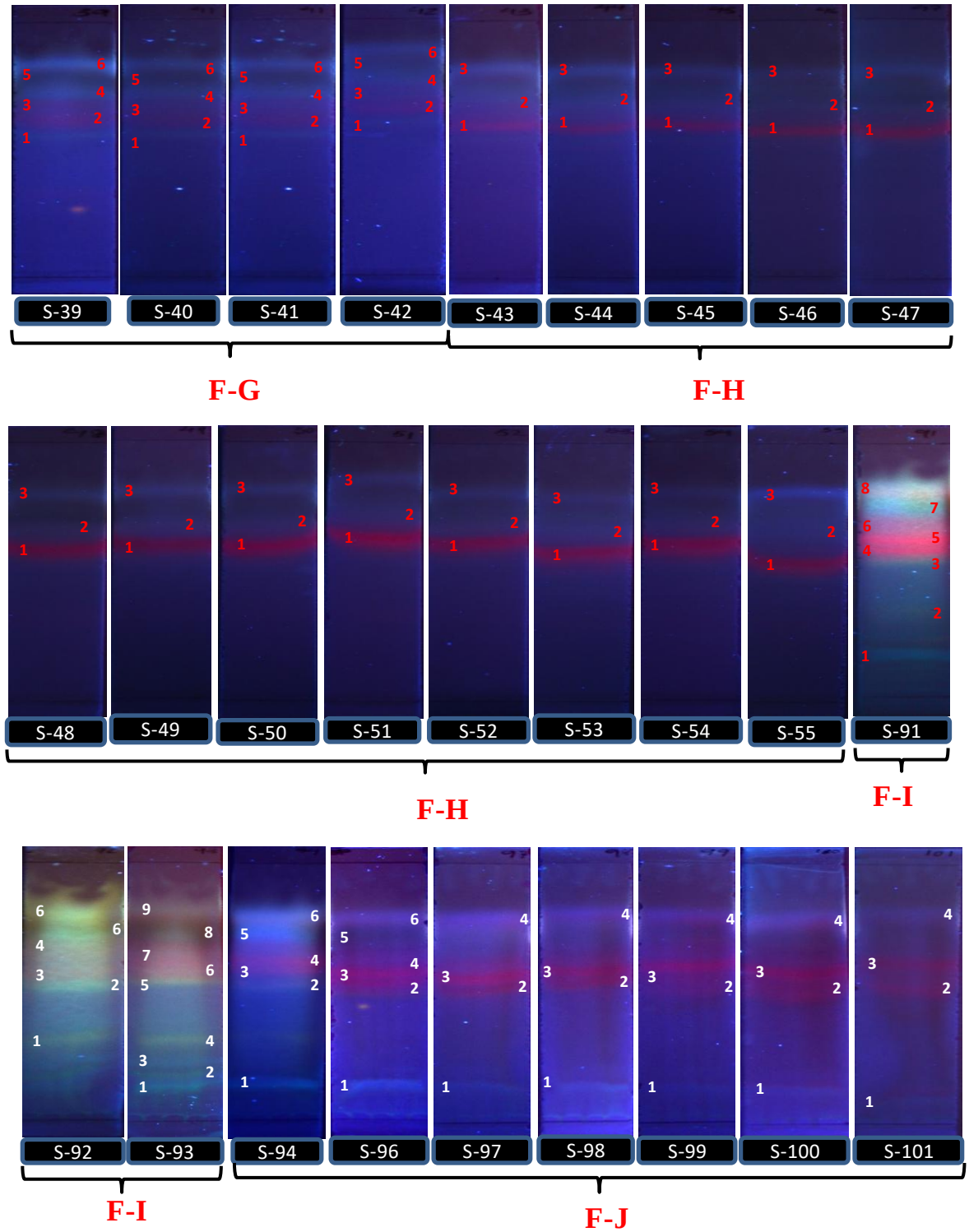


Fig 5.7.4 : TLC profiling of bioactive sub-fractions of *M. paleacea* obtained from first column chromatograph (Continuation)

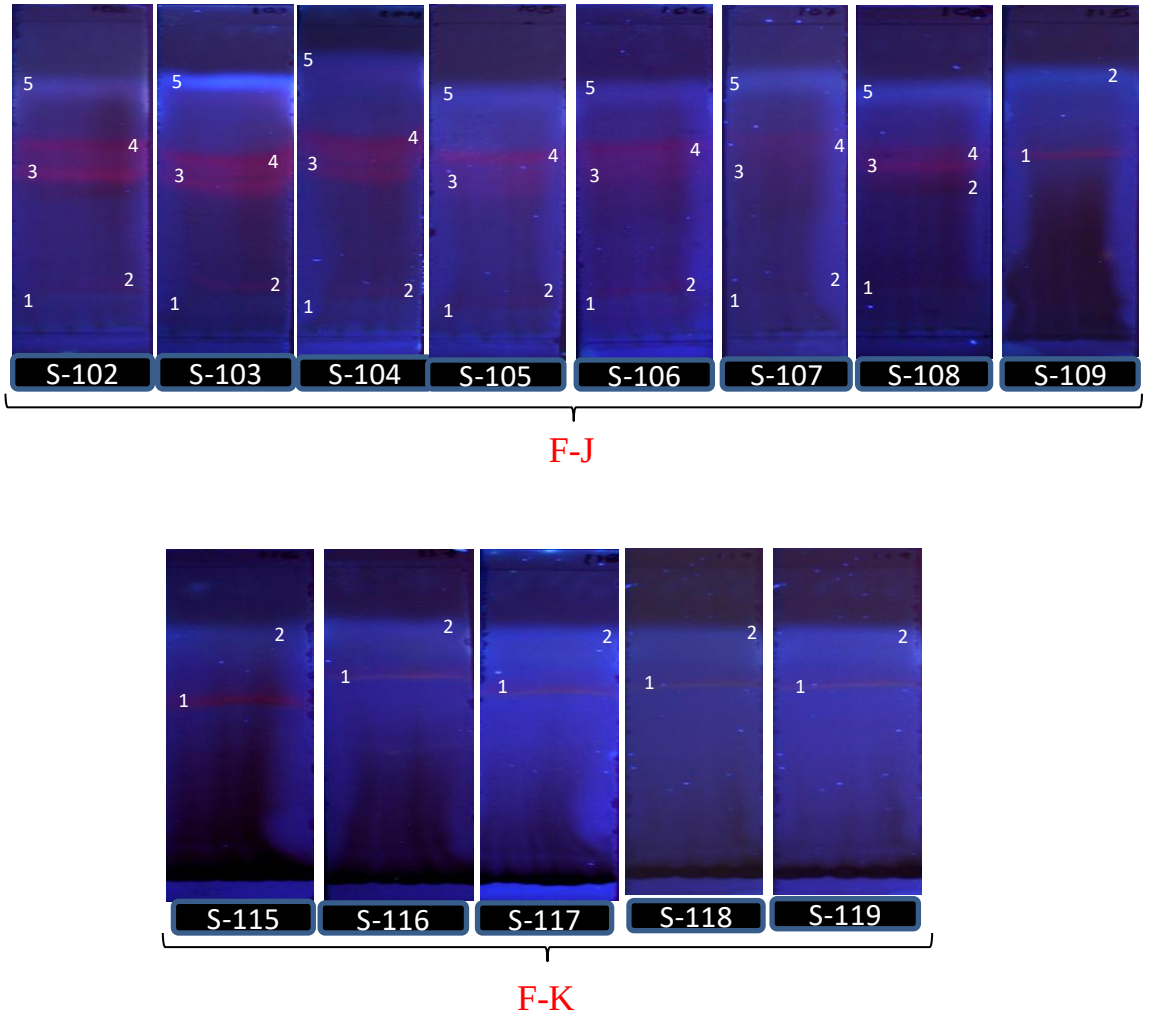


Fig 5.7.4: TLC profiling of bioactive sub-fractions of *M. paleacea* obtained from first column chromatograph (Continuation)

Table 5.3: Retardation factors of different analytes present in bioactive fractions of *M. paleacea* obtained from first column chromatograph of *M. paleacea*

Sub fractions	***Identity of bands***	**Rf**	***Sub fractions***	***Identity of bands***	**Rf**
12	1 2	0.65 0.77	22	1 2 3 4 5	0.25 0.34 0.37 0.42 0.51
13	1 2 3	0.48 0.65 0.77	23	1 2 3 4 5	0.23 0.31 0.41 0.47 0.51
14	1 2 3 4 5 6 7	0.17 0.34 0.43 0.6 0.51 0.65 0.75	24	1 2 3 4 5	0.25 0.34 0.37 0.45 0.51
15	1 2 3 4 5	0.48 0.5 0.53 0.67 0.77	25	1 2 3 4 5	0.25 0.34 0.37 0.42 0.51
17	1 2 3 4 5	0.15 0.34 0.48 0.56 0.65	26	1 2 3 4 5	0.15 0.17 0.24 0.27 0.51
18	1 2 3 4 5	0.15 0.34 0.48 0.56 0.65	27	1 2 3 4 5	0.15 0.17 0.24 0.27 0.50
19	1 2 3 4 5 6	0.3 0.37 0.41 0.43 0.48 0.52	28	1 2 3 4 5	0.46 0.51 0.68 0.74 0.81
20	1 2 3 4 5 6	0.3 0.37 0.41 0.43 0.48 0.52	29	1 2 3 4 5	0.46 0.51 0.68 0.74 0.81
21	1 2 3 4 5	0.29 0.36 0.41 0.45 0.51	30	1 2 3 4 5	0.46 0.51 0.68 0.74 0.81

Table 5.3: Retardation factors of different analytes present in bioactive fractions of *M. paleacea* obtained from first column chromatograph of *M. paleacea* (continuation)

Sub fractions	***Identity of bands***	***Rf***	***Sub fractions***	***Identity of bands***	***Rf***
31	1	0.48	40	1	0.51
	2	0.53		2	0.55
	3	0.58		3	0.60
	4	0.68		4	0.65
	5	0.74		5	0.71
	6	0.81		6	0.81
32	1	0.48	41	1	0.51
	2	0.53		2	0.55
	3	0.58		3	0.60
	4	0.68		4	0.65
	5	0.74		5	0.71
	6	0.81		6	0.81
33	1	0.46	42	1	0.51
	2	0.56		2	0.55
	3	0.60		3	0.60
	4	0.65		4	0.65
	5	0.68		5	0.71
	6	0.75		6	0.81
	7	0.81			
34	1	0.46	43	1	0.59
	2	0.56		2	0.72
	3	0.60		3	0.82
	4	0.65			
	5	0.68			
	6	0.75			
	7	0.81			
35	1	0.46	44	1	0.59
	2	0.56		2	0.72
	3	0.60		3	0.82
	4	0.65			
	5	0.68			
	6	0.75			
	7	0.81			
36	1	0.51	45	1	0.59
	2	0.55		2	0.72
	3	0.60		3	0.82
	4	0.65			
	5	0.70			
	6	0.81			
37	1	0.51	46	1	0.59
	2	0.55		2	0.72
	3	0.60		3	0.82
	4	0.65			
	5	0.71			
	6	0.81			
38	1	0.51	47	1	0.58
	2	0.55		2	0.64
	3	0.60		3	0.82
	4	0.65			
	5	0.71			
	6	0.81			
39	1	0.51	48	1	0.58
	2	0.55		2	0.64
	3	0.60		3	0.82
	4	0.65			
	5	0.71			
	6	0.81			

Table 5.3: Retardation factors of different analytes present in bioactive fractions of *M. paleacea* obtained from first column chromatograph of *M. paleacea* (continuation)

Sub fractions	***Identity of bands***	***Rf***	***Sub fractions***	***Identity of bands***	***Rf***
49	1	0.58	94	1	7.3
	2	0.64		2	0.52
	3	0.82		3	0.64
				4	0.68
				5	0.70
				6	0.78
50	1	0.58	95	1	7.3
	2	0.64		2	0.52
	3	0.82		3	0.64
				4	0.68
				5	0.70
				6	0.78
51	1	0.58	96	1	7.3
	2	0.64		2	0.52
	3	0.82		3	0.64
				4	0.68
				5	0.70
				6	0.78
52	1	0.58	97	1	0.15
	2	0.64		2	0.5
	3	0.82		3	0.6
				4	0.82
53	1	0.58	98	1	0.15
	2	0.64		2	0.5
	3	0.82		3	0.6
				4	0.82
91	1	0.17	99		
	2	0.19			
	3	0.32		1	0.15
	4	0.52		2	0.5
	5	0.56		3	0.6
	6	0.59		4	0.82
	7	0.73			
	8	0.78			
92	1	0.17	100		
	2	0.19		1	0.15
	3	0.32		2	0.5
	4	0.51		3	0.6
	5	0.70		4	0.82
	6	0.78			
93	1	0.14	101	1	0.15
	2	0.19		2	0.5
	3	0.21		3	0.6
	4	0.32		4	0.82
	5	0.52			
	6	0.56			
	7	0.59			
	8	0.73			
	9	0.78			

Table 5.3: Retardation factors of different analytes present in bioactive fractions of *M. paleacea* obtained from first column chromatograph of *M. paleacea* (continuation)

Sub fractions	***Identity of bands***	***Rf***	***Sub fractions***	***Identity of bands***	***Rf***
102	1	0.15		1	0.31
	2	0.5		2	0.48
	3	0.6	108	3	0.55
	4	0.82		4	0.58
				5	0.60
103	1	0.15			
	2	0.5		1	0.58
	3	0.6	109	2	0.79
	4	0.82			
	1	0.31			
	2	0.51		1	0.60
104	3	0.56	115	2	0.79
	4	0.6			
	5	0.77			
	1	0.12			
	2	0.15		1	0.60
105	3	0.56	116	2	0.79
	4	0.6			
	5	0.77			
	1	0.12			
	2	0.15		1	0.60
106	3	0.56	117	2	0.79
	4	0.6			
	5	0.77			
	1	0.12			
	2	0.15		1	0.60
107	3	0.56	118	2	0.79
	4	0.6			
	5	0.77			

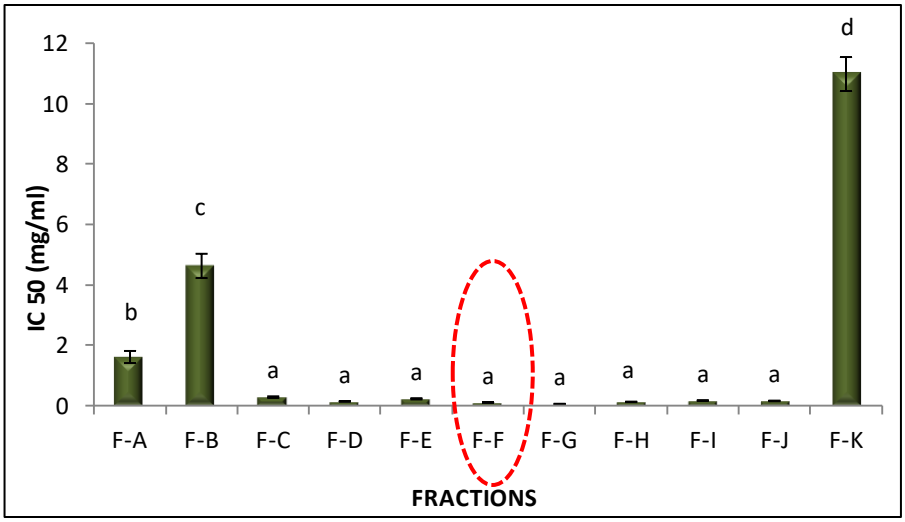


Fig 5.7.5: DPPH$^{\cdot}$ scavenging activity of merged fractions of *M. paleacea* obtained from first column chromatograph. NB: Value with different letters (a, b, c, d etc) differ significantly ($p < 0.05$) from each other by Duncan's Multiple Test (DMRT).

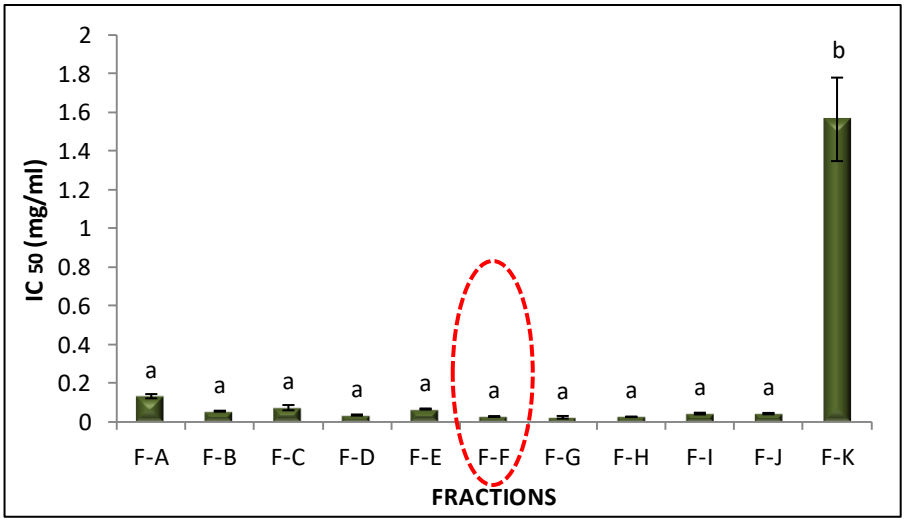


Fig 5.7.6: ABTS^{+} scavenging activity of merged fractions of *M. paleacea* obtained from first column chromatograph. NB: Value with different letters (a, b, c, d etc) differ significantly ($p < 0.05$) from each other by Duncan's Multiple Test (DMRT).

NB: Bar within the ellipse indicates highest DPPH$^{\cdot}$ and ABTS^{+} activity.

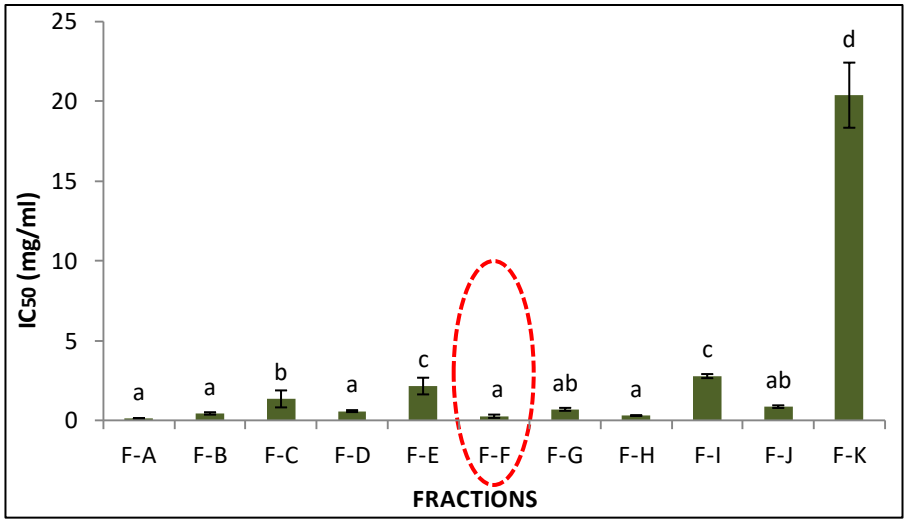


Fig 5.7.7: Anti-diabetic activity (α-glucosidase inhibitory) of merged fractions of *M. paleacea* obtained from first column chromatograph. NB: Value with different letters (a, b, c, d etc) differ significantly ($p < 0.05$) from each other by Duncan's Multiple Test (DMRT).

NB: Bar within the ellipse indicates highest anti-diabetic activity

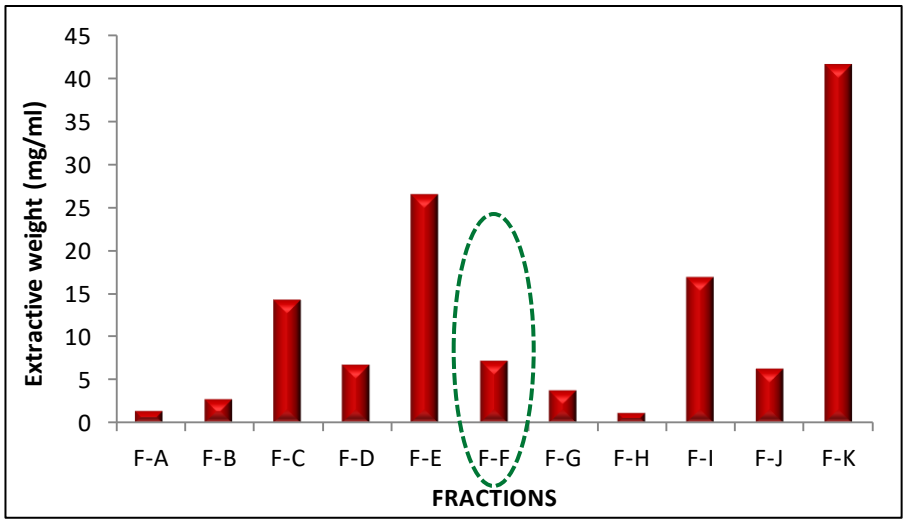


Fig 5.7.8: Extractive yield of the merged fractions of *M. paleacea* obtained from first column chromatograph

NB: Bar within the ellipse indicates highest extractive yield

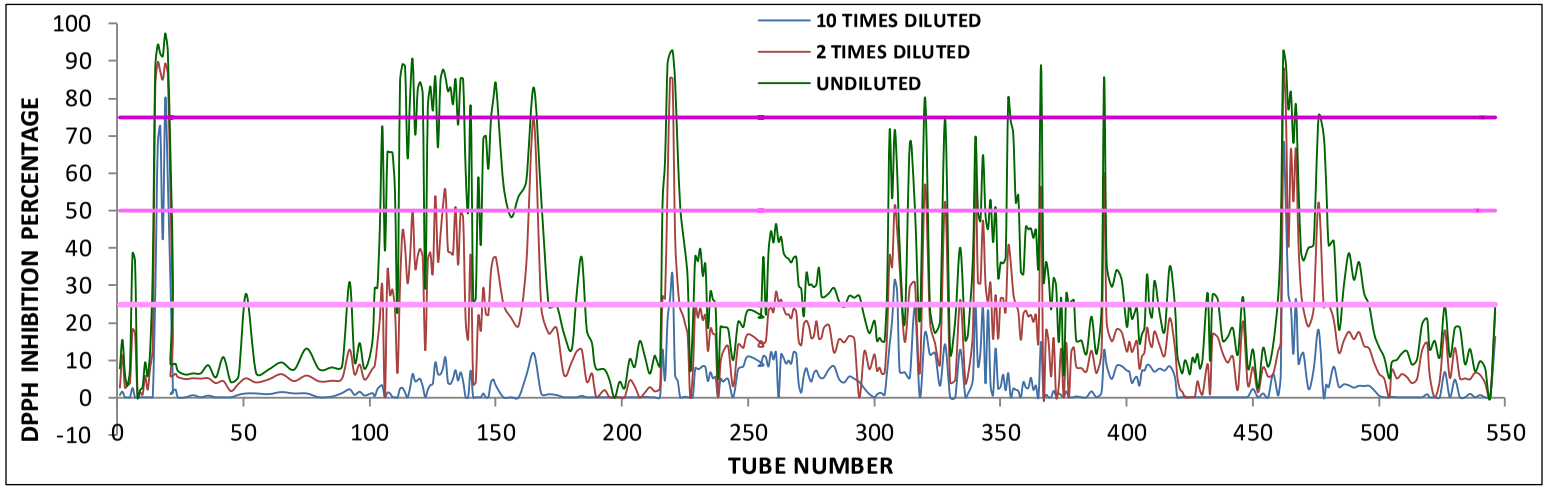


Fig 5.7.9 : DPPH˙ scavenging activity of fractions of *M. paleacea* obtained after second column chromatography

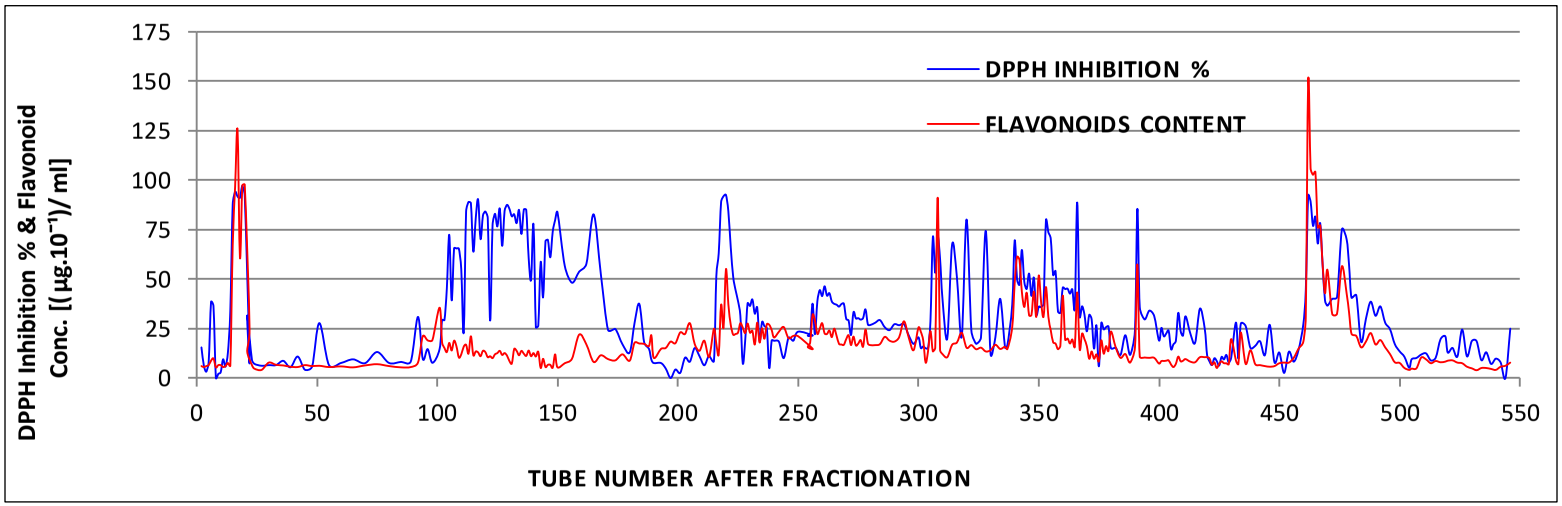


Fig 5.7.10: DPPH˙ scavenging activity and flavonoid content of fractions of *M. paleacea* obtained after second column chromatography

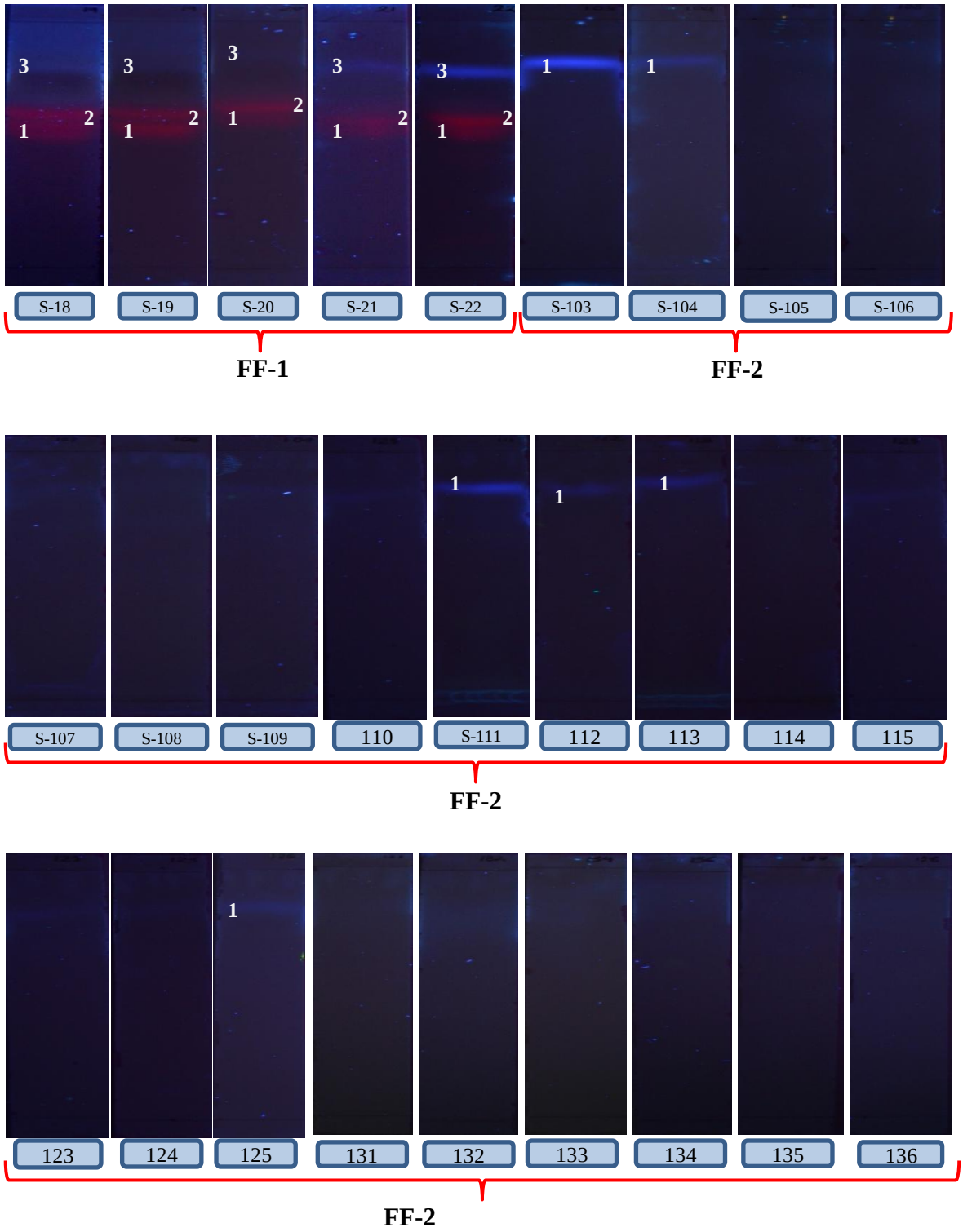


Fig 5.7.11: TLC profiling of bioactive fractions of *M. paleacea* obtained from second column chromatograph

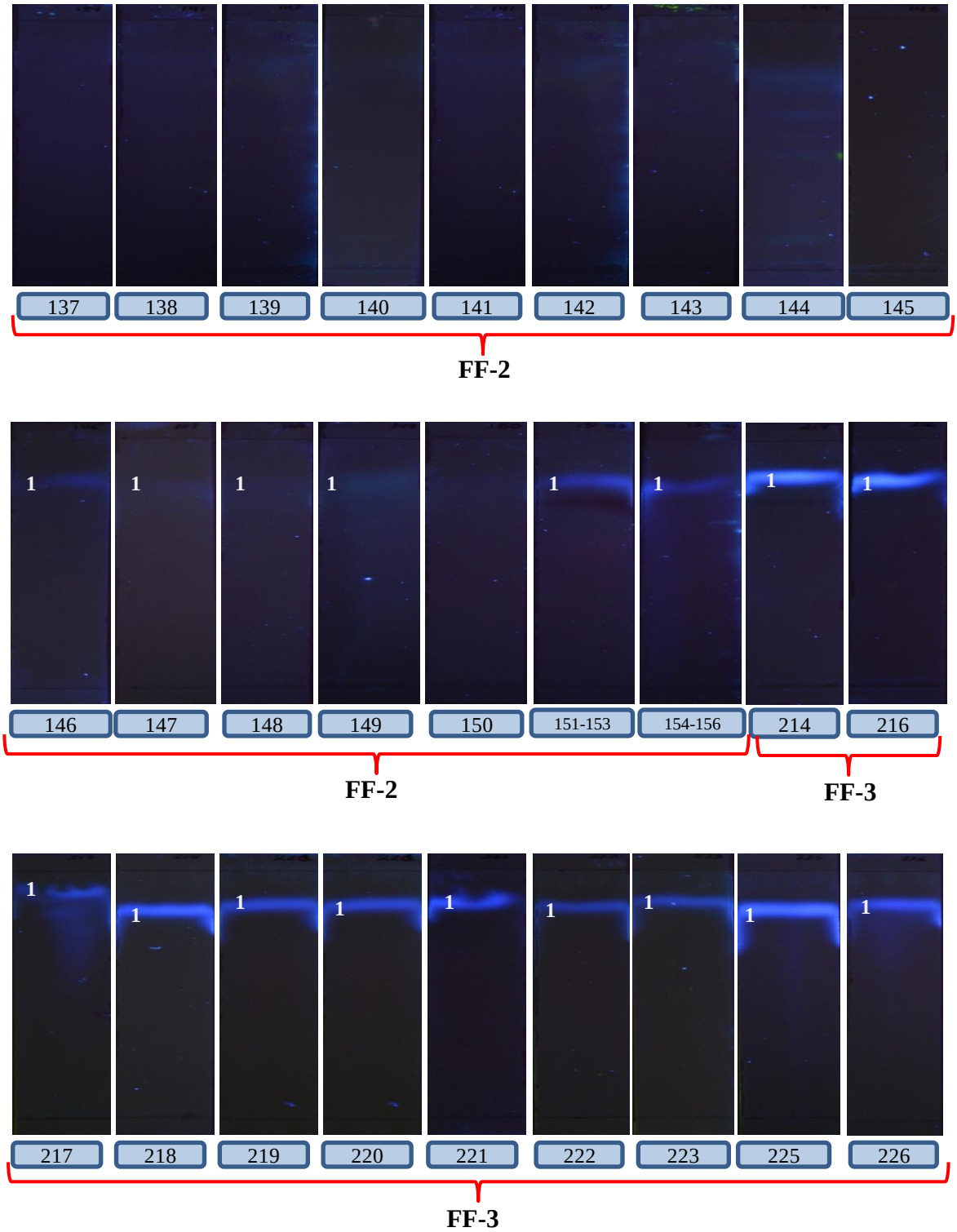


Fig 5.7.11: TLC profiling of bioactive fractions of *M. paleacea* obtained from second column chromatograph (Continuation)

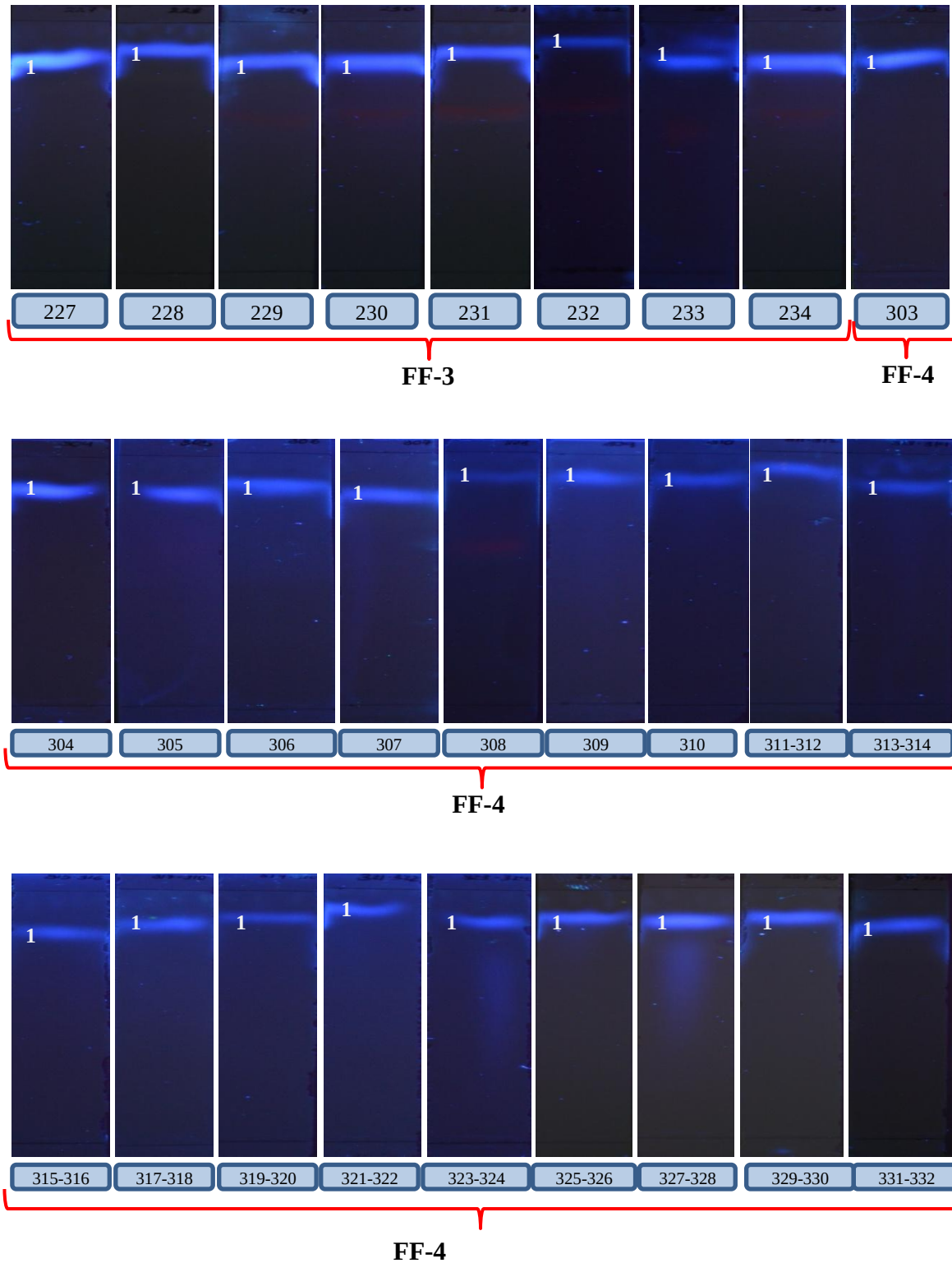
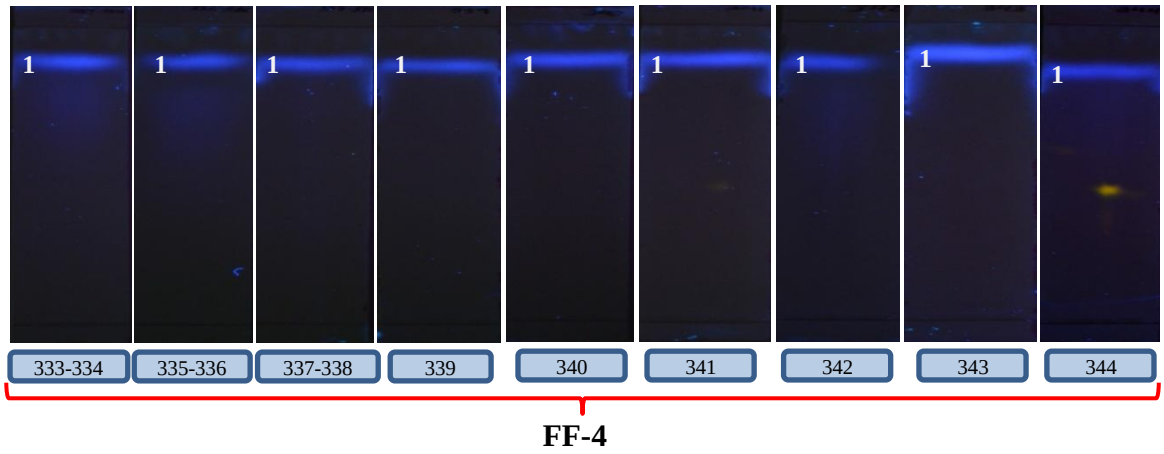
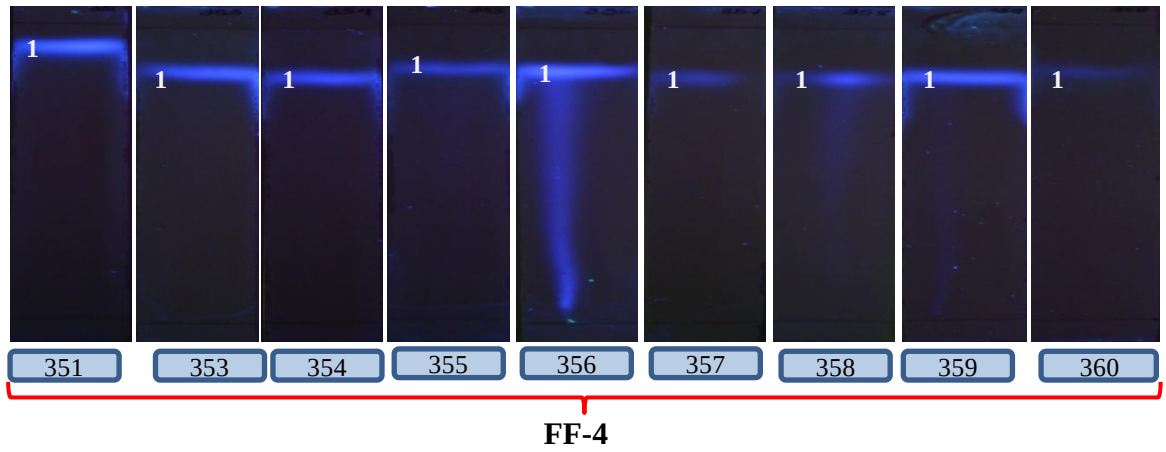


Fig 5.7.11: TLC profiling of bioactive fractions of *M. paleacea* obtained from second column chromatograph (Continuation).





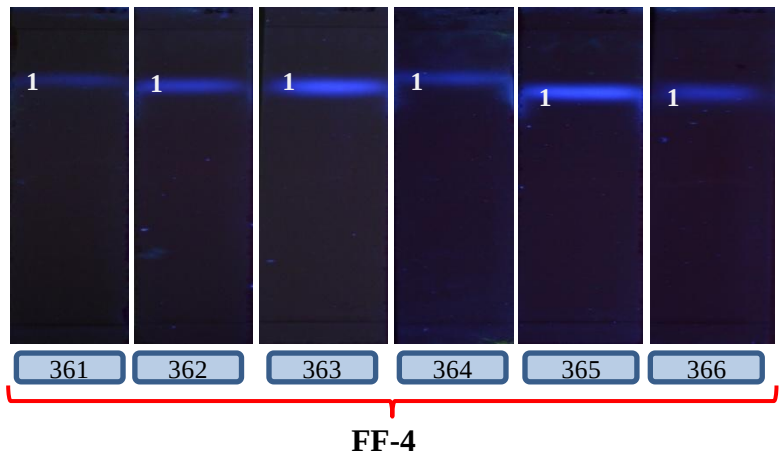
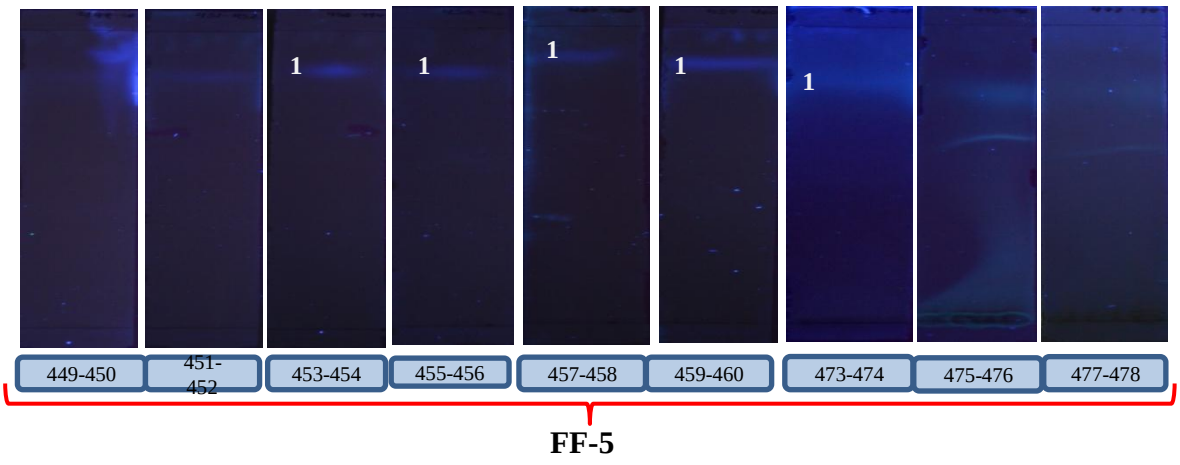


Fig 5.7.11: TLC profiling of bioactive fractions of *M. paleacea* obtained from second column chromatograph (Continuation).



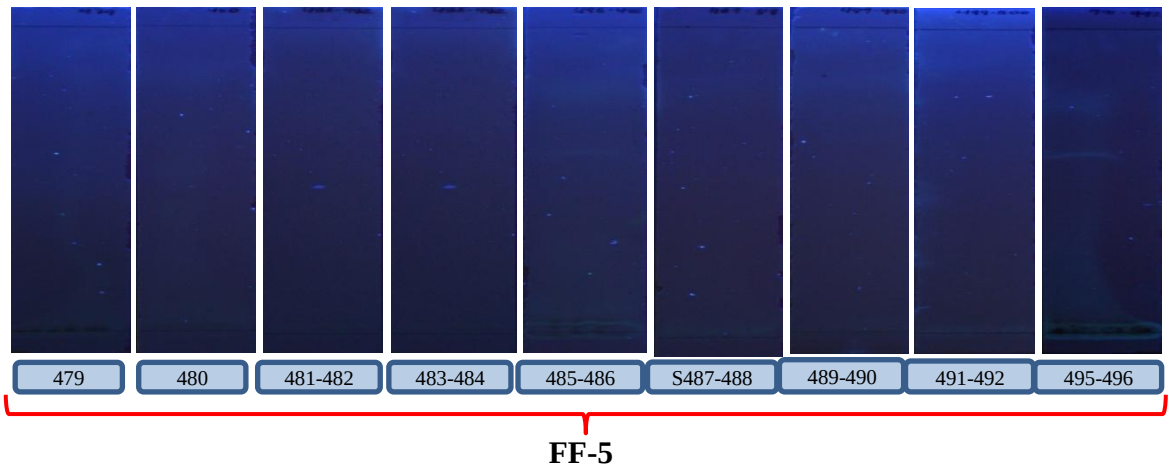


Fig 5.7.11: TLC profiling of bioactive fractions of *M. paleacea* obtained from second column chromatograph (Continuation).

Table 5.4: Retardation factors of different analytes present in bioactive fractions of *M. paleacea* obtained from second column chromatograph

Sub fractions	***Identity of bands***	***Rf***	***Sub fractions***	***Identity of bank***	***Rf***
18	1	0.54	144	1	0.13
	2	0.59		2	0.46
	3	0.79		3	0.63
				4	0.76
19	1	0.54	145	0	No bands
	2	0.59			
	3	0.79			
20	1	0.54	146	1	0.83
	2	0.59			
	3	0.79			
21	1	0.54	147	1	0.83
	2	0.59			
	3	0.79			
22	1	0.54	148	1	0.83
	2	0.59			
	3	0.79			
103	1	0.83	149	1	0.83
104	1	0.83	150	1	0.83
105	0	No bands	151-153	1	0.83
106	0	No bands	154-156	1	0.83
107	0	No bands	214	1	0.83
108	0	No bands	216	1	0.83
109	0	No bands	217	1	0.83
110	0	No bands	218	1	0.83
111	1	0.83	219	1	0.83
112	1	0.83	220	1	0.83
113	0	No bands	221	1	0.83
114	0	No bands	222	1	0.83
115	0	No bands	223	1	0.83
123	0	No bands	224	1	0.83
124	0	No bands	225	1	0.83
125	0	No bands	226	1	0.83
131	0	No bands	228	1	0.83
132	0	No bands	229	1	0.83
133	0	No bands	230	1	0.83
134	0	No bands	231	1	0.83
135	0	No bands	232	1	0.83
136	0	No bands	233	1	0.83
137	0	No bands	234	1	0.83
138	0	No bands	303	1	0.87
139	0	No bands	304	1	0.87
140	0	No bands	305	1	0.87
141	0	No bands	306	1	0.87
142	0	No bands	307	1	0.87
143	0	No bands	308	1	0.87

Table 5.4: Retardation factors of different analytes present in bioactive fractions of *M. paleacea* obtained from second column chromatograph (continuation)

Sub fractions	***Identity of bands***	***Rf***	***Sub fractions***	***Identity of bank***	***Rf***
309	1	0.87	477-478	0	No bands
310	1	0.87	479	0	No bands
311-312	1	0.87	480	0	No bands
313-314	1	0.87	481-482	0	No bands
315-316	1	0.87	483-484	0	No bands
317-318	1	0.87	485-486	0	No bands
319-320	1	0.87	487-488	0	No bands
321-322	1	0.87	489-490	0	No bands
323-324	1	0.87	491-492	0	No bands
325-326	1	0.87	495-496	0	No bands
327-328	1	0.87	497-498	0	No bands
329-330	1	0.87	499-500	0	No bands
331-332	1	0.87			
333-334	1	0.87			
335-336	1	0.87			
337-338	1	0.87			
339	1	0.87			
340	1	0.87			
341	1	0.87			
342	1	0.87			
343	1	0.87			
344	1	0.87			
351	1	0.87			
353	1	0.87			
354	1	0.87			
355	1	0.87			
356	1	0.87			
357	1	0.87			
358	1	0.87			
359	1	0.87			
360	1	0.87			
361	1	0.87			
362	1	0.87			
363	1	0.87			
364	1	0.87			
365	1	0.87			
366	1	0.87			
449-450	0	No bands			
451-452	0	No bands			
453-454	1	0.87			
455-456	1	0.87			
457-458	1	0.87			
459-460	1	0.87			
475-476	0	No bands			

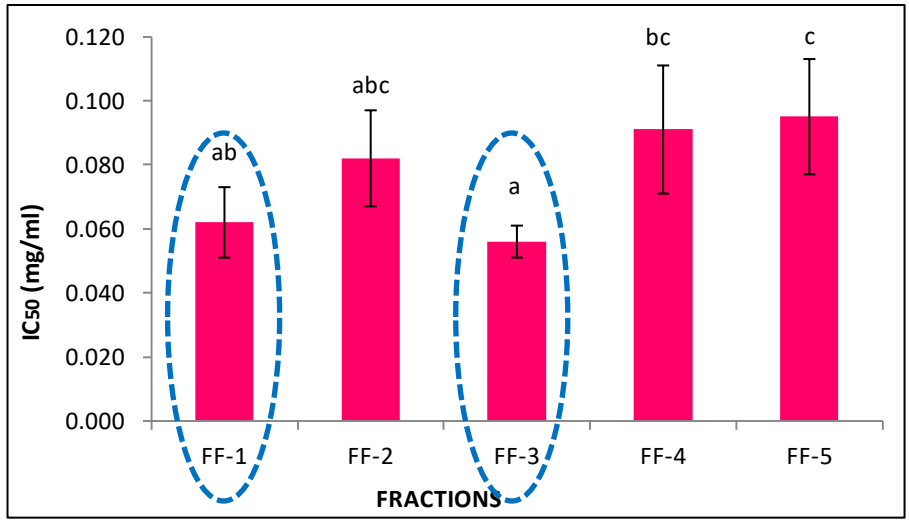


Fig 5.7.12: DPPH˙ scavenging activity of merged fractions of *M. paleacea* obtained from second column chromatograph . NB: Value with different letters (a, b, c, d etc) differ significantly ($p < 0.05$) from each other by Duncan's Multiple Test (DMRT).

NB: Bar within the ellipse indicates highest DPPH˙ scavenging activity

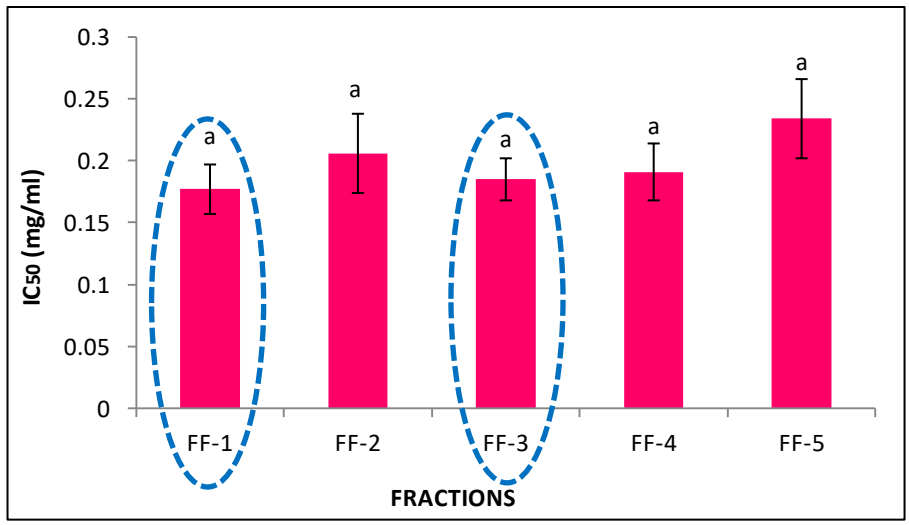


Fig 5.7.13: α-glucosidase inhibitory activity of merged fractions of *M. paleacea* obtained from second column chromatograph . NB: Value with different letters (a, b, c, d etc) differ significantly ($p < 0.05$) from each other by Duncan's Multiple Test (DMRT).

NB: Bar within the ellipse indicates highest DPPH˙ scavenging activity

and active sub fractions were merged together on the basis of their TLC profiles into five main fractions as follows: FF-1, FF-2, FF-3, FF-4 and FF-5 (Fig 5.7.11) Among five main fractions, FF-1 and FF-3 were selected on the basis of their high antioxidant and anti-diabetic activities. After that fractions FF-1 and FF-3 were processed through Gas chromatography mass spectrometry (GC-MS); the chromatogram of the bioactive fractions are presented in Fig 5.7.12 to Fig 5.7.23. The library matching (NIST Library) of mass peaks of fraction FF-1 showed the presence of nine compounds and that of fraction FF-3 showed the presence of nine phyto compounds. Presence of phytochemicals such as phenols, flavonoids, terpenes, alkanes and flavones were found in FF-1 and FF-3 which might be responsible for the antioxidant and anti-diabetic activities displayed by *M. paleacea* (Table 5.5 and Table 5.6). Network pharmacology is an important approach in drug development and discovery process. After the identification of bioactive compound through GC-MS analysis and NIST Library matching, the underlying mechanism of action of these compounds at cellular level was studied by network pharmacology followed by KEGG Metabolic Pathway analysis.

Phenol, 2, 6 bis (1,1-dimethylethyl)-4-ethyl present in fraction FF-3 is a phenylpropanes that are active antioxidants and anti-fungal agents (Varsha *et al.*, 2015). Different forms of terpenes (mono, di, tri, sesqui) were also found to be present in the analyzed fractions. β-Caryophyllene, α-Myrcene, 3-Cyclohexen-1-ol,4-methyl-1-(1-methylethyl), Phytol, Pimaric acid, Palustric acid were detected in the analyzed fractions. Phyto-compounds like 4-H-Benzopyran-4-one,5-hydroxyl-7-methoxy-2-phenyl; 4H-1-Benzopyran-4-one,5-hydroxy-2-(-4 methoxypheny) and 4H-1-Benzopyran-4-one, 7-hydroxy-2 phenyl were also detected which belongs to the class of compound flavonoids.. Apart from this, hydrocarbons like 1-docosene and 1-octadecene were found to be present in the active fractions FF-1 and FF-3. 1-docosene and 1-octadecene detected in the extracts are reported to have important pharmacological activities (Kuppuswamy *et al.*, 2013). Other phytochemical that were present in the fractions FF-1 and FF-3 were 2-Cyclohexen-3,6-diol-1-one, 2-tetradecanoyl; Methyl 2-(4-hydroxy-3-isopropylbenzoyl) benzoate; 2H-1-benzopyran-2-one,7 (diethylamino); Estra-1,3,5 (10), 6-tetraene-3,17-diol, diacetate and Androstan-4-en,3,17-diol-11-one, 9-thiocyanato. No biological activities have been reported for these compounds till date. For drug discovery and development process network pharmacology plays a very important role. After the identification of bioactive compound through GC-MS analysis and NIST Library matching, the underlying mechanism of action of

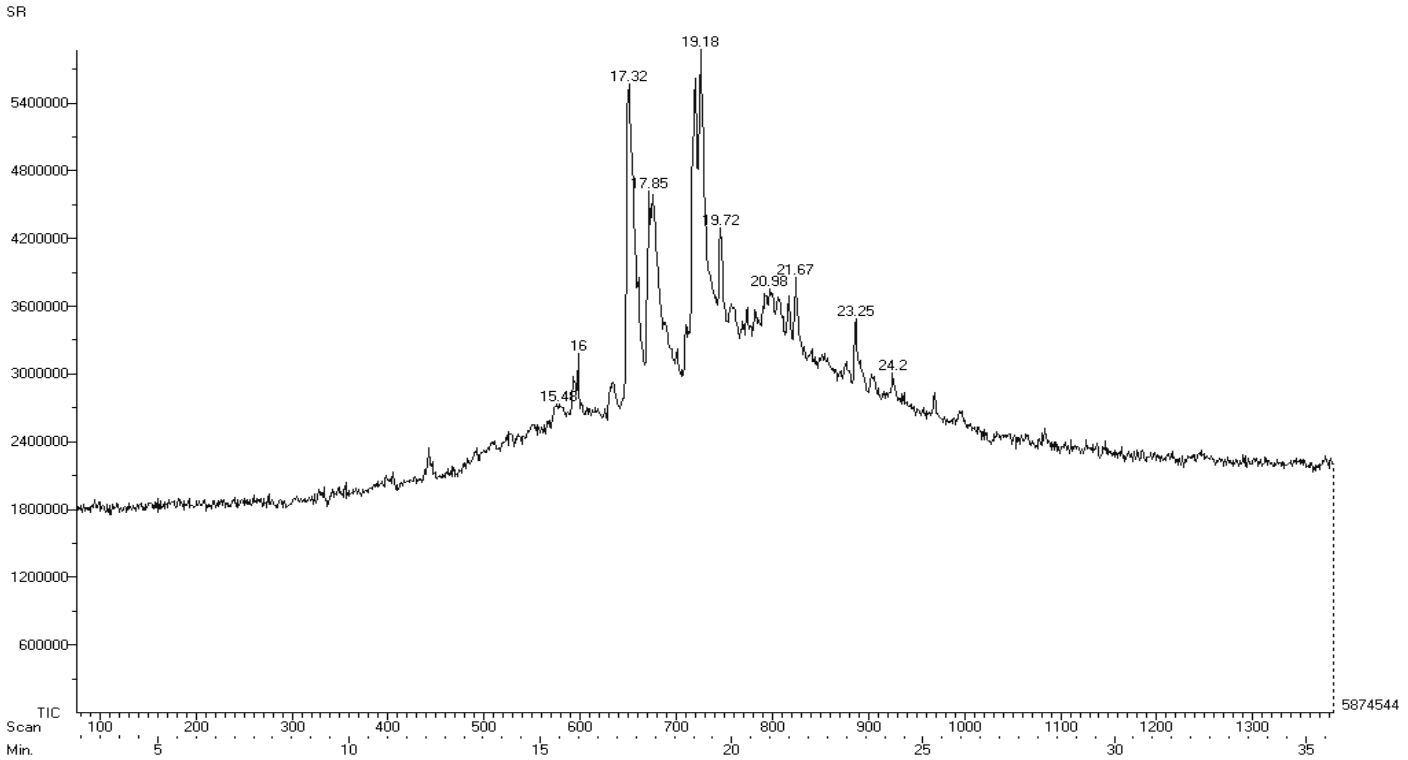


Fig 5.7.14: HR- GCMS scanning chromatogram of fraction FF-1 of *M. paleacea*

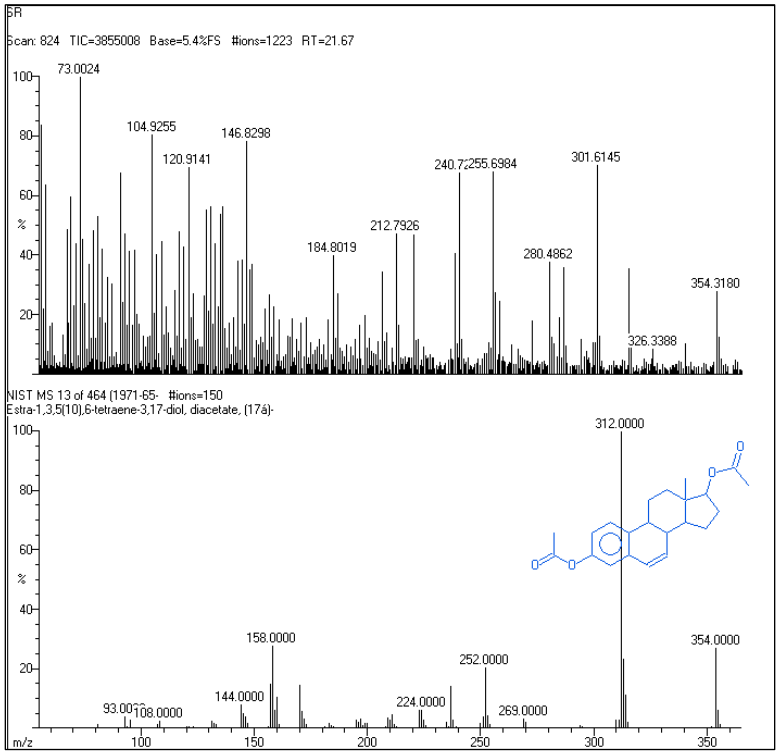


Fig 5.7.15: Structure and MS spectrum of (17á), Estra-1,3,5 (10), 6- tetraene- 3,17- diol diacetate compared with NIST Library Spectral Database

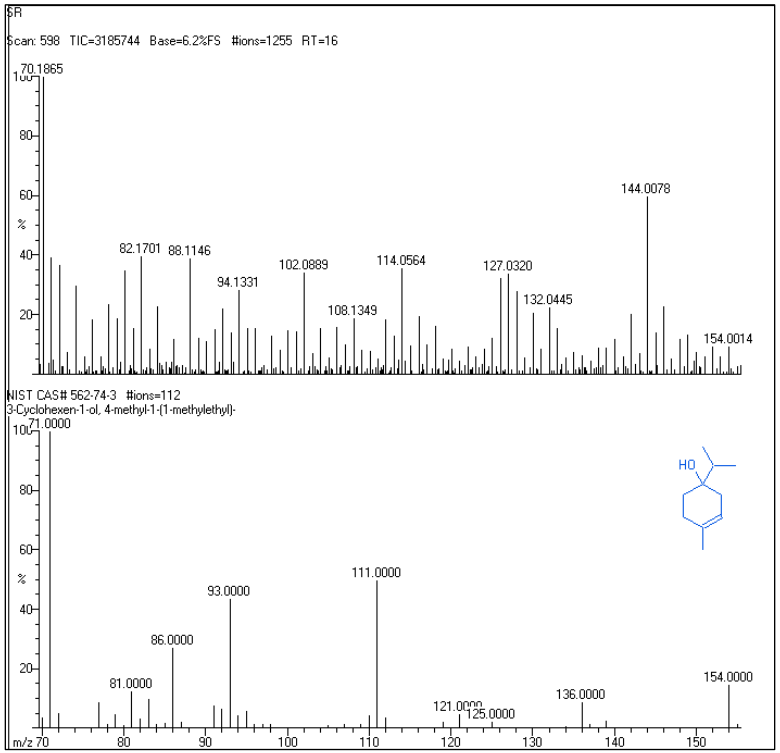


Fig 5.7. 16: Structure and MS spectrum of 4-methyl -1-(1-methylethyl) 3- Cyclohexen -1 ol compared with NIST Library Spectral Database

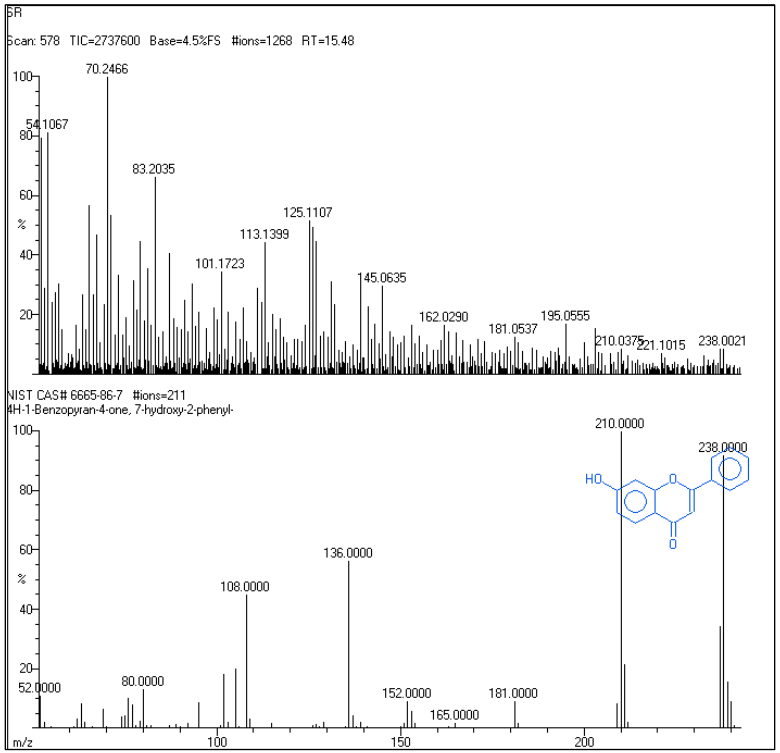


Fig 5.7.17: Structure and MS spectrum of 7-hydroxy-2-phenyl 4H-1- Benzopyran-4- one compared with NIST Library Spectral Database

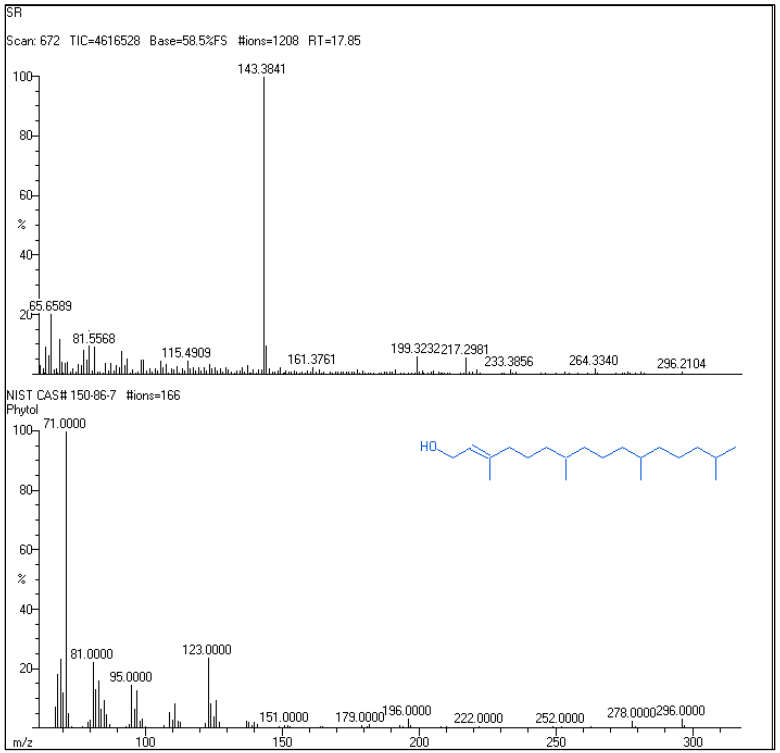


Fig 5.7.18: Structure and MS spectrum of Phytol compared with NIST Library Spectral Database

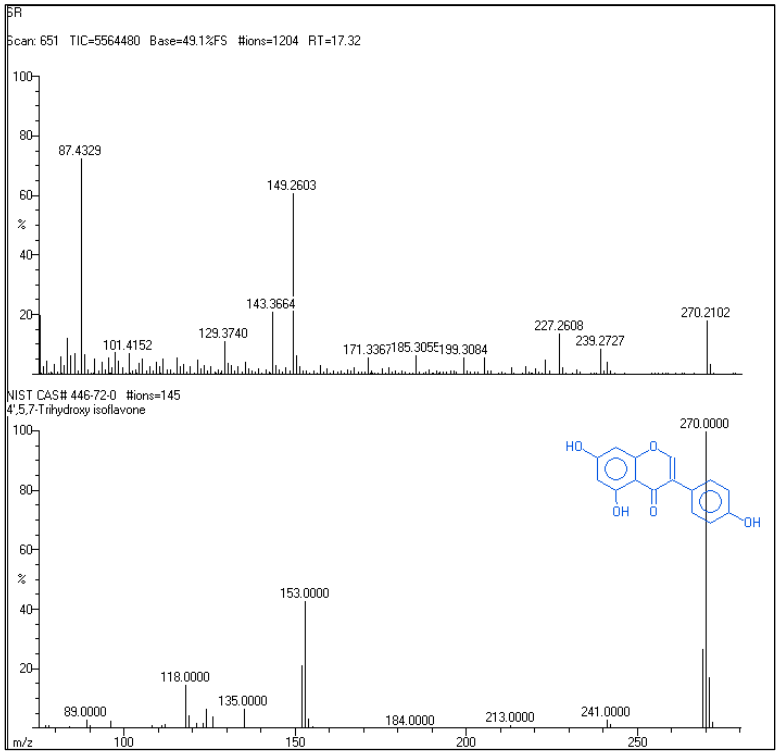


Fig 5.7.19 : Structure and MS spectrum of 4', 5, 7-Trihydroxy isoflavone compared with NIST Library Spectral Database

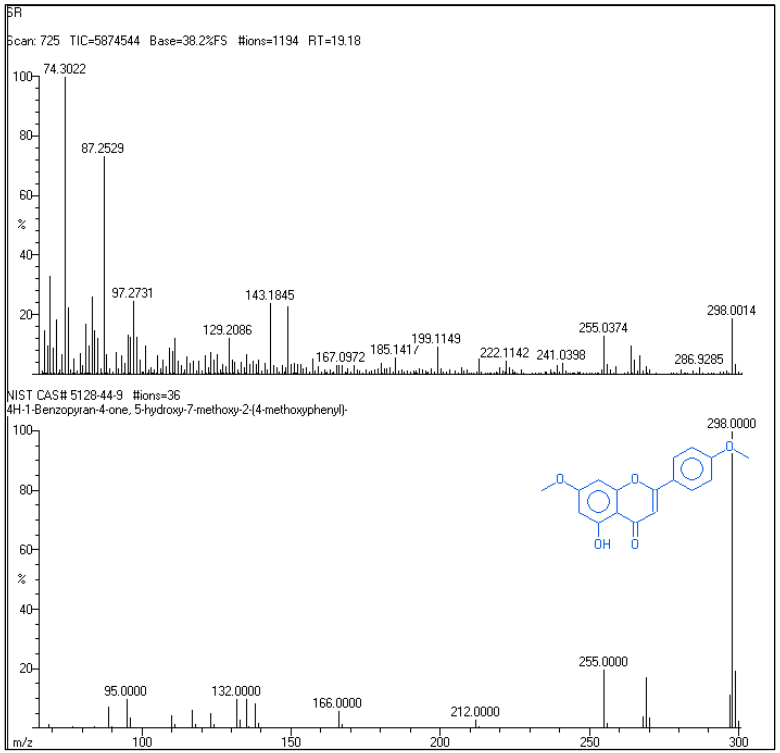


Fig 5.7.20: Structure and MS spectrum of 5- hydroxy-7-methoxy-2-(4-methoxyphenyl) 4H-1- benzopyran-4-one compared with NIST Library Spectral Database

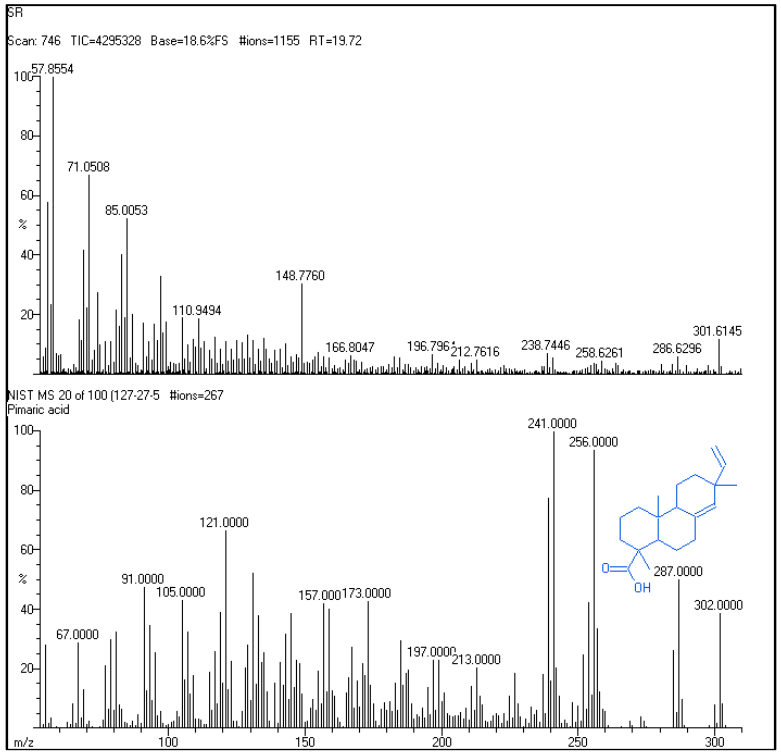


Fig 5.7.21: Structure and MS spectrum of Pimaric acid compared with NIST Library Spectral Database

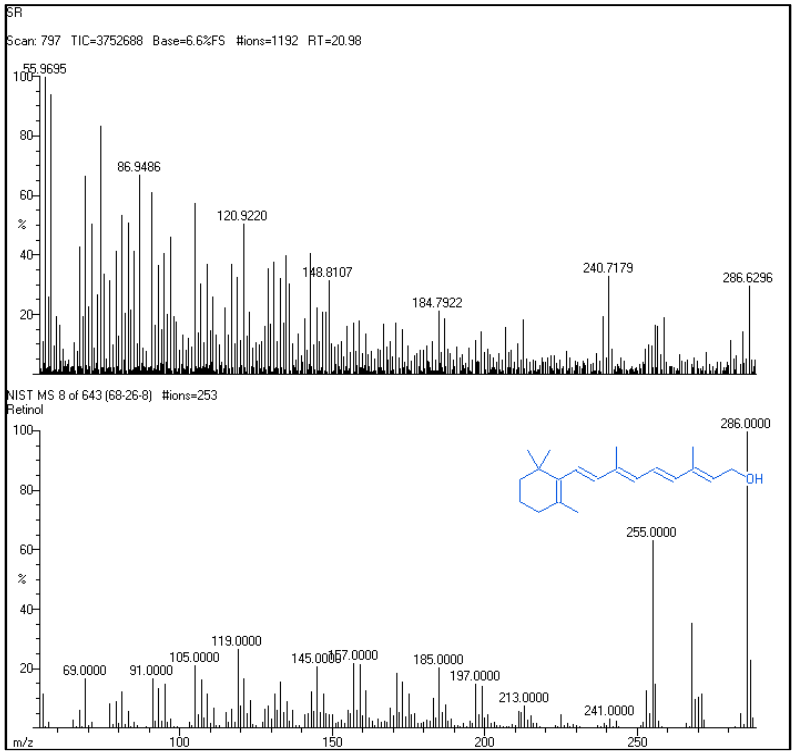


Fig 5.7.22: Structure and MS spectrum of Retinol compared with NIST Library Spectral Database

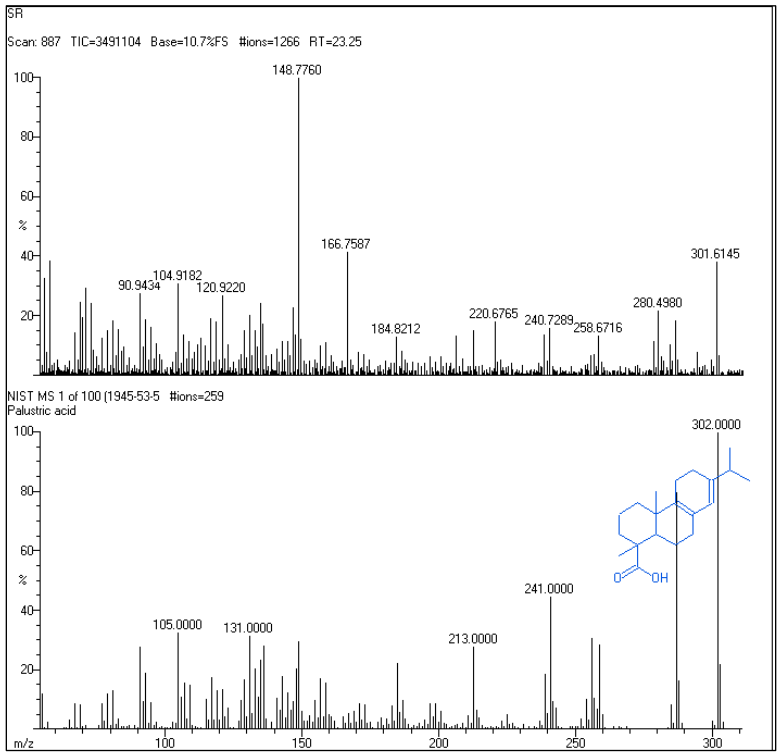


Fig 5.7.23: Structure and MS spectrum of Palustric acid compared with NIST Library Spectral Database

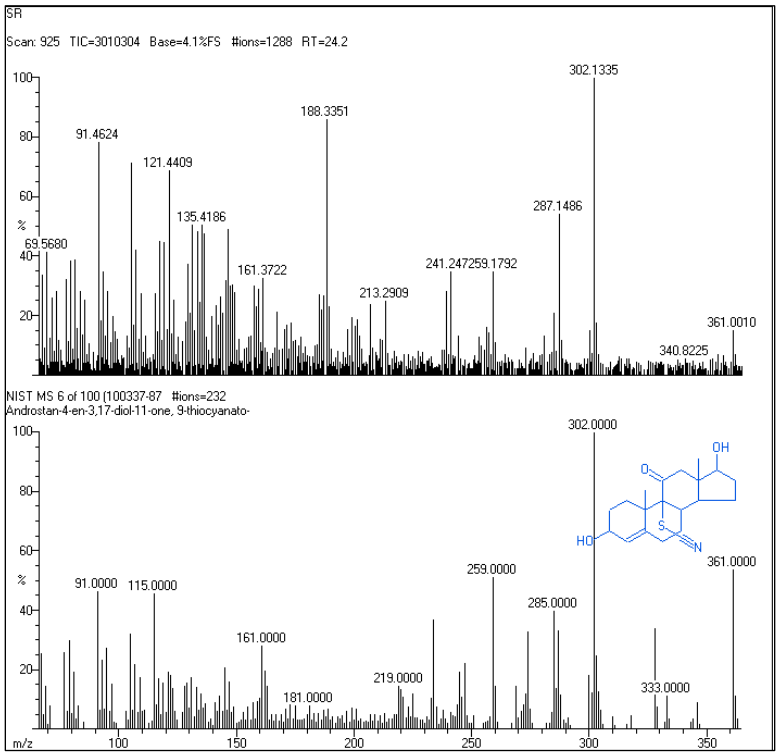


Fig 5.7.24: Structure and MS spectrum of 9-thiocyanato Androstan-4-en-3,17- diol- 11-one compared with NIST Library Spectral Database

Table 5.5: List of phytochemicals present in fraction FF1 of *M. paleacea* and their bioactivities

Sl. no	***Compound name***	***RT***	***Molecular formula***	***Molecular weight***	***Percentage amount***	***Compound nature***	***Bioactivity***
1	(17á), Estra-1,3,5 (10), 6-tetraene-3,17-diol,diacetate	21.67	$C_{22}H_{26}O_4$	354.446	11%	Not found	No activities reported
2	4-methyl-1 -(1-methylethyl) 3-cyclohexen-1-ol	16	$C_{10}H_{18}O$	154.249	9%	Monoterpenoids.	antioxidant (Chung IM, 2011)
3	7-hydroxy-2 phenyl 4H-1-Benzopyran-4-one	15.48	$C_{15}H_{10}O_3$	238.242	7%	Isoflavone	estrogenic activity (Vitale DC, 2013)
4	Phytol	17.85	$C_{20}H_{40}O$	296.539	13%	Diterpene	antinociceptive (Santos *et* al., 2013) antioxidant (Santos *et* al., 2013
5	4',5,7 trihydroxy isoflavone	17.32	$C_{15}H_{10}O_5$	270.24	15%	Isoflavone	antioxidant (Dowling *et al*., 2010)
6	5-hydroxy-7-methoxy-2-(4-methoxyphenyl)-4H-1-Benzopyran-4-one	19.18	$C_{17}H_{14}O_5$	298.290	16%	flavonoid	estrogenic activity (Vitale DC, 2013); antioxidant (Dowling *et al*., 2010)
7	Pimaric acid	19.72	$C_{20}H_{30}O_2$	302.458	12%	Diterpene	antimicrobial (Ali *et al*., 2012)
8	Palustric acid	23.25	$C_{20}H_{30}O_2$	302.458	10%	Diterpene	anti-microbial activity (Savluchinske-Feio *et al*., 2006)
9	9-thiocyanato Androstan-4-en-3,17-diol-11-one	24.2	$C_{20}H_{27}NO_3S$	361.498	8%	Not found	No activities reported

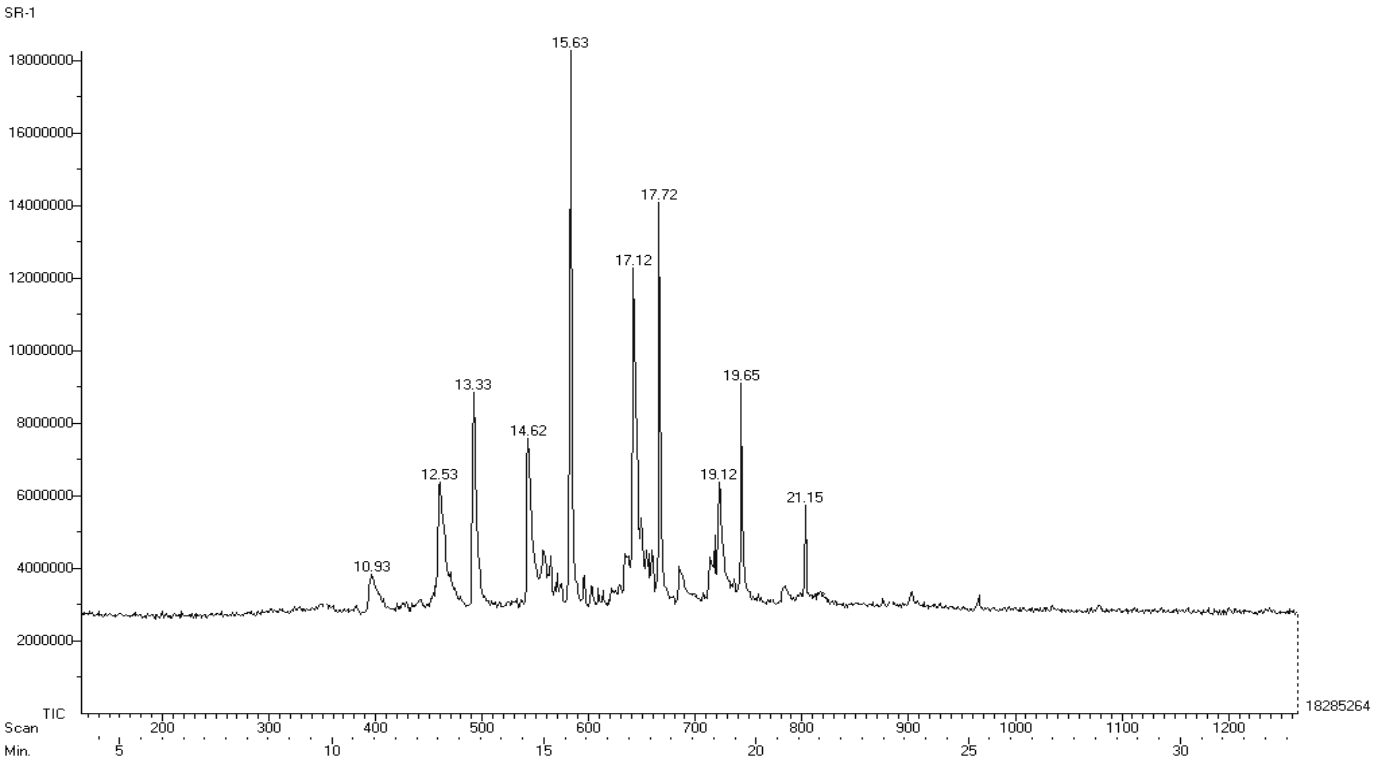


Fig 5.7.25: HR- GCMS scanning chromatogram of fraction FF-3 of *M. paleacea*

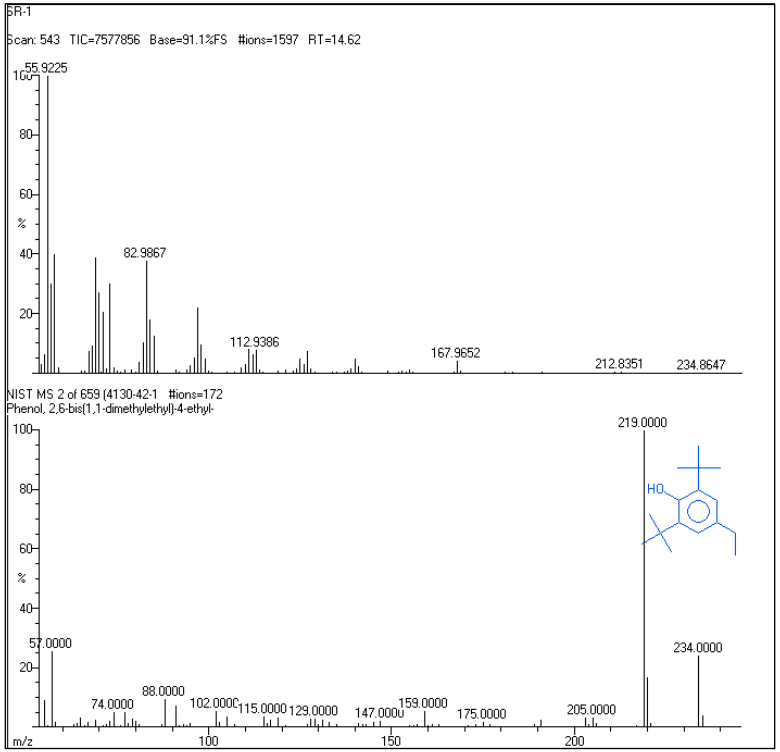


Fig 5.7.26: Structure and MS spectrum of 2,6-bis (1,1-dimethylethyl)-4- ethyl Phenol compared with NIST Library Spectral Database

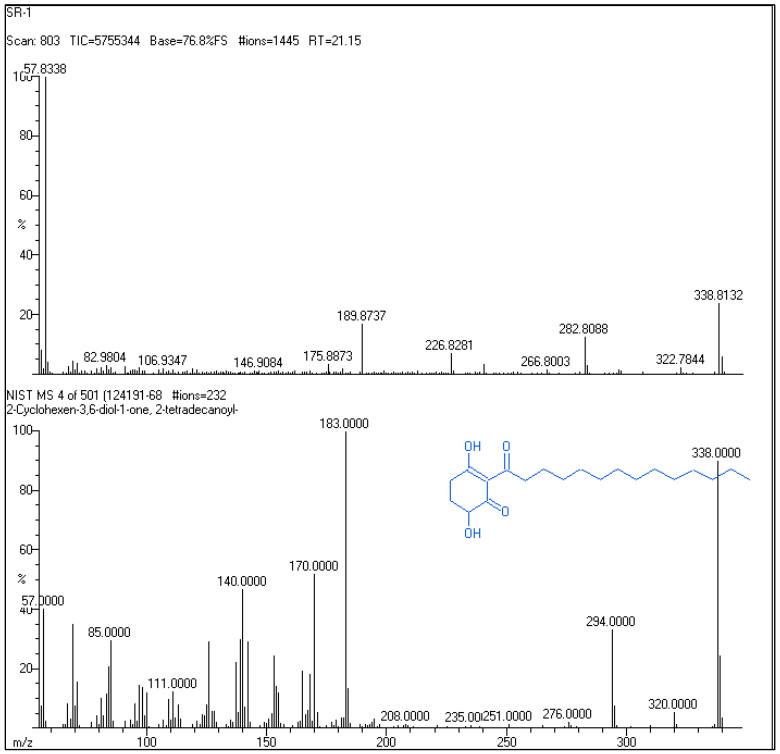


Fig 5.7.27: Structure and MS spectrum of 2-tetradecanoyl-2-Cyclohexen-3,6-diol-1-one compared with NIST Library Spectral Database

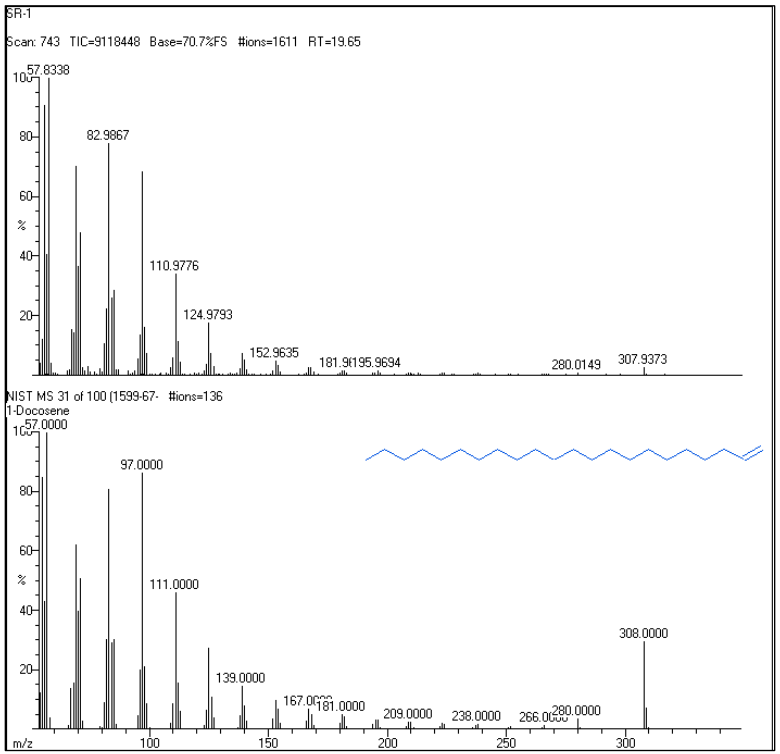


Fig 5.7.28: Structure and MS spectrum of 1-Docosene compared with NIST Library Spectral Database

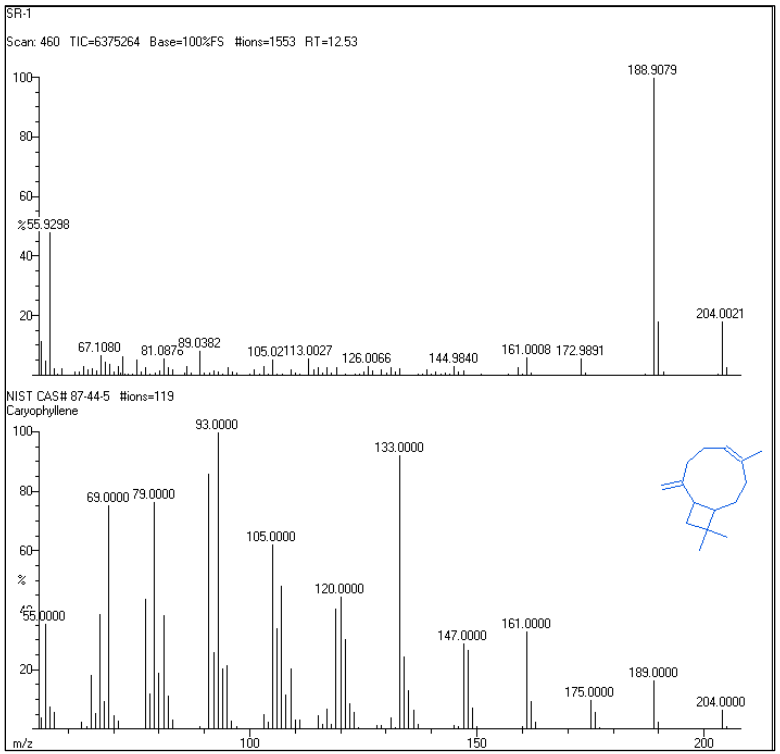


Fig 5.7.29: Structure and MS spectrum of Caryophyllene compared with NIST Library Spectral Database

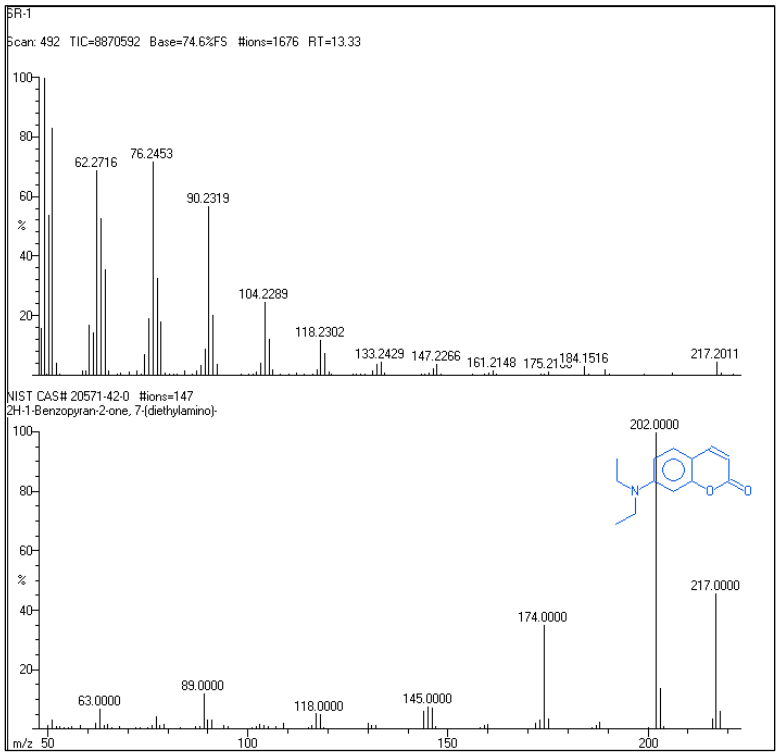


Fig 5.7.30: Structure and MS spectrum of 7-(diethylamino) -2H-1-Benzopyran-2-one compared with NIST Library Spectral Database

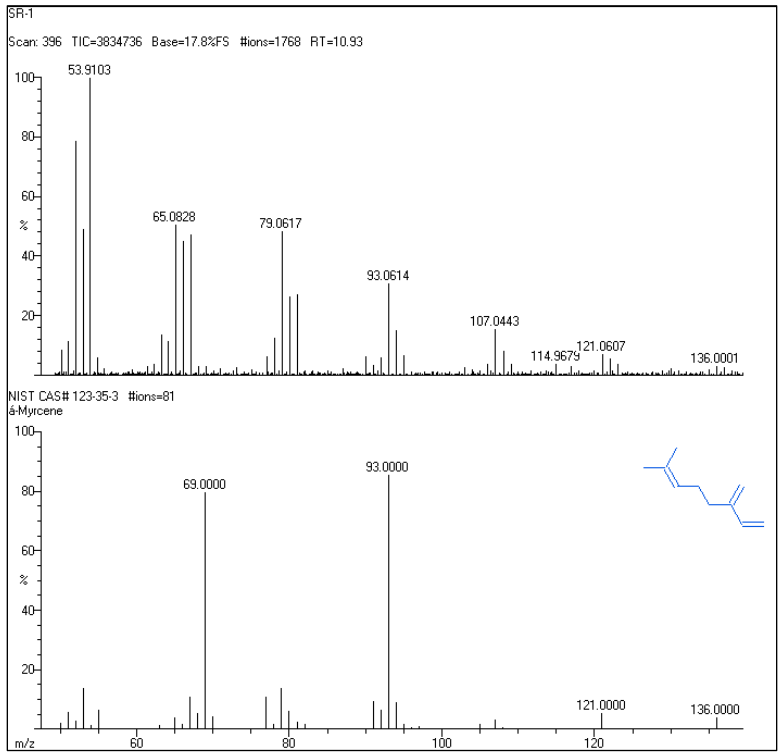


Fig 5.7.31: Structure and MS spectrum of α-Myrcene compared with NIST Library Spectral Database

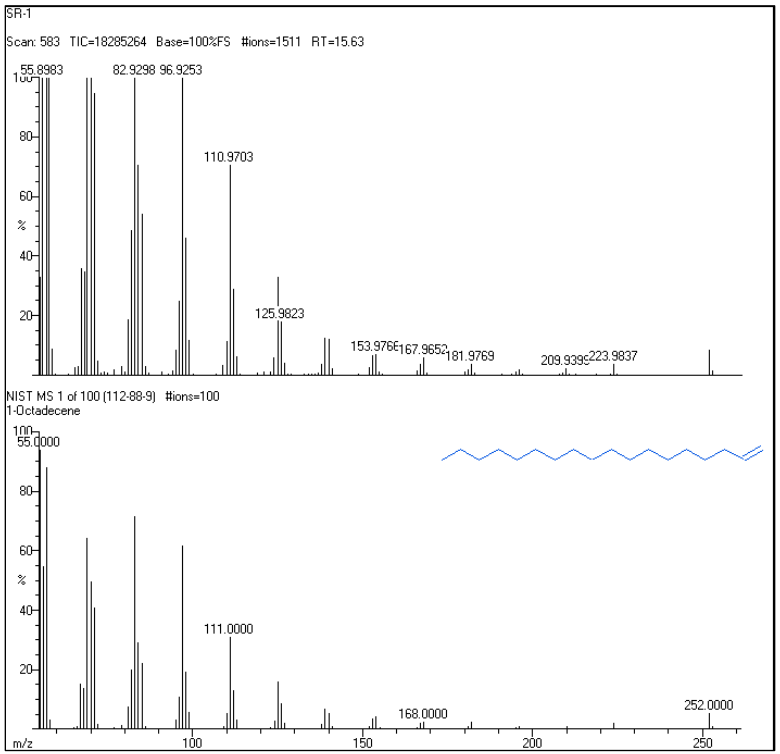


Fig 5.7.32: Structure and MS spectrum of 1-Octadecene compared with NIST Library Spectral Database

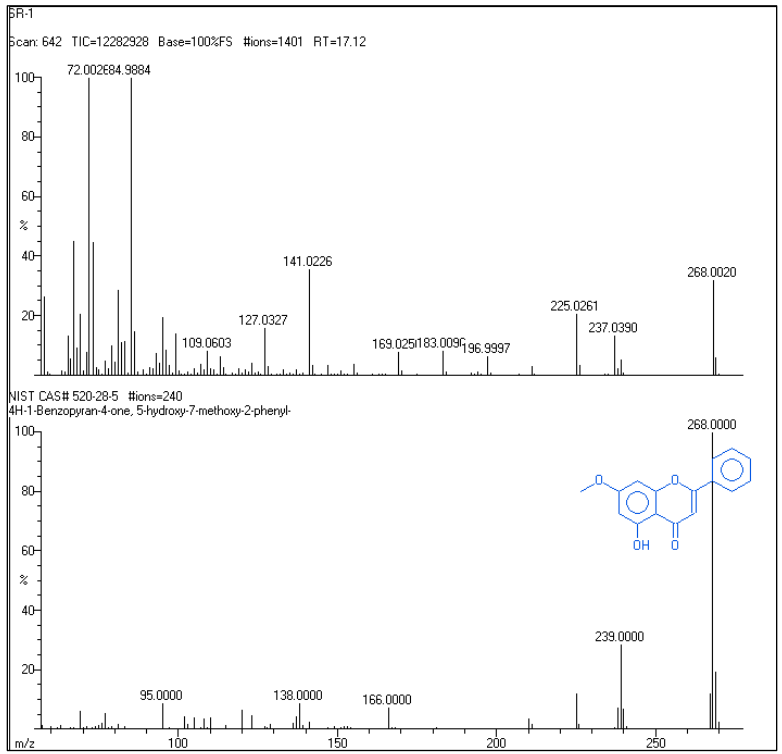


Fig 5.7.33: Structure and MS spectrum of 4H-1-Benzopyran-4-one, 5-hydroxy-7-methoxy-2-phenyl compared with NIST Library Spectral Database

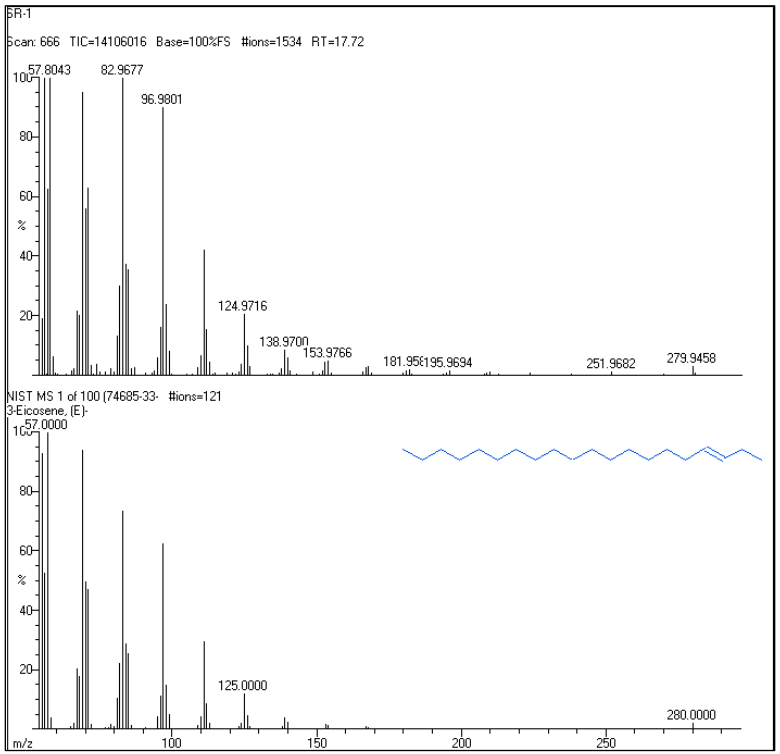


Fig 5.7.34: Structure and MS spectrum of (9E), 3-Eicosene compared with NIST Library Spectral Database

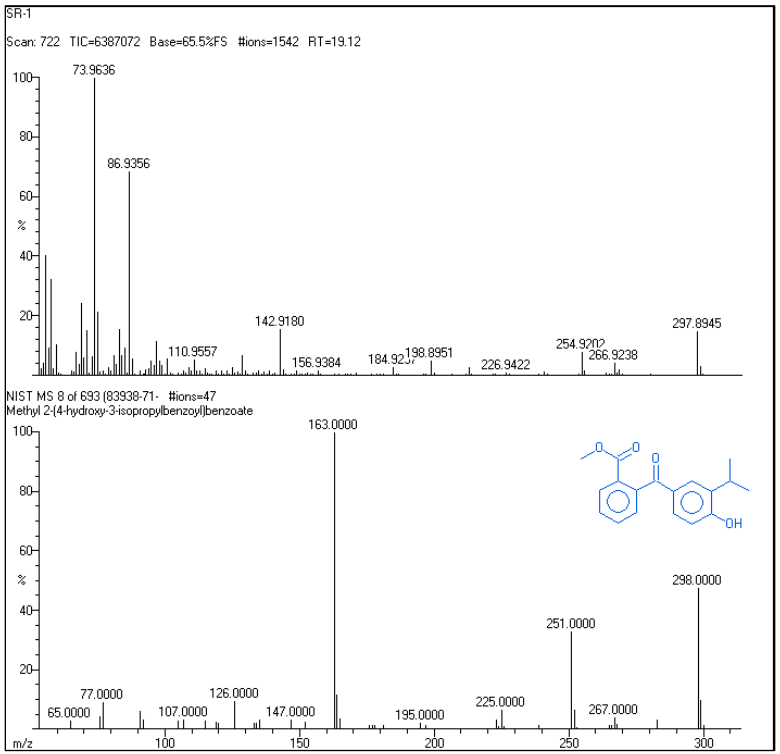


Fig 5.7.35: Structure and MS spectrum of Methyl 2-(4-hydroxy-3-isopropyl) benzoate compared with NIST Library Spectral Database

Table 5.6: List of phytochemicals present in fraction FF3 of *M. paleacea* and their bioactivities

Sl no	***Compound name***	***RT***	***Molecular formula***	***Molecular weight***	***Percentage amount***	***Compound nature***	***Bioactivity***
1	2,6-bis (1,1-dimethylethyl)-4-ethyl phehol	14.62	$C_{16}H_{26}O$	234.377	8%	Phenylpropanes	Antioxidant (KKet al., 2015
2	2-tetradecanoyl-2-Cyclohexen-3,6-diol-1-one	21.15	Not found	Not found	6%	Not found	No activities reported
3	1-docosene	19.65	$C_{22}H_{44}$	308.594	10%	aliphatic hydrocarbons.	No activities reported
4	beta-Caryophyllene	12.53	$C_{15}H_{24}$	204.357	7%	sesquiterpene	anticancer activities
5	7-(diethylamino) 2H-1-Benzopyran-2-one	13.33	$C_{15}H_{17}O_3$	259.305	10%	Not found	No activities reported
6	Myrecene	10.93	$C_{10}H_{16}$	136.238	4%	Myrcene	anti-inflammatory properties [22] and has been widely used in pharmaceutical and cosmetic product12)
7	1-octadecene	15.63	$C_{18}H_{36}$	252.486	20%	alkane	anti-bacterial (20,21)
8	5-hydroxy-7-methoxy-2-phenyl 4H-1-benzopyran 4 one	17.12	$C_{18}H_{12}O_4$	268.264	13%	flavonoids	anti-mitotic (Hadjeri et al., 2004), anti-bacterial (Tracanna et al., 2010)
9	3-Eicosene	17.72	$C_{20}H_{40}$	280.531	15%	aliphatic hydrocarbons	anti-microbial (Kuppuswamy et al., 2013)
10	Methyl 2-(4-hydroxy-3-isopropyllbenzoyl) benzoate	19.12	$C_8H_7NO_5$	197.146	7%	Not found	No activities reported

these compounds at cellular level was studied by network pharmacology followed by KEGG Metabolic Pathway analysis.

5.7.1 Network pharmacology

5.7.1a Identification of phytochemical compound

Active phytochemicals present on *M. paleacea* were identified by Gas Chromatography-Mass Spectrometry (GC-MS) of bioactive fractions (FF-1 and FF-3) obtained from column chromatography. In total 19 phyto-compounds have been identified from the two bioactive fractions of *M. paleacea*. Their name, unique ID and canonical smiles are presented in table 5.7 and table 5.8.

5.7.1b Protein target compilation

The therapeutic potential of any phyto-compound depends on the number of proteins that the particular compound targets. To determine the therapeutic potential of identified compounds a network analysis of phytochemicals and their protein targets was performed. In total 81 unique human proteins was targeted by the phytochemicals. Among ninteen phytochemicals under study, human protein was potentially targeted by fifteen phytochemicals. All the protein targets of studied phytochemicals were represented in table 5.9 along with their gene ID. Interaction between target proteins of bioactive phytochemicalas by suing STRING Interactome Database is presented figure 5.7.3

Table 5.7: List of phytochemicals present in FF1 of *M. paleacea* and their canonical smile and Pubchem ID

Sl. No	*Phytochemical*	*Canonical smile*	*PubChem CID*	*Compound Class*
1	(17α)-estra-1,3,5(10),6-tetraene-3,17-diol, diacetate	[H]C12CCC3C(C=CC4=C3C=CC(OC(C)=O)=C4)C1CCC2OC(C)=O	Not found	Not found
2	4-methyl-1 -(1-methylethyl), 3-cyclohexen-1-ol	CC1=CCC(CC1)(C(C)C)O	11230	Monoterpenoids
3	7-hydroxy-2-phenyl, 4H-1-Benzopyran-4-one	C1=CC=C(C=C1)C2=CC(=O)C3=C(O2)C=C(C=C3)O	5281894	Isoflavone
4	Phytol	CC(C)CCCC(C)CCCC(C)CCCC(=CCO)C	5280435	Diterpene
5	4',5,7-Trihydroxy isoflavone	C1=CC(=CC=C1C2=COC3=CC(=CC(=C3C2=O)O)O)O	5280961	Isoflavone
6	5-hydroxy-2-(4-methoxyphenyl) 4-H-1 Benzopyran-4-one	COC1=CC=C(C=C1)C2=CC(=O)C3=C(C=C(C=C3O2)OC)O	5281601	Flavonoid
7	Pimaric acid	CC1(CCC2C(=C1)CCC3C2(CCCC3(C)C(=O)O)C)C=C	220338	Diterpene
8	Retinol	CC1=C(C(CCC1)(C)C)C=CC(=CC=CC(=CCO)C)C	445354	Not found
9	Palustric Acid	CC(C)C1=CC2=C(CC1)C3(CCCC(C3CC2)(C)C(=O)O)C	443613	Diterpene
10	9-thiocyanato, androstan-4-en 3,17-diol-11-one	Not found	Not found	Not found

Table 5.8: List of phytochemicals present FF3 of *M. paleacea* and their canonical smile and Pubchem ID

Sl. no	***Phytochemical***	***Canonical smile***	***PubChem CID***	***Compound Class***
1	2,6-bis(1,1-dimethylethyl)-4-ethyl-Phenol	CCC1=CC(=C(C(=C1)C(C)(C)C)O)C(C)(C)C	20087	Phenylpropanes
2	2-tetradecanoyl-2-Cyclohexen-3,6-diol-1-one	Not found	Not found	Not found
3	1-Docosene	CCCCCCCCCCCCCCCCCCCCC=C	Not found	Aliphatic hydrocarbons
4	beta-Caryophyllene	CC1=CCCC(=C)C2CC(C2CC1)(C)C	5281515	Sesquiterpene
5	7-diethylamino,2H-1-Benzopyran-2-one	CCN(CC)C1=CC2=C(C=C1)C=CC(=O)O2	88598	Not found
6	a-Myrcene	CC(=CCCC(=C)C=C)C	Not found	Myrcene
7	1-Octadecene	CCCCCCCCCCCCCCCCCC=C	Not found	Alkane
8	5 hydroxy-7-methoxy-2-phenyl, 4H-1-Benzoyran-4-one	COC1=CC(=C2C(=C1)OC(=CC2=O)C3=CC=CC=C3)O	5281954	Flavonoids
9	3-Eicosene	Not found	Not found	Aliphatic hydrocarbons
10	Methyl 2-(4-hydroxy-3-isopropyllbenzoyl) benzoate	Not found	Not found	Not found

Table 5.9: List of human proteins targeted by the phytochemicals

Sl.no.	***Target proteins (human)***	***Gene ID***
1	3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA)	HMGCR
2	Androgen Receptor	AR
3	Estrogen receptor	ESR1
4	Liver X receptor beta (LXRB)	NR1H2
5	LXR-alpha	NR1H3
6	12-Lipoxygenase (12-LOX)	ALOX12
7	5-lipoxygenase/FLAP	ALOX5
8	Acetylcholinesterase	ACHE
9	Adenosine receptor	ADORA1
10	Adenosine Receptor A3	CA7
11	Aldose reductase	AKR1B1
13	Arachidonate 15-lipoxygenase	ALOX15
14	Arachidonate 5-lipoxygenase	ALOX5
15	Aromatase (CYP19)	CYP19A1
16	Aryl hydrocarbon receptor	AHR
17	AXL receptor tyrosine kinase	AXL
18	Beta amyloid A4 protein	APP
19	Butyrylcholine esterase	BCHE
20	Carbonic anhydrase	CA12
21	Carbonic anhydrase 2	CA2
22	Carbonic Anhydrase IV	CA4
23	Casein kinase II alpha/beta	CSNK2A1
24	Cyclin-dependent kinase	CDK1
25	Cyclooxygenase	PTGS2
26	Cystic fibrosis transmembrane conductance regulator	CFTR
27	D(2) dopamine receptor	DRD2
28	Delta-type opioid receptor	OPRD1
29	DNA topoisomerase 1	TOP1
30	Estradiol 17-beta-dehydrogenase 1 (17beta-HSD1)	HSD17B1
31	Estradiol 17-beta-dehydrogenase 2	HSD17B2
33	Glycogen Synthase Kinase-3, beta	GSK3B
34	Glyoxalase I	GLO1
35	Insulin-like growth factor 1 receptor (IGF1R)	IGF1R
36	Matrix metalloproteinase-12 (MMP12)	MMP12
37	Monoamine oxidase A (MAO-A)	MAOA
38	Monoamine oxidase B (MAO-B)	MAOB
39	Multidrug resistance protein 1 (MRP1)	ABCC1
40	Insulin-like growth factor 1 receptor (IGF1R)	IGF1R
41	Lck	LCK
42	Sucrase-isomaltase	SI

Table 5.9: List of human proteins targeted by the phytochemicals (continuation)

Sl.no.	*Target proteins (human)*	*Gene ID*
42	NADPH oxidase 4	NOX4
43	Neurotrophic tyrosine kinase receptor type 2	NTRK2
44	Placenta growth factor	PGF
45	Protein E6	E6
46	RXR alpha/PPAR gamma	PPARG
47	Serine/threonine-protein kinase Aurora-B	AURKB
48	Sigma opioid receptor	SIGMAR1
49	Telomerase reverse transcriptase	TERT
50	Transthyretin (TTR)	TTR
51	Vascular endothelial growth factor A	VEGFA
52	Xanthine oxidase	XDH
53	Aldehyde dehydrogenase (ALDH2)	ALDH2
54	Aldehyde oxidase	AOX1
55	Epidermal growth factor receptor (EGFR)	EGFR
56	Estrogen-related receptor alpha	ESRRA
57	Macrophage migration inhibitory factor (MIF)	MIF
58	MER intracellular domain/EGFR extracellular domain chimera	EGFR
59	Glucocorticoid receptor	NR3C1
60	Testis-specific androgen-binding protein	SHBG
61	11-beta-hydroxysteroid dehydrogenase 1	HSD11B1
62	11-beta-hydroxysteroid dehydrogenase 2	HSD11B2
63	Dual specificity phosphatase Cdc25B	CDC25B
64	Prostaglandin E synthase	PTGES
65	Protein-tyrosine phosphatase 1B (PTP1B)	PTPN1
66	RXR alpha/PPAR gamma	RXRA
67	Carbonic Anhydrase IX	CA9
68	D(4) dopamine receptor	DRD4
69	Progesterone receptor	PGR
70	Adenosine Receptor A3	ADORA3
71	Adenosine Receptors A2a (A2a)	ADORA2A
72	Arachidonate 12-lipoxygenase	ALOX12
73	ATP-binding cassette sub-family G member 2	ABCG2
74	Beta-secretase 1	BACE1
75	Cyclin-dependent kinase	CDK1
76	Cyclooxygenase-1 (COX-1)	PTGS1
77	Cytochrome P450 1B1	CYP1B1
78	Tyrosine-protein kinase Met	MET
79	Cannabinoid receptor	CNR2
80	GABA receptor alpha-1/beta-2/gamma-2 subunit	GABRA1
81	Dopamine Transporter (DAT)	SLCA3

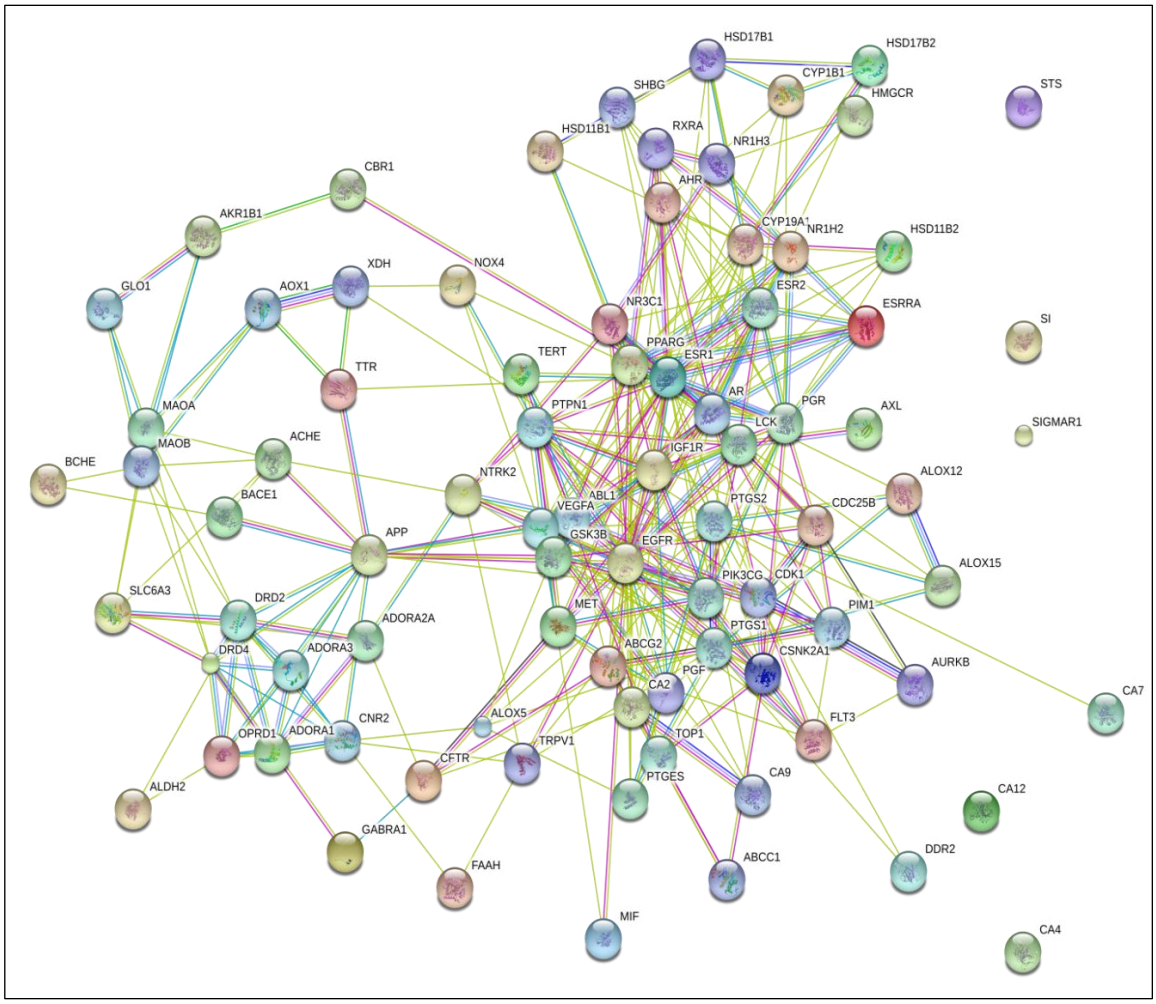


Fig 5.7.36: Interaction between target proteins of bioactive phytochemicals by using STRING Interactome Database

5.7.1c Phytochemical, Protein target and targeted disease

To determine the diseases targeted by *M. paleacea*, a tripartite network was constructed using its phytochemicals, their protein targets and associated diseases via Cytoscape. A total of 180 disease targets (Table 5.10) were identified and were classified into 22 broad categories (Fig 5.7.35). Figure 5.7.36 represents the interaction between phytochemicals, proteins and disease targets.. Among the diseases targeted by the proteins, 26% percent of the total diseases belonged to the class malignant neoplasm, 13% belonged to endocrine, nutritional and metabolic diseases, 9% belonged to disease of circulatory system and 8% belonged to certain infectious and parasitic diseases. Each of the remaining disease classes constituted 1-5% of all the disease targets. In this work phytochemicals obtained by *in-vitro* bioassay guided purification. The assays used for extraction of phytochemicals were *in-vitro* antioxidant and anti-diabetic assays. Network analysis of phytochemicals-protein targets-associated diseases revealed that the endocrine, nutritional and metabolic diseases were associated with the protein target of studied phytochemicals which proved that phytochemicals extracted using *in-vitro* anti-diabetic activity efficiently targets the proteins involved in endocrine nutritional and metabolic pathways. Second highest number of disease associated with the target proteins was disease of endocrine, nutritional and metabolic diseases (13%), highest being the diseases belonging to the disease class malignant neoplasm.

The aim of this work was to isolate phytochemicals having anti-diabetic activity and to validate the therapeutic relevance of these compounds through network analysis. So, the further network analysis was mainly focused on disease class endocrine, nutritional and metabolic diseases.

Table 5.10: List of diseases associated with target proteins

Disease	*Disease ID*	*Class*	*Disease*	*Disease ID*	*Class*
Diabetic complications	ICD10: E08-E13	**ENDOCRINE, NUTRITIONAL AND METABOLIC DISEASES**	Alzheimer's disease	ICD10: G30, G31.84	**DISEASE OF NERVOUS SYSTEM**
Diabetic neuropathy	ICD10: E08-E13		Motor neurone disease	ICD10: G12.2	
Diabetic retinopathy	ICD10: H36		Parkinson's disease		
Non-insulin-dependent diabetes mellitus	ICD10: E08-E13		Analgesics	ICD10: R52	
Cystic fibrosis	ICD10: E84, L90.5		Vascular diseases	ICD10: G45-G46	
Endocrine disease	ICD10: E00-E35		Neurodegenerative diseases	ICD10: G30-G32	
Hormone deficiency	ICD10: E00-E90		Cardiovascular disease	ICD10: G45-G46, I00-I99, I10, I11, I12	
Hypogonadism	ICD10: E23.0, E28.3, E29.1		Disabling peak-dose dyskinesias	ICD10: G24.9	
Male hormonal deficiencies	ICD10: E20-E35		Neurological diseases	ICD10: G00-G99	
Testosterone deficiency	ICD10: E29.1		Lateral sclerosis	ICD10: G12.2	
Peripheral precocious puberty	ICD10: E22.8, E30.1		Motor neurone disease	ICD10: G12.2	
Obesity	ICD10: E66		Dyskinesia	ICD10: G24.9	
Amyloidosis	ICD10: E85		Neuropathic pain	ICD10: G64, G90.0	
TTR amyloidosis	ICD10: E85				
Diabetic angiopathy	ICD10: E10.5, E11.5, E13.5		Insomnia	ICD10: F51.0, G47.0	
Diabetes mellitus	ICD10: E23.2		Melanoma	ICD10: C43	**MELIGNANT MELANOMA OF SKIN**
Inflammation	ICD10: E08-E13, E10.2		Diarrhea	ICD10: A09, K59.1	**CERTAIN INFECTIOUS AND PARASITIC DISEASE**
Hyperlipidemia	ICD10: E78				
Metabolic disease	ICD10: E70-E89		HIV-associated diarrhoea	ICD10: A09	
Diabetes mellitus type 2	ICD10: E11		Streptococcus pneumoniae infections	ICD10: A09	
Dyslipidemia	ICD10: E78		HIV infections	ICD10: A00-B99	
Hypercholesterolemia	ICD10: E78.1		HCV infection	ICD10: B17.1, B18.2	
Hypertriglyceridemia	ICD10: E78.1		Septic shock	ICD10: A41.9	
Metabolic disorders	ICD10: E70-E89				

Table 5.10: List of diseases associated with target proteins (continuation)

Disease	*Disease ID*	*Class*	*Disease*	*Disease ID*	*Class*
Adult respiratory distress syndrome	ICD10: J80	**DISEASE OF RESPIRATORY SYSTEM**	Tumors	ICD10: C00-C75, C7A, C7B, D10-D36, D3A	**MALIGNANT NEOPLASM**
Allergic rhinitis	ICD10: J00, J30, J31.0, T78.4		Cancer	ICD10: C00-C96	
Asthma	ICD10: J45		Bladder cancer	ICD10: C00-C96, C67	
Pulmonary fibrosis	ICD10: J84.1		Hormone refractory prostate cancer	ICD10: C00-C96, C61	
Emphysema	ICD10: J43		Metastasis	CD10: C00-C97	
Respiratory tract inflammation	ICD10: J00-J99		Salivary glands cancer	ICD10: C00-C96	
Allergic diseases	ICD10: T78.4	**INJURY, POISONING AND CERTAIN OTHER CONSEQUENCES OF EXTERNAL CAUSES**	Breast cancer	ICD10: C50	
Allergic rhinitis	ICD10: J00, J30, J31.0, T78.4		Multiple myeloma	ICD10: C90.0	
Antigen-induced decrease in coronary flow and cardiac anaphylaxis	ICD10: T78.2		Myeloma	ICD10: C90.0	
Brain injury	ICD10: S09.90		Solid tumours	ICD10: C00-C75	
Sepsis	ICD10: A40, A41, T78		Inflammatory diseases	ICD10: K75.9	
			Colorectal cancer	ICD10: C18-C21	
Bronchiolar carcinoma	ICD10: C34	**MALIGNANT NEOPLASM**	Lung cancer	ICD10: C33-C34	
Cervical cancer	ICD10: C00-C96		Hormone-sensitive breast cancer	ICD10: C00-C96	
Chronic myeloid leukemia	ICD10: C92.1		Endocrine independent cancer	ICD9: 140-229 ICD10: C00-C96	
Gastrointestinal cancers	ICD10: C15-C26		Osteosarcoma	ICD10: C40-C41	
Pancreatic cancer	ICD10: C00-C96		Ewing's sarcoma	ICD10: C41	
Urological cancers	ICD10: C00-C96		Medulloblastoma	ICD10: C71	
Peripheral neuroectodermal tumor	ICD10: C00-C75		Renal cell carcinoma	ICD10: C64	
Non-small cell lung cancer	ICD10: C00-C96		Brain cancer	ICD10: C71, D33	

Table 5.10: List of diseases associated with target proteins (continuation)

Disease	*Disease ID*	*Class*	*Disease*	*Disease ID*	*Class*
Ovarian cancer	ICD10: C00-C96	**MALIGNANT NEOPLASM**	Myocardial infarction	ICD10: I21	**DISEASES OF THE CIRCULATORY SYSTEM**
Acute myeloid leukemia	ICD10: C92.0		Cardiomyopathy	ICD10: I42.0	
Leukemia	ICD10: C91-C95		Angiogenesis disorder	ICD10: I00-I99	
MLL-rearranged acute lymphoblastic leukemias	ICD10: C91-C95		Coronary artery disease	ICD10: I20-I25	
Metastatic colorectal cancer	ICD10: C18-C21		Ischemia reperfusion injuries	ICD10: I99.8	
Metastatic prostate cancer	ICD10: C00-C96		Stroke	ICD10: I61-I63	
Melanoma	ICD10: C43		Coronary heart disease	ICD10: I25.1	
Chronic myelogenous leukemia	ICD10: C91-C95, C92.1		Essential hypertension	ICD10: I10, I11, I12, I13, I15	
Colon adenocarcinoma	ICD10: C00-C75		Pulmonary hypertension	ICD10: I27.0, I27.2	
Philadelphia-positive leukemia	ICD9: 208.9 ICD10: C91-C95		Subarachnoid hemorrhage	ICD10: I60, P10.3, S06.6	
Acute leukaemia	ICD10: C91-C95		Prostate hyperplasia	ICD10: N40	**DISEASES OF THE GENITOURINARY SYSTEM**
Glioblastoma multiforme	ICD10: C71		Glomerulonephritis	ICD10: N00, N01	
Head and neck tumors	ICD10: C00-C75		Endometriosis	ICD10: N80	
Squamous cell carcinoma	ICD10: C44		Male infertility	ICD10: N46	
ER beta-positive prostate tumors	ICD10: C00-C75		Renal failure	ICD10: N17, N18, N19	
Head and neck squamous cell carcinomas	ICD10: C44		Stroke	ICD10: I61-I63, I80-I82	
Cerebral vasospasm	ICD10: I73.9	**DISEASES OF THE CIRCULATORY SYSTEM**	Postmenopausal symptoms	ICD10: N95.0	
Myocardial ischemia	ICD10: I99.8		Renal diseases	ICD10: N00-N39	
Cardiovascular disease	ICD10: I00-I99		Menopause	ICD10: N95.0	
Heart failure	ICD10: I21, I22, I50		Inflammatory bowel disease	ICD10: K50, K51	**DISEASES OF THE DIGESTIVE SYSTEM**
Atherosclerosis	ICD10: I70		Chronic ileitis	ICD10: K50-K52	
Ischemia	ICD10: I99.8		Ulcerative colitis	ICD10: K51	
Coronary atherosclerosis	ICD10: I70		Crohn's disease	ICD10: K50	

Table 5.10: List of diseases associated with target proteins (continuation)

Disease	*Disease ID*	*Class*	*Disease*	*Disease ID*	*Class*
Inflammatory diseases	ICD10: C00-C96	**DISEASES OF THE DIGESTIVE SYSTEM**	Delusional disorder	ICD10: F22.0	
Gastro-intestinal ulcers	ICD10: K25		Erectile dysfunction	ICD10: F52.2	
Inflammatory response in alcoholic liver disease	ICD10: K70-K77		Parkinson's disease	[ICD10: F02.3	
Magnesium deficiency dermatitis	ICD10: L20-L30	**DISEASES OF THE SKIN AND SUBCUTANEOUS TISSUE**	Bipolar affective disorder	ICD10: F31	
Psoriasis	ICD10: L40		Social phobias	ICD10: F40.1	
Acne vulgaris	ICD10: L70.0		Major depressive disorder	ICD10: F32, F33, M32	
Dermatological disease	ICD10: L00-L99		Chronic alcoholism	ICD10: F10.2]	
Fibrosis	ICD10: L90.5		Drug dependence	ICD10: F10-F19	
Alopecia	ICD10: L65.9		Alopecia	ICD10: L65.9	**NONSCARRING HAIR LOSS**
Systemic lupus erythematosus	ICD10: L51-L54		Contraception	ICD10: Z30	**FACTORS INFLUENCING HEALTH STATUS AND CONTACT WITH HEALTH SERVICES**
Osteoarthritis	ICD10: M15-M19, M47	**DISEASES OF THE MUSCULOSKELETAL SYSTEM AND CONNECTIVE TISSUE**	Pain	ICD10: G64	**PAIN**
Muscle atrophy	ICD10: M62.5		Analgesics	ICD10: R52	
Osteoporosis	ICD10: M80-M81		Glaucoma	ICD10: H40-H42	**DISEASES OF THE EYE AND ADNEXA**
Spinal and bulbar muscular atrophy	ICD10: M62.5		Ocular hypertension	ICD10: H40.0	
Gout	ICD10: M10		Ocular inflammation	ICD10: H00-H59	
Rheumatoid arthritis	ICD10: M05-M06		Macular degeneration	ICD10: H35.3	
Sjogren-Larsson syndrome	ICD10: Q87.1	**CONGENITAL MALFORMATIONS, DEFORMATIONS AND CHROMOSOMAL ABNORMALITIES**	Oxygen-induced retinopathy	ICD10: H35-H35.2	
Ulcerative colitis	ICD10: K51	**NON-INFECTIVE ENTERITIS AND COLITIS**	Nausea and vomiting	ICD10: R11	**SYMPTOMS AND SIGNS INVOLVING THE DIGESTIVE SYSTEM AND ABDOMEN**
Cognitive deficits	ICD10: F01-F07, F04, R41.3	**MENTAL, BEHAVIOURAL AND NEURO-DEVELOPMENTAL DISORDERS**	Vomiting	ICD10: R11	
Depression	ICD10: F32		Cough	ICD10: R05	**SYMPTOMS AND SIGNS INVOLVING THE CIRCULATORY AND RESPIRATORY SYSTEMS**
Dementia	ICD10: F01-F07, G30		Dyspnea	ICD10: R06.0	
Schizophrenia	ICD10: F20, M32		Immunodeficiency	ICD10: D84.9	**DISEASES OF THE BLOOD AND BLOOD-FORMING ORGANS AND CERTAIN DISORDERS INVOLVING THE IMMUNE MECHANISM**
Anxiety disorders	ICD10: F40-F42		Angioedema	ICD10: D84.1	
Attention deficit hyperactivity disorder	ICD10: F90		Autoimmune diseases	ICD10: D84.9	
Cocaine dependence	ICD10: F14.2, F32		Male infertility	ICD10: N46	**DISEASES OF MALE GENITAL ORGANS**

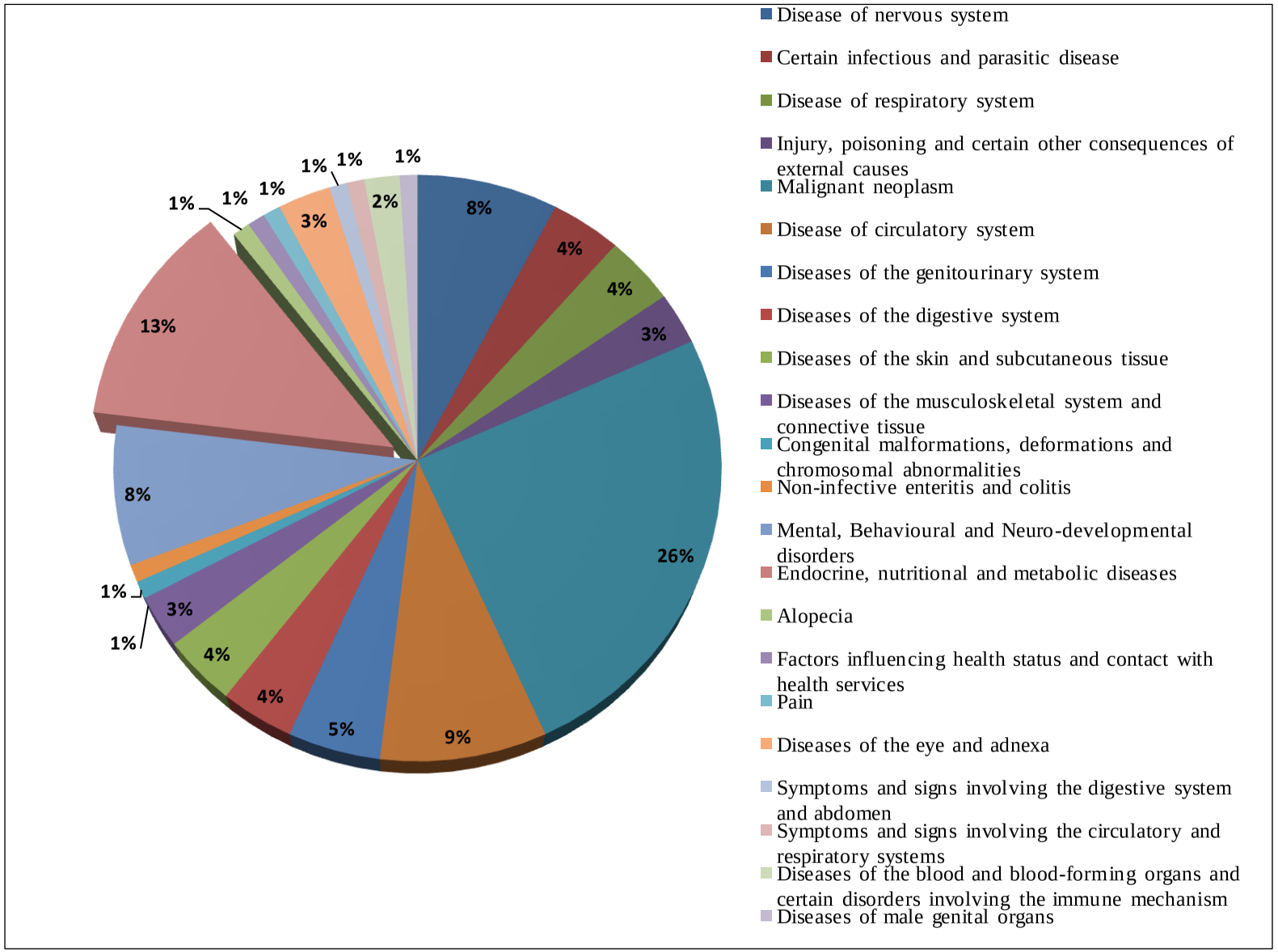


Fig 5.7.37: Pie chart representing the disease classes associated with target protein.

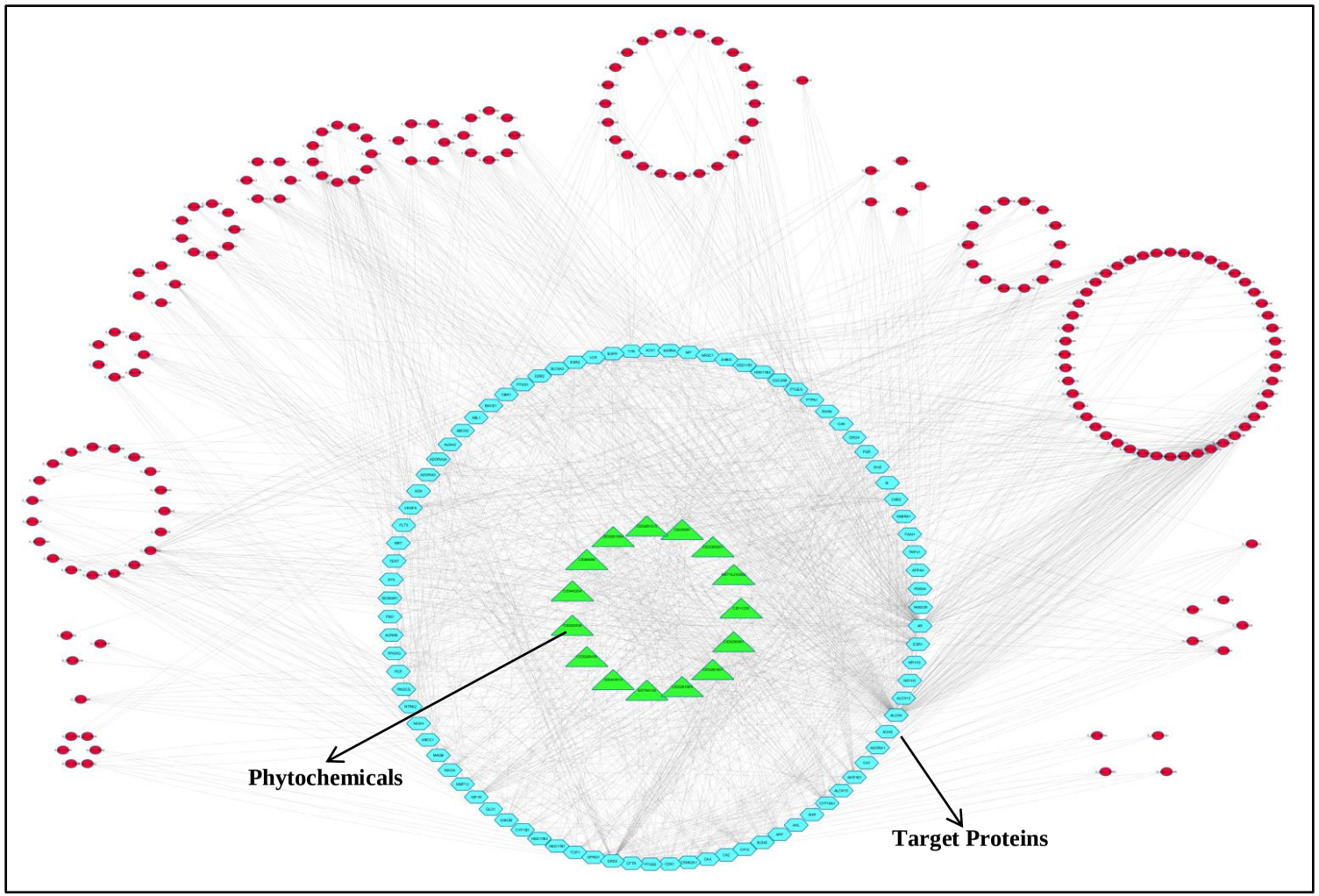


Fig 5.7.38: Whole tripartite network of phytochemicals, target proteins and associated disease classes.

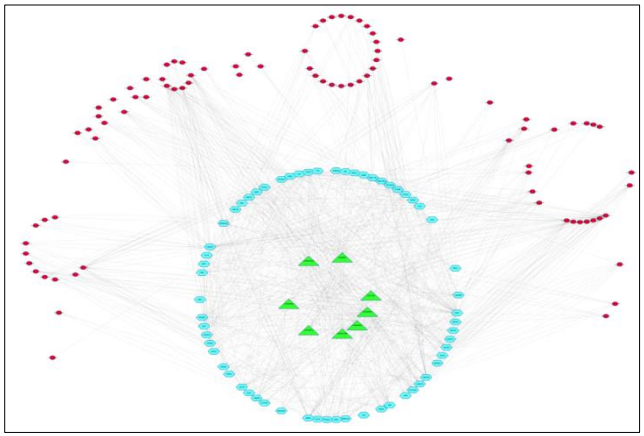
5.7.1d Network robustness Study

Network robustness was analyzed by the elimination of proteins from the sub network on the basis of Degree × Betweenness × Closeness value. For breaking the robust structure of network, about 30% of targeted proteins were deleted from network through gradual elimination of 5% proteins for 6 times (Fig 5.7.37). Degree centrality is the number of links incident upon a node (Freeman, 1979). The average length of the shortest path between a node and all other nodes in a network is Closeness centrality of a node. So if the node is at the centre, closer it will be to all the other nodes (Bavelas, 1950). The total number of times a node bridges the shortest path between other two nodes of a network is its Betweenness centrality. It is a centrality measure of a vertex within a network (Freeman, 1977).

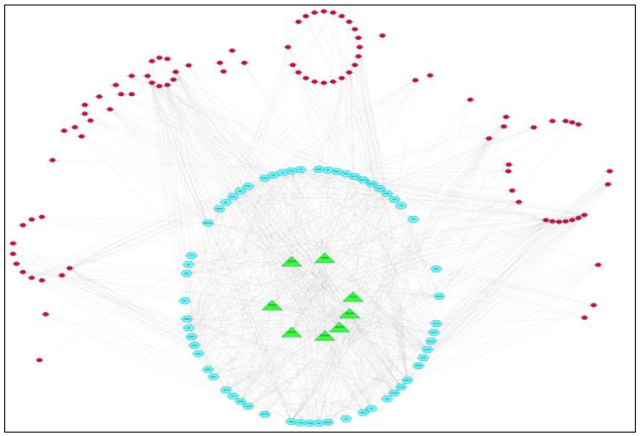
Degree × Betweenness × Closeness (indicators of centrality) identifies the most important vertices in the network. In this work, vertices represent the target proteins. Thus the Network Robustness helps to resolve the complexness of network by removing the centralized targets. Network robustness analysis found twenty protein targets of phytochemicals isolated from *M. paleacea* to be involved in endocrine, nutritional and metabolic diseases. List of the protein targets is presented in table 5.11

5.7.1e Network parameters and their relation

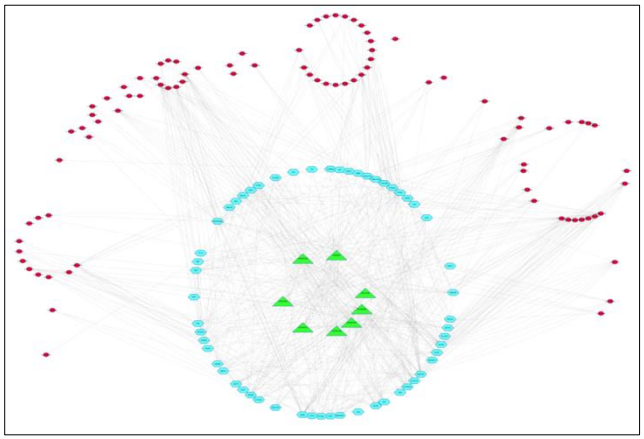
Different parameters like isolated nodes, characteristic path length, clustering coefficient and network diameter were studied for network analysis and are presented in Fig 5.7.38. Parameters like isolated nodes and characteristic path length was found to increase, while decrease in clustering co-efficient was noticed. Diameter of the network first increased and then remained constant.



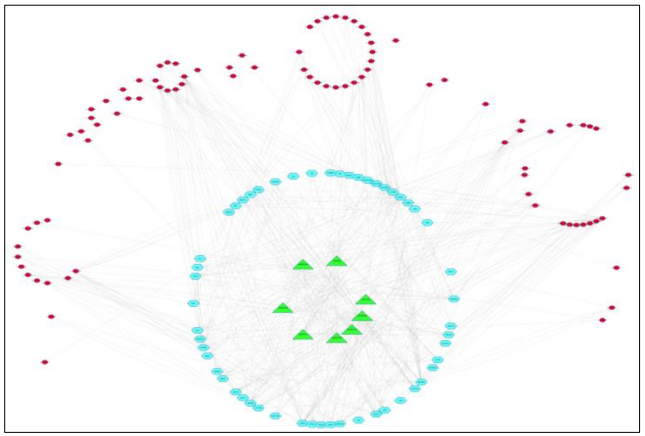
1.5 % protein elimination by Network Perturbation



5 % protein elimination by Network Perturbation

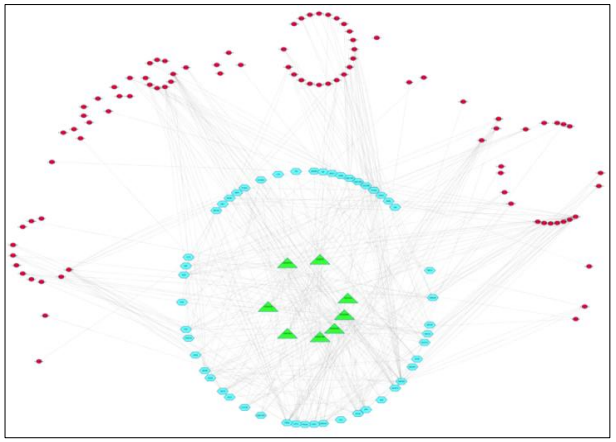


10% protein elimination by Network Perturbation

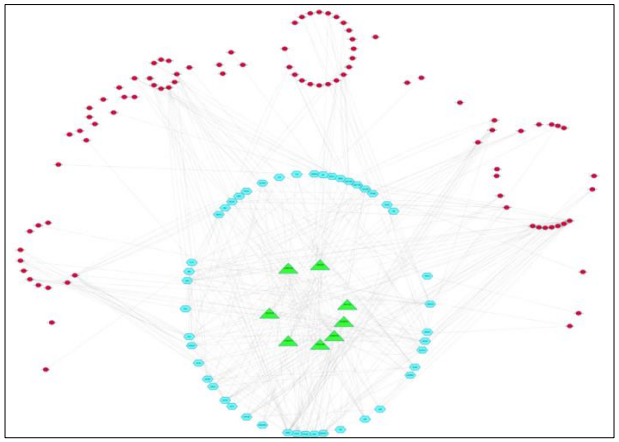


15% protein elimination by Network Perturbation

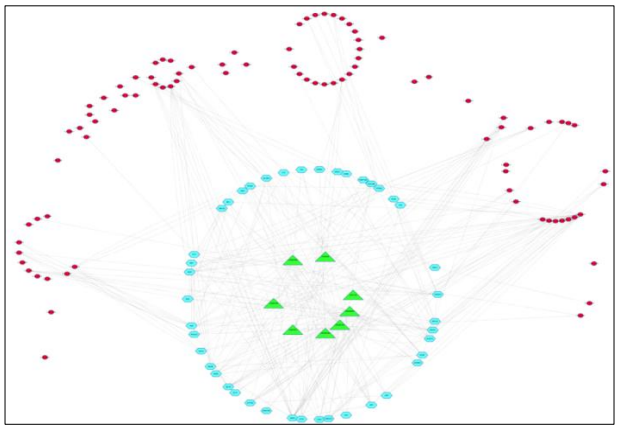
Fig 5.7.39: Protein elimination by Network Perturbation



20% protein elimination by Network Perturbation



26% protein elimination by Network Perturbation



32% protein elimination by Network Perturbation

Fig 5.7.39: Protein elimination by Network Perturbation (continuation)

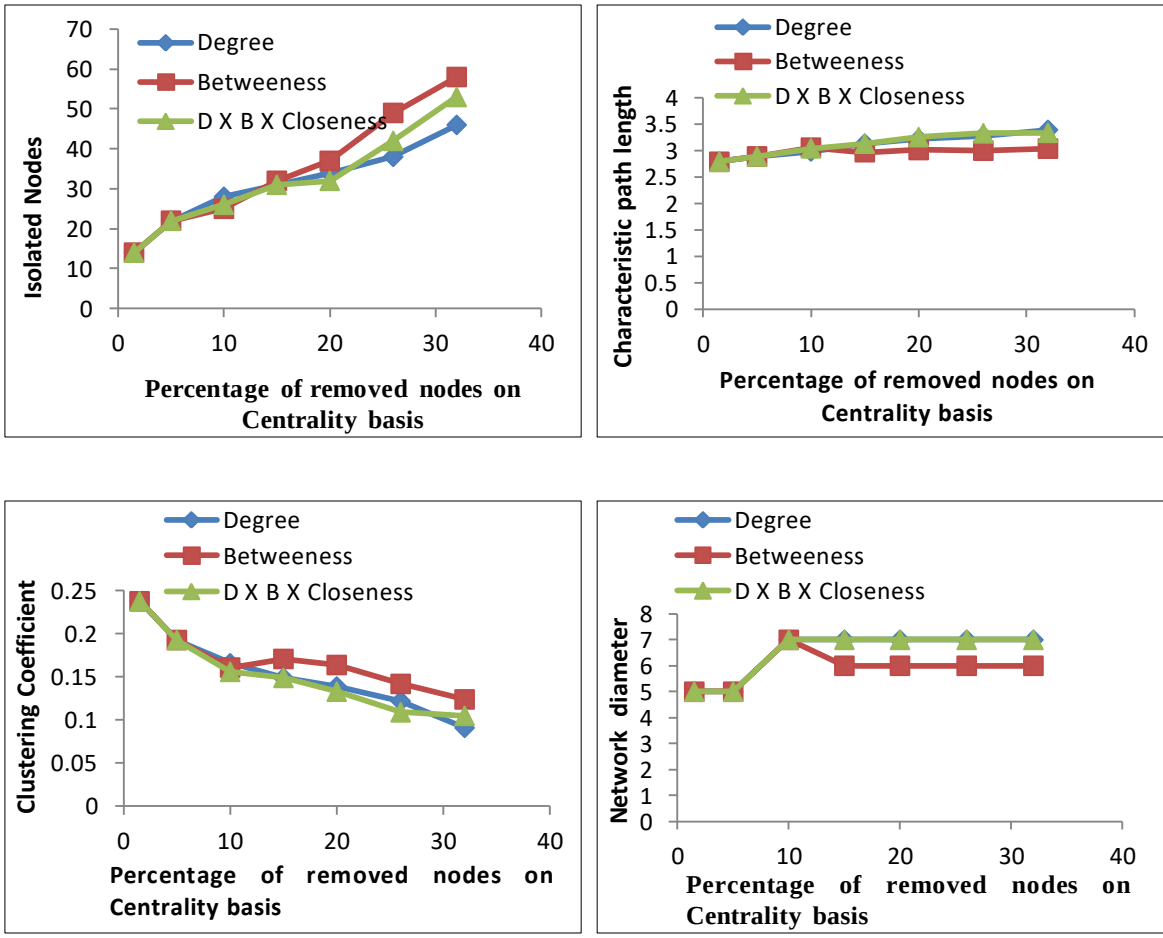


Fig 5.7.40: Network parameters versus percentage of network perturbation

Table 5.11: Network list of first 30% of total targets analyzed through tripartite network

Gene	*Betweenness Centrality*	*Closeness Centrality*	*Clustering Coefficient*	*Degree*	*D × B × Close*
PPARG	0.072	0.518	0.309	30	1.122
VEGFA	0.125	0.501	0.195	59	3.704
AKR1B1	0.042	0.381	0.153	25	0.409
PTPN1	0.043	0.435	0.2	24	0.450
HMGCR	0.048	0.367	0.030	12	0.214
EGFR	0.070	0.519	0.258	38	1.392
HSD11B1	0.026	0.380	0.075	17	0.172
MIF	0.063	0.380	0.060	12	0.290
BCHE	0.022	0.386	0.076	19	0.166
GSK3B	0.049	0.456	0.187	26	0.593
ESR1	0.152	0.523	0.122	70	5.578
CYP19A1	0.017	0.473	0.411	30	0.246
AR	0.242	0.523	0.105	142	18.03
ALOX15	0.033	0.397	0.219	19	0.253
BACE1	0.017	0.396	0.145	17	0.118
DRD2	0.018	0.382	0.269	23	0.163
NTRK2	0.040	0.431	0.166	26	0.451
ESR2	0.023	0.459	0.307	43	0.458
ADORA1	0.068	0.405	0.098	29	0.801
ALOX5	0.028	0.407	0.179	38	0.438

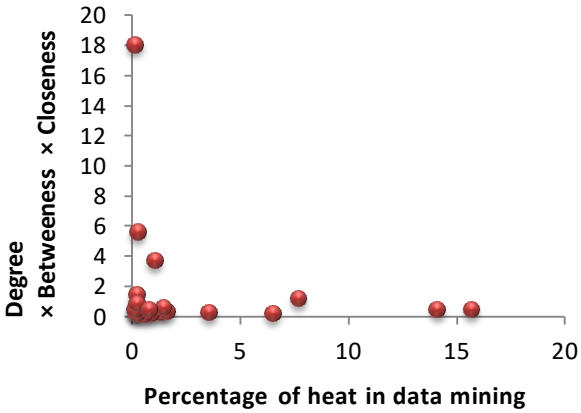


Fig 5.7.41: Degree × betweenness × clossness analysis of 20 protein targets

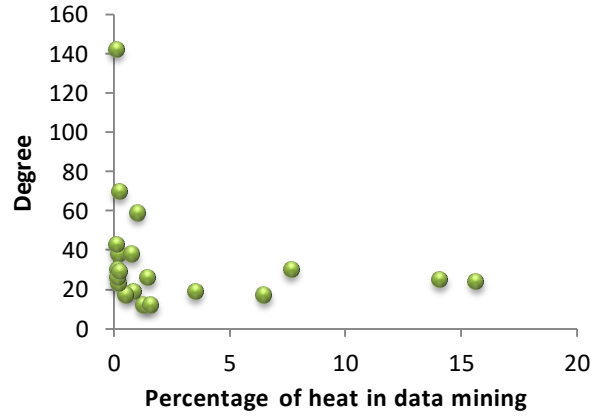


Fig 5.7.42: Degree analysis of 20 protein targets

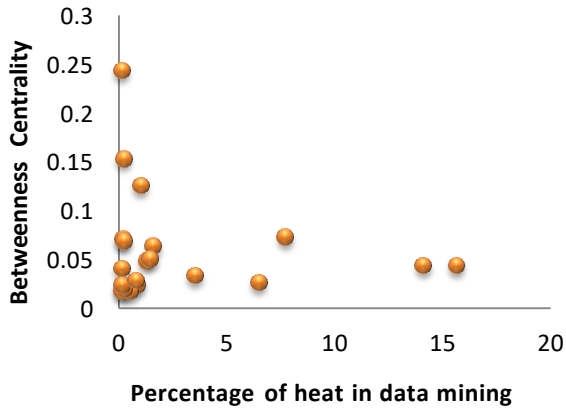


Fig 5.7.43: Betweenness centrality analysis of 20 protein targets

5.7.1f. Enrichment (KEGG) Analysis of Centralized Target Proteins

Betweenness Centrality analysis and percentage of heat from CooLGen data mining had identified eight targets having high betweenness centrality and heat percentage from earlier identified twenty targets by robustness analysis (Fig 5.7.39 to Fig 5.7.41). These targets were PTPN1, AKR1B1, PPARG, ADORA1, MIF, GSK3B, HMGCR and VEGFA (Fig 5.7.43). The functional enrichments of isolated centralized proteins were done through KEGG pathway to find out the role of these targets in initiating endocrine, nutritional and metabolic diseases. Biological pathways in which these targets are involved are described sequentially in the following section.

VEGFR: associated with MAP kinase pathway in cancer.

HSDII: involved in steroid hormone metabolism pathway.

MIF: involved in tyrosin metabolism pathway

BCHE: Butylcholineesterase inhibitor

AKR1B1: mainly associated with steroid hormone synthesis pathway. Insulin when binds with the insulin receptor of ovary it triggers the biosynthesis of AKR1B1. It then utilizes cholesterol inside ovary and produces hormone like testesteron and projesterone etc.

ESR1: in ovarian system it acts as estrogen receptor when leuteinising hormone is received by the leutonising hormone receptor.

CYP1: when insulin binds with insulin receptor in ovary during ovarian steroidogenesis, CYP1 converts cholesterol present in ovary into testerone and projesterone hormones and insulin signalling pathway particularly enhances the expression of CYP1.

AR: in protrate epithelial cell testerone binds with androgen receptor and subsequently genes required for cell proliferation are going to be expressed, which might create prostate cancer.

ALOX15: associated with linolic acid metabolism.

BACE1: In Alzemier disease, BACE1 inhibits acetylcholine.

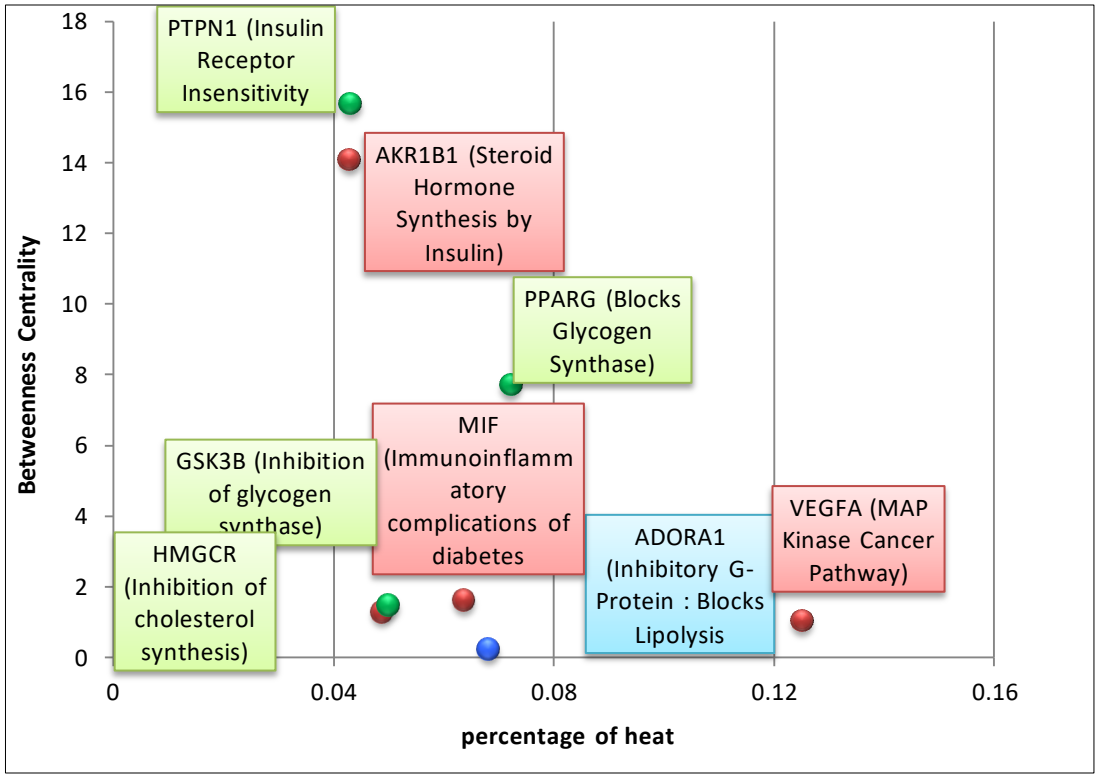


Fig 5.7.44: Function of eight protein target having high betweenness centrality value and percentage of heat

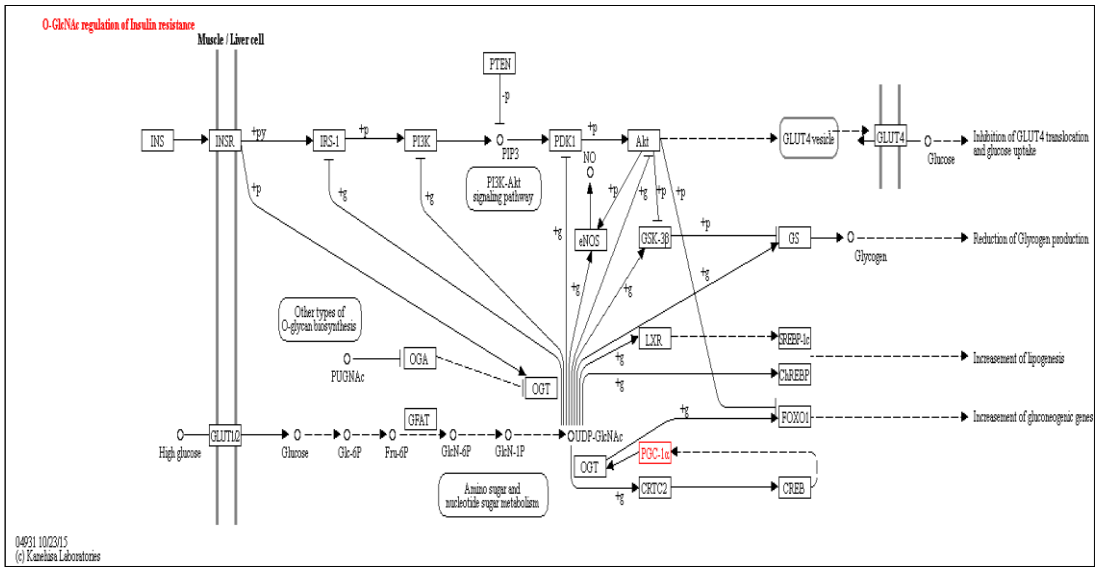


Fig 5.7. 45: Insulin signaling pathway (hsa04931) obtained from KEGG database. Genes present in tripartite network is marked red

ALOX5: in ovarian tissue ALOX is activated by arachidonic acid which is produced through phospholipase A by stimulatory G_{α} and cyclic AMP. ALOX is a type of lypooxygenase which produce different arachidonic metabolites and express the genes of steroidal sex hormone biosynthesis like testerone and projesterone.

PPARG: responsible for insulin resistance

PTPN1: responsible for insulin receptor insensitivity

GSK3: inhibits glycogen synthase

ADORA1: inhibits the process of fatty acid degradation and lipolysis.

From Enrichment analysis of centralized target by KEGG pathway, it was found that four protein targets were mainly involved in the pathways associated with diabetic diseases. Entire pathway and involved genes of these targets are presented in the following section.

PPARG: Insulin resistance pathway

PPAR nuclear receptors and winged-helix-forkhead box class O (FOXO) are most important transcription factors that regulate GLUT4 gene expression. In absence of ligand, PPARG forms a heterodimer with retinoid X receptor (RXR) and binds to PPAR response elements (PPREs) of target gene (GLUT4). The PPARG-RXR heterodimer then binds with co-repressor and represses the expression of GLUT4 gene. However, in presence of ligand the corepressor will be replaced from PPARG by a coactivator complex and leads to transcriptional activation of GLUT4.

PTPN1: Insulin signalling pathway

PTPN1 is responsible for insulin receptor insensitivity as it blocks insulin receptor and insulin receptor substrate 1 which results in the inhibition of the process of glycogenesis.

ADORA1: Insulin signalling pathway

ADORA1 induces production of inhibitory G_i protein and inhibits adenyl cyclase. Adenyl cyclase when present in the active form is able to activate the protein Kinase A

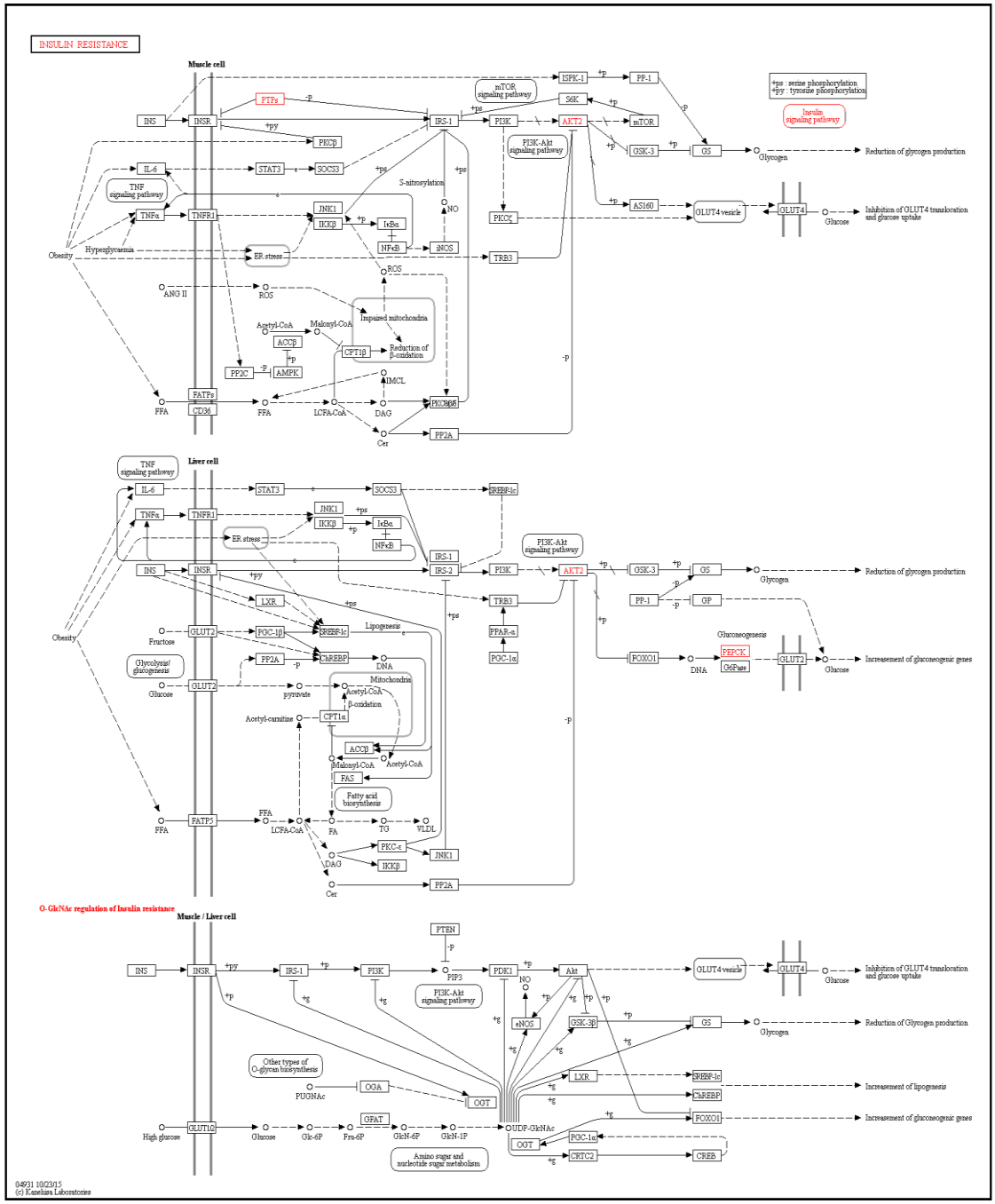


Fig 5.7.46: Insulin resistance pathway (hsa04931) obtained from KEGG database. Genes present in tripartite network are marked in red

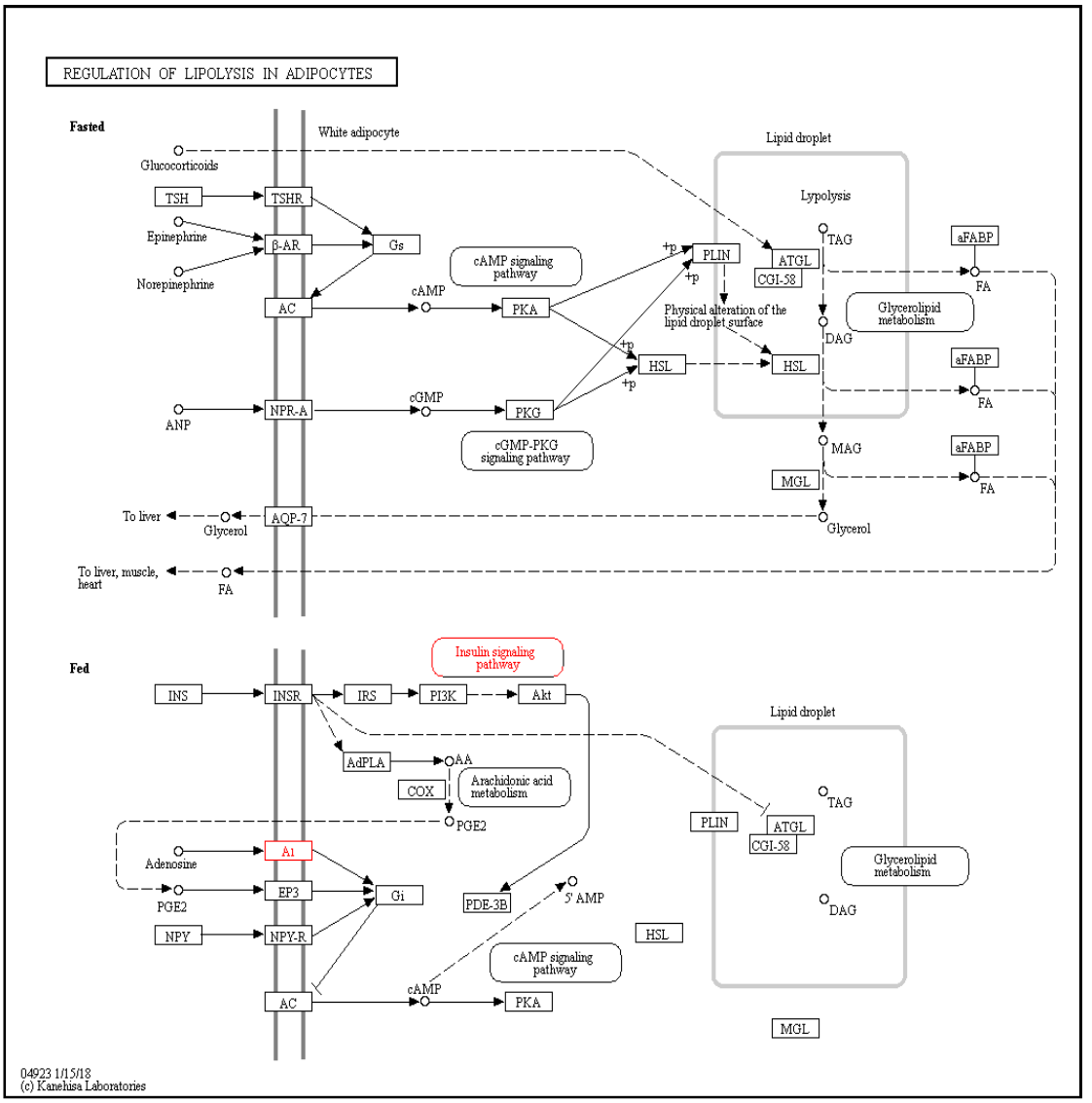


Fig 5.7.47: Insulin signaling pathway (has 04923) obtained from KEGG database. Genes present in tripartite network are marked in red

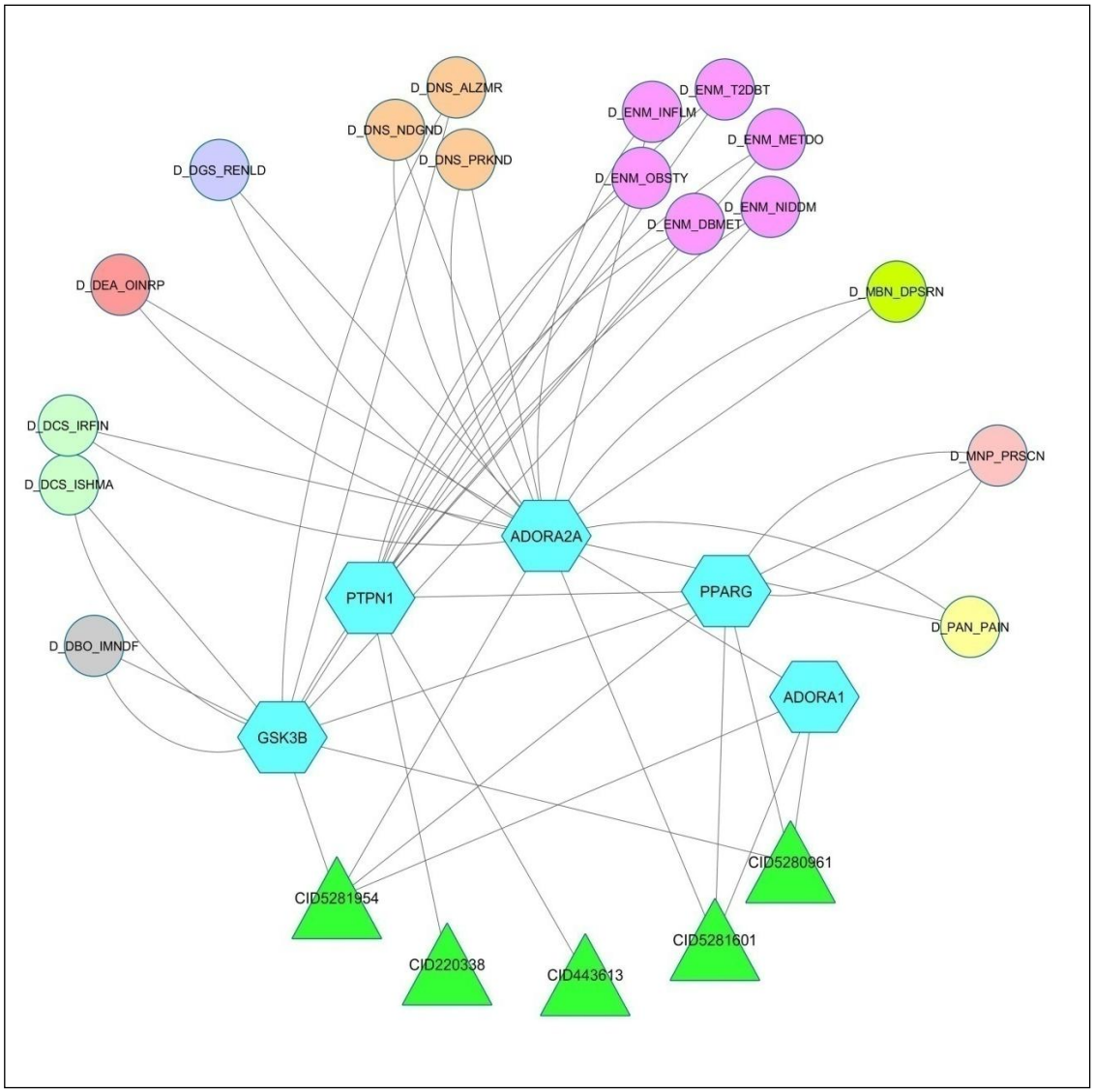


Fig 5.7.48: Final tripartite network of five most important protein targets participating in diabetes pathway

(PKA) through generation of secondary messenger cAMP. Activated PKA then degrades fatty acid and causes lipolysis in adipose tissue. Thus ADORA1 inhibits the process of fatty acid degradation and lipolysis by inhibiting the PKA activation.

5.7.1g Druggability Analysis and ADMET Study

Druaggability analysis is important for drug discovery process. Estimation of ADMET properties is highly important for considering a drug candidate for further clinical trials (Van De Waterbeemd and Gifford *et al*., 2003). Analysis of ADMET properties of five phytochemicals of *M. paleacea* that are found to be good candidate for preventing diabetes was studied. *In silico* test showed that the intestinal absorption percentage of studied five phytochemicals was more than 90%. $Caco_2$ cell permeability was also high i.e. more than 0.9 of the concerned phytochemicals. It is important to consider the capability of a drug to cross blood brain barrier (BBB) for reducing side effects and toxicities or improve efficiency drug acting within brain. Studied phytochemicals showed good BBB permeability (logBB values were more than -1) [Table 5.12.1]. Similarly, these phytochemicals also showed the Central Nervous System (CNS) penetrating potential, the CNS penetration values were less than -3 (Table 5.12.2). Another important parameter to be considered in the drug discovery is their clearance from the body. Organic Cation Transporter 2 is a transporter (OCT2) that helps in renal clearance of drugs and other compounds. Drugs capable of being transported by OCT2 provide information about its clearance. Phytochemicals 5-hydroxy-2-(4-methoxyphenyl), 4-H-1 Benzopyran-4-one and Palustric acid found to be capable of acting as a substrate of OCT2 (Table 5.12.5).

The study of toxic potency of a drug is highly important. Ames test is used to access the mutagenic potential of a drug. *In silico* Ames test showed that the analyzed phytochemicals have no mutagenic effect. Estimation of maximum recommended tolerated dose (MRTD) estimates the threshold toxic dose of chemicals. Studied phytochemicals found to have low maximum recommended tolerated dose. Human ether-a-go-go gene (hERG) codes for the potassium channels. Blocking of these channel are the main cause of development of fatal arrhythmia. Studied phytochemicals was found to have hERG I and II inhibitory activity. Drug induced liver damage is of high concern in drug development and is one of the main cause of drug attrition. 5-hydroxy-2-(4-methoxyphenyl), 4-H-1 Benzopyran-

4-one, 5 hydroxy-7-methoxy-2-phenyl, 4H-1-Benzoyran-4-one and 4',5,7-Trihydroxy isoflavone showed no hepatotoxicity in *in silico* test while pimaric acid and palustric acid showed hepatoxicity. Evaluating whether a drug can induce allergic contact dermatitis and cause skin sensitisation is highly important. No skin sensitization is induced by the studied five phytochemicals (Table 5.12.4).

Lipinski's "rule of five" criterion measures the likeliness of the compounds to act as a drug (Lipinski *et al*., 1997). Criteria for Lipinski's rule are: (i.) molecular weight less than 500 Dalton, (ii.) Number of hydrogen bond donors less than 5, (iii.) Number of hydrogen bond acceptors less than 10, (iv.) logP value (octanal-water partition coefficient) less than 5. All tested phytochemicals found to maintain the entire criteria (Table 5.12.6).

Table 5.12 .1: Druggability analysis of phytochemicals for absorption ability

Property	***unit***	***Predicted value***					
		Data prediction	***4',5,7-Trihydroxy isoflavone***	***5-hydroxy-2-(4-methoxyphenyl) ,4-H-1 Benzopyran-4-one***	***5 hydroxy-7-methoxy-2-phenyl, 4H-1-Benzoyran-4-one***	***Palustric acid***	***pimaric acid***
Water solubility	Numeric (log mol/L)	The higher the better	-3.595	-3.714	-3.641	-3.88	-3.683
Caco2 permeability	Numeric (log Papp in 10^{-6} cm/s)	> 0.90 = high Caco2 permeability	0.9	1.106	1.248	1.621	1.742
Intestinal absorption (human)	Numeric (% Absorbed)	Absorbence of less than 30 % is considered to be poorly absorbed	93.387	95.453	95.229	97.612	98.196
Skin Permeability	Numeric (log Kp)	> -2.5 = Low skin permeability	-2.735	-2.666	-2.758	-2.715	-2.727
P-glycoprotein substrate	Categorical (Yes/No)		Yes	No	Yes	No	No
P-glycoprotein I inhibitor	Categorical (Yes/No)		No	No	No	No	No
P-glycoprotein II inhibitor	Categorical (Yes/No)		No	Yes	Yes	No	No

Table 5.12 .2: Druggability analysis of phytochemicals for distribution

Property	***unit***	***Predicted value***					
		Prediction	***4',5,7-Trihydroxy isoflavone***	***5-hydroxy-2-(4-methoxyphenyl), 4-H-1 Benzopyran-4-one***	***5 hydroxy-7-methoxy-2-phenyl, 4H-1-Benzoyran-4-one***	***Palustric acid***	***pimaric acid***
VDss (human)	Numeric (log L/kg)	Low below 0.71 and high above 2.81	0.094	-0.1	-0.047	-0.546	-0.645
Fraction unbound (human)	Numeric (Fu)	For a given compound the predicted fraction that would be unbound in plasma will be calculated.	0.087	0.156	0.119	0.073	0.081
BBB permeability	Numeric (log BB)	> 0.3 = readealy cross the blood brain barrier, < -1 = poorly distributed in brain	-0.71	-0.458	0.003	0.324	0.273
CNS permeability	Numeric (log PS)	> -2 = penetrate the central nervous system, <-3= unable to penetrate the CNS	-2.048	-2.144	-1.992	-2.022	-2

Table 5.12.3: Druggability of phytochemicals for metabolism

Property	***unit***	***Predicted value***				
		4',5,7-Trihydroxy isoflavone	***5-hydroxy-2-(4-methoxyphenyl),4-H-1 Benzopyran-4-one***	***5 hydroxy-7-methoxy-2-phenyl, 4H-1-Benzoyran-4-one***	***Palustric acid***	***pimaric acid***
CYP2D6 substrate	Categorical (Yes/No)	No	No	No	No	No
CYP3A4 substrate	Categorical (Yes/No)	No	Yes	Yes	Yes	Yes
CYP1A2 inhibitior	Categorical (Yes/No)	Yes	Yes	Yes	No	No
CYP2C19 inhibitior	Categorical (Yes/No)	Yes	Yes	Yes	Yes	No
CYP2C9 inhibitior	Categorical (Yes/No)	No	Yes	Yes	Yes	Yes
CYP2D6 inhibitior	Categorical (Yes/No)	No	No	No	No	No
CYP3A4 inhibitior	Categorical (Yes/No)	No	Yes	Yes	No	No

Table 5.12.4: Druggability analysis of phytochemicals for toxicity

Property	***unit***	***Predicted value***					
		Prediction	***4',5,7-Trihydroxy isoflavone***	***5-hydroxy-2-(4-methoxyphenyl),4-H-1 Benzopyran-4-one***	***5 hydroxy-7-methoxy-2-phenyl, 4H-1-Benzoyran-4-one***	***Palustric acid***	***pimaric acid***
AMES toxicity	Categorical (Yes/No)		No	No	No	No	No
Max. tolerated dose (human)	Numeric (log mg/kg/day)	Low = Less than and equal to 0.477, high = greater than 0.477	0.478	0.193	-0.105	-0.086	0.008
hERG I inhibitor	Categorical (Yes/No)		No	No	No	No	No
hERG II inhibitor	Categorical (Yes/No)		No	No	No	No	No
Oral Rat Acute Toxicity (LD50)	Numeric (mol/kg)	The Lower the better	2.268	2.085	2.042	1.869	1.838
Oral Rat Chronic Toxicity (LOAEL)	Numeric (log mg/kg_bw/day)	The higher the better	2.189	1.432	0.744	2.344	2.207
Hepatotoxicity	Categorical (Yes/No)		No	No	No	Yes	Yes
Skin Sensitisation	Categorical (Yes/No)		No	No	No	No	No
T.Pyriformis toxicity	Numeric (log ug/L)	> -0.5 = toxic	0.377	0.567	0.638	0.388	0.31
Minnow toxicity	Numeric (log mM)	Below (<) 0.5 =high acute toxicity	1.941	0.609	-0.04	-0.491	-0.35

Table 5.12.5: Druggability analysis of phytochemicals for excretion

Property	***unit***	***Predicted value***					
		Prediction	***4',5,7-Trihydroxy isoflavone***	***5-hydroxy-2-(4-methoxyphenyl),4-H-1 Benzopyran-4-one***	***5 hydroxy-7-methoxy-2-phenyl, 4H-1-Benzoyran-4-one***	***Palustric acid***	***pimaric acid***
Total Clearance	Numeric (log ml/min/kg)	The higher the better	0.151	0.737	0.457	0.917	0.941
Renal OCT2 substrate	Categorical (Yes/No)		No	Yes	No	Yes	No

Table 5.12.6: Druggability analysis according to Lipinski's criterion

Property	***Values***				
	4',5,7-Trihydroxy isoflavone	***5-hydroxy-2-(4-methoxyphenyl),4-H-1 Benzopyran-4-one***	***5 hydroxy-7-methoxy-2-phenyl, 4H-1-Benzoyran-4-one***	***Palustric acid***	***pimaric acid***
Molecular weight	270.24	298.29	268.27	302.46	302.46
Log P	2.58	3.18	3.17	5.35	5.21
Number of hydrogen bond donors	3	1	1	1	1
Number of hydrogen bond acceptor	5	5	4	1	1

5.8 *IN VITRO* PROPAGATION AND COMPARISON OF BIOACTIVITIES OF AXENICALLY AND NATURALLY GROWN LIVERWORT *LUNULARIA CRUCIATA*

Result of antioxidant activities revealed that liverworts *Plagiochasma cordatum, Asterella wallichiana, Lunularia cruciata, Marchantia paleacea* and *Plagichila nepalensis* have high potential to scavenge free radicals. Considering the bio prospective value of the plant and their lower availability in nature, axenic culture was started with gemmae of liverwort *Lunularia cruciata*. As sporophytes were not found in proper stage of development, an attempt was made to initiate axenic culture from the gemmae. Germination of gemmae started after 7-8 days. Gemmae increased in size absorbing moisture and the viable gemmae were turned green in colour. Half strength Murashige and Skoog (MS) medium in alternate light (L) and dark condition (D) of 14hrs L/ 12hrs D was found suitable, supporting maximum gemmae germination. Germination of few gemmae was noticed in Knop's micronutrient medium also, but no further growth and development was observed. No germination was noticed in half and full strength Gamborg B-5 medium. Continuous illumination of 4000-5000 lux and temperature 21^0C $\pm 2^0$C was found to be favourable. Among different concentration of sterilants used either 1% sodium hypochlorite with exposure time of 4 minutes or 4% sodium hypochlorite with exposure time of 1 minute proved effective as gemmae remained alive and maximum microbes were killed. Exposure to higher concentration of sterilant solution even for few seconds was found to be toxic to plant material. Similarly, treatment of the explants in lower concentration given for longer time was also found to be lethal.

The early sign of germination was the change in the colour of gemmae from brown to green. Highest percentage (90%) of germination was observed in half strength MS media followed by half strength Knop's medium (20%), while no germination was noticed in full strength MS media, Knop's medium, Gamborg B5 medium and half strength Gamborg G5 medium (figure 5.8.1). Gemmae then started to increase in size and green colour became more prominent. Young thalli started developing from gemma after 10-12 days. After 2 weeks of development of thalli, gemmae turned into dark blackish green coloured undifferentiated tissue called callus. At this stage profound effect of growth regulators like Benzylaminopurine and Indole acetic acid was noticed. Effect of different combination of growth regulator concentrations was studied which confirmed 2 mg/L BAP and 0.5 mg/L

	NAA (0.5 mg/L)	NAA (1 mg/L)	NAA (2 mg/L)
BAP (1 mg)	No germination	No germination	No germination
BAP (2 mg)	Germination	No germination	No germination
BAP (3 mg)	No germination	No germination	No germination

Table 5.13: Effect of BAP/NAA on gemmae germination

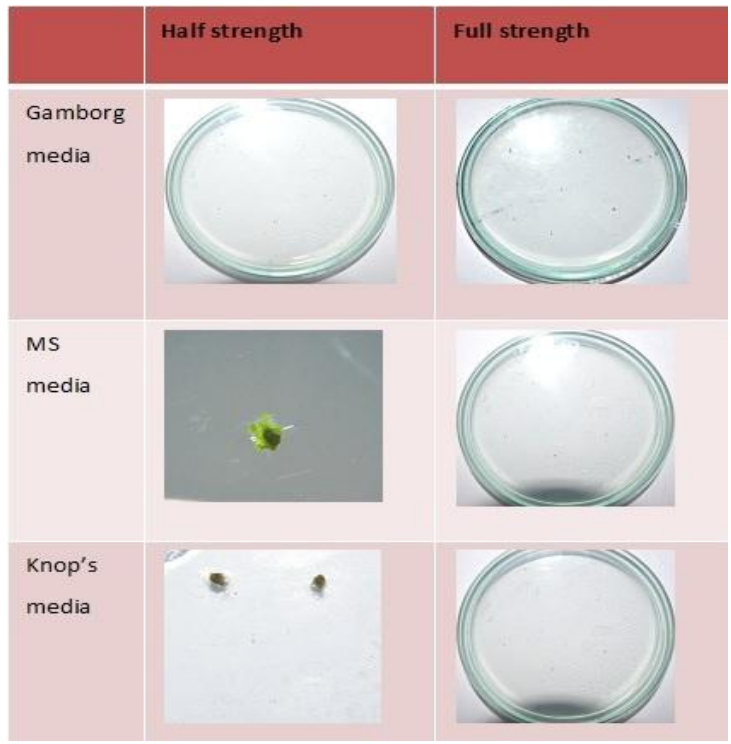


Fig 5.8.1: Effect of different growth medium on gemmae germination

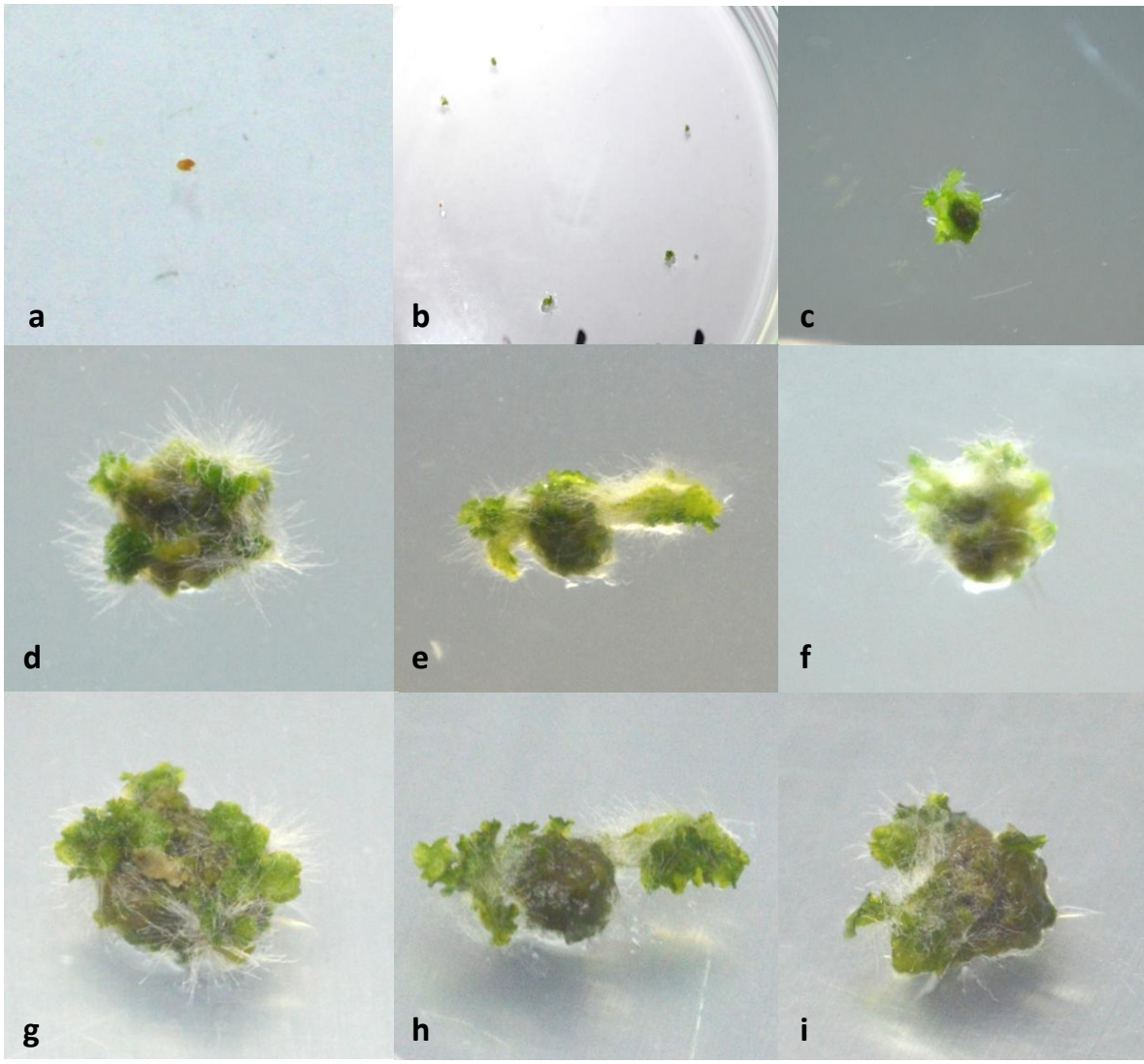


Fig 5.8.2.: **In-vitro growth of *Lunularia cruciata***. **a.** Gemmae placed on MS media **b-c.** germinating gemmae after 16-17 days **d-f.** thallus and rhizoids developed from gemmae **g-i.** well developed thalli after repeated sub culturing **j-l.** control of excessive growth of rhizoids by alternating hormone ratio and growth of dichotomously branched thalli

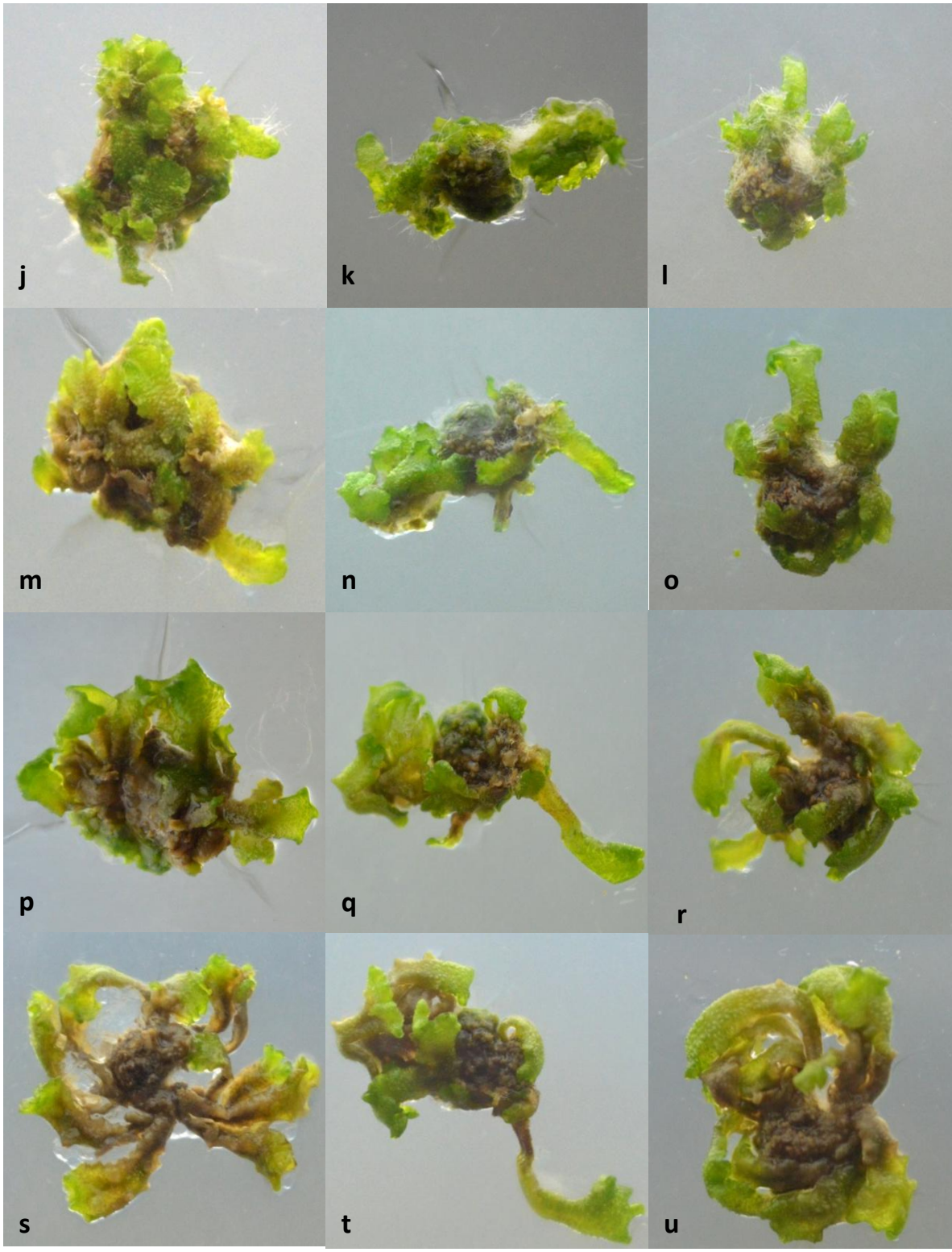


Fig 5.8.2.: ***In-vitro* growth of *Lunularia cruciata*** . **j-l** control of excessive growth of rhizoids by alternating hormone ratio and growth of dichotomously branched thalli **m-u** growth and maturation of thalli (continuation).

NAA to be most efficient for differentiation (Table 5.13). Higher concentration of growth regulators had shown detrimental effect on callus. Large number of rhizoids growing from all part of callus was noticed in the media containing 0.5 mg/L NAA hindering further differentiation of tissues. The transfer of callus in the media containing 4 mg/L BAP and 0.2 mg/L IAA lead to the development of thallus from undifferentiated tissue with much reduced number of rhizoids on the dorsal side of the thallus. With illumination of 4000-5000 lux under alternate light and dark condition of 14 hrs and 10 hrs and temperature maintained at $21^0C \pm 2^0C$ the thallus further continued to branch and grow (Fig 5.8.2). After the maturation of thallus an *in-vitro* antioxidant and anti-diabetic activities of this *in-vitro* grown liverwort was compared with its naturally growing counterparts to check if there occurs any change in the biological activities when liverworts are grown in artificial culture medium.

5.8.1 Comparison of free radical scavenging potential of *in-vitro* and naturally grown *Lunularia cruciata*

5.8.1a *DPPH$^{\bullet}$ scavenging activity*

The result of DPPH scavenging assay revealed substantial ability of both the extracts to scavenge DPPH radical (Fig 5.8.3). *In-vitro* and *in-vivo* plant extract showed only slight variation in the magnitude of radical scavenging activity. *In-vivo* plant extract showed 73%, 40% and 24% inhibition at 650 µg, 350 µg and 250 µg concentrations respectively. Extract from *in vitro* source showed 48%, 35% and 22% inhibition of DPPH at 650 µg, 350 µg and 250 µg concentrations respectively.

5.8.1b *$ABTS^+$ scavenging activity*

This assay is widely used in *in-vitro* studies to assess the antioxidant property. Free radical scavenging property of extracts was further confirmed by $ABTS^+$ scavenging assay (Fig 5.8.4). 98% and 88% inhibition of ABTS radical was shown by *in-vitro* and *in-vivo* extracts respectively at 2 mg/ml concentration. Data highlighted slightly better $ABTS^+$ scavenging property of *in-vitro* plant extract than that of *in-vivo* plant extract.

5.8.1c *Metal chelating assay*

Variation between *in-vitro* and *in-vivo* grown plant extracts was recorded for metal chelating activity. A reduction of 85% in free ferrous ion concentration was shown by 4.5 mg

in-vivo plant extract while *in-vitro* plant extracts showed 46% reduction at 4.5 mg concentration (Fig 5.8.5).

5.8.2 Comparison of anti-diabetic activity of *in-vitro* and naturally grown *Lunularia cruciata*

5.8.2a *α-Glucosidase inhibitory activity*

Result of α-glucosidase inhibitory assay revealed the property of *in-vitro* and *in-vivo* plant extracts to inhibit the α-glucosidase enzyme activity to an impressive level. *In-vitro* plant extract showed IC_{50} value 0.24 mg/ml and *in-vivo* plant showed IC_{50} value 0.51 mg/ml (Fig 5.8.6). Lower IC_{50} value of *in-vitro* plant extract indicates its higher α-glucosidase inhibitory activity than the *in-vivo* grown plants.

5.8.2b *α-amylase inhibitory activity*

In-vitro and *in-vivo* grown plants both showed α-amylase enzyme inhibitory activity. IC_{50} value of 0.48 mg/ml and 0.40mg/ml was recorded for *in-vitro* and *in-vivo* grown plant extract respectively (Fig 5.8.7).

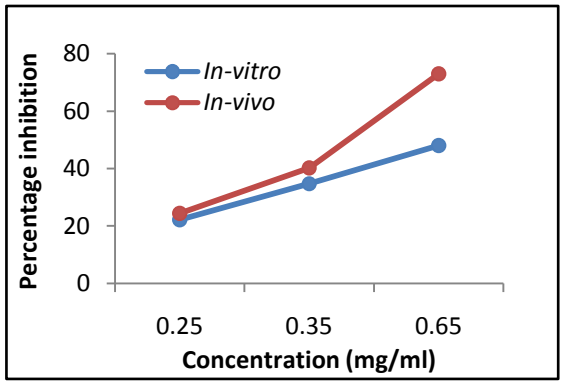


Fig 5.8.3: DPPH· scavenging activity of *in-vitro* and naturally grown *L. cruciata*

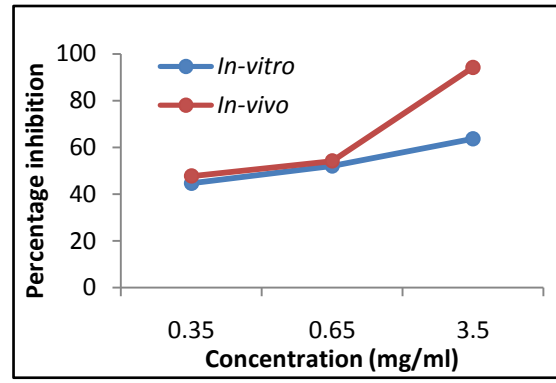


Fig 5.8.4: ABTS· scavenging activity of *in-vitro* and naturally grown *L. cruciata*

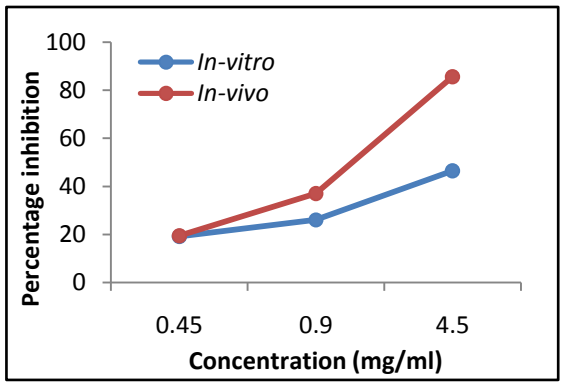


Fig 5.8.5: Metal chelating activity of *in-vitro* and naturally grown *L. cruciata*

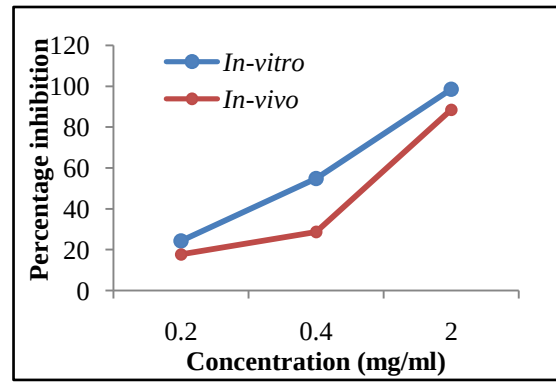


Fig 5.8.6: α-glucosidase inhibitory activity of *in-vitro* and naturally grown *L. cruciata*

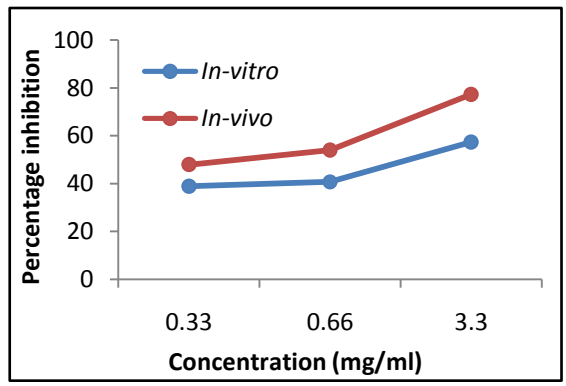


Fig 5.8.7: α-amylase inhibitory activity of *in-vitro* and naturally grown *L. cruciata*

5.8.3 Phytochemical analysis of *in-vitro* and naturally grown *Lunularia cruciata*

GC-MS analysis was performed to study the variation in chemical profile of *in-vitro* and naturally grown plants and also to characterize the possible sources of liverwort extracts for biological activity. Methanolic extract of *in-vitro* and naturally grown plants showed the presence of different types of alkaloids, flavonoids, terpenes, fatty acid, aliphatic hydrocarbon and acyclic alkanes on analyses. Total of 9 compounds have been identified from naturally grown plant methanolic extract, this includes thujopsene; flavones; cyclopentaneudecanoic acid, methyl ester; cyclopentaneundecanoic acid; 13,16-octadecadienoic acid; methyl ester; phytol; deoxyaspidodispermine; 13-docosenoic acid; methyl ester and 4-piperidieacetic acid,1-acetyl-5-ethyl-2-[3(2-hydroxyethyl)-1H-indol-2-yl]-a-methyl and methyl ester (Table 5.14). Methanolic extract of *in-vitro* grown plant also showed the presence of 9 compounds *namely*, piperidine, 1-butyl; 2-cyclohexen-1-one, 3hydroxy-2-[(5-oxo-1-cyclopenten-1-yl) methyl]; flavones; 4H-1-benzopyran-4-one, 3 hydroxy-2-phenyl, tetradecanoic acid, ethyl ester; phytol; Z-13-octadecen-1-yl acetate; Isopropyl stearate and tricosane-2,4-dione (Table 5.15).

GC-MS analysis has showed the present of phytol and flavones in plants grown in both natural and artificial growth condition. These compounds showed similar retention time as well. Alkaloid group was present in both naturally and artificially grown *L. cruciata* but in different forms. 1-butyl Piperidine was found in *in-vitro* grown plants while slightly modified form, 4-piperdineacetic acid, 1 acetyl 5 ethyl-2[3-(2 hydroxy ethyl)-1H-indol-2-yl]-a methyl, methyl ester was found in grown grown plants. Fatty acid and their esters were also detected in plants grown in both the habitats. Ethyl tetradecanoate, Isopropyl stearate were fatty acids present in *in-vitro* grown plants and Cyclopentaneudecanoic acid, Cyclopentaneudecanoic acid, methyl ester, 13-Decosenoic acid, methyl ester, 13,16-Octadecanoic acid, methyl ester are form of fatty acids that were present in naturally grown plants. However, presence of aliphatic hydrocarbon and alkanes were found only in *in-vivo* grown plants (Table 5.16).

Table 5.14 : Phytochemicals present in *in-vitro* grown *Lunularia cruciata* methanolic extract and their biological activities

Name	***RT***	***TIC***	***Compound nature***	***Biological activities***
1-1-butyl Piperidine	12.08	481975	alkaloid	Anti-microbial (Kumar *et al*., 2009); cytotoxic (Rinaldi *et al*., 2017); Antimalarial (Wirasathien *et al*., 2006)
3-Hydroxy-2-[(5-oxo-1-cyclopenten-1-yl)methyl]-2-cyclohexen-1-one	14.17			No activities reported
Flavone	16.5	596325	Flavonoid	Antioxidant (Pietta, 2000), Hepatoprotective (Akachi *et al*., 2010), Antibacterial (Cushnie and Lamb, 2005), Anti-Inflammatory (Serafini *et al*., 2010), Anticancer (Zhang *et al*., 2008), Antiviral Activity (Lani *et al*., 2016)
3-hydroxy-2-phenyl 4H-1-Benzopyran-4-one	17.27	1412798	Flavonols	Antioxidant (Pietta, 2000), Hepatoprotective (Akachi *et al*., 2010), Antibacterial (Cushnie and Lamb, 2005), Anti-Inflammatory (Serafini *et al*., 2010), Anticancer (Zhang *et al*., 2008), Antiviral Activity (Lani *et al*., 2016)
Tetradecanoic acid, ethyl ester	18.03	632812	Fatty acid	No activities reported
Phytol	18.95	2122402	Diterpene alcohol	Antimicrobial (Cardoso *et al*., 2012), Anti-inflammatory (Khiev *et al*., 2011), Anti cancer (Islam, 2017), Cardiovascular and diuretic activity (Somova *et al*., 2001)
Z-13-octadecen-1yl acetate	20.43	1229732	Aliphatic hydrocarbon	No activities reported
Isopropyl stearate	22	833849	Fatty acid ester	No activities reported
Tricosane 2,4-dione	23.17	607218	acyclic alkanes	No activities reported

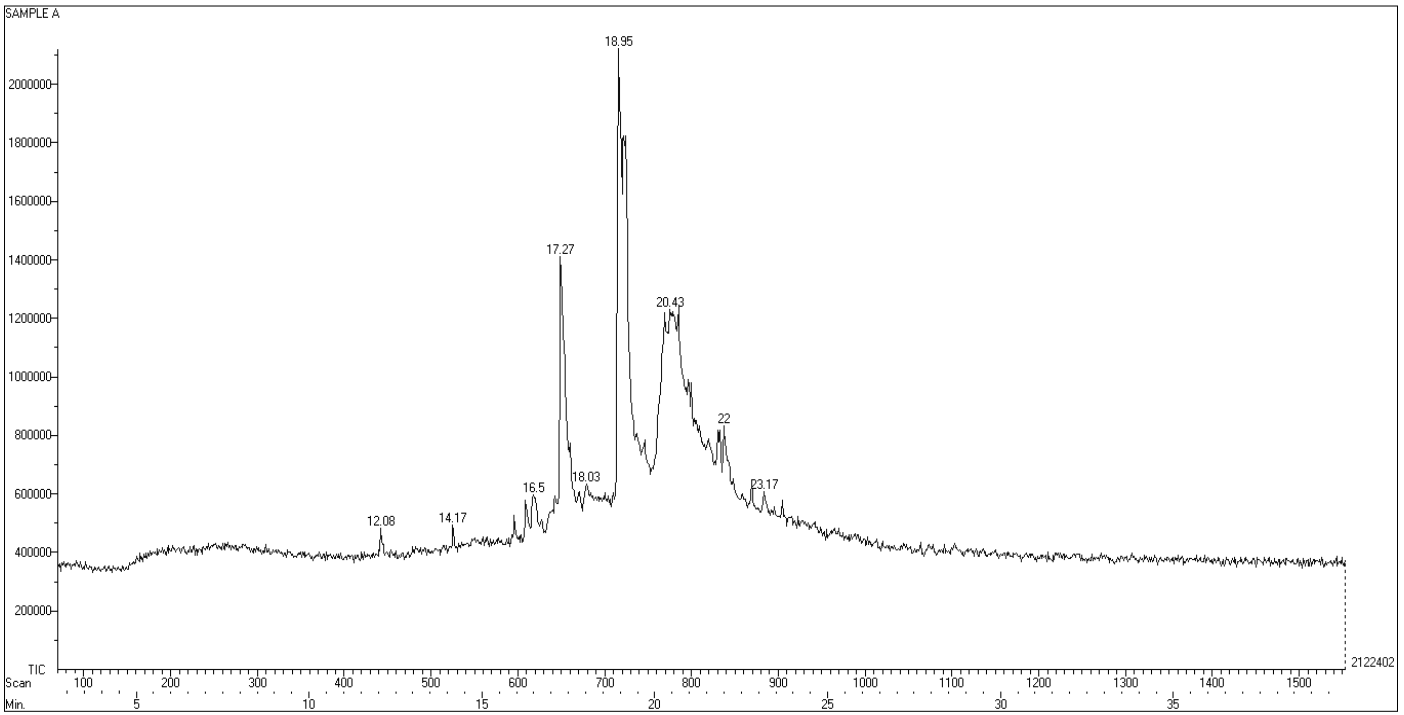


Fig 5.8.8: Gas chromatogram with mass-spectrometric detection of the methanolic extract of *in-vitro* grown *Lunularia cruciata*

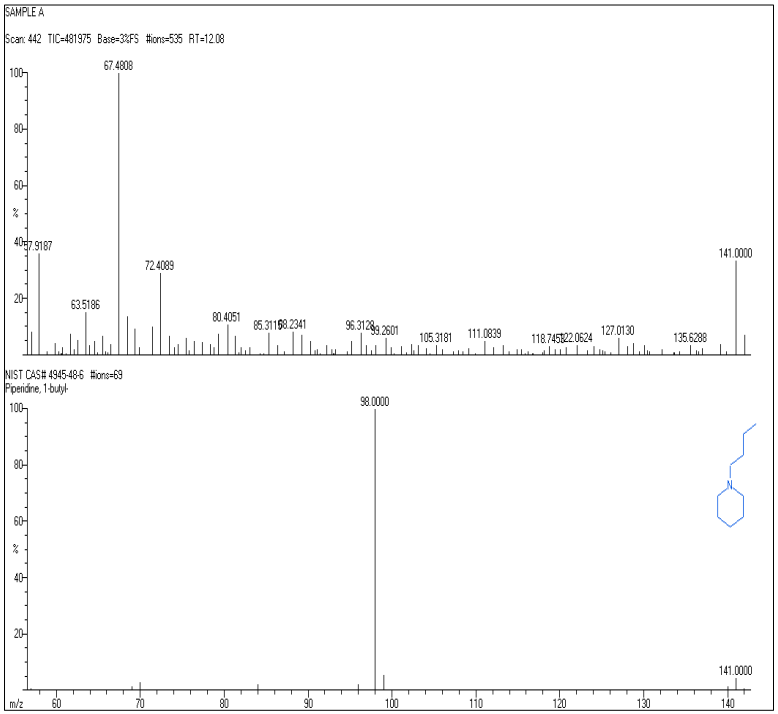


Fig 5.8.9: Structure and MS spectrum of 1-butyl-Piperidine compared with NIST Library Spectral Database

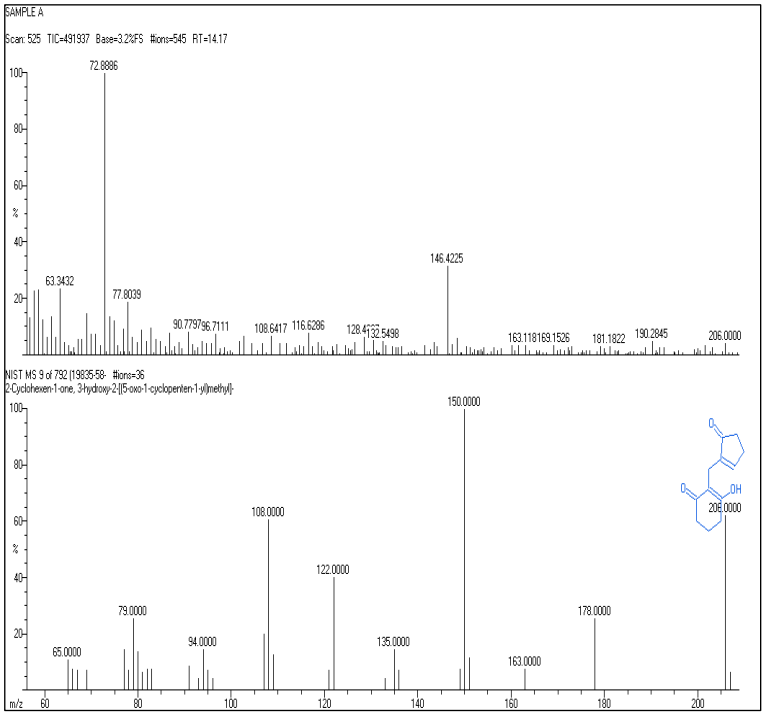


Fig 5.8.10: Structure and MS spectrum of 3-Hydroxy-2-[(5-oxo-1-cyclopenten-1-yl)methyl]-2-cyclohexen-1-one compared with NIST Library Spectral Database

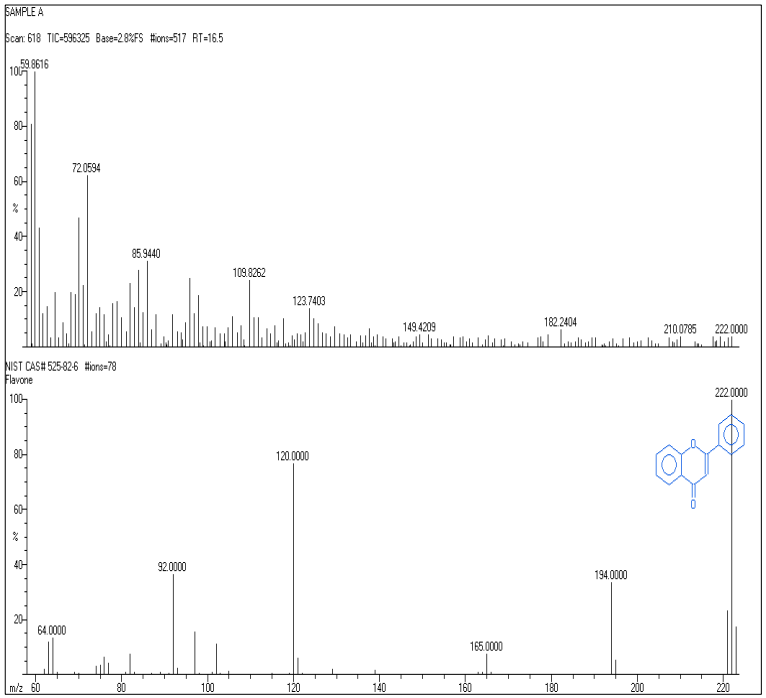


Fig 5.8.11: Structure and MS spectrum of flavone compared with NIST Library Spectral Database

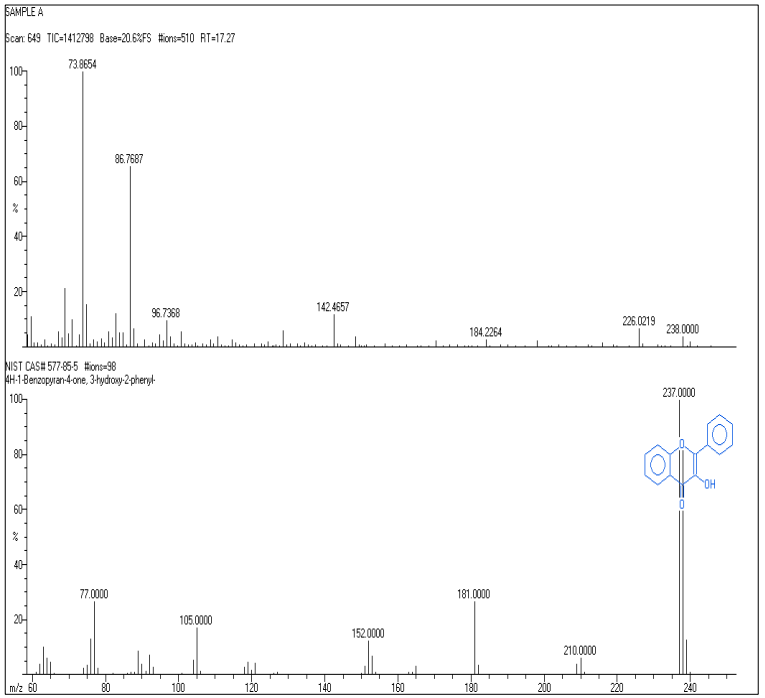


Fig 5.8.12: Structure and MS spectrum of 4H-1-Benzopyren-4-one, 3-hydroxy-2-phenyl compared with NIST Library Spectral Database

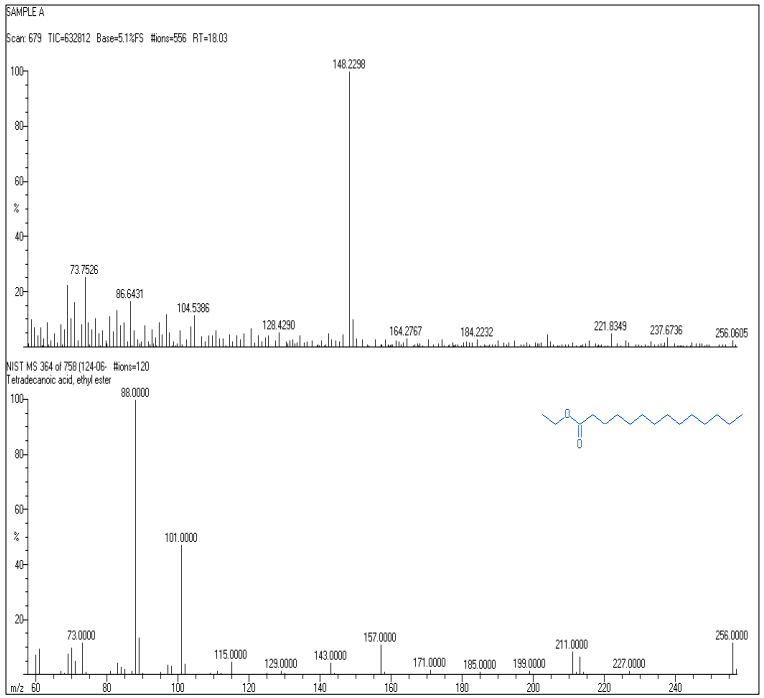


Fig 5.7.13: Structure and MS spectrum of Tetradecanoic acid, ethyl ester compared with NIST Library Spectral Database

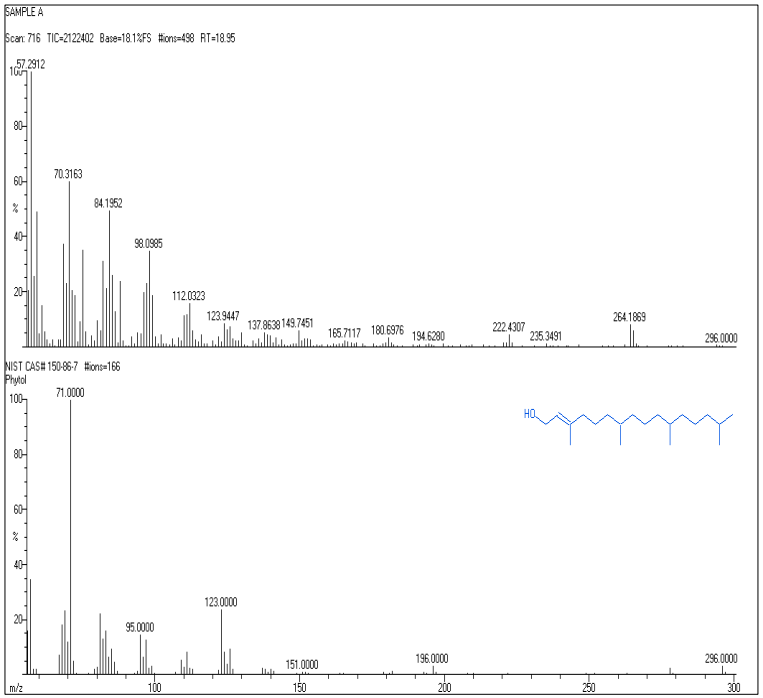


Fig 5.7.14: Structure and MS spectrum of Phytol compared with NIST Library Spectral Database

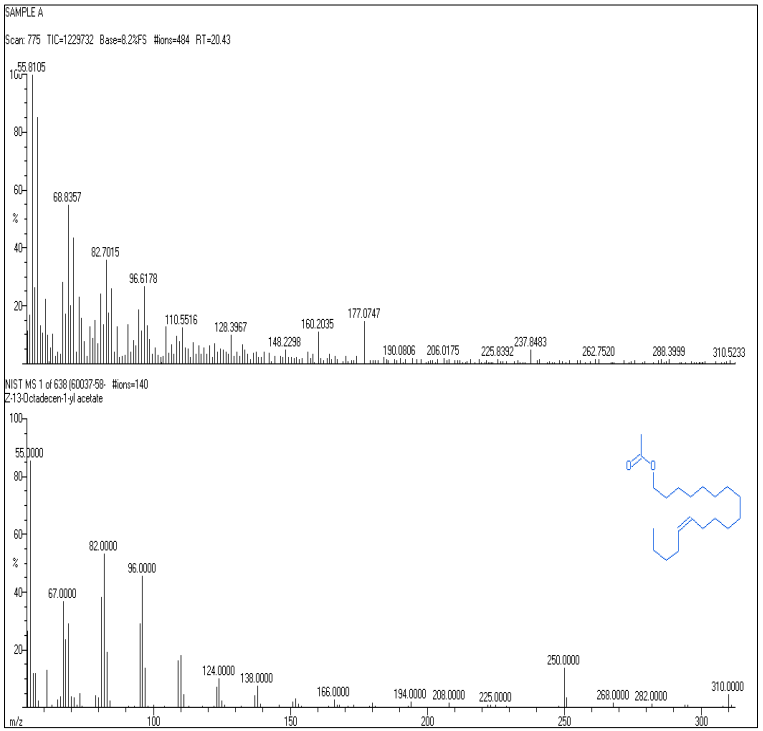


Fig 5.7.15: Structure and MS spectrum of 2-13-octadecen-1-yl acetate compared with NIST Library Spectral Database

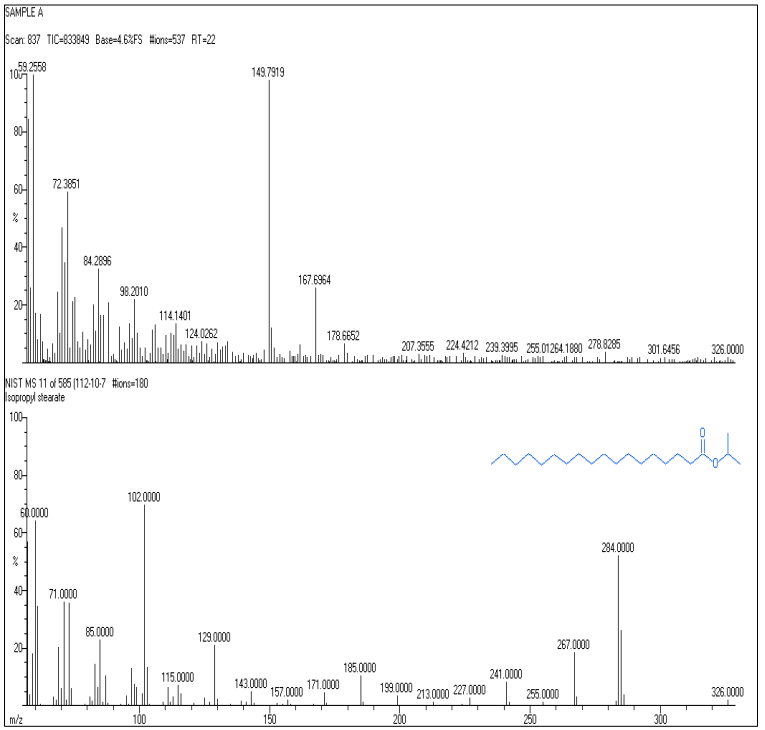


Fig 5.7.16: Structure and MS spectrum of Isopropyl stearate compared with NIST Library Spectral Database

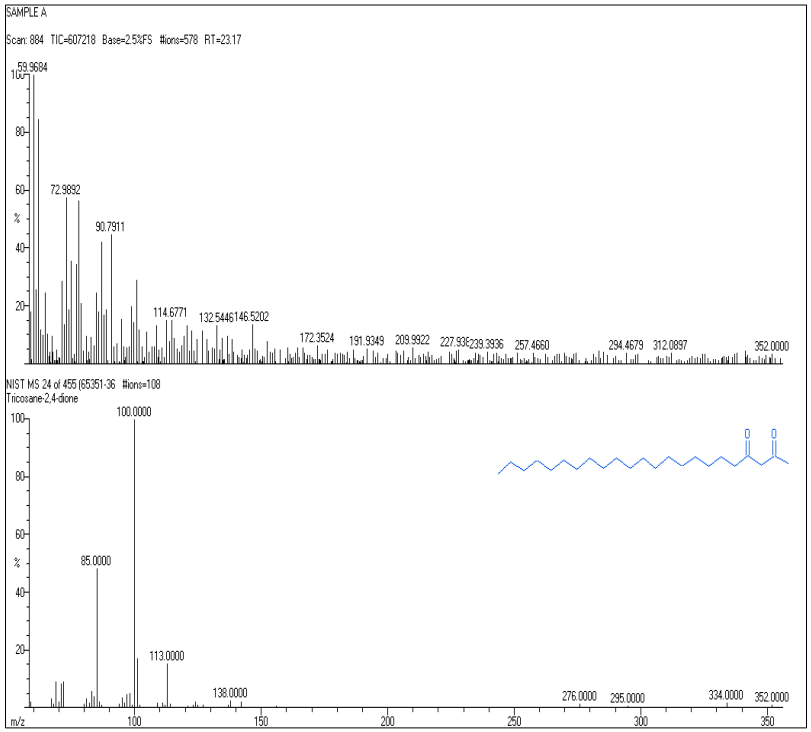


Fig 5.8.17: Structure and MS spectrum of Tricosane -2,4- dione compared with NIST Library Spectral Database

Table 5.15 : Phytochemicals present in naturally grown *Lunularia cruciata* methanolic extract and their biological activities

Name	***RT***	***TIC***	***Compound nature***	***Biological activities***
Thujopsene-[12]	14.03	873686	sesquiterpene	Antimicrobial (Cardoso *et al*., 2012), Anti-inflammatory (Khiev *et al*., 2011), Anti cancer (Islam 2017), Cardiovascular and diuretic activity (Somova *et al*., 2001)
Flavone	15.75	1138027	Flavonoid	Antioxidant (Pietta, 2000), Hepatoprotective (Akachi *et al*., 2010), Antibacterial (Cushnie and Lamb, 2005), Anti-Inflammatory (Serafini *et al*., 2010), Anticancer (Zhang *et al*., 2008), Antiviral Activity (Lani *et al*., 2016)
Cyclopentaneudecanoic acid, methyl ester	17.17	1822103	Fatty acid ester	No activities found
Cyclopentaneudecanoic acid	18.08	1274201	Fatty acid	No activities found
13,16-Octadecanoic acid, methyl ester	18.85	2536991	Fatty acid ester	No activities found
Phytol	19.03	2799556	Diterpene alcohol	Antimicrobial (Cardoso *et al*., 2012), Anti-inflammatory (Khiev *et al*., 2011), Anti cancer (Islam 2017), Cardiovascular and diuretic activity (Somova *et al*., 2001)
Deoxy aspidodispermine	20.43	2148295	alkaloid	No activities found
13-Decosenoic acid, methyl ester	21.77	1202092	Fatty acid ester	No activities found
1 acetyl 5 ethyl-2[3-(2 hydroxy ethyl)-1H-indol-2-yl]-a methyl, 4-piperdineacetic acid, methyl ester	21.95	1105543	Alkaloid	Anti-microbial (Kumar *et al*., 2009); cytotoxic (Rinaldi *et al*.,2017); Antimalarial (Wirasathien *et al*., 2006)

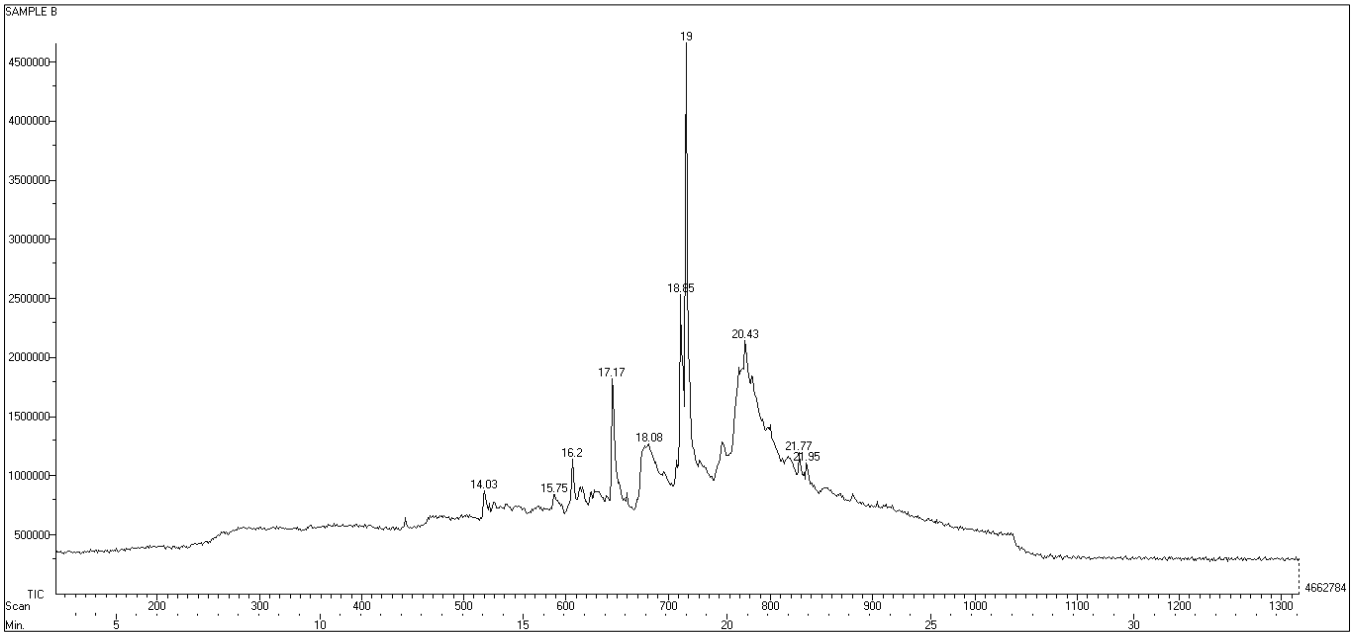


Fig 5.8.18: Gas chromatogram with mass-spectrometric detection of the methanolic extract of naturally grown *Lunularia cruciata*

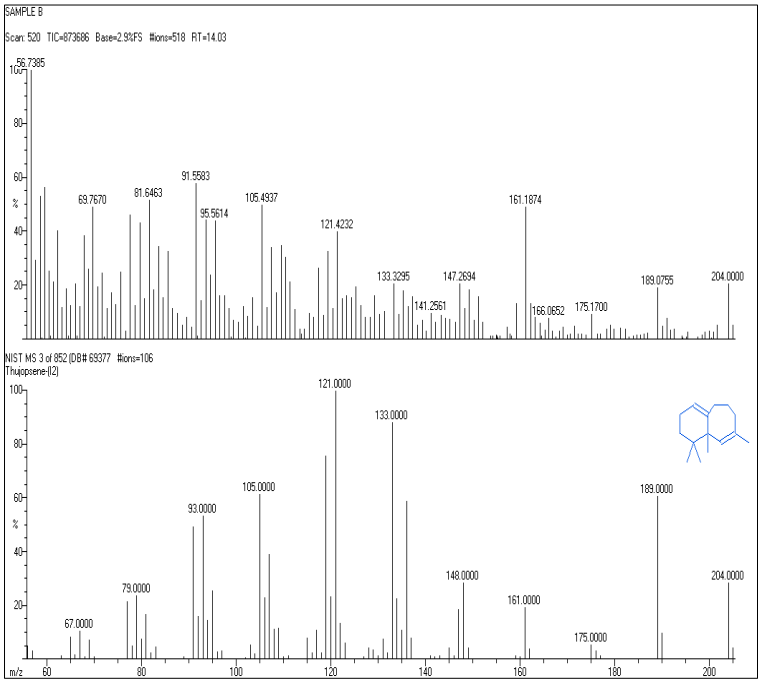


Fig 5.8.19: Structure and MS spectrum of Thujopsene-(12) compared with NIST Library Spectral Database

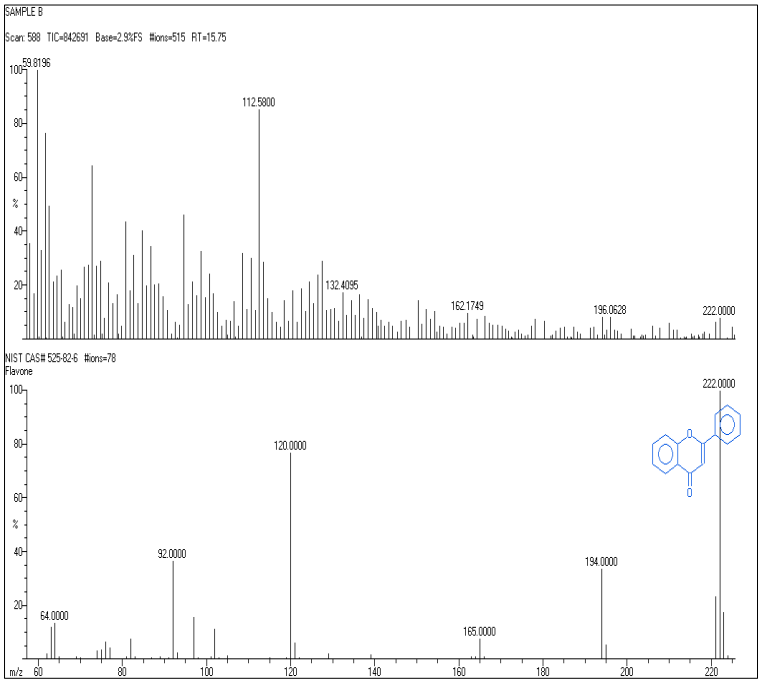


Fig 5.8.20 : Structure and MS spectrum of Flavone compared with NIST Library Spectral Database

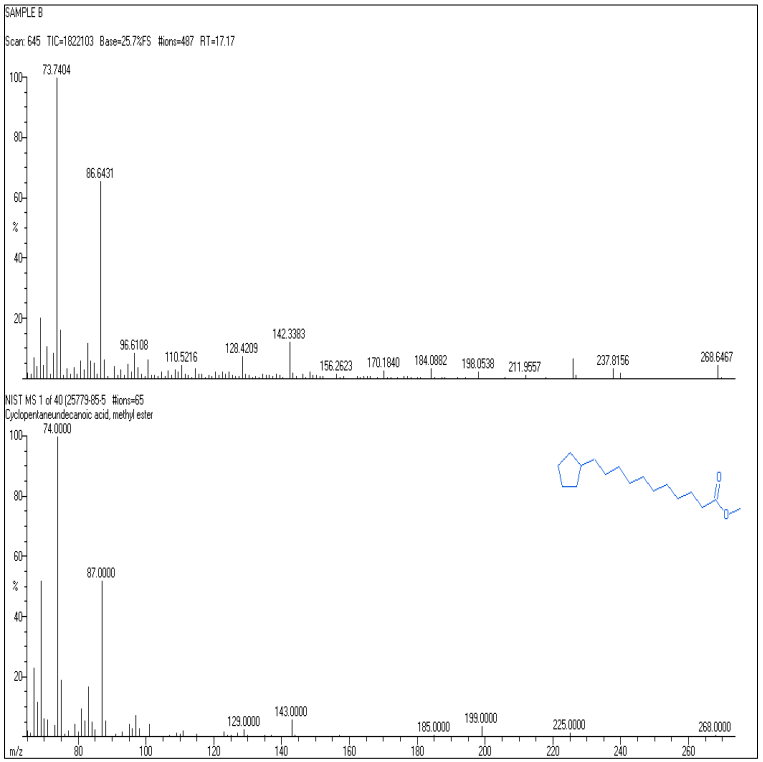


Fig 5.8.21: Structure and MS spectrum of Cyclopentaneudecanoic acid, methyl ester compared with NIST Library Spectral Database

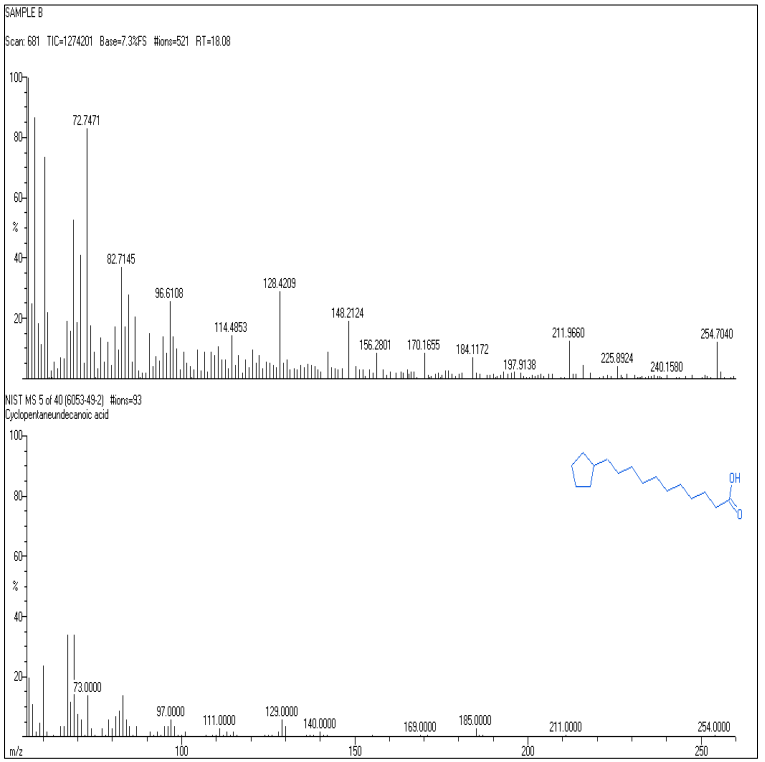


Fig 5.8.22: Structure and MS spectrum of Cyclopentaneudecanoic acid compared with NIST Library Spectral Database

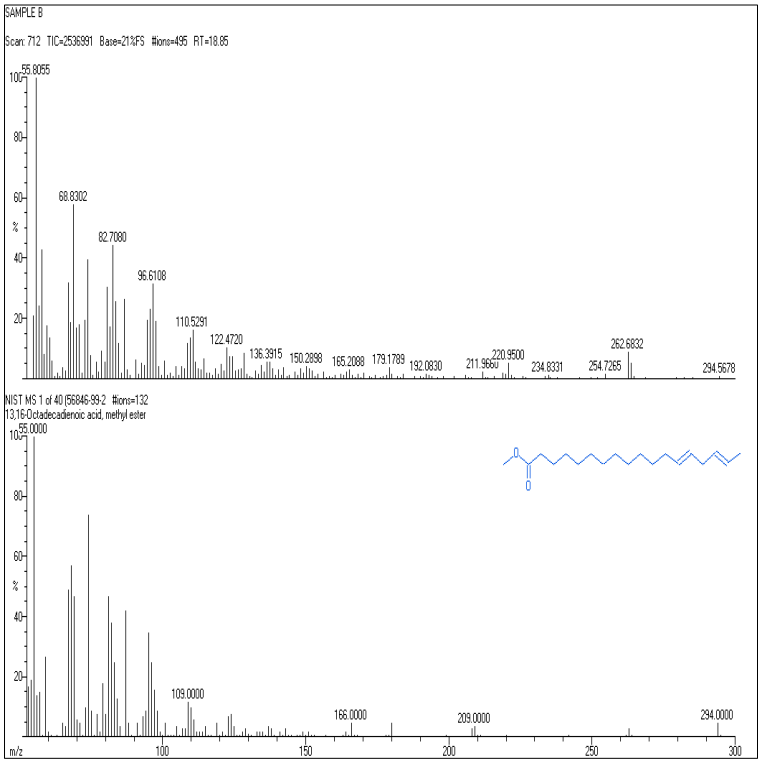


Fig 5.8.23: Structure and MS spectrum of 13,16-Octadecadienoic acid, methyl ester compared with NIST Library Spectral Database

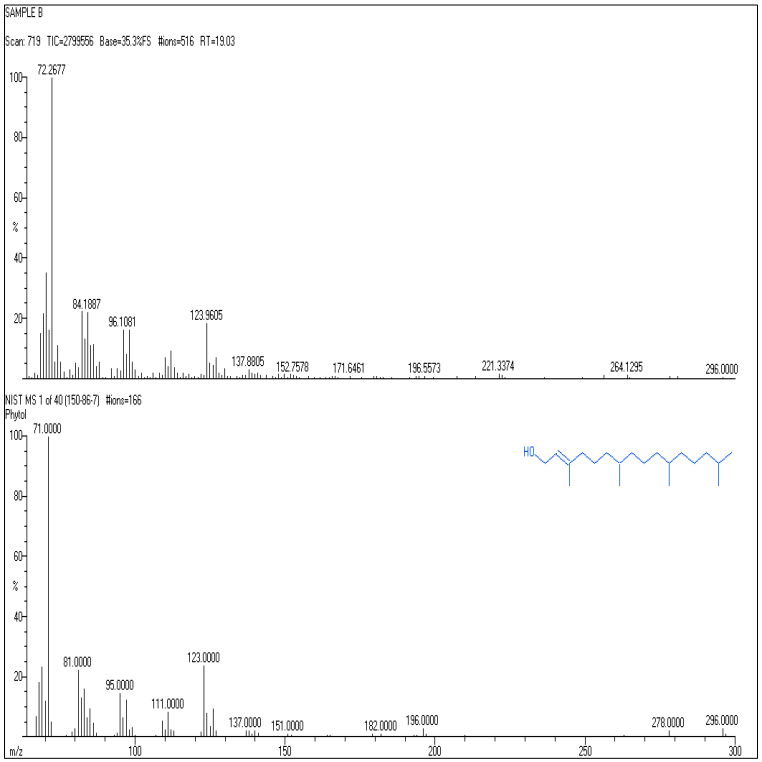


Fig 5.8.24 : Structure and MS spectrum of Phytol compared with NIST Library Spectral Database

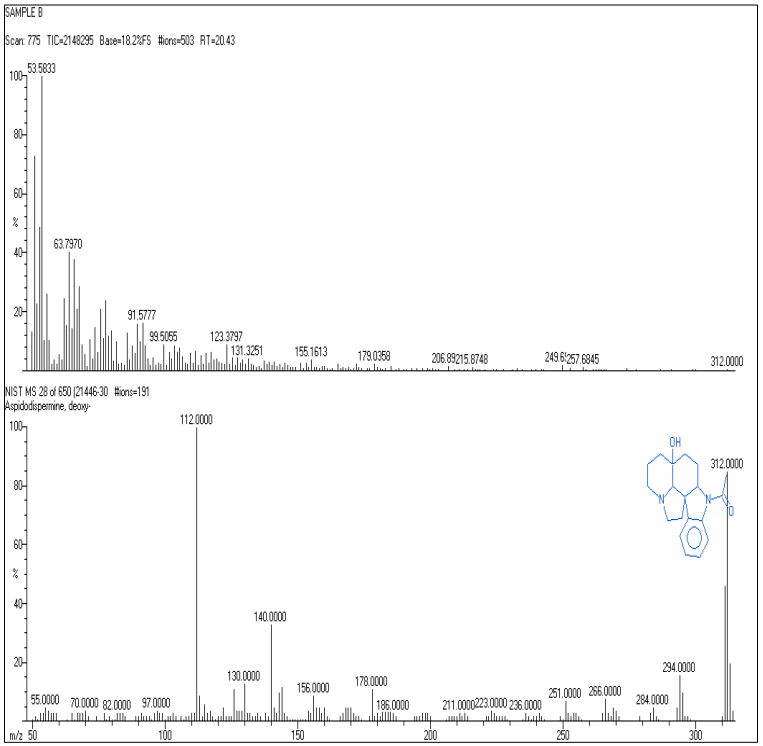


Fig 5.8.25: Structure and MS spectrum of deoxy-Aspidodispermine compared with NIST Library Spectral Database

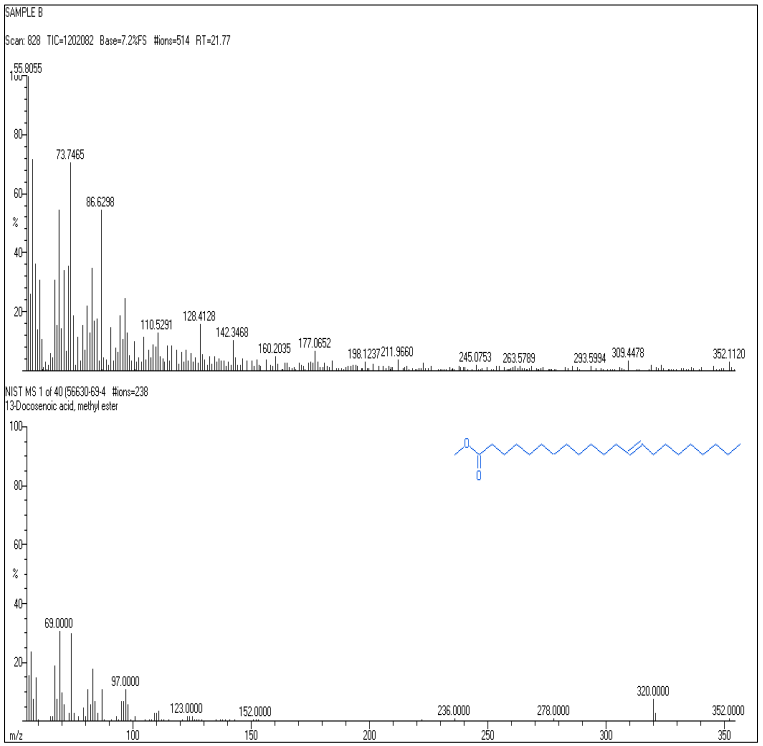


Fig 5.8.26: Structure and MS spectrum of 13-Docosenoic acid, methyl ester compared with NIST Library Spectral Database

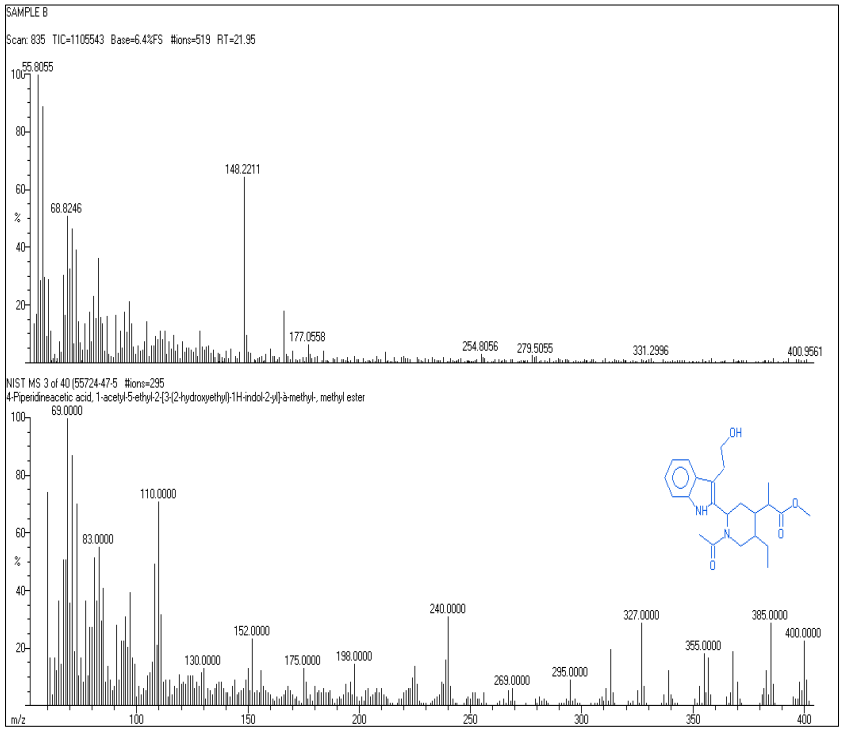


Fig 5.8.27: Structure and MS spectrum of 1-acetyl-5-ethyl-2-[3-[2-hydroxyethy]-1H-indol-2-yl]-α-methyl 4-Piperidineacetic acid, methyl ester compared with NIST Library Spectral Database

Table 5.16: Comparison of phytochemicals present in *in-vitro* and *in-vivo* grown Lunularia cruciata

Sl. No.	*Compound class*	*Plant growth condition*			*Retention time*	*Relative abundance*
		In-vitro	*Relative abundance*	*Naturally grown*		
1	**Alkaloid**	1-butyl Piperidine	23%	-	12.08	
		-		4-piperdineacetic acid,; 1 acetyl 5 ethyl-2[3-(2 hydroxy ethyl)-1H-indol-2-yl]-a methyl, methyl ester	21.95	40%
2	**Terpenes**	Phytol	100%	Phytol	18.95 (*in-vitro*), 19.03 (*in-vivo*)	100%,
		-		Thujopsene-[12]	14.03	31%
3	**Flavonoid**	Flavone ;	28%	Flavone	16.5	71%
		3-hydroxy-2-phenyl 4H-1-Benzopyran-4-one,	67%	-	16.80	
4	**Fatty acid**	Ethyl tetradecanoate	30%	-	18.03	
				Cyclopentaneudecanoic acid	18.08	46%
5	**Fatty acid ester**	Isopropyl stearate	39%		22	
		-		Cyclopentaneudecanoic acid, methyl ester ;	17.17	65%
		-		13,16-Octadecanoic acid, methyl ester ;	18.85	91%
		-		13-Decosenoic acid, methyl ester	21.77	43%
6	**Aliphatic hydrocarbon**	Z-13-octadecen-1yl acetate	58%	-	20.43	
7	**Alkanes**	Tricosane 2,4-dione	29%	-	23.17	

5.9.1 VARIATION IN THE ABUNDANCE OF EPIPHYTIC LIVERWORTS IN RELATION PHYSICO-CHEMICAL ATTRIBUTES

This study demonstrates the effects of different environmental factors and biochemical characteristics of *Cryptomeria japonica* tree bark on the relative abundance of the epiphytic liverworts. Liverwort flora on *C. japonica* bark consisted mainly of three species *viz, Bazzania oshimensis*, *Ptycanthus striatus* and *Pellia epiphylla* belonging to families Lepidoziaceae, Lejeuneaceae and Peliaceae respectively. According to abundance percentage, *B. oshimensis* was the most dominant species while *Pellia epiphylla* was found scarcely.

The study on epiphytic liverworts' abundance in Senchal forest showed moisture content of the substrate (bark) to be best correlated with the epiphytic cover in positive manner showing coefficient of correlation value of 0.648 with $R^2 = 0.420$. Barks with moisture content ranging between 52.24% and 84.48% favours luxurious growth of epiphytic liverworts while, their frequency of occurrence declined drastically below 50% moisture level (Fig 5.9.1). Among analyzed trees, maximum trees showed 65–71% moisture content in their bark (Fig 5.9.2). Light intensity on the studied sites ranged between 1620 lux and 15200 lux. Data suggested that light intensity in the canopy and epiphytic liverwort cover were inversely related with each other. Pearson correlation matrix value of -0.77 signifies inverse relationship between two variables. In the studied sites, maximum trees favouring epiphytic liverwort cover experienced 2800–3700 lux light intensity (Fig 5.9.4). High epiphytic liverwort abundance was recorded in light intensity range between 1900 to 4600 lux (Fig 5.9.3).

The pH of bark of *C. japonica* was slightly acidic (pH = 3.0 – 5.1). In our study, pH of the bark didn't affect the abundance of the epiphytic liverwort on the tree trunk [$R^2 = 0.0043$] (Table 5.20). Maximum trees had pH ranging between 3.2–3.4 (Fig 5.9.6). Presence of epiphytic liverworts was noticed on moderate to old aged trees, while they were completely absent on the young trees (Fig 5.9.5). Coefficients of determination (R^2) value of $R^2 = 0.602$ between abundance and dbh indicates positive correlation between the studied variables (Pearson Correlation matrix = 0.776). Epiphytic liverwort cover was noticed on trees having girth above 100 cm, while no growth were observed on trees having girth size below this level (Fig 5.9.7).

In studied trees, phenol content ranged between 36–844 µg gallic acid equivalent (eq.) / mg fresh weight tissue (fwt). Maximum trees under study showed phenol content in a range of 85–170 µg gallic acid eq. / mg fwt (Fig 5.9.10). Epiphytic liverwort cover was noticed on trees with phenol content ranging between 36–510 µg gallic acid eq./ mg fwt. Higher content of phenol showed inhibitory activity against liverwort proliferation (Fig 5.9.9) with few exceptions. However, Pearson correlation test didn't indicate any direct correlation between phenol content and bryophyte cover (Table 5.19).

Flavonoid content of the tree also didn't affect the liverwort proliferation. Co-efficient of determination and Pearson correlation value indicated no correlation between two variables. Epiphytic liverwort cover was noticed on trees having flavonoid content above 5.7 µg Quercetin equivalent/ mg fwt (Fig 5.9.11). Maximum epiphytic liverwort proliferation was noticed on trees containing flavonoid in the range 11.3–14.2 µg quercetin eqv. / mg fwt (Fig 5.9.12). Similar to flavonoids, no correlation was found between ortho-dihydric phenol content and liverwort cover by Coefficient of determination and Pearson correlation analysis (Table 5.19). Epiphytic liverwort abundance didn't increase or decrease with the content of orthodihydric phenol (Fig 5.9.13). Maximum percentage of epiphytic liverwort abundance was noticed on trees having 3.8–4.5 µg catechol eqv./ mg fwt ortho-dihydric phenol (Fig 5.9.14). Phenol, flavonoid and ortho-dihydric phenol content of the studied trees varied irrespective of tree age, suggesting no effect of age on phenolic compound content of plants. Another important class of secondary metabolite synthesized by plants is terpenoids.

Terpenoids are the primary constituents of pine oleoresin (Rodrigues-Correa *et al.*, 2013). Co-efficient of determination value, R^2= 0.938 suggests direct correlation between terpenoid content and liverwort cover (Table 5.20); Pearson correlation value of -0.959 (Table 5.18) suggested correlation to be negative between two variables. Coefficient of determination (Table 5.20); R^2= 0.654 (Pearson correlation matrix = - 0.851) suggested that terpenoid content in the studied trees decreased with the increase in tree age (Table 5.18). Maximum epiphytic growth was observed on the tree having terpenoid content in a range between 1.32 mg to 13.2 mg fwt. However, increase in the concentration of terpenoid affected the proliferation of liverworts in a negative manner.

Two more biochemical variables, total sugar and tannin content of the bark were studied to understand their possible role in epiphytic liverwort distribution. Optimal

proliferation of epiphytic liverworts was noticed on trees containing sugar in a range between 0.19 - 1.16 µg glucose eqv. /mg fwt (Fig: 5.9.17). Coefficient of determination test value R^2= 0.05 (Table 5.20) and Pearson correlation test value -0.087 (Table 5.18) indicates no direct or indirect correlation between sugar content of the bark and epiphytic cover. Similarly tannin also didn't affect the epiphytic distribution on the tree bark (R^2= 0.07). Maximum growth of epiphytic liverworts was noticed on trees having 0.3–0.15 µg tannic acid eqv. / mg fwt tannic acid (Fig 5.9.20).

5.9.2 VARIATION IN THE ABUNDANCE OF LIVERWORTS GROWING ON SOIL IN RELATION TO PHYSICAL FACTOR AND SOIL PROPERTIES

There was significant influence of light on the abundance of liverworts growing on soil. Coefficient of determination test value R^2= 0.877 indicated direct correlation between the two attributes (Fig 5.9.23). It was noticed that light and bryophyte abundance were correlated negatively each other. Moisture content of the soil also significantly influenced the bryophyte abundance (Coefficient of determination test value R^2=0.983). Liverwort abundance was positively correlated with the moisture content of the soil (Fig: 5.9.24). No influence of soil pH was found on the abundance of liverworts in this work (0.006). Similarly no correlation between the liverwort abundance and other physico chemical properties of soil like organic carbon (R^2=0.130) [Fig 5.9.27], nitrogen (R^2=0.203) [Fig 5.9.28], phosphorous (R^2=0.014) [Fig 5.9.30], potassium (R^2=0.001) [Fig 5.9.32] and sulphur content (R^2=0.006) [Fig 5.9.33] was found in this work. While, carbon and nitrogen ratio of the soil was found to be positively correlated with the liverwort abundance, although weakly so (R^2=0.579) [Fig 5.9.31].

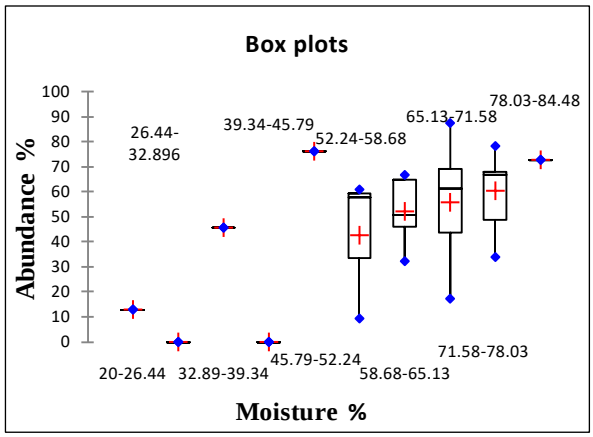


Fig 5.9.1: Variation in abundance % according to moisture content of bark

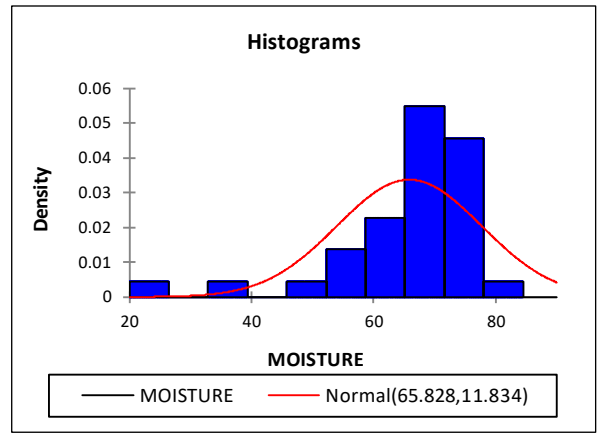


Fig 5.9.2: Normal probability curve of distribution of liverworts related with moisture content of the bark

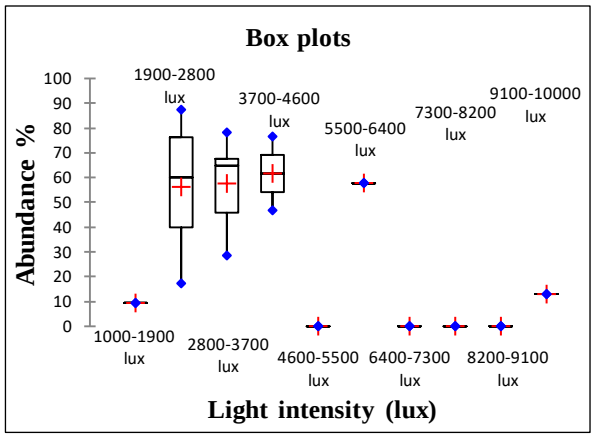


Fig 5.9.3: Variation in abundance % according to light intensity

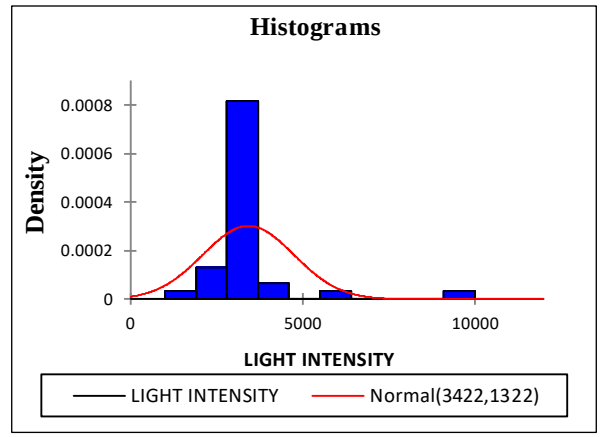


Fig 5.9.4: Normal probability curve of distribution of liverworts related with light intensity

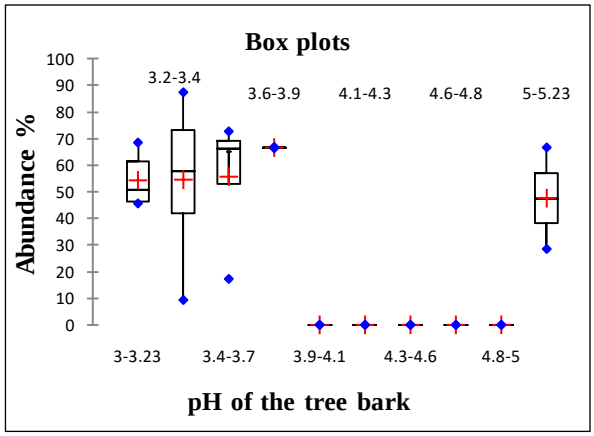


Fig 5.9. 5: Variation in abundance % according to pH of the bark

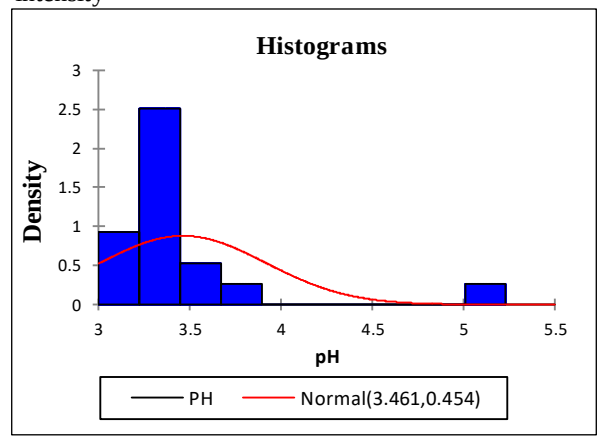


Fig 5.9.6: Normal probability curve of distribution of liverworts related with pH bark

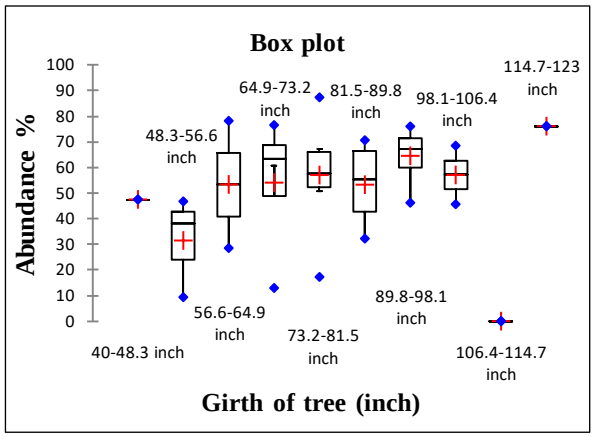


Fig 5.9 7: Variation in abundance % according to girth of the tree

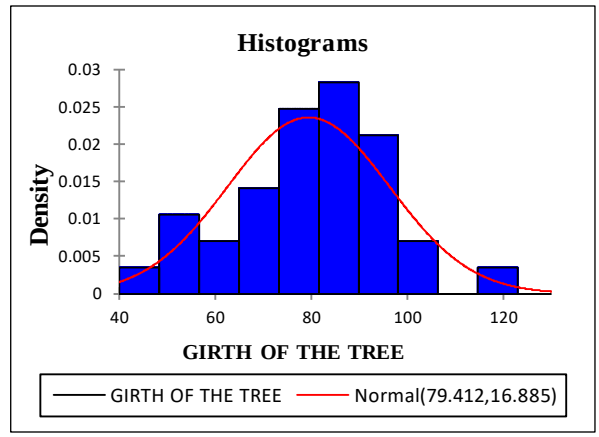


Fig 5.9.8: Normal probability curve of distribution of liverworts related with girth of the tree

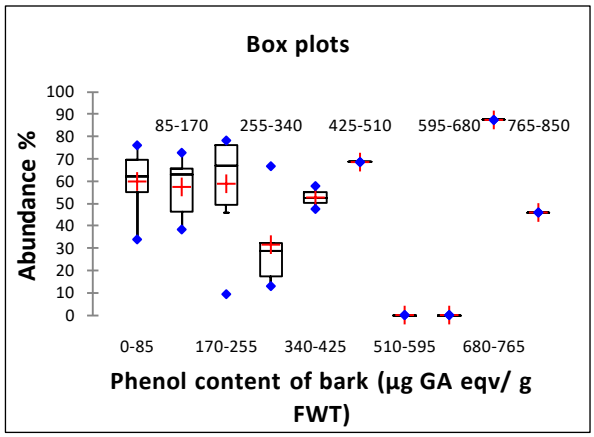


Fig 5.9 .9: Variation in abundance % according to phenol content of bark

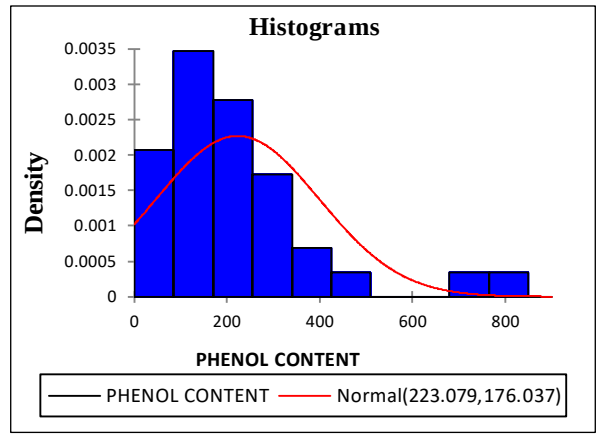


Fig 5.9.10: Normal probability curve of distribution of liverworts related with phenol content of the bark

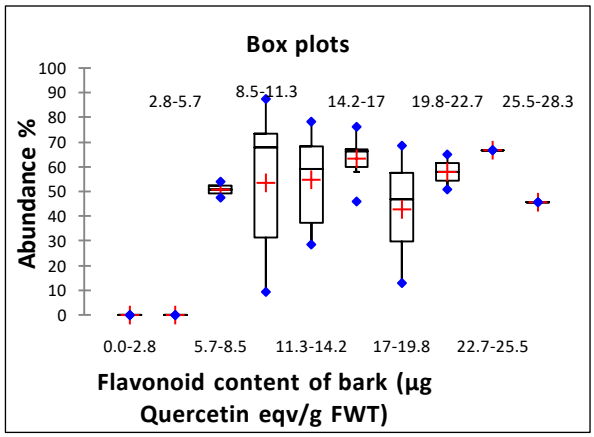


Fig 5.9.11: Variation in abundance % according to flavonoid content of bark

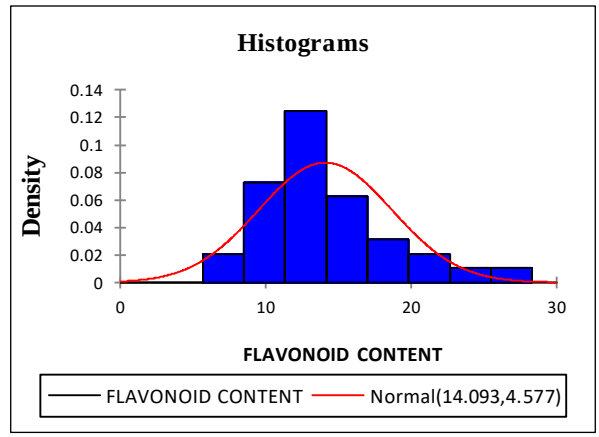


Fig 5.9.12: Normal probability curve of distribution of liverworts related with flavonoid content of bark

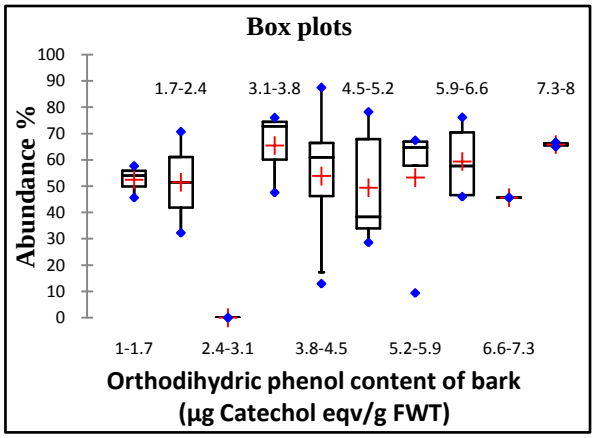


Fig 5.9.13: variation in abundance % according to ortho-dihydric phenol content

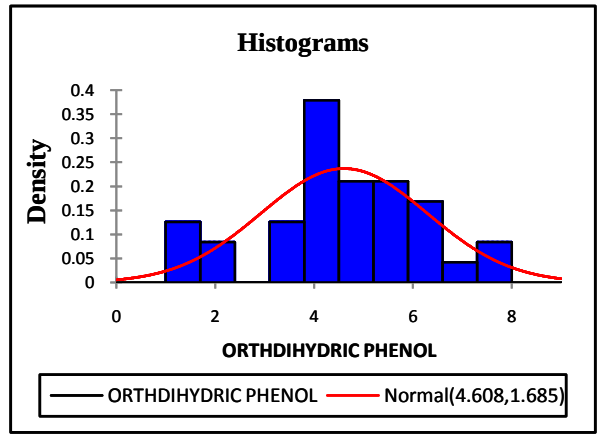


Fig 5.9.14: Normal probability curve of distribution of liverworts related with ortho-dihydric phenol content of bark

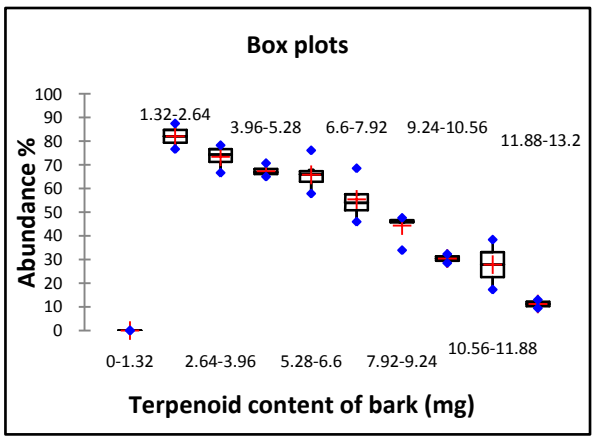


Fig 5.9.15: Variation in abundance % according to terpenoid content of bark

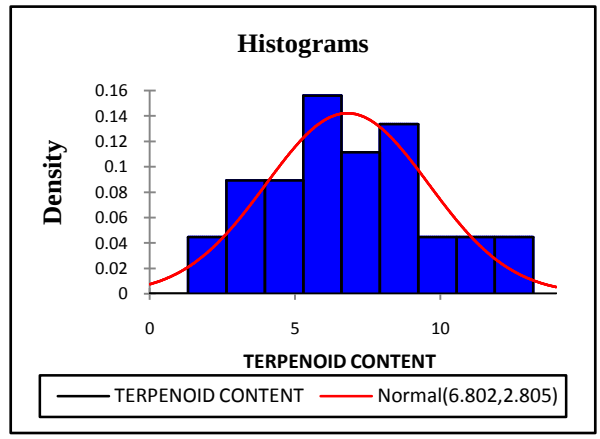


Fig 5.9.16: Normal probability curve of distribution of liverworts related with terpenoid content of bark

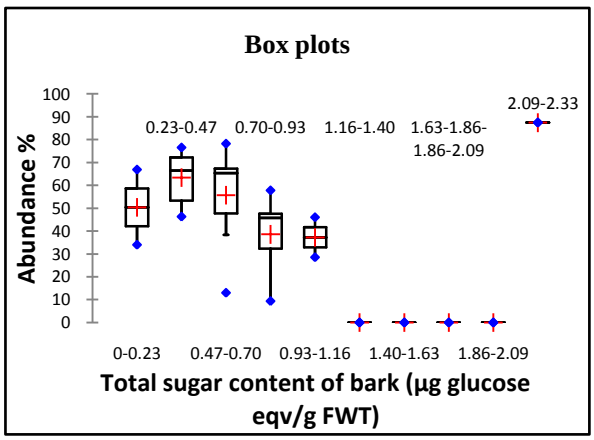


Fig 5.9.17: Variation in abundance % according to total sugar content of bark

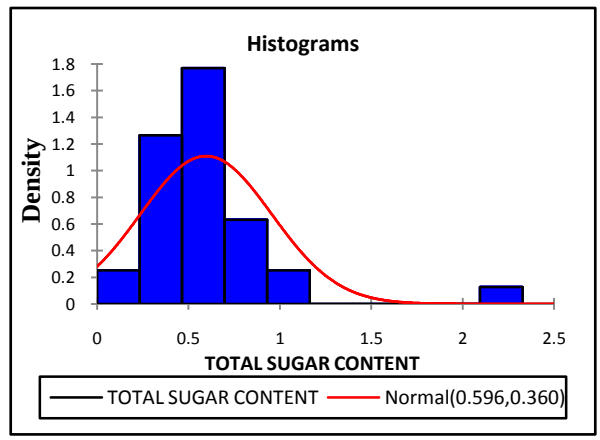


Fig 5.9.18: Normal probability curve of distribution of liverworts related with sugar content of bark

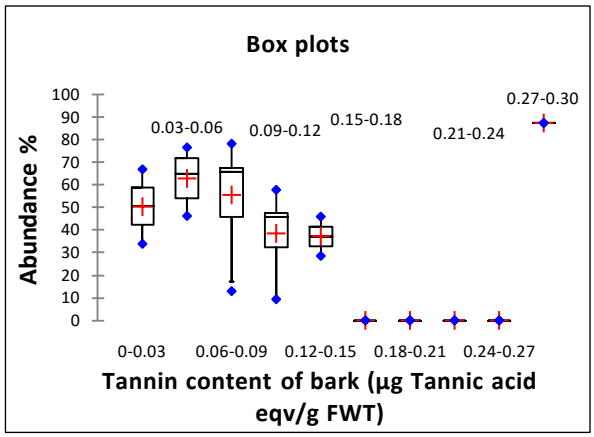


Fig 5.9.19: Variation in abundance % according to tannin content of bark

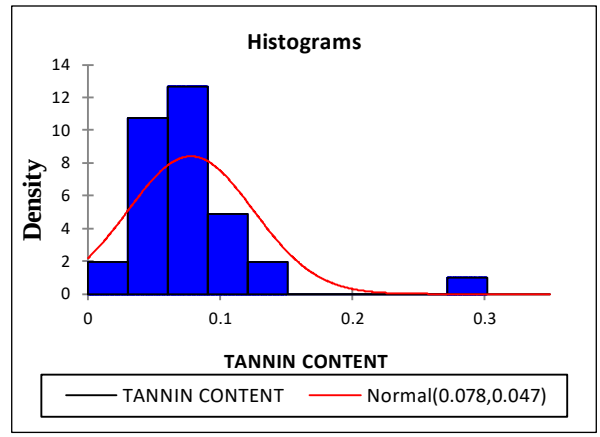


Fig 5.9.20: Normal probability curve of distribution of liverworts related with tannin content of bark

Table 5.17: Correlation between epiphytic liverwort abundance and the physiological factors like moisture content of bark, pH of the bark and light intensity. **. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed).

	Abundance	***Moisture***	***Ph***	***Light Intensity***
Abundance	1	**0.648****	0.065	**-0.769****
Moist ure	**0.648****	1	0.220	**-0.606****
Ph	0.065	0.220	1	-0.123
Light intensity	**-0.769****	**-0.606****	-0.123	1

Table 5.18: Correlation between epiphytic liverwort abundance, terpenoid content of the tree barks and tree age. **. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed).

	Abundance	***Terpenoid content***	***Tree age***
Abundance	1	**-0.959****	**0.937****
Terpenoid content	**-0.959****	1	**-0.836****
Tree age	**0.937****	**-0.836****	1

Table 5.19: Correlation between epiphytic liverwort abundance and phenol, flavonoid, orthodihydric phenol, tannin and sugar content of the studied tree bark. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed)

	Abun	***Gir***	***Phen***	***Flav***	***Odp***	***Tann***	***Sug***
Abun	1	**0.776****	-0.137	0.007	-0.066	-0.084	-0.087
Gir	**0.776****	1	-0.100	0.037	-0.101	0.005	-0.005
Phen	-0.137	-0.100	1	**0.675****	**0.679****	-0.213	-0.227
Flav	0.007	0.037	**0.675****	1	**0.647****	**-0.335***	**-0.347***
Odp	-0.066	-0.101	**0.679****	**0.647****	1	-0.145	-0.176
Tann	-0.084	0.005	-0.213	**-0.335***	-0.145	1	**0.996****
Sug	-0.087	-0.005	-0.227	**-0.347***	-0.176	**0.996****	1

NB: Abun= abundance, Gir= girth, Phen= phenol, Flav= flavonoid, Odp= orthodihydric phenol, Tann= tannin, Sug= total sugar

Table 5.20: Co-efficient of determination (R^2)

	Abun	***Gir***	***Phen***	***Flav***	***Odp***	***Moist***	***Phen***	***Lig***	***Sug***	***Tann***
Gir	0.602									
Phen	0.006	0.000								
Flav	0.000	0.001	0.008							
Odp	0.004	0.010	0.017	0.419						
Moist	0.420	0.246	0.034	0.017	0.012					
Ph	0.004	0.003	0.004	0.027	0.023	0.048				
Lig	0.591	0.523	0.003	0.017	0.019	0.368	0.015			
Sug	0.005	0.000	0.603	0.115	0.030	0.007	0.004	0.000		
Tann	0.007	0.000	0.619	0.114	0.026	0.008	0.004	0.001	0.996	
Terpen	0.938	0.654	0.001	0.000	0.005	0.422	0.015	0.684	0.001	0.002

NB: Abun= abundance, Gir= girth, Phen= phenol, Flav= flavonoid, Odp= orthodihydric phenol, Moist= moisture, Lig= light, Sug= total sugar, Tann= tannin, Terpen= terpenoid

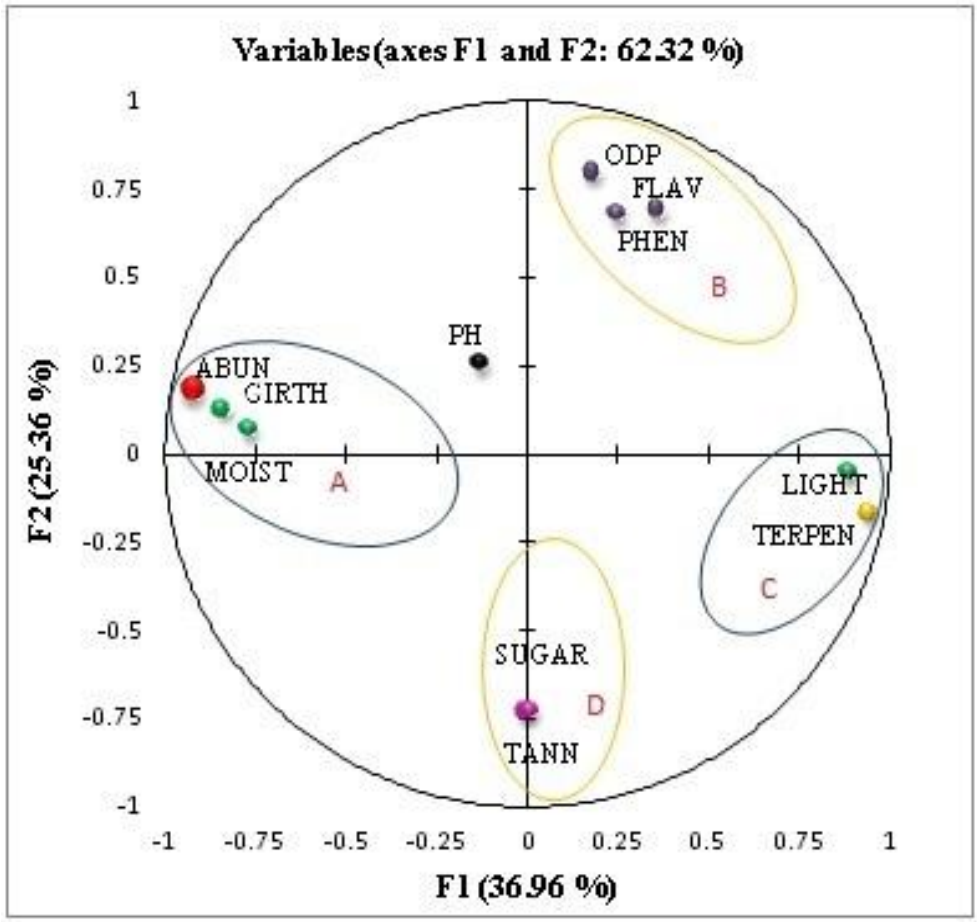


Fig 5.9.21: Principle component analysis of epiphytic liverwort abundance and different physical factors (light, moisture) and biochemical characteristics of plants (pH, girth, phenol, flavonoid, orthodihydric phenol, sugar, tannin and terpenoid conent).

NB: **ABUN= abundance, GIR= girth, PHEN= phenol, FLAV= flavonoid, ODP= orthodihydric phenol, MOIST= moisture, LIG= light, SUG= total sugar, TAN= tannin, TERPEN= terpenoid**

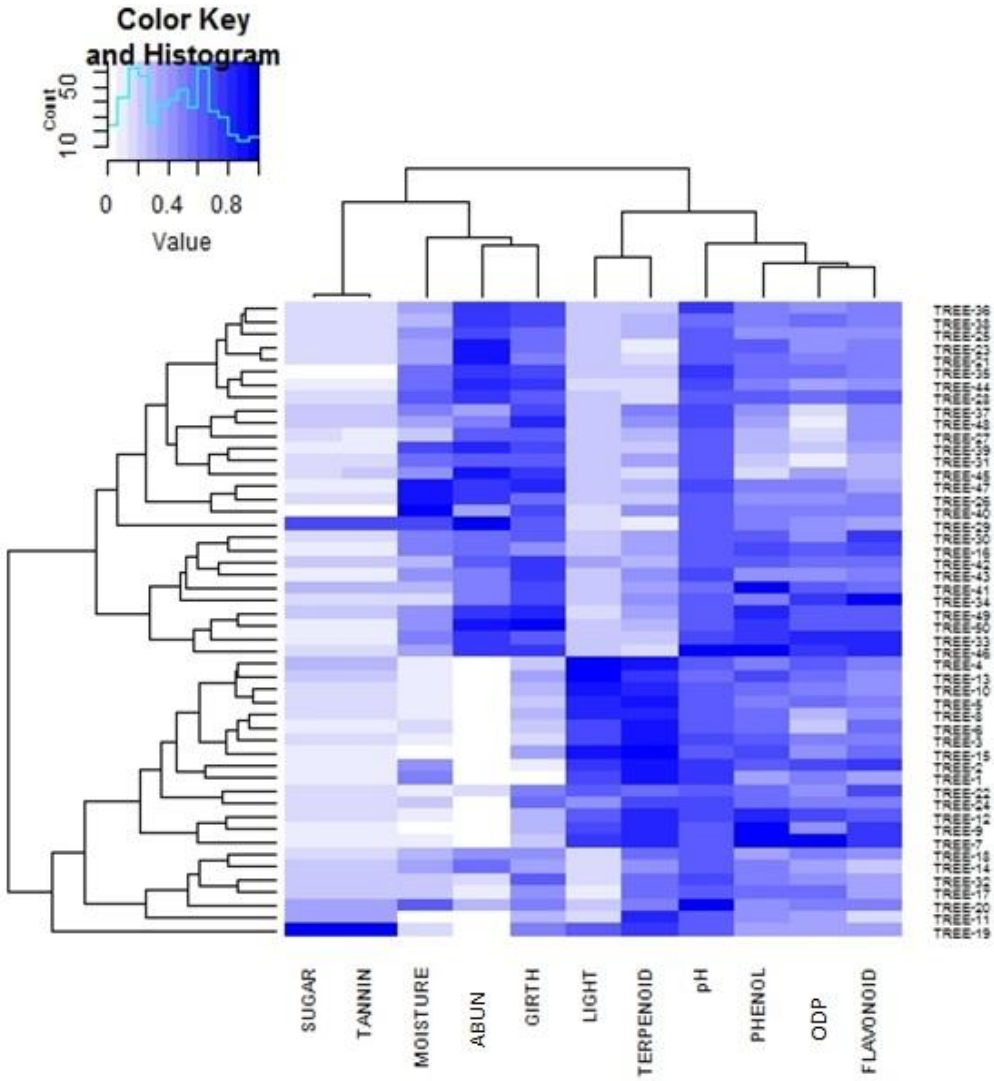


Fig 5.9.22: Z-score heatmap of the epiphytic liverwort density and different physical and biochemical factors.

NB: ABUN= abundance, ODP= orthodihydric phenol, TREE= Trees on which the abundance of epiphytic liverwort was studied

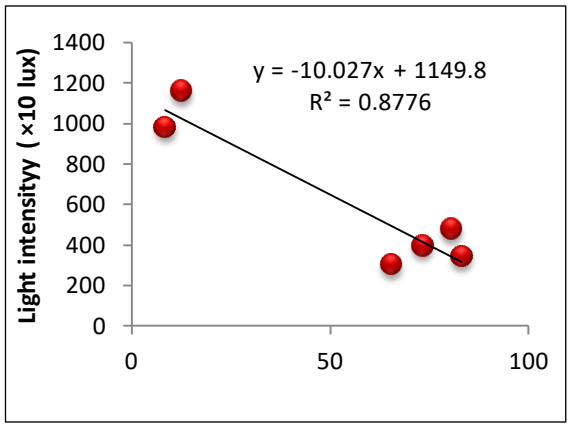


Fig 5.9.23: Influence of light intensity on abundance % of liverworts

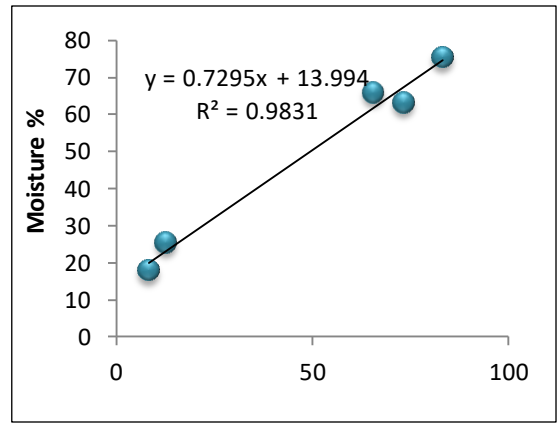


Fig 5.9.24 : Influence of soil moisture % on abundance % of liverworts

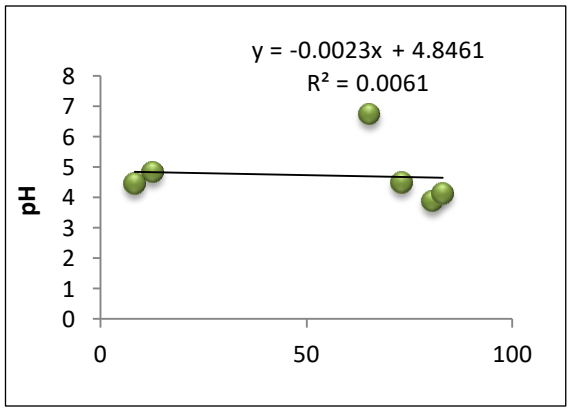


Fig 5.9.25 : Influence of soil pH on abundance % of liverworts

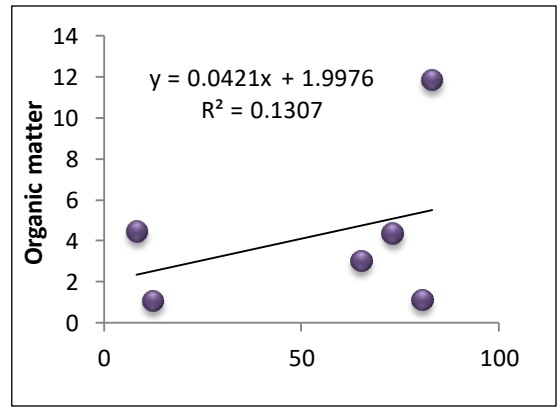


Fig 5.9.26: Influence of organic matter on abundance % of liverworts

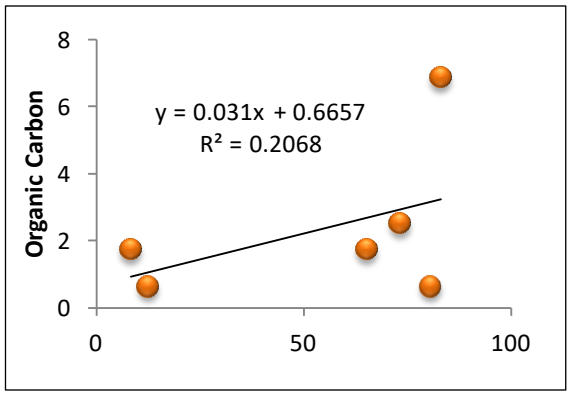


Fig 5.9.27: Influence of organic carbon on abundance % of liverworts

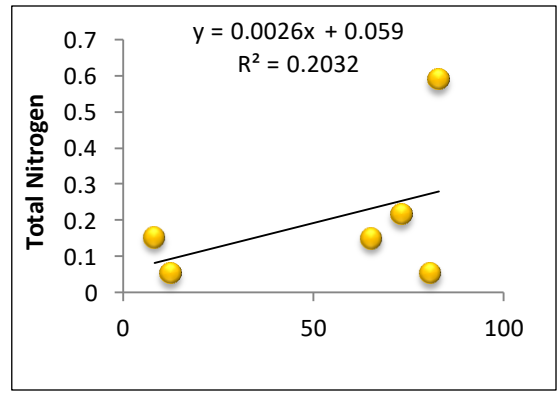


Fig 5.9.28: Influence of total nitrogen on abundance % of liverworts

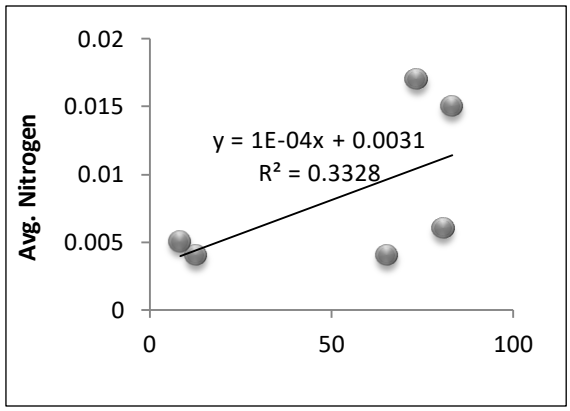


Fig 5.9.29: Influence of avg. nitrogen on abundance % of liverworts

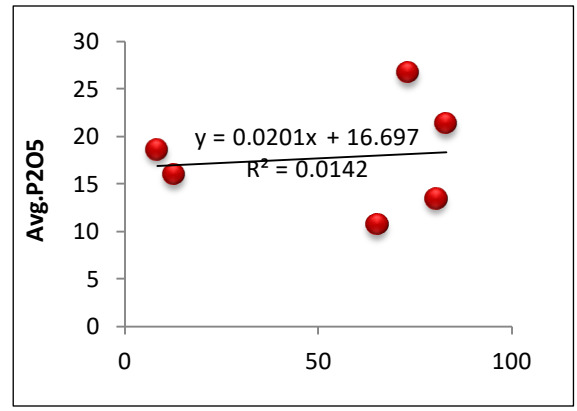


Fig 5.9.30: Influence of avg. P_2O_5 on abundance % of liverworts

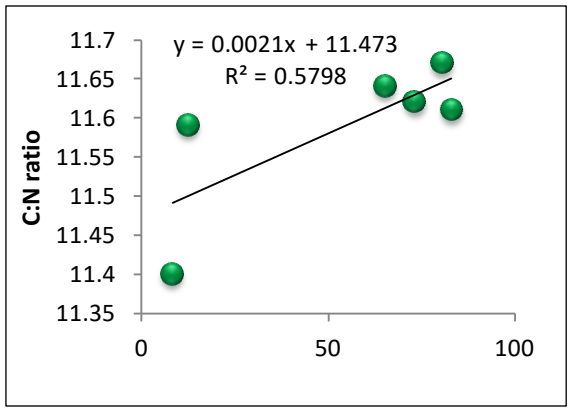


Fig 5.9.31: Influence of C:N ratio on abundance % of liverworts

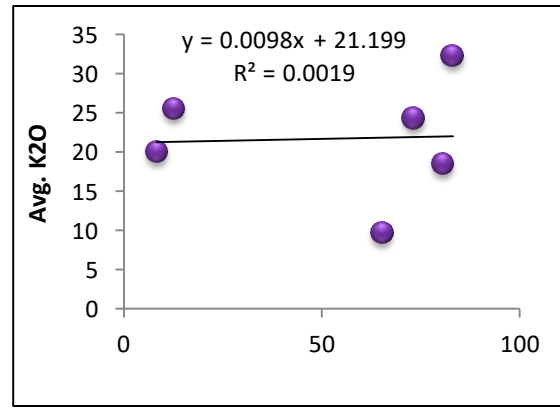


Fig 5.9.32: Influence of avg. K_2O on abundance % of liverworts

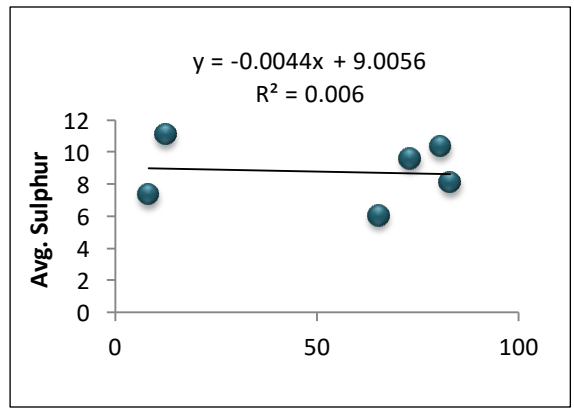


Fig 5.9.33: Influence of avg. sulphur on abundance % of liverworts