4. RESULTS

OBJECTIVE 1: Collection and Identification of Traditionally Used Anti-diabetic Medicinal Plants from Kokrajhar District

4.1. Survey, identification, and documentation of medicinal plants

Kokrajhar is the headquarters of the Bodoland Territorial Region of Assam. The district comprises of 1,068 villages, divided into 11 community development blocks (CDBs). In the present study, 54 traditional healers (known as Kaviraja) were interviewed from 54 different villages, covering all the CDBs of the district. Figure 4.1a displays the entire map of Kokrajhar district and the surveyed villages during the study period, with red dots representing the data collection sites. Out of the 54 traditional healers interviewed, only 15 could provide information about the traditional herbal practices for antidiabetic medicines. The locations of these 15 villages are shown as red dots in Figure 4.1b. Table 4.1 lists the names of the 11 CDBs and the 54 villages surveyed, along with their geographical locations. During the survey, out of the 54 villages, Kachugaon CDB has the highest number of informants constituting 12 individuals from 12 villages, followed by Kokrajhar (11 villages), Dotma (9 villages), Debitola (8 villages), Gossaigaon (7 villages), Rupsi (3 villages), Chapar-Salkocha (2 villages), Hatidhura (1 village), and Mahamaya (1 village). No data was collected from Golokganj (Part) and Bilasipara (part) because of fewer villages and the absence of tribal communities in the blocks. Regarding diabetic healers, the highest data was collected from Kokrajhar CDB with five informants, followed by three informants each from Dotma and Gossaigaon CDBs. The names of the specific villages from where antidiabetic healers responded positively were Borghola, Baoraguri, Dotma Bazar, Narenguri, Karikar F.V., Kumtola F.V., Chilaguri, Mahendrapur, Mawriagaon-II, Phagriguri, Sutharpara, Banglabari, Gossaigaon -I, Singimari-II, and Kazigaon.

In terms of the demographics of the informants, out of the total 54, 37 were males and 17 were females (Table 4.2). Most of the traditional knowledge bearers were elderly, aged over 50 years. About 74% of the total informants were above 50 years old, followed by 26% in the age group of 40 to 50 years. Our survey did not encounter any traditional healer below 40 years. In terms of literacy, most informants were literates (□67%), where, 48% of the informants had a school-level education, whereas 19% had

a college-level education, followed by 33% with no formal education at all. Among the 54 informants, only four were employed in government jobs, all of whom were male, while the rest engaged in agriculture-based livelihoods. The study also observed that most informants from Kokrajhar and Kachugaon CDBs were literate, while those from other CDBs were illiterate. It was also observed that the number of plants cited by literate (4.6 citations) and illiterate (5 citations) informants was almost similar. About 72% of the informants were professional traditional healers who practiced traditional healing daily for their livelihood, while 28% were elderly knowledgeable individuals who were not involved in traditional healing practices or any income-generating activities. Similarly, out of the 15 informants knowledgeable about antidiabetic medicinal plants, 11 were males and four were females. Most of the traditional healers who knew about antidiabetic medicinal plants were aged above 40 years. Of the total informants, 60% were between 40 to 50 years of age, and 40% were above 50 years. The study did not encounter young individuals having ethnomedicinal information.

In terms of the literacy rate of the informants (diabetic healers), it was found that most of them are literates (80%), 46.66% had a school-level education. In comparison, 33.3% had at least a college-level education, and 20% had no formal education at all (Table 4.3). The number of plants cited by literate informants (4.16 plant citations) and illiterate informants (2 plant citations) varied. Nevertheless, the information shared by the illiterate individuals was more or less similar to that of the literate ones. All the informants were local healers who had been practicing the traditional medicine system for a long time. It was also found that most of the knowledge about medicinal plants had been passed down to them by their parents, grandparents, or other relatives who possessed vast knowledge about diseases and their cures. The name of the plant species, its local names, and the traditional formulation methods practiced by traditional healers are shown in Table 4.4. The present survey revealed that 37 species of medicinal plants belonging to 24 Families and 33 Genera are used in the traditional herbal preparation for the treatment of diabetes. The survey found that most of the plants cited by the informants are locally available, with some being wild, and others cultivated by individuals for easy access and conservation of valuable medicinal resources. However, traditional healers have noted a decrease in the availability of medicinal plants, observing that species abundant several years ago have now diminished in number.

Figure 4.1. Map of the survey site with red dots indicating the survey sites. (a) Total and location of 54 villages where the survey was conducted, and traditional healers were interviewed, and (b) the location of 15 villages where ethnomedicinal data was collected during the survey

Table 4.1. List of villages where antidiabetic medicinal plants were collected along with the geographical location

Sl. no.	CDBs	List of villages	Geographical location
1.	Kokrajhar	Athiabari	26°58'48.66"N 90°20'38.75"E
2.		Bashbari	26°35'09.16"N 90°12'13.84"E
3.		Batipara	26°22'40.17"N 90°15'23.14"E
4.		Mahendrapur*	26˚36˙09.86″N 90˚14˙24.07″E
5.		Chilaguri*	26°28'35.26"N 90°12˙04.06"E
6.		Latagaon	26°29'03.41"N 90°20'36.29"E
7.		Mawriagaon-II*	26°27'04.83"N 90°08'40.14"E
8.		Pakriguri*	26°31'00.58"N 90°14'32.44"E
9.		Ranighuli	26°19'53.71"N 90°16'11.16"E
10.		Shamthaibari	26°22'40.96"N 90°18'22.35"E
11.		Sutarpara*	26°29'36.69"N 90°20'38.95"E
12.	Chapar-Salkocha (Pt.)	Bamungaon Pt.IV	26°29'05.81"N 90°28'05.83"E
13.		Borghola*	26°17'04.23"N 90°18'20.32"E
14.	Dotma	Baghmar	26°28'38.78"N 90°08'48.01"E
15.		Bauti	26°29'29.72"N 90°09'19.26"E
16.		Bhalukmari	26°29'56.05"N 90°12'12.12"E
17.		Boraguri*	26°27'07.21"N 90°08'36.27"E
18.		Dangarkhuti	26°27'21.23"N 90°12'21.58"E
19.		Dotma Bazar*	26°28'06.87"N 90°09'02.61"E
20.		Ghoramar	26°32'54.97"N 90°48'20.83"E
21.		Narenguri*	26°29'19.30"N 90°05'23.74"E
22.		Dumuriguri	26°26'11.96"N 90°04'59.02"E
23.	Gossaigaon	Balapara-Siljhar	26°32'24.60"N 90°10'40.43"E
24.		Banglabari*	26°39'90.55"N 90°03'27.32"E
25.		Bhowraguri	26°36'06.15"N 90°14'24.83"E
26.		Gossaigaon-I*	26°42'84.87"N 89°99'88.33"E
27.		Madati	26°41'84.03"N 90°02'27.54"E
28.		Mowamari	26°42'46.90"N 90°10'77.73"E

29.		Singimari-II*	26°36'04.09"N 89°99'01.02"E
30.	Kachugaon	Balagong	26°58'17.06"N 90°07'53.78"E
31.		Chengmari FV	26°32'55.30"N 89°56'37.73"E
32.		Gombariguri	26°51'13.51"N 90°11'98.16"E
33.		Haldibari FV	26°36'08.48"N 89°54'08.78"E
34.		Jonaligaon FV	26°64'69.75"N 89°94'36.99"E
35.		Karikar FV*	26°32'49.27"N 90°05'12.04"E
36.		Kumguri FV	26°53'98.81"N 90°15'97.63"E
37.		Kumtola FV*	26°32'52.96"N 90°03'23.55"E
38.		Lotamari FV-II	26°56'89.20"N 90°10'86.83"E
39.		Salbari FV	26°56'50.57"N 90°00'08.17"E
40.		Takampur FV	26°60'29.82"N 90°03'51.70"E
41.		Kathalguri	26°52'17.60"N 90°00'58.82"E
42.	Debitola (Pt.)	Borshijhora Pt-II	26°21'65.21"N 89°96'23.53"E
43.		Daibari Pt.-I	26°26'92.97"N 89°97'80.08"E
44.		Daimaguri-I	26°30'46.58"N 90°05'36.79"E
45.		Dampur –II	26°12'20.56"N 90°02'53.42"E
46.		Debitola Pt.-II	26°15'76.43"N 90°00'75.26"E
47.		Dholagaon Pt.-I	26°20'61.94"N 90°02'04.14"E
48.		Duligaon Pt.-III	26°25'98.01"N 90°07'24.97"E
49.		Kazigaon Pt.-I*	26°19'21.79"N 89°99'46.81"E
50.	Mahamaya (Pt.)	Silbari	26°13'52.68"N 89°97'82.28"E
51.	Rupsi (Pt.)	Basbari	26°18'93.98"N 89°90'26.52"E
52.		Dukhisukhijhar Pt.-I	26°16'60.90"N 89°89'27.91"E
53.		Malatijhora	26°16'97.28"N 89°95'99.30"E
54.	Hatidhura (Pt.)	Hatidhura	26°29'14.89"N 90°15'41.86"E

*Part (Pt.) means some villages of Debitola CDB comes under Dhubri district and some under Kokrajhar district. FV - forest village. *Villages where antidiabetic ethnomedicinal data was collected

Table 4.2. Demographic characteristics of informants interacted in Kokrajhar district Community Development Blocks

	Literacy			Age group		Informants		
Block Names	**School**	**College**	**Illiterate**	**>50**	**40-50**	**Kaviraja**	**Older**	**Total**
Kokrajhar	3	6	2	9	2	8	3	11
Kachugaon	7	2	3	9	3	10	2	12
Dotma	3	2	4	6	3	4	5	9
Debitola	4	0	4	4	4	7	1	8
Gossaigaon	4	0	3	5	2	5	2	7
Rupsi	2	0	1	3	0	2	1	3
Chapar-Salkocha	2	0	0	2	0	1	1	2
Hatidhura	0	0	1	1	0	1	0	1
Mahamaya	1	0	0	1	0	1	0	1
Total	**26**	**10**	**18**	**40**	**14**	**39**	**15**	**54**

Table 4.3. Demographic characteristics of informants of Kokrajhar district Community Development Blocks

	Literacy			Informants	
Block	**School**	**College**	**Illiterate**	**>50**	**40-50**
Chapar-Salkhocha	1	-	-	-	1
Dotma	2	1	-	1	2
Debitola	1	-	-	-	1
Gossaigaon	1	1	1	1	2
Kokrajhar	1	3	1	2	3
Kachugaon	1	-	1	2	-
Total	**7**	**5**	**3**	**6**	**9**

Of the 24 plant families, the most popular were Apocynaceae (16.6%), followed by Moraceae, Combretaceae, and Myrtaceae (12.5% each), and Cucurbitaceae, Acanthaceae, Apiaceae, and Lamiaceae (8.3% each). The study also observed that traditional healers mostly use parts of trees (40.54%) rather than herbs (29.72%), shrubs (16.21%), and climbers (13.51%). The most commonly used antidiabetic plant was found to be *Hodgsonia heteroclita*, with the highest frequency of citation (FC = 6; RFC = 0.40; FIV = 46.67%), followed by *Andrographis paniculata* (FC = 5; RFC = 0.33; FIV = 33.33), *Rauvolfia tetraphylla* (FC = 3; RFC = 0.20; FIV = 20%), etc. Out of the 37 reported plant species, 26 (70.27%) were mentioned once by the informants, while 11 plants showed repeated citations, with *H. heteroclita* being the most cited plant (Figure 4.2). In terms of the popularity of families among informants, Cucurbitaceae had the highest Family Importance Value (FIV) at 40%, followed by Acanthaceae (33%) and Asparagaceae (20%). Traditional healers utilize different parts of medicinal plants for remedy preparation. The types of plant parts used in the preparation of traditional herbal medicines are depicted in Figure 4.3. The dominant plant part used in preparation is leaves (51.35%), followed by fruit (16.21%), flowers (13.50%), roots (8.10%), bark (5.40%), seeds (5.40%), and stems (5.40%) (Figure 4.3). It has come to our observation that out of 37 plant species, in 16% of plants, more than one part is used to prepare traditional medicine.

The study also collected the information related to the traditional formulation practices followed during the preparation. The traditional medicines were prepared in different methods such as decoction, infusion, and raw. Decoction is the process by which the plant parts are ground, smashed, and boiled. The boiled mixture is then filtered by a strainer and the liquid or soup is consumed as a medicine. Infusion mode of preparation is the process where the parts of the plants are soaked overnight in water without smashing it, and the liquid is consumed. Raw mode of use is generally for tender leaves which can be consumed directly. Based on the preparation method, the survey observed that decoction (54.05%) is the most used method adopted by traditional healers for the preparation of herbal medicine (Figure 4.4). Our study also found that most of the plants (29.73%) are being prepared as raw and some are soaked (16.22%) as a whole and the water is consumed as an herbal medicine. Figure 4.4 shows the methods adopted by traditional healers.

Figure 4.2. List of plants and citations by traditional healers

Figure 4.3. Frequency of plant parts used during preparation of herbal medicines. mop - more than one part, wp - whole plant

Figure 4.4. Traditional formulations adopted by the traditional healers in the preparation of herbal remedies.

We have also performed a literature survey for all the plants cited by the informants based on secondary data, and found that 64.87% of the plants have one or more published literature on antidiabetic properties. However, 35.13 % of the plants and the parts cited by the traditional healers were found to have no scientific literature, particularly on antidiabetic medicinal plants.

Table 4.4. Name of the plants, parts used, traditional formulation, and habit of plants

Sl. No.	Scientific Name & Voucher Number	Family	Local name (Bodo)	Parts Used	Traditional Formulation	Habit	RFC	FIV
1.	*Tinospora cordifolia* (Willd.) Meirs [BUBH2018024]	Menispermaceae	amar lotha	stem/leaves	decoction	climber	0.133	13.33
2.	*Phyllanthus emblica* L. [BUBH2018023]	Euphorbiaceae	amla	fruit	raw	tree	0.066	6.66
3.	*Terminalia arjuna* (Roxb. Ex DC.) Wigt & Arn [BUBH2018066]	Combretaceae	arjun	bark	infusion	tree	0.133	26.66
4.	*Musa balbisiana* Colla [BUBH2018067]	Musaceae	athia thalir	aerial stem	decoction	shrub	0.066	6.66
5.	*Phlogacanthus thyrsiformes* Mabb. [BUBH2018028]	Acanthaceae	basikhar	flower	decoction	shrub	0.066	33.33
6.	*Aegle marmelos* (L.) Corrêa [BUBH2018068]	Rutaceae	bell	leaves	decoction	tree	0.066	6.66
7.	*Terminalia bellirica* (Gaertn.) Roxb. [BUBH2018069]	Combretaceae	bhaora	fruit	raw	tree	0.066	26.66
8.	*Paspalum fimbriatum* Kunth [BUBH2018070]	Poaceae	dapsa	whole plant	decoction	herb	0.066	6.66

9.	*Syzygium jambos* (L.) Alston [BUBH2018071]	Myrtaceae	godjaam	tender leaves	raw	tree	0.066	2.67
10.	*Calotropis gigantea* (L.) R.Br. ex Schult. [BUBH2018072]	Apocynaceae	gogondo	leaves	decoction	shrub	0.066	33.33
11.	*Rosa alba* L. [BUBH2018073]	Rosaceae	golab gufur	flower	infusion	shrub	0.066	6.67
12.	*Syzygium cumini* (L.) Skeels BUBH2018074]	Myrtaceae	gwswm jamboo	leaves	raw	tree	0.133	2.67
13.	*Hodgsonia heteroclita* (Roxb.) Hook.f. & Thomson [BUBH2018075]	Cucurbitaceae	hagrani jwgwnar	fruit	decoction	climber	0.400	46.67
14.	*Artocarpus heterophyllus* Lam. [BUBH2018076]	Moraceae	khanthal	leaves	raw	tree	0.066	20
15.	*Ficus racemosa* L. [BUBH2018077]	Moraceae	dumburu	fruit/leaves	decoction	tree	0.066	20
16.	*Alpinia galanga* (L.) Willd. [BUBH2018078]	Zingiberaceae	jermao	tuber	raw	herb	0.066	6.67
17.	*Andrographis paniculata* (Burm.f.) Nees [BUBH2018009]	Acanthaceae	kalmith	leaves	raw	herb	0.333	33.33
18.	*Oroxylum indicum* (L.) Kurz [BUBH2018012]	Bignoniaceae	kharong khandai	leaves	decoction	tree	0.066	6.67

19.	*Rauvolfia tetraphylla* L. [BUBH2018013]	Apocynaceae	kharwkha	root	decoction	shrub	0.133	33.33
20.	*Syzygium aromaticum* (L.) Merr. &L.M.Perry [BUBH20180079]	Myrtaceae	long	flower bud	decoction	tree	0.066	2.67
21.	*Centella asiatica* (L.) Urb. [BUBH2018020]	Apiaceae	manimuni gidir	leaf	decoction	herb	0.066	6.67
22.	*Hydrocotyle sibthorpioides* Lam. [BUBH2018019]	Apiaceae	manimuni fisa	whole plant	decoction	herb	0.066	6.67
23.	*Trigonella foenum-graecum* L. [BUBH2018080]	Fabaceae	methi	seed	infusion	herb	0.133	13.33
24.	*Clerodendrum infortunatum* L. [BUBH2018047]	Lamiaceae	mwkhwna	tender leaf	decoction	shrub	0.066	13.33
25.	*Lindernia crustacea* (L.) F. Muell. [BUBH2018048]	Linderniaceae	na bikhi	whole plant	raw	herb	0.066	13.33
26.	*Azadirachta indica* A. Juss. [BUBH2018051]	Meliaceae	neem	leaf	raw	tree	0.133	13.33
27.	*Asparagus racemosus* Willd. [BUBH2018063]	Asparagaceae	nilikhor	roots	decoction	climber	0.200	20
28.	*Catharanthus roseus* var. *albus* G. Don. [BUBH2018081]	Apocynaceae	parboti	flower/leaves	decoction	herb	0.133	33.33

29.	*Bryophyllum pinnatum* (Lam.) Oken [BUBH2018057]	Crassulaceae	path gaja	leaves	infusion	herb	0.066	6.67
30.	*Ficus religiosa* L. [BUBH2018082]	Moraceae	phakhri	leaves	decoction	tree	0.066	20
31.	*Nelumbo nucifera* Gaertn. [BUBH2018083]	Nelumbonaceae	podophul	stem	infusion	herb	0.066	6.67
32.	*Terminalia chebula* Retz. [BUBH2018062]	Combretaceae	selekha	fruit	raw	tree	0.133	26.67
33.	*Nyctanthes arbor-tristis* L. [BUBH2018084]	Oleaceae	sewali	flower	decoction	tree	0.066	6.67
34.	*Piper longum* L. [BUBH2018085]	Piperaceae	simfri	fruit	raw	climber	0.066	6.67
35.	*Alstonia scholaris* (L.) R.Br. [BUBH2018040]	Apocynaceae	sithona	bark	infusion	tree	0.066	33.33
36.	*Ocimum tenuiflorum* L. [BUBH2018045]	Lamiaceae	tulsi	roots	decoction	herb	0.066	13.33
37.	*Momordica charantia* L. [BUBH2018086]	Cucurbitaceae	udasi	tender leaves	decoction	climber	0.066	46.66

4.2. Preparation of alcoholic crude extract

The methanolic crude extracts of all the sample plants have been shown in the Table 4.5. The 11 tested plants showed high in moisture content. From 1000 g of wet weight of the plant parts, the highest dry weight was obtained in *Phlogacanthus thyrsiformis* and the lowest in *Ficus racemosa*. The moisture content ranged from 43% to 90%. Among the plant parts, the highest moisture content was observed in *F. racemosa* fruits with about 90% of the wet weight, followed by *An. paniculata* (88.7%). The average moisture content was found to be 66.67%. Among the leaves, the moisture content ranges from (59.10%-88.7%). *Andrographis paniculata* possessed the highest moisture content followed by *Oroxylum indicum*. In terms of bark of tree, the moisture content is 76.39±7.11%. In flower extract, the moisture content was found to be as high as 71.47±6.98 and as low as 43±13.29%. The corm extract was seen to have moisture content of 75.30±4.52%. *Lindernia crustacea* showed the highest moisture content in terms of whole plant used. Among the plant parts, the highest moisture content was seen in fruit extract and lowest moisture present was seen in flower extract. The average crude extract yield was 7.73 g per 100 g of plant powder. The highest crude extract yield was seen in whole plant extract of *Lindernia crustacea* followed by *Oroxylum indicum* (leaves). The lowest crude extract yield was seen in whole plant extract of *Paspalum fimbriatum*. Among the plant parts the most yielded crude extract was seen in whole plant extract (18.88 g), followed by leaves (16.84 g and 11.94 g). However, the lowest crude extract yield was also seen in whole plant extract (1.49 g).

4.3. Qualitative Phytochemical study

The qualitative study of all the plants was done to test for the presence of carbohydrates, protein, phenol, flavonoid, saponins, tannins, glycosides and alkaloids. The result is shown in Table 4.6. In all the plant extracts the presence of protein and carbohydrate content was found. The plants also showed the presence phenol and flavonoids. The presence of saponin was seen in all the plant extract except *Phlogacanthus thyrsiformes* and *Alstonia scholaris.* All the plants showed presence of alkaloids, glycosides except *Alstonia scholaris, Rauvolfia tetraphylla,* and *Clerodendrum infortunatum*, showing the absence of tannins.

PHOTO PLATE: III

A-*Alstonia scholaris*; **B**- *Andrographis paniculata;* **C**- *Clerodendrum infortunatum;* **D**- *Ficus racemosa;* **E**- *Hydrocotyle sibthorpioides* **F**- *Lindernea crustacea*.

PHOTO PLATE: IV

G- *Musa balbisiana*; **H**-*Oroxylum indicum*; **I**-*Paspalum fimbriatum*; **J**-*Phlogacanthus thyrsiformes*; **K**- *Rauvolfia tetraphylla*

Table 4.5. Medicinal plants, wet weight, dried weight and the crude extract obtained

Sl. no.	Name of the plant	Plant part used	Dried weight	Moisture content	Crude extract
1.	*Alstonia scholaris*	Bark	236.09±19.9	76.39±7.11	6.64±0.23
2.	*Rauvolfia tetraphylla*	Root	485.71±27.82	51.42±15.40	5.23±1.11
3.	*Hydrocotyle sibthorpioides*	Whole Plant	308.47±15.44	69.15±7.33	3.17±0.78
4.	*Oroxylum indicum*	Leaves	278.93±12.21	71.47±6.98	16.84±1.93
5.	*Phloganthus thyrsiformis*	Flower	570.00±32.97	43.00±13.29	6.29±1.06
6.	*Musa balbisiana*	Corm/rhizome	246.94±17.39	75.30±4.52	2.35±0.28
7.	*Clerodendrum infortunatum*	Leaves	343.67±18.34	65.00±13.94	5.86±1.12
8.	*Lindernia crustacea*	Whole plant	131.46±13.20	86.85±6.80	18.83±3.20
9.	*Andrographis paniculata*	Leaves	112.34±9.89	88.70±8.19	11.94±2.78
10.	*Paspalum fimbriatum*	Whole plant	408.94±35.78	59.10±4.53	1.49±0.88
11.	*Ficus racemosa*	Fruit	100.00±6.84	90.00±6.12	6.39±0.76

Dry weights were represented in g/kg wet weight; Crude extracts were represented as mg/100 g dry powder); Moisture content in %. Values are expressed as mean ± SD with three experimental replicates (n = 3)

Table 4.6. Qualitative phytochemical test of methanolic crude extracts of plants

Test	Methodology	Observation	Plants										
			AS	RT	HS	MB	PF	FR	PT	AP	OI	LS	CV
Protein	Ninhydrin test	*Violet color*	+	+	+	+	+	+	+	+	+	+	+
Carbohydrate	Fehling's test	*Red brick ppt.*	+	+	+	+	+	+	+	+	+	+	+
Phenol	Folin-phenol	*Blue-green color*	+	+	+	+	+	+	+	+	+	+	+
Flavonoid	Acids test	*Orange color*	+	+	+	+	+	+	+	+	+	+	+
Saponin	Vigorous shaking	*Constant foam*	-	+	+	+	+	+	-	+	+	+	+
Tannin	Ferric-chloride test	*Blue-black, green or blue green.*	-	-	+	+	+	+	+	+	+	+	-
Alkaloids	Mayer's test	*Yellow cream ppt.*	+	+	+	+	+	+	+	+	+	+	+
Glycosides	Salkowski test	*Upper layer blueish red to violet color lower layer yellow to green*	+	+	+	+	+	+	+	+	+	+	+

*AS - *Alstonia scholaris,* RT - *Rauvolfia tetraphylla,* HS - *Hydrocotyle sibthorpioides,* MB - *Musa balbisiana,* PF - *Paspalum fimbriatum,* FR - *Ficus racemosa,* PT - *Phlogacanthus thyrsiformis,* AP - *Andrographis paniculata,* OI - *Oroxylum indicum,* LS - *Lindernia crustacea,* CI – *Clerodendrum infortunatum.* '+' = presence and '-' = absence of the phytocompounds

OBJECTIVE 2: PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT STUDY OF THE PLANTS

4.4. Quantitative phytochemical study

Quantitative studies were conducted to estimate the crude protein content, carbohydrate content, total phenolics, and flavonoid contents of all 11 plants. For the analysis of protein, a standard curve was plotted against the standard chemical, BSA (Annexure I A). Spectrophotometric analysis revealed that the protein content of plants ranged from 30.45±7.68 to 401.82±11.68 µg protein/mg plant extract (Figure 4.5), with an average of 230.84±134.67 µg protein/mg plant extract. The highest protein content was found in *Alstonia scholaris* (bark), followed by leaves of *Clerodendrum infortunatum*. *Andrographis paniculata* exhibited the lowest protein content among the tested plants. Among the plant parts, bark showed the highest protein content (401.82±11.68 µg/mg extract), compared to other parts such as flowers (323.4±8.62 µg/mg extract), roots (306.45±7.91 µg/mg extract), leaves (262.57±149.86 µg/mg extract), fruit (159.51±2.84 µg/mg extract), whole plant (153.30±17.49 µg/mg extract), and corm (79.65±1.22 µg/mg extract) respectively (Figure 4.5). Statistical analysis among the 11 plants showed that almost all the plants differ significantly from each other in terms of protein content at $P \leq 0.05$. However, *Ficus racemosa*, *Hydrocotyle sibthorpioides*, and *Lindernia crustacea* had almost similar protein content without any significant difference in their values.

The crude extract of the 11 plants was analyzed for its carbohydrate content. Glucose was used as standard and standard curve was obtained (Annexure I B). The carbohydrate content of all the plant extract ranged from 10.76±0.53 to 384.29±14.05 µg glucose/mg plant extract. The average carbohydrate content of the plant extract is 133.15±101.56 µg glucose/mg plant extract. The highest carbohydrate content was found in *Alstonia scholaris* followed by *Phlogacanthus. thyrsiformis*. *Musa balbisiana* was reported to have the lowest carbohydrate content carbohydrate content of all the plants were represented in Figure 4.6. Statistical analysis showed that carbohydrate content of almost all the plants differ significantly among each other at $P \leq 0.05$. However, *Hydrocotyle sibthorpiodes*, *Andrographis paniculata* and *Lindernia crustacea* showed no statistical difference among themselves. Also, *Musa balbisiana* and

Paspalum fimbriatum showed no statistical difference at P≤0.05 while, differ significantly with the other plants.

For the analysis of phenolic content, a standard curve was prepared using gallic acid as the standard (Annexure I C). The phenol content of the tested plants ranged from 123.68±2.95 to 10.73±0.97 µgGAE/mg plant extract (Figure 4.7). The average phenolic content was 59.46±39 µgGAE/mg plant extract. The highest phenolic content was found in *Phlogacanthus thyrsiformis* with 123.68±2.95 µgGAE/mg plant extract followed by *Oroxylum indicum* (102.65±4.43 µgGAE/mg plant extract) and *Clerodendrum infortunatum* (98.69±3.15 µgGAE/mg plant extract). The lowest phenolic content was seen in *Andrographis paniculata*.

The standard curve of flavonoid content was drawn using quercetin as the standard (Annexure I D). The flavonoid content ranged from 45.85±1.26 to 4.72±0.33 µgQE/mg of plant extract (Figure 4.8). The average flavonoid content was 17.59±11.84 µgQE/ mg plant extract. The highest flavonoid content was found in *Phlogacanthus thyrsiformis* with 45.85±1.26 µgQE/mg of plant extract. At P≤0.05 level, the mean of protein and phenol, protein and flavonoids, carbohydrate and flavonoids are significant. In contrast, protein and carbohydrate, carbohydrate and phenol, and phenol and flavonoids significantly differ.

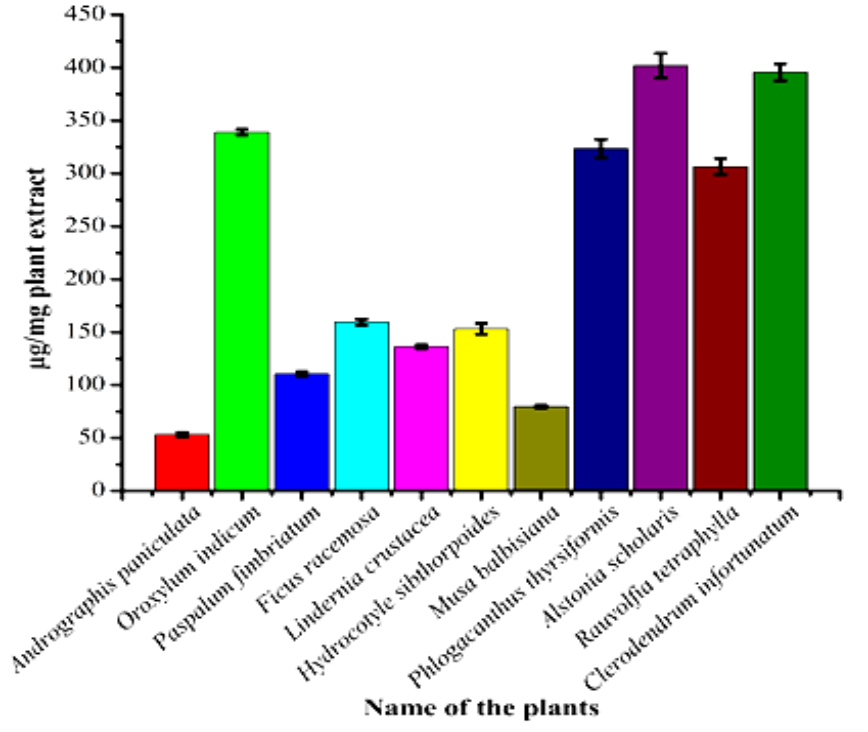

Figure 4.5. Crude protein content of plant extracts. Values are represented as mean ± SD, n = 3 (number of experiments)

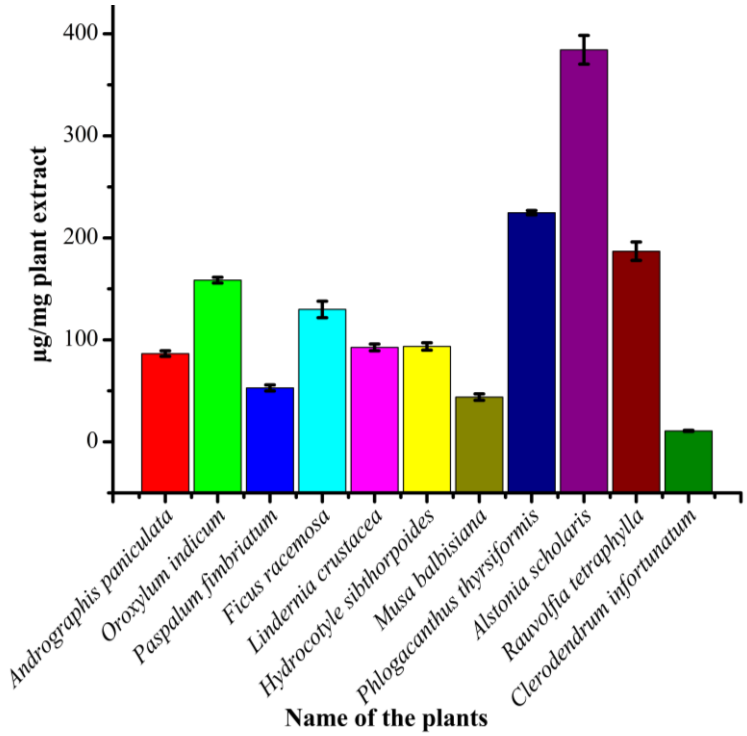

Figure 4.6. Carbohydrate content of plant extracts. Values are represented as mean ± SD, n = 3 (number of experiments)

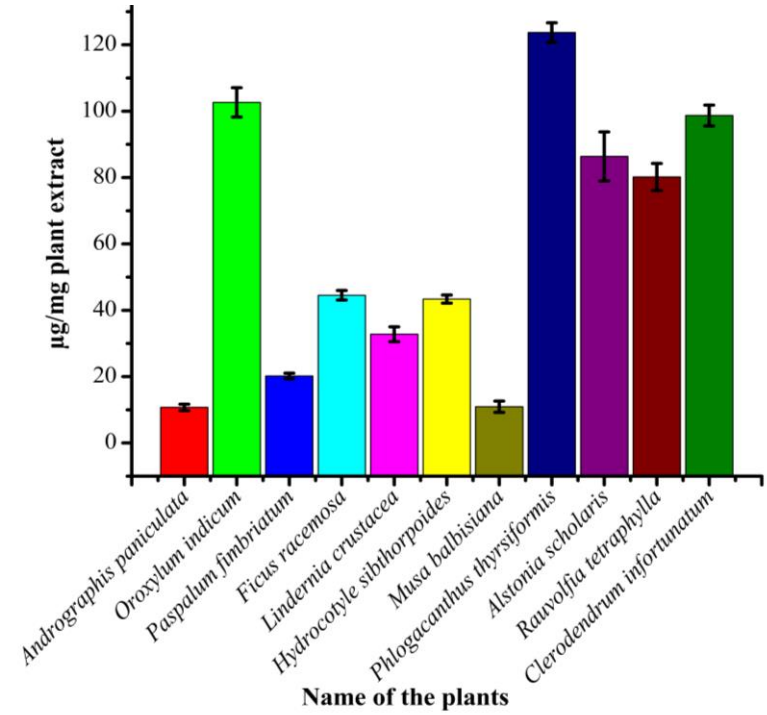

Figure 4.7. Total phenolic content of plant extracts. Values are represented as mean ± SD, n = 3 (number of experiments)

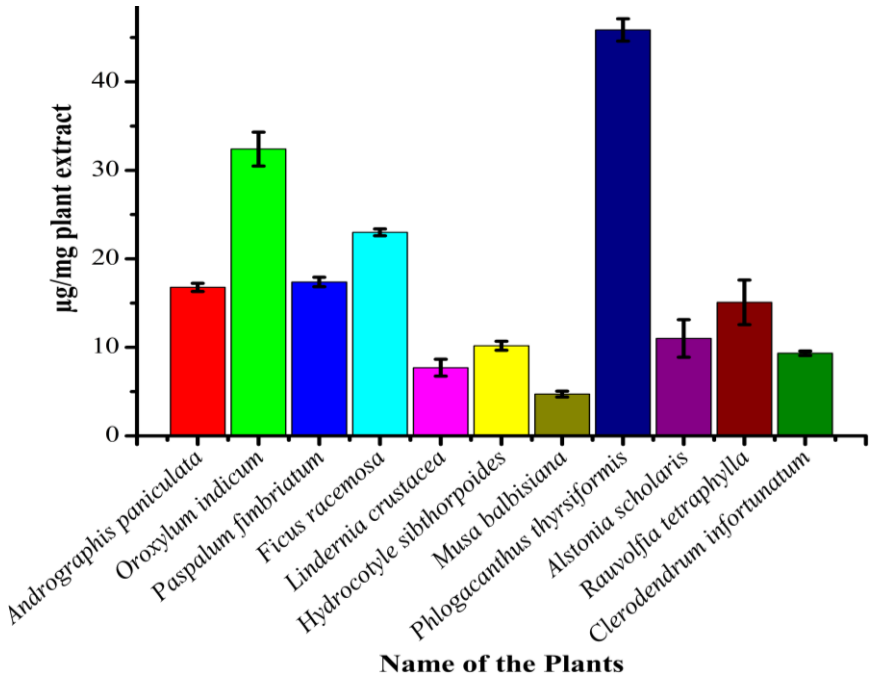

Figure 4.8. Total flavonoid content of plant extracts. Values are represented as mean ± SD, n = 3 (number of experiments)

4.5. Antioxidant Study

Antioxidant assays were conducted to understand the free radical scavenging properties of the plants. The methanolic crude extract of all the 11 plants was tested for its antioxidant property. Five different antioxidant tests were conducted in the present study: total antioxidant capacity (TAC) (phosphomolybdate assay), ferric reducing power (FRAP), DPPH, ABTS, and TBARS assays. The standard curves for the estimation of TAC and FRAPS assays are given in Annexure I (E & F). The TAC content ranged from 11.89±0.22 µg to 118.7±3.5 µg AAE/mg extract. A standard curve was drawn against ascorbic acid (Annexure I E). The highest TAC was seen in *Phlogacanthus thyrsiformis*, while the lowest was seen in *Andrographis paniculata* (Figure 4.9). At P≤0.05, *Lindernia crustacea* and *Alstonia scholaris, Alstonia scholaris,* and *Oroxylum indicum, Andrographis paniculata* and *Paspapalum fimbriatum, Rauvolfia tetraphylla,* and *Ficus racemosa, Rauvolfia tetraphylla* and *Oroxylum indicum, Musa balbisiana*, and *Ficus racemosa* do not differ significantly.

For FRAP assay, a standard curve against $FeSo_4$ was plotted (Annexure I F). The highest FRAP was observed in *Oroxylum indicum* with 745. 15±10.03 µg FE/mg plant extract followed by *Ficus racemosa* (432.02±6.26 µg FE/mg plant extract) and the lowest activity was observed in *Andrographis paniculata* (44.42±3.51 µg FE/mg plant extract). The FRAP reducing potential of all the plants is shown in the figure 4.10. At P≤0.05, All the mean of all the plants were significant except, *Lindernia crustacea* and *Hydrocotyle sibthorpioides, Clerodendrum infortunatum* and *Alstonia scholaris* and *Rauvolfia tetraphylla* and *Phlogacanthus thyrsiformis*.

All the plants showed a concentration-dependent antioxidant activity of the plants. IC_{50} values of antioxidant tests were evaluated. For DPPH the IC_{50} values of the tested plants ranged from 23.34 to 340.33 µg/mL. The highest DPPH scavenging property was seen in *Phlogacanthus thyrsiformis*, slightly lower than the standard chemical ascorbic acid 3.44±0.20 µg/mL. The percentage inhibition of all the plants and the standard chemical is shown in Figure 4.11. At P≤0.05 level, *Alstonia scholaris, Rauvolfia tetraphylla, Lindernia crustacea,* and *Hydrocotyle sibthorpioides* does not differ. Also, *Musa balbisiana, Rauvolfia tetraphylla* and *Lindernia crustacea* shows no statistical difference. Along with that, *Ficus racemosa* does not differ with *Alstonia scholaris, Hydrocotyle sibthorpioides, Phlogacanthus thyrsiformis, Oroxylum indicum,*

and *Clerodendrum infortunatum*. *Oroxylum indicum* also showed no statistical difference with *Hydrocotyle sibthorpioides, Phlogacanthus thyrsiformis,* and *Clerodendrum infortunatum.* Likewise, *Clerodendrum infortunatum* shows no difference with *Hydrocotyle sibthorpioides* and *Phlogacanthus thyrsiformis*.

For the ABTS assay, the IC_{50} values of the plant extract range from 7.62 to 490.03 μg/mL (Figure 4.12). *Oroxylum indicum* exhibits strong antioxidant activity among all the tested plants. Gallic acid shows an IC_{50} value of 1.76±0.05 μg/mL. The percentage inhibition for the ABTS assay for all the plants and the standard chemical is shown in Figure 4.12. At $P \leq 0.05$, *Phlogacanthus thyrsiformis* and *Oroxylum indicum* do not differ from the standard chemical gallic acid. Few plant extracts such as *Hydrocotyle sibthorpioides, Clerodendrum infortunatum,* and *Ficus racemosa* do not differ from each other. Likewise, *Lindernia crustacea*, *H. sibthorpioides,* and *C. infortunatum* do not differ. Additionally, the IC_{50} of *Musa balbisiana* and *Alstonia scholaris,* and *Musa balbisiana* and *Ficus racemosa*, do not differ. Furthermore, no difference was observed in *Oroxylum indicum* and *Phlogacanthus thyrsiformis, Ficus racemosa*. The IC_{50} value of *Paspalum fimbriatum* and *Rauvolfia tetraphylla*, and *Clerodendrum infortunatum* and *P. thyrsiformis* do not differ.

The IC_{50} value range for TBARS assay is 27.28 to 209.33±15.98 μg/mL. The highest lipid scavenging activity was seen in the crude extract of *Oroxylum indicum* even better than the standard chemical ascorbic acid which has an IC_{50} value of 37.1±0.13 μg/mL. The percentage inhibition of all the plants and the standard chemical is shown in Figure 4.13. At $P \leq 0.05$ level, *Musa balbisiana* and *Clerodendrum infortunatum*, *Hydrocotyle sibthorpioides* and *Phlogacanthus thyrsiformis*, *Paspalum fimbriatum* and *Musa balbisiana* does not differ. Likewise, no difference was observed among the IC_{50}s of *Alstonia scholaris*, *Andrographis paniculata* and *Phlogacanthus thyrsiformis*. Moreover, *Clerodendrum infortunatum* and *Oroxylum indicum* does not differ from the standard chemical ascorbic acid.

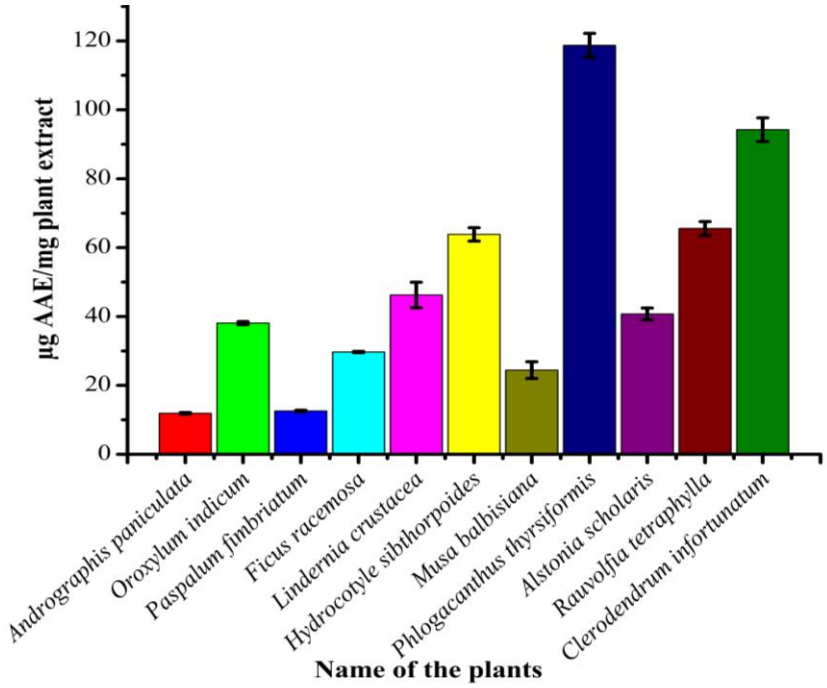

Figure 4.9. Total antioxidant activity of eleven plants. Values are represented as mean ± standard deviation, n = 3 (number of experiments)

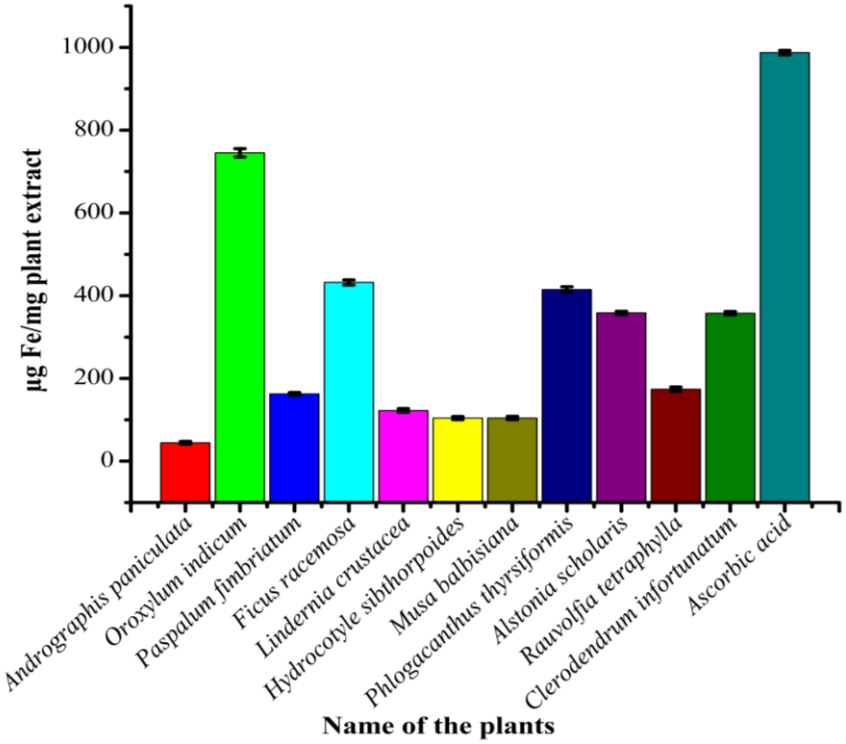

Figure 4.10. FRAP activity of eleven plants and reference chemical, ascorbic acid. Values are represented as mean ± standard deviation, n = 3 (number of experiments)

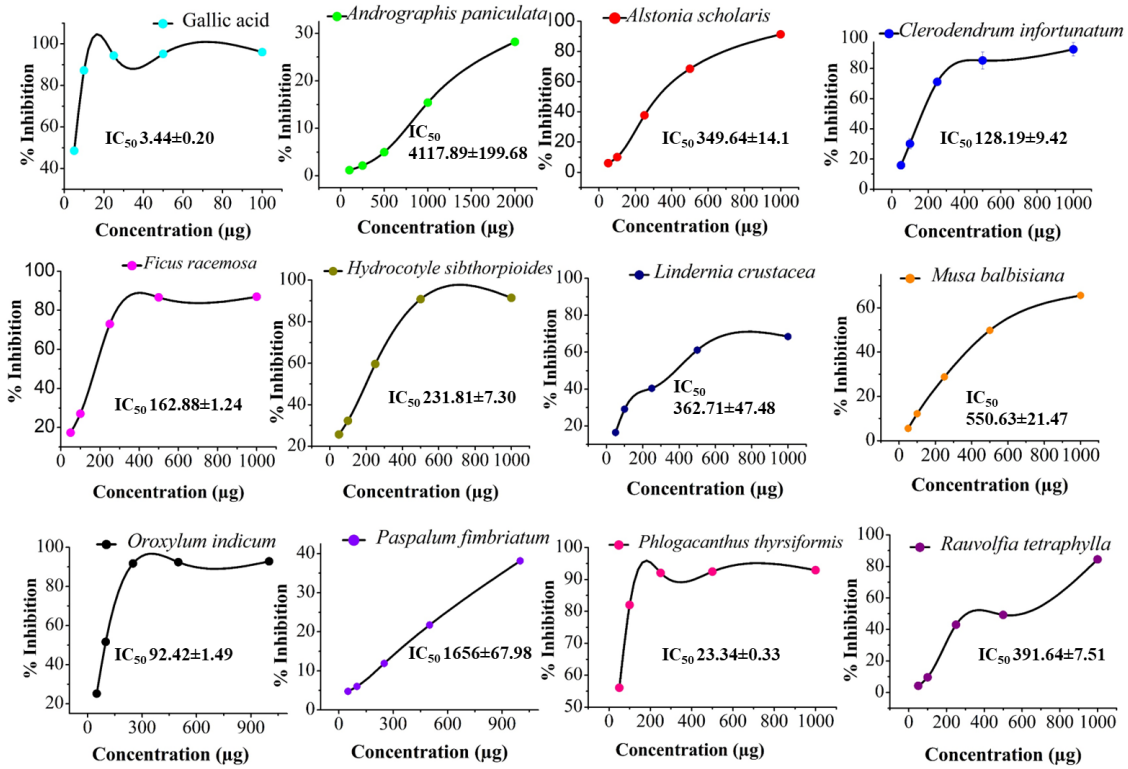

Figure 4.11. Dose-dependent percentage inhibition of DPPH free radical scavenging activity of plants Values are represented as mean ± standard deviation, n = 3 (number of experiments)

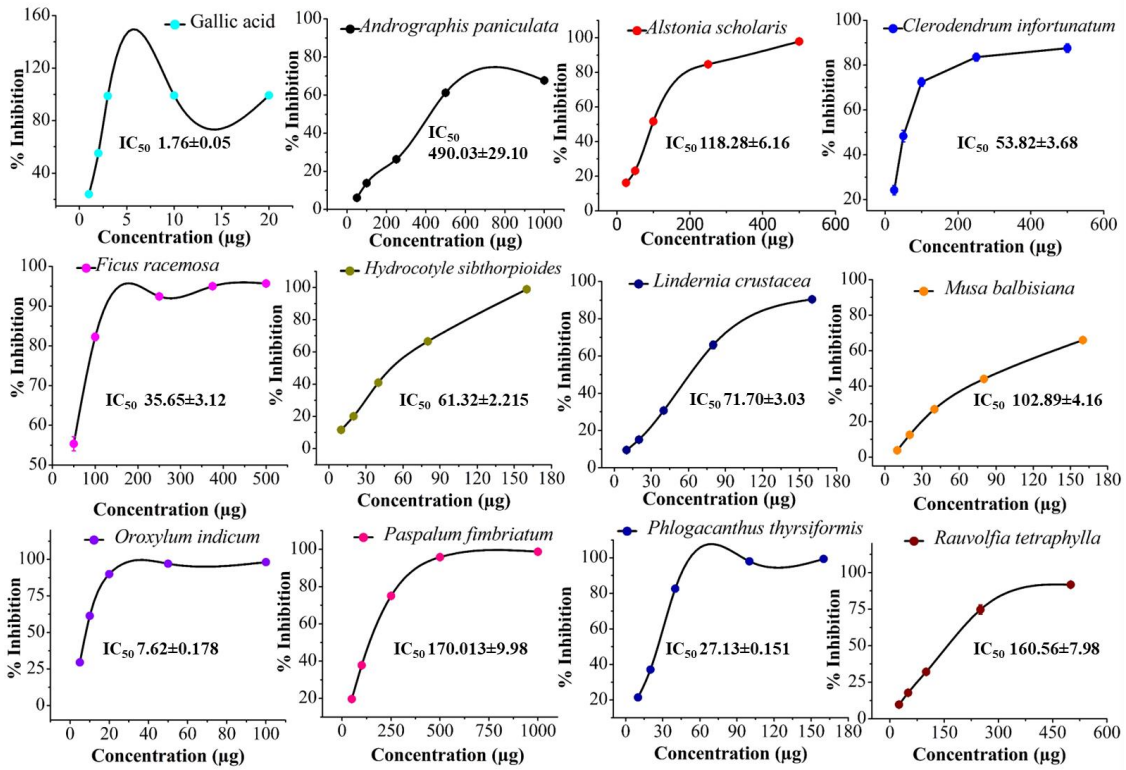

Figure 4.12. Dose-dependent percentage inhibition of ABTS free radical scavenging activity of plants. Values are represented as mean ± standard deviation, n = 3 (number of experiments)

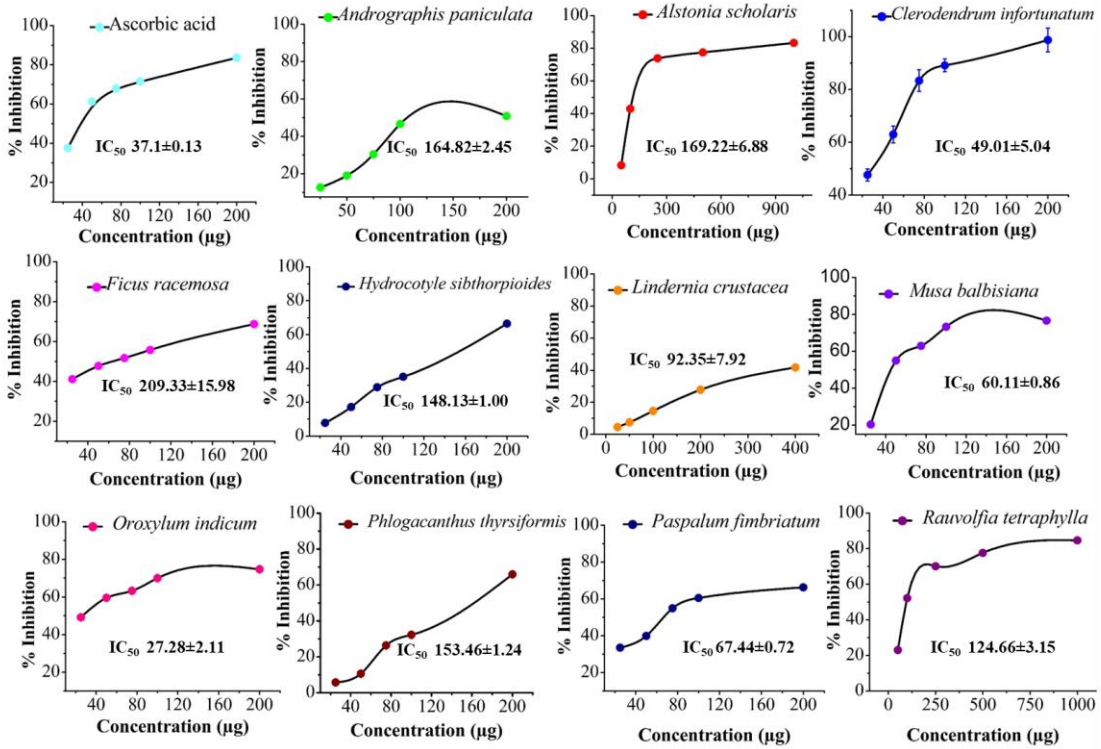

Figure 4.13. Percentage inhibition of lipid peroxide scavenging activity Values is represented as mean ± standard deviation, n = 3 (number of experiments).

4.6. Elemental Analysis

Heavy metal refers to any metallic chemical element with high density and is toxic or poisonous at low concentrations. Exposures to these metals at higher levels at shorter periods can lead to various health-related problems, including lung diseases, increased heart rate, diarrhea etc. A total of 11 medicinal plants were studied for seven toxic elements (Table 4.7) and estimated their heavy metal content. The toxic levels were determined by Atomic Absorption Spectroscopy, and the values were represented as parts per million.

The present study did not observe Cd in all the plants. Similarly, Cr, Ni, and Pb were also found to be absent in many plants. Cr is not detected in *Musa balbisiana, Clerodendrum infortunatum, Lindernia crustacea, Alstonia scholaris,* and *Paspalum fimbriatum,* while other plants extract showed the least content. *Ficus racemosa* showed the highest Cr content. Pb was not detected in *Phlogacanthus thyrsiformis, Musa balbisiana,* and *Ficus racemosa.* In others, it has been found in very minute amounts (Table 4.4). Ni is not detected in *Oroxylum indicum* and *Clerodendrum infortunatum,* whereas for others, it ranges from 0.03 ppm to 0.1974 ppm. Mn concentration ranges from 0.0095 ppm to 0.085 ppm. Zn is an essential heavy metal required for normal physiological functions of the body. Zn was found in all the tested plants ranging from 0.25 ppm to 1.18 ppm. Cu was also reported in all the tested plants ranging from 0.0073 ppm to 0.054 ppm in concentration.

Table 4.7. Trace element concentration of medicinal plants (in ppm)

Sample name	Cd	Mn	Cr	Zn	Pb	Ni	Cu
Phlogacanthus thyrsiformis	Nd	0.01	0.0262	0.323	nd	0.042	0.0161
Musa balbisiana	Nd	0.0121	Nd	0.299	nd	0.03	0.0124
Oroxylum indicum	Nd	0.0095	0.0324	0.31	0.068	nd	0.054
Hydrocotyle sibthorpoides	Nd	0.040	0.0459	0.971	0.0228	0.047	0.0299
Clerodendrum infortunatum	Nd	0.058	Nd	0.3069	0.1139	nd	0.0613
Ficus racemosa	Nd	0.023	0.1606	0.3111	nd	0.1974	0.0336
Andrographis paniculata	Nd	0.011	0.0852	0.523	0.1595	0.0729	0.0088
Lindernia crustacea	Nd	0.085	Nd	1.1837	0.0911	0.1116	0.0212
Alstonia scholaris	Nd	0.009	Nd	0.1102	0.1823	0.1373	0.0073
Paspalum fimbriatum	Nd	0.0368	Nd	0.3941	0.0911	0.0686	0.0095
Rauvolfia tetraphylla	Nd	0.0137	0.111	0.2567	0.1139	0.1373	0.0212

nd - Not detected, values are expressed in parts par millions, Cd- Cadmium; Mn- Manganese; Cr- Chromium; Zn- Zinc; Pb- Lead; Ni- Nickel; Cu- Copper

4.7. GC-MS Analysis

A total of 11 medicinal plants were studied for its compound study. Those compounds that has an Reverse Search Index (RSI) value of 850 or greater in a value of 1000 are identified and listed for the plant sample. The RSI factors near or below 800 are not identified. The possible compounds of the plants were listed below (Table 4.8 - 4.18). The chromatogram and spectrum of the plants are given in the figures (Figure 4.14-4.24). The presence of eight phytocompounds in methanolic extract of *Andrographis paniculata* leaf extract was analysed by GC-MS system. The GC-MS chromatogram of the compounds identified from the *A. paniculata* is shown in Figure 4.14. Peak structure with retention time (RT) 22. 620 showed the highest percentage area, followed by RT-23.78, compared to other structures. The names of the probable phytocompounds with the RT and m/z data have been provided in Table 4.8.

GC-MS analysis of *Alstonia scholaris* showed the presence of six possible compounds. Peak structure with retention time (RT) 15.975 showed the highest percentage height. 4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene) tyramine being the major compound (Table 4.9). Likewise, GC-MS analysis of *Clerodendrum infortunatum* showed three compounds. RT 7.65 showed the highest percentage of height, making Picolinyl-14-octadecenoate the major compound (Table 4.10). GC-MS analysis of *Hydrocotyle sibthorpioides* showed three compounds. RT 10.482 showed the highest percentage of height making Cyclopropanecarboxylic acid, 2,2-dichloro-1-methyl, the major compound (Table 4.11).

GC-MS analysis of *Ficus racemosa* showed the presence of six possible compounds. Peak structure with retention time (RT) 3.980 showed the highest percentage height Silane, dimethyl-(2,3,5,6-tetrachlorophenoxy) octadecyloxy- being the major compounds (Table 4.12). A total of 10 possible compounds were identified from *Lindernea crustacea*. Peak structure with retention time (RT) 19.501 showed the highest percentage height. Table 4.13 shows the identified compounds from *L. crustacea.*

GC-MS analysis of *Musa balbisiana* showed the presence of five possible compounds. Peak structure with retention time (RT) 5.785 showed the highest percentage height. Table 4.14 shows the chromatogram of the GC-MS analysis. GC-MS analysis of *Oroxylum indicum* showed the presence of seven possible compounds. Peak

structure with retention time (RT) 12.28 showed the highest percentage height (Table 4.15). Four possible compounds were detected from *Paspalum fimbriatum*, six from *Phlogacanthus thyrsiformis* and four from *Rauvolfia tetraphylla* with peak structure of 22.451(RT), 4.169(RT) and 19.000 (RT) being the highest percentage of height, respectively (Table 4.16 to 4.18).

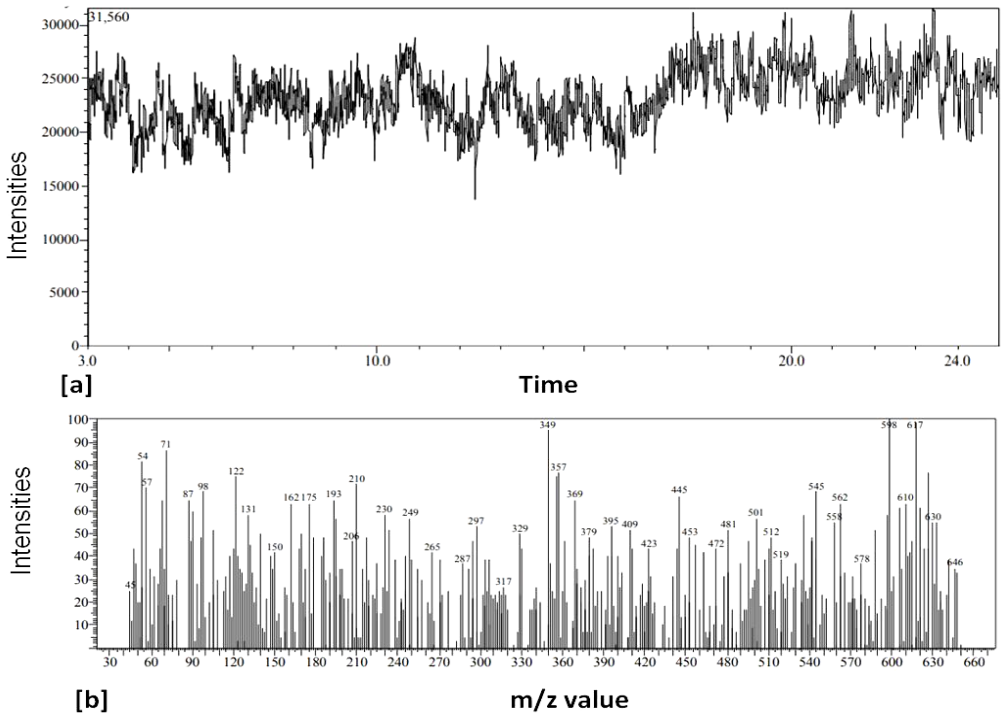

Figure 4.14. GC-MS chromatogram of *Andrographis paniculata* methanolic extract. (a) Chromatogram showing the retention time in min and (b) Chromatograms showing the m/z value of the phytocompounds

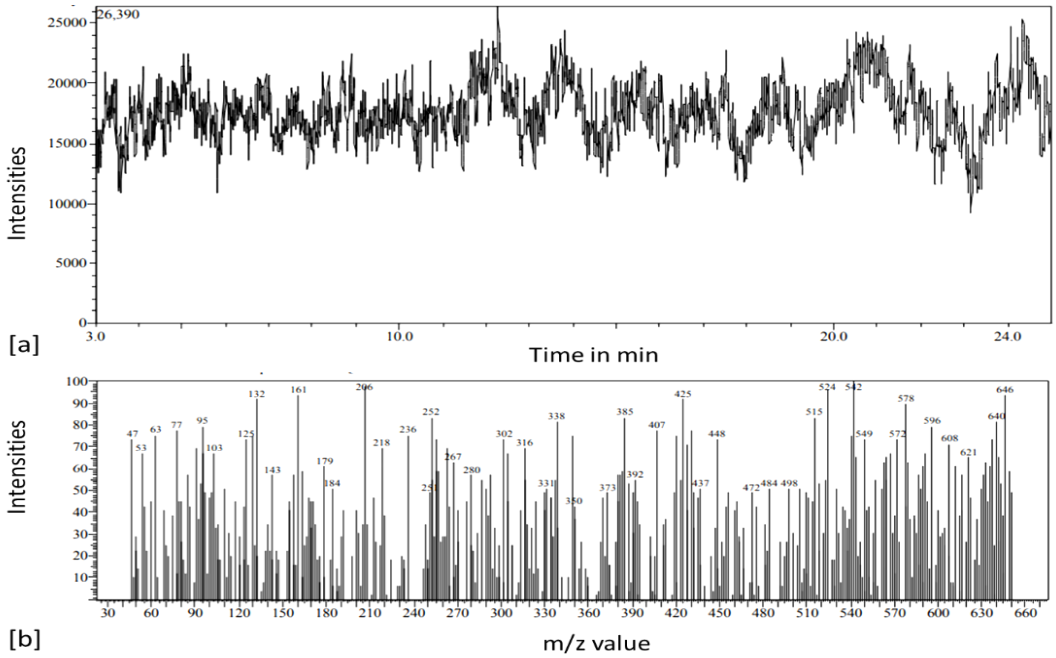

Figure 4.15. GC-MS chromatogram of *Alstonia scholaris* methanolic extract. Chromatogram showing the retention time in min and (b) Chromatograms showing the m/z value of the phytocompounds

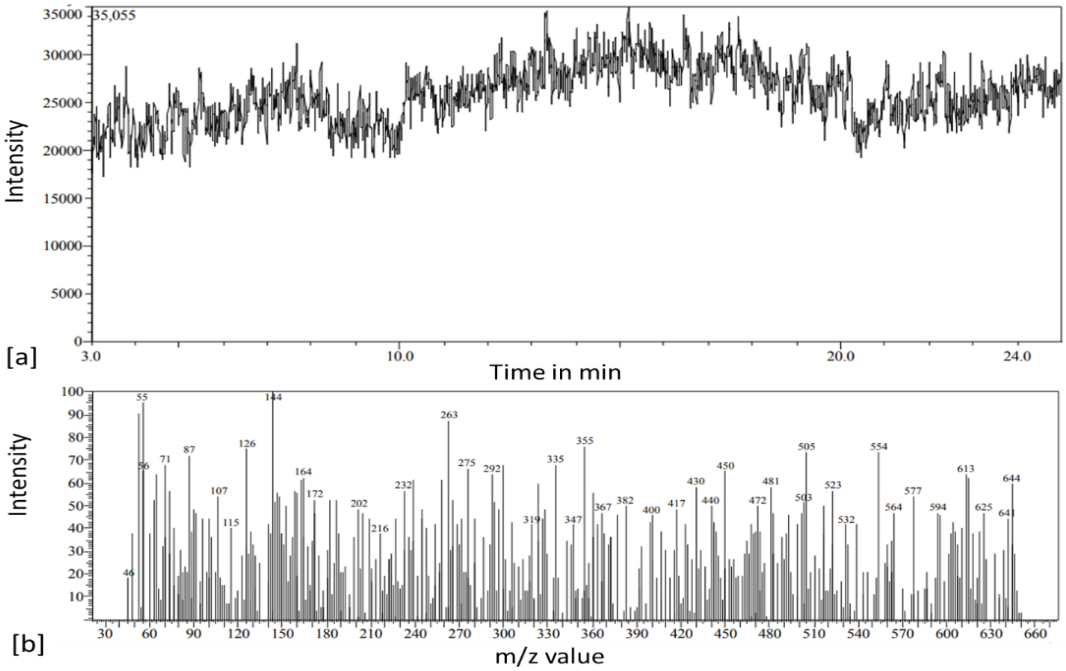

Figure 4.16. GC-MS chromatogram of *Clerodendrum infortunatum* methanolic extract. Chromatogram showing the retention time in min and (b) Chromatograms showing the m/z value of the phytocompounds

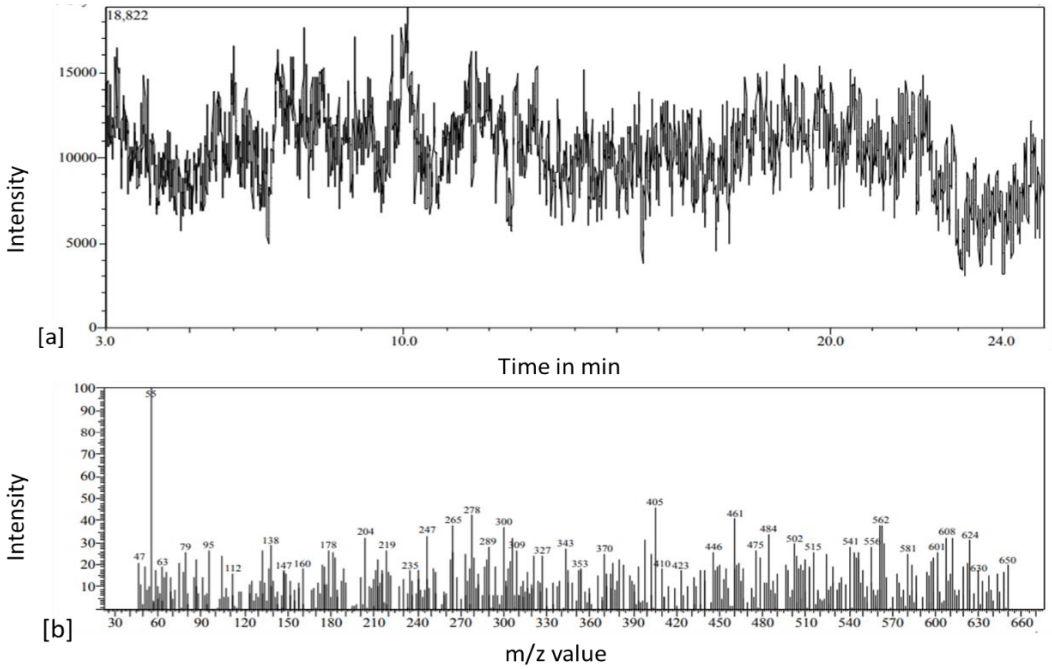

Figure 4.17. GC-MS chromatogram of methanolic extract of *Hydrocotyle sibthorpioides.* Chromatogram showing the retention time in min and (b) Chromatograms showing the m/z value of the phytocompounds

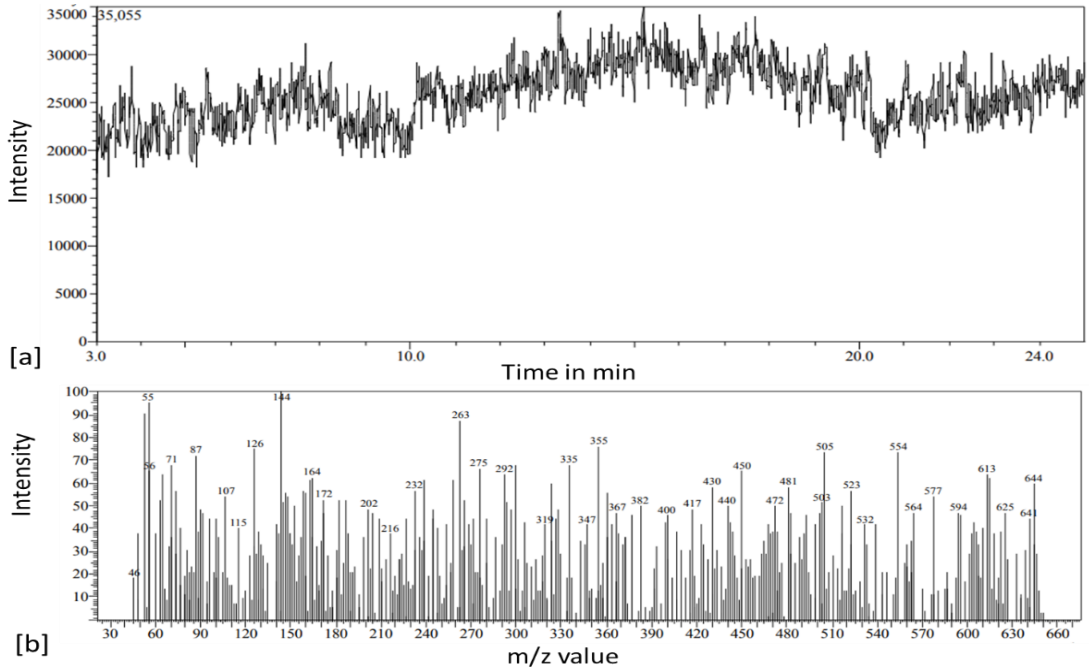

Figure 4.18. GC-MS chromatogram of methanolic extract of *Ficus racemosa.* Chromatogram showing the retention time in min and (b) Chromatograms showing the m/z value of the phytocompounds

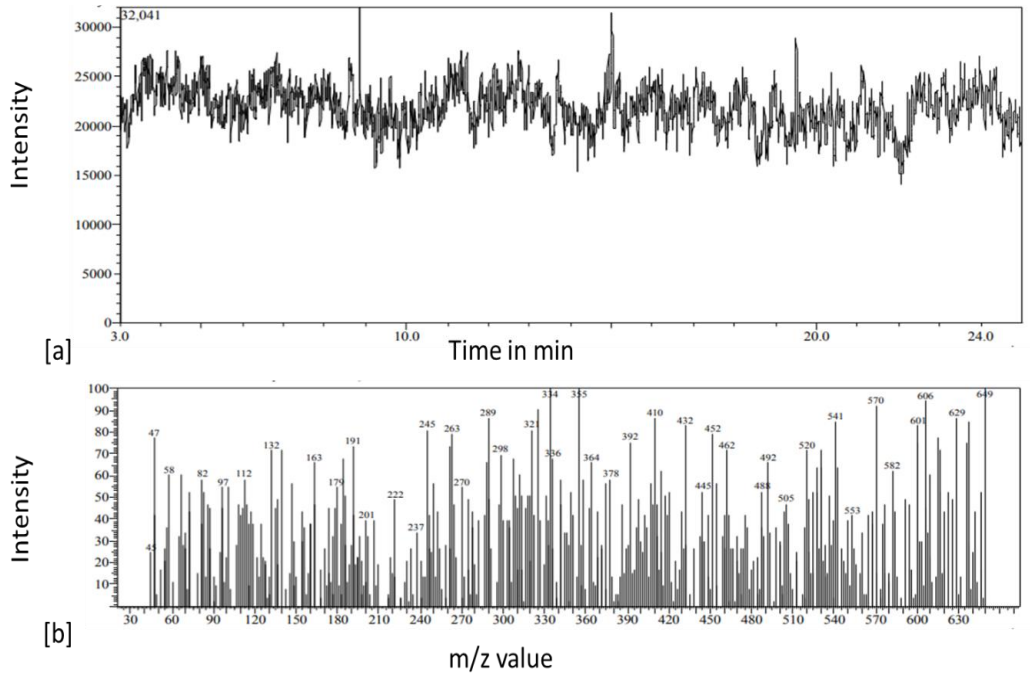

Figure 4.19. GC-MS chromatogram of methanolic extract of *Lindernia crustacea.* Chromatogram showing the retention time in min and (b) Chromatograms showing the m/z value of the phytocompounds

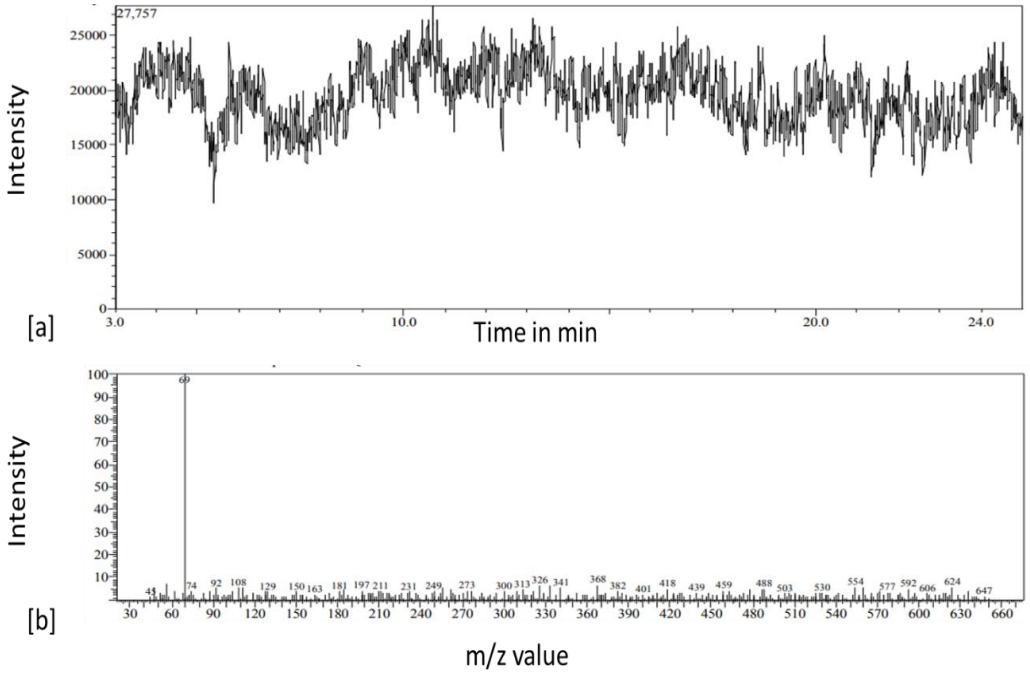

Figure 4.20. GC-MS chromatogram of methanolic extract of *Musa balbisiana.* Chromatogram showing the retention time in min and (b) Chromatograms showing the m/z value of the phytocompounds

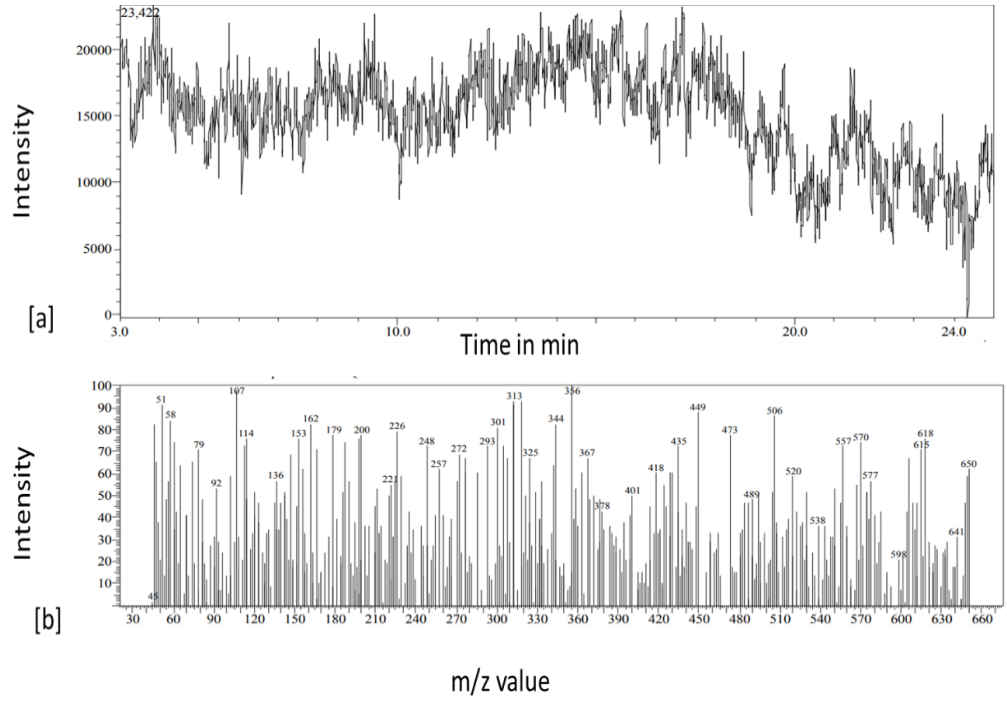

Figure 4.21. GC-MS chromatogram of methanolic extract of *Oroxylum indicum.* Chromatogram showing the retention time in min and (b) Chromatograms showing the m/z value of the phytocompounds

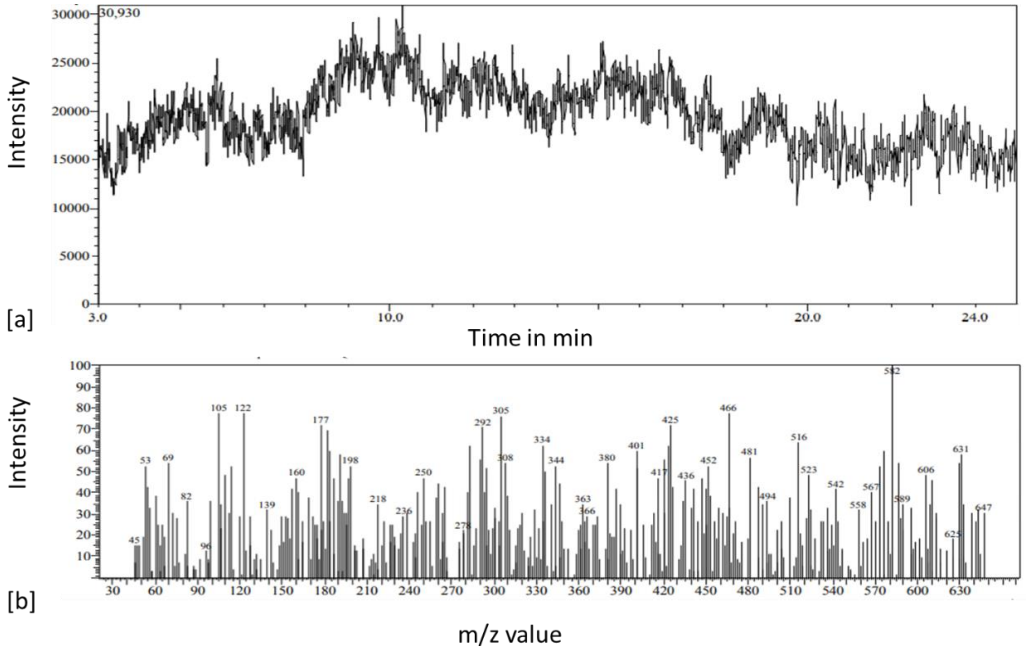

Figure 4.22. GC-MS chromatogram of methanolic extract of *Paspalum fimbriatum.* Chromatogram showing the retention time in min and (b) Chromatograms showing the m/z value of the phytocompounds

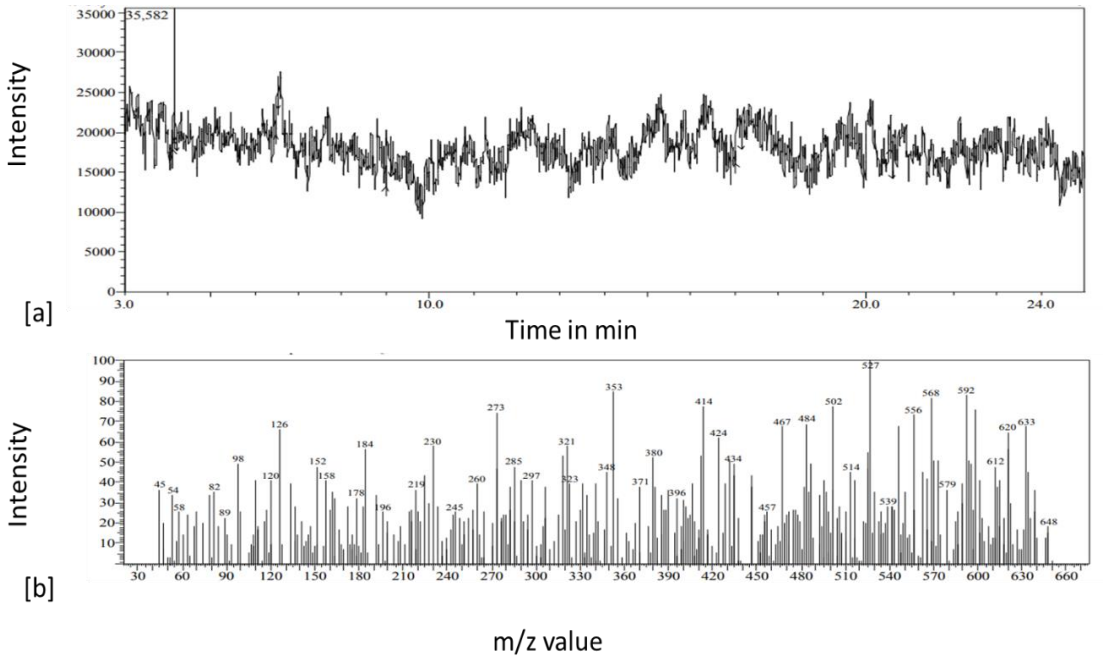

Figure 4.23. GC-MS chromatogram of *Phlogacanthus thyrsiformis.* Chromatogram showing the retention time in min and (b) Chromatograms showing the m/z value of the phytocompounds

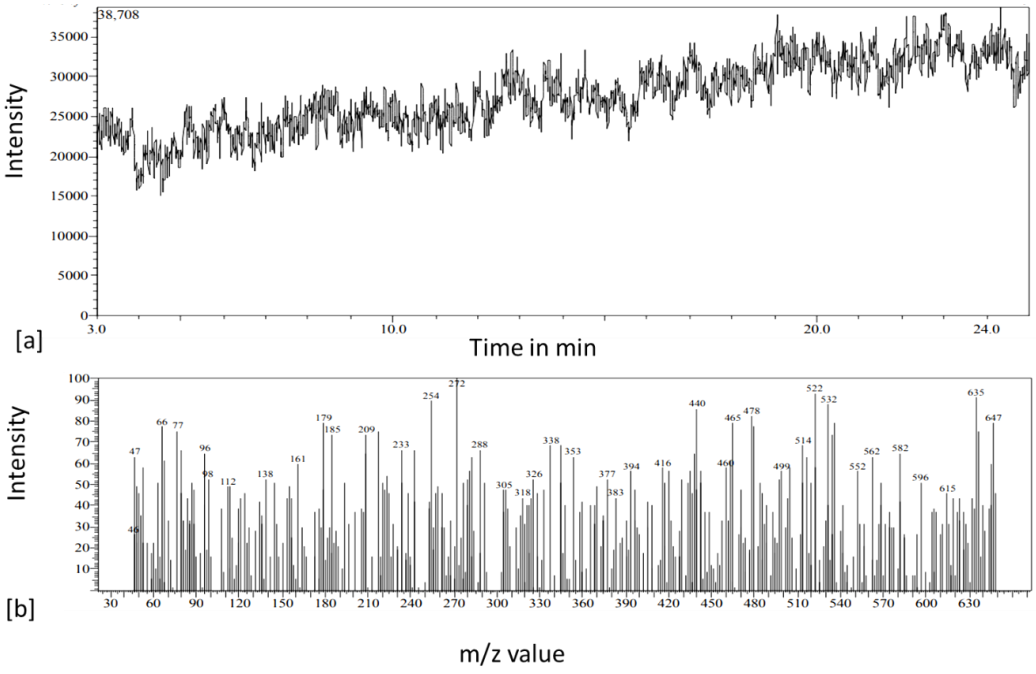

Figure 4.24. GC-MS chromatogram of methanolic extract of *Ravoulfia tetraphylla.* Chromatogram showing the retention time in min and (b) Chromatograms showing the m/z value of the phytocompound

Table 4.8. GC-MS profiles of the compounds identified from *Andrographis paniculata*

Sl. no.	Name of the compound	RT	MW	Area	Height	m/z	MF
1.	Dimethyl 2-octylundecane-1,11-dioate	6.450	356.5	9.06	10.97	598.00	$C_{21}H_{40}$
2.	Benzene, hexakis(trifluoromethyl)-	7.774	486.09	2.91	9.95	483.00	$C_{12}F_{18}$
3.	2,7-Dibromo-9-formylacridine	19.830	365.02	13.19	15.74	285.00	$C_{14}H_7Br$
4.	Sarpagan-16-carboxylic acid, 17-(acetyloxy)-1-methyl-, methyl ester, (16.xi.)-	21.509	408.49	6.51	10.82	263.00	$C_{24}H_{28}$
5.	4,5,6,7-Tetraacetoxydecyl isothiocyanate	22.277	431.50	16.28	13.06	208.00	$C_{19}H_{29}$
6.	(5S,8R,8aS)-5-Allyl-8-butyloctahydroindolizine	22.620	221.38	25.41	14.97	179.65	$C_{15}H_{27}$
7.	3,4-Diacetoxy-9,12-dimethoxy-5,10-dimethyldibenzo[c,kl]xanthene	23.196	472.48	8.86	12.69	596.00	$C_{28}H_{24}$
8.	Propenoic acid, 2-cyano-3-(2-thiazolylamino)-, ethyl ester	23.785	223.25	17.78	11.80	549.00	$C_9H_9N_3$

MW – molecular weight (g/mol); MF – molecular formula; RT- Retention time in seconds; Height in percentage (%)

Table 4.9. GC-MS profiles of the compounds identified from *Alstonia scholaris*

Sl. no.	Name of the compound	RT	MW	Area	Height	m/z	MF
1.	N-(2,4-Dinitrophenyl) morpholine	4.091	253.21	10.15	16.92	542	$C_{10}H_{11}N_3O_5$
2.	Acetamide, 2-(4-morpholylcarbonylmethyl) thio-N-phenyl-	9.725	294.37	16.71	15.55	149	$C_{14}H_{18}N_2O_3S$
3.	1,4-Naphthalenedione, 3-hydroxy-6-methyl-2-(1-methylethyl)-5-(4-methyl-3-oxopentyl)-	13.085	328.402	8.39	17.24	342	$C_{20}H_{24}O_4$
4.	Androstan-3-ol, (3.beta.,5.alpha.)-	13.824	276.5	10.57	16.78	482	$C_{19}H_{32}O$
5.	4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene) tyramine	15.975	298.29	30.23	20.32	207	$C_{16}H_{14}N_2O_4$
6.	Conocarpan	20.315	266.3	23.96	13.20	120	$C_{18}H_{18}O_2$

MW – molecular weight (g/mol); MF – molecular formula; RT- Retention time in seconds; Height in percentage (%)

Table 4.10. GC-MS profiles of the compounds identified from *Clerodendrum infortunatum*

Name of the compound	RT	MW	Area	Height	m/z	MF
Carbonic acid, 2-chloroethyl 2-pentyl ester	3.409	194.656	47.13	33.07	144	$C_8H_{15}ClO_3$
Picolinyl 14-octadecenoate	7.655	373.6	43.10	34.61	310	$C_{24}H_{39}NO_2$
1,1'-Biphenyl, 2,2',3,3',4,4',5,6,6'-nonachloro-	22.946	464.2	9.77	32.32	194	$C_{12}HCl_9$

MW – molecular formula; MF – molecular formula; RT- Retiontion time in seconds; Height in percentage.

Table 4.11. GC-MS properties of compounds identified from *Hydrocotyle sibthorpioides*

Sl. no.	Name of the compounds	RT	M/Z	Area	Height	MW	MF
1.	8-Methoxyoctanoic acid, methyl ester	3.199	475	31.92	28.26	188.26	$C_{10}H_{20}O_3$
2.	17.alpha.-Ethynyl-5(10)-estrene-3.alpha.,17.beta.-diol-	3.565	249	30.44	33.54	444.8	$C_{26}H_{44}O_2Si_2$
3.	Cyclopropanecarboxylic acid, 2,2-dichloro-1-methyl-, m	10.482	314	37.65	38.20	169	$C_5H_6Cl_2O_2$

Table 4.12. GC-MS profiles of the compounds identified from *Ficus racemosa*

Sl. no.	Name of the compounds	RT	M/Z	Area	Height	MW	MF
1.	Silane, dimethyl(2,3,5,6-tetrachlorophenoxy) octadecyloxy-	3.980	55	12.67	18.41	558.5	$C_{26}H_{44}Cl_4O_2Si$
2.	Indole-3-carboxylic acid, 1-(2-acetylaminoethyl)-6-bromo-5-methoxy-2-methyl-, ethyl ester	6.275	123	10.66	15.58	397.3	$C_{17}H_{21}BrN_2O_4$
3.	1H-4-Oxabenzo(f)cyclobut(cd)inden-8-ol, 1a-.alpha.,2,3,3a,8b-alpha,8c-alpha-hexahydro-1,1,3a-trimethyl-6-pentyl-	15.485	230	11.68	15.60	314.5	$C_{21}H_{30}O_2$
4.	Meclofenamic acid di-methyl derivative	20.310	317	29.76	14.59	324.2	$C_{16}H_{15}Cl_2NO_2$
5.	2-(3,3-Diethoxypropyl)-6-methoxypyridine	21.580	194	23.62	18.13	239.31	$C_{13}H_{21}NO_3$
6.	beta.-Alanine, N-cyclohexylcarbonyl	22.065	521.00	11.60	17.70	199.25	$C_{10}H_{17}NO_3$

MW – molecular weight (g/mol); MF – molecular formula; RT- Retention time in seconds; Height in percentage (%)

Table 4.13. GC-MS profiles of the compounds identified from *Lindernia crustacea*

Sl. No.	Name of the compounds	RT	MW	Area	Height	m/z	MF
1.	1-(4-Hydroxybenzoyl)-6,7-dimethoxyisoquinoline	8.868	309.31	6.44	14.97	638	$C_{18}H_{15}$
2.	Molybdenum, [(1,2,3,4, 5-.eta.)-1-(1,1-dimethylethyl)-2,4-cyclopentadien-1-yl]bis(.eta.3-2-propenyl)-	9.295	309.32	6.35	7.06	264	$C_{18}H_{15}NO_4$
3.	1-Propylpentachlorotriphosphazene	12.020	299.30	9.22	6.22	453	$C_{15}H_{23}Mo$
4.	Terephthalic acid, 2,2-dichloroethyl undecyl ester	14.820	355.3	6.96	8.78	427	$C_3H_7Cl_5N_3P_3$
5.	N-Ethyl-N'-isopropyl-6-phenoxy-[1,3,5]triazine-2,4-diamine	14.925	417.4	2.91	5.79	50	$C_{21}H_{30}Cl_2O_4$
6.	Manganese, pentacarbonyl(2,3,3,4,4,5,5,6,6-nonafluoro-1-cyclohexen-1-yl)-	15.007	273.33	6.35	9.42	94	$C_{14}H_{19}N_5O$
7.	Thiophene, 3-methyl-5-octadecyl-2-pentadecyl-	16.220	438.04	15.15	7.97	560	$C_{11}F_9MnO_5$
8.	Bis(t-butyldimethylsilyl) selenite	16.615	561	16.16	6.52	208	$C_{38}H_{72}S$
9.	Phenol, pentabromo-	19.501	357.5	10.89	16.03	592	$C_{12}H_{30}O_3SeSi_2$
10.	6-Methoxy-9H-purine tbdms	20.359	488.59	8.16	9.63	208	C_6Br_5OH

MW – molecular weight (g/mol); MF – molecular formula; RT- Retention time in seconds; Area & Height in percentage (%)

Table 4.14. GC-MS profiles of the compounds identified from *Musa balbisiana*

Sl. No.	Name of the compound	RT	m/z	Area	Height	MW	MF
1.	Difluoroisocyanatophosphine	5.78	69.25	8.77	24.94	110.987	CF_2NOP
2.	2'-Methoxy-2,3',4,4'-tetrabromodiphenyl ether	9.79	569.00	10.42	20.49	515.8	$C_{13}H_8Br_4O_2$
3.	Isophthalic acid, ethyl 6-ethyloct-3-yl ester	15.10	177.00	30.20	21.14	334.4	$C_{20}H_{30}O_4$
4.	Phthalic acid, 2-(4-chlorophenoxy)ethyl hexyl ester	20.46	193.00	29.98	16.67	404.9	$C_{22}H_{25}ClO_5$
5.	Pseudodiosgenin diacetate	23.58	81.00	20.63	16.76	498.7	$C_{31}H_{46}O_5$

MW – molecular weight (g/mol); MF – molecular formula; RT- Retention time in seconds; Area & Height in percentage (%)

Table 4.15. GC-MS profiles of the compounds identified from *Oroxylum indicum*

Sl. No.	Name of the compound	RT	MW	Area	Height	M/Z	MF
1.	Benzo[b]thiophene, octahydro-2-methyl- (2.alpha., 3a.alpha., 7a.alpha.)-	5.975	156.29	16.94	14.98	356.00	$C_9H_{16}S$
2.	2-Benzimidazolemethanol, .alpha.-(p-bromophenyl)-	6.105	303.15	6.65	9.00	600.00	$C_{14}H_{11}BrN_2O$
3.	Tricyclo[8.4.1.1(4,9)]hexadeca-4,6,8,10,12,14-hexaene, 2,3-bis(2,6-dimethylphenylimino)-, anti-	6.137	156.29	5.07	10.99	442.00	$C_{32}H_{30}N_2$
4.	1-(3',5'-Dibromo-4'-hydroxyphenyl)-5,5-dibromo-2,4-dioxohexahydropyrimidine	9.159	521.78	11.67	15.38	119.00	$C_{10}H_6Br_4N_2O_3$
5.	Dithiocarbamate, S-methyl-, N-(2,3-dimethyl-4-oxo-2-pentyl)-	12.288	219.4	40.07	19.75	480.00	$C_9H_{17}NOS_2$
6.	Pregnane-3,7,20-trione, (5. alpha.)-	21.419	330.5	8.06	13.33	330.00	$C_{21}H_{30}O3$
7.	Methylenebis(ethyl thioglycolate)	24.304	252.4	11.54	16.56	459.00	$C_9H_{16}O_4S_2$

MW – molecular weight (g/mol); MF – molecular formula; RT- Retention time in seconds; Area & Height in percentage (%)

Table 4.16. GC-MS profiles of the compounds identified from *Paspalum fimbriatum*

Sl. No.	Name of the compound	RT	MW	Area	Height	m/z	MF
1.	Methanone, (5-bromo-2-thienyl)(5-hydroxy-4-dimethylaminomethyl-3-benzofuryl)	5.855	503.4	19.53	18.65	45	C_2Br_6
2.	5-beta-Androstan-17-one, 11beta-hydroxy-3alpha-(trimethylsiloxy)-	7.793	380.3	9.31	16.16	449	$C_{16}H_{14}BrNO_3S$
3.	Amoxapine acetate II	9.110	378.6	15.52	16.29	235	$C_{22}H_{38}O_3Si$
4.	1,2,4-Benzenetricarboxylic acid, 5-methyl-, trimethyl ester	17.688	355.8	8.17	15.36	235	$C_{19}H_{18}ClN_3O_2$
5.	Cyclononasiloxane, octadecamethyl-	22.451	266.25	32.16	21.44	147	$C_{13}H_{14}O_6$

MW – molecular weight (g/mol); MF – molecular formula; RT- Retention time in seconds; Area & Height in percentage (%)

Table 4.17. GC-MS profiles of the compounds identified from *Phlogacanthus thyrsiformis*

Sl. No.	Name of the compound	RT	MW	Area	Height	m/z	MF
1.	Tungsten, dicarbonyl-bis(eta.-4-R(+)-pulegone)	4.130	544.33	4.81	9.00	527.00	$C_{22}H_{32}O_4W$
2.	Chromium (III) tris(undecane-5,7-dione)	4.169	604.8	15.20	39.27	603.00	$C_{33}H_{60}CrO_6$
3.	6-Chloro-12H-tetrachlorodibenzo[d,g][1,3,2]phosphorin-6-sulfide	6.500	434.489	6.73	8.72	122.00	$C_{13}H_6Cl$
4.	Ethyl 4,4,6,6-tetramethyl-9-oxo-3,5,7,10-tetraoxa-4,6-disiladodecan-1-oate	9.014	338.50	33.37	16.26	265.00	$C_{12}H_{26}$
5.	Cyclopenta[d,E]anthracene, 5,7-dichloro-1,2(1H,2H)-dioxo-	17.076	301.1	30.79	14.411	278.00	$C_{16}H_6Cl_2O_2$
6.	Ruthenium, tricarbonyl[(3, 4-.eta.)-4,5-diethyl-2,2-dimethyl-3-(1-methylethenyl)-1-selena-2-sila-5-boracyclopent-3-ene]-	20.573	501.2	9.09	12.35	400.00	$C_{16}H_6BOSSi$

MW – molecular weight (g/mol); MF – molecular formula; RT- Retention time in seconds; Area & Height in percentage (%)

Table 4.18. GC-MS profiles of the compounds identified from *Rauvolfia tetraphylla*

Sl. No.	Name of the compound	RT	MW	Area	Height	m/z	MF
1.	8-(Dimethylamino)-7-(3-(4-ethylphenoxy)-2-hydroxypropyl)-3-methyl-3,7-dihydro-1H-purine-2,6-dione	12.28	430.511	8.71	23.61	272.00	$C_{21}H_{30}N_6O_4$
2.	Chlorflurenol	19.050	274.70	47.97	33.43	193.00	$C_{15}H_{11}ClO_3$
3.	2-(4-Nitropyrazol-1-yl) propion ic acid, hydrazide	20.831	199.17	36.88	21.65	114.00	$C_6H_9N_5O_3$
4.	9-(2-Methoxyethyl)carbazole	24.960	225.291	6.43	21.31	179.75	$C_{15}H_{15}NO$

MW – molecular weight (g/mol); MF – molecular formula; RT- Retention time in seconds; Area & Height in percentage (%)

OBJECTIVE 3: Preliminary anti-hyperglycemic studies of different crude extracts and selection of the best fraction

4.8. Enzyme Inhibition Assays

The crude methanolic extract of 11 plants was investigated for their in vitro α-amylase and α-glucosidase inhibitory activity. The α-amylase inhibition activity of all the plants is presented in Table 4.19 and Figure 4.25. The methanolic crude extracts of the plants showed concentration-dependent inhibition in both the enzyme activities. In the case of α-amylase enzyme activity, three plants, namely, *Ficus racemosa* (1.17±0.02 mg/mL), *Oroxylum indicum* (1.21±0.04 mg/mL) and *Hydrocotyle sibthorpioides* (1.23±0.05 mg/mL), showed better inhibitory activity compared to reference acarbose at $P \leq 0.05$. (Figure 4.25). Few plant extracts such as *Musa balbisiana*, *Andrographis paniculata,* and *Alstonia scholaris* have shown a strong inhibitory property against the enzyme, showing no significant difference with the reference inhibitor acarbose. *Clerodendrum infortunatum* shows the least inhibiting property than other plant extracts (Table 4.19).

In the case of α-glucosidase, two plants, namely, *Ficus racemosa* and *Musa balbisiana* showed better activity and significant difference with the reference chemical acarbose ($P \leq 0.05$). Meanwhile, *Lindernia crustacea*, *Hydrocotyle sibthorpioides* and *Andrographis paniculata* showed similar activity as that of reference chemical acarbose (Figure 4.26). The IC_{50} of all the plants are given in Table 4.19. On the other hand, both the reference inhibitor and plant extract showed much more potent inhibition against α-glucosidase enzyme activity than amylase (Figures 4.25 and 4.26). Out of the eleven plants, *Ficus racemosa* showed the most substantial inhibitory property against both the enzymes. The best active plant, *Ficus racemosa* was further carried out for solvent fractionation. Four solvent systems were used according to the polarity index *viz.* hexane, diethyl ether, ethyl acetate, and methanol. All four fractions were then analysed for their phytochemical content, antioxidant activity and enzyme inhibitory activity.

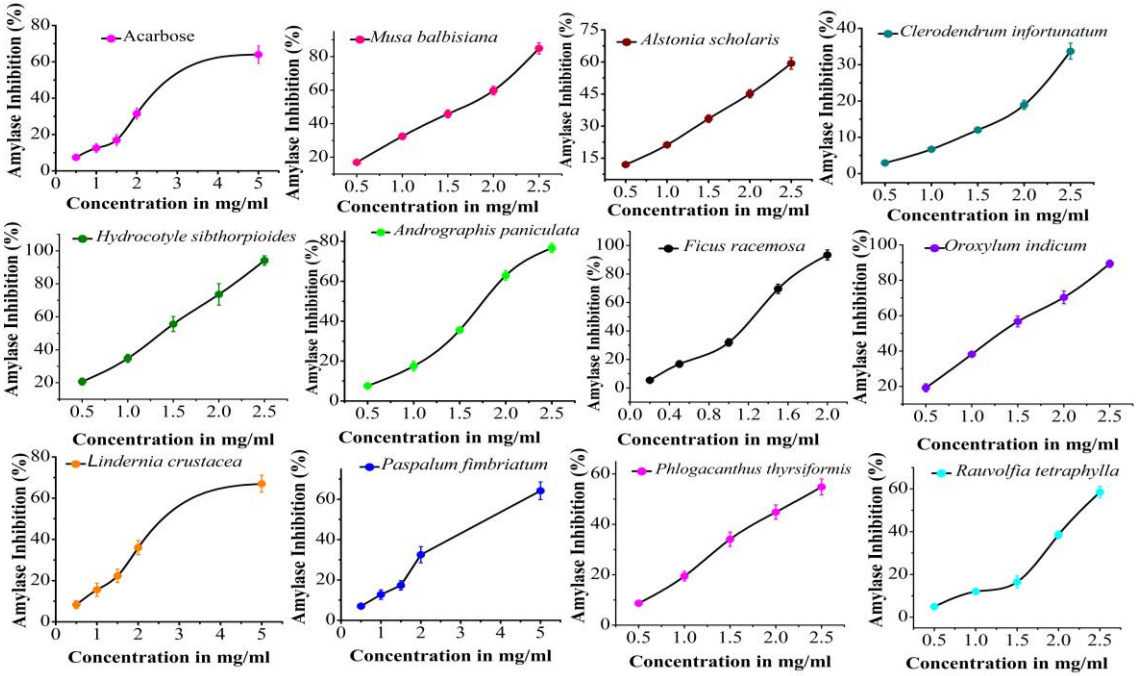

Figure 4.25. Activity of α-amylase on exposed to methanolic crude extracts of plant and reference chemical, acarbose. Values are expressed as mean ± SD, n = 3 (number of experiments)

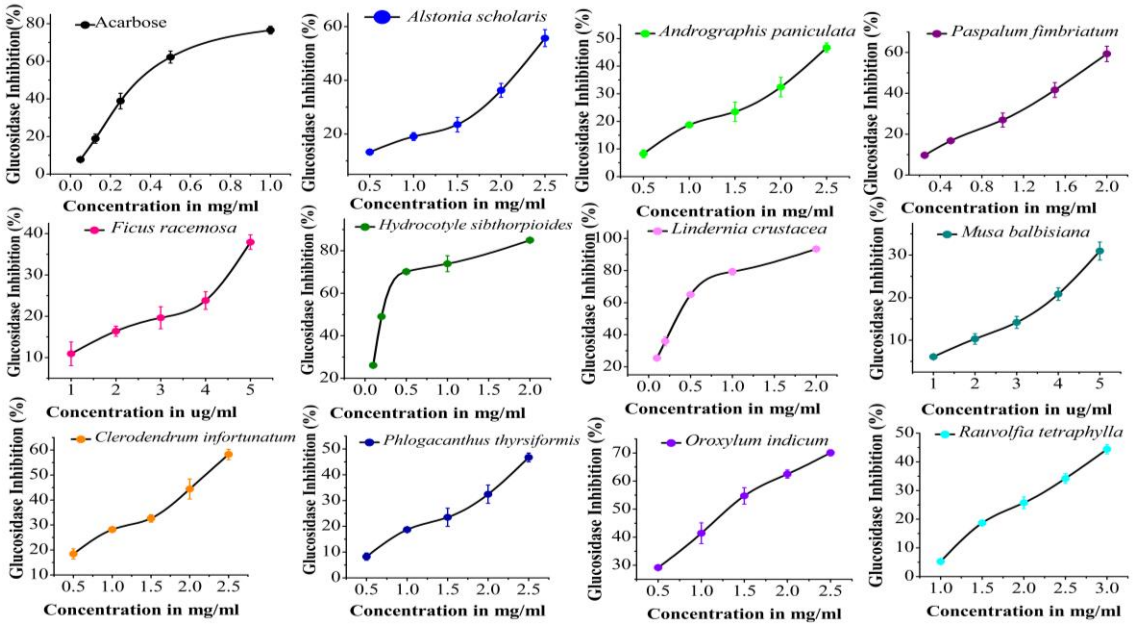

Figure 4.26. Activity of α-glucosidase on exposed to methanolic crude extracts of different plant and reference chemical, acarbose. Values are expressed as mean ± SD, n = 3 (number of experiments)

Table 4.19. IC_{50} values of different enzyme activity assays of methanolic plant extracts

Sl. no.	Name of the plant	α-amylase (mg)	α-glucosidase (mg)
1.	Standard, acarbose	1.71±0.11	0.36±0.03
2.	*Hydrocotyle sibthorpioides*	1.23±0.05	0.24±0.141*
3.	*Musa balbisiana*	1.52±0.04*	10.55±0.86**
4.	*Ficus racemosa*	1.17±0.02	9.75±1.14****
5.	*Oroxylum indicum*	1.21±0.04	1.24±0.112
6.	*Lindernia crustacea*	2.24±0.04	0.3±0.004*
6.	*Ravoulfia tetraphylla*	2.29±0.06	3.37±0.152
7.	*Clerodendrum infortunatum*	3.46±0.32	2.27±0.18
8.	*Paspalum fimbriatum*	3.45±0.23	1.75±0.048
9.	*Alstonia scholaris*	2.15±0.07*	2.53±0.10
10.	*Andrographis paniculata*	1.72±0.03*	0.301±0.008*
11.	*Phlogacanthus thyrsiformis*	2.24±0.06	0.300±0.616

Values are expressed as mean ± SD, n = 3 (number of experiments), ** values in µg; * indicate no significant difference with the standard

4.9. Phytochemical content analysis of different solvent extracts

The four fractions of *Ficus racemosa* Linn. was analyzed for its phytochemical content. The protein content of the four fractions ranged from 49.16±2.80 to 214.64 µg protein/mg plant extract (Figure 4.27 a). Diethyl ether fractions showed the highest protein content followed by ethyl acetate. At P≤0.05 level, all the value differs significantly. The carbohydrate content ranged from 23.69±3.77 to 154.29±16.50 µg glucose/mg plant extracts. The highest carbohydrate content was seen in methanol fraction of *F. racemosa* followed by hexane, diethyl ether fraction and ethyl acetate fraction of *F. racemosa* showed the least carbohydrate content (Figure 4.27 b). At P≤0.05 level, diethyl fraction does not differ significantly from hexane fraction and ethyl acetate fraction.

The phenolic content ranged from 5.29±1.46 to 193.74±5.09 µg GAE/milligram plant extract. Diethyl ether fraction showed the highest phenolic content followed by

ethyl acetate (Figure 4.27 c). The flavonoid content ranged from 5.73±1.43 to 32.12±1.50 µgQE/mg plant extract (Figure 4.27 d). The highest flavonoid content was seen in the methanol fraction followed by diethyl ether, hexane and the lowest were seen in the ethyl acetate fraction (Figure 4.27 d). At P≤0.05, each of the fractions of *F. racemosa* differs significantly both in total phenolic content and total flavonoid content.

4.10. Antioxidant assays of different solvent extracts

The four fractions were also tested for their antioxidant property. Five different antioxidant tests were conducted, such as FRAP, TAA, DPPH, ABTS, and TBARS. In terms of TAA, diethyl ether fraction of *F. racemosa* showed strongest activity, followed by ethyl acetate, hexane and methanol (Figure 4.28 a). At P≤0.05 level, all the four fractions of *F. racemosa* differ significantly from each other, except ethyl acetate and hexane, where no statistical difference was observed. In case of FRAP activity, among the crude extract solvent fractions, diethyl fractions show the strongest activity when compared with other fractions of *F. racemosa* after Ascorbic acid (Figure 4.28 b). However, no significant difference was observed between ethyl acetate fraction and diethyl ether fraction.

For, DPPH, ABTS and TBARS assays, IC_{50} values of antioxidant tests were evaluated. For DPPH, the IC_{50} values ranged from 5.54±0.20 µg/mL to 13.725±1.071 mg/mL (Figure 4.29). Diethyl ether fraction of *F. racemosa* showed a strong DPPH scavenging activity than the other fractions followed by ethyl acetate fractions. At p≤ 0.05 level, IC_{50} values of gallic acid and diethyl ether fraction of *F. racemosa* and gallic acid and ethyl acetate fraction of *F. racemosa* does not differ.

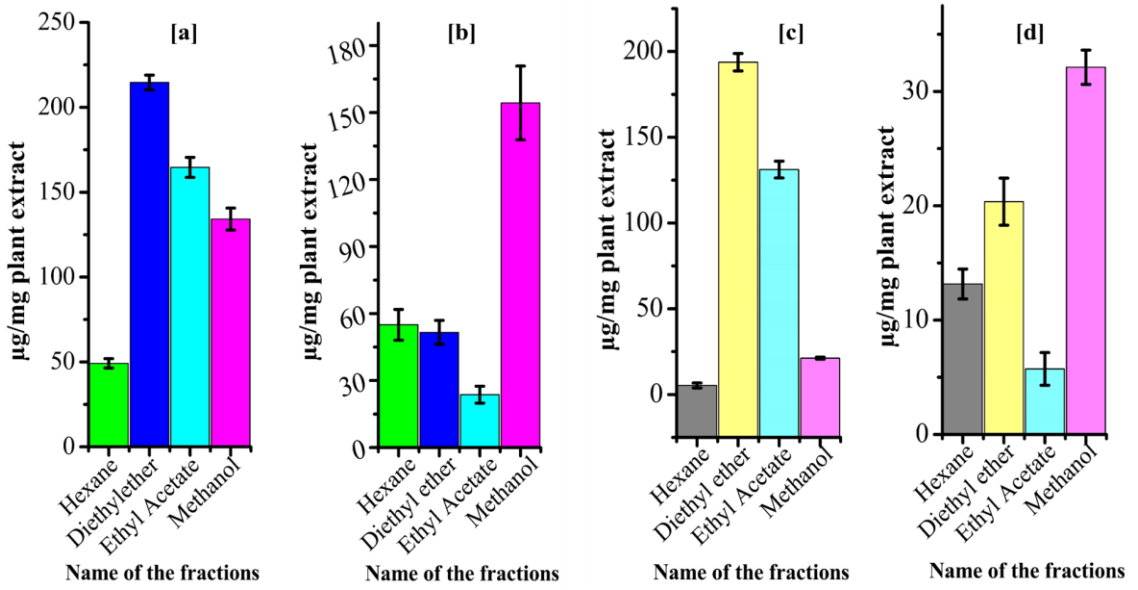

Figure 4.27. Phytochemical content of different solvent fractions of *Ficus racemosa.* a) Protein content, (b) Carbohydrate content, (c) Phenolic content and (d) Flavonoid content. Values are expressed as mean ± SD, n = 3 (number of experiments)

For ABTS assay, the IC_{50} value range from 1.19±0.03 to 1244.65 µg/mL. Diethyl ether extract showed the strongest ABTS scavenging activity than other fractions (Figure 4.30). However, all the three fraction *viz.*, diethyl ether, ethyl acetate, methanol and standard chemical showed no significant difference at P≤0.05 level. The lowest activity was observed in hexane fraction with IC_{50} value of 1.24±0.06 mg/mL plant extract. For TBARS assay, the IC_{50} value range from 24.59±0.49 to 280.78±9.53 µg/mL plant extract. The highest lipid scavenging activity was observed in diethyl fraction of *F. racemosa* showing similar activity with ascorbic acid at P≤0.05 (Figure 4.31). The lowest activity was observed in hexane fraction of *F. racemosa*. All in all, diethyl fraction of *F. racemosa* showed the most substantial antioxidant property among the four fractions.

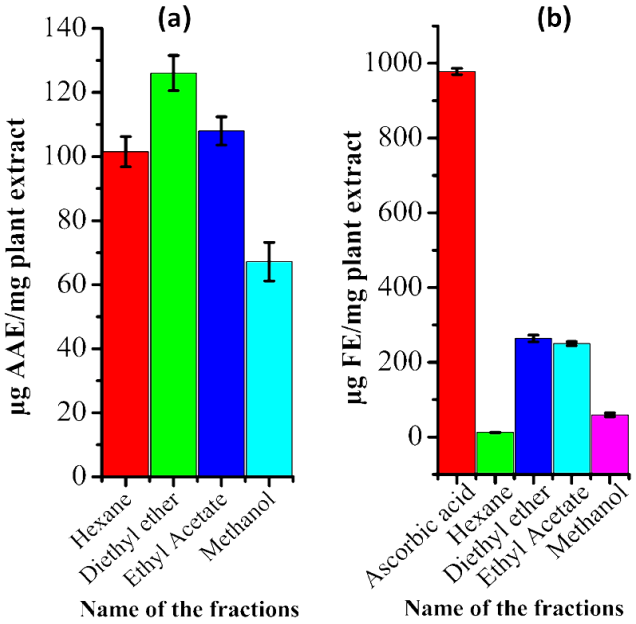

Figure 4.28. (a) Total antioxidant activity (b) Graph showing FRAP activity of solvent fractions of *Ficus racemosa*. Values are expressed as mean ± SD, n = 3 (number of experiments)

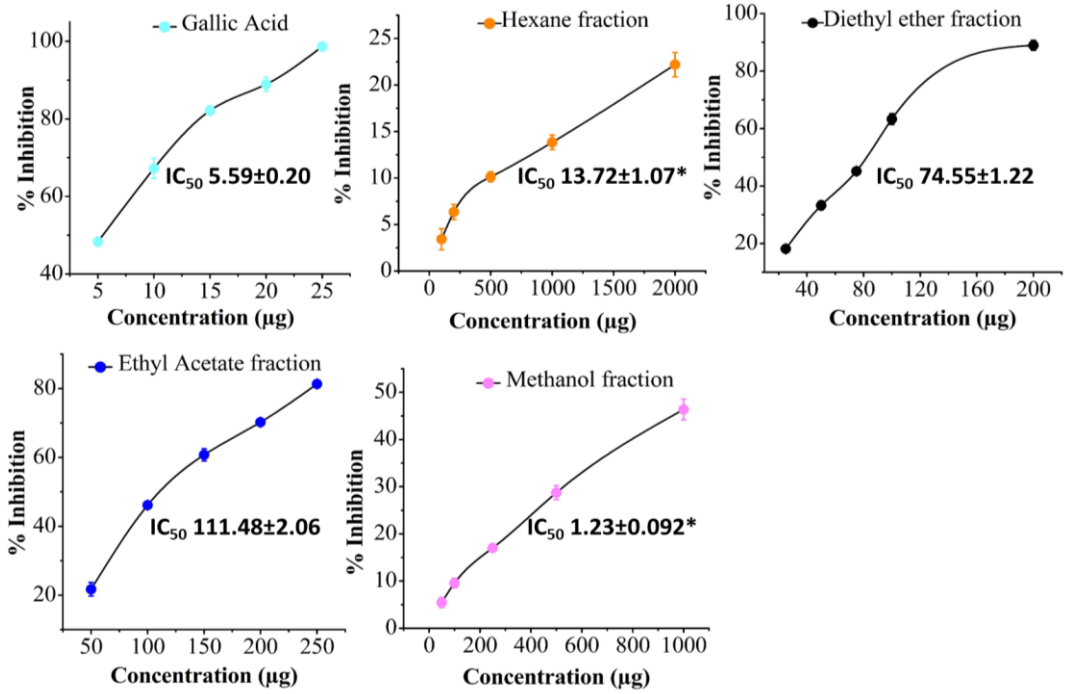

Figure 4.29. DPPH free radical scavenging activity of different solvent fractions of *Ficus racemosa.* Values are expressed as mean ± SD; n = 3 (number of experiments); *represents values in mg/mL

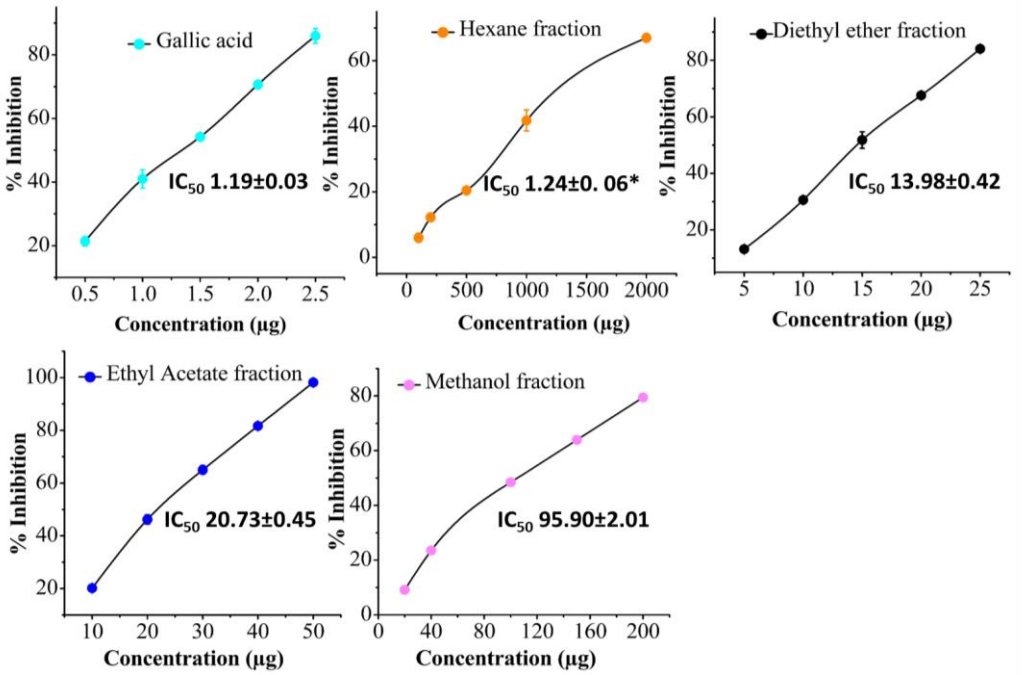

Figure 4.30. ABTS free radical scavenging activity of different solvent fractions of *Ficus racemosa*. Values are expressed as mean ± SD, n = 3 (number of experiments); *IC_{50} values in µg/mL, except hexane fraction (mg/mL)

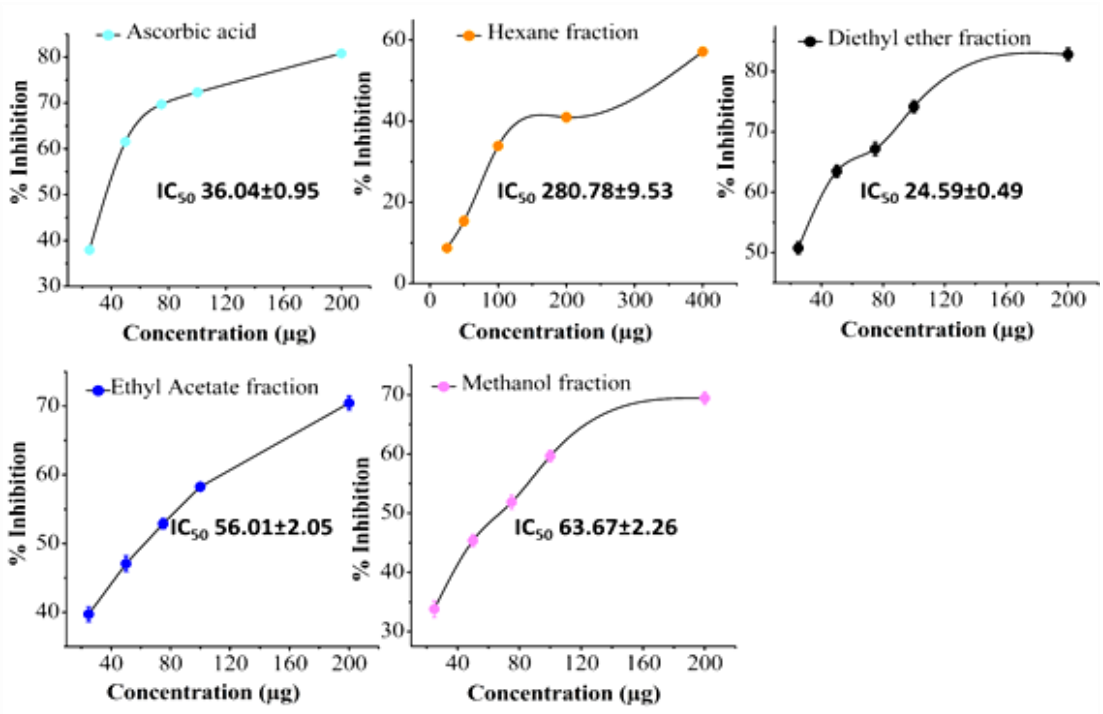

Figure 4.31. Lipid peroxidation inhibition activity of different solvent fractions of *Ficus racemosa*. Values are expressed as mean ± SD, n = 3 (number of experiments)

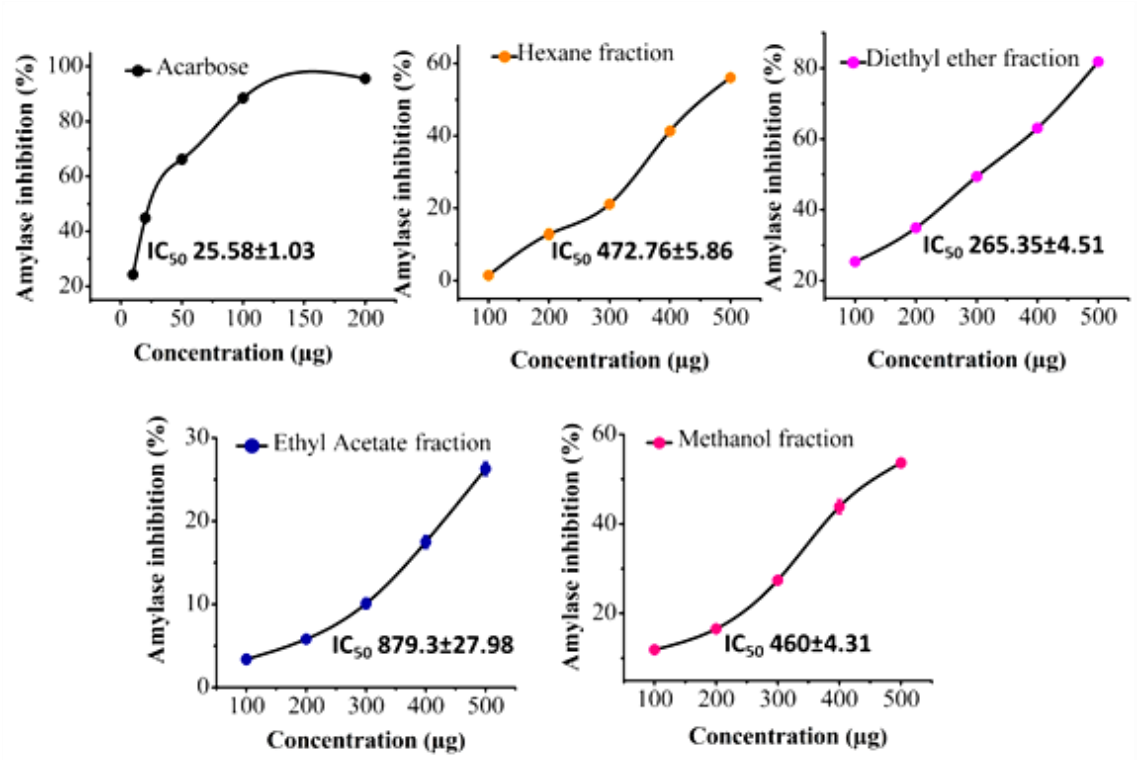

Figure 4.32. Activity of α-amylase on exposed to *Ficus racemosa* different fractions and acarbose Values are expressed as mean ± SD, n = 3 (number of experiments)

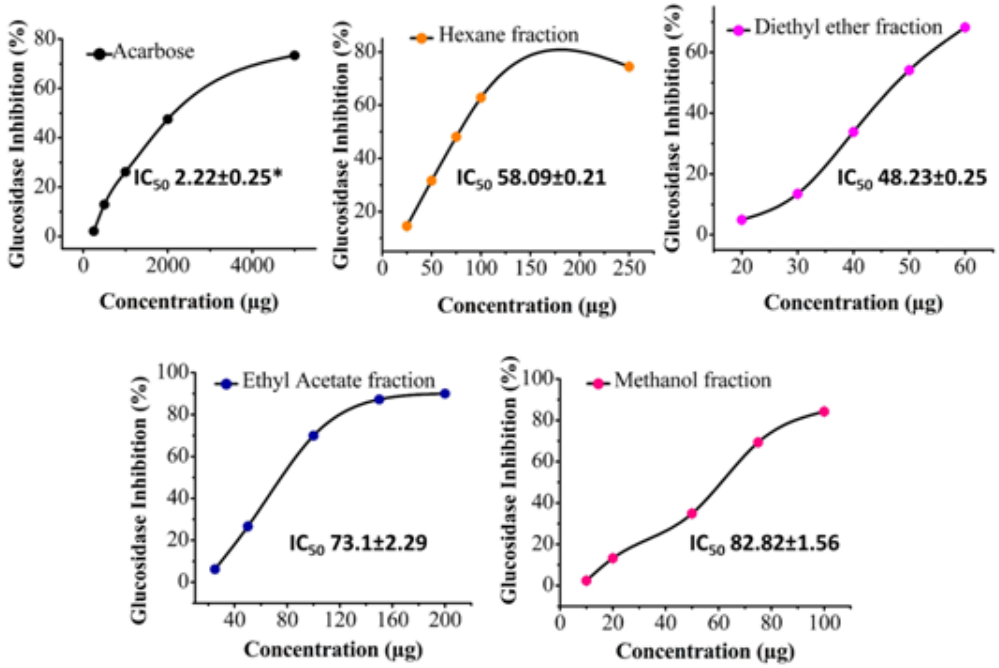

Figure 4.33. Activity of α-glucosidase on exposed to *Ficus racemosa* different fractions and acarbose. Values are expressed as mean ± SD, n = 3 (number of experiments)

4.11. Enzyme inhibition assays of different solvent extracts

The α-amylase inhibition activity of all the fractions of *F. racemosa* is presented in Figure 4.32. The methanolic crude extracts of the plants showed concentration-dependent inhibition in both the enzyme activities. Diethyl ether fraction showed better inhibitory activity compared to the other fractions, followed by methanol fractions. Statistical analysis showed that at 95% confidence level all the values differ from each other. The IC_{50} value ranges from 25.58±1.03 to 870.3± 27.98 μg/mL (Figure 4.32). In the case of α-glucosidase, among the four fractions, diethyl ether fraction has shown better activity than the three fractions ($P \leq 0.05$) (Figure 4.33). The IC_{50} values α-glucosidase ranged from 2.22±0.25 to 82.82±1.56 μg/mL. Diethyl ether fraction of *F. racemosa* showed the most potent α-amylase and α-glucosidase activity after the standard chemical. On the other hand, both the reference inhibitor and plant extract showed much stronger inhibition in α-glucosidase enzyme activity compared to amylase. Out of the four fractions of *F. racemosa*, diethyl ether fraction showed stronger inhibitory property against both the enzyme compared to other fractions. Taking into account of phytochemical, antioxidant and α-amylase and α-glucosidase activity, diethyl ether fraction of *F. racemosa* has been chosen for further study or preceded for toxicity and antidiabetic study in in-vivo system.

4.12. Toxicity studies in in-vivo system

I) Acute and Sub-acute oral toxicity

Acute toxicity study observed that the extract of *F. racemosa* did not show any sign of toxicity. There was no sign of behavioural changes in the rat. All three doses of *F. racemosa* diethyl ether fraction (FRDF) did not show any sign of toxicity. No significant weight gain or loss was observed in the rat groups after 28 days of observations. On the 28^{th} day, the rats were subjected to gross necroscopy, and blood was collected and used for hematological and biochemical analysis. The haematological parameters of all 4 groups of rats are presented in Table 4.20. Like behavioural changes, there were no significant alterations in the blood parameters in the FRDF-treated rats. Small changes were observed in the plant extract-treated groups. Increased RBC, PLT, and PCT were observed in rat groups treated with FRDF ($P \leq 0.05$). No significant

changes were observed in WBC, neutrophil, lymphocyte, monocyte, eosinophil, basophil, immunoglobulin, HGB, MCV, MCH, MCHC, RDW-SD, MPV, PDW, and NRBC when compared with the control ($P \leq 0.05$). An elevated level of PLCC and RDW was observed in the rat group treated with 200 mg, while 100 and 500 mg did not show any alterations. A decrease in PLCR was seen in 100 mg FRDF-receiving rat groups, while 200 mg FRDF and 500 mg FRDF-receiving rats shows no alterations. Similarly, 100 and 200 mg FRDF-treated groups showed an increase in HCT, while 500 mg/kg bw did not show any alteration (Table 4.20).

The present study observed that the AST activity ranged from 98 to 157 U/L. No significant difference was observed between normal control and FRDF-treated groups. Similarly, ALT activity ranges from 52 to 95 U/L. Statistical analysis showed no significant difference between the control and treated groups. A similar condition was seen in ALP activity and bilirubin content where rat groups exposed to FRDF doses did not show any difference from the normal control group. Creatinine and albumin are important biomolecules for proper kidney functioning. The present study revealed a significant decrease in creatinine levels in rat groups treated with 200 and 500 mg/kg bw compared to the control group. Meanwhile, no significant changes were observed in the albumin level of the rats (Table 4.21).

Table 4.20. Hematological profile of FRDF-treated rat groups on sub-acute toxicity study

Sl. No.	Control	Drug Concentration (mg/kg bw)		
		100	100	500
WBC (10^3/µL)	7.43±0.03	5.83±1.66	8.73±2.11	5.21±1.36
Neutrophil (%)	3.34±0.17	1.87±0.34	3.51±1.45	2.29±0.69
Lymphocyte (%)	2.14±0.21	1.24±0	3.83±0.97	2.54±0.50
Monocyte (%)	0.52±0.08	0.58±0	1.01±0.29	0.35±0.10
Eosinophill (%)	0.28±0.03	0.1±0.01	0.29±0.10	0.159±0.05
Basinophill (%)	0.003±0.005	0.006±0.004	0.01±0.005	0.006±0.004
Imunoglobulin	0.01±0.005	0.01±0	0.006±0.005	0.01±0.004
RBC (10^6/µL)	5.24±0.16	7.44±0.30*	8.36±0.502*	6.76±0.42*

HGB (g/dL)	11.16±0.67	13.26±0.33	14.96±0.31	11.733±1.84
HCT (%)	30.4±2.38	42.08±2.00*	44.73±1.03*	33.53±5.10
MCV (fL)	54.06±2.003	52.1±1.04	53.63±2.8	53.5±0.53
MCH (pg)	20±2.08	17.56±0.14	17.9±0.85	18.2±0.18
MCHC g/dL	33.66±0.16	34.3±0.44	33.46±0.16	33.70±0.39
RDW-CV (%)	13.56±0.60	14.3±0.37	15.46±0.12*	14.66±0.53
RDW-SD (%)	26.8±2.80	26.03±1.13	28.96±0.12	26.55±0.20
PLT (10^3/µL)	416.66±25.01	676.66±21.12*	774±95.01*	634±40.40*
MPV (%)	8.3±0.65	7.16±0.17	7.56±0.04	7.83±0.42
PDW (%)	15.1±0.15	15±0	15.13±0.12	15.367±0.38
PCT (µg/L)	0.325±0.015	0.50±0.017*	0.58±0.07*	0.48±0.03*
PLCC (%)	62.33±5.52	63±7.07	89±9.74*	61.66±1.77
PLCR (%)	15.46±2.50	8.93±0.88*	11.53±0.12	12.73±2.07
NRBC (%)	0.646±0.005	0.37±0.10	0.50±0.13	0.56±0.04

Values are presented in mean ± SD, n = 3, WBC - White Blood corpuscles; RBC - Red Bood Corpuscles; HCB - Hemoglobin HCT - Hematocrit; MCV - Mean corpuscular volume; MCH - Mean corpuscular hemoglobin; MCHC - Mean corpuscular hemoglobin concentration; RDW-CV - Red cell distribution width coefficient variant; RDW-SD - Red cell distribution width size distribution; PLT - Platelet count; MPV - Mean platelet Volume; PDW - Platelet distribution width; PCT - Procalcitonin test PLCC-Platelet large cell ratio; PLCR - Platelet large cell ratio; NRBC - Nucleated Red blood cells. *Indicates significant differences with the control at P≤ 0.05 level.

Serum Lipid Profile

Various parameters such as TC, HDL, LDL, VLDL, and Triglyceride levels were analyzed to know if there are any changes in the lipid profile of the FRDF-treated groups. No significant changes were seen in Total cholesterol, Triglyceride, and VLDL. However, 200 mg and 500 mg FRDF showed a significant increase in HDL and a decrease in LDL when compared to the control at P≤0.05 level (Table 4.22).

Table 4.21. Biochemical parameters of FRDF-treated rat groups in the sub-acute toxicity study

Sl. No.	Parameters	Control	Drug concentration (mg/kg bw)		
			100	200	500
1.	Creatinine (mg/dL)	0.31±0.04	0.25±0.02	0.22±0.004*	0.17±0.01*
2.	Albumin (g/dL)	2.53±0.07	2.47±0.12	2.40±0.12	2.46±0.06
3.	AST (U/L)	114.33±3.39	134±35.32	141.33±5.93	117.66±0.94
4.	ALT (U/L)	60.66±6.34	69.33±5.79	72.33±2.85	76.66±17.01
5.	ALP (U/L)	451±15.12	322.66±72.02	394.66±44.22	358.66±23.79
6.	Total bilirubin (mg/dL)	1.15±0.04	0.93±0.12	0.93±0.10	1.10±0.16

Values were presented in mean ± SD of three replicates (n = 3). AST- Aspartate aminotransferase; ALT - Alanine transaminase; ALP - Alkaline phosphatase; U/L – Unit/litre; *Significant differences with the control at P≤0.05

Table 4.22. Lipid profile of normal and FRDF-treated rats in the sub-acute toxicity study

Parameters	Control	Drug concentration (mg/kg)		
		100	200	500
Total cholesterol	70.41±0.9	69.93±2.02	72.06±1.10	71.5±2.15
HDL	50.01±3.45	49.60±1.61	53.3±0.85*	53.00±0.73*
LDL	14.83±0.30	13.33±1.52	12.03±0.25*	12.26±0.76*
VLDL	13.03±1.38	13.4±0.91	13.93±0.80	13.56±0.65
Triglyceride	101.96±3.59	103.66±2.51	103.5±2.21	102.66±3.51

Values were presented in mean ± SD of three replicates (n = 3), HDL - High-density lipoprotein; LDL - Low-density lipoprotein; VLDL - Very low-density lipoprotein, *indicates significant differences with the control at P≤0.05 level

Assessment of cardiovascular risk index

Few risk indexes were calculated to know the risk factor for the cardiovascular system. Our study observed a decrease in Castelli risk index-2 in 200 and 500 mg FRDF-treated groups. However, there were no changes in CR1, AIP, and AC factors.

Table 4.23. Cardiovascular risk index

Parameters	Control	Drug concentration (mg/kg)		
		100	200	500
CR-I	1.40±0.05	1.40±0.05	1.35±0.02	1.34±0.05
CR-II	0.29±0.01	0.26±0.03	0.22±0.01*	0.23±0.01*
AIP	0.30±0.01	0.31±0.01	0.30±0.01	0.28±0.01
AC	0.40±0.05	0.40±0.05	35±0.02	0.34±0.05

Values were presented in mean ± SD of three replicates (n = 3). CR-I- Castelli risk index 1; CR-II- Castelli risk index 2; AIP-Atherogenic index of plasma. AC-Atherogenic co-efficient *indicates significant differences with the control at P≤0.05 level

4.13. Oral Glucose Tolerance Test

OGTT measures the body's ability to metabolize sugar and clear it from the bloodstream. It is a standard assay to evaluate insulin resistance and glucose tolerability. The result of OGTT is depicted in Figure 4.34. In all the normal and diabetic rats, the fasting blood glucose level was measured. The fasting blood sugar (FBS) of normal (non-diabetic) and diabetic rat groups ranged from 84 − 102 mg/dL and 274 − 301 mg/dL, respectively. In the normal control and diabetic control group, glibenclamide-treated rat groups showed the highest reduction of blood glucose of about 44% and 58% respectively, 30 min after the administration of drugs. This is followed by the 500 mg-treated group which shows a reduction of 36% both in normal and diabetic rats, respectively. A significant blood glucose decrease was observed in all the animal groups treated with plant extract and glibenclamide compared to normal control and diabetic control (P≤0.05). In both the diabetic and normal groups, when a 2 g/kg bw glucose was loaded, there is a decrease in blood glucose levels in 200 and 500 mg FRDF, and

glibenclamide-treated groups when compared with the control rats ($P \leq 0.05$). Meanwhile, 100 mg treated rats in non-diabetic groups, do not show any difference with the control rats, while the same is opposite in diabetic groups. All the doses (100, 200, and 500 mg/kg bw) of FRDF and glibenclamide showed decrease in blood glucose levels in diabetic groups after the glucose load. After 30 min of glucose load, a spike in glucose level was seen in both the control groups of diabetic and non-diabetic rats showing a 36% and 41% increase in nondiabetic and diabetic groups from the FBS level. 100 mg FRDF-treated group is seen to control the spike of glucose showing only 20% and 35% increase in glucose from the initial FBS in non-diabetic and diabetic rats, respectively. Similarly, in both groups, a slower rise of blood glucose was seen in 200, 500 mg, and glibenclamide-treated rat groups. After 30 min of glucose injection, both the diabetic and non-diabetic groups showed significant differences compared to the control group ($P \leq 0.05$). At 60 min, in non-diabetic rat groups, a rise of 28%, 13%, 2%, 8%, and 11% were seen in control, 100, 200, and 500 mg extract, and glibenclamide treatment compared to the initial FBS. In diabetic rats, an increase in 33%, 25%, 18%, 21%, and 21% was seen in control, 100, 200, and 500 mg FRDF, and glibenclamide-treated rat groups, respectively compared to the initial FBS. At 60 min, all the treated rats (diabetic and non-diabetic) showed decrease in glucose levels. The rat group treated with 200 mg FRDF showed stronger reduction both in nondiabetic and diabetic rat groups. At 90^{th} min, an increase in blood glucose level was observed in both non-diabetic (19%, 2% 5%) and diabetic rat groups (25% 18%, and 9%) in control, 100 and 500 mg FRDF-treated groups compared to initial FBS values. While a decrease of 5% and 16% (non-diabetic) and 2% and 53% (diabetic rats) blood glucose was seen in 200 mg and glibenclamide-treated rat groups. In both the diabetic and non-diabetic groups, glibenclamide and 200 mg FRDF-treated groups showed higher glucose-lowering potential. At 120^{th} min, in non-diabetic rat groups, 500 mg-treated groups showed no difference from the control. The other two doses (100 and 200 mg FRDF) and glibenclamide showed significant differences from the control. When compared with the initial FBS, glibenclamide and 200 mg FRDF decreases the blood glucose level even below the previous FBS ($P \leq 0.05$). In diabetic rats, when compared with the initial FBS, all the groups except control (10% increase), show a reduction of glucose,

glibenclamide (38%) being the best-lowering agent followed by 200 mg FRDF (23%), 100 mg FRDF (5%), and 500 mg FRDF (2%).

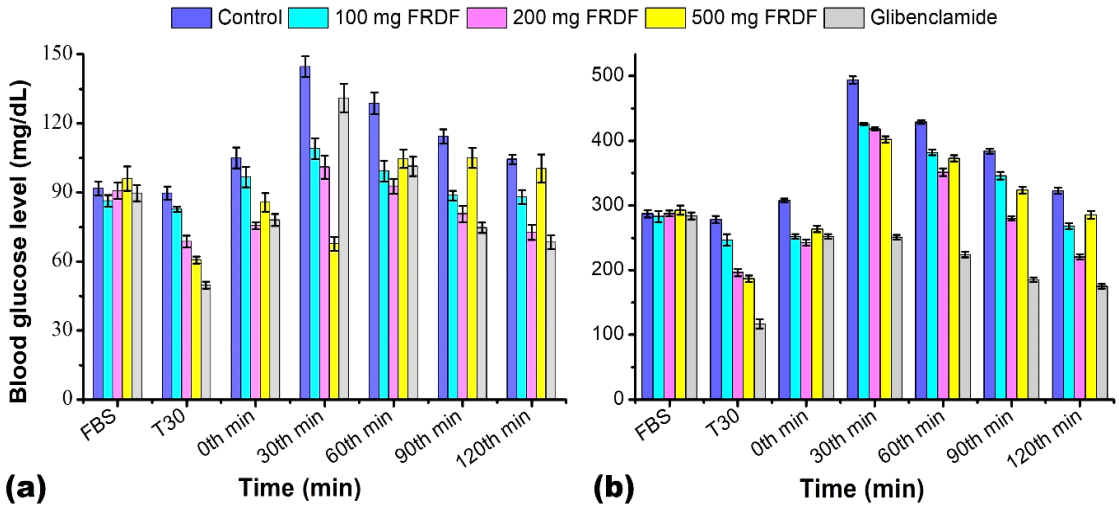

Figure 4.34. Evaluation of oral glucose tolerance test in glucose-loaded rats when treated with FRDF, a) OGTT test on normal control rats, b) OGTT on diabetic rats. Values were represented in mean ± SD

OBJECTIVE 4: Biochemical and histochemical analysis of antidiabetic effects of the most active fraction of plant

4.14. Study of antihyperglycemic effect of the best fraction of the plant

I) Serum Glucose level

On the administration of STZ, the blood glucose levels of diabetic rats increased (400-500 mg dL^{-1}) as compared to that of the normal control rats (85-115 mg/dL), indicating diabetes induction. The blood glucose level of all the rats were monitored and recorded on weekly basis, i.e. on 0^{th}, 7^{th} 14^{th} 21^{st} and 28^{th} day. A considerable improvement in terms of blood glucose has been observed in all the groups except for diabetic control and normal control groups (Table 4.24). Statistical analysis showed that on 7^{th} day, there is no significant difference among the three groups (Diabetic control, 100 mg FRDF, and glibenclamide), but showed a difference with 200 mg FRDF treated group at $P \leq 0.05$. On 14^{th} and 21^{st} day, all the treated groups showed a considerable difference

with the diabetic control groups and normal control groups. On the 28th day, both the crude extracts 100 mg FRDF (238.66±21.57 mg/dL) and 200 mg FRDF treated rat groups (122.33±20.50 mg/dL) showed a decrease in the blood glucose compared to the Diabetic control (466±30.78 mg/dL). It has also been observed that the 200 mg FRDF treated group showed better antihyperglycemic property than the standard drug, Glibenclamide treated group (180.33±21.36 mg/dL). On 28th day, all the treated groups caused a significant reduction of blood glucose level when compared with the diabetic control group ($P \leq 0.05$). Meanwhile, it has been seen that, the 200 mg FRDF treated rat group does not show significant difference from normal control group indicating their return to normalcy.

II) Body Weight

From 0 to 28th day the body weight of the normal control groups did not show significant differences ($P \leq 0.05$). The average total body weights were seen to be decreased in diabetic rats (□25% of body weight in Diabetic Control) from the initial day of the experiment (238.66±8.08 g). The average body weight was also seen to be slightly decreased in glibenclamide treated groups (182.33±12.09 g) but no significant difference was observed at $P \leq 0.05$ in the same group in 28 days' time period. 100 mg FRDF (194±8.71 mg/dL) treated rats however showed a significant difference of weight loss at 28th day from the initial day of treatment. On 28th day, the body weight of the rats has seen to be slightly increased, almost attaining their normal weight (Table 4.25). Statistical analysis showed that there is no significant decrease of body weight on 200 mg treated groups in 28 days from the initial day of treatment ($P \leq 0.05$).

The food consumption decreased in the diabetic rats, while the water consumption increased drastically during the first few days. 200 mg treated groups decreased the water consumption by the end of 28th days.

III) Liver Function Test on serum of normal and diabetic rats

Enzymes indicating liver function were analyzed from the serum of the animals. Diabetes increases the risk of liver diseases, so liver function test is inevitable. Few parameters including AST, ALT, ALP Bilirubin and creatinine measures the functionality of liver. Alkaline phosphatase is a group of metabolic enzymes that catalyzes wide variety of phosphate esters at alkaline pH. Serum ALP is found to be higher in diabetic groups compared to normal control group. The highest ALP level was

seen in Diabetic Control group followed by Glibenclamide treated groups and 100 mg FRDF treated group (Table 4.26). Statistical analysis shows that 100 mg and 200 mg FRDF treated group shows a no significant difference with normal control group at $P \leq 0.05$.

In terms of level of serum AST, a surge of level of AST was seen in diabetic rat groups. In diabetic control rat group, the increase is up to two-fold i.e., 161.67±17.78 U/L when compared to the normal control rats (70±18.24 U/L). (Table 4.26). However, 200 mg treated group showed a lower in AST level (281±20.41 U/L) almost like normal control rats (197.67±12.50 U/L). Among the treated dose, 200 mg/kg bw was found to be more effective in lowering the AST level (83.33±14.01 U/L), better than the standard glibenclamide (126.33±20.25 U/L) and 100 mg treated groups (133±12.49 U/L). Statistical analysis showed there is a significant difference between the level of AST in normal control rats and Diabetic control rats, glibenclamide treated, 100 mg FRDF (Table 4.26). However, 200 mg FRDF treated groups does not differ significantly from the normal control group ($P \leq 0.05$).

A rise in ALT level was seen in diabetic rats (except 200mg FRDF treated rat groups) compared to normal control rat groups (Table 4.26). ALT level of 200 mg treated groups had no significant difference with the normal control groups ($P \leq 0.05$). Diabetic control rats showed the highest serum bilirubin level among all the five groups. Bilirubin level has seen to be slightly decreased in glibenclamide treated groups, than 100 mg treated groups. The serum bilirubin level was seen to be lowered in 200 mg FRDF treated groups and had no significant difference with the normal control rats (Table 4.26).

IV) Lipid profile of normal and diabetic rats

The cholesterol level in normal control rats was found to be 74.25±19.51 mg/dL. In diabetic rats the value increases almost two-fold compared to the normal control rats i.e., 156.33±4.50 mg/dL. There was a decrease in total cholesterol in diabetic rat groups due to administration of the crude extracts and glibenclamide (Table 4.27). 200 mg FRDF treated group has lowest cholesterol (106.66±17.03 mg/dL) among all other diabetic groups and does not show any significant difference with the normal control rats.

Table 4.24. Effect of diethyl ether fraction of *Ficus racemosa* on blood glucose

Groups	Blood glucose (mg/dL)				
	Day 0	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Control	103.33±12.66	105.33±6.42	99.33±11.01	103±11.53	95.66±10.96
Diabetic Control	474.33±30.89[a]	485±48.86[a]	458.66±41.25[a]	465±22.06 [a]	466±30.78 [b]
Diabetic+ Glibenclamide	448.33±30.53 [a]	381±40.70[a]	270.33±43.98 [a,b]	216.33±28.43 [a,b]	180.33±21.36 [a,b]
Diabetic + 100 mg FRDF	459±39.15 [a]	391.33±16.50[a]	380.33±19.34[a,b]	299.66±19.55 [a,b]	238.66±21.57 [a,b]
Diabetic + 200 mg FRDF	457±36.51 [a]	298.66±16.74 [a,b]	260.66±21.38[a,b]	196.66±30.53 [a,b]	122.33±20.50 [b]

'a' represents significant difference with the normal control rats; 'b' represents significant difference with the diabetic control. 'a,b' shows significant differences both with the normal control and diabetic control rat groups at P≤0.05 level

Table 4.25. Effect of diethyl ether fraction of *Ficus racemosa* on body weight

Groups	Body weight (gm)				
	Day 0	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Control	232±4.00	235.33±10.01	230.66±3.51	236.66±5.68	234.66±3.05
Diabetic Control	238.66±8.08	216±8.18*	200±5.56*	186.33±8.73*	177.66±10.50*
Diabetic+ Glibenclamide	209.66±16.26	199.33±16.28	191.66±15.88	184.33±11.84	182.33±12.09
Diabetic + 100 mg FRDE	231.33±16.77	216±16.82	204±13.22	197.33±5.50	194±8.71*
Diabetic + 200 mg FRDE	230.33±19.34	223.66±16.19	220.33±16.77	218.33±16.74	221±14.73

*Represents statistical difference from 0^{th} day among the same groups at P≤0.05 level

An elevated triglyceride level was seen in diabetic rat groups compared to the normal control rats. In diabetic control group, triglyceride level increases up to three-fold (270.33±16.41 mg/dL) compared to the normal control rat group (90.7±6.54 mg/dL). However, the triglyceride level was seen to been lower (196.66±11.93 mg/dL) in glibenclamide treated rats, 100 mg FRDF treated rats (157.86±25.25 mg/dL). An improvement of triglyceride level has been seen in 200 mg FRDF treated rat groups which shows no significant difference with the normal control rat groups (Table 4.27).

In terms of LDL and VLDL there is an increase in VLDL in diabetic rats as compared to normal control rats. An increase of serum VLDL was seen higher in glibenclamide treated rat group than the diabetic rat group and show significant difference with the normal control groups. Meanwhile, VLDL level was seen to be less affected in FRDF treated rat groups and does not differ statistically from the normal control rat groups ($P \leq 0.05$). In terms of LDL, the diabetic rat group exhibit a high level of serum LDL level compared to the normal control rats. All the diabetic rat groups except group treated with 200 mg FRDF showed a significant difference with the normal control rats at $P \leq 0.05$ (Table 4.27). HDL level in diabetic rat groups dropped down in all the diabetic groups. It was seen that there is an improvement in HDL level in 100 mg FRDF, 200 mg FRDF and glibenclamide treated rat groups when compared to diabetic control rats.

V) Kidney profile of normal and diabetic rats

Diabetic groups showed a high creatinine level which is much higher than the normal range. The creatinine level increases almost four-fold in diabetic control group (0.95±0.11 mg/dL) when compared to normal control groups (0.20±0.02 mg/dL). However, creatinine level was found to be significantly lowered in glibenclamide treated diabetic rats (0.76±0.05 mg/dL), 100 mg FRDF treated diabetic rats (0.81±0.07 mg/dL), and 200 mg FRDF treated diabetic rats (0.46±0.06 mg/dL). 200mg FRDF treated rat groups does not differ from the normal control groups (Table 4.28). Diabetic rats showed an elevated level of serum urea when compared to the normal control rats. In diabetic control groups the serum urea increased to 136.03±8.18 mg/dL which decreased to almost half in 200 mg FRDF treated rats (67.33±6.65 mg/dL). Glibenclamide and 100 mg FRDF treated rats showed a high serum urea level but lower than the diabetic control rats (Table 4.28).

Table 4.26. Liver function test of the normal and diabetic rats

Groups	AST (U/L)	ALT(U/L)	ALP(U/L)	BILIRUBIN (µg/dL)
Control	197.67±12.50	70±18.24	427±30.19	2.52±0.2
Diabetic Control	536.67±70.57	161.67±17.78	1006±43.55	4.4±0.17
Diabetic+ Glibenclamide	578±11	126.33±20.25	791.33±57	3.73±0.19
Diabetic + 100 mg FRDF	628±41.01	133±12.49	525.66±20.79*	3.32±0.34
Diabetic + 200 mg FRDF	281±20.41	83.33±14.01*,	436±39.50*,	2.84±0.32*

AST – Aspartate aminaotransferase ALT- Alanine transaminase ALP- Alkaline phosphatise, *Indicates no significant differences at P≤0.05 with the normal control rats. All experiments are replicated for three times (n = 3)

Table 4.27. Effect of diethyl ether fraction of *Ficus racemosa* on lipid moieties

Groups	Cholesterol (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)	HDL (mg/dL)	Triglycerides (mg/dL)
Control	74.25±19.51	12.33±2.08	18.33±1.52	42±2.2	90.7±6.54
Diabetic Control	156.33±4.50	24.33±1.52	27±0.36*	19±2	270.33±16.41
Diabetic+ Glibenclamide	141.66±4.04	21.33±2.51	36±5.56	28.67±3.51	196.66±11.93
Diabetic + 100 mg FRDE	147.33±3.78	23.33±3.05	28.66±3.78*	22±3.60	157.86±25.25
Diabetic + 200 mg FRDE	106.66±17.03	12.33±2.62*	28±4.58*	33.6±3.07	89.8±15.16*

LDL - low density lipoprotein; VLDL- very low-density lipoprotein; HDL- High density lipoprotein, *Indicates no significant differences at P≤0.05 with the normal control rats. All experiments are replicated for three times (n = 3)

Table 4.28. Kidney function test in normal and diabetic rats

Groups	Creatinine (mg/dL)	Serum Urea (mg/dL)
Control	0.20±0.02	45.33±6.65
Diabetic Control	0.95±0.11	136.03±8.18
Diabetic+ Glibenclamide	0.76±0.05	103.33±10.96
Diabetic + 100 mg FRDF	0.81±0.07	82.33±9.01
Diabetic + 200 mg FRDF	0.46±0.06*	67.33±6.65

*Indicates no significant differences at P≤0.05 with the normal control rats, All experiments are replicated for three times (n = 3)

VI) Antioxidant marker enzymes tests

A) Glutathione-S-transferase (GST)

Antioxidant enzymes are the indicator of health of tissue and organs in the body. For detoxification, metabolism, storage and excretion, liver and kidney are the vital organs involved and are particularly vulnerable to oxidative damage. The enzyme glutathione-S-transferase (GST) neutralises reactive oxygen species (ROS) by enzymatically conjugating with the peptide glutathione. Our study revealed a high GST activity in the liver compared to the kidney. The GST activity in the liver was found to be 363.33±25.15 µM/min/mg tissue protein in normal control group. In diabetic control groups, the activity rises to 582.41±15.36 µM/min/mg tissue protein showing almost 60% increase in the activity. Meanwhile, glibenclamide treated diabetic rat group showed decreased activity (471.58±17.49 µM/min/mg protein). Both the doses of the plant extract showed a decrease in GST activity compared to diabetic control. In 100 mg FRDF treated rat groups, the activity decreased upto 25% from diabetic control rat groups. Similarly, 200 mg FRDF treated rat group showed almost 35% decreased activity compared to diabetic control group (Table 4.29). Statistical analysis showed no significant difference between 200 mg FRDF treated group and normal control group (P≤0.05). In contrast to liver, GST activity in kidney was found to be lower in diabetic control (288.63±24.05 µM/min/mg protein) than that of normal control rats (385.67±19.25 µM/min/mg protein). There is a decrease of 25.16% GST activity in

diabetic control rat groups compared to the normal control rats. In glibenclamide treated rat groups, there is an increase of 9.15 % GST activity compared to diabetic control rats. 100 mg treated rats showed an increase of 6.05% GST activity compared to diabetic control rats. However, 200 mg FRDF treated rat groups had a higher GST activity than those of other diabetic rat groups ($P \leq 0.05$). There is an increase of 34.76% GST activity in 200 mg FRDF treated rat groups than that of the normal control rats almost returning to the normalcy. Also, 200 mg FRDF treated rats showed no difference with the normal control groups ($P \leq 0.05$) (Table 4.29).

B) Catalase

Catalase activity showed increased activity in diabetic rat group when compared to normal control groups. In liver tissues, an increase of 42.14% activity was observed compared to normal control. Upon treatment with standard drug and crude extracts the catalase activity was seen to be lower than that of the non- treated diabetic rats. No significant difference was observed for catalase activity between diabetic control rat group and 200 mg FRDF treated rat group. A significant difference was observed in gliblenclamide and 200 mg FRDF treated rat groups with diabetic control rat groups. There is a decrease of almost 25.35% in glibenclamide-treated rat group, when compared to the diabetic control rat group. In both the doses of FRDF (100 mg and 200 mg) treated rat groups, the catalase activity was found to be reduced (9.79% and 18.22% respectively) from the diabetic control rats. When compared to the normal control rats, glibenclamide treated rat group does not differ significantly at $P \leq 0.05$ level. 200 mg FRDF treated rat groups also showed a recovery of catalase activity showing only 18.22% rise from the normal control rat group.

In kidney, the highest catalase activity was observed in diabetic control rats while the lowest activity was observed in normal control rats. The activity rises up to 17% in diabetic control rats when compared to the normal control rats. However, after the treatment with standard chemical glibenclamide and two doses of FRDF the catalase activity reduced from the diabetic control rat groups. Glibenclamide treated rat group showed 7.6%, reduction from the diabetic control rat groups and overall increase of 8.6% from the normal control rat group. Meanwhile, 100 mg FRDF treated rat group showed 11.50% reduction from diabetic control rat group and only increase of about

4.10% from the normal control rat group. Similarly, 200 mg FRDF treated rat group showed a huge decrease of catalase activity from the diabetic control rat group (12.49%) and only 2.93% increase from the normal control rat group, almost returning to their normalcy (Table 4.29). Statistical analysis revealed that, the catalase activity of glibenclamide, 100 mg FRDF and 200 mg FRDF treated rat groups does not differ from the normal control rat group at $P \leq 0.05$.

C) Lipid Peroxidation Inhibition Assay

In liver, MDA level increases up to two-fold in diabetic control rat groups. The increase was seen to persist even after the treatment of glibenclamide with 117.19% increase from normal control rat groups which is 7.33% less than the rise of diabetic rat groups. However, in both the doses of FRDF, there is 44.75% and 60.18% decrease from diabetic control rat in 100 mg and 200 mg FRDF treated rat groups, respectively almost returning to normalcy. Statistical analysis showed that, at $P \leq 0.05$, MDA level of diabetic control rat groups significantly differ from normal control, 100 mg and 200 mg FRDF treated rat group. Additionally, the MDA level of glibenclamide and diabetic rat groups does not differ. Furthermore, 100 mg and 200 mg FRDF treated rat groups does not differ from normal control rat groups.

In kidney, in diabetic control groups, MDA level increases 254.94% from the normal control rat groups. The increase is lower in Glibenclamide treated rat groups with 78% from the normal control rat groups thereby decreasing about 49.85% from the diabetic rat group MDA level. In 100 mg FRDF treated rat groups; there is more decrease of MDA level (60.76%) when compared to the diabetic control rat group and less increase from the normal control group (39.28%). However, on the contrary to other treated rat groups, there is slight decrease of MDA level in 200 mg FRDF treated rat groups (8.74%). Statistical analysis showed that, normal control rat groups, 100 mg and 200 mg treated rat groups do not differ (Table 4.29).

Table 4.29 Antioxidant marker enzymes from tissue of normal and diabetic rats

Groups	GST (µM/min/mg protein)		CAT (µM/min/mg protein)		MDA (n mol^{-1}/mg protein)	
	Liver	Kidney	Liver	Kidney	Liver	Kidney
Control	363.33±25.15	385.67±19.25	31.92±1.20	79.53±5.12	58.04±3.52	153.35±8.26
Diabetic Control	582.41±15.36	288.63±24.05	45.38±3.31	93.56±2.05	115±1.81	544.31±30.31
Diabetic+ Glibenclamide	471.58±17.49	315.061 ±10.96	33.87±2.44*	86.41±1.85*	114±0.004	272.96±46.07
Diabetic + 100 mg FRDF	433.14±29.40	306.1224±10.69	40.93±1.26	81.86±1.93*	65.60±5.46*	213.59±6.70*
Diabetic + 200 mg FRDF	375.05±25.44 *	388.98±19.28*	39.45±1.28	81.86±1.93*	47.01±6.91*	139.94±11.06*

GST- Glutathione-S-transferase; CAT- Catalase, MDA – Malondialdehyde'*'Indicates no significant differences at P≤0.05 ** with the normal control rats

VII) Histological study

Histology study provides the knowledge of microscopic anatomy and visualization of structures of tissue and changes it might have undergone during the experiment. Histology plays a crucial role in knowing the amount of damage and features of healing of tissues before and after the application of drugs. In our study, tissue sections of liver and kidney was examined using H and E staining in normal and diabetic rat groups. The microscopic liver architecture of normal control rats showed general structures preserved. In the hepatic parenchyma of normal control rats, normal hepatic lobules are observed, normal hepatocytes are surrounded by sinusoids which are radially arranged around a central vein containing Kupffer cells and red blood cells (Figure 4.35 a). Upon examination of liver cells of diabetic rat groups, numerous pathological alterations were visible including distortion of normal architecture of liver, irregular arrangement of hepatocytes, enlargement of sinusoidal space, dilated sinusoids, and deposition of collagen was observed (Figure 4.35 b). In case of glibenclamide treated STZ rats, dilation of hepatic lobules, sinusoids and loss of organ structure was observed. The hepatocytes were swollen, inflammatory cells and steatosis is observed (Figure 4.35 c). Meanwhile no fatty deposition was observed. In 100 mg FRDF treated rat groups, no swollen hepatocytes were observed, and however restoration of the normal architecture of the liver was observed. Fatty degeneration was still seen to be present even after the treatment of the rats with 100 mg FRDF for 28 days. Sinusoids were seen to be normal (Figure 4.35 d). In 200 mg FRDF treated rat groups; restoration of normal architecture of liver was seen after 28 days of treatment. Hepatocytes distributed radially towards the centrilobular veins. Decrease of collagen deposits were also observed in 28 days of treatment (Figure 4.35 e).

Histological study of kidney of normal control rat group revealed normal proximal and distal convoluted tubule, Glomerulus surrounded by Bowman’s capsule, normal parietal and visceral layer of Glomerulus and normal Bowman’s space (Figure 4.36 a). In STZ diabetic rats, shrinkage of glomerulus, distorted visceral layer of glomerulus, slightly thickened parietal layer and basement membrane was observed (Figure 4.36 b). In Glibenclamide treated rat group, distorted visceral layer of glomerulus was not seen. However, the glomerulus was seen to be degenerated and the Bowman’s space was seen to be enlarged but less than the diabetic control rat group. No

thickening of parietal layer was observed in glibenclamide treated rat group (Figure 4.36 c). In 100 mg FRDF treated rat group, thickening of the parietal layer, enlarged Bowman's space was still observed even after the treatment of rats with 100 mg FRDF for 28 days. However, the visceral layer was seen to be normal when compared with the normal control rat group. The group treated with 200 mg FRDF showed features of healing i.e. normal parietal and visceral layer of glomerulus, normal glomerulus, and normal Bowman's space.

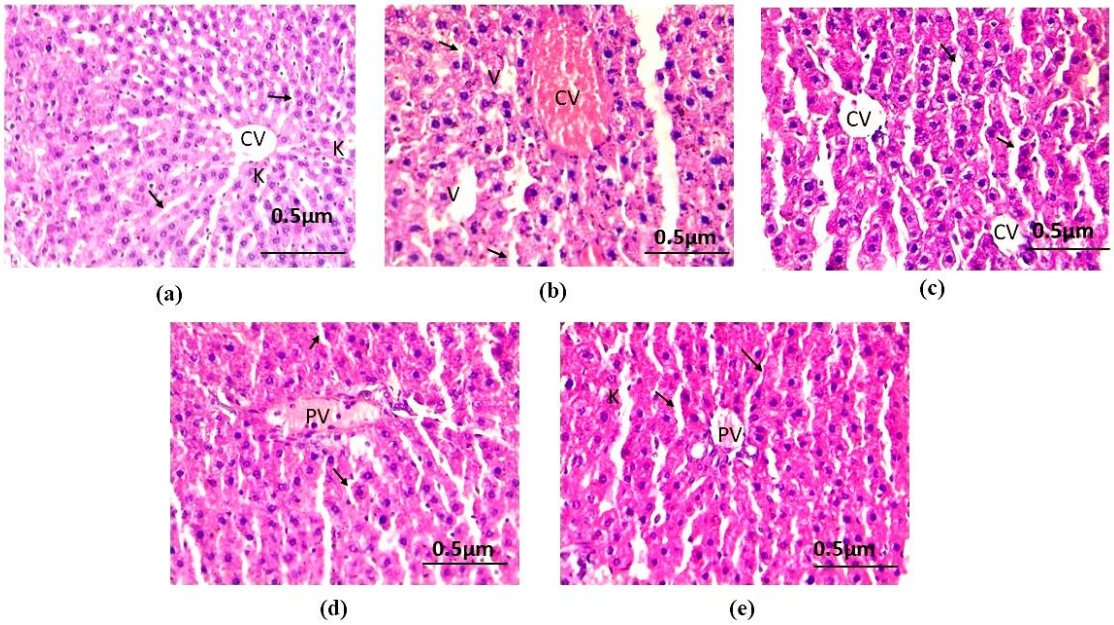

Figure 4.35. Histological pattern of liver in experimental rats in 40x magnification, H&E stain. a) normal control rats b) diabetic control rats. c) diabetic rats treated with glibenclamide d) diabetic rats treated with 100 mg FRDF e) Diabetic rats treated with 200 mg FRDF. 'v' vacuolation; '□' sinusoids; 'K' Kupffer cells; CV- Central vein; PV- Portal vein; '*' represents inflammatory cells and steatosis.

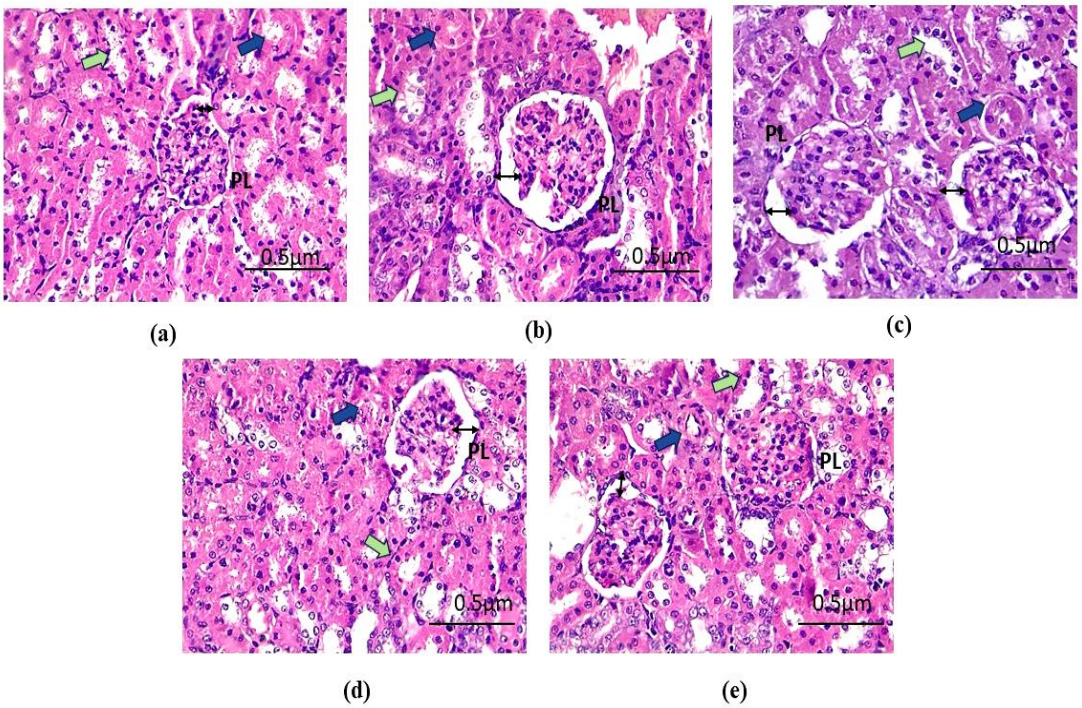

Figure 4.36. Morphological changes in kidney in experimental rats 40x magnification H&E stain. a) Normal control rats b) Diabetic control rats. c) Diabetic rats treated with glibenclamide d) Diabetic rats treated with 100 mg FRDF e) Diabetic rats treated with 200 mg FRDF. Green arrow- Distal convoluted tubule; blue arrow- Proximal convoluted tubule; PL- Parietal layer of glomerulus; '↔' represents Bowman's space.