CHAPTER- V

5. RESULTS

5.1 Hepatoprotective medicinal plants

In the survey of hepatoprotective medicinal plants utilized traditionally by local tribe, a total of 40 plants (**Table 1** & ***Appendix-G***) from 26 families and 40 different genus have been identified (having authentication number- BSI/ERC/2014/Plant authentication/538; BSI/ERC/ Tech./ Plant Iden./ 2015/68; BSI/ERC/ Tech./ Plant Iden./ 2015/513; BSI/ERC/ Tech./ Plant Iden./ 2017/309) and documented. From the survey (**Figure 1**) it was revealed that the tribe uses 29 types of leaves, 10 types of roots, 3 types of whole shoot, 2 types of flowers and fruit, one type of bark and seed respectively. For the process of making herbal medicine, formulations may depend on traditional healers (Ojha), who may use single or combination of different plants or plant parts for various liver disorders. From the **Table 1,** it was evident that the traditional healers mostly prefer single plant formulation, whereas only 3 different formulations of plant combinations were recorded.

Name of places under Kokrajhar district area with GPS location where the plant samples have been collected:

1. Bashbari:	Lat: 26˚33’42.6”N,	Lon: 90˚16’17.2”E
2. Karigaon:	Lat: 26˚33’7.71”N,	Lon: 90˚20’34.36”E
3. Tinali:	Lat: 26˚29’11.92”N,	Lon: 90˚18’32.28”E
4. Khagrabari:	Lat: 26˚28’3.66”N,	Lon: 90˚13’27.13”E
5. Khagrabari(Part1):	Lat: 26˚28’3.65”N,	Lon: 90˚13’27.14”E
6. Salbari:	Lat: 26˚23’22.07”N,	Lon: 90˚12’46.54”E
7. Sukhanjhara:	Lat: 26˚23’26.18”N,	Lon: 90˚12’18.61”E
8. Kokrajhar Main Bazar:	Lat: 26˚23’55.46”N,	Lon: 90˚16’3.06”E
9. Naigaon:	Lat: 26˚21’57.80”N,	Lon: 90˚22’28.32”E
10. Khargaon:	Lat: 26˚24’46.66”N,	Lon: 90˚17’8.70”E
11. Bowbazar:	Lat: 26˚24’50.00”N,	Lon: 90˚16’34.12”E
12. Purana Titaguri:	Lat: 26˚26’43.44”N,	Lon: 90˚17’16.07”E
13. Choto gendrabil:	Lat: 26˚23’0.58”N,	Lon: 90˚17’5.33”E
14. Debargaon:	Lat: 26˚28’11.62”N,	Lon: 90˚17’42.92”E
15. Tipkai Doloagaon:	Lat: 26˚17’12.59”N,	Lon: 90˚1’56.07”E
16. Sundaari:	Lat: 26˚31’26.79”N,	Lon: 90˚27’51.80”E

Table 1: The medicinal plants with vernacular name, family name, scientific name, parts used and formulation for medicines are described below:

Family Name	Scientific name	Vernacular name	Parts Used	Mode of Use
1.Moraceae	*Morus indica* L.	Gonger thaisib	Leaves & Roots	Small pieces of roots are made hollow cylinder by removing the mid portion and are put around the neck with white/red thread. Leaves are used in bathing with mixture of other medicinal plant leaves (Herbal formulation 3) after boiling. Roots mixture with other plant roots are also used (Herbal formulation 2).
2.Moraceae	*Ficus religiosa* L.	Fakhri fifang	Bark	Bark is cut 1 inch deep in V shape & dipped in water along with three tulsi leaves, $2^{1/2}$ black pepper & kept overnight. Extract along with raw milk (kept overnight) are mixed 1:1 ratio & drink in the morning.
3.Moraceae	*Artocarpus heterophyllus* Lam.	Khanthal fifang	Leaves	Leaves are boiled in water with mixture of other medicinal plant leaves (Herbal formulation 3) and used for bathing.
4.Rutaceae	*Glycosmis pentaphyla* (Retz.)DC	Amai fifang	Leaves	1 cup water extract is mixed with rock sugar & allowed to drink 2-3 times a day for 3 days.
5.Rutaceae	*Murraya koenigii* (L.) Spreng.	Nwrshing	Leaves	Leaves are boiled in combination with other plant leaves and are used in bath.
6.Rutaceae	*Citrus medica* L.	Nareng lebu	Leaves	Leaves are used in jaundice. Mixed with leaves of other plants & boiled. Boiled water is used for bathing.
7. Rubiaceae	*Paederia foetida* L.	Khifi bendwng	Leaves	Leaves are boiled in water with mixture of other medicinal plant leaves (Herbal formulation 3) and are used in bathing. It is also eaten as vegetable.
8. Rubiaceae	*Oldenlandia diffusa* Willd. (Roxb)	Dausri athing	Leaves	Leaves are boiled in water with mixture of other medicinal plant leaves (Herbal formulation 3) and the patient is advised to take bath.
9. Apiaceae	*Hydrocotyle sibthorpioides* Lam.	Mani-muni fisa	Leaves	Boiled in water with mixture of other medicinal plant leaves (Herbal formulation 3) and used in bathing. Also eaten fresh in early morning.
10. Apiaceae	*Centella asiatica* L.	Mani-muni gidir	Leaves	Leaves are boiled with mixture of other medicinal plant leaves (Herbal formulation 3) and are used in bathing. It can be also eaten fresh early morning.
11. Acanthaceae	*Justicia*	Barsikha gufur	Leaves, roots	Root powders are mixed with other plants root (Herbal formulation 1) &

	adhatoda L.		and flowers	rice grain powder and were advised to eat 2-3 times a day. Leaves are boiled with mixture of other medicinal plant leaves (Herbal formulation 3) and are used in bathing. Flowers are edible and are good for jaundice.
12. Acanthaceae	*Phlogacanthus thyrsiflorus* Nees.	Barsikha gwja	Leaves, roots and flowers	Leaves are used for bathing after boiling with mixture of other medicinal plant leaves (Herbal formulation 3). Root powders are mixed with rice grain & other plant roots (Herbal formulation 1) and are eaten 2-3 times/day. Flowers are edible as vegetable.
13. Solanaceae	*Solanum indicum* L.	Khunthai nara	Roots.	Root powder along with other plant roots (Herbal formulation 1) & rice grain powder is mixed and advised to take 2-3 times per day.
14. Solanaceae	*Physalis minima* L.	Ganga thofa	Roots.	Root powder along with other plant roots (Herbal formulation 2) are grounded together, placed inside the cocoon of Eri worm and tied in red thread and are put in neck for one complete week.
15. Menispermaceae	*Stephania japonica* (Thunb.) Miers	Phanel khuga	Whole shoot	Leaves are separated and only shoots are allowed to put around neck before sleep at night and must be thrown in next morning. It can be also placed bellow the pillow at night instead of wearing in neck. The leaves can be boiled with mixture of other medicinal plant leaves (Herbal formulation 3) and also be used in bathing.
16. Cuscutaceae	*Cuscuta reflexa* Roxb.	Gwmw bendwng	Whole plant	Whole plant is boiled with mixture of other medicinal plant leaves (Herbal formulation 3) & used for bathing in specific day like Tuesday and Saturday only.
17. Dilleniaceae	*Dillenia indica* L.	Thaigir fifang	Leaves & Fruits	Leaves are boiled in combination with the leaves of other plants (Herbal formulation 3) and are advised to take bath before sunrise and after sunset. Fruits are boiled & filtered fruit water is taken fresh.
18. Verbenaceae	*Clerodendrum cordatum* (D.Don)	Lwkhwna	Leaves & roots	The leaves are boiled with mixture of other medicinal plant leaves (Herbal formulation 3) & used in bathing. In other formulation, dried roots are used along with other plant roots (Herbal formulation 1 & 2).
19. Costaceae	*Costus*	Buri thokon	Roots	Rhizome juice is used as medicine for jaundice. The juice is allowed to

	speciosus Koen ex. Retz			drink in empty stomach early in the morning. Its young shoots are also eaten as vegetable by local tribe.
20. Plumbaginaceae	*Plumbago zeylanica* L.	Agar sitha	Roots	Roots are grounded in water and a white thread is mixed with the powder and advised to put in right hand. The remaining grounded powder is applied in forehead for 3 hours. Its roots are also used with other plants root (Herbal formulation 2).
21.Hypericaceae	*Hypericum japonicum* Thunb.	Sona puli	Leaves	Boiled leaves along with other plant leaves (Herbal formulation 3) are used for bathing.
22. Carryophyllaceae	*Stellaria media* L.	Na bikhi	Leaves	Leaves are used in taking bath after boiling with leaves of other plants (Herbal formulation 3).
23. Lamiaceae	*Leucas indica* (L.) R.Br ex. Vatke	Kangsinsha	Leaves	Leaves are boiled in water with mixture of other medicinal plant leaves (Herbal formulation 3) & are used for bathing. It is also used as vegetable.
24. Myrtaceae	*Psidium guajava* L.	Sumfram	Leaves	Leaves are boiled in combination with other plant leaves (Herbal formulation 3) and are allowed to take bath. Fresh young leaves are also eaten to cure stomach ailment.
25. Asteraceae	*Artemisia vulgaris* L.	Na deona	Leaves	Leaves are boiled in water in combination with leaves of other medicinal plants (Herbal formulation 3) & used for bathing.
26. Lamiaceae	*Pogostemon plectranoides* Desf.	Swimakhitangth-ari	Leaves	Leaves are boiled in water with the mixture of other plant leaves (Herbal formulation 3) and are used for taking bath.
27. Scrophulariaceae	*Scoparia dulcis* L.	Sini fifang	Leaves	Leaves of the plant along with other plant leaves (Herbal formulation 3) are boiled together and the patient is advised to take bath.
28. Saururaceae	*Houttuynia cordata* Thunb.	Maisundari	Leaves	Leaves are boiled in water with the mixture of other plant leaves (Herbal formulation 3). The boiled water is used for bathing.
29. Meliaceae	*Azadirachta indica* A. Juss.	Neem fifang	Leaves	Leaves are boiled in water along with the leaves of other plants (Herbal formulation 3) & are used for bathing.
30. Oxalidaceae	*Averrhoa carambola* L.	Khwrdwi / Khambrenga	Fruits, leaves & roots	Ripe fruits are eaten fresh & also taken as curry. Leaves are boiled with other plants leave (Herbal formulation 3) & used in bathing. Root water extract is taken twice after meal.

31. Anacardiaceae	*Mangifera indica* L.	Thaijwo fifang	Leaves & seeds	Leaves are mixed with other medicinal plant leaves (Herbal formulation 3) & boiled in water and advised to take bath.
32. Thelypteridaceae	*Amphineuron opulentum* (Kaulf.)	Bis-dingkia	Roots.	Dried root powder along with roots of other plants (herbal formulation 1) are mixed with rice grain powder and are advised to eat 2-3 times/day.
33. Clusiaceae	*Garcinia cowa* Roxb.	Thaikha	Leaves	Leaves are boiled in combination with other plant leaves (Herbal formulation 3) and are advised to take bath with it.
34. Verbenaceae	*Premna herbacea* Roxb.	Kheradapkhini	Leaves	Leaves are boiled in water along with mixtures of other plant leaves (Herbal formulation 3) and are allowed to take bath. The leaves are also taken as vegetable.
35. Fabaceae	*Cajanus cajan* L. Millsp.	Khokling	Leaves	Fresh leaves are ground and allowed to drink.
36. Asteraceae	*Spilanthes paniculata* Wall. ex DC.	Usumwi	Leaves	Leaves are boiled in water along with mixtures of other plant leaves (Herbal formulation 3) and are allowed to take bath. The leaves are also taken as vegetable.
37. Acanthaceae	*Hygrophila phlomoides* Nees.	Rwda gangra	Root	Fresh roots are put on the hand.
38. Rubiaceae	*Morinda angustifolia* Roxb.	Asho	Leaves	Leaves are used but no proper formulation was obtained.
39. Molluginaceae	*Mollugo pentaphylla* L.	Rupa fuli	Whole shoot	Leaves are boiled in water along with mixtures of other plant leaves (Herbal formulation 3) and are allowed to take bath.
40. Acanthaceae	*Andrographis paniculata* (Burm.f.) Nees	Khalmegh	Whole shoot	Whole shoots are eaten fresh.

5.2 Some herbal formulation

1. Roots (dried powder) of *Phlogacanthus thyrsiflorus*, *Amphineuron opulentum*, *Justicia adhatoda*, *Clerodendrum cordatum* and *Solanum indicum* were mixed along with rice grain powder and were advised to take 2-3 times a day.
2. In some other formulation, the roots of *Morus indica, Plumbago zeylanica, Clerodendrum cordatum, Stephania japonica* and *Physalis minima,* are ground together. The ground powder are placed inside the cocoon of Eri worm and tied around the neck with the help of red thread for one week.
3. In another formulation the leaves of *Garcinia cowa*, *Justicia adhatoda*, *Hydrocotyle sibthorpioides, Centella asiatica, Averrhoa carambola, Morus indica, Artocarpus*

heterophyllus, Mangifera indica, Azadirachta indica, Stephania japonica, Scoparia dulcis, Psidium guajava, Murraya koenigii, Leucas indica, Stellaria media, Citrus medica, Houttuynia cordata, Oldenlandia diffusa, Hypericum japonicum, Pogostemon plectranoides, Artemisia vulgaris, Dillenia indica Clerodendrum cordatum, Cuscuta reflexa, Mollugo pentaphylla, Paederia foetida, Permna herbacea, Spilanthes paniculata and Phlogacanthus thyrsiflorus were washed and boiled together. After that, the patient is advised to take bath before sunrise or after sunset.

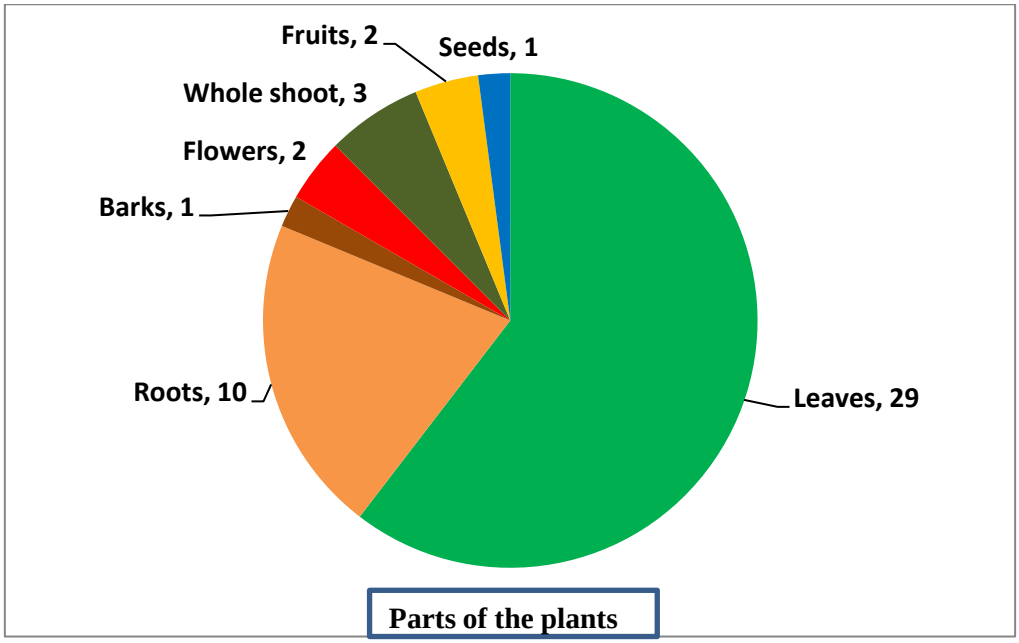


Fig 1: Hepatoprotective medicinal plant parts used.

5.3 Extract yield

The roots of *Morus indica* L., *Phlogacanthus thyrsiflorus* Nees. and *Averrhoa carambola* L., produced a total of 16.5%, 12% and 15.76% (w/w) yield from the ethanolic extracts and 11.78%, 9% and 10.2% (w/w) yield from that of acetone extract respectively.

5.4 Phytochemical screening

Qualitative screening showed positive for phenols, flavonoids, tannins, resins, terpenoids, glycosides and steroids in all the root extracts of *P. thyrsiflorus* (RoPt), *M. indica* (RoMi) and *A. carambola* (RoAc). Presence of alkaloids was seen in ethanolic extracts of RoMi, ethanolic and acetone extracts of RoPt. Cardiac glycosides was absent in both extracts of RoMi. Reducing sugar was detected only in acetone extract of RoMi. Except in the extracts of RoPt, presence of anthraquinone were seen in all RoMi & RoAc extracts. All the extracts showed positive for saponin except acetone extract of RoPt, **Table 2**.

Table 2: Qualitative tests

Phytochemical Tests	**RoPt**		**RoMi**		**RoAc**	
	70% EE	AE	70% EE	AE	70% EE	AE
Phenols	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Resins	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+
Alkaloids	+	+	+	—	—	—
Glycosides	+	+	+	+	+	+
Cardicac Glycosides	+	+	—	—	+	+
Reducing sugar	—	—	—	+	—	—
Anthraquinone	—	—	+	+	+	+
Saponins	+	—	+	+	+	+
Steroids	+	+	+	+	+	+

NB: (+) represents presence and (⁻) indicates absence of phytochemical compound.

5.5 Total phenolic and flavonoid contents

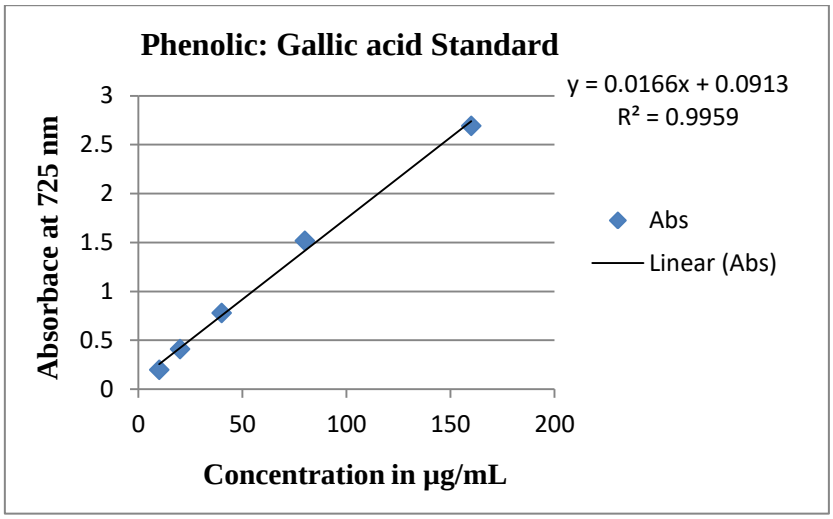


Fig 2: Total phenolic content: standard linear graph of gallic acid.

Result for the total phenolic content (extracts of RoMi, RoAc and RoPt) are presented in **Figure 3.** Phenolic content in the ethanolic and acetone extracts were determined from linear curve of standard gallic acid (y = 0.0166x + 0.0913; R^2 = 0.9959), **Figure 2**. Highest content of TPC were found in ethanolic extract of RoAc with 235.26 ± 11.91 mg GAE/g, followed by ethanolic extracts of RoMi with 214.71 ± 2.21 mg GAE/g of dried extract. The lowest content was found in acetone extract of RoPt with 84.21 ± 4.82 mg GAE/g of dried extract. Other results are RoAc-AE: 213.91 ± 11.18; RoMi-AE: 190.61 ± 2.88 and RoPt-EE: 101.26 ± 2.52 mg GAE/g of dried extract.

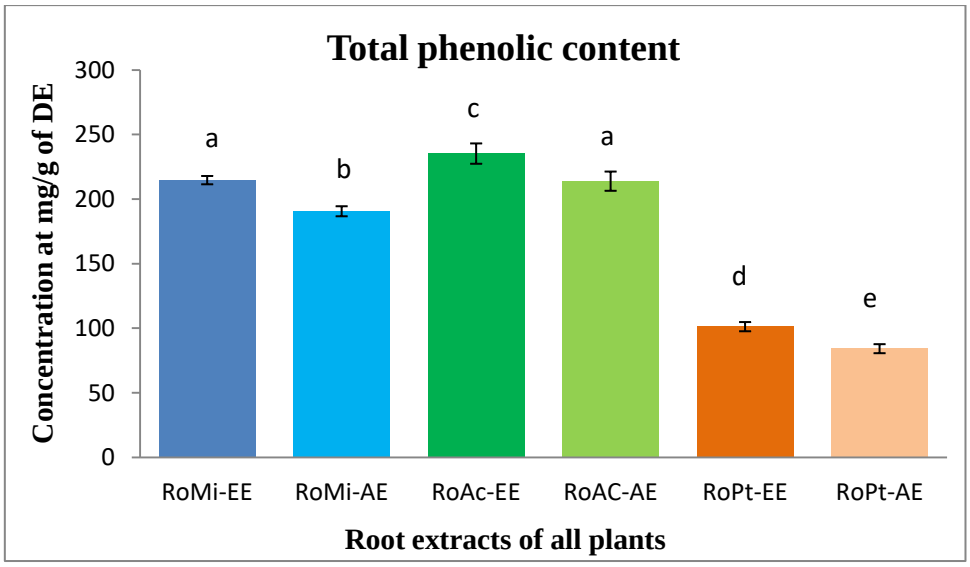


Fig 3: Showing the TPC in all extracts of RoMi, RoAc & RoPt. Results are mean value of ±SD (3n). Different letters indicate statistically significant (ANOVA-Tukey, $p<0.05$).

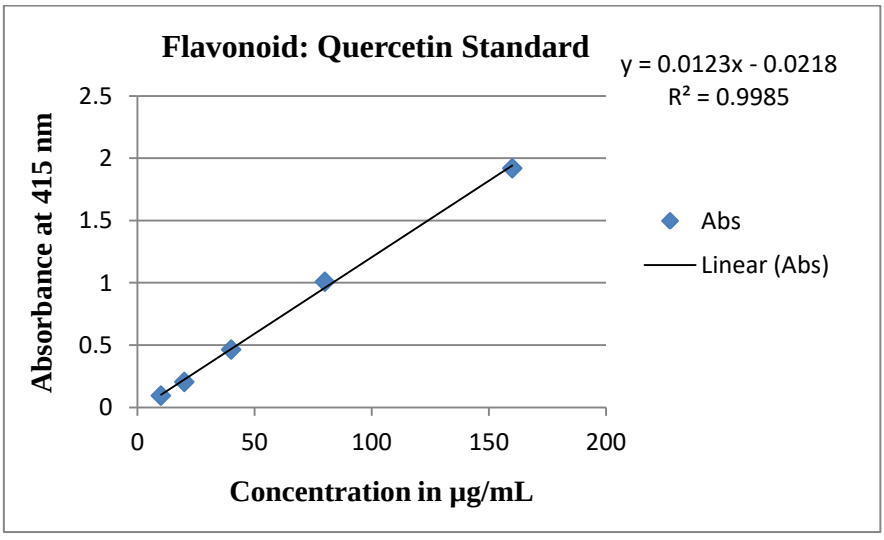


Fig 4: Total flavonoid content showing linear curve of Quercetin standard.

Flavonoid content in the ethanolic and acetone extracts were determined from linear curve of standard quercetin (y = 0.0123x - 0.0218; R^2 = 0.9985), **Figure 4**. Result for the total flavonoid contents are presented in **Figure 5**. Highest TFC was found in RoMi-EE with 123.39 ± 2.04 followed by RoMi-AE with 113.09 ± 7.25 and the least amount of flavonoid was observed in RoPt-AE with 68.22 ± 4.82 mg QE/g of dried extract respectively. Flavonoid contents of other extracts showed RoAc-EE: 101.96 ± 6.87; RoPt-EE: 99.92 ± 5.49 and RoAc-AE: 82.81 ± 5.94 mg QE/g of dried extract.

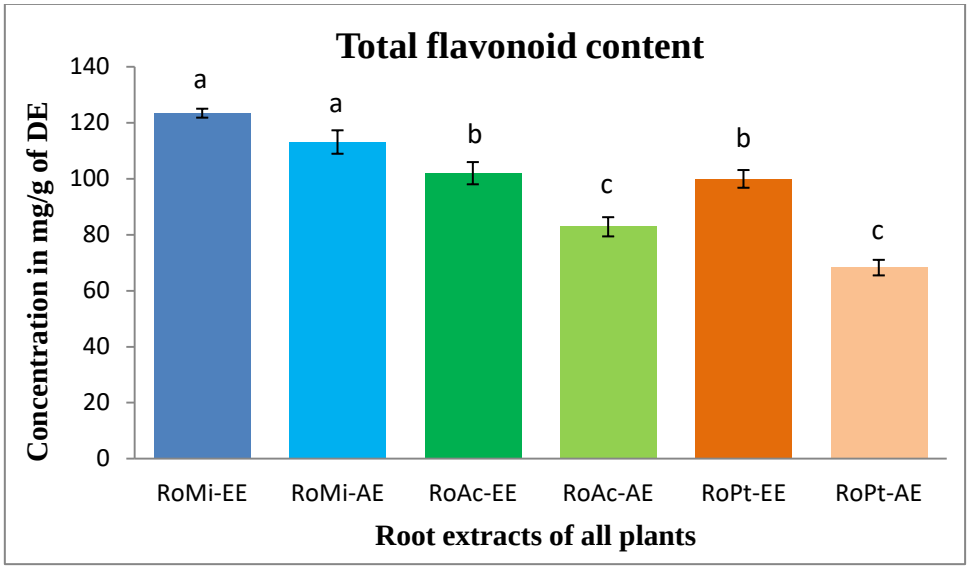


Fig 5: Showing TFC in all extracts of RoMi, RoAc & RoPt. Results are mean value of ±SD (3n). Different letters indicate statistically significant (ANOVA-Tukey, $p<0.05$).

5.6 Total reducing power assay

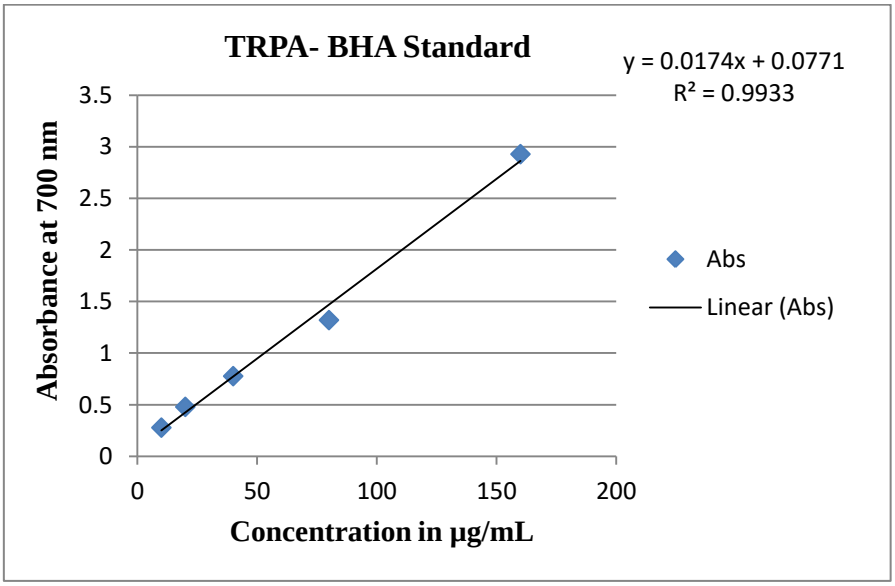


Fig 6: Total reducing power assay showing linear curve of BHA standard.

The reducing power activity of the extracts were determined from the standard linear curve of butylated hydroxyanisole (y= 0.0174x + 0.0771; R^2 = 0.9933), **Figure 6**. Reducing power assay of the extracts was carried out by taking three different concentrations (50, 100 and 200 µg/mL). RoAc-EE showed highest reducing power activity with the absorbance 0.907 ± 0.015 followed by RoMi-EE with 0.878 ± 0.035 and the lowest reducing power assay was found in RoPt-AE which showed 0.395 ± 0.015 absorbance value at the concentration of 200 µg/mL of dried extract. The standard BHA was found to be better reducing power activity than the extracts which showed absorbance of 2.928 at only 160 µg/mL of concentration. The results of reducing power assay were presented in the **Table 3**.

Table 3: Showing reducing power activity of all extracts of RoMi, RoAc and RoPt.

Concentration	RoMi-EE	RoMi-AE	RoAc-EE	RoAc-AE	RoPt-EE	RoPt-AE
50 µg/mL	0.324 ± 0.021 a	0.162 ± 0.016 b	0.355 ± 0.006 a	0.265 ± 0.008 c	0.175 ± 0.011 b	0.149 ± 0.009 b
100 µg/mL	0.541 ± 0.016 a	0.293 ± 0.023 b	0.605 ± 0.007 c	0.472 ± 0.014 d	0.268 ± 0.013 b	0.246 ± 0.012 b
200 µg/mL	0.878 ± 0.035 a	0.498 ± 0.03 b	0.907 ± 0.015 a	0.732 ± 0.014 c	0.421 ± 0.013 d	0.395 ± 0.015 d

Result represents mean ± SD value of triplicate experiment. Different letters indicate statistically significant in the same row (ANOVA-Tukey, $p<0.05$).

5.7 Total antioxidant property as per phosphomolybdate assay.

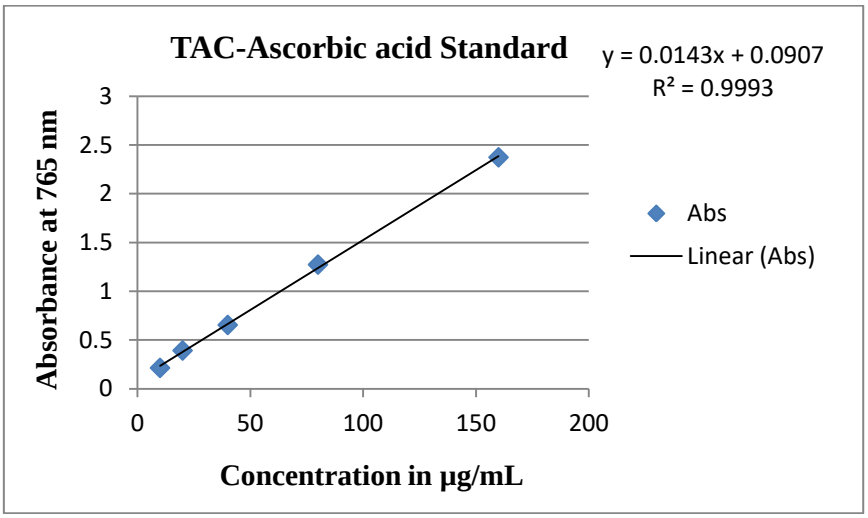


Fig 7: Total antioxidant property showing linear curve of ascorbic acid standard.

The total antioxidant property of root extracts were determined from the standard linear curve of ascorbic acid (y= 0.0143x + 0.0907; R^2 = 0.9993), **Figure 7**. In the present study, the highest antioxidant property was found in RoMi-EE having 584.98 ± 22.28 and is

followed by RoAc-EE with 512.87 ± 29.72 in respect to RoPt-AE which showed lowest value of only 189.94 ± 16.72 mg Ascorbic acid equivalent (AAE)/g of the dried extracts. The data obtained are presented in the **Figure 8**. The other plant extracts showed RoAc-AE: 478.57 ± 24.99; RoMi-AE: 287.3 ± 17.3 and RoPt-EE: 198.35 ± 18.25 mg AAE/g of dried extracts.

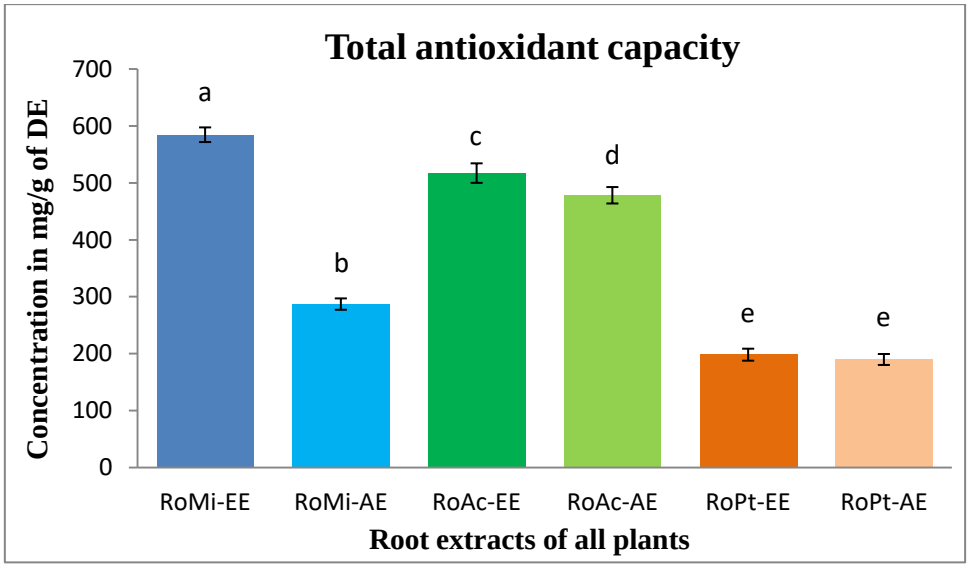


Fig 8: Showing TAC in all extracts of RoMi, RoAc & RoPt. Results are mean value of ±SD (3n). Different letters indicate statistically significant (ANOVA-Tukey, $p<0.05$).

5.8 DPPH radical scavenging activity

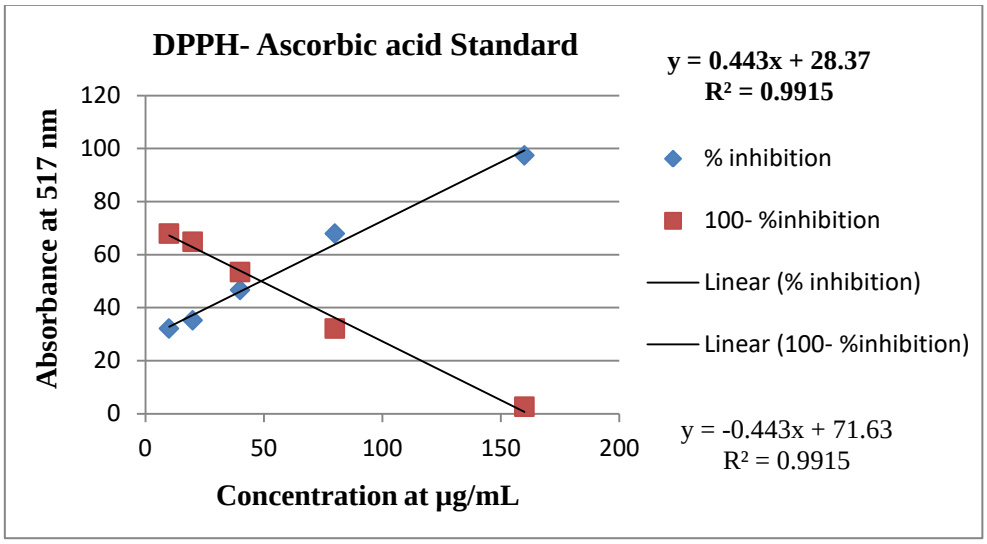


Fig 9: Showing DPPH free radical scavenging activity of ascorbic acid.

Increase in DPPH radical scavenging activity was observed notably with increased concentration of the extracts/standard. Among the extracts highest percent inhibition of 54.36 ± 2.15% was seen in the 160 µg/mL concentration of RoMi-EE (with IC_{50} value of 130.57 ±

12.46 µg/mL) and the RoPt-AE showed lowest inhibition of 30.05 ± 3.56% with IC_{50} value 302.55 ± 35.68 µg/mL. The IC_{50} values & increased in percent inhibition with increase in concentration of the extracts and ascorbic acid are furnished in the **Table 4** & **Figure 10**. Results obtained in this study suggest that DPPH scavenging activity of ascorbic acid is quite better than all extracts which showed 97.62% inhibition at 160 µg/mL concentration with 48.93 µg/mL IC_{50} value. Result of IC_{50} values of other plant extracts are RoAc-EE (138.66 ± 11.41), RoAc-AE (174.1 ± 21.18), RoMi-AE (233.92 ± 14.46) & RoPt-EE (265.87 ± 17.58).

Table 4: Presenting the DPPH radical scavenging activity by extracts/standard.

Samples	% inhibition at different concentration of sample/standard at µg/mL					
	10 µL	20 µL	40 µL	80 µL	160 µL	IC_{50}
AA	31.87	34.84	46.73	68.13	97.62	48.93
RoMi-EE	25.76 ±1.91	28.22 ±2.13	33.81 ± 2.1	42.77 ±2.86	54.36 ±2.15	130.57 ±12.46
RoMi-AE	16.09 ±0.92	17.95 ±1.13	22 ± 0.97	28.7 ± 1.1	38.41 ±1.25	233.92 ±14.46
RoAc-EE	25.01 ± 3.53	27.35 ±3.94	31.94 ±3.23	40.98 ±3.34	52.91 ±1.63	138.66 ±11.41
RoAc-AE	29.69 ± 3.22	31.03 ±3.05	34.36 ±2.32	39.52 ±2.79	47.8 ± 2.68	174.1 ± 21.18
RoPt-EE	14.35 ± 2.11	15.5 ± 1.98	17.68 ±1.69	25.13 ±1.91	34.88 ±1.89	265.87 ±17.58
RoPt-AE	9.35 ± 2.39	10.9 ± 2.58	15.42 ±2.18	20.89 ±3.15	30.05 ±3.56	302.55 ±35.68

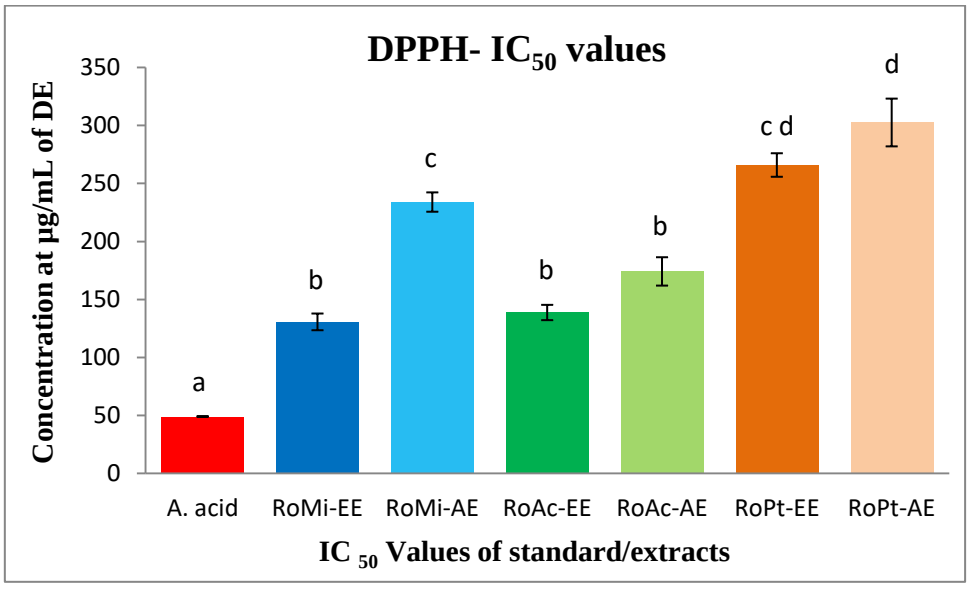


Fig 10: Showing DPPH IC_{50} values of all extracts of RoMi, RoAc, RoPt & Ascorbic acid. Results are mean value of ±SD (3n). Different letters indicate statistically significant (ANOVA-Tukey, $p<0.05$).

5.9 ABTS radical scavenging activity

The percent inhibition and IC_{50} values of ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] free radical scavenging activity of both the extracts and standard butylated hydroxytoluene (BHT) are plotted in **Table 5** and **Figure 12**.

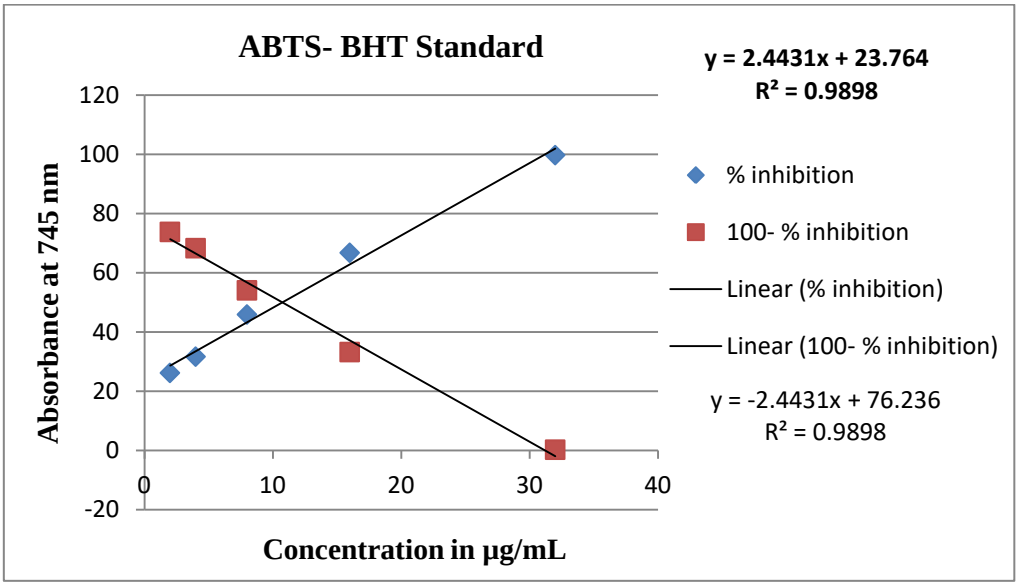


Fig 11: ABTS radical scavenging activity of standard BHT.

Table 5: Presenting the ABTS radical scavenging activity by extracts/standard.

Samples	% inhibition at different concentration of sample/standard at µg/mL					
	2 µL	4 µL	8 µL	16 µL	32 µL	IC_{50}
BHT	33.8	42.11	57.18	74.1	99.72	7.04
RoMi-EE	28.85 ±3	37.59 ±3.08	53.3 ±3.39	72.53 ±3.49	97.29 ±2.79	8.82 ± 1.42
RoMi-AE	15.43 ±4.61	25.6 ±4.18	42.08 ±2.65	66.68 ±5.88	95.65 ±3.81	12.75 ±1.61
RoAc-EE	30.87 ±3.08	40.41 ±2.35	54.96 ±2.91	74.03 ±3.45	97.2 ± 2.28	7.94 ± 1.33
RoAc-AE	29.77 ±3.49	38.1 ±2.88	52.7 ±3.28	71.99 ±5.04	96.34 ± 3.6	8.81 ± 1.66
RoPt-EE	18.78 ±1.14	26.81 ±1.28	35.35 ±1.39	49.06 ±1.36	72.82 ±3.39	17.89 ±1.18
RoPt-AE	19.15 ±1.62	26.34 ±1.69	31.74 ±1.55	41.46 ±2.53	58.97 ±2.19	24 ±1.61

The BHT showed highest scavenging activity at 32 µg/mL concentration, which inhibited 99.72% of ABTS free radicals and having the IC_{50} value of only 7.04 µg/mL. While in the same concentration the RoAc-EE showed high percent inhibition of 97.2 ± 2.28 showing best IC_{50} value of 7.94 ± 1.33 µg/mL and is followed by RoMi-EE with percent

inhibition of 97.29 ± 2.79 having IC_{50} value of 8.82 ± 1.42 µg/mL. The acetone extract of RoPt showed lowest percent inhibition of 58.97 ± 2.19 with IC_{50} value of 24 ± 1.61 µg/mL. Concentration depended inhibition were observed in all the extract as well as standard.

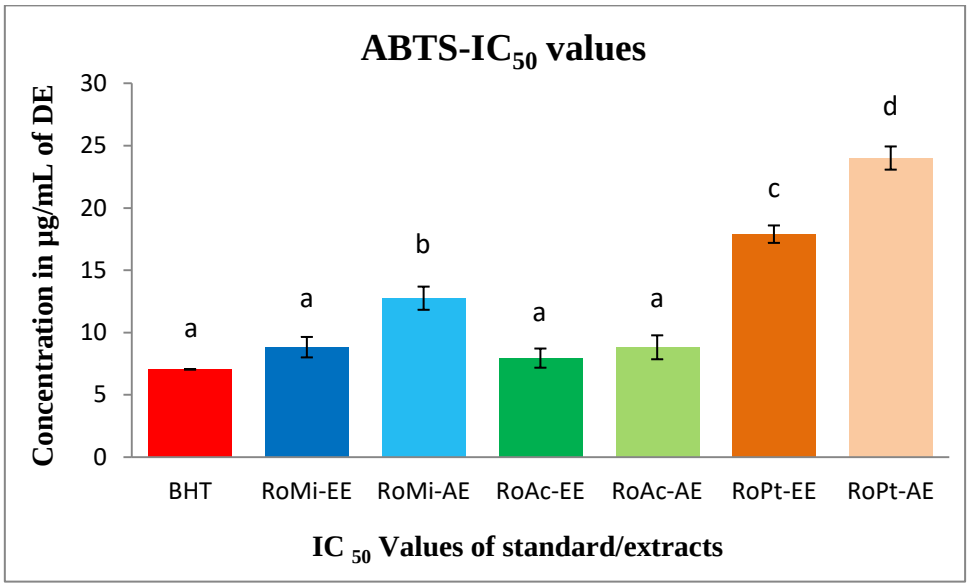


Fig 12: Showing ABTS IC_{50} value of all extracts of RoMi, RoAc, RoPt & BHT. Results are mean value of ±SD (3n). Different letters indicate statistically significant (ANOVA-Tukey, $p<0.05$).

5.10 Iron chelating capacity

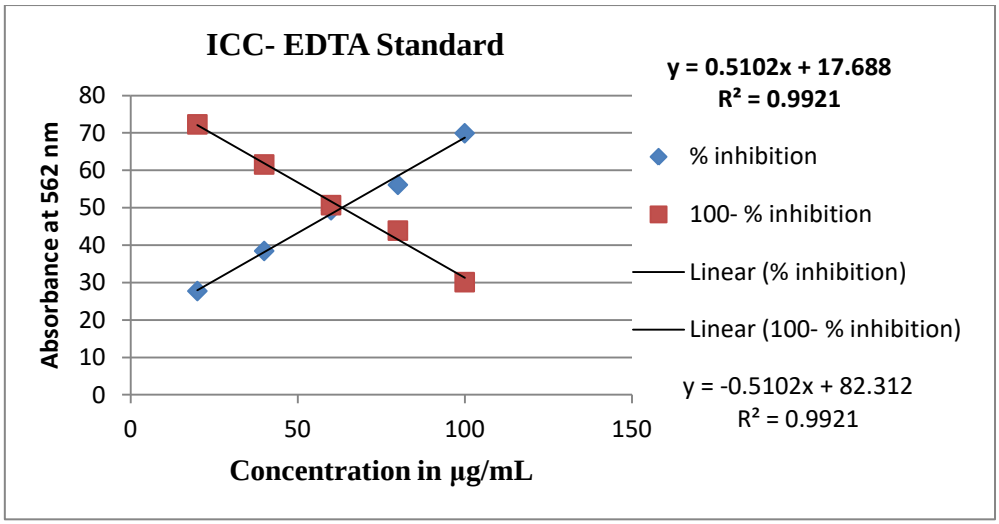


Fig 13: Showing iron chelating capacity of EDTA standard.

The result obtained in the study was presented in **Table 6** & **Figure 14**. The result indicates that highest chelating activity was observed in RoPt-EE at 1000 µg/mL having 72.06 ± 6.69% chelating capacity which also showed very good EC_{50} value with 535.16 ±

121.56 µg/mL followed by RoMi-EE with 50.06 ± 6.08 percent inhibition & having 1038.6 ± 143.97 µg/mL EC_{50} value, whereas lowest chelating activity was observed in RoMi-AE with 24.65 ± 1.91 percent inhibition and having EC_{50} value of 2006.9 ± 170.4 µg/mL of dried extract respectively. The chelating ability of extracts increases with increased concentration of extracts/EDTA. The EC_{50} value of EDTA was found to be lowest with 63.33 µg/mL, showing 69.9% inhibition at only 100 µg/mL concentration.

Table 6: Presenting the ICC radical scavenging activity by extracts/standard.

Samples/ Standard	% inhibition at different concentration of sample/standard at µg/mL					
	200 /20 µL	400 /40 µL	600 /60 µL	800 /80 µL	1000/100µL	EC_{50}
EDTA	27.72	38.44	49.32	56.12	69.9	63.33
RoMi-EE	21.31 ±4.51	27.02 ±4.29	31.27 ±3.91	41.77 ± 4.1	50.06 ± 6.08	1038.6 ±143.97
RoMi-AE	3.72 ±0.49	7.1 ±0.35	11.64 ±1.51	18.16 ±0.99	24.65 ± 1.91	2006.9 ± 170.4
RoAc-EE	3.68 ±0.97	8.8 ±1.07	16.84 ±1.39	24.02 ±2.76	32.78 ± 2.83	1500.43 ±130.1
RoAc-AE	2.85 ±1.04	7.2 ±0.97	13.39 ±1.93	21.18 ±2.93	25.72 ± 2.83	1817.3 ±183.26
RoPt-EE	34.58 ±3.14	44.56 ±5.66	54.11 ±6.97	61.65 ±8.32	72.06 ± 6.69	535.16 ±121.56
RoPt-AE	3.06 ±0.61	10.33 ±2.12	19.1 ± 2.25	25.83 ± 1.9	32.12 ± 2.15	1471.32 ±91.7

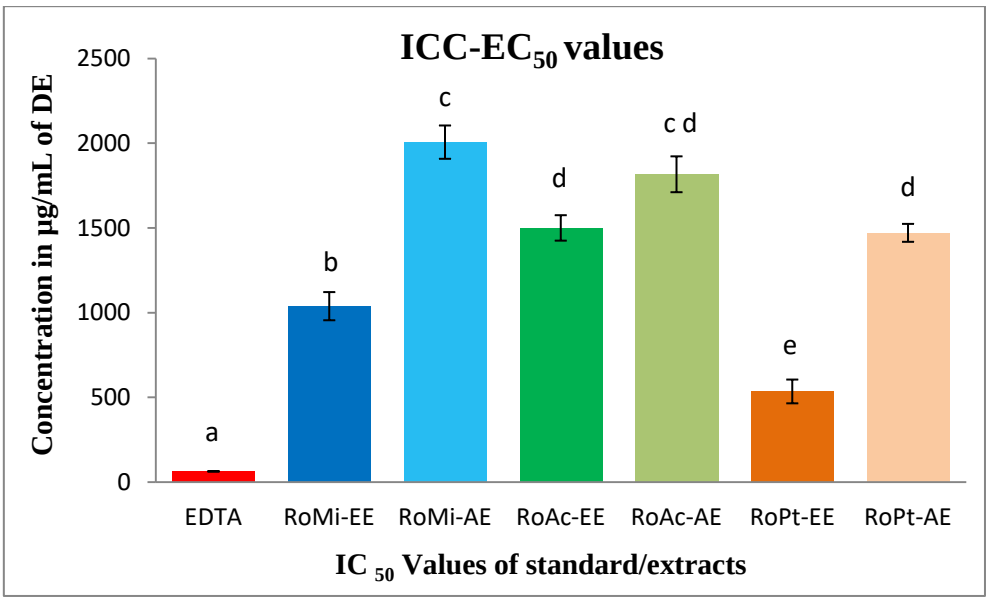


Fig 14: Showing ICC EC_{50} value of all extracts of RoMi, RoAc, RoPt & EDTA. Results are mean value of ±SD (3n). Different letters indicate statistically significant (ANOVA-Tukey, $p<0.05$).

5.11 H_2O_2 radical scavenging activity

The ability of ethanolic & acetone extracts of RoMi, RoAc and RoPt to scavenge hydrogen peroxide radicals is presented in **Table 7** & **Figure 16**, using BHA as standard. The **Figure 15** shows the ability of Butylated hydroxyanisole to scavenge the free radicals of hydrogen peroxide.

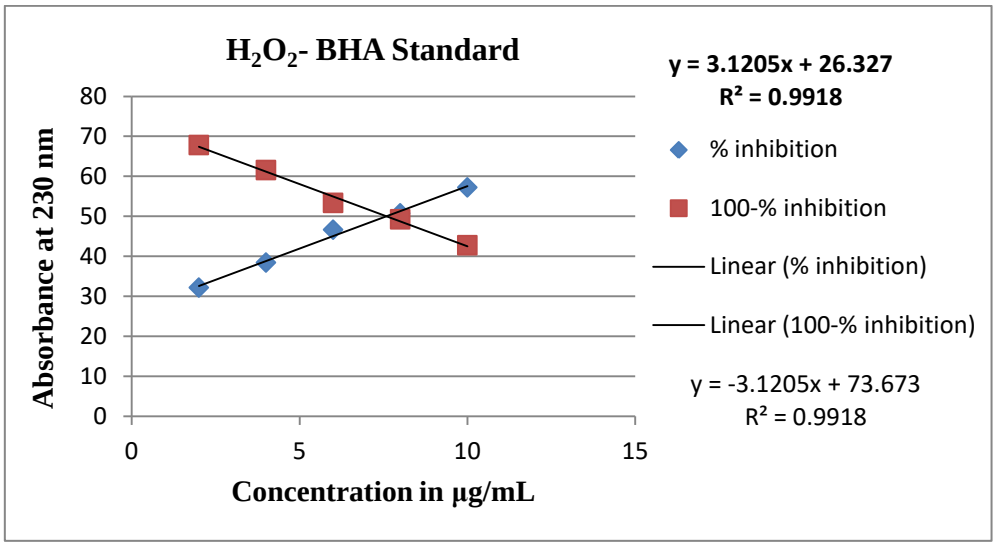


Fig 15: Showing hydrogen peroxide radical scavenging activity of Butylated hydroxyanisole.

Table 7: Presenting the H_2O_2 radical scavenging activity by extracts/standard.

Samples	% inhibition at different concentration of sample/standard at µg/mL					
	2 µL	4 µL	6 µL	8 µL	10 µL	IC_{50}
BHA	32.18	38.44	46.65	50.75	57.23	7.59
RoMi-EE	4.89 ±2.38	13.75 ±4.11	22.87 ±2.35	29.89 ±2.81	37.85 ±6.23	12.88 ±1.54
RoMi-AE	3.67±2.4	10.56 ±3.52	16.2 ±3.9	22.62 ±4.29	28.75 ±4.01	16.9 ±1.8
RoAc-EE	30.6 ±2.29	32.52 ±1.61	38.12 ±2.26	41.75 ±2.04	44.8 ±2.93	12.67 ±1.58
RoAc-AE	20.74 ±1.42	27.09 ±0.79	29.51 ±1.12	34.01 ±1.71	38.2 ±3.3	15.82 ±2.13
RoPt-EE	16.56 ±1.65	21.38 ±3	23.97 ±2.86	28.51 ±2.19	34.13 ±1.89	17.89 ±1.05
RoPt-AE	12.82 ±1.83	18.43 ±1.87	22.75 ±0.66	27.14 ±1.39	31.68 ±0.54	18 ±1.87

The result indicated a concentration dependent activity in BHA and all the root extracts with highest inhibition of 57.23% (at only 10 µg/mL concentration) was observed in BHA having IC_{50} value of only 7.59 µg/mL. Among the plant extracts at same concentration, the highest inhibition of 44.8 ± 2.93% was found in RoAc-EE which showed the IC_{50} value

of 12.67 ± 1.58, followed by RoMi-EE which showed 37.85 ± 6.23 percent inhibition (at 10 µg/mL concentration) with IC_{50} value of 12.88 ± 1.54 µg/mL of dried extract. The lowest IC_{50} value of 18 ± 1.87 µg/mL was obtained in RoPt-AE. The IC_{50} values of hydrogen peroxide radical scavenging activity of other extracts are RoAc-AE (15.82 ± 2.13), RoMi-AE (16.9 ± 1.8) and RoPt-EE (17.89 ± 1.05).

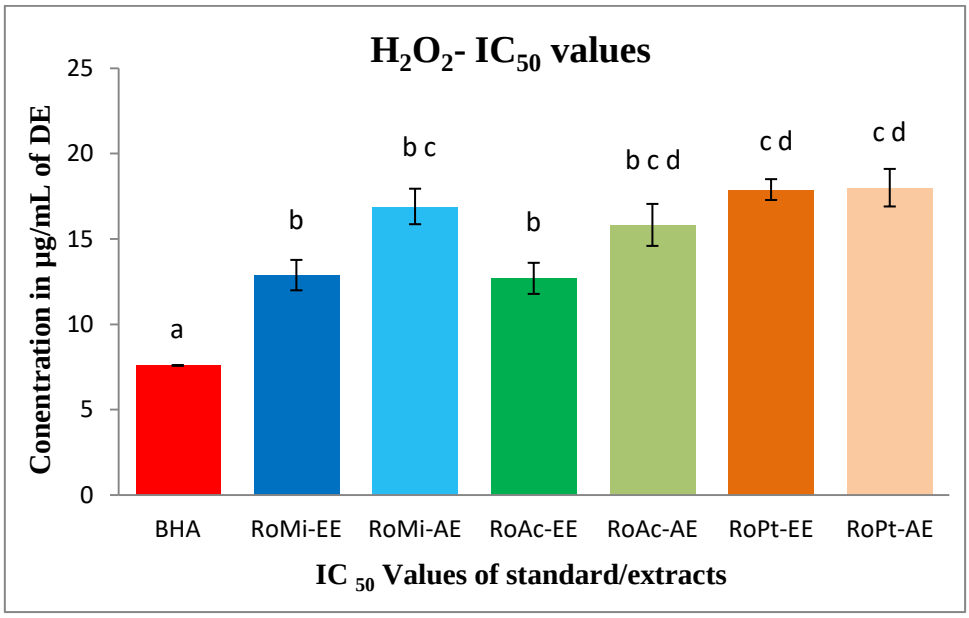


Fig 16: Showing H_2O_2 IC_{50} values of all extracts of RoMi, RoAc, RoPt & BHA. Results are mean value of ±SD (3n). Different letters indicate statistically significant (ANOVA-Tukey, $p<0.05$).

5.12 Ferric reducing antioxidant property

In FRAP assay, the antioxidant capacity was evaluated on the basis of the extract's ability to reduce ferric (III) ion to ferrous (II) ion. The result of ferric reducing antioxidant properties were evaluated from the standard linear curve of $FeSO_4.7H_2O$ ($0.0051x - 0.0408$; $R^2 = 0.9965$) **Figure 17**.

The results of FRAP antioxidant capacity is presented in **Figure 18**. Present study have revealed that acetone extract of RoAc showed better antioxidant capacity with 2512.7 ± 157.37 µmol Fe^{2+}/g followed by RoAc-EE having 2484.27 ± 135.3 µmol Fe^{2+}/g of dried extract. The lowest ferrous ion concentration was observed in RoPt-EE with 751.67 ± 85.48 µmol Fe^{2+}/g of dried extract. Result for other root extracts showed ferrous ion concentration of 1116.4 ± 98.56 in RoMi-EE, 1027.9 ± 115.03 in RoMi-AE and RoPt-AE with 820 ± 110.63 µmol Fe^{2+}/g of dried extract.

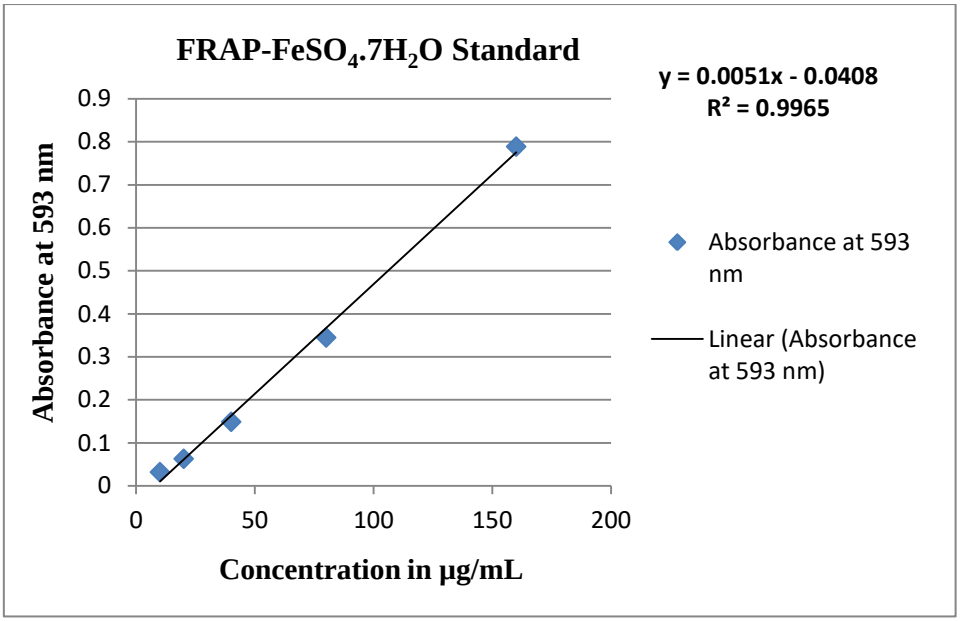


Fig 17: FRAP radical scavenging activity showing linear curve of $FeSO_4.7H_2O$ standard.

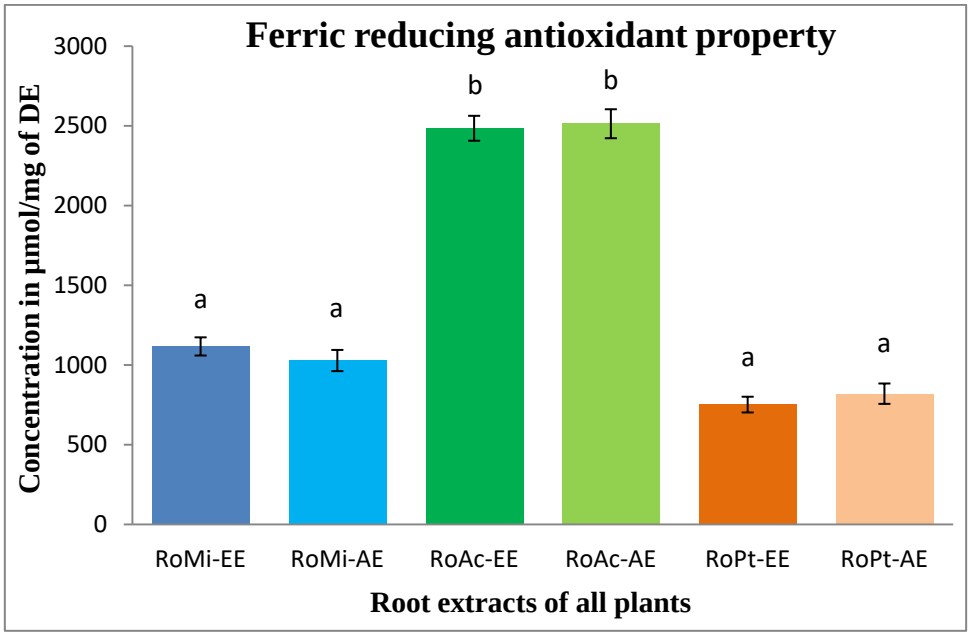
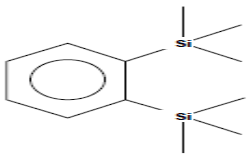
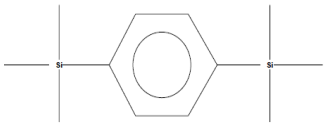
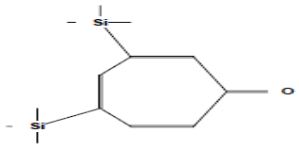
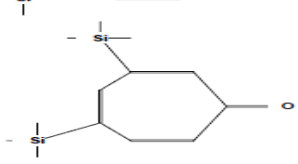
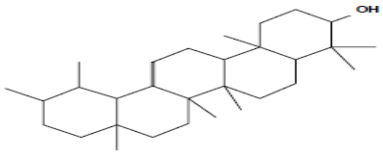


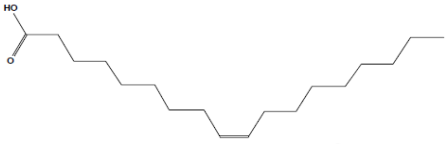
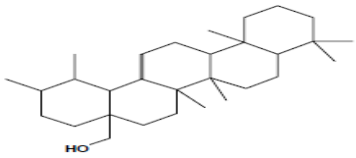
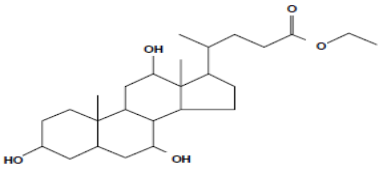
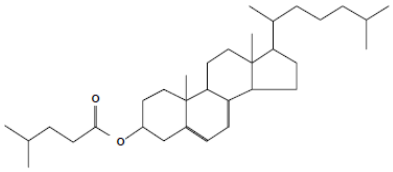
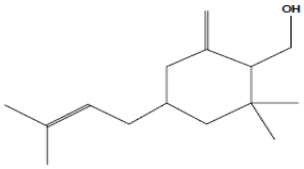
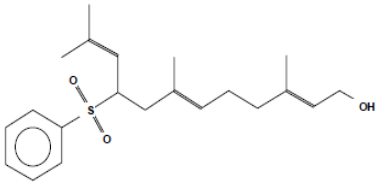
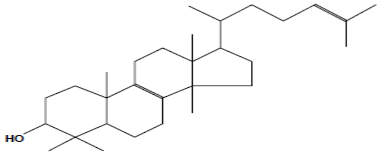
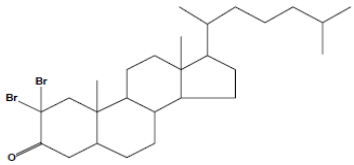
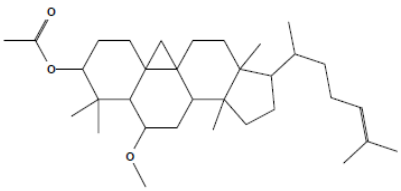
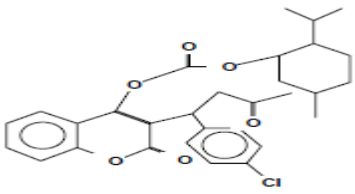
Fig 18: Extracts showing $FeSO_4.7H_2O$ concentration in µM/mg of DE. Results are mean value of ±SD (3n). Different letters indicate statistically significant (ANOVA-Tukey, $p<0.05$).

5.13 GC-MS analysis

The GC-MS chromatograms of hydro-alcoholic extract of *Morus indica* root are presented in ***Appendix-B***. A set of peaks were observed that indicates the existence of the diverse phytochemical components. The active components, molecular weight, retention time, peak area in percentage and pubchem CID are presented in **Table 8**.

Table 8: Bioactive compounds identified by GC/MS analysis of RoMi-EE.

Compound names (RoMi-EE)- 1st Chromatogram.	2D Structure	PubChem ID	MW	RT	Area (%)
1,2-Bis(Trimethylsilyl) Benzene (CAS: 17151-09-6)		519794	222	30.609	6.953
Silane, 1,4-Phenylenebis [Trimethyl- (CAS: 13183-70-5)		25771	222	30.634	5.191
2,4,6-Cycloheptatrien-1-One, 3,5-Bis-Trimethylsilyl- (CAS: 900161-21-8)		610038	250	30.674	5.699
2,4,6-Cycloheptatrien-1-One, 3,5-Bis-Trimethylsilyl- (CAS: 900161-21-8)		610038	250	31.035	27.563
Alpha.-Amyrin (CAS: 638-95-9)		73170	426	31.755	54.594
Results from the 2nd chromatogram					
Octadecanoic acid, ethyl ester (111-61-5)		8122	312	18.175	2.276
N-Hexadecanoic Acid (CAS: 57-10-3)		985	256	18.815	14.195
Eicosanoic Acid- (CAS: 506-30-9)		10467	312	19.040	1.993
9,12-Octadecadienoic Acid (Z,Z)- (CAS: 60-33-3)		3931	280	19.455	4.831
1-Octadecyne (CAS: 629-89-0)		69425	250	20.300	14.539
Pentadecanoic Acid- (CAS: 1002-84-2)		13849	242	20.465	4.612

Oleic Acid- (CAS: 112-80-1)		445639	282	20.585	2.097
Urs-12-En-28-Ol- (CAS: 10153-88-5)		22213452	426	25.918	6.621
Ethyl Iso-Allocholate- (CAS: 900043-05-3)		6452096	436	26.318	1.878
7-Dehydrocholesteryl Isocaproate- (CAS: 900251-07-6)		312789	484	28.408	3.384
1-Methylene-2b-Hydroxy methyl-3,3-Dimethyl-4b-(3-Methylbut-2-Enyl)-C (CAS: 900144-10-6)		550196	222	28.889	3.849
2,6,10-Dodecatrien-1-Ol, 3,7,11-Trimethyl-9-(Phenylsulfonyl)-, (E,E) (CAS: 57683-67-7)		5368759	362	29.034	6.163
Lanosterol- (CAS: 79-63-0)		246983	426	29.339	5.678
2,2-Dibromo- Cholestanone (CAS: 97370-79-1)		22212696	542	29.884	2.343
3-O-Acetyl-6- Methoxy -Cycloartenol- (CAS: 900286-40-9)		537607	498	30.114	7.021
2-Isopropyl-5- Methylcyclo hexyl -3-(1-(4-Chlorophenyl)-3-Oxobutyl)-C- (CAS: 900143-59-5)		537118	524	30.409	1.976

5.14 *In-vivo* hepatoprotective activity

5.14.1 Acute toxicity study

The RoMi ethanolic extract was administered orally and no fatalities were examined in the investigational animals at 2000 mg/kg dose. Therefore, $1/10^{th}$ (200 mg/kg) of the maximum dose and $1/20^{th}$ (100 mg/kg) were considered safe for the *in-vivo* studies. There were no sign of clinical or toxicological symptoms or delay death in experimental animals observed after 14 days.

5.14.2 Effect of interventions on Biochemical parameters

5.14.2.1 Effect of RoMi on the indices of hepatotoxicity by liver marker enzymes

Administration of CCl_4 markedly increased the levels of liver serum enzymes such as ALT, AST, and ALP in the control group as compared with the normal group (**Table 9**). Elevation in the secretion of these enzymes was significantly decreased with the treatment of RoMi-EE; however, the higher concentration (200 mg/kg) showed better results.

5.14.2.2 Effect of RoMi on TC, TG, HDL, LDL, and VLDL level

The administration of CCl_4 has increased TC (6.40 ± 0.02 mmol/L), TG (1.78 ± 0.01 mmol/L), LDL (162.00 ± 10.81 mg/dL), and VLDL (29.01 ± 0.87 mg/dL), and decreased HDL (27.11 ± 1.42 mg/dL); whereas in the groups that received silymarin and test drug RoMi-EE, the levels of TC, TG and LDL have significantly decreased and that of HDL was increased slightly (**Table 9**). Higher concentration of RoMi-EE shows better results when compared with the low concentration group. The major fold changes were seen in LDL (1.2 folds) followed by TC (1.1 folds), VLDL (0.99 folds) and TG (0.73 folds) after the treatment with a higher dose of RoMi-EE as compared to the CCl_4 treated animals. The level of HDL increased with the administration of RoMi-EE and showed better result at higher concentration (34.20 ± 2.83 mg/dL). These results of high dose treatment are also comparable with the standard drug silymarin which was effective in reverting the biochemical parameters in diseased animals.

5.14.2.3 Effect of interventions on Creatinine

The serum biochemical assay of current investigation has been tabulated in **Table 9**. In normal group, creatinine level was noted to be 0.68 ± 0.01 mg/dL. CCl_4 administration significantly increased the plasma creatinine level (0.79 ± 0.01) as compared to the normal group. Treatment with CCl_4 shoot up the creatinine level by almost 1.2 folds. When the

experimental rats were treated with standard drug silymarin and different dosage of RoMi-EE, it was noticed that in the case of silymarin the creatinine level almost clocked back to the normal (0.7 ± 0.01 mg/dL). Upon treatment with RoMi-EE, the high dose was found more effective (0.71 ± 0.04 mg/dL) than the lower dose (0.77 ± 0.02 mg/dL) and was found statically significant in both of the cases.

Table 9: Biochemical parameters of Wistar rat blood from control and treatments

Tests	Normal	Negative Control	Silymarin (25 mg)	RoMi-EE (100 mg)	RoMi-EE (200 mg)
Albumin (g/dL)	39.14 ±1.21	25.7 ±1.76a	29.6 ±1.98b	26.02 ±1.90a	30.25 ±0.25b
ALT (U/L)	26.5 ±0.98	118 ±9.43a	88.1 ±9.31b	108.3 ±11.42a	66.33 ±4.73b
AST (U/L)	27.5 ±1.31	208 ±24.94a	98.2 ±10.21b	149.67 ±11.93c	101.33 ±11.2c
ALP (U/L)	14 ±0.45	27 ±1.20a	15.5 ±0.8b	22.33 ±2.52a	21 ±1.31b
TC (mmol/L)	4.1 ±0.03	6.4 ±0.02a	4.3 ±0.2b	5.6 ±0.01c	4.9 ±0.01b
TG (mmol/L)	1.09 ±0.01	1.78 ±0.01a	1.31 ±0.01b	1.5 ±0.01a	1.38 ±0.01b
HDL (mg/dL)	51.19 ±0.06	27.11 ±1.42a	37.92 ±0.3b	29.12 ±0.39a	34.2 ±2.83b
LDL (mg/dL)	44.98 ±0.21	162 ±10.81a	91.00 ±11.91b	131.00 ±11.3c	109.0 ±11.4b
VLDL (mg/dL)	10.90 ±0.09	29.01 ±0.87a	21.91 ±1.31b	28.90 ±0.02a	23.41 ±1.92b
Bilirubin (mg/dL)	0.6 ±0.01	1.88 ±0.02a	1.09 ±0.01b	1.51 ±0.01a	1.29 ±0.01b
GGT (U/L)	4.53 ±0.12	11.87 ±1.10a	6.81 ±0.31b	9.01 ±0.43c	8.93 ±0.8b
Creatinine (mg/dL)	0.68 ±0.01	0.79 ±0.01a	0.7 ±0.01b	0.77 ±0.02a	0.71 ±0.04b
Total Protein (g/dL)	6.16 ±0.23	5.98 ±0.11a	6.11 ±0.34b	6.09 ±0.05b	6.13 ± 0.17b

Values are mean ± S.E. (n= 6 animals /group). Statistical analysis was done by one-way ANOVA between groups and values were considered significant at $p<0.05$. Those which are not sharing the same letters in a row are significantly different. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alanine phosphatase; TC: Total Cholestrol; TG: Triglyceride; HDL: High-density lipoproteins; LDL: Low-density lipoproteins; VLDL: Very-low-density lipoproteins; GGT: γ-glutamyl transferase

5.14.2.4 Effect of interventions on Total Protein and Albumin

As shown in **Table 9**, CCl_4 administration decreased the albumin and total protein levels, although the decrease in total protein was not significant. The albumin was decreased to 65.6% (as compared to the normal group). Treatment with RoMi-EE at a higher dose (200 mg/kg) and silymarin significantly increased the albumin to 77.3% and 75.62% respectively. There were no significant changes (p<0.05) was observed in the levels of total protein in the treatment groups as compared to the normal group.

5.14.2.5 Effect of interventions on Bilirubin and GGT

The control group that received CCl_4 showed an elevation in the levels of serum bilirubin (313.3%) and GGT (262.03%) as compared to the normal group (**Table 9**). After the treatment, the bilirubin was lessened to 251.6% (100 mg of RoMi-EE), 215% (200 mg of RoMi-EE) and 181.6% (25 mg silymarin); whereas, GGT was reduced to 198.9% (100 mg of RoMi-EE), 197.1% (200 mg of RoMi-EE), and 150.3% (25 mg silymarin).

5.14.3 Effect of RoMi on the parameters of oxidative stress in liver

5.14.3.1 Effect of interventions on antioxidant enzymes SOD, CAT and GPx

The administration of CCl_4 resulted in a decrease of hepatic antioxidant enzymes such as SOD, CAT, and GPx in the liver (**Figure 19-21**).

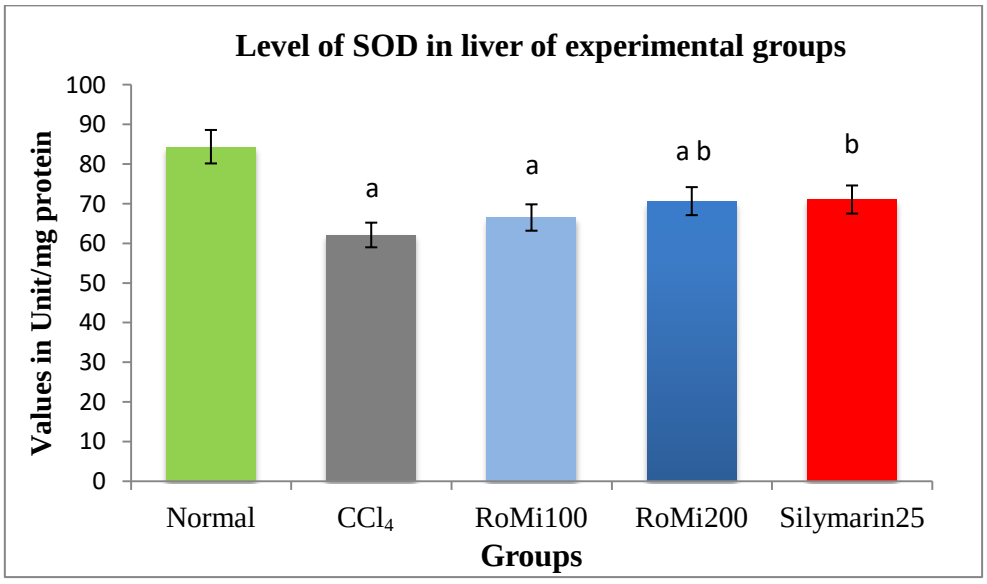


Fig 19: Effect of RoMi-EE on the levels of Superoxide dismutase. Values are mean ±SE (n= 6 animals /group). Statistical analysis was done by one-way ANOVA between groups and values were considered significant at p<0.05. Those which are not sharing the same letters are significantly different.

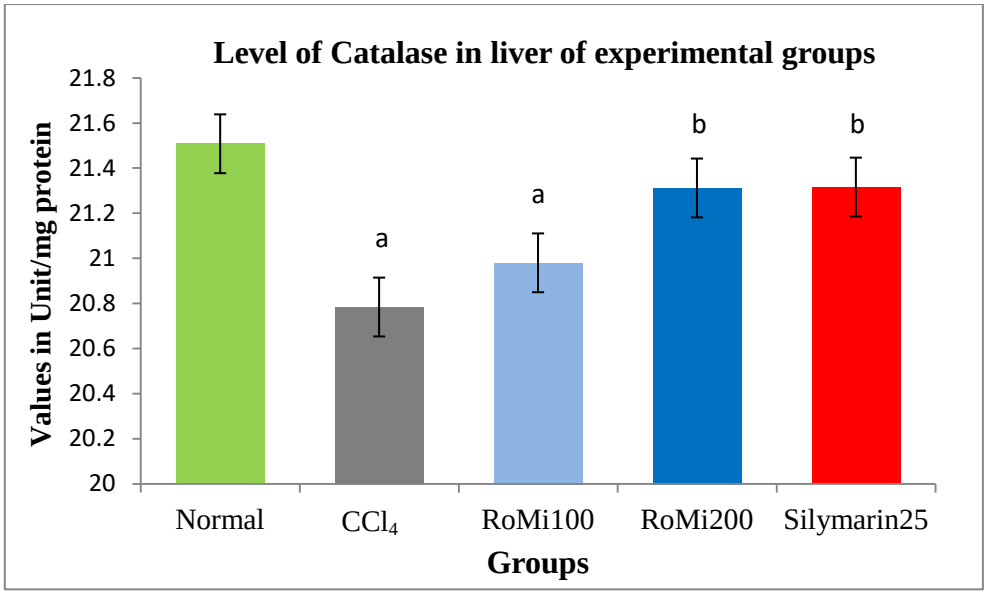


Fig 20: Effect of RoMi-EE on the levels of Catalase. Values are mean ±SE. (n= 6 animals /group). Statistical analysis was done by one-way ANOVA between groups and values were considered significant at $p<0.05$. Those which are not sharing the same letters are significantly different.

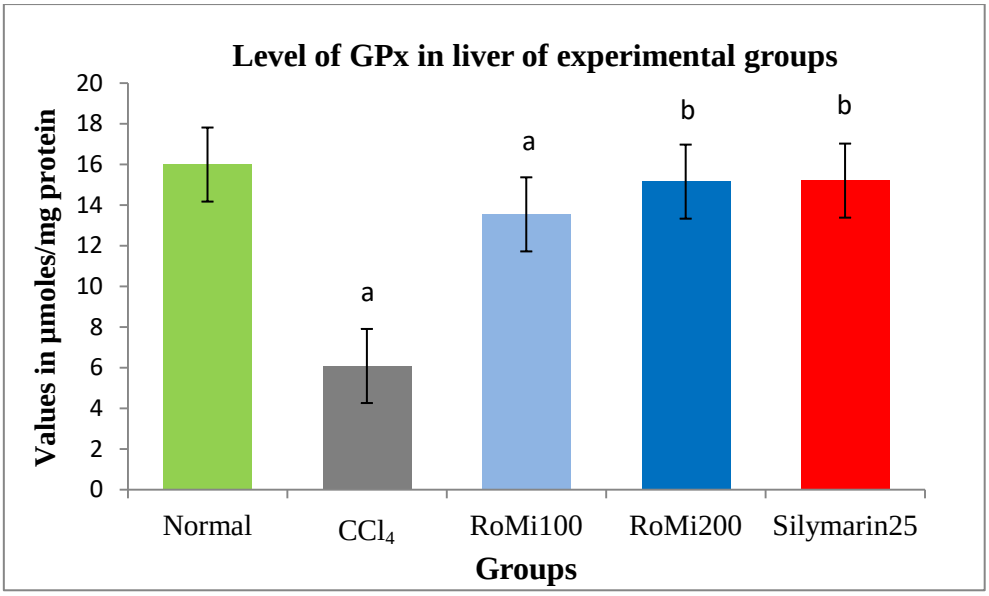


Fig 21: Effect of RoMi-EE on the levels of Glutathione peroxidase. Values are mean ±SE. (n= 6 animals /group). Statistical analysis was done by one-way ANOVA between groups and values were considered significant at $p<0.05$. Those which are not sharing the same letters are significantly different.

The activities of SOD, CAT, and GPx were decreased respectively by 27.3%, 3.53%, and 63.1%. The groups treated with RoMi-EE at two different doses and silymarin

significantly increased ($p<0.05$) in the activities of the enzymes, and the protective effect of RoMi-EE treatment at 200 mg/kg was similar to that of silymarin treatment.

5.14.3.2 Effect of interventions on the activity of GSH

From **Figure 22**, it is evident that CCl_4 reduced the activity of GSH by 25% as compared to the normal group. The activity was ameliorated upon treatment with RoMi-EE at both the concentrations as well as silymarin group. The 100 mg RoMi-EE showed 9.11% increase in GSH concentration. However, 200 mg RoMi-EE have better GSH concentration that showed 12.42% increase and was comparable to that of silymarin group with 12.83% increase when compared to the CCl_4 group. The higher concentration of RoMi-EE and silymarin showed significant increase ($p<0.05$).

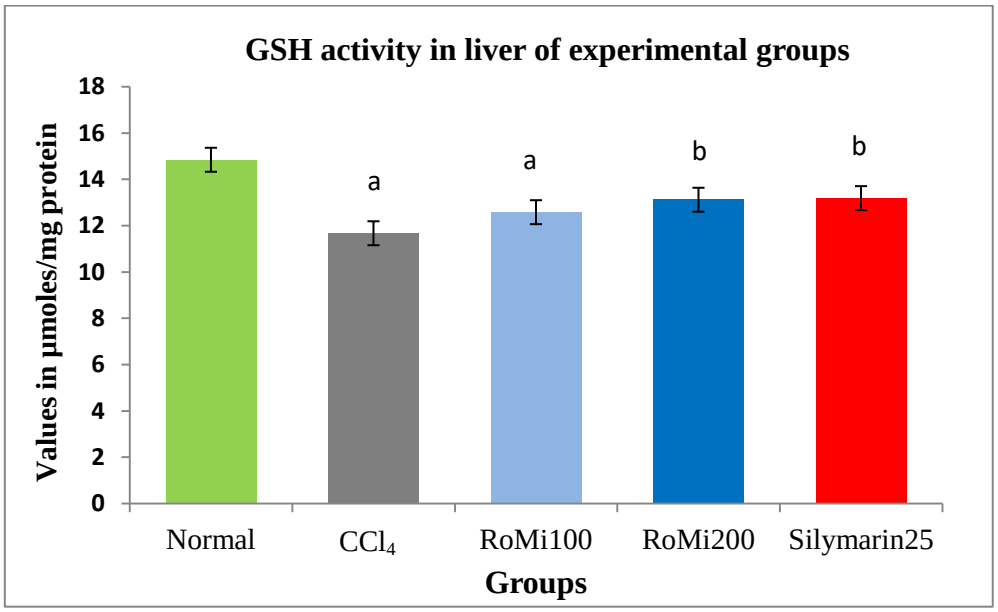


Fig 22: Effect of RoMi-EE on the levels of GSH. Values are mean ±SE. (n= 6 animals /group). Statistical analysis was done by one-way ANOVA between groups and values were considered significant at $p<0.05$. Those which are not sharing the same letters are significantly different.

5.14.3.3 Effect of interventions on Lipid Peroxidation

Lipid peroxidation was accessed by evaluating the level of Malondialdehyde (MDA) and was expressed in nmol/mg of protein. **Figure 23**, shows the effects RoMi-EE treatment on the CCl_4-induced alteration of MDA level. The MDA content was significantly elevated by 48.53% in the CCl_4-treated group when compared with that of the normal group ($p<0.05$). However, the treatment with RoMi-EE and silymarin significantly reduced MDA level.

Lower concentration of RoMi-EE have reduced the elevated level of MDA by 19.12%. However, RoMi ethanolic extract at higher concentration markedly decreased the CCl_4-induced elevation of lipid peroxidation by 33.82% showing notably better result than the silymarin group which reduced the same by 22.08% ($p<0.05$).

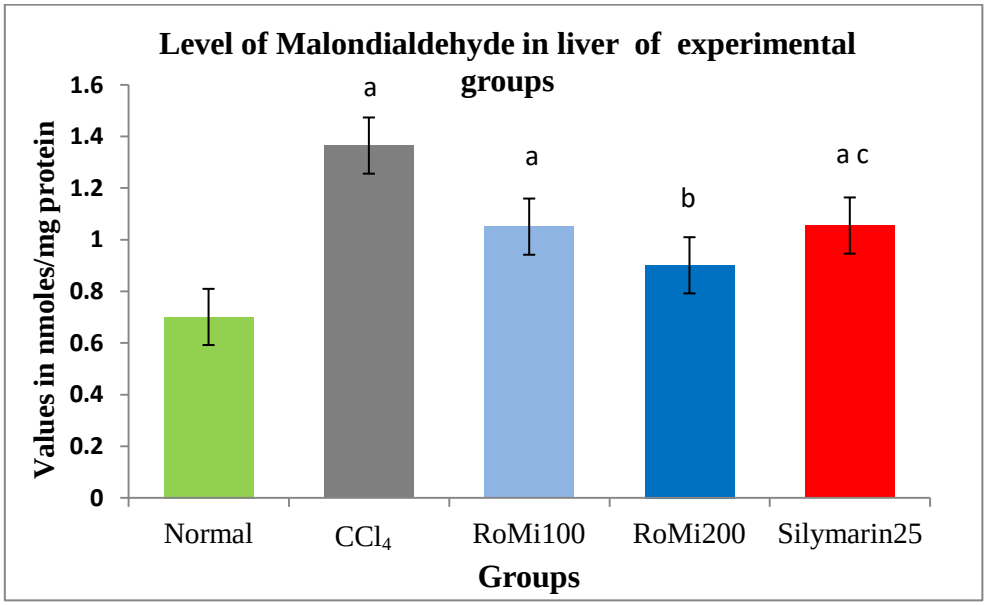


Fig 23: Effect of RoMi-EE on lipid peroxidation (the level of MDA). Values are mean ±SE. (n= 6 animals /group). Statistical analysis was done by one-way ANOVA between groups and values were considered significant at $p<0.05$. Those which are not sharing the same letters are significantly different.

5.15 Effect of interventions on Histopathological changes in Liver

Histopathological evaluation of the rat livers (**Figure 24**) showed that the hepatocytes of a healthy rat had a normal architecture (**Figure 24-A**), whereas, in contrast, the CCl_4 induced severe hepatocyte necrosis, inflammation, hemorrhage, biliary cirrhosis, vacuolation, microvesicular steaosis and broad infiltration of kupffer cells around the central vein (**Figure 24-B**). After treatment with RoMi ethanolic extract, the severity of CCl_4-induced liver intoxication was reduced in a dose-dependent manner. Animal treated with CCl_4 and lower concentration of RoMi-EE showed mild sinusoidal dialatation in centrizonal area, necrosis recovery around the central vein and the regenerating hepatocytes are observed (**Figure 24-C**). Animal treated with CCl_4 and higher concentration (200 mg) of RoMi-EE showed no centrilobular necrosis, higher recovery of hepatocytes around the central vein and proper sinusoid texture (**Figure 24-D**), although the treatment with silymarin showed much better result showing normal hepatocytes and proper central vein. (**Figure 24-E**).

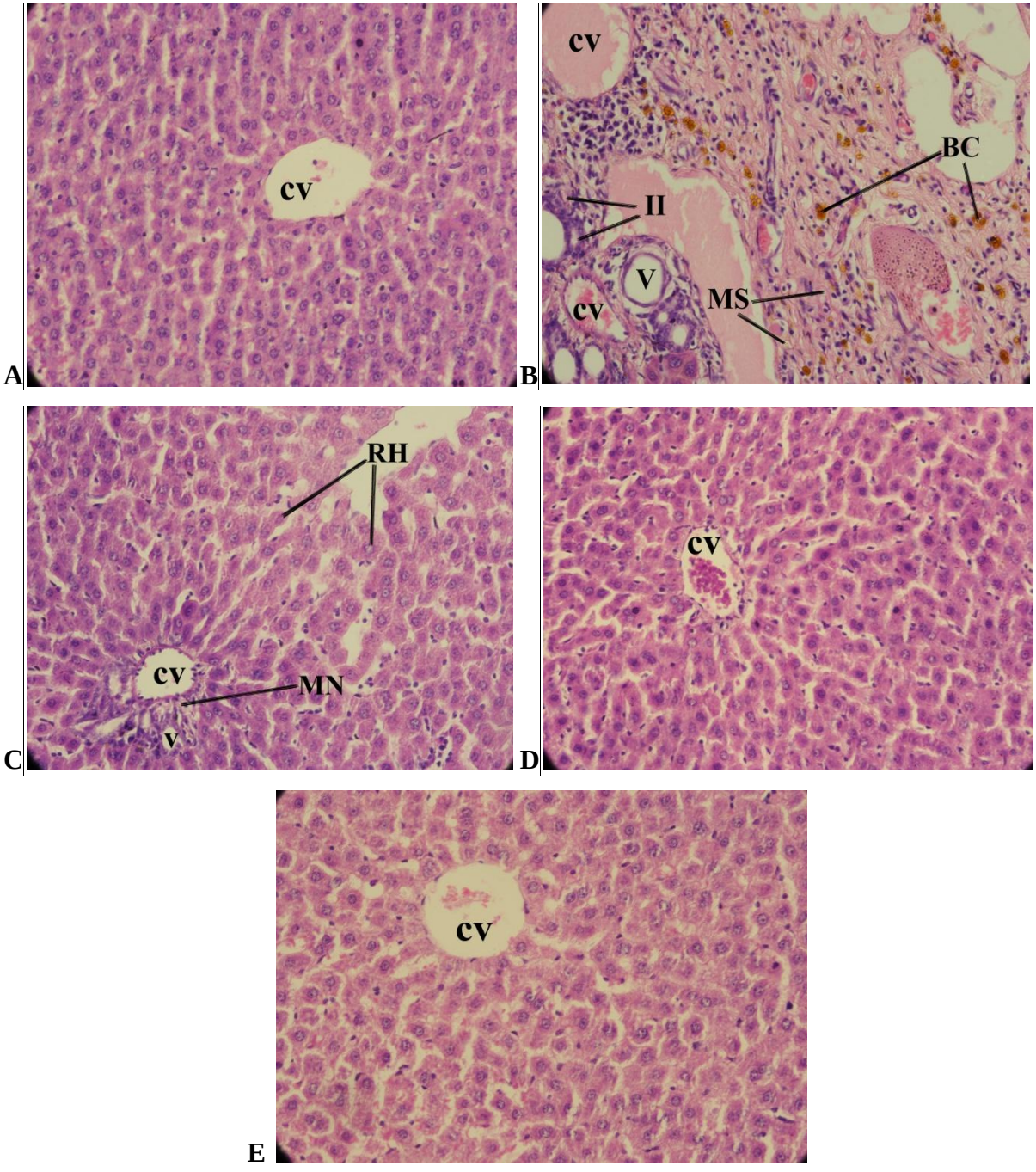


Fig 24: Histopathologic section of liver (40× magnification): A. Normal group: showing arrangement of hepatocytes in the liver lobule. B. CCl_4 group: showing hepatocyte necrosis, inflammatory infiltration (II), biliary cirrhosis (BC), microvesicular steatosis (MS), broad infiltration of kupffer cells and vacuolation (V). C. CCl_4 & 100 mg treated group: showing mild sinusoidal dialatation in centrizonal area, necrosis recovery/ mild necrosis (MN) around the central vein (CV) and regenerative hepatocytes (RH). D. CCl_4 & 200 mg treated gFroup: showing no centrilobular necrosis, hepatocytes with normal texture around the central vein and proper sinusoid texture. E. CCl_4 & silymarin treated group: showing normal hepatocytes and proper central vein.

5.16 Effect of interventions on Histopathological changes in kidney

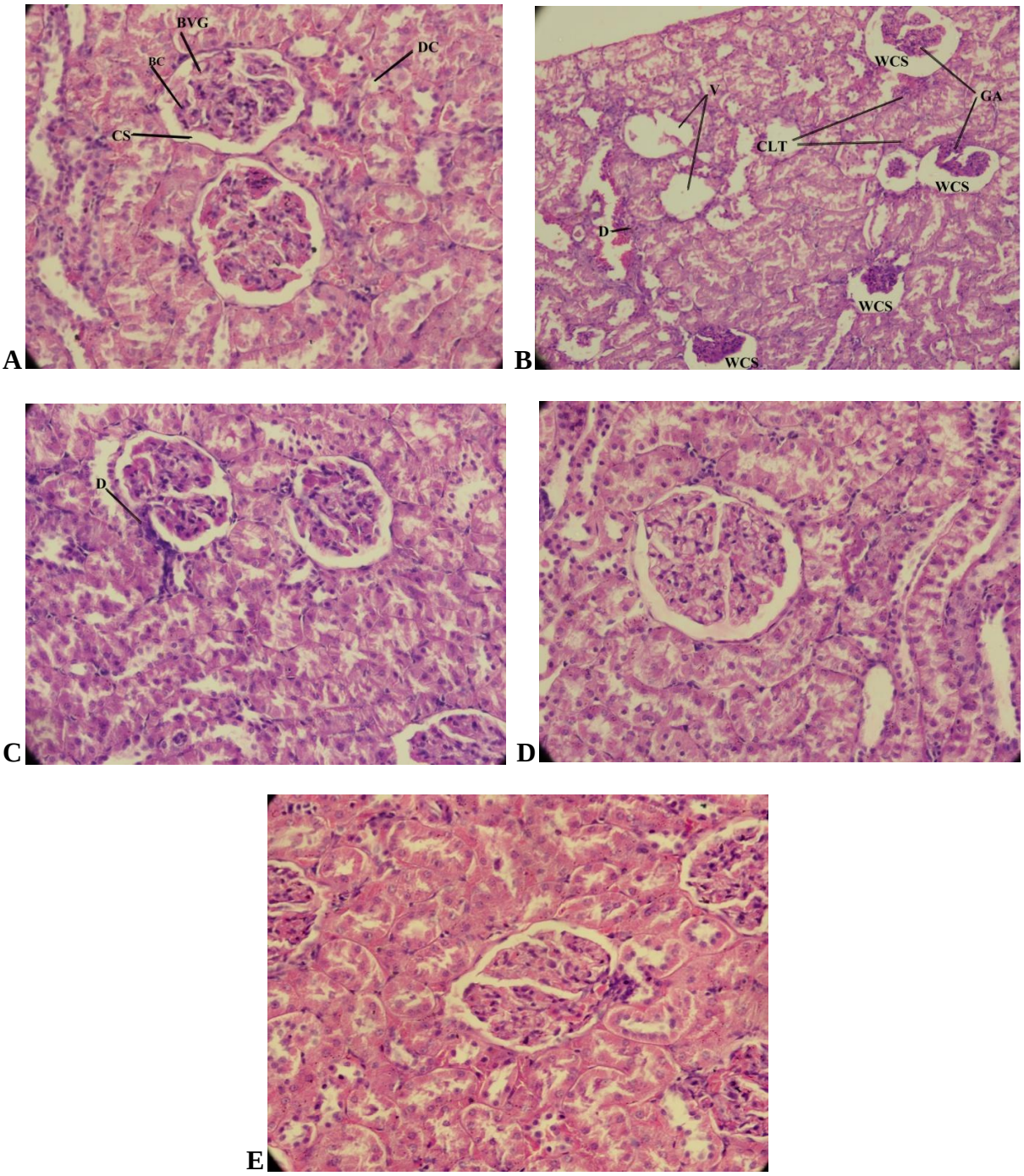


Fig 25: Cross section through kidney cortex (40× magnification): A. Positive control B. Negative control (CCl_4 induced), C. CCl_4 & 100 mg, D. CCl_4 & 200 mg and E. CCl_4 & 25 mg Silymarin. Normal cell structure with blood vessel glomeruli (BVG), distal convoluted tubule (DC), capsule space (CS) and bowman's capsule was observed in A, D & E. Disrupted cells with vacuolation (V), glomerular atrophy (GA), widening of capsule space (WCS), Cell layer thickening and degeneration of cells was seen in B. Recovery of glomerular atrophy, capsule space decrease and degeneration (D) of cell was observed in C.

The histopathology of cross section through wistar rat cortex kidney in CCl_4 group showed vacuolation, glomerular atrophy, widening of capsule space, cell layer thickening and degeneration of cells. The recovery of glomerular atrophy, decrease in capsule space and less degeneration of cell was observed in 100 mg RoMi-EE experimental drug and the 200 mg RoMi-EE experimental drug. Whereas silymarin showed identical structure and was similar to that of normal group.

5.17 Docking analysis of NFκB (1NFK) protein

5.17.1 Receptor Sitemap and site score of 1NFK

Since there is no information about the binding site or standard ligand in a target of interest (reference protein), a putative binding site has been identified by computational means and the druggability of the target was also identified by druggability score [**Table 10**].

A.

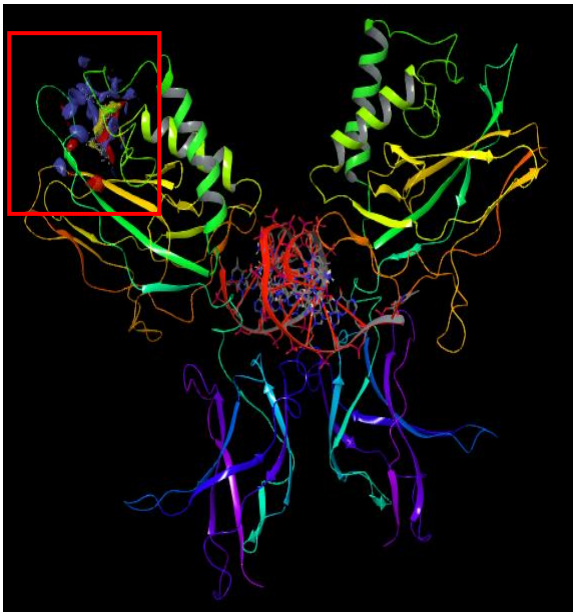


Fig 26: 1NFK protein, A. Showing Sitemap (Site 4) in the protein NFκB.

Table 10: Site score of the 1NFK protein

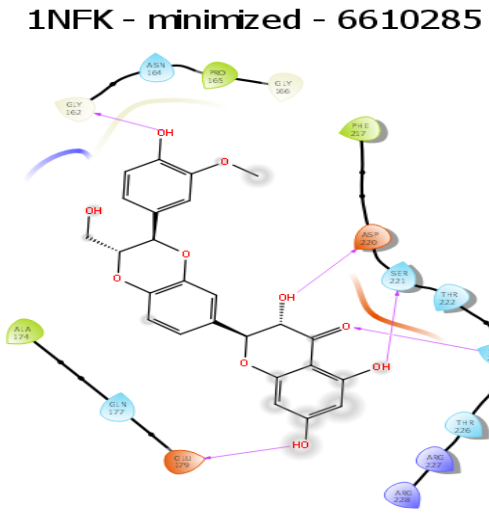
Sites of 1NFK	Site Score	Drug ability Score
Site 1	1.043	0.986
Site 2	0.981	1.015
Site 3	0.993	1.04
Site 4	1	1.04
Site 5	0.868	0.674

The results of site score (**Table 10**) shows that Site 4 as best since the site is showing site score and drug ability score of ≥ 1. Hence the Site 4 was docked with selected ligands.

5.17.2 Ligand docking with 1NFK

As a result of docking a number of values of consensus scoring functions has been obtained. These values assess the quality and energy of binding of molecules studied with the 1NFK-having residues of Site-4 (Chain B): 92, 161, 162, 163, 164, 165, 166, 167, 174, 176, 177, 178, 179, 180, 181, 182, 183, 217, 223, 224, 225, 226, 227, 228. The values of docking score of all compounds with 1NFK and binding energy were given in **Table 11**. The values obtained were compared with the values of hepatoprotective compound silymarin. 3D image of silymarin and ligand (of best docking score) are presented in **Figure 27 & 28**.

A.



B.

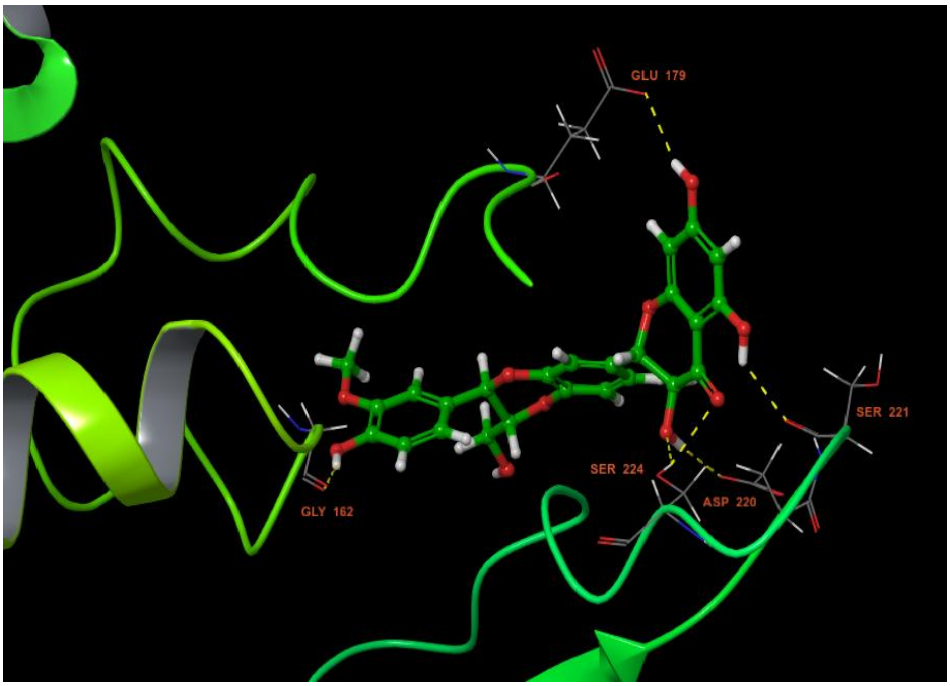
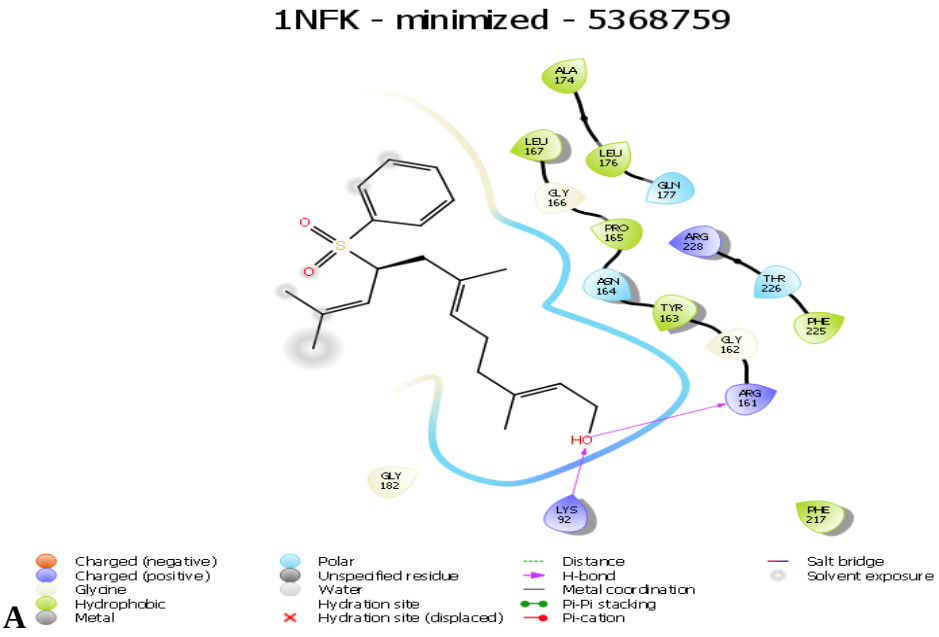
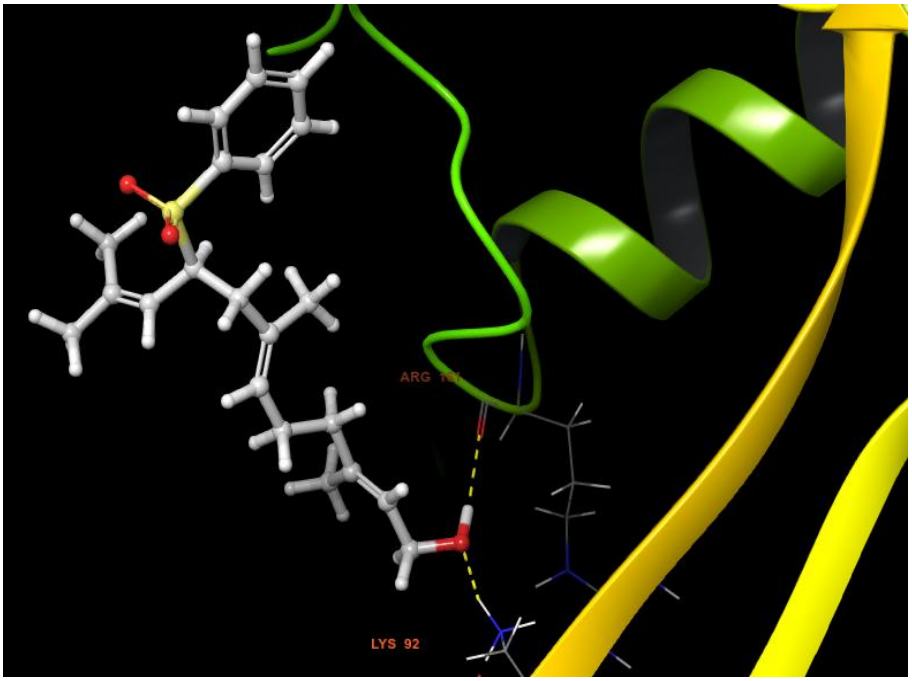


Fig 27: A. 2D & B. 3D image of silymarin binding with 1NFK site-4.



A



B

Fig 28: A. 2D & B. 3D image of ligand CID: 5368759 [2,6,10-Dodecatrien-1- Ol, 3,7,11-Trimethyl-9-(Phenylsulfonyl)-, (E,E)] binding with 1NFK site-4.

Results obtained from the docking studies showed that silymarin have highest docking score of -5.956 and highest binding energy (ΔG) -54.79 and showed five hydrogen bonding with the Gly 162, Glu 179, Asp 220, Ser 221 and Ser 224 residues of 1NFK. 2,6,10-Dodecatrien-1- Ol, 3,7,11-Trimethyl-9-(Phenylsulfonyl)-, (E,E)- [CID: 5368759] showed best docking result among the ligands selected having docking score of -4.958 and binding energy (ΔG) of -45.35, the 3D image shows two hydrogen bonding with the Lys 92, and Arg 161 residues of active site (4) of 1NFK.

5.18 Docking analysis of COX-2 (3LN1) protein

2D and 3D image of ligand (5368759) docking with 3LN1 protein is presented in **Figure 30** (A & B). The 3D image of protein 3LN1 is presented in the **Figure 29**.

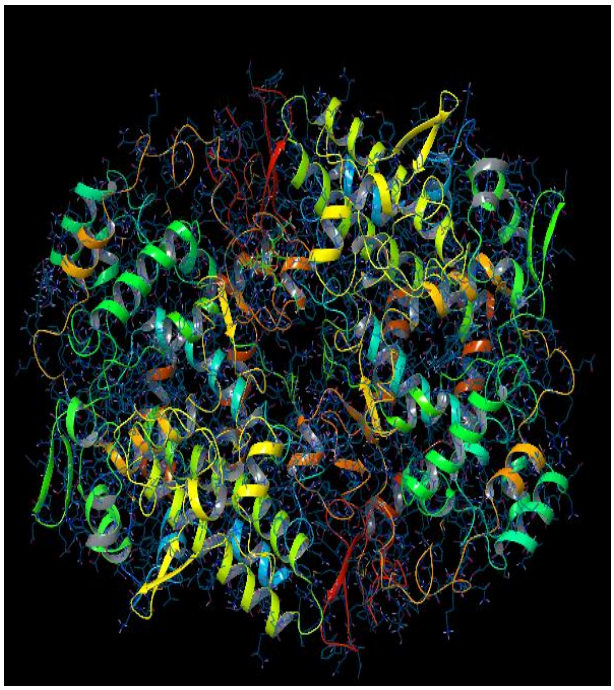
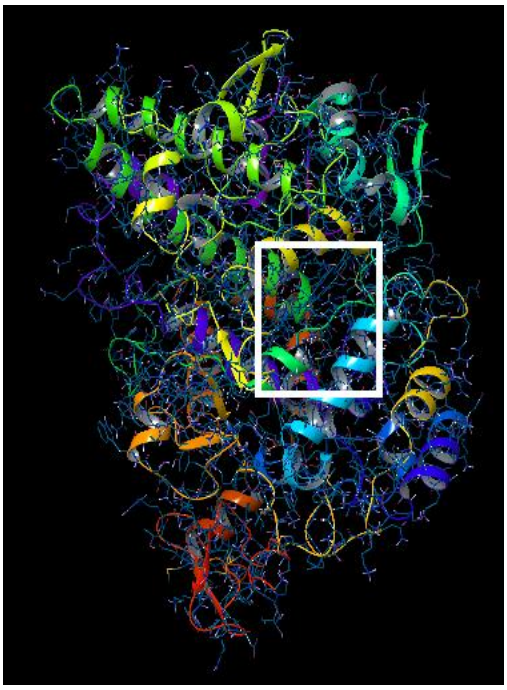
A.  **B.** 

Fig 29: A. 3D image of 3LN1 (COX-2 protein) & B. 3D image of 3LN1 (β subunit).

A.

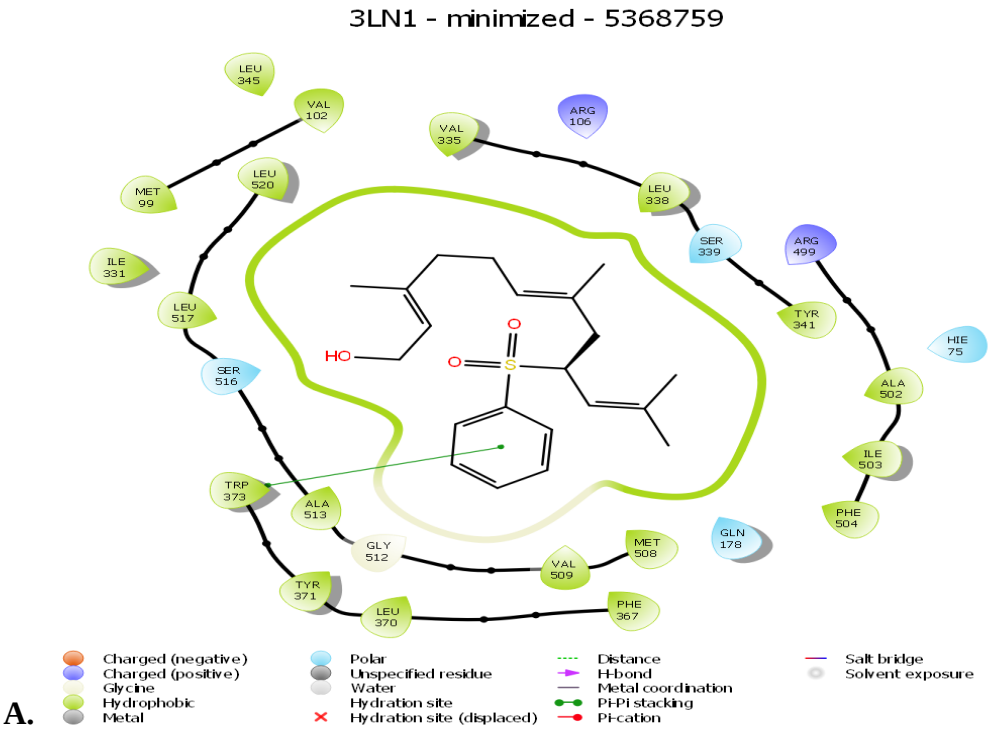


Fig 30: A. 2D image of CID-5368759 (2,6,10-Dodecatrien-1- Ol, 3,7,11-Trimethyl-9-(Phenylsulfonyl)-, (E,E)) binding with 3LN1 (β subunit) showing pi-pi interaction.

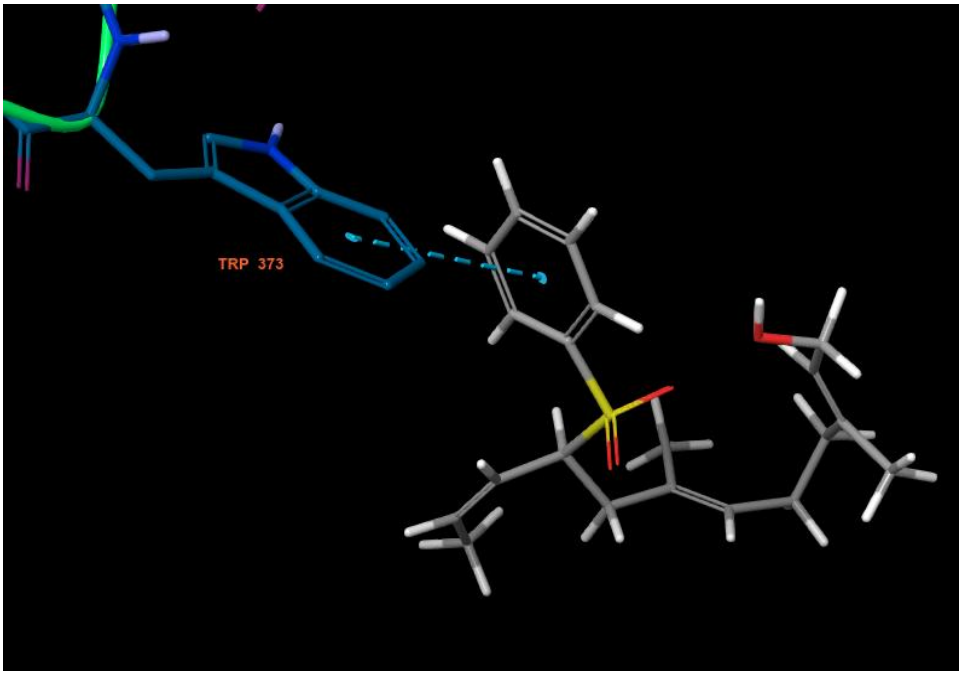
B. 

Fig 30: B. 3D image of CID-5368759 (2,6,10-Dodecatrien-1- Ol, 3,7,11-Trimethyl-9-(Phenylsulfonyl)-, (E,E)) binding with 3LN1 (β subunit) showing pi-pi interaction.

The docking study with 3LN1 crystallized protein shows that the compound: 2,6,10-Dodecatrien-1- Ol, 3,7,11-Trimethyl-9-(Phenylsulfonyl)-, (E,E) showed the Pi-Pi stacking interaction between the aromatic ring of the ligand with another aromatic ring of Tryptophan (TRP 373) residue of the target receptor. This interaction also was the best interaction among the selected ligands which showed best docking score -9.78 and ΔG binding affinity of -27.8173 kcal/mol, following by the compound 9,12-Octadecadienoic Acid (Z,Z)- (CID: 3931), which showed second best docking score of -8.047 and ΔG binding affinity of -21.081 kcal/mol. The compound 2,6,10-Dodecatrien-1- Ol, 3,7,11-Trimethyl-9-(Phenylsulfonyl)-, (E,E), also bound to 1NFK protein with best docking score and highest ΔG binding affinity among the selected ligands. However, the silymarin which showed best docking score with 1NFK, did not show any binding affinity to the 3LN1 protein.

The docking score of other ligands were in order of CID: 3931 (-8.047) > 550196 (-7.569) > 610038 (-7.559) > 519794 (-6.472) > 13849 (-5.995) > 69425 (-5.496) > 445631 (-5.355) > 8122 (-5.028) > 985 (-4.838). The values of docking score of all compounds (ligands) with 1NFK and 3LN1 protein with their binding energy were given in **Table 11**. The compounds CID: 6452096 and 73170 did not show any interaction with the 3LN1 protein, whereas the compounds CID: 3931, 25771, 13849, 69425, 445631 and 985 did not show any interaction with the 1NFK protein.

Table 11: Presenting best docking NFκB (1NFK) and COX-2 (3LN1) score (below -1.5), and MMGBSA ΔG binding affinity of the molecules.

Sl no.	PubChem CID	Compound Name	NFκB docking (1NFK)		COX-2 docking (3LN1)	
			Docking Score	MMGBSA ΔG binding	Docking Score	MMGBSA ΔG binding
1.	6610285	Silymarin	-5.956	-54.79	---	---
2.	5368759	2,6,10-Dodecatrien-1-Ol, 3,7,11-Trimethyl-9-(Phenylsulfonyl)-, (E,E)	-4.958	-45.35	-9.78	-27.8173
3.	8122	Octadecanoic acid, ethyl ester	-4.528	-33.85	-5.028	-1.44194
4.	6452096	Ethyl Iso-Allocholate-	-3.679	-39.5	---	---
5.	550196	1-Methylene-2b-Hydroxy methyl-3,3-Dimethyl-4b- (3-Methylbut-2-Enyl)-C	-3.277	-5.67	-7.569	-10.189
6.	519794	1,2-Bis(Trimethylsilyl) Benzene	-3.001	-29.05	-6.472	2.080533
7.	73170	Alpha.-Amyrin	-1.629	-27.59	---	---
8.	610038	2,4,6-Cycloheptatrien-1-One, 3,5-Bis-Trimethylsilyl-	-1.595	-26.24	-7.559	-10.7121
9.	3931	9,12-Octadecadienoic Acid (Z,Z)-	---	---	-8.047	-21.081
10.	25771	Silane, 1,4-Phenylenebis [Trimethyl-	---	---	-7.025	-3.6666
11.	13849	Pentadecanoic Acid-	---	---	-5.995	-18.4921
12.	69425	1-Octadecyne	---	---	-5.496	-19.8241
13.	445631	Oleic Acid-	---	---	-5.355	-1.51702
14.	985	N-Hexadecanoic Acid	---	---	-4.838	11.81801

5.19 ADME property of ligands

Physically significant and pharmaceutically significant properties of all the lead molecules were analysed by using “molsoft” prediction tool. Molecular weight, H-bond donors, log P Octanol/water partition coefficient, H-bond acceptors, Mol Log S and their

positions according to Lipinski's rule of five were presented in **Table 12**. Almost all the compounds were in the acceptable range of Lipinski's rule of five, indicating their potential for use as drug-like molecules.

Table 12: Physical properties of molecules calculated by Lipinski's rule of five.

Sl no.	PubChem CID	Molecular weight[a]	Number of HBA[b]	Number of HBD[c]	Mol Log P[d]	Mol Log S[e]
1.	6610285	482.12	10	5	2.59	-6.19
2.	5368759	362.19	3	1	5.61	-5.37
3.	*8122*	*312.3*	*2*	*0*	*8.45*	*-7.21*
4.	6452096	436.32	5	3	4.67	-5.06
5.	550196	222.2	1	1	4.77	-4.16
6.	519794	222.13	0	0	2.6	-3.96
7.	*73170*	*426.39*	*1*	*1*	*9.21*	*-8.11*
8.	610038	250.12	1	0	1.73	-2.73
9.	*3931*	*280.24*	*2*	*1*	*6.75*	*-5.44*
10.	25771	222.13	0	0	2.72	-2.77
11.	13849	242.22	2	1	6.17	-5.24
12.	*69425*	*250.27*	*0*	*0*	*7.69*	*-7.34*
13.	*445639*	*282.26*	*2*	*1*	*7.15*	*-5.97*
14.	*985*	*256.24*	*2*	*1*	*6.65*	*-5.66*

a. Molecular weight of the molecule (160 to 500)

b. Hydrogen bonds that would be accepted by the solute from water molecules in an aqueous solution (not more than 10).

c. Hydrogen bonds that would be donated by the solute to water molecules in an aqueous solution (not more than 5).

d. Log P for octanol/water (−2.0 to 6.5).

e. Water solubility, log S (−6.5 to 0.5).

Most of the ligands were under the acceptable range of molecular weight, hydrogen bond acceptor, hydrogen bond donor and water solubility (other than CID: 8122, 73170 & 69425) of Lipinski rules. The log P value of the ligands (except CID: 8122, 73170, 3931, 69425 and 445639) also suggests that it cannot cross the blood–brain barrier and hence it can be used as drug in other organs or body parts without affecting the brain tissue.