Chapter 4

RESULTS

4. RESULTS

4.1 SURVEY AND DOCUMENTATION OF ETHNOMEDICINE USED BY THE TRADITIONAL HERBAL PRACTITIONERS

Sikkim has four districts viz. East, West, North and South. The area of our study covers the West district since it was the least explored district in terms of ethnomedicinal survey. This district comprises of four sub divisions, viz. Gyalshing, Soreng, Dentam and Yuksom. Some villages were shortlisted from these areas which is given in table 4.1.1. The image of the villages where the survey was conducted along with the verbal interaction with the herbal practitioners is presented in figure 4.1.1.a to 4.1.1.d. The list of herbal practitioners who were interviewed is given in table 4.1.1 along with their gender and age. Due to advancement of modern medication and establishment of primary healthcare centers nearby, it was observed that traditional herbal therapy was depleting gradually.

In total, 14 traditional practitioners in total agreed to be interviewed. Out of them, 28.5% were female and 71.43% were male (figure 4.1.2). It was observed that maximum numbers of the healers were elderly people (senior citizens) above the age of 60 years old with 42.86 % falling between the ages of 60-69 years and 35.75 % between 70-79 years. The younger generation was observed to be less interested in the traditional medicine. The educational qualification of the herbal healers is an important aspect as it is necessary to be aware about pros and cons of the use of medicinal plants as well as to understand the role of ethnomedicine in the modern world. From figure 4.1.2, we could also analyze that most of the herbal healers were illiterate (64.29%) and those who had studied were also not above the secondary level. Since the practice of traditional therapy has less scope for income, we also surveyed the occupation of the healers. It was found that most of them were farmers (51.12%) and were dependent on agriculture to earn for their living (figure 4.1.3). All the female practitioners appeared to be homemakers (28.57 %). Only one person among them had a government job and one was a restaurant owner.

From the survey, 36 medicinal plants were used which we collected under the supervision of the herbal healers. These plants were identified and the

herbariums were prepared which are presented in figure 4.1.4 and 4.1.5. There were ethnomedicines which could also be stored in dry powder form for few months while some of the formulations were prepared instantly and used in fresh condition. Total 46 herbal formulations were documented out of which 9 were polyherbal and 37 were monoherbal formulations (table 4.1.2). All the medicinal plants belonged to 29 different families (figure 4.1.6) with each of the plants belonging to each different family except for Polygonaceae, Oleaceae, Combretaceae and Asparagaceae with 2 medicinal plants in each of these families. To prepare these formulations, different parts of the plants were used like roots (48 %), shoot (19%), whole plant (13%), leaf (13%), flower, bark and seed with 2% each (figure 4.1.7). All of these formulations had different mode of administration which is illustrated in figure 5.1.8. Most of the formulations were used in the form of juice (29.73%) after which they were used in powder form (27.03%), 18.92% were used in their fresh or raw form, 16.22% were used as paste, 5.41 % were used after decoction and 2.70% were used after infusion. All the ailments cured by the herbal healers of West Sikkim were categorized in 19 numbers in which it was observed that there were majority of 6 formulations for gastritis and stomach related disorders (table 4.1.3). There were other ailments such as tonsillitis (5 formulations), arthritis and bone related disorders (4 formulations), food poisoning (3 formulations) etc. All the plants involved in preparation of these formulations were collected for identification and accession numbers were taken. Depending upon their effectiveness in traditional system of medicines as suggested by the herbal healers, some of the formulations (both polyherb and monoherb) were shortlisted for literature review. Only those formulations were selected which lack appropriate scientific study. The selection of the formulations for further study was also dependent on the sufficient availability of the plant parts used for the preparation of ethnomedicines used by the healers.

After all the study, 11 formulations were selected, out of which 4 were polyherbal formulations and 7 were monoherbal formulations. The list of selected formulations is given in table 4.1.4 along with the diseases involved and each of the formulations was given a particular abbreviations.

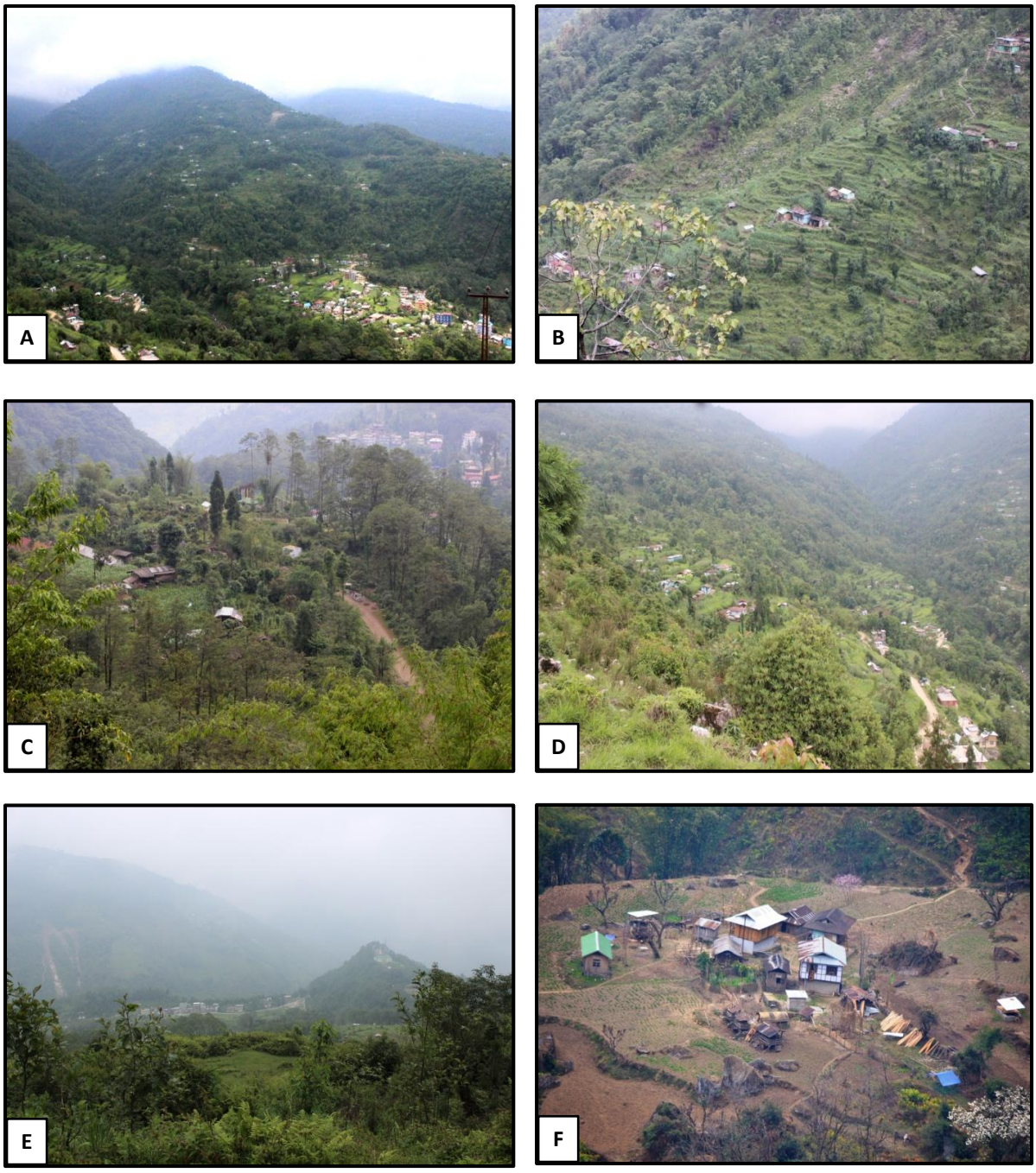


Figure 4.1.1.a: Areas under survey. **A**: Dentam Tar; **B**: Sankhu; **C**: Yuksom; **D**: Hee Yangthang; **E**: Uttarey; **F**: Tesenthang

Figure 4.1.1.b: Areas under survey (**A**: Malbasey; **B**: Darap) and the herbal practitioners who were interviewed for traditional knowledge of herbal medicines (**C**: Mr. Nar Bahadur Subba; **D**: Mr. Suk Bahadur Subba; **E**: Mr. Monarath Dahal; **F**: Mr. Tulshi Ram Sharma)

Figure 4.1.1.c: The herbal practitioners who were interviewed for traditional knowledge of herbal medicines. **A**: Buddhi Maya Subba; **B**: Dilmaya Subba; **C**: Yadu Ram Chettri; **D**: Bahadur Basnett; **E**: Lek Bahadur Subba; **F**: Ram Maya Basnett.

Figure 4.1.1.d: Herbal practitioners (**A**: Lt. Phiprani Subba; **B**: Lt. Lakpa Lepcha); **C** and **D**: Conservation of medicinal plants in nursery; **E**: Herbal formulations stored in powder form; **F**: Traditional wooden grinder used to crush / grind medicinal plants for preparation of herbal formulations.

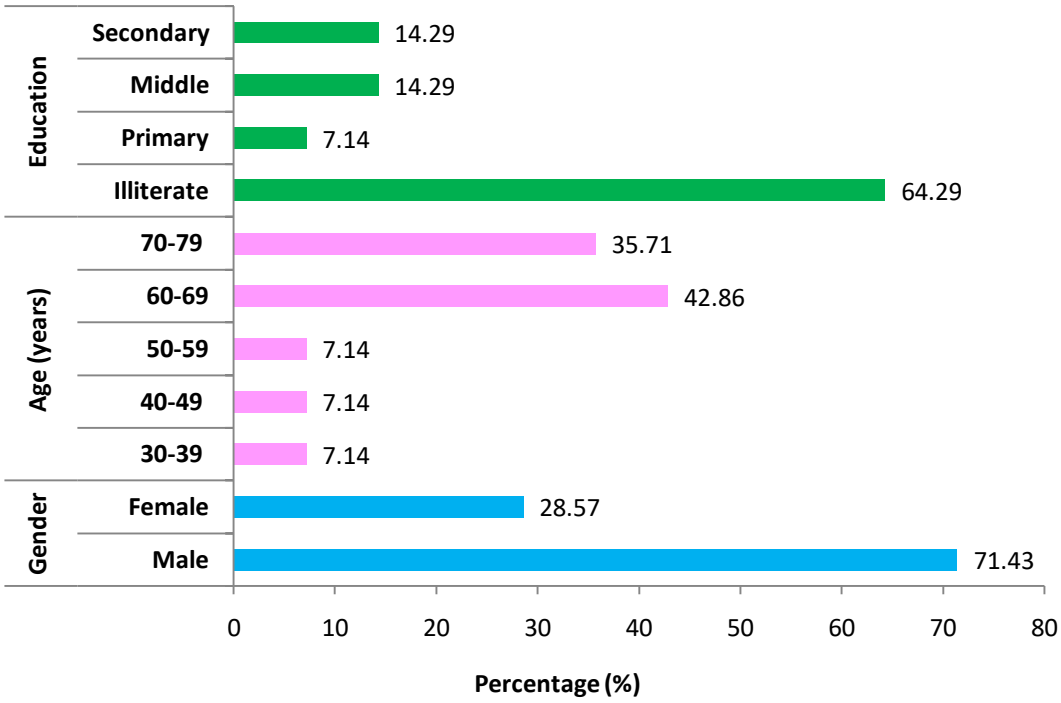


Figure 4.1.2: Information of the traditional herbal practitioners about their age, gender and educational qualifications

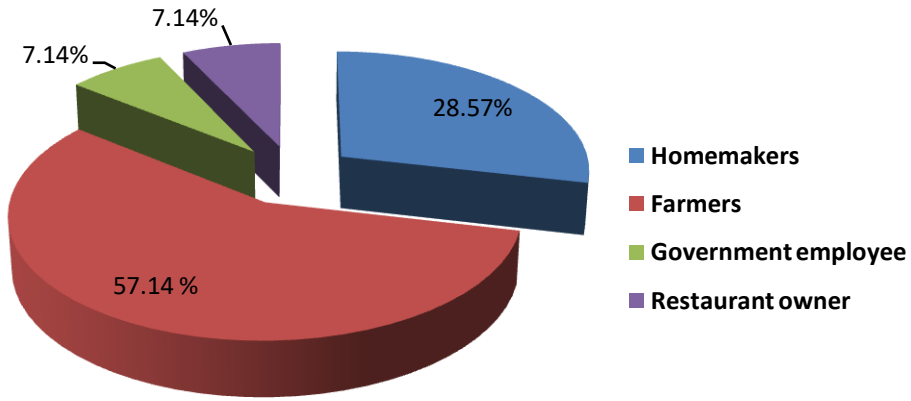


Figure 4.1.3: Occupational status of the herbal practitioners

Achyranthes aspera L.

Aconitum heterophyllum Wall. ex. Royle

Asparagus filicinus Buch.-Ham. ex. D.Don

Asparagus officinalis L.

Astilbe rivularis Buch.-Ham. ex D. Don

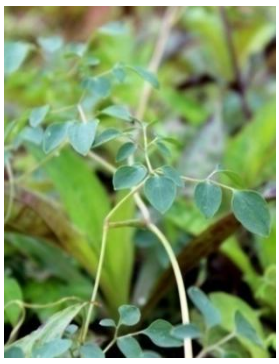
Berberis asiatica Roxb. ex DC.

Boenninghausenia albiflora (Hook.) Rchd. ex Meisn.

Clematis buchanania DC.

Costus speciosus (J.Koenig)Sm.

Cynodon dactylon (L.) Pers.



Dicentra scandens (D.Don)Walp.

Drymaria diandra Blume

Fallopia convolvulus (L)A. Love

Fraxinus floribunda Wall.

Gloriosa superba L.

Continued...

Gonostegia hirta (Blume ex Hassk.)Mq.

Hedyotis scandens Roxb.

Houttuynia cordata Thunb.

Malvaviscus arboreus Cav.

Nyctanthes arbor-tristis L.

Ocimum americanum L.

Paris polyphylla Sm.

Phytolacca acinosa Roxb.

Plantago erosa Wall.

Plumbago zeylanica L.

Rheum acuminatum Hook. f. & Thomson

Rubia cordifolia L.

Rubus ellipticus Sm.

Saurauia napaulensis DC.

Stephania glabra (Roxb.) Miers

Terminalia bellirica (Gaertn.) Roxb.

Continued…

Terminalia chebula Retz.

Trigonella foenum-graecum L.

Valeriana jatamansi Jones

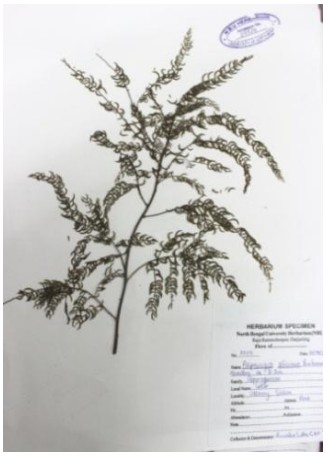
Viscum articulatum Burm. f.

Zingiber officinale Rosc.

Figure 4.1.4.: Medicinal plants collected from the survey area which were used to prepare the herbal formulations

Achyranthes aspera L.

Aconitum heterophyllum Wall. ex. Royle



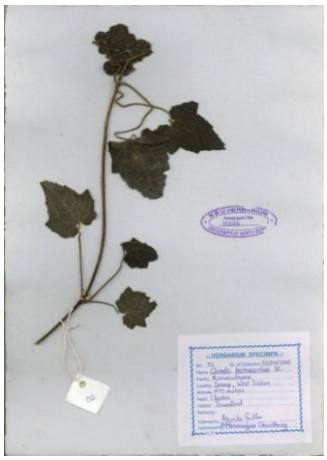
Asparagus filicinus Buch.-Ham. ex. D.Don

Asparagus officinalis L.

Astilbe rivularis Buch.-Ham. ex D. Don

Berberis asiatica Roxb. ex DC.

Boenninghausenia albiflora (Hook.) Rchd. ex Meisn.



Clematis buchanania DC.

Costus speciosus (J.Koenig)Sm.

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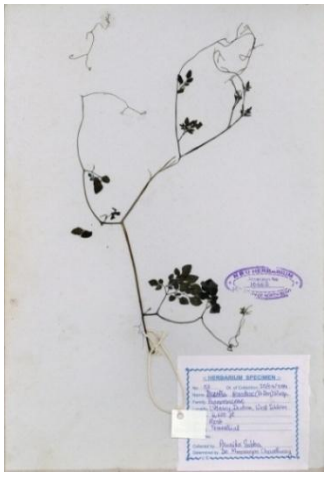
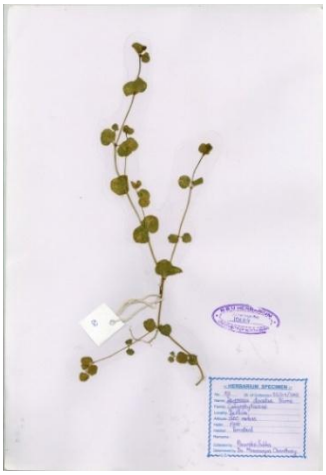
Cynodon dactylon (L.) Pers.

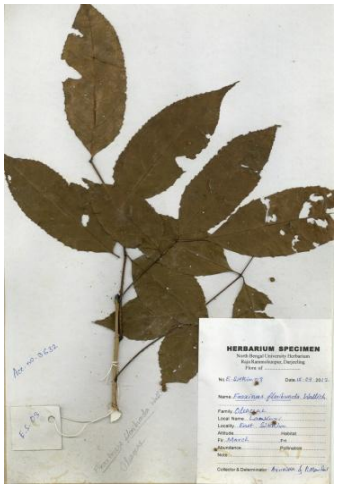


Dicentra scandens (D.Don)Walp.

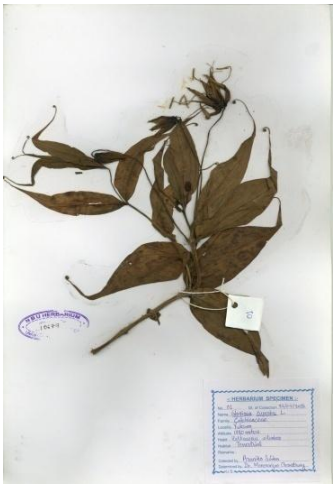


Drymaria diandra Blume

Fallopia convolvulus (L)A. Love



Fraxinus floribunda Wall.



Gloriosa superba L.

Gonostegia hirta (Blume ex Hassk.)Mq.

Hedyotis scandens Roxb.

Houttuynia cordata Thunb.

Continued….

Malvaviscus arboreus Cav.

Nyctanthes arbor-tristis L.

Ocimum americanum L.

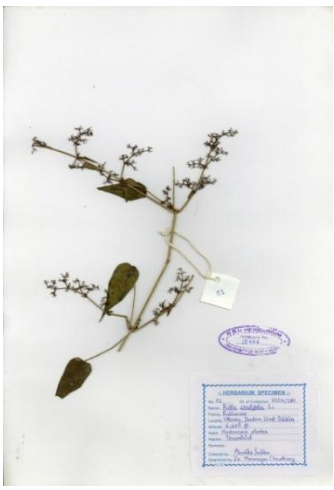
Paris polyphylla Sm.

Phytolacca acinosa Roxb.

Plantago erosa Wall.

Plumbago zeylanica L.

Rheum acuminatum Hook. f. & Thomson



Rubia cordifolia L.

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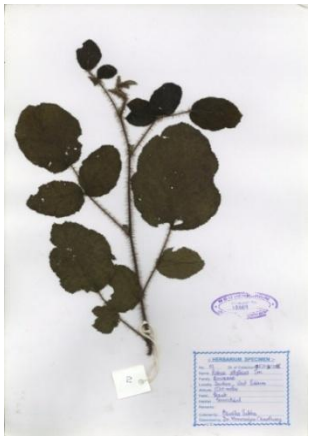
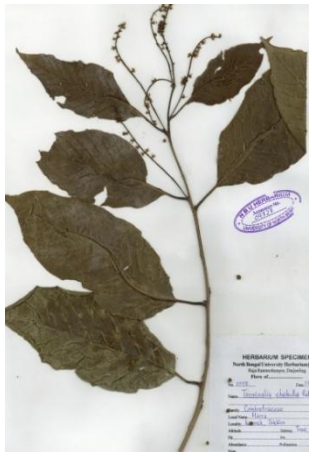


Rubus ellipticus Sm.

Saurauia napaulensis DC.

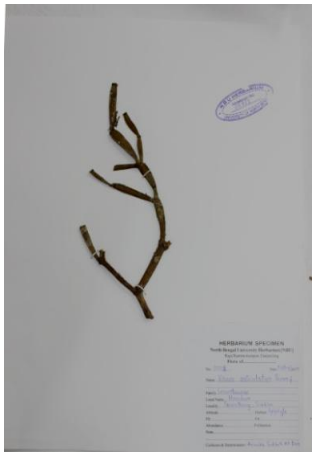
Stephania glabra (Roxb.) Miers

Terminalia bellirica (Gaertn.) Roxb.



Terminalia chebula Retz.

Valeriana jatamansi Jones



Viscum articulatum Burm. f.

Figure 4.1.5.: Digital herbarium with authenticated accession numbers of medicinal plants collected during survey submitted at the Taxonomy & Environmental Biology Laboratory, Department of Botany, University of North Bengal

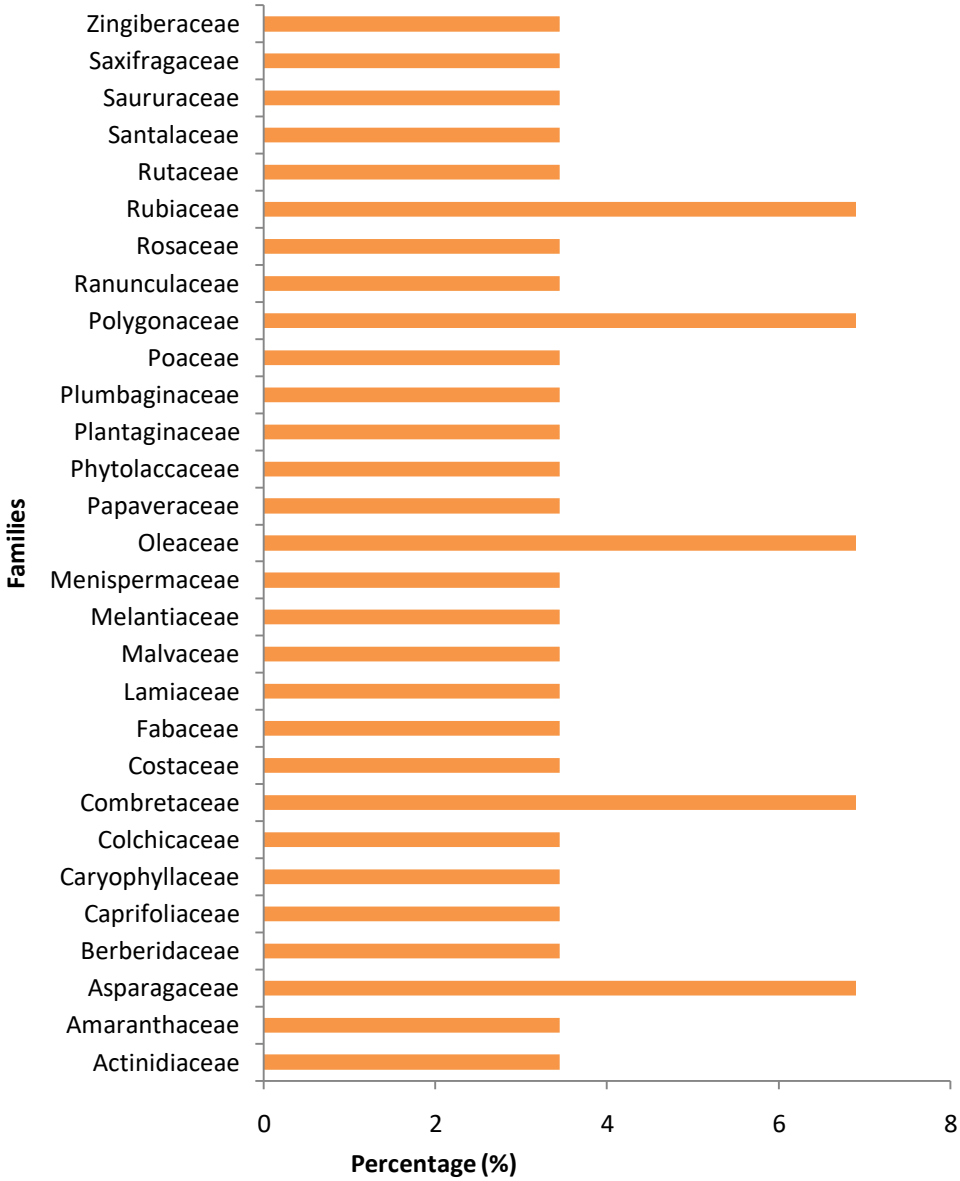


Figure 4.1.6: List of the families of all the medicinal plants collected during survey used for the preparation of herbal formulations

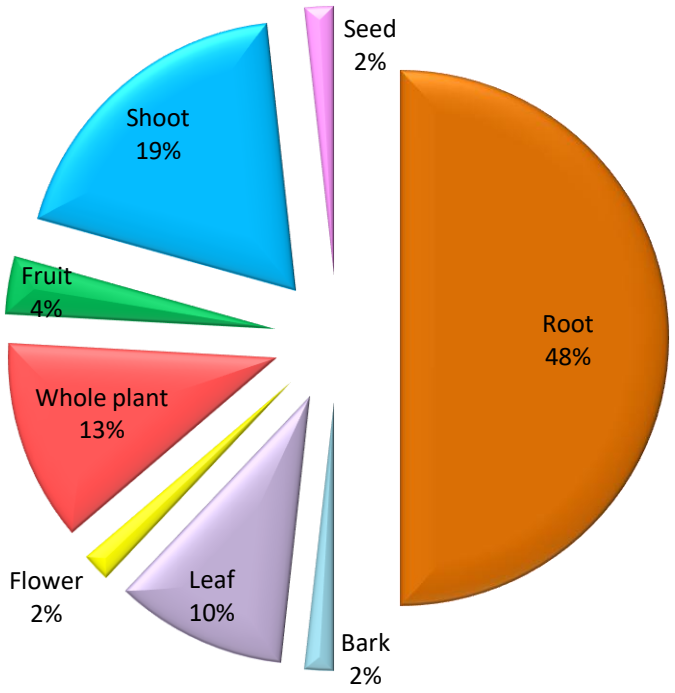


Figure 4.1.7: Plant parts used for the preparation of herbal formulations

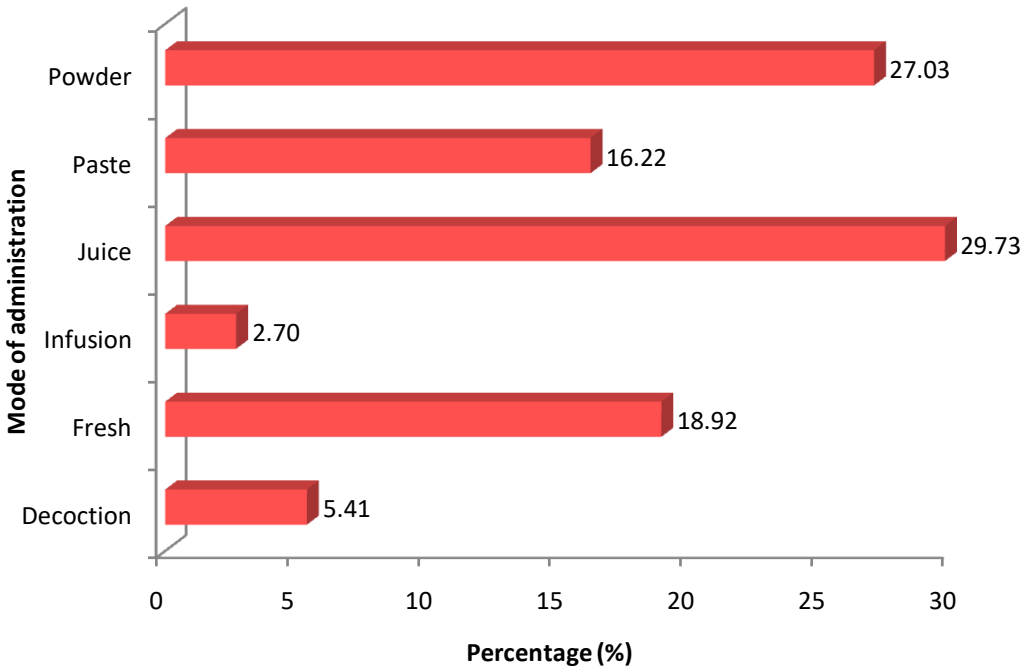


Figure 4.1.8: Mode of administration of the herbal formulations

Table 4.1.1: List of the herbal healers interviewed from the different villages of West Sikkim

Block	Village	Name of herbal practitioners	Gender	Age
Dentam	Dentam Tar	Lakpa Lepcha	Male	70
	Dentam Tar	Monarath Dahal	Male	65
	Sankhu	Tulshi Ram Sharma	Male	60
	Hee Yangthang	Phiprani Subba	Female	72
	Uttarey	Nar Bahadur Subba	Male	61
Yuksom	Yuksom busty	Mangal Bir Subba	Male	60
Soreng	Soreng bazar	Yadu Ram Chettri	Male	36
	Malbasey	Ram Maya Basnett	Female	73
	Malbasey	Lek Bahadur Subba	Male	61
	Malbasey	Bahadur Basnett	Male	60
Gyalsing	Darap	Suk Bahadur Subba	Male	59
	Darap	Phul Bir Limbu	Male	73
	Darap	Buddhi Maya Subba	Female	40
	Tesenthang	Dil Maya Subba	Female	70

Table 4.1.2: List of ethnomedicine used by the herbal practitioners of West Sikkim

Sl. No.	Ailment	No. of plants used	Scientific name and accession number	Family	Local name	Part used	Mode of treatment
1	Acidity	1	*Fallopia convolvulus* (L)A. Love 09720	Polygonaceae	Rato tamarke	Root	Root is washed, cut into small pieces and crushed into powder and consumed with water
2	Acne	1	*Rubia cordifolia* L. 10666	Rubiaceae	Majito	Shoot along with leaf	Stem and leaf of the plant is dried and crushed to powder. The dried powder is soaked in a small amount of water to make a paste which is applied on the affected area.
3	Arthritis	3	a) *Viscum articulatum* Burm. f. 09727	a) Santalaceae	a) Harchur	a) Whole plant	The parts of all the three plants are washed thoroughly, cut into small pieces and sundried. The dried parts are then crushed to powder form.
			b) *Rheum acuminatum* Hook. f. & Thomson 6501	b)Polygonaceae	b) Khokim	b) Root	
			c) *Astilbe rivularis* Buch.-Ham. ex D. Don 6502	c) Saxifragaceae	c) Buro okhati	c) Rhizome	
4	Asthma	2	a) *Asparagus filicinus* Buch.-Ham. ex. D.Don 09724	a) Asparagaceae	a) Sotar	a) Root	Roots of the plants are washed, cut and sundried. The dried parts are then ground to powder form and consumed with water.
			b) *Fallopia convolvulus* (L)A. Love 09720	b) Polygonaceae	b) Rato tamarke	b) Root	

5	Black fever	1	*Aconitum heterophyllum* Wall. ex Royle 10673	Ranunculaceae	Bikhuma	Root	The roots are cleaned and cut into small pieces and dried to be stored.
6	Bodyache	1	*Astilbe rivularis* Buch.-Ham. ex D. Don 6502	Saxifragaceae	Buro okhati	Rhizome	The rhizome is cleaned, the outer layer is peeled off and the inner part is chewed.
7	Cuts and wounds	3	a) *Plantago erosa* Wall. 10667	a) Plantaginaceae	a) Makai jhar	a) Shoot	All the plant parts mentioned are crushed and applied on the cut areas and covered with a cloth.
			b) *Drymaria diandra* Bl. 10664	b) Caryophyllaceae	b) Abijalo	b) Shoot	
			c) *Cynodon dactylon* (L.) Pers. 10680	c) Poaceae	c) Dubo	c) Whole plant	
8	Cuts and wounds	1	*Gonostegia hirta* (Blume ex Hassk.)Mq. 10670	Urticaceae	Chipley jhar	Leaf	The fresh leaves are crushed and applied on the cut area and covered with a cloth.
9	Cuts and wounds	1	*Gonostegia hirta* (Blume ex Hassk.)Mq. 10670	Urticaceae	Chipley jhar	Whole plant	The fresh plant is taken and crushed to obtain juice which is applied on the affected area for small cuts and wounds.

10	Diabetes	1	*Fraxinus floribunda* Wall. 9632	Oleaceae	Lakuri	Bark	The bark of the plant is washed in tap water, cut into small pieces and boiled in water. The aqueous decoction of the bark is consumed orally.
11	Diabetes	1	*Nyctanthes arbor-tristis* L. 10660	Oleaceae	Parijat	Leaves	Fresh leaves are washed and crushed to obtain juice which is consumed orally.
12	Food poisoning	1	*Plumbago zeylanica* L. 10659	Plumbaginaceae	Dudey	Root	Root of the plant is washed thoroughly and dried under sunlight which is later crushed into powder form to be consumed orally with water.
13	Food poisoning	3	a) *Hedyotis scandens* Roxb. 10657	a) Rubiaceae	a) Kali lahara	a) Root	Roots of all the three plants are taken in equal proportion, washed properly and crushed to obtain juice. The freshly prepared juice is consumed orally.
			b) *Aconitum heterophyllum* Wall. ex Royle 10673	b) Ranunculaceae	b) Bikhuma	b) Root	
			c) *Phytolacca acinosa* Roxb. 10663	c) Phytolaccaceae	c) Jaringo	c) Root	
14	Food poisoning	1	*Aconitum heterophyllum* Wall. ex Royle 10673	Ranunculaceae	Bikhuma	Root	The cut pieces are then dried and stored to be used.

15	Gastritis	4	a) *Terminalia chebula* Retz. 09727	a) Combretaceae	a) Harra	a) Fruit	All the plant parts are washed and dried completely. They are crushed to powder in okhli (a wooden traditional grinder). The powder is consumed orally with warm water.
			b) *Terminalia bellirica* (Gaertn.) Roxb. 09726	b) Combretaceae	b) Barra	b) Fruit	
			c) *Zingiber officinale* Rosc.	c) Zingiberaceae	c)Adhuwa	c) Rhizome	
			d) *Trigonella foenum-graecum* L.	d) Fabaceae	d)Methi	d) Seed	
16	Gastritis	1	*Rubus ellipticus* Sm. 10669	Rosaceae	Aiselu	Tender shoot	The tender shoot of the plant is taken and the outer layer is peeled and the inner part is chewed to consume the juicy part.
17	Heart palpitation	2	a) *Asparagus filicinus* Buch.-Ham. ex. D.Don. 09724	a) Asparagaceae	a) Sotar	a) Root	Roots of both the plants are washed, dried, ground into powder and mixed together into 1:1 ratio and orally consumed with warm water.
			b) *Asparagus officinalis* L. 09723	b) Asparagaceae	b) Kurilo	b) Root	
18	High blood pressure	1	*Berberis asiatica* Roxb. ex DC. 09722	Berberidaceae	Tinsurey	Whole plant	Whole plant is washed and cut into small pieces. After complete drying under sunlight, the plant is crushed into fine powder. The powder is consumed with water.
19	High blood pressure	1	*Nyctanthes arbor-tristis* L. 10660	Oleaceae	Parijat	Leaves	Fresh leaves of the plant were soaked in water. The juice obtained is consumed orally.

20	Indigestion, dysentery	1	*Valeriana jatamansi* Jones 10675	Caprifoliaceae	Jatamansi	Root	The dried roots are crushed to powder which is consumed orally.
21	Indigestion	1	*Hedyotis scandens* Roxb. 10657	Rubiaceae	Kali lahara	Root	The roots are washed and cut into small pieces and dried.
22	Jaundice	1	*Stephania glabra* (Roxb.) Miers 10661	Menispermaceae	Pahelo tamarke	Root	Root is cut into small pieces and dried which is chewed directly.
23	Jaundice	1	*Boenninghausenia albiflora* (Hook.) Rchd. ex Meisn. 09724	Rutaceae	Mirmirey jhar	Whole plant	Fresh plant is washed and crushed with little amount of water. The juice obtained is orally consumed.
24	Jaundice	1	*Boenninghausenia albiflora* (Hook.) Rchd. ex Meisn. 09724	Rutaceae	Mirmirey jhar	Whole plant	Fresh plant is washed and crushed with little amount of water. The juice obtained is orally consumed.
25	Jaundice	1	*Stephania glabra* (Roxb.)Miers 10661	Menispermaceae	Pahelo tamarkey	Twig	The twigs are washed and chewed
26	Loss of appetite	1	*Rubia cordifolia* L. 10666	Rubiaceae	Meda	Stem	Stem of the plant is washed and dried under sunlight. The dried stem is crushed to powder which is consumed orally.

27	Pneumonia	3	a) *Drymaria diandra* Blume 10664	a) Caryophyllaceae	a)Abijalo	a)Whole plant	All the fresh plant parts are washed and crushed to obtain juice which is consumed orally.
			b) *Achyranthes aspera* L. 10658	b) Amaranthaceae	b)Rato aankhley	b) Root	
			c) *Plantago erosa* Wall. 10667	c) Plantaginaceae	c) Kaney jhar	c) Root	
28	Pneumonia	1	*Drymaria diandra* Blume 10664	Caryophyllaceae	Abijalo	Root	Fresh roots are washed, crushed and soaked in water for few hours. The root is consumed along with water.
29	Septic	1	*Gloriosa superba* L. 10677	Colchicaceae		Root	Fresh root is collected and crushed to make paste which is applied on the affected area and is bandaged.
30	Sinusitis	1	*Clematis buchanania* DC. 10656	Ranunculaceae	Pinase lahara	Stem	The outer layer of the stem is peeled off and the inner part is cleaned and chewed in raw form
31	Skin allergy	1	*Ocimum americanum* L. 10681	Lamiaceae	Babari	Shoot	The fresh shoot is crushed to make a paste and applied on the skin at the affected area.

32	Skin infection	1	*Houttuynia cordata* Thunb. 10678	Saururaceae	Gandey jhar	Leaves	Fresh leaves are crushed and applied on the affected area and covered with a cloth.
33	Skin rashes in infants	1	*Drymaria diandra* Blume 10664	Caryophyllaceae	Abijalo	leaves	The fresh leaves are crushed applied on the affected area.
34	Sprain	2	a) *Rheum acuminatum* Hook. f. & Thomson 6501	a) Polygonaceae	a) Khokim	a) Rhizome	The parts of these two plants are dried and ground to powder form and is consumed orally.
			b) *Viscum articulatum* Burm. f. 09727	b) Santalaceae	b) Harchur	b) Whole plant	
35	Stomachache	1	*Saurauia napaulensis* DC. 10668	Actinidaceae	Gagoon	Tender shoot	The bark of the tender shoot is removed and the inner shoot is chewed and the juice is swallowed.
36	Sprain	1	*Rheum acuminatum* Hook. f. & Thomson 6501	Polygonaceae	Khokim	Rhizome	Root is dried and ground to powder and stored for use.
37	Throat pain	1	*Rubus ellipticus* Sm. 10669	Rosaceae	Aiselu	Root	Root of the plant is washed, crushed and boiled to obtain decoction.

38	To boost the immune system	1	*Paris polyphylla* Sm. 10674	Melanthiaceae	Paris	whole plant	Whole plant is washed and crushed to obtain juice which is orally consumed. Mode of treatment: The juice is obtained once a day.
39	Tonsilitis	1	*Boenninghausenia albiflora* (Hook.) Rchd. ex Meisn. 09724	Rutaceae	Mirmirey	Root	Root of the plant is washed, cut into small pieces and sun dried until the moisture is completely drained off. The dried pieces are chewed and swallowed without water.
40	Tonsilitis	1	*Achyranthes aspera* L. 10658	Amaranthaceae	Rato aankhley	Root	The fresh roots of the plant are cleaned and chewed to swallow the juice.
41	Tonsilitis	1	*Rubus ellipticus* Sm. 10669	Rosaceae	Aiselu	Tender shoot	The tender shoot of the plant is taken and the outer layer is peeled and the inner part is chewed to consume the juicy part.
42	Tonsilitis	3	a) *Achyranthes aspera* L. 10658	a) Amaranthaceae	a) Rato ankhley	a) Root	Plant parts are washed and crushed to make paste. The paste is soaked in water for 2-3 hours and filtered through strainer. The juice is consumed orally.
			b) *Rubus ellipticus* Sm. 10669	b) Rosaceae	b) Aiselu	b) Root	
			c) *Drymaria diandra* Blume 10664	c) Caryophyllaceae	c) Abijalo	c) Leaf	
43	Toothache	1	*Malvaviscus arboreus* Cav. 10665	Malvaceae	Rato phool	Flower	Fresh flower is chewed.

44	Ulcer in throat or stomach	1	*Dicentra scandens* (D.Don)Walp. 10662	Papaveraceae	Angurey	Root	Root of the plant is washed in tap water and dried under sunlight. The dried root is crushed to powder and consumed with water
45	Urinary infection	1	*Costus speciosus* (J.Koenig)Sm. 10682	Costaceae	Bet lauri	Stem	Stem is crushed with water and the juice is consumed orally.
46	Urinary tract infection	1	*Costus speciosus* (J. Koeing)Sm. 10682	Costaceae	Bet lauri	Root	Root is washed and crushed then soaked in water for a night. The juice is taken orally.

Table 4.1.3: List of number of formulations used for the treatment of various ailments by the traditional healers of West Sikkim

Sl. NO.	LIST OF AILMENTS	NUMBER OF FORMULATIONS
1	Gastritis, stomachache and indigestion	6
2	Sore throat and tonsilitis	5
3	Arthritis, body ache and sprain	4
4	Food poisoning	3
5	Skin diseases	3
6	High blood pressure	2
7	Urinary infection	2
8	Cuts and wounds	2
9	Diabetes	2
10	Toothache	1
11	Heart palpitation	1
12	Pneumonia	1
13	Septic	1
14	Loss of appetite	1
15	Weak immune system	1
16	Jaundice	1
17	Asthma	1
18	Sinusitis	1
19	Black fever	1

Table 4.1.4: List of the herbal formulations selected for analysis

SL NO	AILMENTS	NAME OF THE PLANTS	ABBREVIATION
1	Heart palpitation	*i) Asparagus filicinus* Buch.-Ham. ex. D.Don *ii) Asparagus officinalis* L.	HP
2	Arthritis	*i) Viscum articulatum* Burm. f. *ii) Rheum acuminatum* Hook. f. & Thomson *iii) Astilbe rivularis* Buch.-Ham. ex D. Don	AR
3	Gastritis	*i) Terminalia chebula* Retz. *ii) Terminalia bellirica* (Gaertn.) Roxb. *iii) Zingiber officinale* Rosc. *iv) Trigonella foenum-graecum* L.	GS
4	Asthma	*i) Asparagus filicinus* Buch.-Ham. ex. D.Don *ii) Fallopia convolvulus* (L.) A. Love	AS
5	High blood pressure	*Berberis asiatica* Roxb. ex DC.	BP
6	Food poisoning	*Plumbago zeylanica* L.	FP
7	Tonsilitis	*Boenninghausenia albiflora* (Hook.) Rchd. ex Meisn.	TS
8	Diabetes	*Fraxinus floribunda* Wall.	FF
9	Indigestion and stomachache	*Hedyotis scandens* Roxb.	HS
10	Sinusitis	*Clematis buchanania* DC.	CB
11	Throat infection	*Achyranthes aspera* L.	AA

4.2.PHARMACOGNOSTIC STUDY

4.2.1Organoleptic study

Organoleptic test is a basic and a simple parameter to authenticate any herbal formulations in their powder form by merely using our senses. The result of organoleptic test of powder formulations is given in figure 4.2.1 and table 4.2.1.

4.2.2 Powder microscopy

Power microscopy is another simple method to authenticate any crude drugs in their powder form. Under a compound microscope, various plant tissues can be observed with or without the treatmentthrough particular staining procedure. From the results, sample HP revealed the presence of collenchyma, some starch granules, spiral xylem vessels, stone cells and cork cells (figure 4.2.2.a). In figure 4.2.2.b, powder microscopy of AR revealed the presence of spiral xylem vessels, medullary rays with fibers and starch grainswith many clusters of calcium oxalate crystals prominently scattered. Sample AS showed the presence of plenty of sclereids and some crystals, epidermal cells, stone cells and parenchymatous cells were also observed (figure 4.2.2.c). Sample GS showed the presence of parenchymatous cells, xylem vessels, stone cells, starch grains and crystals (figure 4.2.2.d). There were plenty of sclereids in sample BP along with cork cells, parenchymatous cells, stone cell, xylem vessels and starch granules (figure 4.2.2.e). In sample TS, starch granules were abundantly scattered and some xylem vessels as well as stones cells were also observed (figure 4.2.2.f). Sample FP revealed the presence of scalariform tracheid stained with phluroglucinol, stone cells, pitted vessels, starch grains, and oil globule (figure 4.2.2.g). In sample FF, sclereids, stone cells, cork cells and medullary rays were observed (figure 4.2.2.h). Powder microscopy of AA is presented in figure 4.2.2.i, which showed the presence of stone cells, numerous pitted vessels, starch grains, calcium oxalate crystals in cluster form and some phloem fibers. Powder microscopy of CB showed the presence of starch granules with prominent hilum, spiral xylem vessels, trichome, phloem fibers and parenchymatous cells (figure 4.2.2.j). In figure 4.2.2.k, powder microscopy of HS revealedthe presence of starch grains, xylem vessels, stone cells, pitted vessels and parenchymatous cells.

Figure 4.2.1: Herbal formulations in dry powder form for organoleptic study.
HP: heart palpitaion; **AR**: Arthritis; **GS**: Gastritis; **AS**: Asthma; **BP**: High blood pressure; **FP**: Food poisoning; **TS**: Tonsilitis; **FF**: *Fraxinus floribunda* for diabetes; **HS**: *Hedyotis scandens* for food poisoning; **CB**: *Clematis buchanania* for sinusitis; **AA**: *Achyranthes aspera* for throat infection

Table 4.2.1: Organoleptic study of herbal formulations

Sl. No.	Formulation	Colour	Odour	Taste	Texture
1	HP	Blackish brown	Characteristic smell	Slightly bitter	Coarse
2	AR	Light brown	No odour	Slightly bitter and sour	Coarse
3	GS	Blackish brown	No odour	Tasteless	Coarse
4	AS	Dark green	No odour	Slightly bitter	Coarse
5	BP	Brown and green	Characteristic smell	Bitter	Coarse
6	FP	Brown	No odour	Slightly bitter	Coarse
7	TS	Grey	No odour	Tasteless	Coarse
8	FF	Light brown	Characteristic smell	Slightly bitter	Coarse
9	HS	Yellowish brown	No odour	Tasteless	Coarse
10	CB	Copper brown	No odour	Tasteless	Coarse
11	AA	Blackish brown	No odour	Tasteless	Coarse

HP: Heart palpitation; **AR**: Arthritis; **BP**: High blood pressure; **AS**: Asthma; **TS**: Tonsilitis; **GS**: Gastritis; **FP**: Food poisoning; **FF**: *Fraxinus floribunda* (Diabetes); **HS**: *Hedyotis scandens* (Indigestion and stomachache); **AA**: *Achyranthes aspera* (Throat infection); CB: *Clematis buchanania* (Sinusitis).

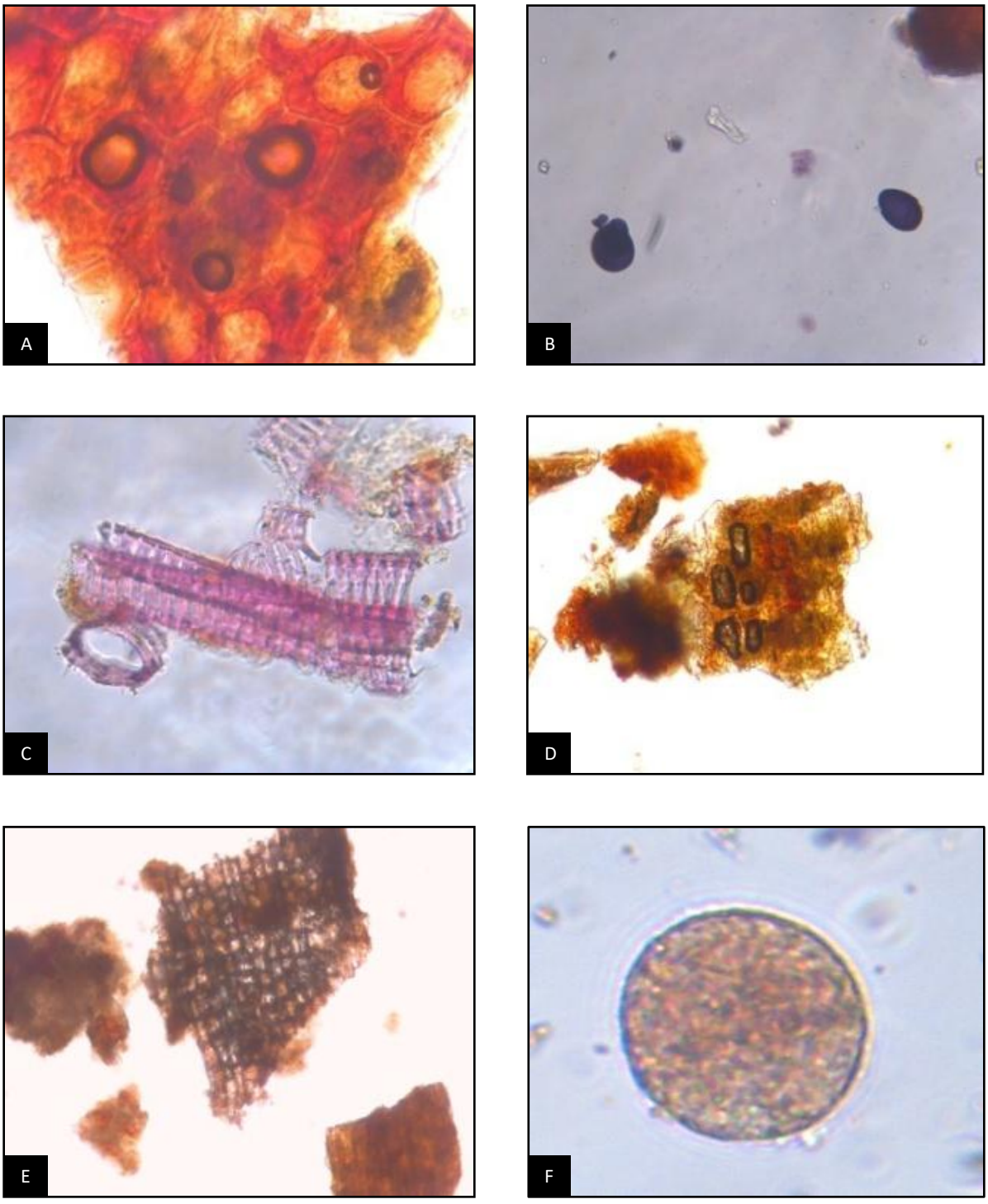


Figure 4.2.2.a: Observation in powder microscopy of a herbal formulation used for the treatment of heart palpitation which is abbreviated as **HP.**
A: Collenchyma cells; **B**: Starch granules; **C**: Xylem vessels; **D**: Stone cells; **E**: Cork cells; **F**: Oil globule.

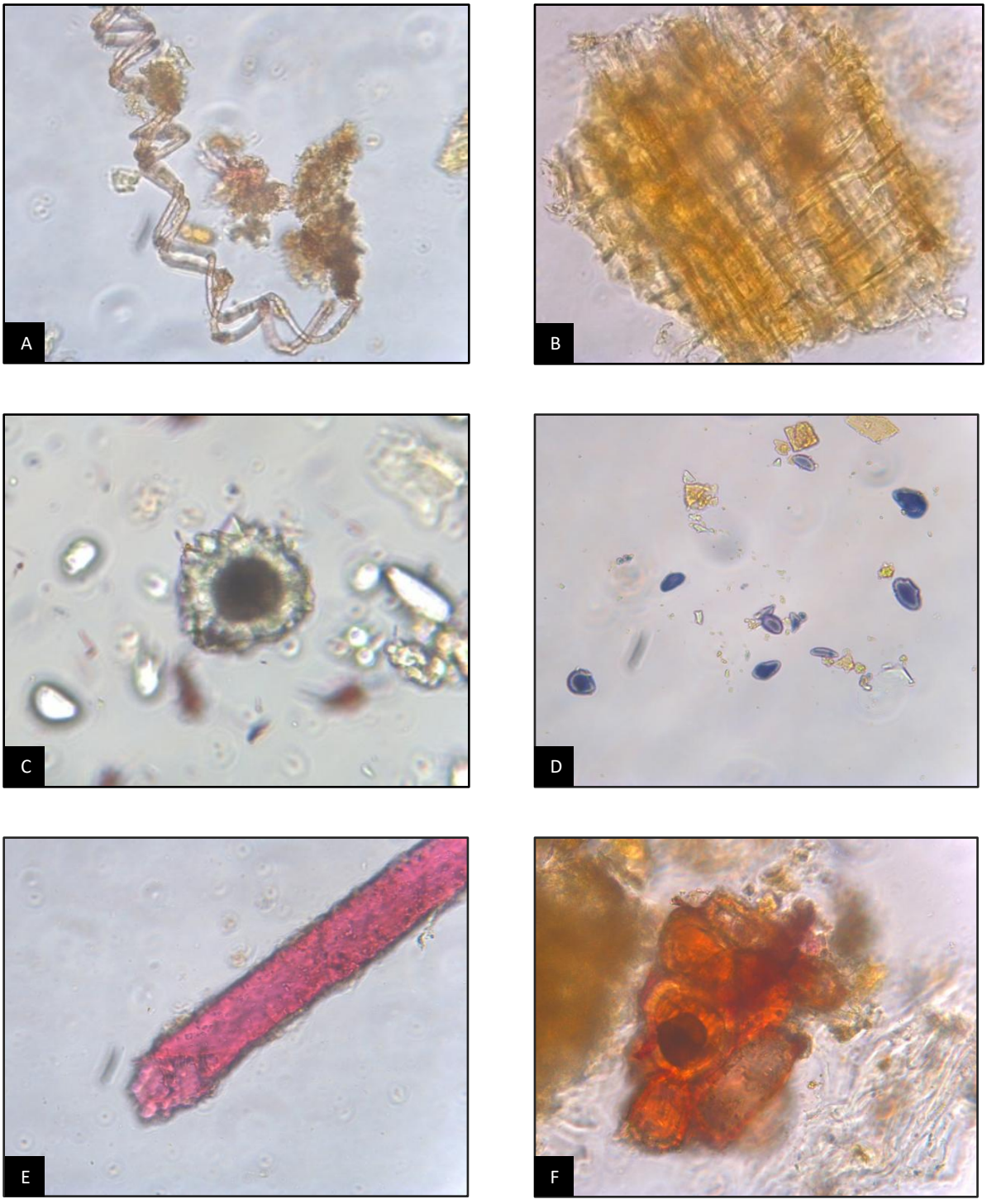


Figure 4.2.2.b: Observation in powder microscopy of a herbal formulation used for the treatment of arthritis which is abbreviated as **AR**.
A: Spiral xylem vessels; **B**: Medullary ray with fibers; **C**: Calcium oxalate crystal; **D**: Starch grains; **E**: Vessels with pits; **F**: Stone cells.

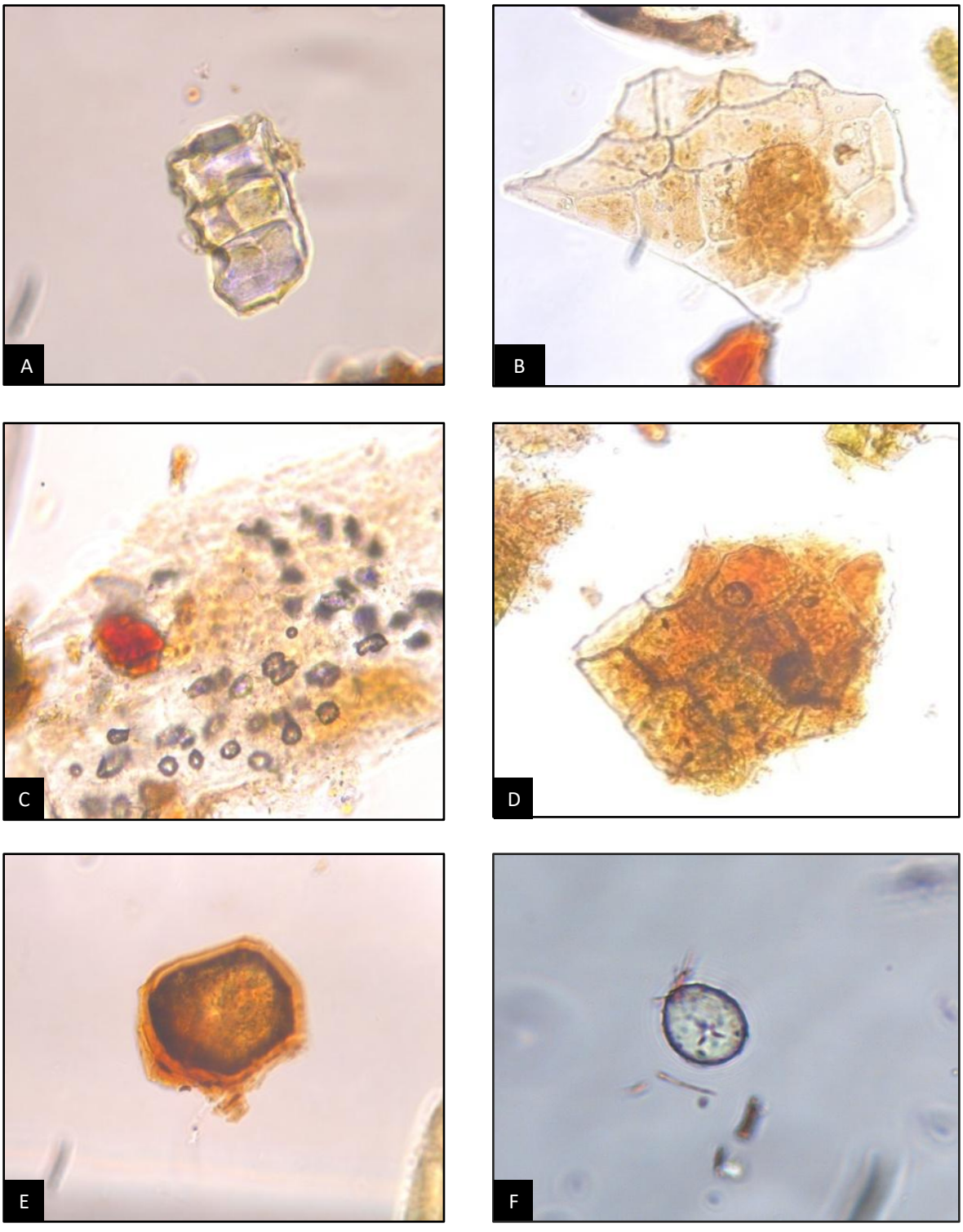


Figure 4.2.2.c: Observation in powder microscopy of a herbal formulation used for the treatment of asthma which is abbreviated as **AS**.
A: Calcium oxalate crystal; **B**: Epidermal cells; **C**: Sclereids; **D**: Parenchymatous cells; **E**: Stone cells; **F**: Starch grain.

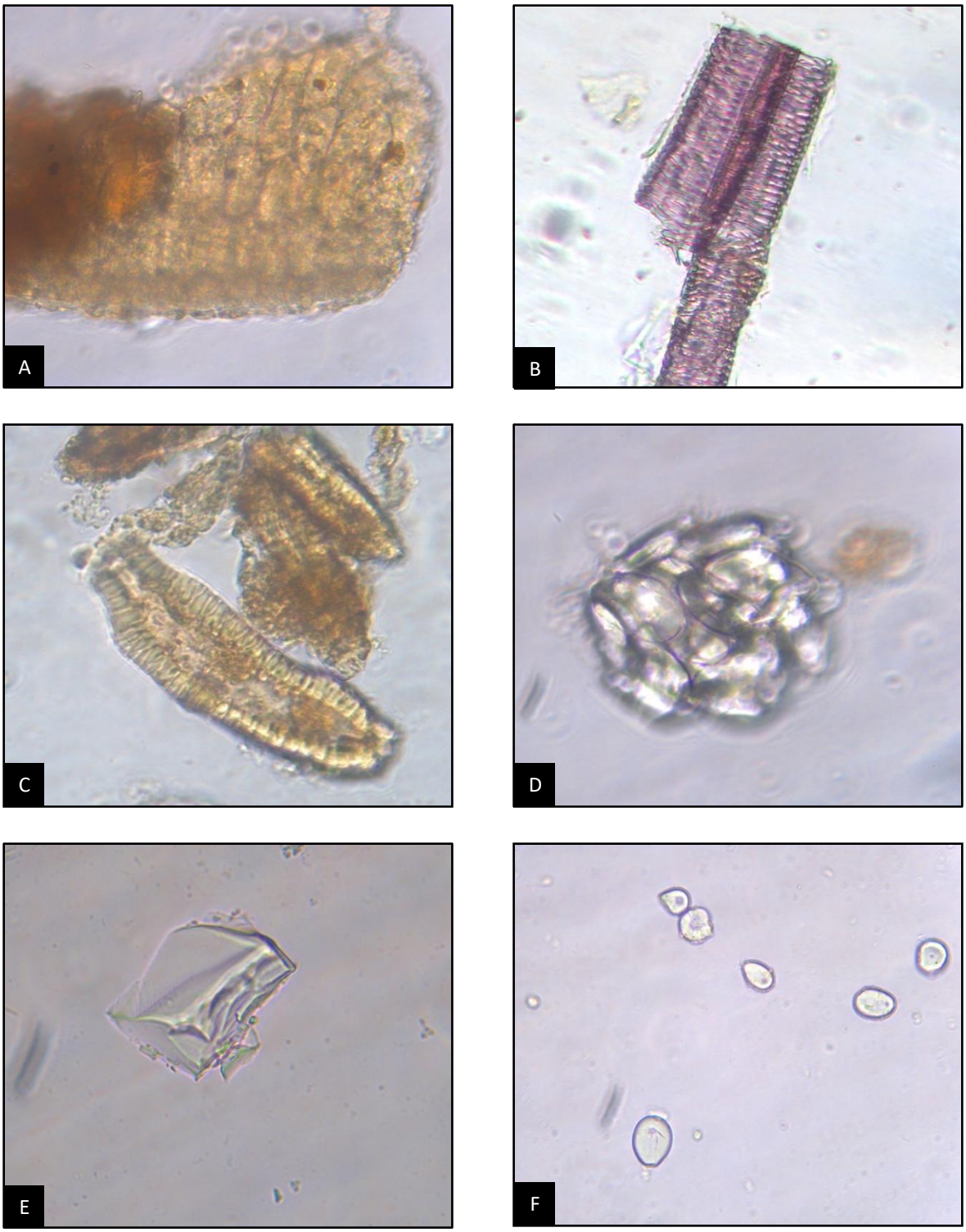


Figure 4.2.2.d: Observation in powder microscopy of a herbal formulation used for the treatment of gastritis which is abbreviated as **GS**.
A: Parenchymatous cell; **B**: Spiral xylem vessel; **C**: Stone cells; **D**: Clustered starch grains; **E**: Calcium oxalate crystal; **F**: Starch granules.

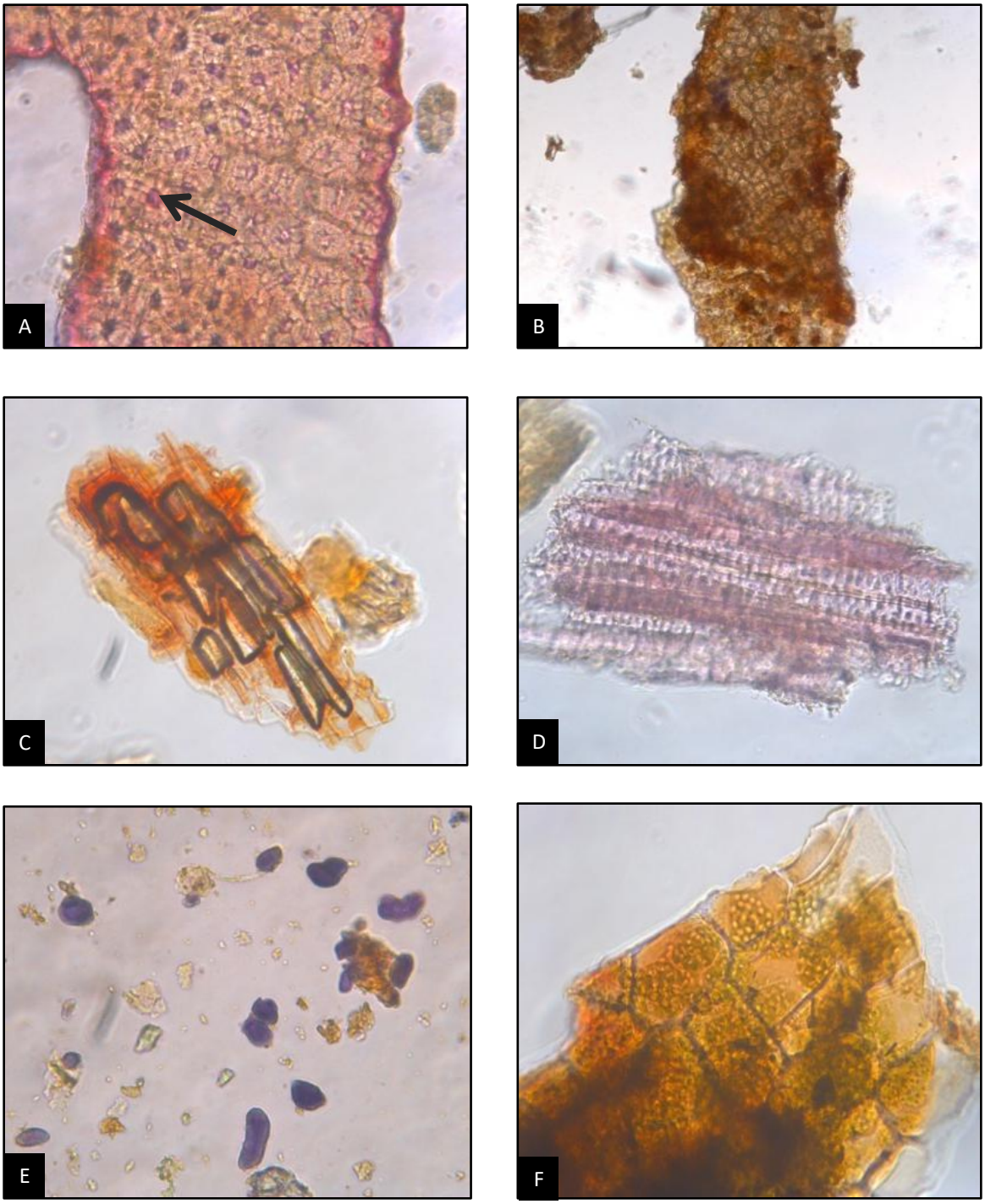


Figure 4.2.2.e: Observation in powder microscopy of a herbal formulation used for the treatment of high blood pressure which is abbreviated as **BP**.
A: Sclereids; **B**: Cork cells; **C**: Stone cells; **D**: Xylem vessels; **E**: Starch granules; **F**: Parenchymatous cells.

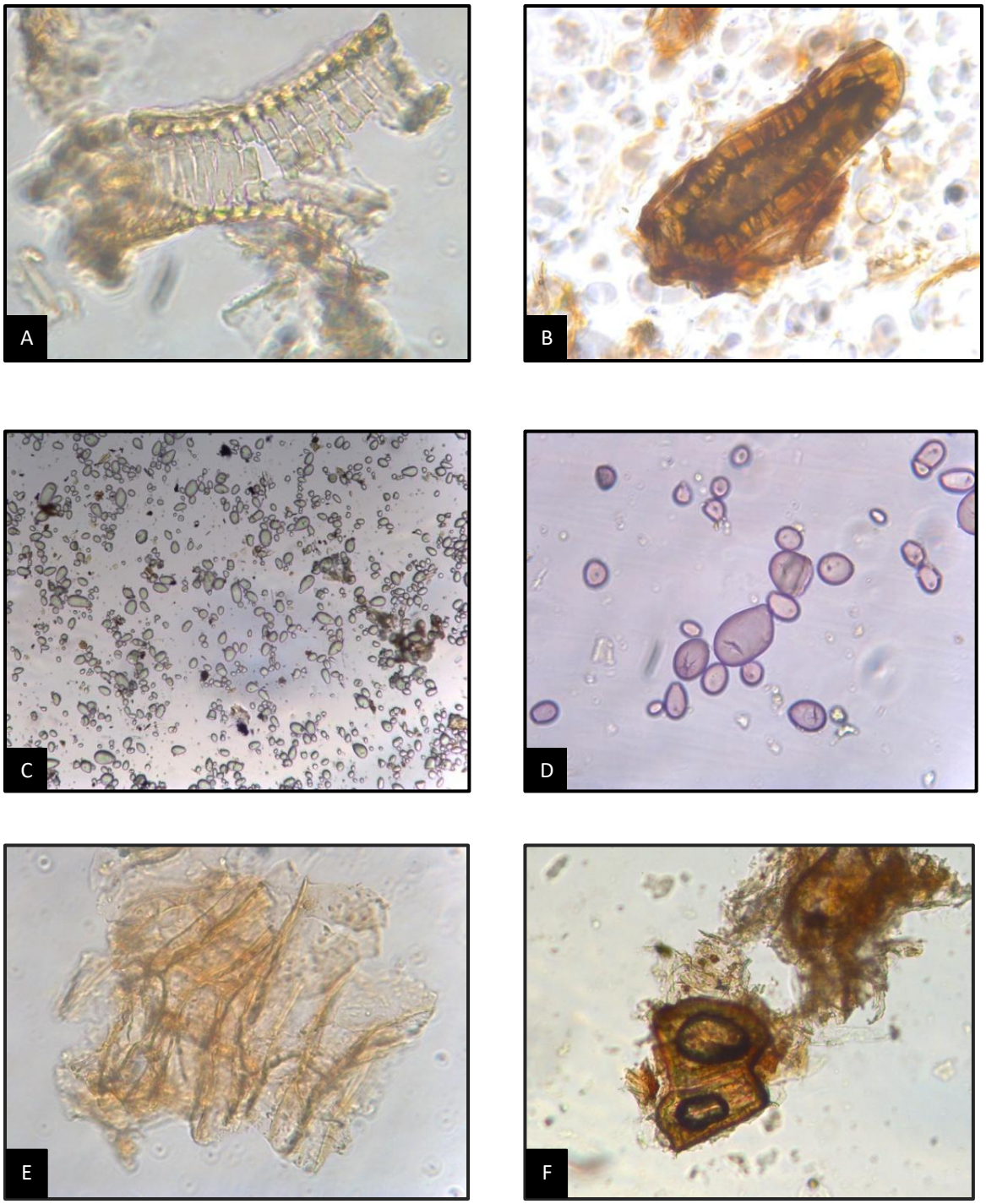


Figure 4.2.2.f: Observation in powder microscopy of a herbal formulation used for the treatment of tonsilitis which is abbreviated as **TS**.
A: Scalariform tracheids; **B**: Stone cells; **C**: Starch granules; **D**: Starch granules (magnified); **E**: Parenchymatous cells; **F**: Sclereids.

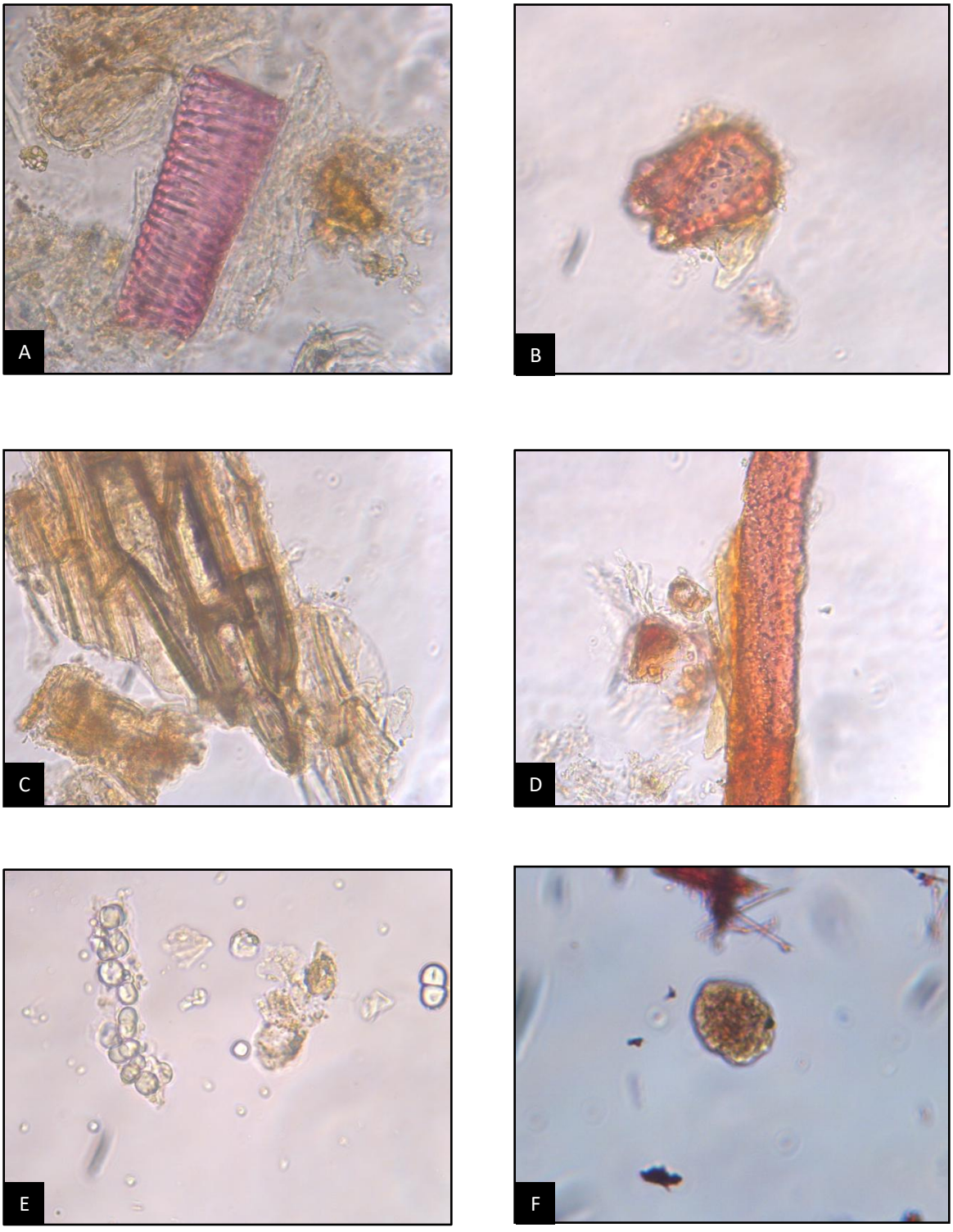


Figure 4.2.2.g: Observation in powder microscopy of a herbal formulation used for the treatment of food poisoning which is abbreviated as **FP**.
A: Scalariform tracheids; **B**: Stone cells; **C**: Parenchymatous cells; **D**: Pitted vessels; **E**: Clustered starch granules; **F**:Oil globule.

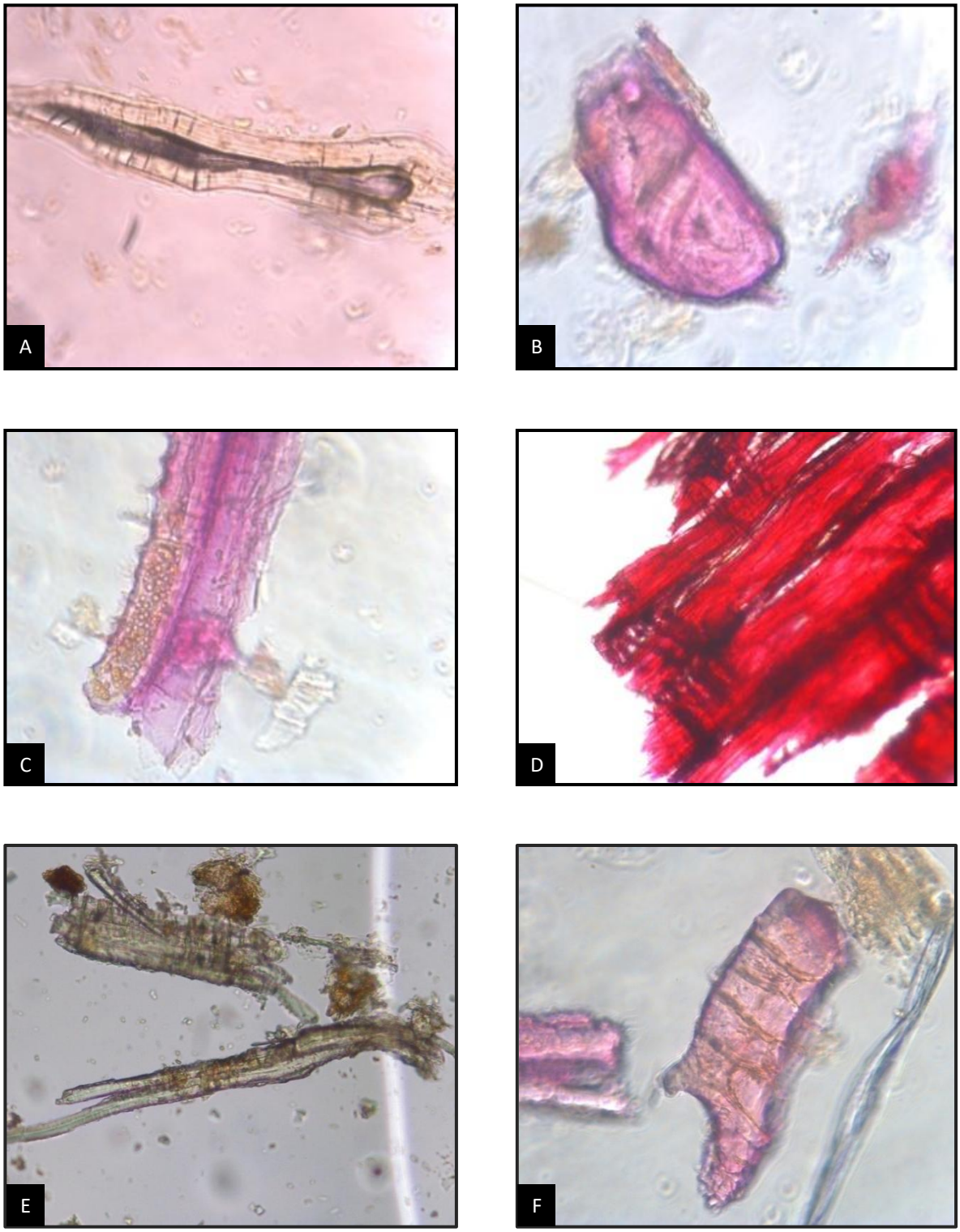


Figure 4.2.2.h: Observation in powder microscopy of *Fraxinus floribunda* bark used for the treatment of diabetes which is abbreviated as **FF**.
A: Sclereid; **B**: Stone cells; **C**: Cork cells; **D**: Medullary rays with fibers; **E**: Vessels; **F:** Parenchymatous cells.

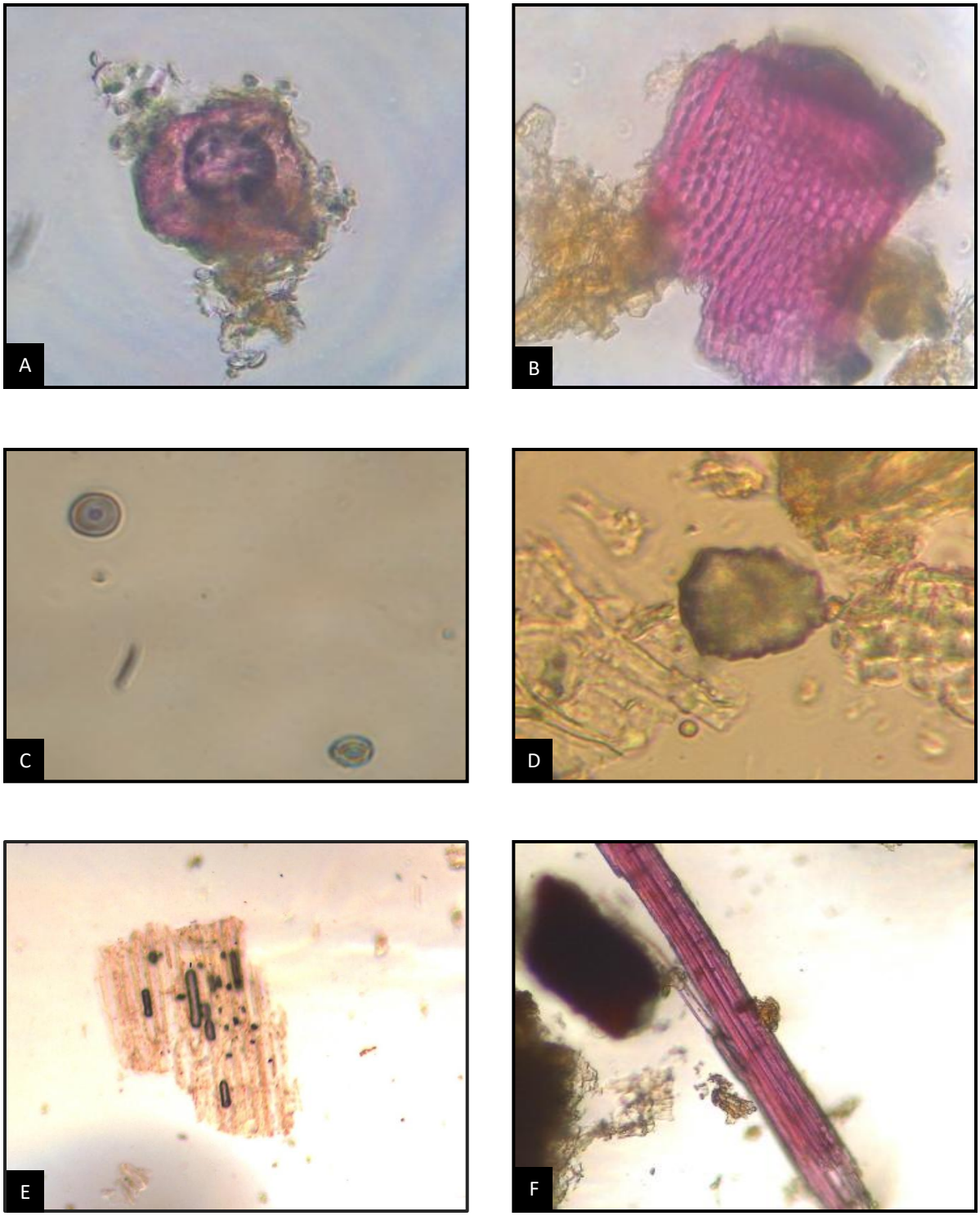


Figure 4.2.2.i: Observation in powder microscopy of *Achyranthes aspera* root used for the treatment of throat infection which is abbreviated as **AA**.
A: Stone cells; **B**: pitted vessels; **C**: Starch grains; **D**: Calcium oxalate crystals in a cluster; **E**: Sclereids; **F**: Phloem fiber.

Figure 4.2.2.j: Observation in powder microscopy of *Clematis buchanania* root used for the treatment of sinusitis which is abbreviated as **CB**.
A: Starch grain; **B**: Spiral xylem vessel; **C**: Pitted vessel; **D**: Phloem fiber; **E**: Vessel; **F**: Trichome.

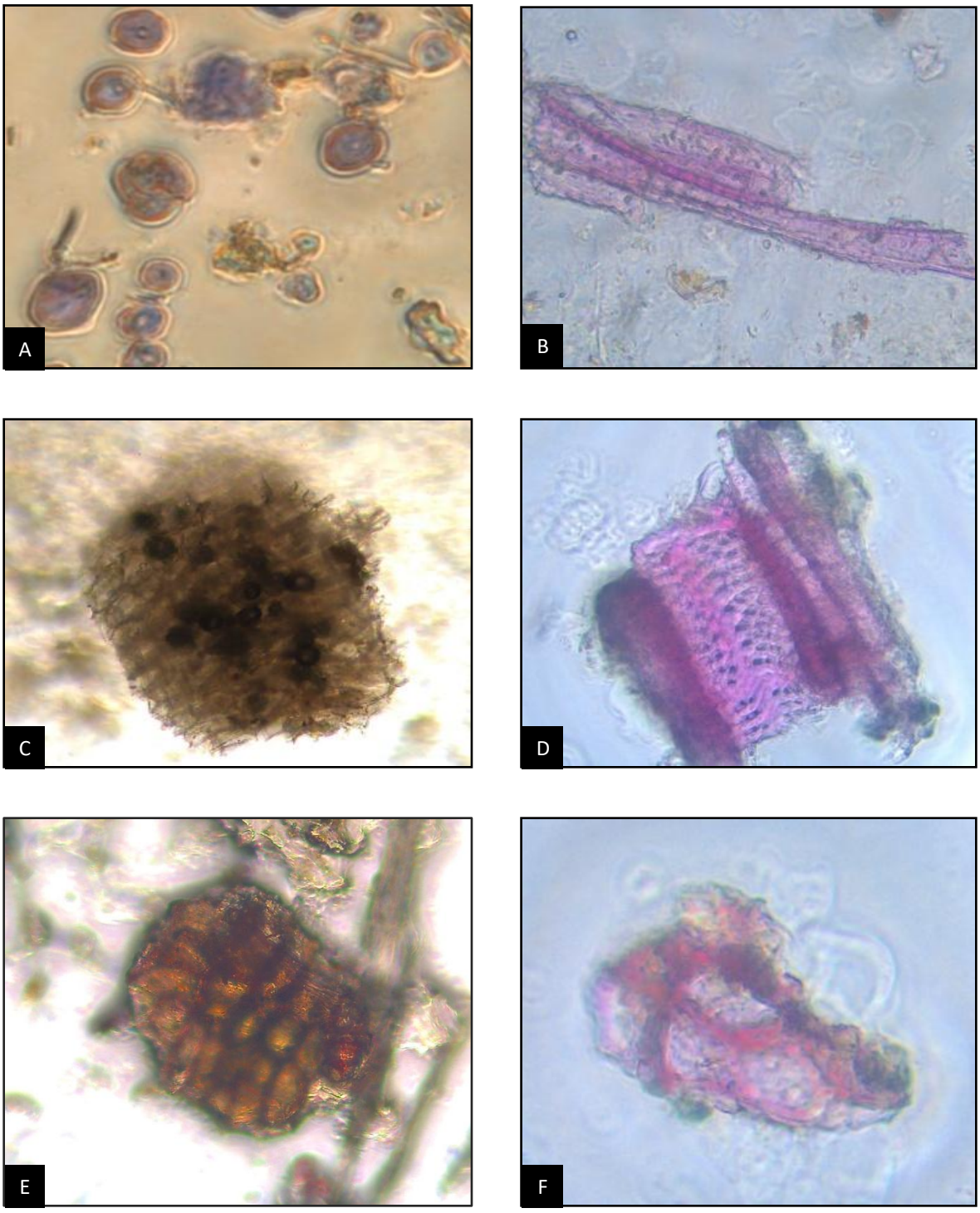


Figure 4.2.2.k: Observation in powder microscopy of *Hedyotis scandens* root used for the treatment of indigestion and stomachache which is abbreviated as **HS**.
A: Starch grain; **B**: Xylem vessel; **C**: Stone cells; **D**: Pitted vessels; **E**: Cork cells; **F**: Parenchymatous cells.

4.2.3 Physicochemical analysis

The result of physicochemical analysis is presented in table 4.2.3. Determination of ash values is necessary for the evaluation of the purity of crude drugs. From the table, it was revealed that the total ash values of the powder formulations varied from approximately 12 to 29% which indicated the presence of some impurities or adulterants which might have been present due to carelessness during the preparation. Acid insoluble ash indicated the presence of earthy matter or silica in the sample which was found to be less than 10% in all the samples except in case of HP (16.01 %) and AR (19.55 %). Water soluble ash indicates the presence ofinorganic matters in the sample which was clearly highest in AS (21.34 %) and FF (21.33 %). The lowest water soluble ash was in sample AA (2.93%). Extractive value mainly shows the better solvent for the extraction of any samples to obtain higher amount of bioactive compounds. Water and alcohol was used in this case as these are two commonly used solvents for extraction. In the samples, water soluble extractive values were higher than alcohol soluble extractives indicating water as ideal solvent for extraction of these samples. Loss on drying percentage is evaluated to determine the moisture content in the crude drugs. It was revealed that all the samples had less than 14% moisture content because it is standard moisture content and if it exceeds more than that, it is not suitable for oral consumption as there might be a probability of microbial growth in the sample. To find whether the formulation is suitable for consumption, pH of the samples was determined. The pH of the formulations ranged between 4 to 7 which was not very acidic and could not be harmful for oral consumption. The percentage of foreign matter present in the powder formulations was all less than 1.5 %.

4.2.4. Fluorescence study

All the powder formulations were subjected to fluorescence analysis under UV short wavelength (254 nm), UV long wavelength (365 nm) and visible light. All the samples exhibited variation of colours under these lights which is illustrated in details from table 4.2.4.a to table 4.2.4.k. The different chemical reagents exhibited different colours as well as fluorescence under different wavelengths of light. This method is useful for the authentication of powder drugs because all the chemical

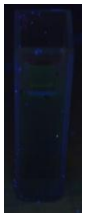
Table 4.2.3: Physicochemical analysis of the herbal formulations

Parameters	HP	AR	GS	AS	BP	FP	TS	FF	HS	CB	AA
Total Ash(%) w/w	20.24	28.60	17.73	19.73	20.84	21.52	21.25	20.35	12.58	19.59	13.73
Acid insoluble ash(%) w/w	16.01	19.55	3.24	2.20	6.45	1.17	3.34	2.00	0.15	0.12	1.36
Water soluble ash(%) w/w	14.01	14.68	13.30	21.34	18.71	18.12	13.29	21.22	12.42	11.08	2.93
Alcohol soluble extractive value(%) w/w	0.44	3.16	3.99	0.85	7.50	3.02	1.03	0.94	2.05	0.95	1.18
Water soluble extractive value(%) w/w	3.21	6.21	8.91	1.93	7.63	6.20	2.82	1.82	5.78	2.08	5.05
Loss on drying(%) w/w	10.52	8.61	7.38	4.44	8.83	3.77	3.8	3.03	3.05	6.13	3.44
pH (1% w/v)	6.79	6.38	4.17	6.48	4.25	5.15	4.1	6.34	6.21	6.26	5.14
pH (10% w/v)	6.38	6.02	4.07	6.06	6.75	4.82	6.41	5.63	6.39	5.13	4.67
Foreign matter (%)	0.8	0.6	0.4	1.1	0.9	0.7	0.8	1.4	0.5	0.8	0.6

HP: Heart palpitation; **AR**: Arthritis; **BP**: High blood pressure; **AS**: Asthma; **TS**: Tonsilitis; **GS**: Gastritis; **FP**: Food poisoning; **FF**: *Fraxinus floribunda* (Diabetes); **HS**: *Hedyotis scandens* (Indigestion and stomachache); **AA**: *Achyranthes aspera* (Throat infection); CB: *Clematis buchanania* (Sinusitis).

Table 4.2.4.a: Fluorescence analysis of herbal formulation, HP (treatment of Heart palpitation)

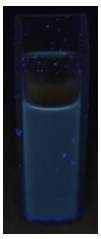
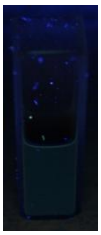
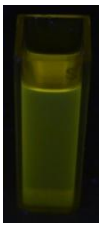
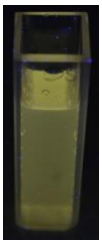
Sl. No.	REAGENTS	VISIBLE	UV-254	UV-365
1	Powder + distilled water			
		Green beige	Azure blue	Brilliant blue
2	Powder + 50% KOH			
		Red orange	Colourless	Black green
3	Powder + Benzene			
		Sulphur yellow	Curry yellow	Broom yellow
4	Powder + 50% Benzene			
		Fern green	Colourless	Raspberry red
5	Powder +Chloroform			
		Colourless	Colourless	Distant blue
6	Powder + 50% Chloroform			
		Fern green	Pearl blackberry	Rose

Sl. No.	REAGENTS	VISIBLE	UV-254	UV-365
7	Powder + Nitric acid (conc.)			
		Broom yellow	Colourless	Colourless
8	Powder + 50% Nitric acid			
		Broom yellow	Colourless	Colourless
9	Powder + 10% Ferric chloride			
		Broom yellow	Colourless	Colourless
10	Powder + Methanol			
		Colourless	Cobalt blue	Ultramine blue
11	Powder + 50% Methanol			
		Khaki grey	Grey blue	Gentian blue

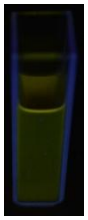
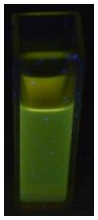
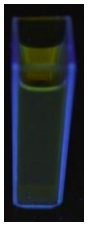
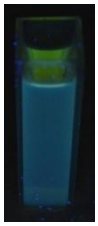
Sl. No.	REAGENTS	VISIBLE	UV-254	UV-365
12	Powder + Ethanol			
		Colourless	Cobalt blue	Ultramine blue
13	Powder + 50% Ethanol			
		Olive grey	Pearl night blue	Traffic blue
14	Powder + Glacial acetic acid (conc.)			
		Reed green	Yellow olive	Brown beige
15	Powder + 50% Glacial acetic acid			
		Green beige	No colour	Ultramine blue
16	Powder + Sulphuric acid (conc.)			
		Ochre brown	Colourless	Blue grey

Sl. No.	REAGENTS	VISIBLE	UV-254	UV-365
17	Powder + 50% sulphuric acid			
		Ochre yellow	Colourless	Grey blue

Table 4.2.4.b: Fluorescence analysis of herbal formulation, AR (treatment of arthritis)

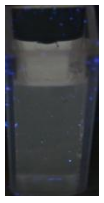
SL NO	REAGENTS	VISIBLE	UV-254 nm	UV-365nm
1	Powder + distilled water			
		Yellow orange	Colourless	Violet blue
2	Powder + 50% KOH			
		Brown red	Colourless	Fir green
3	Powder + Benzene			
		Colourless	Colourless	Signal violet
4	Powder + 50% Benzene			
		Lemon yellow	Honey yellow	Broom yellow
5	Powder + Chloroform			
		Lemon yellow	Broom yellow	Ochre yellow

SL NO	REAGENTS	VISIBLE	UV-254 nm	UV-365nm
6	Powder + 50% Chloroform			
		Broom yellow	Curry yellow	Grey beige
7	Powder + Nitric acid (conc.)			
		Daffodil	Colourless	Colourless
8	Powder + 50% Nitric acid			
		Honey yellow	Colourless	Colourless
9	Powder + 10% Ferric chloride			
		Curry yellow	Colourless	Colourless
10	Powder + Methanol			
		Honey yellow	Curry yellow	Grey beige

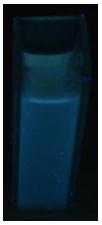
SL NO	REAGENTS	VISIBLE	UV-254 nm	UV-365nm
11	Powder + 50% Methanol			
		Broom yellow	Brilliant blue	Violet blue
12	Powder + Ethanol			
		Daffodil yellow	Curry yellow	Grey beige
13	Powder + 50% Ethanol			
		Broom yellow	Sapphire blue	Ultramine blue
14	Powder +Glacial acetic acid (conc.)			
		Lemon yellow	Curry yellow	Broom yellow
15	Powder + 50% Glacial acetic acid			
		Lemon yellow	Honey yellow	Azure blue

SL NO	REAGENTS	VISIBLE	UV-254 nm	UV-365nm
16	Powder + Sulphuric acid (conc.)			
		Copper brown	Granite grey	Azure blue
17	Powder + 50% Sulphuric acid			
		Beige	Capri blue	Azure blue

Table 4.2.4.c: Fluorescence analysis of herbal formulation, GS (treatment of gastritis)

SL NO	REAGENTS	VISIBLE	UV-254 nm	UV-365 nm
1	Powder + Distilled water			
		Broom yellow	Colourless	Violet blue
2	Powder + 50% KOH			
		Yellow orange	Colourless	Fir green
3	Powder + Benzene			
		Colourless	Colourless	Violet blue
4	Powder + 50% Benzene			
		Colourless	Colourless	Violet blue
5	Powder + Chloroform			
		Colourless	Colourless	Concrete grey

SL NO	REAGENTS	VISIBLE	UV-254 nm	UV-365 nm
6	Powder + 50% Chloroform			
		Ochre yellow	Colourless	Sapphire blue
	Powder + Nitric acid (conc.)			
		Ochre brown	Colourless	Colourless
8	Powder + 50% nitric acid			
		Honey yellow	Colourless	Colourless
9	Powder + 10% Ferric chloride			
		Green beige	Colourless	Colourless
10	Powder + Methanol			
		Broom yellow	Colourless	Sapphire blue

SL NO	REAGENTS	VISIBLE	UV-254 nm	UV-365 nm
11	Powder + 50% Methanol			
		Lemon yellow	Colourless	Gentian blue
12	Powder + Ethanol			
		Pale green	Colourless	Traffic blue
13	Powder + 50% Ethanol			
		Honey yellow	Colourless	Violet blue
14	Powder + Glacial acetic acid (conc)			
		Olive green	Grey blue	Brilliant blue
15	Powder + 50% Glacial acetic acid			
		Honey yellow	Colourless	Sapphire blue

SL NO	REAGENTS	VISIBLE	UV-254 nm	UV-365 nm
16	Powder + Sulphuric acid			
		Khaki grey	Chrome green	Pearl gentian blue
17	Powder + 50% Sulphuric acid			
		Sand yellow	Colourless	Green blue

Table 4.2.4.d: Fluorescence analysis of herbal formulation, AS (treatment of asthma)

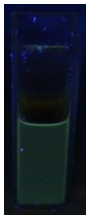
SL NO	REAGENTS	VISIBLE	UV-254nm	UV-365nm
1	Powder + Distilled water			
		Colourless	Grey blue	Violet blue
2	Powder + 50% KOH			
		Pastel yellow	Colourless	Pearl gentian blue
3	Powder + Benzene			
		Green beige	Wine red	Raspberry red
4	Powder + 50% Benzene			
		Fern green	Red violet	Raspberry red
5	Powder + Chloroform			
		Colourless	Colourless	Traffic purple

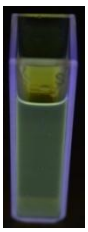
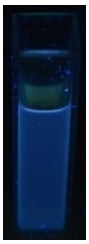
SL NO	REAGENTS	VISIBLE	UV-254nm	UV-365nm
6	Powder + 50% chloroform			
		Fern green	Claret violet	Raspberry red
7	Powder + Nitric acid (conc.)			
		Broom yellow	Colourless	Colourless
8	Powder + 50% Nitric acid			
		Colourless	Colourless	Colourless
9	Powder + 10% ferric chloride			
		Lemon yellow	Colourless	Colourless
10	Powder + Methanol			
		Pale green	Colourless	Raspberry red

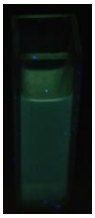
SL NO	REAGENTS	VISIBLE	UV-254nm	UV-365nm
11	Powder + 50% Methanol			
		Colourless	Sapphire blue	Gentian blue
12	Powder + Ethanol			
		Colourless	Ultramine blue	Ultramine blue
13	Powder + 50% Ethanol			
		Colourless	Sapphire blue	Signal blue
14	Powder + Glacial acetic acid (conc.)			
		Curry yellow	Colourless	Grass green
15	POWDER + 50% GLACIAL ACETIC ACID			
		Green beige	Sapphireblue	Signal blue

16	**Powder + Sulphuric acid (conc.)**			
		Clay brown	**Pine green**	**Azure blue**
17	**Powder + 50% Sulphuric acid**			
		Pale brown	**Colourless**	**Distant blue**

Table 4.2.4.e: Fluorescence analysis of herbal formulation, BP (treatment of high blood pressure)

SL NO	REAGENTS	VISIBLE	UV-254 nm	UV-365 nm
1	Powder + Distilled water			
		Green beige	Azure blue	Violet blue
2	Powder + 50% KOH			
		Yellow orange	Fir green	Pine green
3	Powder + Benzene			
		Colourless	Colourless	Ultramine blue
4	Powder + 50% Benzene			
		Green beige	Violet blue	Blue grey
5	Powder + Chloroform			
		Colourless	Colourless	Silver grey

SL NO	REAGENTS	VISIBLE	UV-254 nm	UV-365 nm
6	Powder + 50% chloroform			
		Broom yellow	Olive yellow	Moss grey
7	Powder + Nitric acid (conc)			
		Ochre brown	Colourless	Colourless
8	Powder + 50% Nitric acid			
		Golden yellow	Colourless	Colourless
9	Powder + 10% Ferric chloride			
		Green brown	Colourless	Colourless
10	Powder + Methanol			
		Curry yellow	Sapphire blue	Signal blue

SL NO	REAGENTS	VISIBLE	UV-254 nm	UV-365 nm
11	Powder + 50% Methanol			
		Ochre yellow	Sapphire blue	Signal blue
12	Powder + Ethanol			
		Green beige	Sapphire blue	Signal blue
13	Powder + 50% Ethanol			
		Ochre brown	Sapphire blue	Signal blue
14	Powder + Glacial acetic acid			
		Pale green	Emerald green	Mouse grey
15	Powder + 50% Glacial acetic acid			
		Ochre brown	Sapphire blue	Capri blue

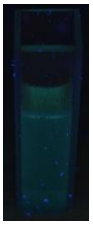
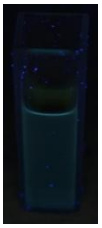
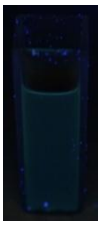
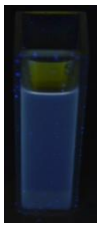
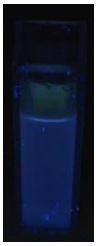
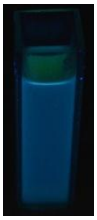
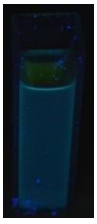
SL NO	REAGENTS	VISIBLE	UV-254 nm	UV-365 nm
16	Powder + Sulphuric acid (conc.)			
		Olive brown	Pine green	Azure blue
17	Powder + 50% sulphuric acid			
		Sand yellow	Colourless	Patina green

Table 4.2.4.f: Fluorescence analysis of herbal formulation, FP (treatment of food poisoning)

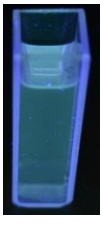
SL NO	REAGENTS	VISIBLE	UV-254nm	UV-365nm
1	Powder + Distilled water			
		Pastel yellow	Colourless	Blue green
2	Powder + 50% KOH			
		Yellow orange	Blue green	Blue green
3	Powder + Benzene			
		Green beige	Colourless	Grey beige
4	Powder + 50% Benzene			
		Lemon yellow	Colourless	Blue lilac
5	Powder + Chloroform			
		Pale green	Pearl violet	Pearl blackberry

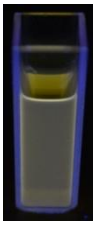
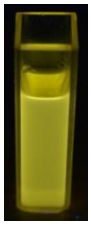
SL NO	REAGENTS	VISIBLE	UV-254nm	UV-365nm
6	Powder + 50% Chloroform			
		Broom yellow	Colourless	Sapphire blue
7	Powder + Nitric acid (conc.)			
		Yellow orange	Colourless	Colourless
8	Powder + 50% Nitric acid			
		Broom yellow	Colourless	Colourless
9	Powder + 10% Ferric chloride			
		Lemonyellow	Colourless	Colourless
10	Powder + Methanol			
		Broom yellow	Colourless	Sapphire blue

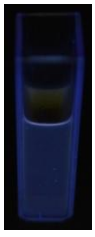
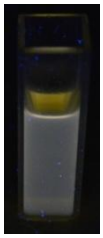
SL NO	REAGENTS	VISIBLE	UV-254nm	UV-365nm
11	Powder + 50% Methanol			
		Ochre brown	Sapphire blue	Gentian blue
12	Powder + Ethanol			
		Broom yellow	Sapphire blue	Violet blue
13	Powder + 50% Ethanol			
		Broom yellow	Sapphire blue	Gentian blue
14	Powder + Glacial acetic acid (conc.)			
		Fern yellow	Violet blue	Azure blue
15	Powder + 50% Glacial acetic acid			
		Ochre brown	Colourless	Blue green

SL NO	REAGENTS	VISIBLE	UV-254nm	UV-365nm
16	Powder + Sulphuric acid (conc.)			
		Green brown	Green blue	Azure blue
17	Powder + 50% Sulphuric acid			
		Beige grey	Colourless	Pearl gentian blue

Table 4.2.4.g: Fluorescence analysis of herbal formulation, TS (treatment of tonsilitis)

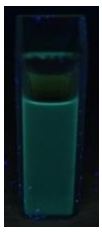
SL NO	REAGENTS	VISIBLE	UV-254nm	UV-365nm
1	Powder + Distilled water			
		Colourless	Sky blue	Traffic blue
2	Powder + 50% KOH			
		Sand yellow	Azure blue	Traffic blue
3	Powder + Benzene			
		Colourless	Steel blue	Capri blue
4	Powder + 50% Benzene			
		Reed green	Colourless	Platinum grey
5	Powder + Chloroform			
		Colourless	Mint turquoise	Concrete grey

SL NO	REAGENTS	VISIBLE	UV-254nm	UV-365nm
6	Powder + 50% Chloroform			
		Curry yellow	Brown beige	Luminous yellow
7	Powder + Nitric acid (conc.)			
		Ochre brown	Colourless	Colourless
8	Powder + 50% Nitric acid			
		Sand yellow	Colourless	Colourless
9	Powder +10% Ferric chloride			
		Broom yellow	Colourless	Colourless
10	Powder + Methanol			
		Pale green	Signal blue	Platinum grey

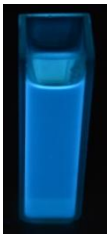
SL NO	REAGENTS	VISIBLE	UV-254nm	UV-365nm
11	Powder + 50% Methanol			
		Sand yellow	Signal blue	Signal blue
12	Powder + Ethanol			
		Colourless	Cobalt blue	Cobalt blue
13	Powder + 50% Ethanol			
		Green beige	Signal blue	Traffic blue
14	Powder + Glacial acetic acid (conc)			
		Khaki grey	Colourless	Olive grey
15	Powder + 50% Glacial acetic acid			
		Khaki grey	Signal blue	Olive grey

SL NO	REAGENTS	VISIBLE	UV-254nm	UV-365nm
16	Powder + Sulphuric acid (conc)			
		Yellow grey	Colourless	Cobalt blue
17	Powder + 50% Sulphuric acid			
		Yellow grey	Colourless	Broom yellow

Table 4.2.4.h: Fluorescence analysis of herbal formulation, FF (*Fraxinus floribunda* for the treatment of diabetes)

SL NO	REAGENTS	VISIBLE	UV-254nm	UV-365nm
1	Powder + Distilled water	 Pale green	 Sky blue	 Light blue
2	Powder + 50% KOH	 Broom yellow	 Colourless	 Patina green
3	Powder + Benzene	 Colourless	 Colourless	 Ultramine blue
4	Powder+ 50% Benzene	 Pale green	 Umbra grey	 Window grey
5	Powder + Chloroform	 Colourless	 Colourless	 Capri blue

SL NO	REAGENTS	VISIBLE	UV-254nm	UV-365nm
6	Powder + 50% Chloroform	 Colourless	 Traffic blue	 Sky blue
7	Powder + Nitric acid (Conc.)	 Pale green	 Colourless	 Colourless
8	Powder + 50% Nitric acid	 Curry yellow	 Colourless	 Colourless
9	Powder + 10% Ferric chloride	 Golden yellow	 Colourless	 Colourless
10	Powder + Methanol	 Colourless	 Sky blue	 Light blue

SL NO	REAGENTS	VISIBLE	UV-254nm	UV-365nm
11	Powder + 50% Methanol	 Pale green	 Sky blue	 Turquoise blue
12	Powder + Ethanol	 Pale green	 Sky blue	 Turquoise blue
13	Powder + 50% Ethanol	 Pale green	 Sky blue	 Turquoise blue
14	Powder + Glacial acetic acid (Conc.)	 Yellow grey	 Gentian blue	 Traffic blue
15	Powder + 50% Glacial acetic acid	 Olive grey	 Capri blue	 Sky blue

SL NO	REAGENTS	VISIBLE	UV-254nm	UV-365nm
16	Powder + Sulphuric acid (Conc.)			
		Copper brown	Colourless	Capri blue
17	Powder + 50% Sulphuric acid			
		Olive grey	Steel blue	Signal blue

Table 4.2.4.i: Fluorescence analysis of herbal formulation, HS (*Hedyotis scandens* for the treatment of indigestion and stomachache)

SL NO	REAGENTS	VISIBLE	UV-254 nm	UV-365nm
1	Powder + Distilled water			
		Yellow orange	Violet blue	Violet blue
2	Powder + 50% KOH			
		Brown red	Colourless	Colourless
3	Powder + Benzene			
		Colourless	Colourless	Signal violet
4	Powder + 50% Benzene			
		Lemon yellow	Honey yellow	Broom yellow
5	Powder + Chloroform			
		Lemon yellow	Broom yellow	Ochre yellow

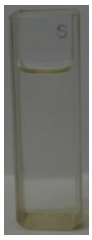
SL NO	REAGENTS	VISIBLE	UV-254 nm	UV-365nm
6	Powder + 50% chloroform			
		Broom yellow	Curry yellow	Grey beige
7	Powder + Nitric acid (Conc.)			
		Daffodil	Colourless	Colourless
8	Powder + 50% Nitric acid			
		Honey yellow	Colourless	Colourless
9	Powder + 10% Ferric chloride			
		Curry yellow	Colourless	Colourless
10	Powder + Methanol			
		Honey yellow	Curry yellow	Grey beige

SL NO	REAGENTS	VISIBLE	UV-254 nm	UV-365nm
11	Powder + 50% Methanol	 Broom yellow	 Brilliant blue	 Violet blue
12	Powder + Ethanol	 Daffodil yellow	 Curry yellow	 Grey beige
13	Powder + 50% Ethanol	 Broom yellow	 Sapphire blue	 Ultramine blue
14	Powder + Glacial acetic acid (Conc.)	 Lemon yellow	 Curry yellow	 Broom yellow
15	Powder + 50% Glacial acetic acid	 Lemon yellow	 Honey yellow	 Azure blue

SL NO	REAGENTS	VISIBLE	UV-254 nm	UV-365nm
16	Powder + Sulphuric acid (Conc.)			
		Copper brown	Granite grey	Azure blue
17	Powder + 50 % sulphuric acid			
		Beige	Capri blue	Azure blue

Table 4.2.4.j: Fluorescence analysis of herbal formulation, CB (*Clematis buchanania* for the treatment of sinusitis)

SL NO	REAGENTS	VISIBLE	UV-254 nm	UV-365nm
1	Powder + Distilled water	 Colourless	 Blue Green	 Opal Green
2	Powder + 50% KOH	 Light Ivory	 Saphire Blue	 Turquoise Green
3	Powder + Benzene	 Colourless	 Steel Blue	 Gentian Blue
4	Powder + 50% Benzene	 Colourless	 Saphire Blue	 Capri Blue
5	Powder + Chloroform	 Colourless	 Steel Blue	 Pearl Night Blue

SL NO	REAGENTS	VISIBLE	UV-254 nm	UV-365nm
6	Powder + 50% Chloroform	 Colourless	 Ultramine Blue	 Signal Blue
7	Powder + Nitric acid (Conc.)	 Light ivory	 Black blue	 Pearl gentian blue
8	Powder + 50% nitric acid	 Light Ivory	 Black Blue	 Blue Green
9	Powder + 10% Ferric chloride	 Dahlia Yellow	 Black Blue	 Black Red
10	Powder + Methanol	 Colourless	 Sky Blue	 Light Blue

SL NO	REAGENTS	VISIBLE	UV-254 nm	UV-365nm
11	Powder + 50% Methanol			
		Colourless	Sky Blue	Light Blue
12	Powder + Ethanol			
		Light Ivory	Sky Blue	Light Blue
13	Powder + 50% Ethanol			
		Light Ivory	Sky Blue	Light Blue
14	Powder + Glacial acetic acid (Conc.)			
		Light Ivory	Pearl Night Blue	Traffic Blue
15	Powder + 50% Glacial acetic acid			
		Light Ivory	Traffic Blue	Sky Blue

SL NO	REAGENTS	VISIBLE	UV-254 nm	UV-365nm
16	Powder + Sulphuric acid (Conc.)			
		Signal Brown	Cobalt Blue	Azure Blue
17	Powder +50 % Sulphuric acid			
		Light Ivory	Signal Blue	Gentian Blue

Table 4.2.4.k: Fluorescence analysis of herbal formulation, AA (*Achyranthes aspera* for the treatment of throat infection)

SL NO	REAGENTS	VISIBLE	UV-254 nm	UV-365nm
1	Powder + Distilled water			
		Colourless	Green blue	Signal blue
2	Powder + 50% KOH			
		Saffron yellow	Black blue	Turquoise green
3	Powder + Benzene			
		Colourless	Jet black	Pearl night blue
4	Powder + 50% Benzene			
		Colourless	Black blue	Signal blue
5	Powder + Chloroform			
		Colourless	Cobalt blue	Gentian blue

SL NO	REAGENTS	VISIBLE	UV-254 nm	UV-365nm
6	Powder + 50% Chloroform	 Colourless	 Black blue	 Pearl night blue
7	Powder + Nitric acid (Conc.)	 Colourless	 Colourless	 Colourless
8	Powder + 50% Nitric acid	 Colourless	 Colourless	 Colourless
9	Powder + 10% Ferric chloride	 Daffodil yellow	 Colourless	 Colourless
10	Powder + Methanol	 Colourless	 Sky blue	 Light blue

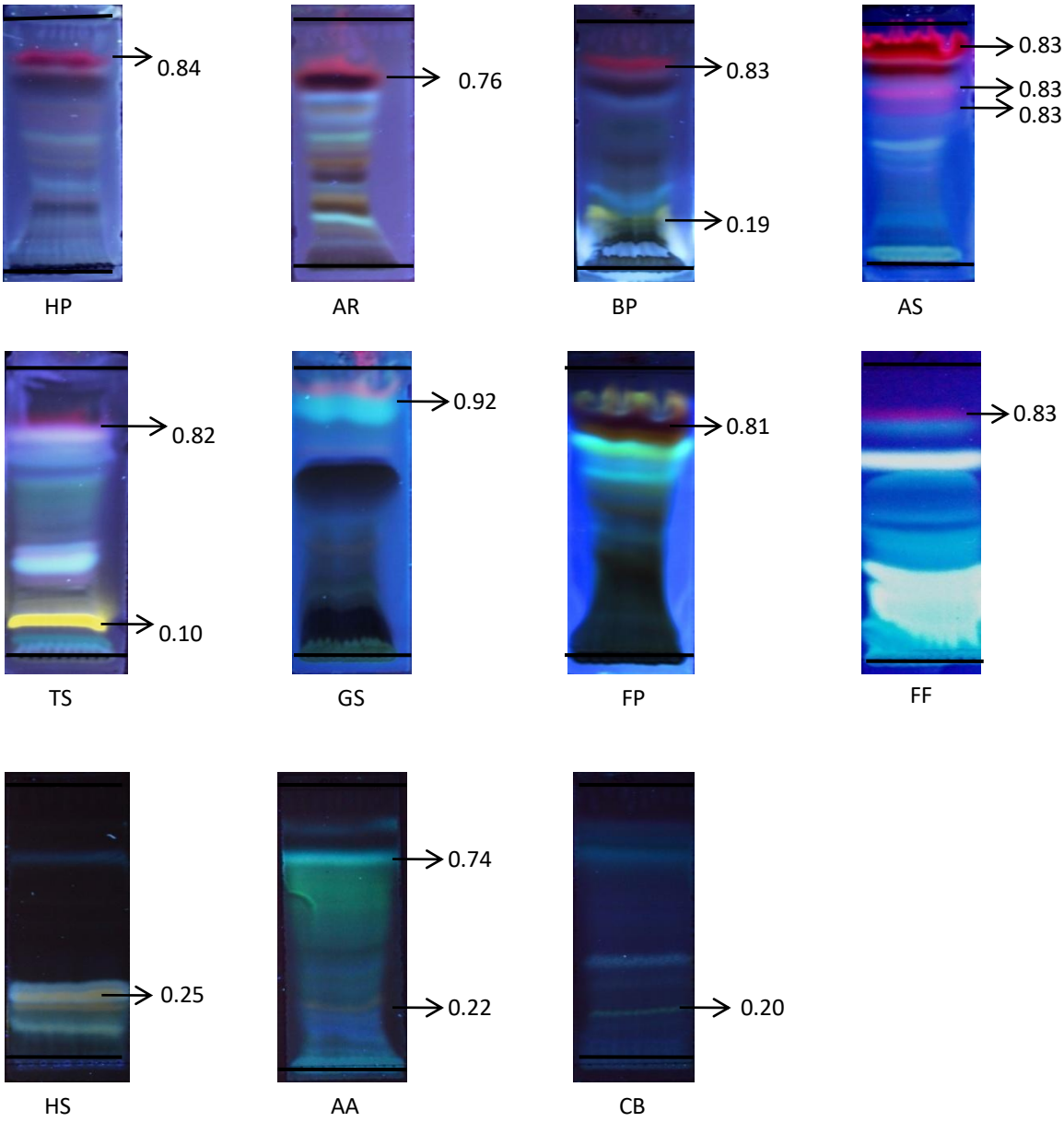
SL NO	REAGENTS	VISIBLE	UV-254 nm	UV-365nm
11	Powder + 50% Methanol	 Colourless	 Sky blue	 Light blue
12	Powder + Ethanol	 Colourless	 Signal blue	 Sky blue
13	Powder + 50% Ethanol	 Colourless	 Signal blue	 Light blue
14	Powder + Glacial acetic acid (conc.)	 Colourless	 Signal blue	 Patina green
15	Powder + 50% Glacial acetic acid	 Curry yellow	 Traffic blue	 Patina green

SL NO	REAGENTS	VISIBLE	UV-254nm	UV-365nm
16	Powder + Sulphuric acid (conc.)			
		Copper brown	Colourless	Colourless
17	Powder + 50% Sulphuric acid			
		Colourless	Cobalt blue	Steel blue

compounds present in plants exhibit a particular colour of fluorescence under different wavelengths of light after treating with certain chemical reagents. This parameter is another simple yet useful tool to check the purity of the any formulations or drugs in their powder form when it is difficult to identify with naked eye. The colours varied from blue, green, red, yellow and some were colourless. All these colours were identified and noted down using the standard colour chart of RAL.

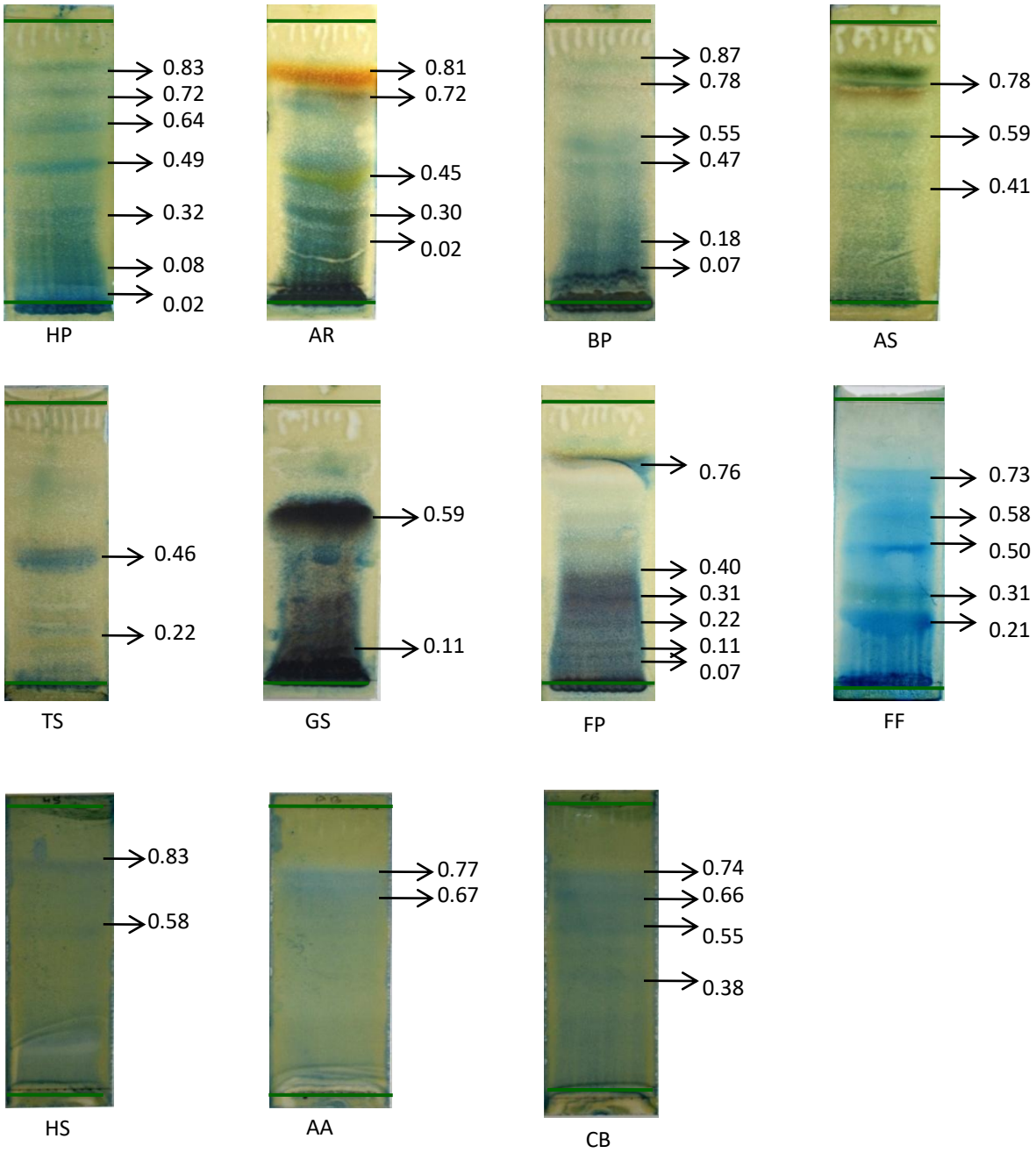
4.2.5. Thin layer chromatography

Thin layer chromatography (TLC) is another method which can be used to check the quality control of the drugs or formulations in their powder form. The extracts of all the herbal formulations were run in TLC plates in specific solvent system followed by spraying with their specific detecting reagents. The colour of the bands was observed under UV and visible light. The R_f values of each bands were noted down which can be used in future to compare with similar formulation for the authentication of the same. In this study, the observation after detection revealed the presence of anthraglycosides in all the herbal formulations (figure 4.2.5.a). The presence of arbutin was observed under visible light as blue bands and it was present in all the formulations with maximum bands in HP, AR, BP, FP and FF (figure 4.2.5.b). Bitter principles were detected with vanillin-sulphuric acid reagent as were observed as blue, yellow, red and orange colour. It was present in all the formulations except HS, AA and CB with more bands found in AR, TS and FF (4.2.5.c). Flavonoids were indicated with green and yellow bands after spraying with NP/PEG reagent and it was present in all the formulations except HS, AA and CB (figure 4.2.5.d). Cardiac glycoside was observed under visible light after detection with antimony tri-chloride reagent which was present in HP, AR, AS, TS, GS and FP (figure 4.2.5.e) Alkaloids was also observed under visible light and it was detected with Dragendorff reagent. It was found to be present in AR, BP, AS, TS, GS and HS with TS showing prominent and abundant bands (figure 4.2.2.f). Coumarins were detected under UV-365 nm after spraying with detecting reagent ethanolic KOH and all the formulations showed the presence of coumarins in blue bands with TS and FF showing maximum number of bands (figure 4.2.2.g).



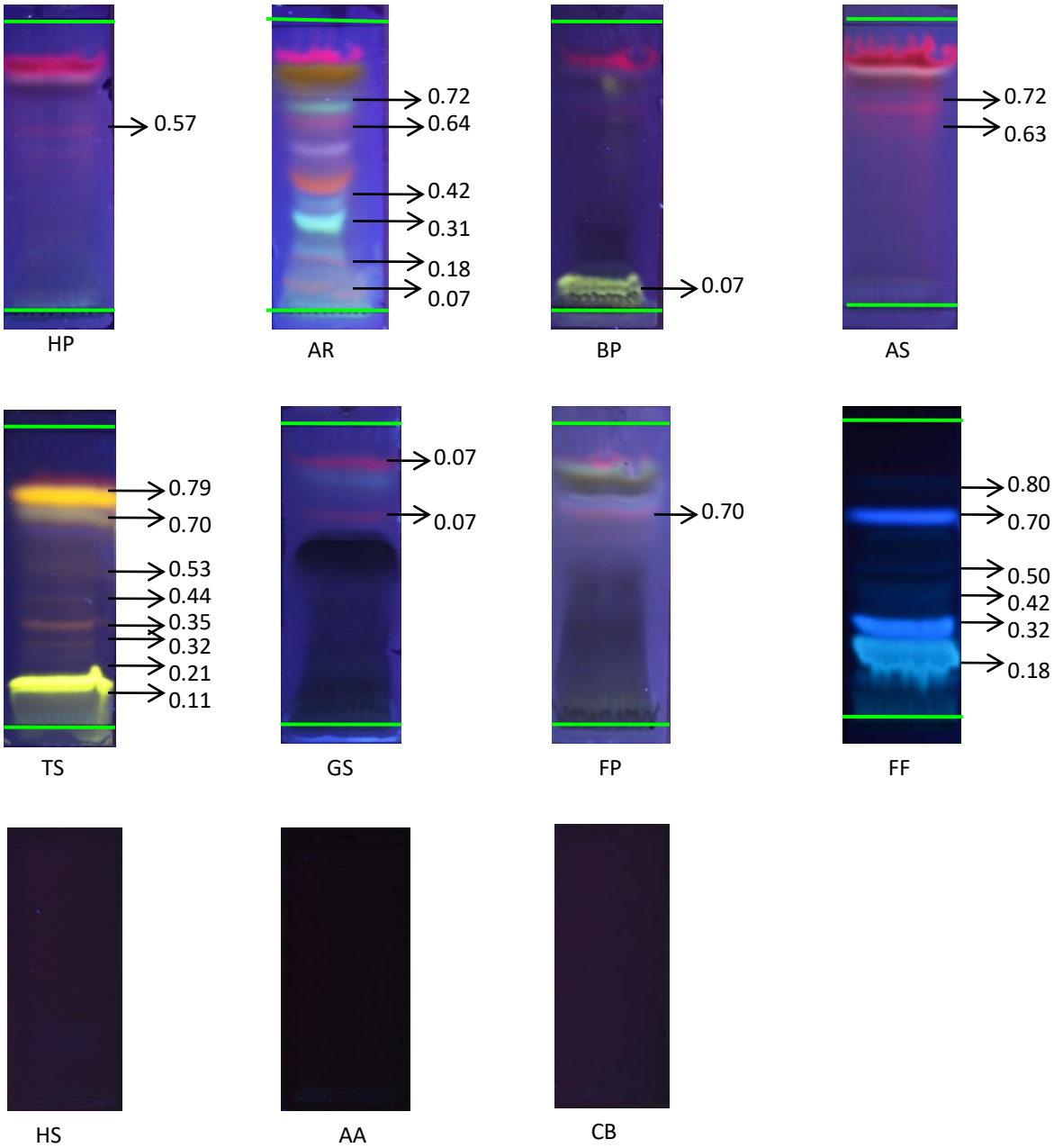
Phyto-constituents: Anthraglycosides
Light: UV-365 nm
Colour of bands: Red and yellow
Solvent system: Ethyl acetate:Methanol:Water (100:13.5:10)
Detecting reagent: 10% ethanolic KOH

Figure 4.2.5.a: Thin layer chromatography of herbal formulations for the detection of anthraglycosides with Rf values.
HP: Heart palpitation; **AR**: Arthritis; **BP**: High blood pressure; **AS**: Asthma; **TS**: Tonsilitis; **GS**: Gastritis; **FP**: Food poisoning; **FF**: *Fraxinus floribunda* (Diabetes); **HS**: *Hedyotis scandens* (Indigestion and stomachache); **AA**: *Achyranthes aspera* (Throat infection); CB: *Clematis buchanania* (Sinusitis).



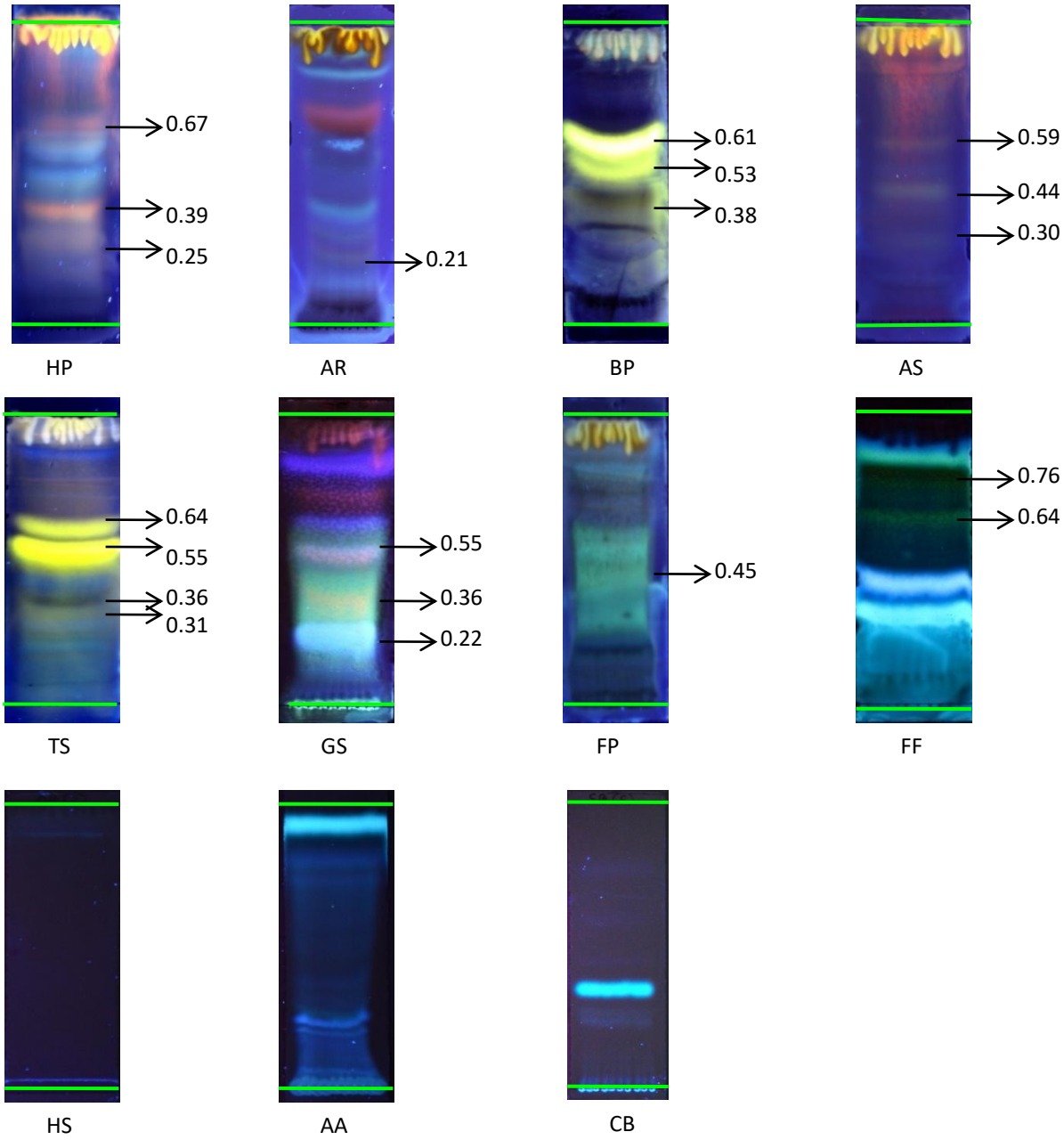
Phytoconstituent: Arbutin
Light: Visible
Colour of bands: Blue
Solvent system: Ethyl acetate:Methanol:Water (100:13.5:10)
Detecting reagent: Berlin blue reagent

Figure 4.2.5.b: Thin layer chromatography of herbal formulations for the detection of arbutin with Rf values.
HP: Heart palpitation; **AR**: Arthritis; **BP**: High blood pressure; **AS**: Asthma; **TS**: Tonsilitis; **GS**: Gastritis; **FP**: Food poisoning; **FF**: *Fraxinus floribunda* (Diabetes); **HS**: *Hedyotis scandens* (Indigestion and stomachache); **AA**: *Achyranthes aspera* (Throat infection); CB: *Clematis buchanania* (Sinusitis).



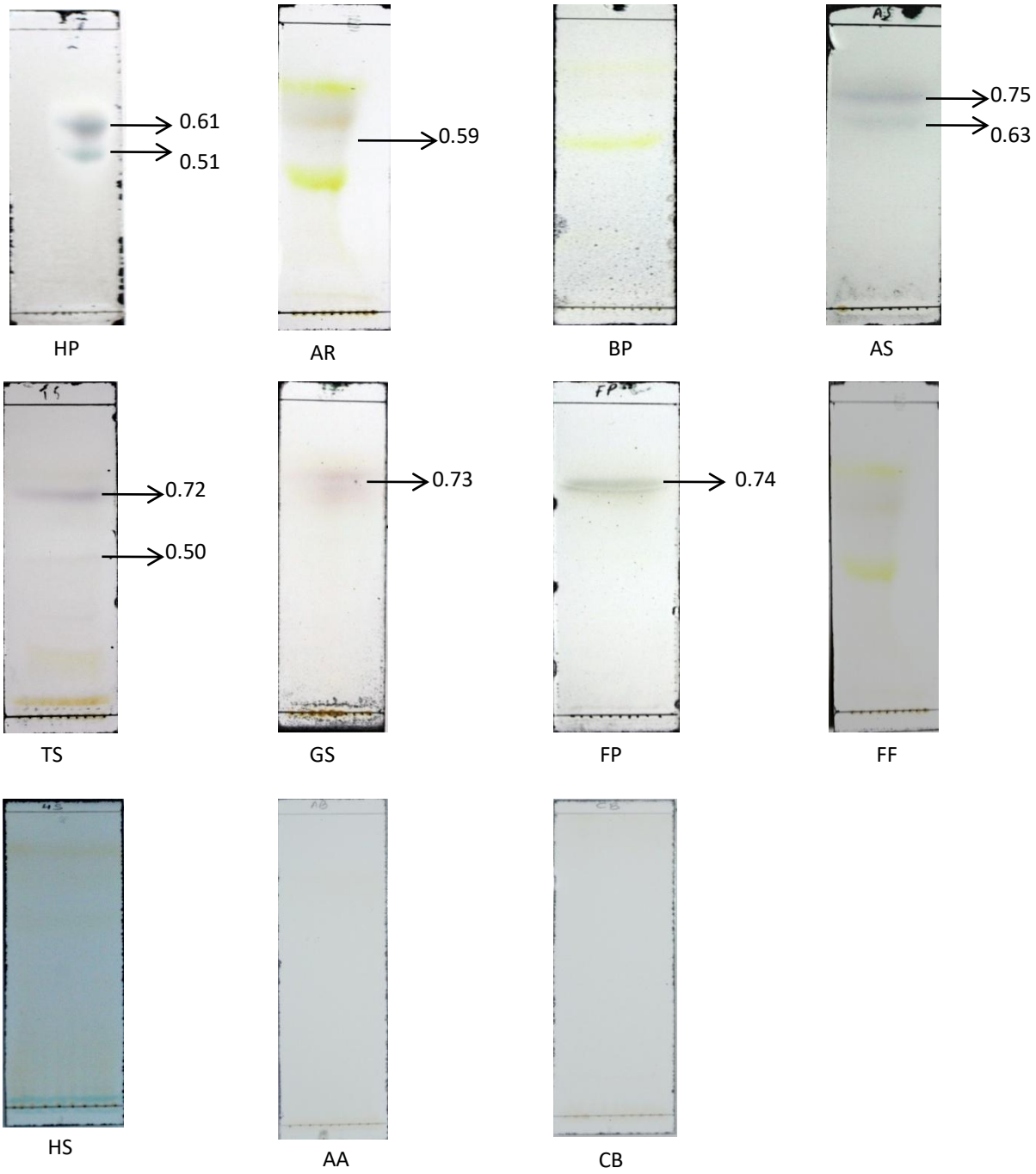
Phytoconstituent: Bitter principles
Light: UV-365 nm
Colour of bands: Blue, yellow, red, orange
Solvent system: Ethyl acetate:Methanol:Water (100:13.5:10)
Detecting reagent: Vanillin sulphuric acid reagent

Figure 4.2.5.c: Thin layer chromatography of herbal formulations for the detection of bitter principles with Rf values.
HP: Heart palpitation; **AR**: Arthritis; **BP**: High blood pressure; **AS**: Asthma; **TS**: Tonsilitis; **GS**: Gastritis; **FP**: Food poisoning; **FF**: *Fraxinus floribunda* (Diabetes); **HS**: *Hedyotis scandens* (Indigestion and stomachache); **AA**: *Achyranthes aspera* (Throat infection); **CB**: *Clematis buchanania* (Sinusitis).



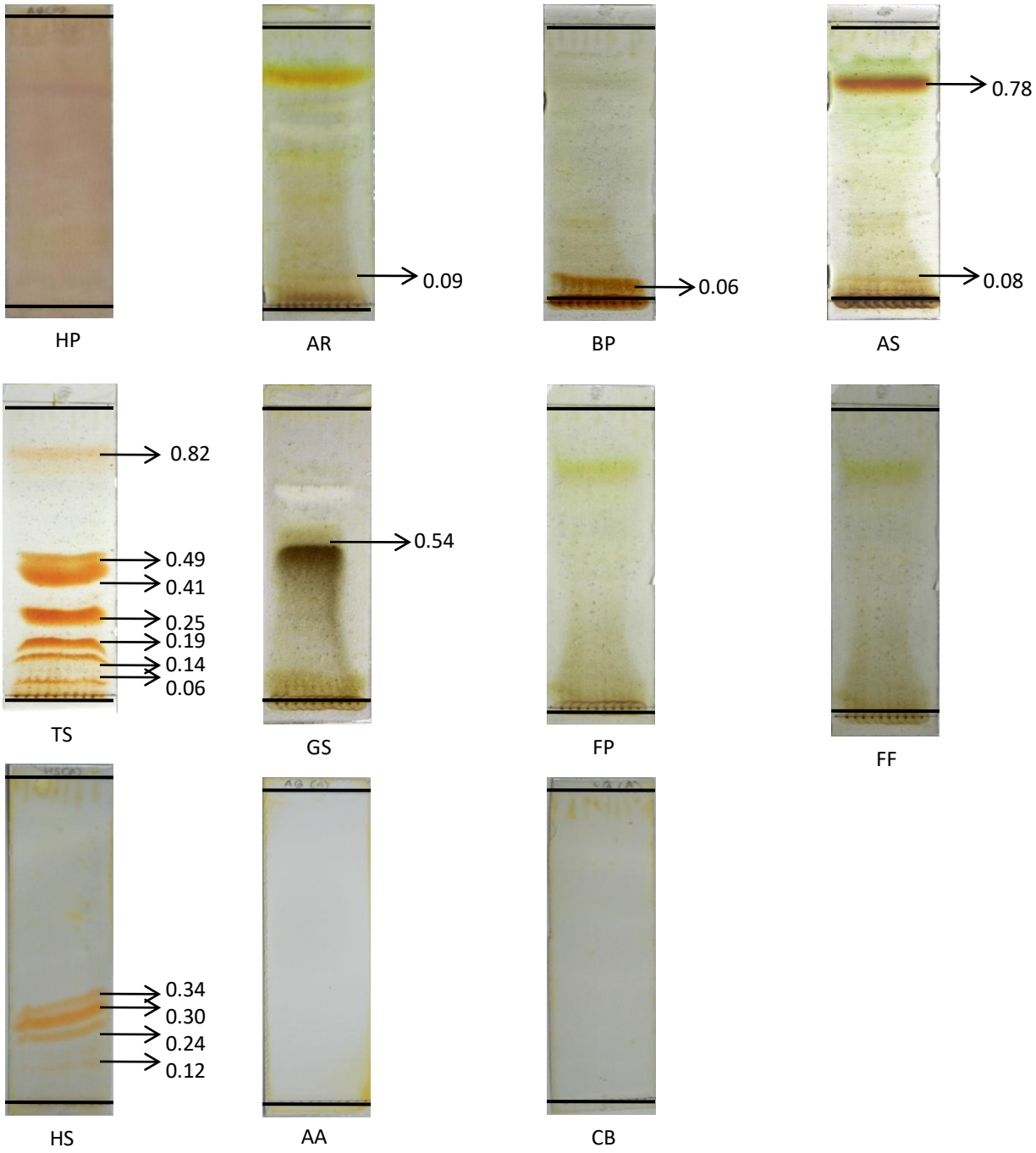
Active phytoconstituent: Flavonoids
Light: UV-365 nm
Colour of bands: Yellow and green
Solvent system: Ethyl acetate:Formic acid:Methanol:Glacial acetic acid (100:11:11:26)
Detecting reagent: NP/PEG [diphenylborinic acid aminoethylester (Natural product) and polyethylene glycol mixture]

Figure 4.2.5.d: Thin layer chromatography of herbal formulations for the detection of flavonoids.
HP: Heart palpitation; **AR**: Arthritis; **BP**: High blood pressure; **AS**: Asthma; **TS**: Tonsilitis; **GS**: Gastritis; **FP**: Food poisoning; **FF**: *Fraxinus floribunda* (Diabetes); **HS**: *Hedyotis scandens* (Indigestion and stomachache); **AA**: *Achyranthes aspera* (Throat infection); **CB**: *Clematis buchanania* (Sinusitis).



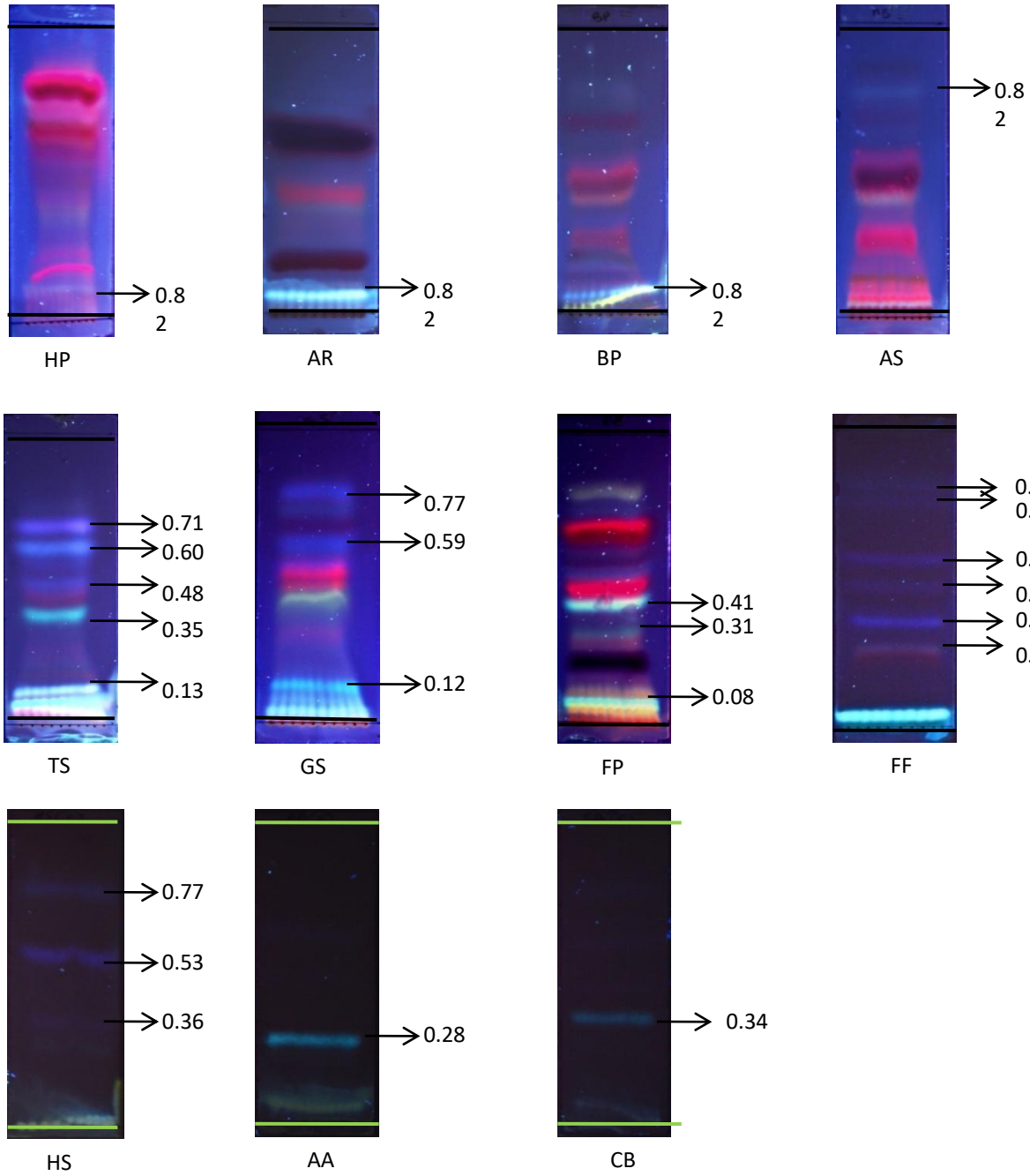
Active phytoconstituent: Cardiac glycosides
Light: Visible
Colour of bands: Blue
Solvent system: Ethyl acetate:Methanol:Water (100:13.5:10)
Detecting reagent: Antimony chloride reagent

Figure 4.2.5.e: Thin layer chromatography of herbal formulations for the detection of cardiac glycosides with Rf values.
HP: Heart palpitation; **AR**: Arthritis; **BP**: High blood pressure; **AS**: Asthma; **TS**: Tonsilitis; **GS**: Gastritis; **FP**: Food poisoning; **FF**: *Fraxinus floribunda* (Diabetes); **HS**: *Hedyotis scandens* (Indigestion and stomachache); **AA**: *Achyranthes aspera* (Throat infection); **CB**: *Clematis buchanania* (Sinusitis).



Active phytoconstituent: Alkaloids
Light: Visible
Colour of bands: Brown and orange
Solvent system: Ethyl acetate:Methanol:Water (100:13.5:10)
Detecting reagent: Dragendorff reagent

Figure 4.2.5.f: Thin layer chromatography of herbal formulations for the detection of alkaloids.
HP: Heart palpitation; **AR**: Arthritis; **BP**: High blood pressure; **AS**: Asthma; **TS**: Tonsilitis; **GS**: Gastritis; **FP**: Food poisoning; **FF**: *Fraxinus floribunda* (Diabetes); **HS**: *Hedyotis scandens* (Indigestion and stomachache); **AA**: *Achyranthes aspera* (Throat infection); **CB**: *Clematis buchanania* (Sinusitis).



Active phytoconstituent: Coumarins
Light: UV-365 nm
Colour of bands: Blue
Solvent system: Toluene: ethyl acetate (93:7)
Detecting reagent: Ethanolic KOH

Figure 4.2.5.g: Thin layer chromatography of herbal formulations for the detection of coumarins with Rf values.
HP: Heart palpitation; **AR**: Arthritis; **BP**: High blood pressure; **AS**: Asthma; **TS**: Tonsilitis; **GS**: Gastritis; **FP**: Food poisoning; **FF**: *Fraxinus floribunda* (Diabetes); **HS**: *Hedyotis scandens* (Indigestion and stomachache); **AA**: *Achyranthes aspera* (Throat infection); **CB**: *Clematis buchanania* (Sinusitis).

The R_f values of all the bands were noted down which can be used as a tool for the authentication of these formulations in future to avoid any kind of adulterations.

4.3. ESTIMATION OF PHYTOCHEMICALS CONTENT

Phytochemicals are the natural bioactive compounds present in plants which are considered to have major contribution in defense system of plants against various diseases and stress conditions which are also the reason for the therapeutic activity of the plants.

4.3.1Qualitative estimation of phytochemicals

Qualitative estimation of phytochemicals was carried out with standardized methods in all the herbal formulations and presented in figure 4.3.1.a to figure 4.3.1.k.Presence of these phytochemicals determines the therapeutic activity of plants thus it is regarded as a preliminary step before exploring their pharmacological activities. All the formulations were successively extracted in ten different solvents according to their non polar to polar nature. The results revealed that the formulation HP is presented in figure 4.3.1.a which contains cardiac glycosides and flavonoids in all the ten extracts with more concentration in HPAc (acetone extract) and HPAq (aqueous extract). Phytosterol was present in HPHp (heptane) and HPAc (acetone) and HPBu (butanol). Triterpenoids was present in HPAc, HPBu, HPEt, HPMt and HPAq while amino acids and resins were present only in HPAq and HPAc respectively. Tannins, glycosides, reducing sugars and alkaloids were absent in HP. The result of phytochemical determination of formulation AR is presented in figure 4.3.1.b. From the figure, it is clear that all the extracts of AR showed the presence of phytosterol and reducing sugars. Triterpenoids was present in all the extracts except ARHp, ARBz and ARCl while cardiac glycosides and flavonoids were preset in all the extracts except ARHp and ARCl. Glycosides were also found in these extracts of AR except for ARHx, ARHp, ARBz and ARCl. Tannins and alkaloids were absent in all the extracts and amino acid was present only in ARAq. The result of phytochemical estimation tests of formulation BP is presented in figure 4.3.1.c. It can be clearly observed that BP contained phytosterol, cardiac glycosdies, glycosides, flavonoids, tannins and reducing sugars. Phytosterol was highly concentrated in BPBz, BPAc and BPBu

while reducing sugar was mainly concentrated in BPBz and BPAc. Amino acid was present only in methanol extract (BPMt) and resin was present in BPBz and BPAq. Triterpenoids and alkaloids were not detected in BP from the tests performed for phytochemical determination. The phytochemical estimation of formulation AS is given in figure 4.3.1.d where it can be observed that active phytochemicals such as phytosterol, triterpenoids, alkaloids, cardiac glycosides, glycosides, flavonoids, tannins, reducing sugars and anthraquinones were present in AS but amino acids and resins were found to be absent in all the extracts. These phytochemicals were highly concentrated in ASEa, ASAc and ASAq. Phytochemical estimation of formulation TS is presented in figure 4.3.1.e. The figure presented that TSCl (chloroform extract) did not show the presence of any of the phytochemicals from the tests performed. Amino acid was absent but alkaloid was present in some non-polar solvent extracts (TSHx, TSBz, TSEa), glycosides was present only in aqueous extract (TSAq) and flavonoids were present in TSBu and TSEt only. Active phytoconstituents such as phytosterol, triterpenoids, cardiac glycosides, tannins and anthraquinone were highly concentrated in benzene (TSBz) and ethyl acetate (TSEa) extracts. Qualitative phytochemical estimation of formulation, GS is presented in figure 4.3.1.f. Phytosterol and reducing sugar were present in all the extracts of GSwith more concentration in GSEa, GSAc and GSEt. Resins were also concentrated highly in GSEa and GSAc but were not observed in hexane, heptanes and benzene extracts. AS also contained other phytochemicals such as tannins, terpenoids (in GSHx, GSBz), cardiac glycosides and flavonoids (in GSEt and GSMt). Glycosides and alkaloids were not observed in GS from the tests performed. Phytochemical estimation of formulation AA is given in figure 4.3.1.g and it was found that only phytosterol was present in all the extracts and phytochemicals such as anthraquinone, cardiac glycosides, glycosides, alkaloids, flavonoids and resins were found to be absent. Tannin and reducing sugar were present in AAMt and AAAq, triterpenoids was present in AAAc, AAEt, AAMt and AAAq, amino acid was present only in AAAq.The result of qualitative phytochemical tests of formulation CB is presented in figure 4.3.1.h which revealed that CB contained phytosterol, tannin, triterpenoids, flavonoids, reducing sugar, resin and amino acid but only in methanolic and aqueous extracts (CBMt and CBAq). Phytochemical estimation of formulation HS is given in figure 4.3.1.i and this formulation also

Phytochemicals	HPHx	HPHp	HPBz	HPEa	HPCl	HPAc	HPBu	HPEt	HPMt	HPAq
Physterol	-	+	-	-	-	++	+	-	-	-
Tannin	-	-	-	-	-	-	-	-	-	-
Triterpenoids	-	-	-	-	-	+	+	+	+	+
Amino acid	-	-	-	-	-	-	-	-	-	+
Resin	-	-	-	-	-	+	-	-	-	-
Glycosides	-	-	-	-	-	-	-	-	-	-
Cardiac glycosides	+	+	+	+	+	++	+	+	++	++
Reducing sugars	-	-	-	-	-	-	-	-	-	-
Flavonoids	+	+	++	++	+	+++	+	+	+	+++
Alkaloids	-	-	-	-	-	-	-	-	-	-

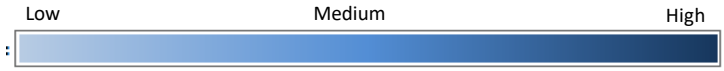


Figure 4.3.1.a: Qualitative estimation of phytochemicals of herbal formulation used for the treatment of heart palpitation (HP)
HPHx: Hexane extract; HPHp: Heptane extract; HPBz: Benzene extract; HPEa: Ethyl acetate extract; HPCl: Chloroform extract; HPAc: Acetone extract; HPBu: Butanol extract; HPEt: Ethanol extract; HPMt: Methanol extract; HPAq: Aqueous extract

Phytochemicals	ARHx	ARHp	ARBz	AREa	ARCl	ARAc	ARBu	AREt	ARMt	ARAq
Phytosterol	+	+	+	++	+	++	+++	++	+++	++
Tannin	-	-	-	-	-	-	-	-	-	-
Triterpenoids	+	-	-	+	-	+	++	+	++	+
Cardiac glycosides	+	-	+	++	-	++	+	+	+	+
Glycosides	-	-	-	+	-	++	+	+	+	+
Alkaloids	-	-	-	-	-	-	-	-	-	-
Flavonoids	+	-	+	++	-	++	++	+	++	+
Reducing sugar	+	+	+	++	+	++	++	++	++	+
Resin	-	-	-	++	-	+	-	-	+	+
Amino acids	-	-	-	-	-	-	-	-	-	+

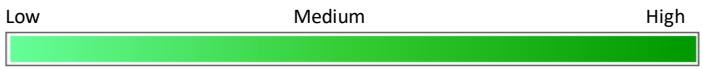


Figure 4.3.1.b: Qualitative estimation of phytochemicals of herbal formulation used for the treatment of arthritis (AR)
ARHx: Hexane extract; ARHp: Heptane extract; ARBz: Benzene extract; AREa: Ethyl acetate extract; ARCl: Chloroform extract; ARAc: Acetone extract; ARBu: Butanol extract; AREt: Ethanol extract; ARMt: Methanol extract; ARAq: Aqueous extract

Phytochemials	BPHx	BPHp	BPBz	BPEa	BPCl	BPAc	BPBu	BPEt	BPMt	BPAq
Amino acid	-	-	-	-	-	-	-	-	+	-
Phytosterol	+	+	+++	+	+	+++	+++	+	+	++
Triterpenoids	-	-	-	-	-	-	-	-	-	-
Alkaloids	-	-	-	-	-	-	-	-	-	-
Cardiac glycosides	+	-	++	+	-	++	++	+	+	+
Glycosides	-	-	+	-	-	+	+	-	+	++
Flavonoids	-	-	++	+	-	+	+	-	+	++
Tannin	-	-	++	-	-	+	-	-	-	++
Reducing sugar	++	-	+++	-	-	+++	++	-	-	+
Resins	-	-	++	-	-	-	-	-	-	+

Figure 4.3.1.c: Qualitative estimation of phytochemicals of herbal formulation used for the treatment of high blood pressure (BP)
BPHx: Hexane extract; BPHp: Heptane extract; BPBz: Benzene extract; BPEa: Ethyl acetate extract; BPCl: Chloroform extract; BPAc: Acetone extract; BPBu: Butanol extract; BPEt: Ethanol extract; BPMt: Methanol extract; BPAq: Aqueous extract

Phytochemicals	ASHx	ASHp	ASBz	ASEa	ASCl	ASAc	ASBu	ASEt	ASMt	ASAq
Amino acid	-	-	-	-	-	-	-	-	-	-
Phytosterol	+	+	+	+++	+	+++	+++	+++	++	+++
Triterpenoids	+	-	+	+++	-	+++	++	++	++	++
Alkaloids	+	-	+	+	+	+	+	-	-	-
Cardiac glycosides	+	-	+	++	+	+++	++	+	++	+++
Glycosides	-	-	-	+	-	++	+	+	+	++
Flavonoids	-	-	-	++	-	++	++	++	+	++
Tannin	+	-	+	+++	-	+++	++	++	++	+++
Reducing sugar	+	-	+	++	-	+++	+	+	+	+
Resins	-	-	-	-	-	-	-	-	-	-
Anthraquinones	-	-	-	++	-	++	+	+	+	++

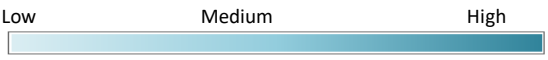


Figure 4.3.1.d: Qualitative estimation of phytochemicals of herbal formulation used for the treatment of asthma(AS)
ASHx: Hexane extract; ASHp: Heptane extract; ASBz: Benzene extract; ASEa: Ethyl acetate extract; ASCl: Chloroform extract; ASAc: Acetone extract; ASBu: Butanol extract; ASEt: Ethanol extract; ASMt: Methanol extract; ASAq: Aqueous extract

Phytochemicals	TSHx	TSHp	TSBz	TSEa	TSCl	TSAc	TSBu	TSEt	TSMt	TSAq
Amino acid	-	-	-	-	-	-	-	-	-	-
Phytosterol	+	+	++	+++	-	++	+	++	++	-
Triterpenoids	+	+	++	+++	-	++	+	+	+	++
Alkaloids	+	-	+	+	-	+	-	-	-	-
Cardiac glycosides	+	+	+++	+++	-	++	-	+	+	-
Glycosides	-	-	-	-	-	-	-	-	-	+
Flavonoids	-	-	-	-	-	-	+	+	-	-
Tannin	+	+	+++	+++	-	++	+	++	++	++
Reducing sugar	-	-	++	++	-	-	-	-	+	+
Resins	+	-	+	++	-	+	-	-	+	+
Anthraquinones	-	-	+	+++	-	+	-	+	+	++

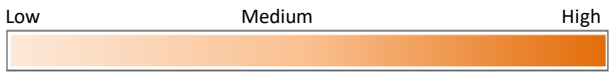


Figure 4.3.1.e: Qualitative estimation of phytochemicals of herbal formulation used for the treatment of tonsilitis (TS)
TSHx: Hexane extract; TSHp: Heptane extract; TSBz: Benzene extract; TSEa: Ethyl acetate extract; TSCl: Chloroform extract; TSAc: Acetone extract; TSBu: Butanol extract; TSEt: Ethanol extract; TSMt: Methanol extract; TSAq: Aqueous extract

Phytochemicals	GSHx	GSHp	GSBz	GSEa	GSCl	GSAc	GSBu	GSEt	GSMt	GSAq
Tannins	-	-	-	++	-	++	+	+	+	+
Phytosterol	++	+	++	+++	+	+++	++	++	++	++
Triterpenoids	+	-	+	-	-	-	-	-	-	-
Cardiac glycosides	+	-	+	++	-	-	++	+	+	+
Flavonoids	-	-	-	++	-	++	++	++	++	+
Alkaloids	-	-	-	-	-	-	-	-	-	-
Amino acids	-	-	-	-	-	-	-	+	+	-
Reducing sugar	++	++	++	++	+	+++	++	+++	++	++
Glycosides	-	-	-	-	-	-	-	-	-	-
Resins	-	-	-	++++	-	+++	+	++	+	++

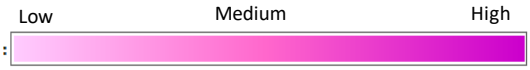


Figure 4.3.1.f: Qualitative estimation of phytochemicals of herbal formulation used for the treatment of gastriti(GS)
GSHx: Hexane extract; GSHp: Heptane extract; GSBz: Benzene extract; GSEa: Ethyl acetate extract; GSCl: Chloroform extract; GSAc: Acetone extract; GSBu: Butanol extract; GSEt: Ethanol extract; GSMt: Methanol extract; GSAq: Aqueous extract

Phytochemicals	AAHx	AAHp	AABz	AAEa	AACl	AAAc	AABu	AAEt	AAMt	AAAq
Phytosterol	+	+	+	+	+	+	+	+	+	+
Anthraquinones	-	-	-	-	-	-	-	-	-	-
Tannin	-	-	-	-	-	-	-	-	+	+
Triterpenoids	-	-	-	-	-	+	-	+	+	+
Cardiac glycosides	-	-	-	-	-	-	-	-	-	-
Glycosides	-	-	-	-	-	-	-	-	-	-
Alkaloids	-	-	-	-	-	-	-	-	-	-
Flavonoids	-	-	-	-	-	-	-	-	-	-
Reducing sugar	-	-	-	-	-	-	-	-	+	+
Amino acids	-	-	-	-	-	-	-	-	-	+
Resins	-	-	-	-	-	-	-	-	-	-

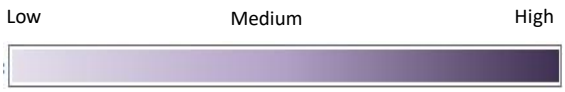


Figure 4.3.1.g: Qualitative estimation of phytochemicals of *Achyranthes aspera* root used for the treatment of throat infection (AA)
AAHx: Hexane extract; AAHp: Heptane extract; AABz: Benzene extract; AAEa: Ethyl acetate extract; AACl: Chloroform extract; AAAc: Acetone extract; AABu: Butanol extract; AAEt: Ethanol extract; AAMt: Methanol extract; AAAq: Aqueous extract

Phytochemicals	CBHx	CBHp	CBBz	CBEa	CBCl	CBAc	CBBu	CBEt	CBMt	CBAq
Phytosterol	-	-	-	-	-	-	-	-	+	+
Anthraquinones	-	-	-	-	-	-	-	-	-	-
Tannin	-	-	-	-	-	-	-	-	-	+
Triterpenoids	-	-	-	-	-	-	-	-	+	++
Cardiac glycosides	-	-	-	-	-	-	-	-	-	-
Glycosides	-	-	-	-	-	-	-	-	-	-
Alkaloids	-	-	-	-	-	-	-	-	-	-
Flavonoids	-	-	-	-	-	-	-	-	-	-
Reducing sugar	-	-	-	-	-	-	-	-	+	+
Resins	-	-	-	-	-	-	-	-	-	+
Amino acids	-	-	-	-	-	-	-	-	+	++

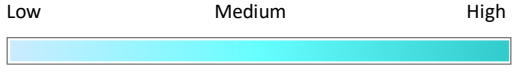


Figure 4.3.1.h: Qualitative estimation of phytochemicals of *Clematis buchananiana* root used for the treatment of sinusitis (CB)
CBHx: Hexane extract; CBHp: Heptane extract; CBBz: Benzene extract; CBEa: Ethyl acetate extract; CBCl: Chloroform extract; CBAc: Acetone extract; CBBu: Butanol extract; CBEt: Ethanol extract; CBMt: Methanol extract; CBAq: Aqueous extract

Phytochemicals	HSHx	HSHp	HSBz	HSEa	HSCl	HSAc	HSBu	HSEt	HSMt	HSAq
Phytosterol	-	-	-	-	-	-	+	+	+	++
Anthraquinones	-	-	-	-	-	-	-	-	-	-
Tannin	-	-	-	-	-	-	-	-	+	+
Triterpenoids	-	-	-	-	-	-	-	-	+	+
Cardiac glycosides	-	-	-	-	-	-	-	-	-	-
Glycosides	-	-	-	-	-	-	-	-	-	-
Alkaloids	-	-	-	-	-	-	-	-	-	-
Flavonoids	-	-	-	-	-	-	-	-	-	-
Reducing sugar	-	-	-	-	-	+	+	+	+	+
Resins	-	-	-	-	-	-	-	-	-	-
Amino acids	-	-	-	-	-	-	-	-	-	-

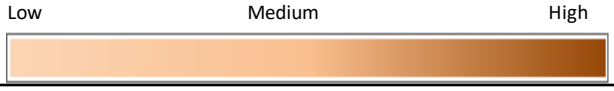


Figure 4.3.1.i: Qualitative estimation of phytochemicals of *Hedyotis scandens* root used for the treatment of indigestion and stomachache (HS)
HSHx: Hexane extract; HSHp: Heptane extract; HSBz: Benzene extract; HSEa: Ethyl acetate extract; HSCl: Chloroform extract; HSAc: Acetone extract; HSBu: Butanol extract; HSEt: Ethanol extract; HSMt: Methanol extract; HSAq: Aqueous extract

Phytochemicals	FFHx	FFHp	FFBz	FFEa	FFCl	FFAc	FFBu	FFEt	FFMt	FFAq
Phytosterol	+	+	+	+	+	++	++	++	++	++++
Anthraquinones	-	-	-	+	-	++	+	+	+	++
Tannin	+	+	+	++	-	+++	+	+	++	+++
Triterpenoids	+	+	+	+	+	+	+	+	+	++
Cardiac glycosides	-	-	-	-	-	+	-	-	+	+
Glycosides	-	-	-	-	-	+	-	-	+	+
Alkaloids	-	-	-	-	-	-	-	-	+	+
Flavonoids	+	+	+	++	+	+++	+	+	++	+++
Reducing sugar	+	+	+	+	-	+	+	+	+	++
Resins	-	-	-	-	-	-	-	-	-	+
Amino acids	-	-	-	-	-	-	-	-	+	+

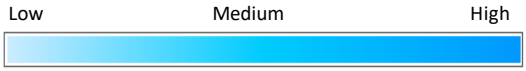


Figure 4.3.1.j: Qualitative estimation of phytochemicals of *Fraxinus floribunda bark* used for the treatment of diabetes (FF)
FFHx: Hexane extract; FFHp: Heptane extract; FFBz: Benzene extract; FFEa: Ethyl acetate extract; FFCl: Chloroform extract; FFAc: Acetone extract; FFBu: Butanol extract; FFEt: Ethanol extract; FFMt: Methanol extract; FFAq: Aqueous extract

Phytochemicals	FPHx	FPHp	FPBz	FPEa	FPCl	FPAc	FPBu	FPEt	FPMt	FPAq
Anthraquinones	+	-	+	++	+	++	+	++	+	++
Phytosterol	++	+	++	+++	+	+++	+	++	+	++
Tannin	++	-	+++	+++	++	+++	++	+++	++	++
Triterpenoids	+++	+	+++	++	++	+++	+	++	+	+
Flavonoids	-	-	-	+	-	++	+	++	+	+
Cardiac glycosides	+	-	++	++	+	+++	+	++	-	++
Amino acid	-	-	-	-	-	-	-	-	-	-
Resins	-	-	-	-	-	-	-	-	-	-
Alkaloids	++	-	++	+	+	+	+	-	-	-
Glycosides	-	-	+	+	-	++	-	+	-	+
Reducing sugar	-	-	+	+	-	++	-	+	-	+

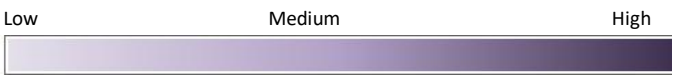


Figure 4.3.1.k: Qualitative estimation of phytochemicals of herbal formulation used for the treatment of food poisoning (FP)
FPHx: Hexane extract; FPHp: Heptane extract; FPBz: Benzene extract; FPEa: Ethyl acetate extract; FPCl: Chloroform extract; FPAc: Acetone extract; FPBu: Butanol extract; FPEt: Ethanol extract; FPMt: Methanol extract; FPAq: Aqueous extract

showed the presence of some phytochemicals in few extracts only. Phytosterol was present in HSBu, HSEt, HSMt and HSAq. Tannin and triterpenoids were present in HSMt and HSAq only. Reducing sugar was observed in HSAc, HSBu, HSEt, HSMt and HSAq. Result of phytochemical determination of formulation FF is presented in figure 4.3.1.j. Phytosterol was present in all the extract but was concentrated highly in aqueous extract (FFAq). Tannin and flavonoids were also present with high concentration in the extracts, FFAc and FFAq. Alkaloids and amino acids were present inFFMt and FFAq only while cardiac glycosides and glycosides were present in FFAc, FFMt and FFAq. Resin was also observed but only in aqueous extract. The phytochemicals present in herbal formulation FP is presented in figure 4.3.1.k. All the tested phytochemicals were present in FP in different solvent extract except for resins and amino acid which were absent.

4.3.2. Quantitative estimation of phytochemicals

Quantitative estimation of some phytochemicals present in ten different solvent extracts of eight studied herbal formulations was carried out and presented in figure 4.3.2.a to 4.3.2.f and table 4.3.2.a to 4.3.2.f. From the figures and tables, it was observed that the aqueous extract of *F. floribunda* (FFAq) showed highest phenol (712.130±0.26mg GAE/g EW), flavonoids (56.330±0.22 mg QE/g EW), tannin (823.450±0.65 mg TAE/g EW) and steroid content (1.29±0.05 mg SE/g EW).Total ortho-dihydric phenol content was found to be highest in heptane extract of HP (88.859±0.018 mg CE/g EW). Alkaloid content was highest in ethyl acetate extract of TS (2.870±0.06 mg SE/g EW). It was observed that alkaloid content was mostly found to be present in non-polar solvent extracts except for sample BP and FF. Only in FF, alkaloid content was detected in all the solvent extracts while most of the herbal formulations revealed the presence of alkaloids mostly from hexane to ethyl acetate.

4.4. ANTIMICROBIAL ACTIVITY

The antimicrobial activities of ethanol and aqueous extracts of eight formulations were screened against two gram negative (*Escherichia coli* and *Salmonella typhi*) and three gram positive bacteria (*Bacillus megaterium*, *Bacillus subtilis* and

Table 4.3.2.a: Total phenol content of studied eight herbal formulations in different solvent extracts (mg Gallic acid eq./g EW)

Solvents used	HP	AR	BP	GS	FP	AS	TS	FF
Hexane	4.353±0.074	NA	46.948±0.119	10.602±0.006	NA	41.078±0.001	38.262±0.001	67.340±0.21
Heptane	361.413±0.003	20.483±0.027	70.063±0.056	NA	NA	98.595±0.001	73.295±0.001	45.210±0.56
Benzene	139.200±0.082	93.887±0.069	152.044±0.236	82.238±0.120	44.014±0.088	74.794±0.002	24.580±0.001	187.450±0.12
Ethyl acetate	176.077±0.239	152.486±0.371	166.166±0.039	618.130±0.121	125.011±0.054	103.617±0.002	15.899±0.002	234.660±0.56
Chloroform	100.849±0.046	91.183±0.082	210.117±0.045	120.342±0.041	45.802±0.036	51.325±0.001	8.972±0.001	65.120±0.93
Acetone	272.988±0.201	321.453±0.556	38.024±0.251	399.231±0.126	95.192±0.032	73.975±0.002	23.298±0.001	456.190±0.11
Butanol	111.389±0.088	188.636±0.516	55.022±0.222	414.122±0.059	162.941±0.049	51.833±0.002	20.300±0.001	397.590±0.07
Ethanol	24.419±0.085	80.781±0.511	360.33±0.148	475.341±0.041	128.524±0.073	47.740±0.002	27.702±0.001	456.200±0.82
Methanol	39.162±0.263	106.796±0.412	105.290±0.306	406.193±0.073	341.637±0.039	41.730±0.001	30.889±0.001	521.320±0.51
Water	122.434±0.169	64.539±0.530	224.080±0.347	279.372±0.091	68.244±0.063	45.624±0.001	22.992±0.001	712.130±0.26

Table 4.3.2.b: Total flavonoid content of studied eight herbal formulations in different solvent extracts (mg quercetin eq./g EW)

Solvents used	HP	AR	BP	GS	FP	AS	TS	FF
Hexane	12.627±0.042	NA	17.847±0.065	1.204±0.008	12.572±0.001	13.169±0.001	8.803±0.001	4.560±0.02
Heptane	NA	14.022±0.025	6.644±0.028	NA	7.342±0.001	NA	3.210±0.001	10.380±0.17
Benzene	15.459±0.046	24.804±0.057	27.654±0.102	7.102±0.002	15.067±0.002	8.904±0.001	7.456±0.001	15.660±0.94
Ethyl acetate	7.326±0.033	34.525±0.069	6.212±0.031	13.050±0.002	28.119±0.001	5.256±0.001	12.387±0.001	23.920±0.37
Chloroform	NA	8.229±0.023	10.563±0.014	2.547±0.017	19.138±0.002	3.215±0.001	NA±	5.010±0.07
Acetone	15.387±0.072	24.680±0.155	4.812±0.063	4.622±0.032	20.409±0.001	7.249±0.001	2.453±0.001	38.560±0.27
Butanol	NA	15.262±0.016	5.903±0.084	9.841±0.032	23.153±0.002	3.044±0.001	NA	25.610±0.48
Ethanol	1.126±0.007	4.578±0.130	16.635±0.036	2.027±0.011	13.952±0.001	1.211±0.001	2.520±0.001	32.110±0.11
Methanol	1.716±0.032	0.120±0.037	0.120±0.087	0.120±0.003	23.748±0.002	0.120±0.002	4.699±0.001	41.780±0.39
Water	10.053±0.112	3.412±0.063	8.166±0.039	1.086±0.009	13.304±0.002	4.309±0.001	1.553±0.001	56.330±0.22

HP: Heart palpitation; **AR**: Arthritis; **BP**: High blood pressure; **GS**: Gastritis; **FP**: Food poisoning;**AS**: Asthma; **TS**: Tonsilitis; **FF**: *Fraxinus floribunda* (Diabetes)

Table 4.3.2.c: Total ortho-dihydric content of studied eight herbal formulations in different solvent extracts (mg catechol eq./g EW)

TOPC	HP	AR	BP	GS	FP	AS	TS	FF
Hexane	18.983±0.011	NA	NA	1.691±0.023	12.371±0.001	14.867±0.001	4.089±0.001	3.410±0.09
Heptane	**88.859±0.018**	0.060±0.01	NA	NA	8.235±0.001	13.306±0.001	6.861±0.001	1.230±0.12
Benzene	14.128±0.004	0.269±0.059	20.589±0.154	7.109±0.019	10.820±0.002	14.061±0.001	3.112±0.001	5.660±0.38
Ethyl acetate	19.021±0.005	0.625±0.065	NA	21.733±0.021	19.834±0.095	3.799±0.002	6.617±0.001	12.940±0.21
Chloroform	33.443±0.02	0.056±0.023	NA	14.868±0.027	16.989±0.001	7.312±0.002	0.390±0.001	1.950±0.29
Acetone	17.406±0.044	1.109±0.006	4.039±0.055	7.743±0.012	12.963±0.032	3.597±0.001	3.273±0.001	28.750±0.14
Butanol	13.628±0.019	1.159±0.009	6.431±0.063	15.501±0.002	22.188±0.007	2.225±0.001	0.738±0.001	21.390±0.33
Ethanol	5.902±0.007	0.264±0.038	19.182±0.018	13.626±0.027	9.656±0.001	1.285±0.001	2.115±0.002	19.010±0.07
Methanol	3.742±0.022	0.488±0.062	12.862±0.070	9.950±0.012	19.692±0.007	1.406±0.001	2.251±0.002	17.840±0.12
Water	13.556±0.077	0.284±0.038	16.116±0.041	6.934±0.006	6.704±0.002	4.339±0.001	1.618±0.002	21.120±0.19

Table 4.3.2.d: Total alkaloid content of studied eight herbal formulations in different solvent extracts (mg solasodine eq./g EW)

Solvents used	HP	AR	BP	GS	FP	AS	TS	FF
Hexane	0.120±0.01	0.340±0.150	NA	0.020±0.001	0.260±0.18	0.450±0.01	1.230±0.01	0.34±0.001
Heptane	0.290±0.002	0.630±0.101	NA	NA	NA	0.760±0.02	2.320±0.12	0.21±0.002
Benzene	0.350±0.007	1.800±0.203	NA	0.020±0.004	0.160±0.01	1.650±0.04	2.560±0.04	0.31±0.008
Ethyl acetate	1.220±0.01	1.240±0.187	NA	0.040±0.002	0.090±0.03	2.310±0.05	**2.870±0.06**	0.49±0.003
Chloroform	NA	NA	NA	NA	NA	NA	NA	0.68±0.004
Acetone	NA	NA	0.010±	0.010±0.006	NA	NA	NA	0.71±0.002
Butanol	NA	NA	0.010±	0.030±0.001	NA	NA	NA	0.61±0.005
Ethanol	NA	NA	0.110±	NA	NA	NA	NA	0.54±0.007
Methanol	NA	NA	0.120±	NA	NA	NA	NA	0.87±0.012
Water	NA	NA	NA	NA	NA	NA	NA	1.12±0.035

HP: Heart palpitation; **AR**: Arthritis; **BP**: High blood pressure; **GS**: Gastritis; **FP**: Food poisoning;**AS**: Asthma; **TS**: Tonsilitis; **FF**: *Fraxinus floribunda* (Diabetes)

Table 4.3.2.e: Total tannin content of studied eight herbal formulations in different solvent extracts (mg tannic acid eq./g EW)

Solvents used	HP	AR	BP	GS	FP	AS	TS	FF
Hexane	86.340±0.12	NA	0.034±0.002	0.027±0.014	0.023±0.035	45.090±	41.240±0.02	76.230±0.21
Heptane	301.290±0.98	77.190±0.010	58.630±0.006	73.002±0.010	NA	112.490±	82.010±0.13	56.090±0.32
Benzene	121.030±1.21	180.619±0.046	98.672±0.191	137.906±0.019	57.954±0.085	83.140±	34.510±0.45	213.160±0.15
Ethyl acetate	188.130±0.45	343.231±0.331	95.981±0.043	249.613±0.065	212.786±0.178	127.810±	20.430±0.28	254.720±0.42
Chloroform	109.110±0.28	191.220±0.017	101.142±0.014	1397.472±0.084	105.613±0.053	65.090±	15.010±0.13	77.090±0.98
Acetone	265.870±0.72	394.423±0.306	19.104±0.148	143.662±0.094	221.411±0.338	82.340±	29.040±0.67	523.280±0.56
Butanol	120.120±0.82	217.096±0.213	38.930±0.106	311.996±0.341	621.674±0.076	59.150±	25.610±0.28	425.080±0.71
Ethanol	19.010±0.38	97.544±0.087	306.214±0.056	388.812±0.187	188.452±0.166	51.020±	37.240±0.17	477.170±0.28
Methanol	45.390±0.19	89.822±0.135	79.188±0.059	370.446±0.234	257.924±0.130	43.160±	43.290±0.33	542.210±0.18
Water	132.200±0.27	6.835±0.017	86.013±0.288	160.946±0.241	72.893±0.166	47.090±	28.180±0.21	**823.450±0.65**

Table 4.3.2.f: Total steroid content of studied eight herbal formulations in different solvent extracts (mg solasodine eq./g EW)

Solvents used	HP	AR	BP	GS	FP	AS	TS	FF
Hexane	0.320±0.001	0.350±0.099	0.210±0.001	0.350±0.01	0.412±0.001	0.220±0.001	0.780±0.02	0.12±0.07
Heptane	0.120±0.01	0.330±0.013	0.110±0.003	0.330±0.002	NA	0.240±0.003	0.990±0.31	0.26±0.006
Benzene	0.270±0.002	0.850±0.063	0.320±0.002	0.850±0.003	0.522±0.002	0.450±0.004	0.590±0.26	0.31±0.09
Ethyl acetate	0.650±0.004	0.250±0.069	0.410±0.001	0.250±0.012	0.303±0.004	0.340±0.002	0.820±0.17	0.72±0.19
Chloroform	0.620±0.001	0.120±0.019	0.100±0.004	0.120±0.032	0.011±0.006	0.120±0.004	0.450±0.31	0.09±0.003
Acetone	0.130±0.008	0.070±0.037	1.230±0.021	0.070±0.02	0.904±0.001	0.040±0.002	1.120±0.19	1.09±0.006
Butanol	0.220±0.005	0.080±0.044	0.980±0.002	0.080±0.011	0.321±0.003	0.270±0.001	0.690±0.28	0.89±0.007
Ethanol	0.340±0.004	0.040±0.043	0.870±0.005	0.040±0.041	0.287±0.02	0.380±0.005	0.360±0.06	0.79±0.03
Methanol	0.250±0.002	0.150±0.044	0.730±0.001	0.150±0.011	0.276±0.03	0.270±0.002	0.810±0.07	0.89±0.02
Water	0.210±0.007	0.030±0.055	0.380±0.005	0.030±0.011	0.102±0.006	0.170±0.005	0.490±0.06	1.29±0.05

HP: Heart palpitation; **AR**: Arthritis; **BP**: High blood pressure; **GS**: Gastritis; **FP**: Food poisoning;**AS**: Asthma; **TS**: Tonsilitis; **FF**: *Fraxinus floribunda* (Diabetes)

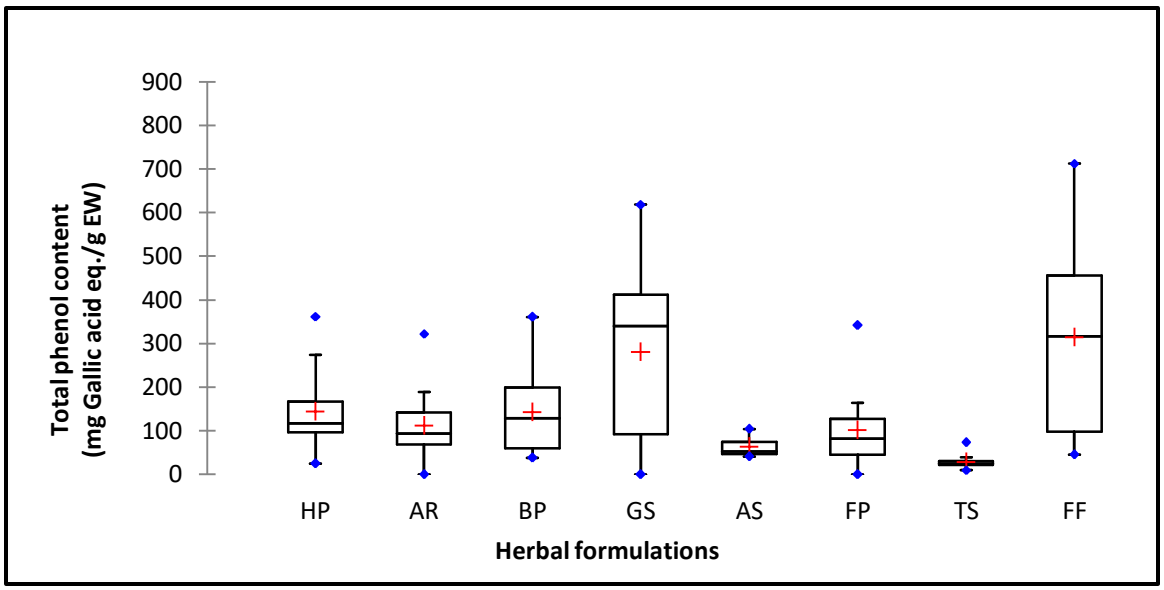


Figure 4.3.2.a : Total phenol content of studied eight herbal formulations.
HP: heart palpitaion; **AR**: Arthritis; **BP**: High blood pressure; **GS**: Gastritis; **AS**: Asthma; **FP**: Food poisoning; **TS**: Tonsilitis; **FF**: *Fraxinus floribunda* for diabetes

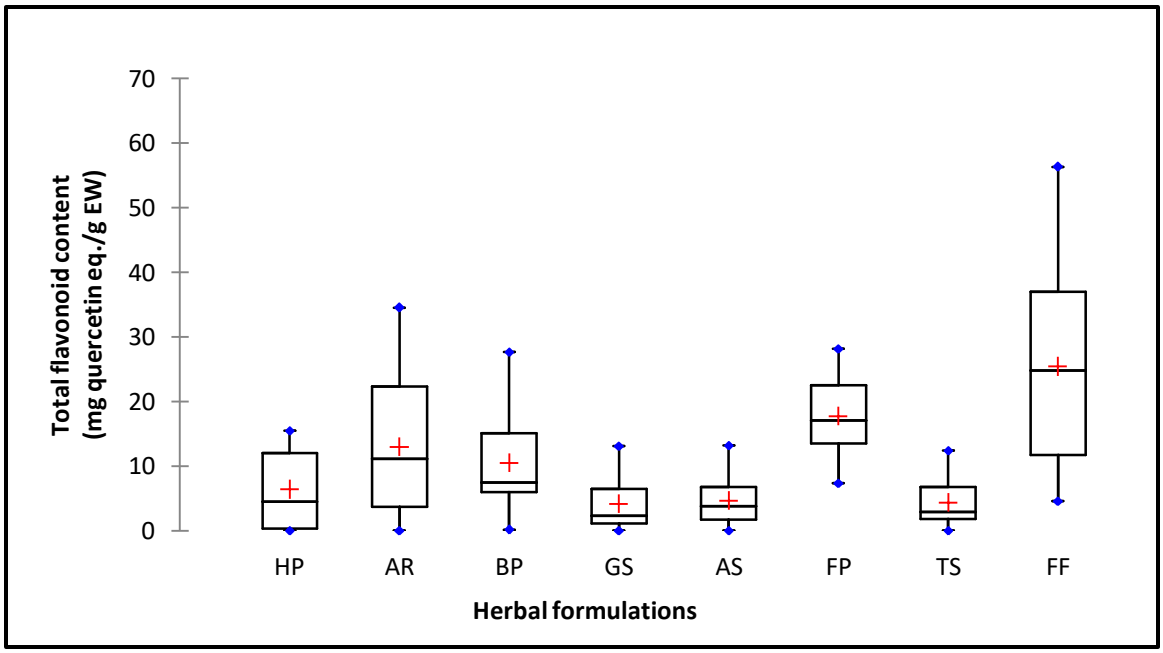


Figure 4.3.2.b : Total flavonoid content of studied eight herbal formulations
HP: heart palpitaion; **AR**: Arthritis; **BP**: High blood pressure; **GS**: Gastritis; **AS**: Asthma; **FP**: Food poisoning; **TS**: Tonsilitis; **FF**: *Fraxinus floribunda* for diabetes

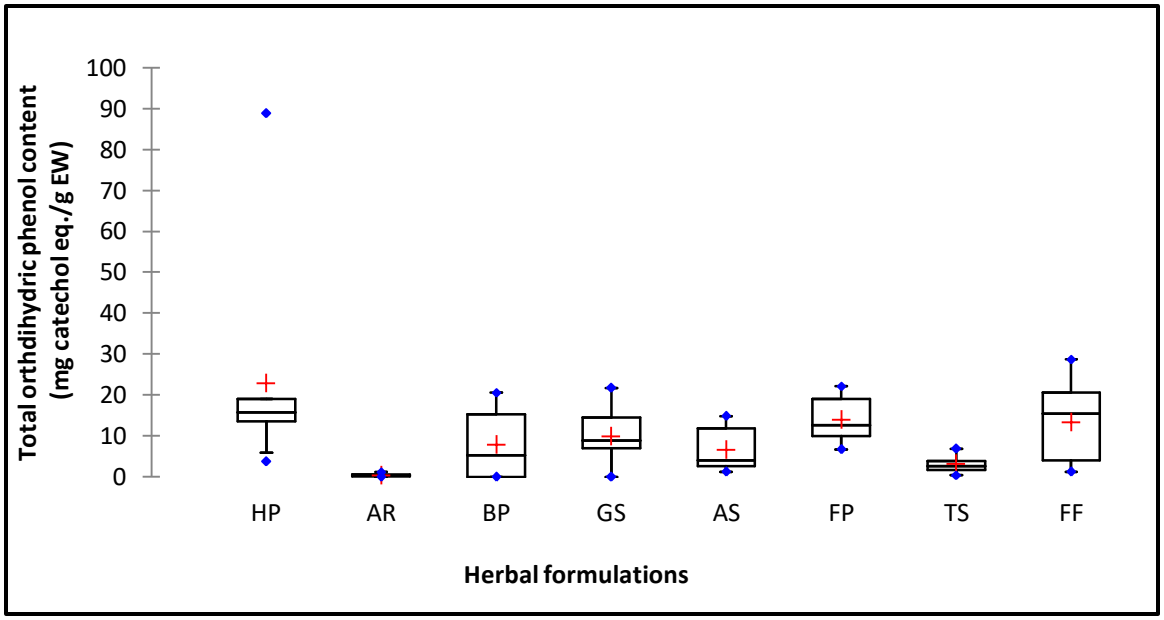


Figure 4.3.2.c: Total orthodihydric phenol content of studied eight herbal formulations
HP: heart palpitaion; **AR**: Arthritis; **BP**: High blood pressure; **GS**: Gastritis; **AS**: Asthma; **FP**: Food poisoning; **TS**: Tonsilitis; **FF**: *Fraxinus floribunda* for diabetes

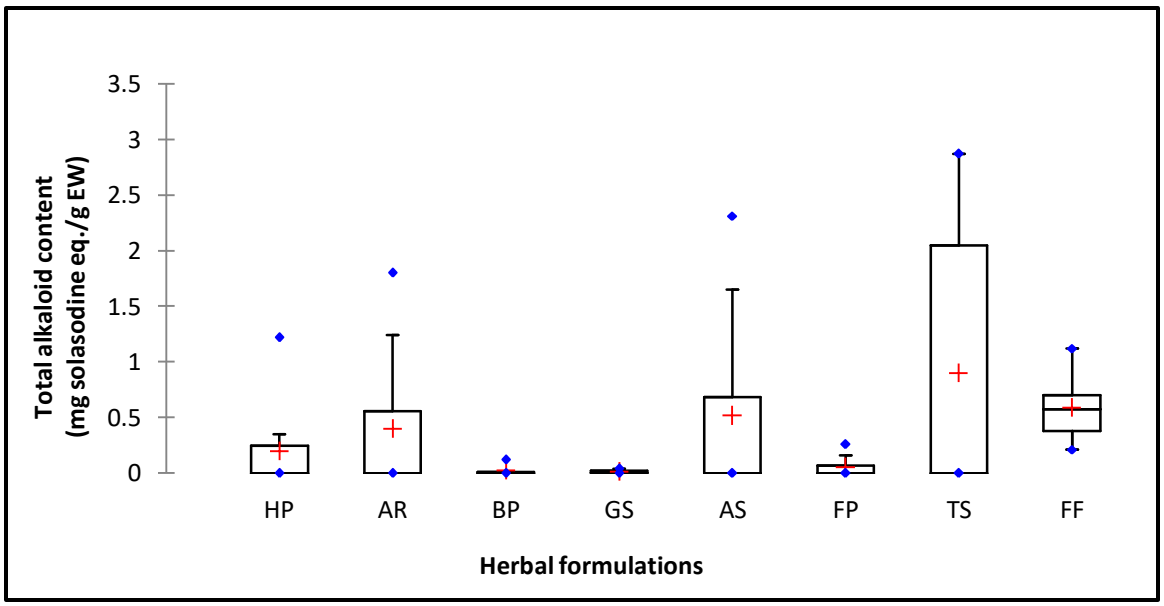


Figure 4.3.2.d: Total alkaloid content of studied eight herbal formulations
HP: heart palpitaion; **AR**: Arthritis; **BP**: High blood pressure; **GS**: Gastritis; **AS**: Asthma; **FP**: Food poisoning; **TS**: Tonsilitis; **FF**: *Fraxinus floribunda* for diabetes

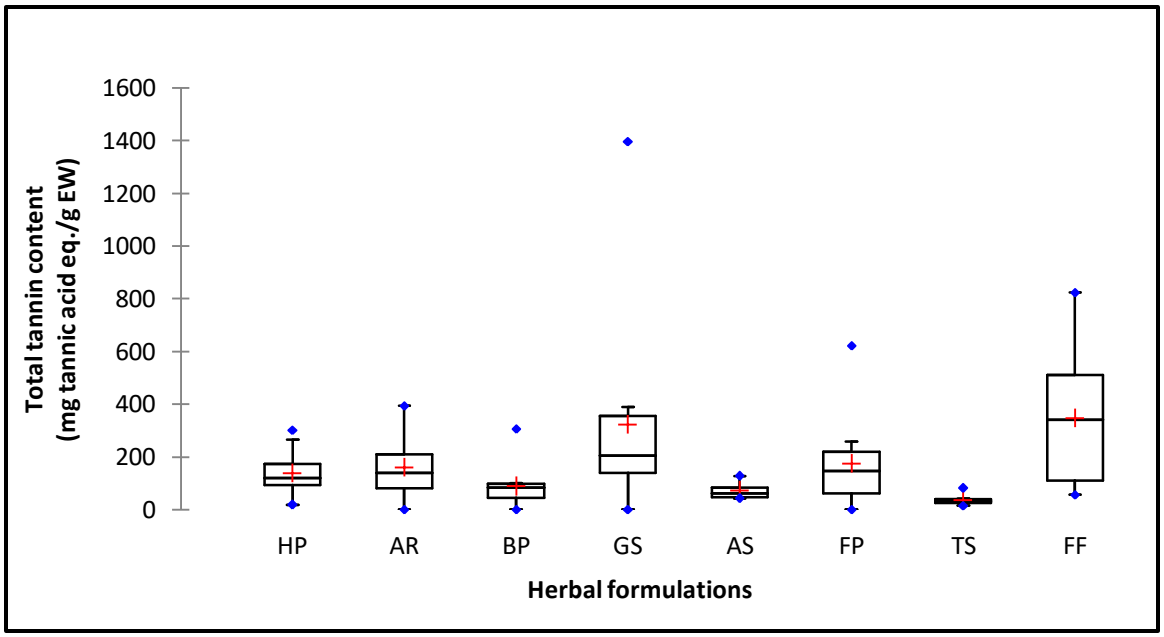


Figure 4.3.2.e: Total tannin content of studied eight herbal formulations
HP: heart palpitaion; **AR**: Arthritis; **BP**: High blood pressure; **GS**: Gastritis; **AS**: Asthma; **FP**: Food poisoning; **TS**: Tonsilitis; **FF**: *Fraxinus floribunda* for diabetes

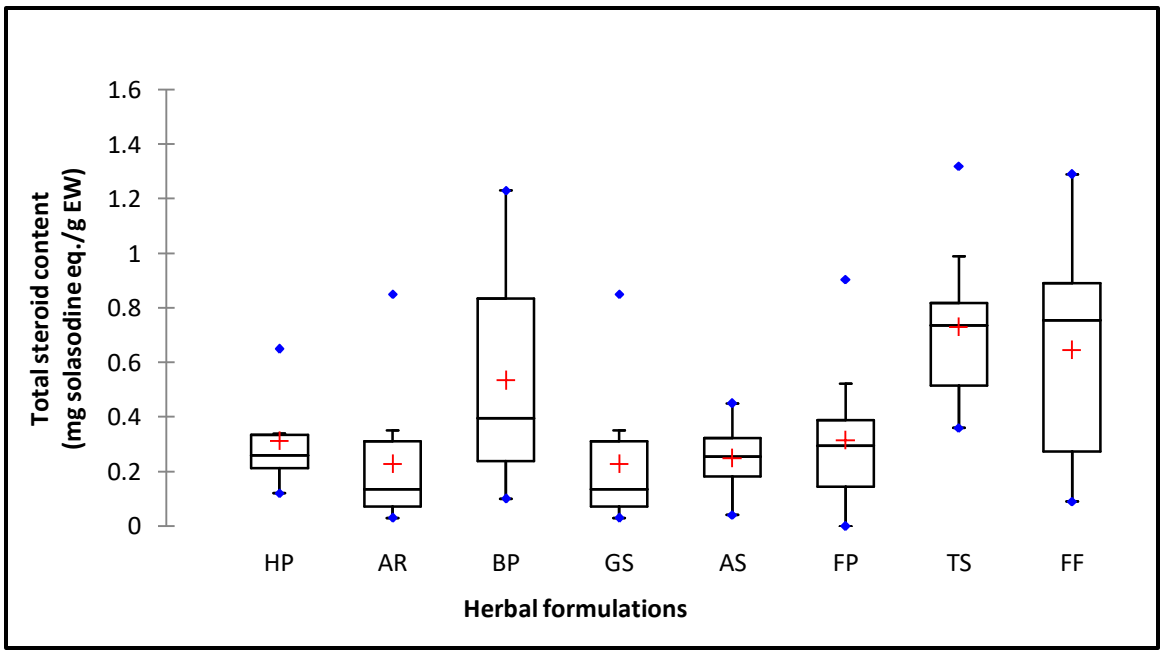


Figure 4.3.2..f: Total steroid content of studied eight herbal formulations
HP: heart palpitaion; **AR**: Arthritis; **BP**: High blood pressure; **GS**: Gastritis; **AS**: Asthma; **FP**: Food poisoning; **TS**: Tonsilitis; **FF**: *Fraxinus floribunda* for diabetes

Staphylococcus aureus).From the results presented in figure 4.4.1 to 4.4.17 and table 4.4, it was found that some extracts were active against the tested bacteria while some showed no inhibition zone or no antimicrobial activity. Antibiotic streptomycin was used as standard and the concentration taken for it were 25 mg/ml, 50 mg/ml, 100 mg/ml and 500 mg/ml. It showed prominent inhibition zones against all the tested bacterial isolates. The highest antimicrobial activity of streptomycin was recorded against *E.coli* as 3.67 cm. It was interesting to observe that both ethanol and aqueous extracts of sample FP showed antimicrobial activity against all the above bacterial isolates with highest activity against *S.typhi* by aqueous extract and against *B. megaterium* by ethanol extract. The sample HPEt, ARAq, BPEt, BPAq, ASEt and TSAq showed no inhibition of anti-bacterial strains up to 500 mg/ml FWT. The aqueous extract of HP showed inhibition against *E.coli* and *B.subtilis*. AREt also showed inhibition against two isolates namely *E. coli* and *S. aureus*. In case of AS, the aqueous extract showed antimicrobial activity against two gram +ve bacteria, *B. subtilis* and *S. aureus*. TSEt and FFAq inhibited *E. coli* only while there was no inhibition for the rest of the isolates. Sample GS was also seen to have potential antimicrobial activity with ethanol extract inhibiting all the bacterial strains except *S. typhi* and the aqueous extract inhibiting all the bacterial isolates except *S. typhi* as well as *B. subtilis*. Overall the inhibition zones did not exceed 2 cm in all the extracts and some herbal formulations showed potential antimicrobial activity especially by sample FP which is also used for the treatment of food poisoning in traditional system.

4.5. *IN VITRO* CYTOTOXIC ACTIVITY

For the study of anti-proliferative activity of the herbal formulations, all the extracts were taken in five different concentrations (50µg/ml, 100µg/ml, 150µg/ml, 200µg/ml, 250 µg/ml) and the cell viability percentage were calculated. MTT assay was performed for this study to determine the cytotoxic effect of the extracts on human liver cell line (WRL-68). WRL-68 cells generally exhibit a similar morphology of hepatocytes and can express several liver-specific enzymes like alkaline phosphatase, gamma-glutamyl transpeptidase, aspartate amino transferase and alanine transferase. Interestingly PCR-based DNA profile study has proved that WRL-68 cell lines are indistinguishable from HeLa and were originally collected

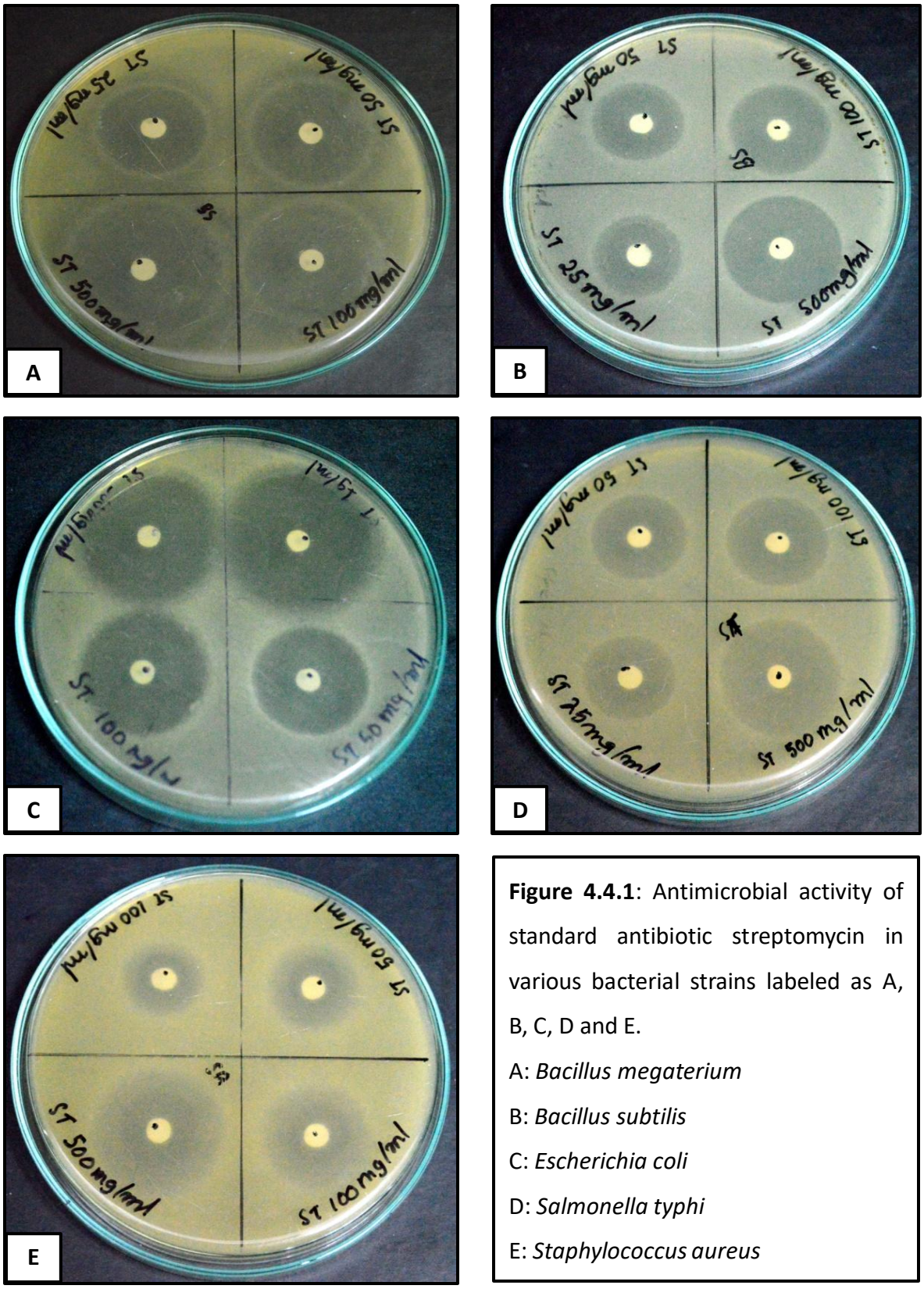


Figure 4.4.1: Antimicrobial activity of standard antibiotic streptomycin in various bacterial strains labeled as A, B, C, D and E.

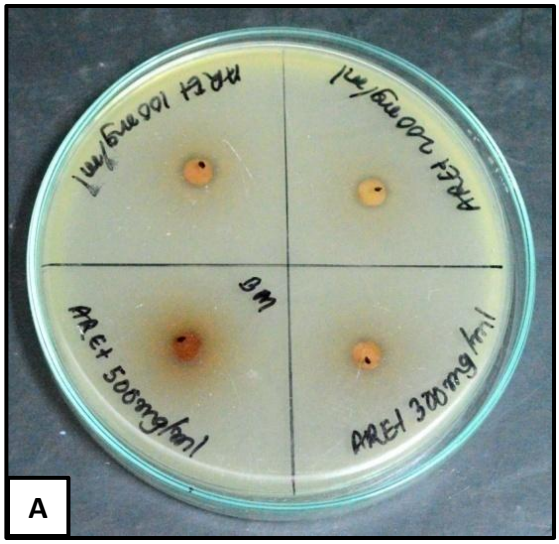
A: *Bacillus megaterium*

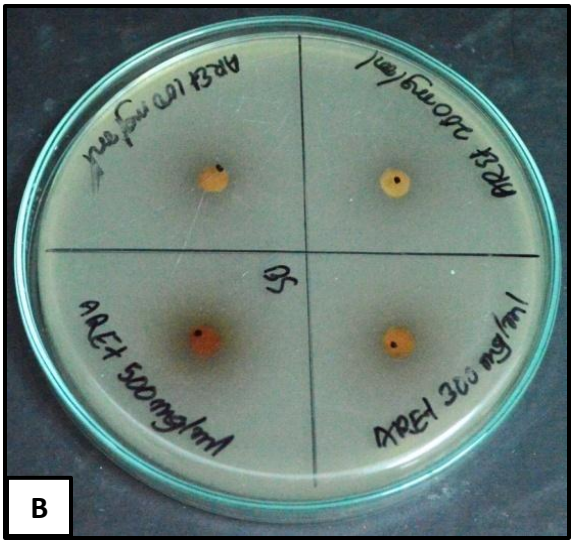
B: *Bacillus subtilis*

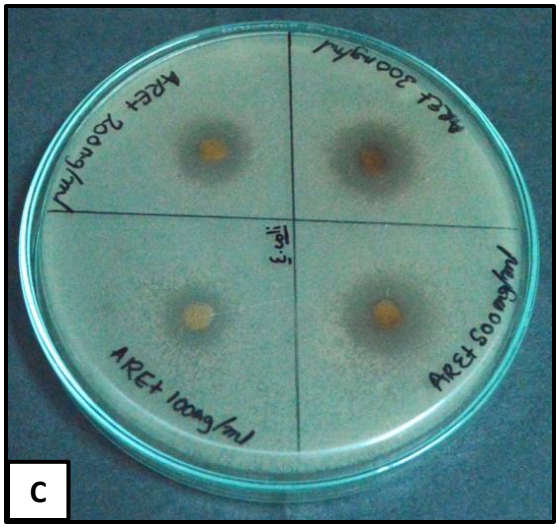
C: *Escherichia coli*

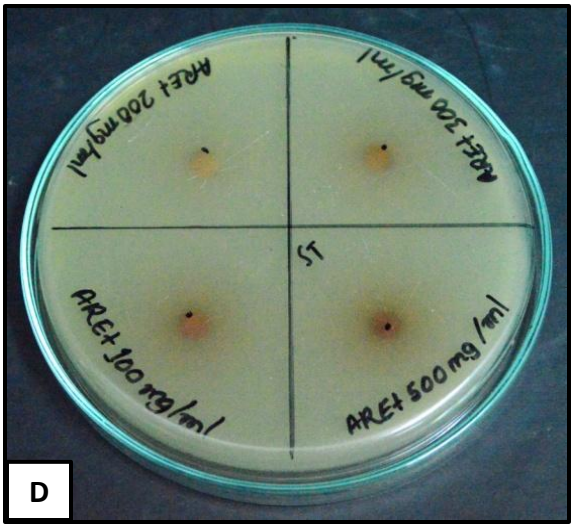
D: *Salmonella typhi*

E: *Staphylococcus aureus*









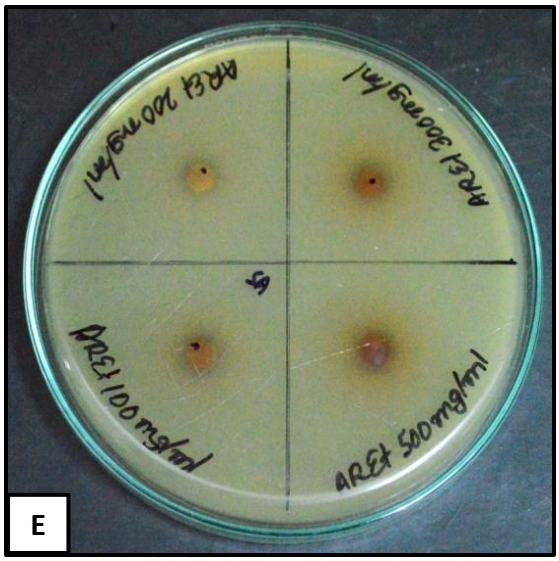


Figure 4.4.2: Antimicrobial activity of ethanolic extract of herbal formulation AR in various bacterial strains labeled as A, B, C, D and E.

A: *Bacillus megaterium*

B: *Bacillus subtilis*

C: *Escherichia coli*

D: *Salmonella typhi*

E: *Staphylococcus aureus*

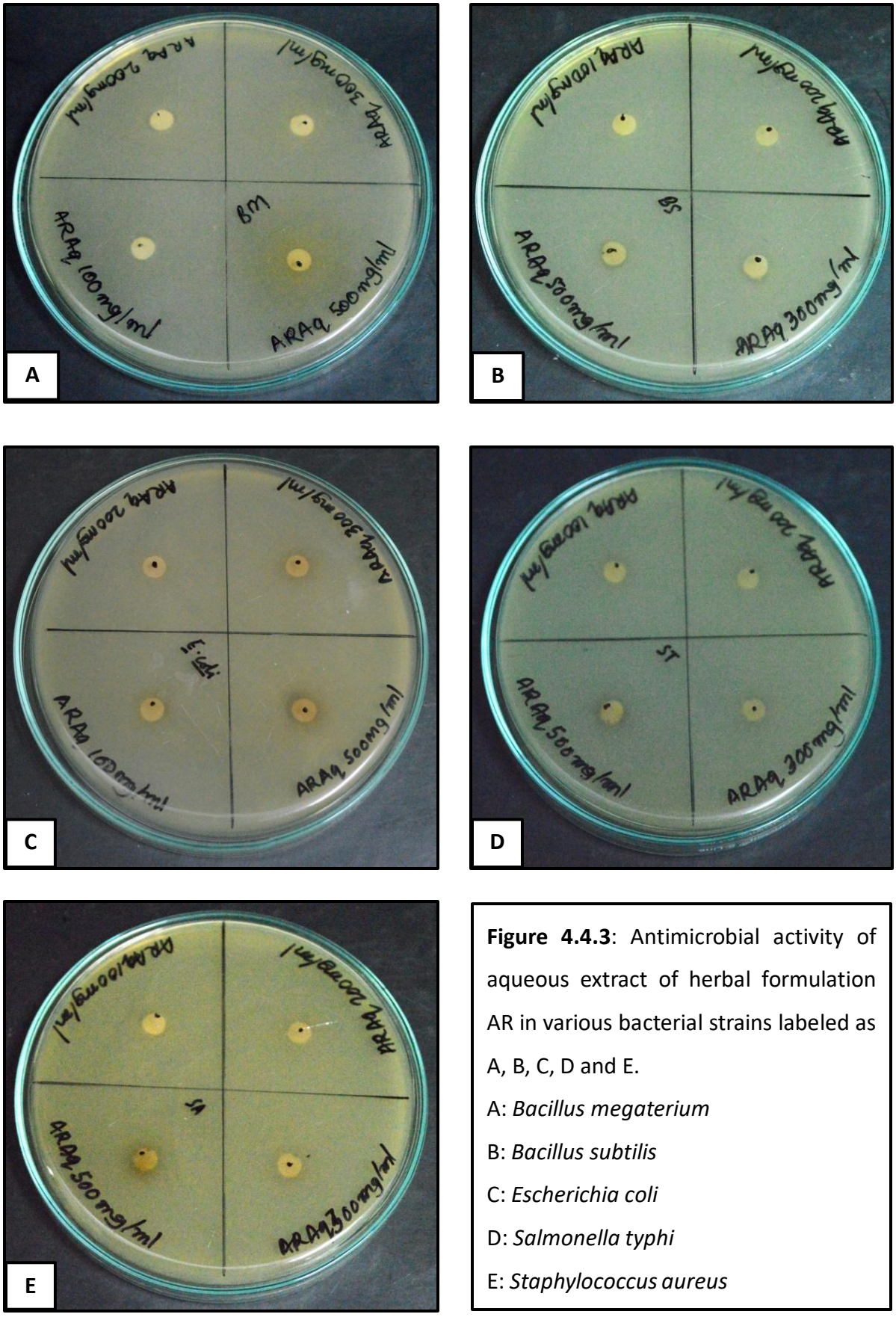


Figure 4.4.3: Antimicrobial activity of aqueous extract of herbal formulation AR in various bacterial strains labeled as A, B, C, D and E.

A: *Bacillus megaterium*

B: *Bacillus subtilis*

C: *Escherichia coli*

D: *Salmonella typhi*

E: *Staphylococcus aureus*

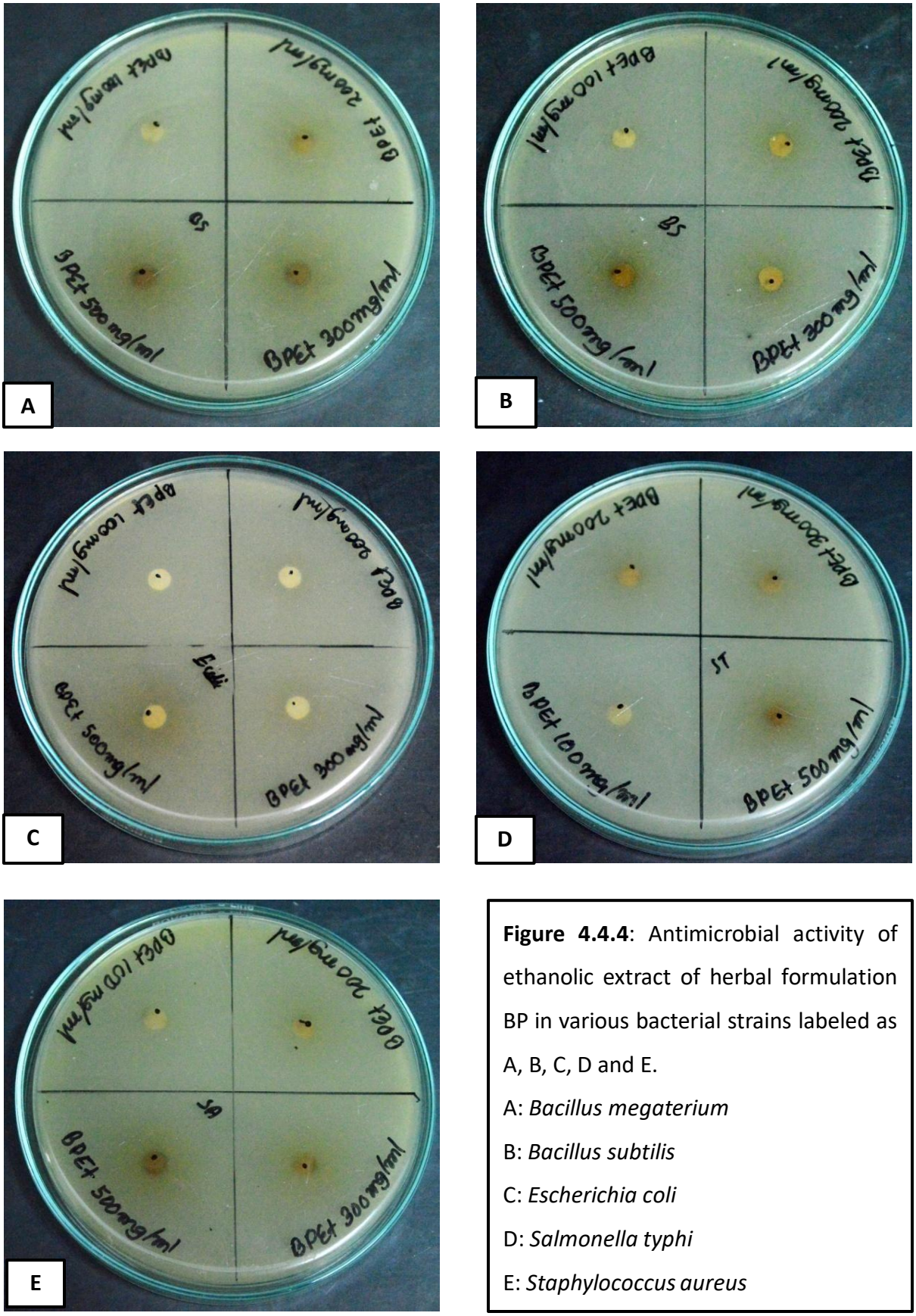


Figure 4.4.4: Antimicrobial activity of ethanolic extract of herbal formulation BP in various bacterial strains labeled as A, B, C, D and E.

A: *Bacillus megaterium*

B: *Bacillus subtilis*

C: *Escherichia coli*

D: *Salmonella typhi*

E: *Staphylococcus aureus*

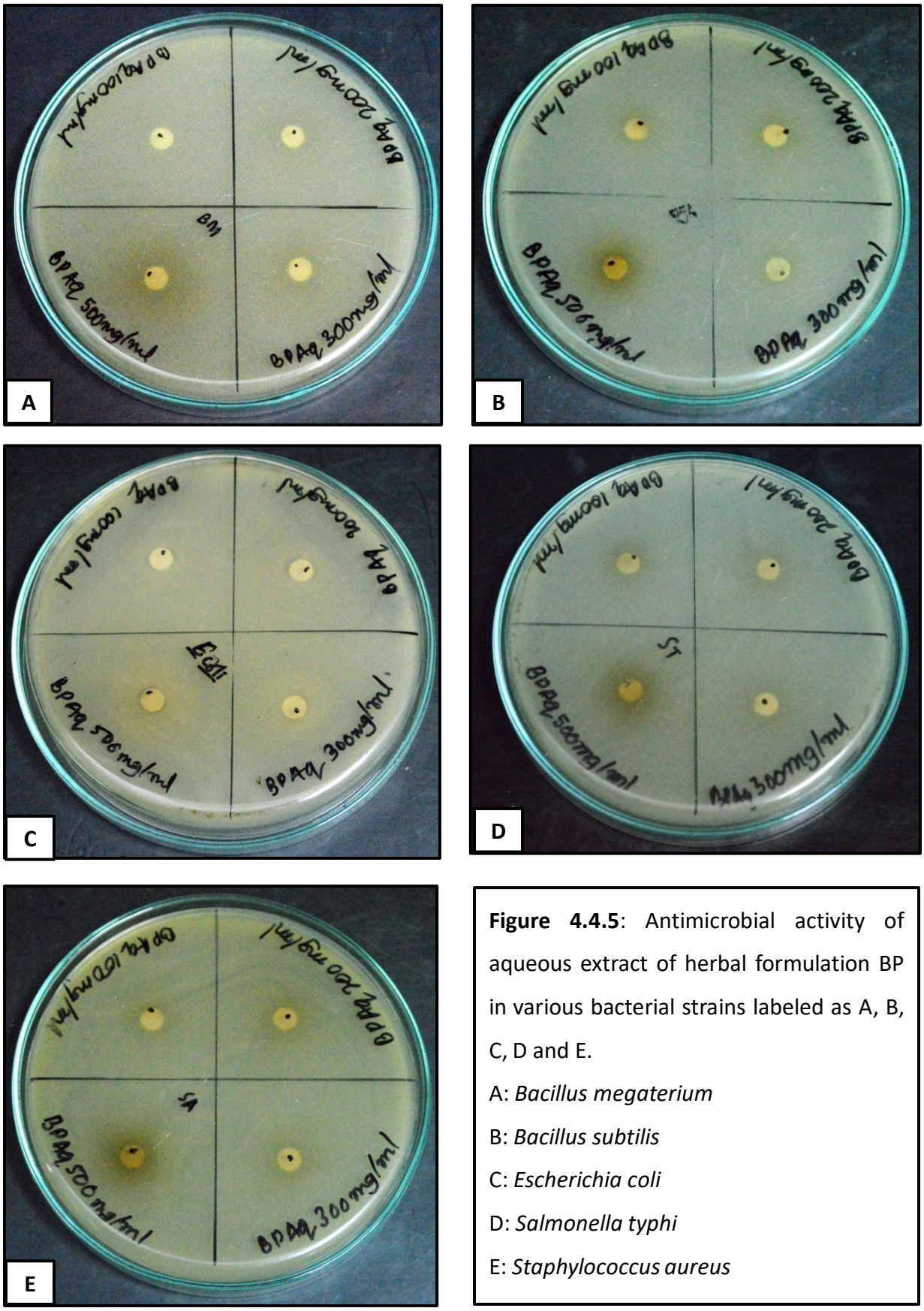


Figure 4.4.5: Antimicrobial activity of aqueous extract of herbal formulation BP in various bacterial strains labeled as A, B, C, D and E.

A: *Bacillus megaterium*

B: *Bacillus subtilis*

C: *Escherichia coli*

D: *Salmonella typhi*

E: *Staphylococcus aureus*

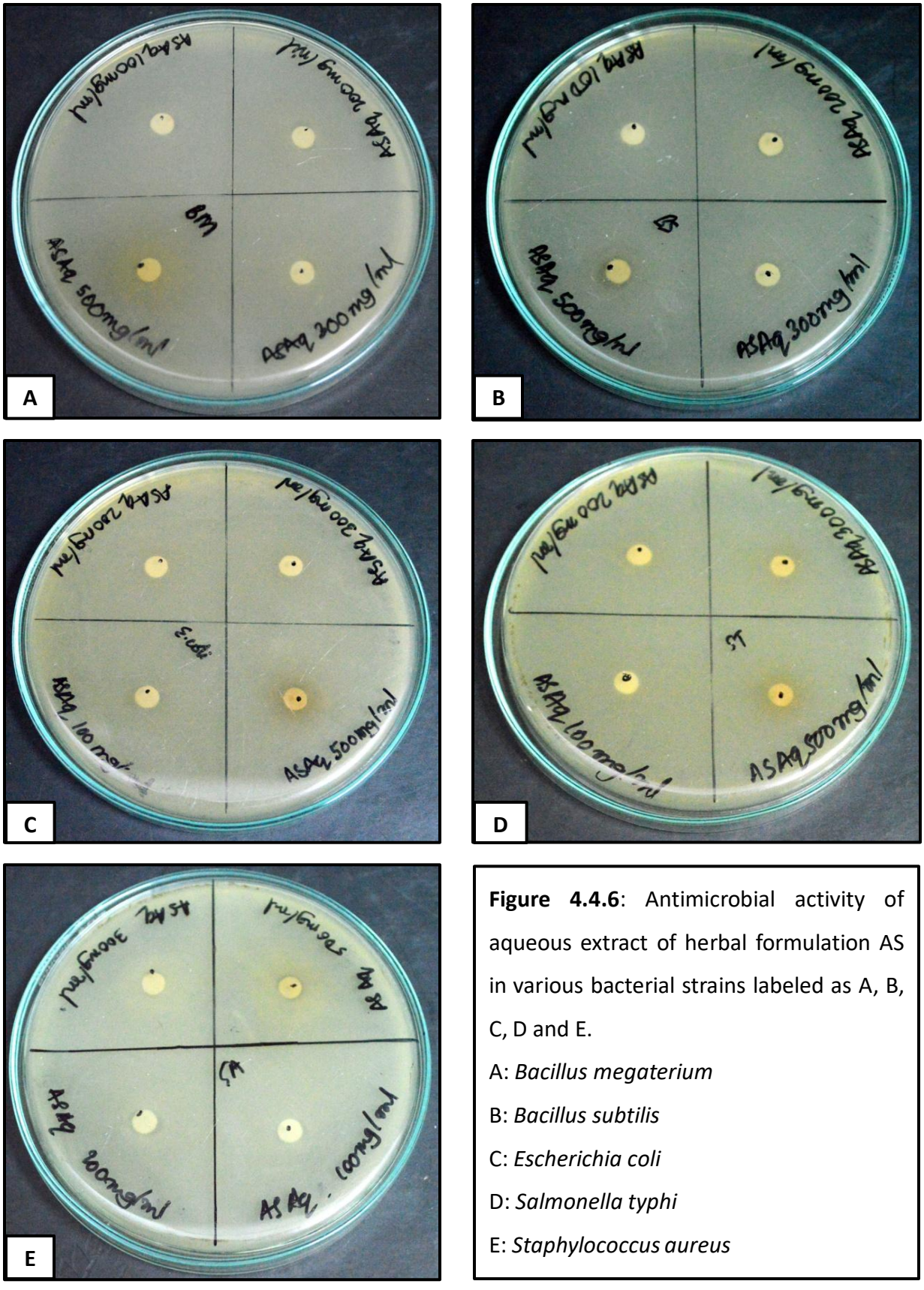


Figure 4.4.6: Antimicrobial activity of aqueous extract of herbal formulation AS in various bacterial strains labeled as A, B, C, D and E.

A: *Bacillus megaterium*

B: *Bacillus subtilis*

C: *Escherichia coli*

D: *Salmonella typhi*

E: *Staphylococcus aureus*

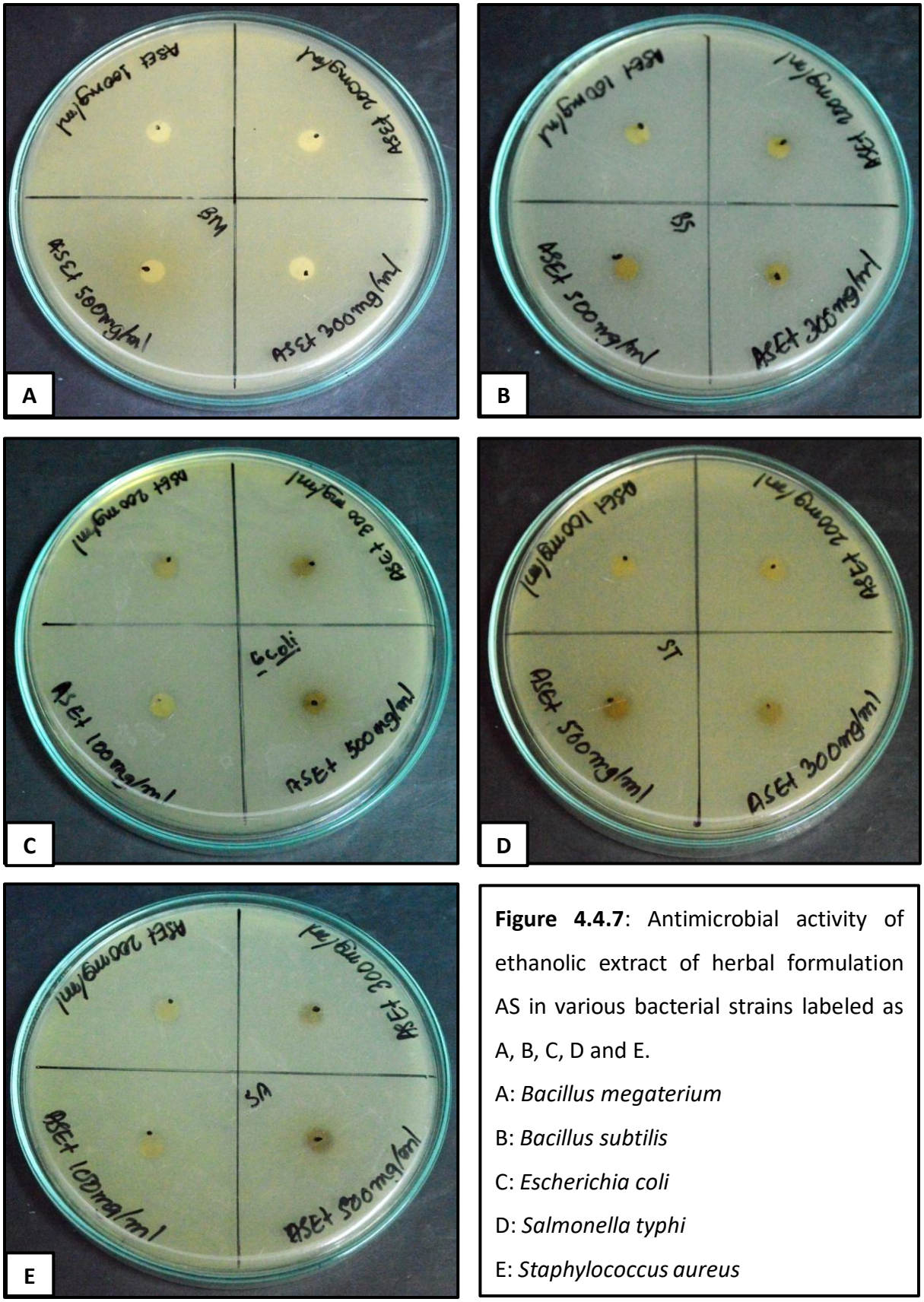


Figure 4.4.7: Antimicrobial activity of ethanolic extract of herbal formulation AS in various bacterial strains labeled as A, B, C, D and E.

A: *Bacillus megaterium*

B: *Bacillus subtilis*

C: *Escherichia coli*

D: *Salmonella typhi*

E: *Staphylococcus aureus*

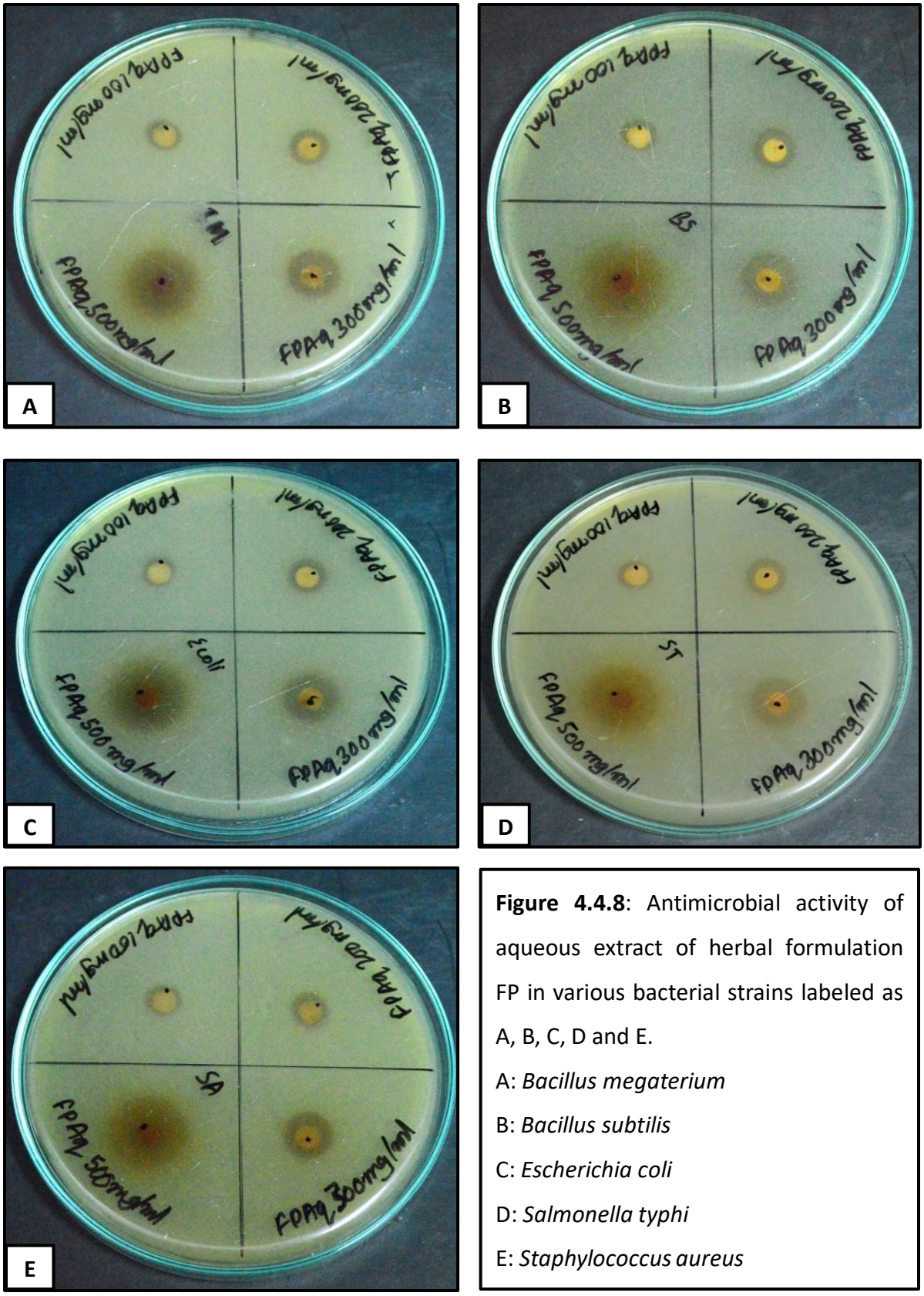


Figure 4.4.8: Antimicrobial activity of aqueous extract of herbal formulation FP in various bacterial strains labeled as A, B, C, D and E.

A: *Bacillus megaterium*

B: *Bacillus subtilis*

C: *Escherichia coli*

D: *Salmonella typhi*

E: *Staphylococcus aureus*

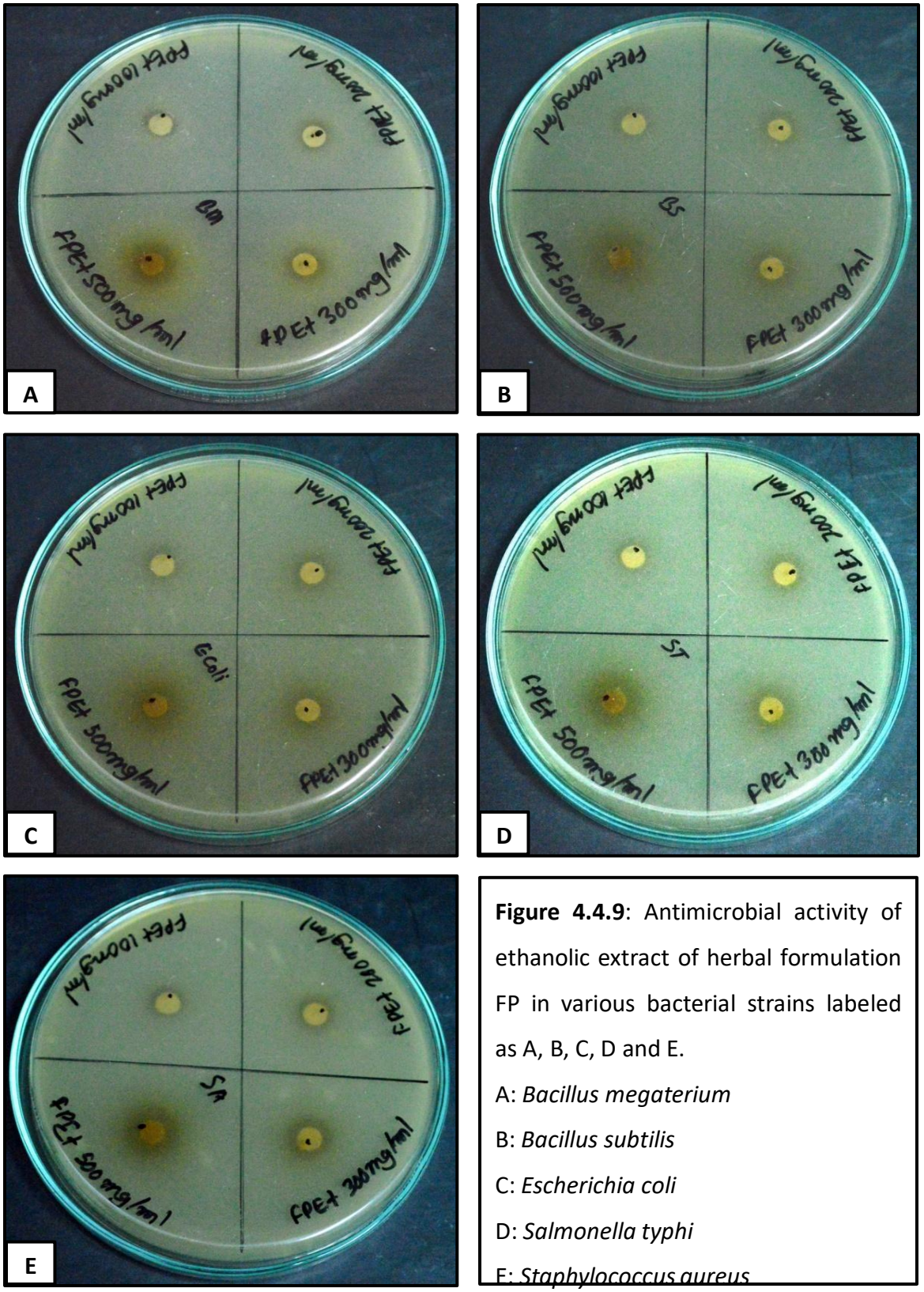


Figure 4.4.9: Antimicrobial activity of ethanolic extract of herbal formulation FP in various bacterial strains labeled as A, B, C, D and E.

A: *Bacillus megaterium*

B: *Bacillus subtilis*

C: *Escherichia coli*

D: *Salmonella typhi*

E: *Staphylococcus aureus*

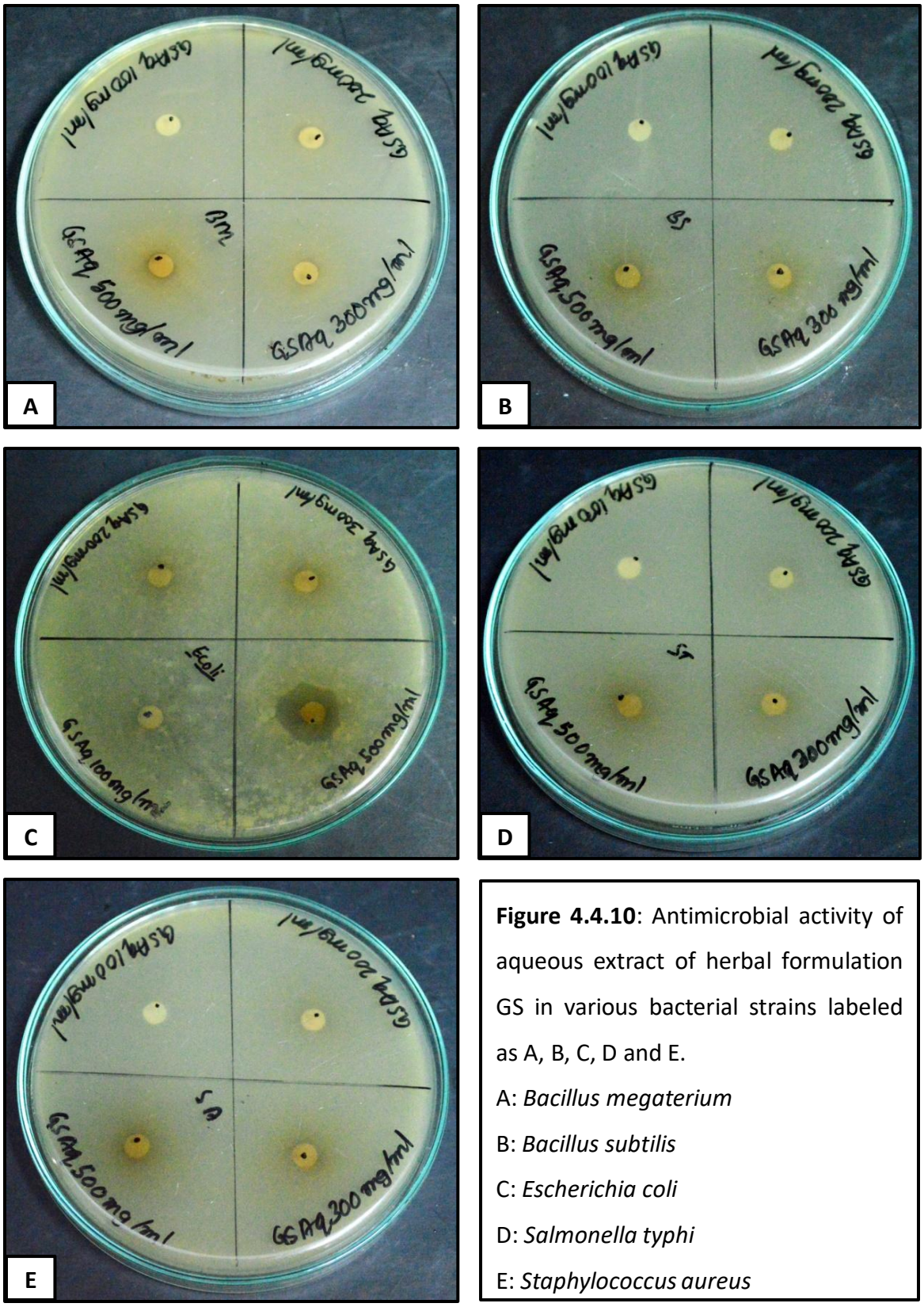


Figure 4.4.10: Antimicrobial activity of aqueous extract of herbal formulation GS in various bacterial strains labeled as A, B, C, D and E.

A: *Bacillus megaterium*

B: *Bacillus subtilis*

C: *Escherichia coli*

D: *Salmonella typhi*

E: *Staphylococcus aureus*

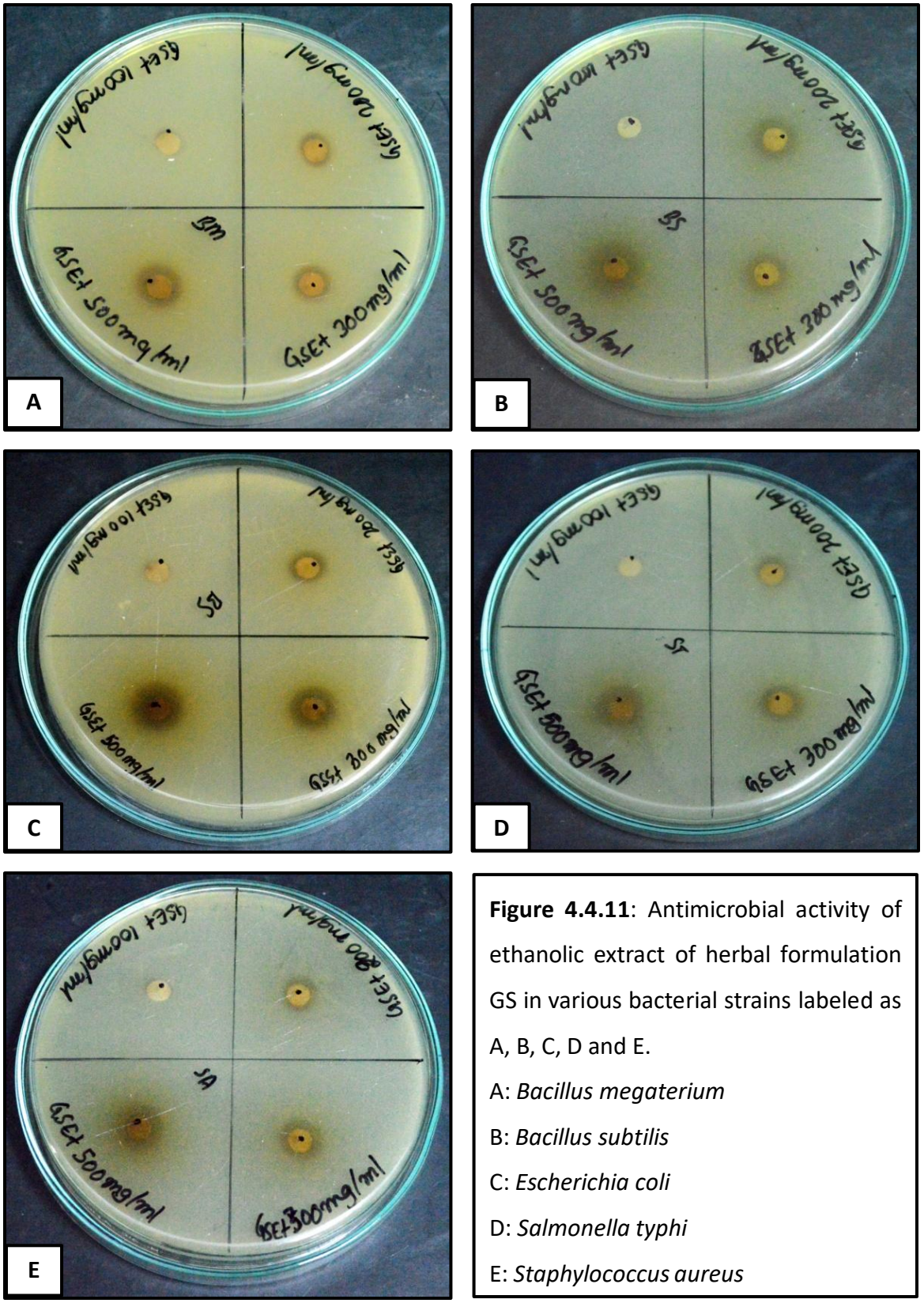


Figure 4.4.11: Antimicrobial activity of ethanolic extract of herbal formulation GS in various bacterial strains labeled as A, B, C, D and E.

A: *Bacillus megaterium*

B: *Bacillus subtilis*

C: *Escherichia coli*

D: *Salmonella typhi*

E: *Staphylococcus aureus*

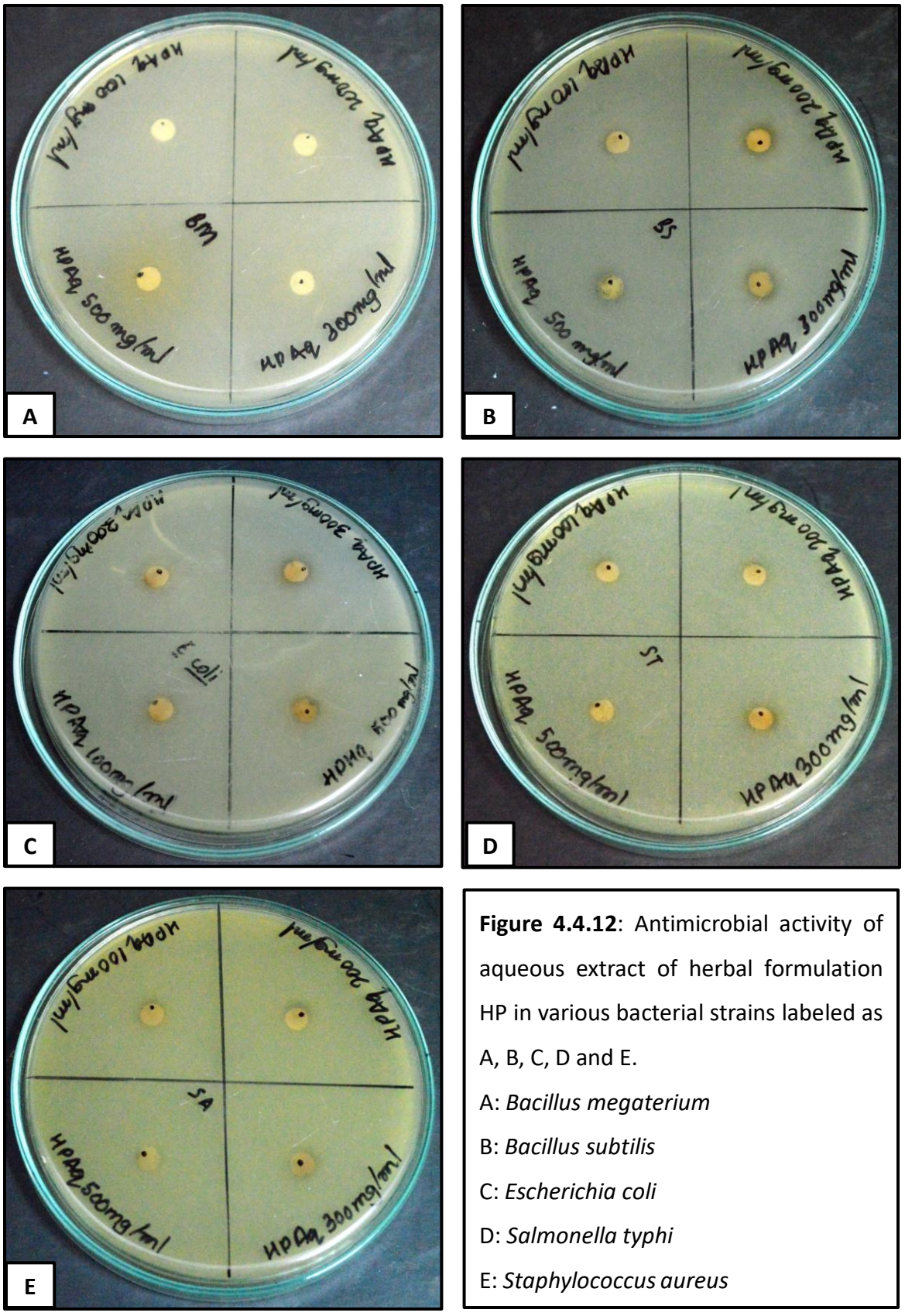


Figure 4.4.12: Antimicrobial activity of aqueous extract of herbal formulation HP in various bacterial strains labeled as A, B, C, D and E.

A: *Bacillus megaterium*

B: *Bacillus subtilis*

C: *Escherichia coli*

D: *Salmonella typhi*

E: *Staphylococcus aureus*

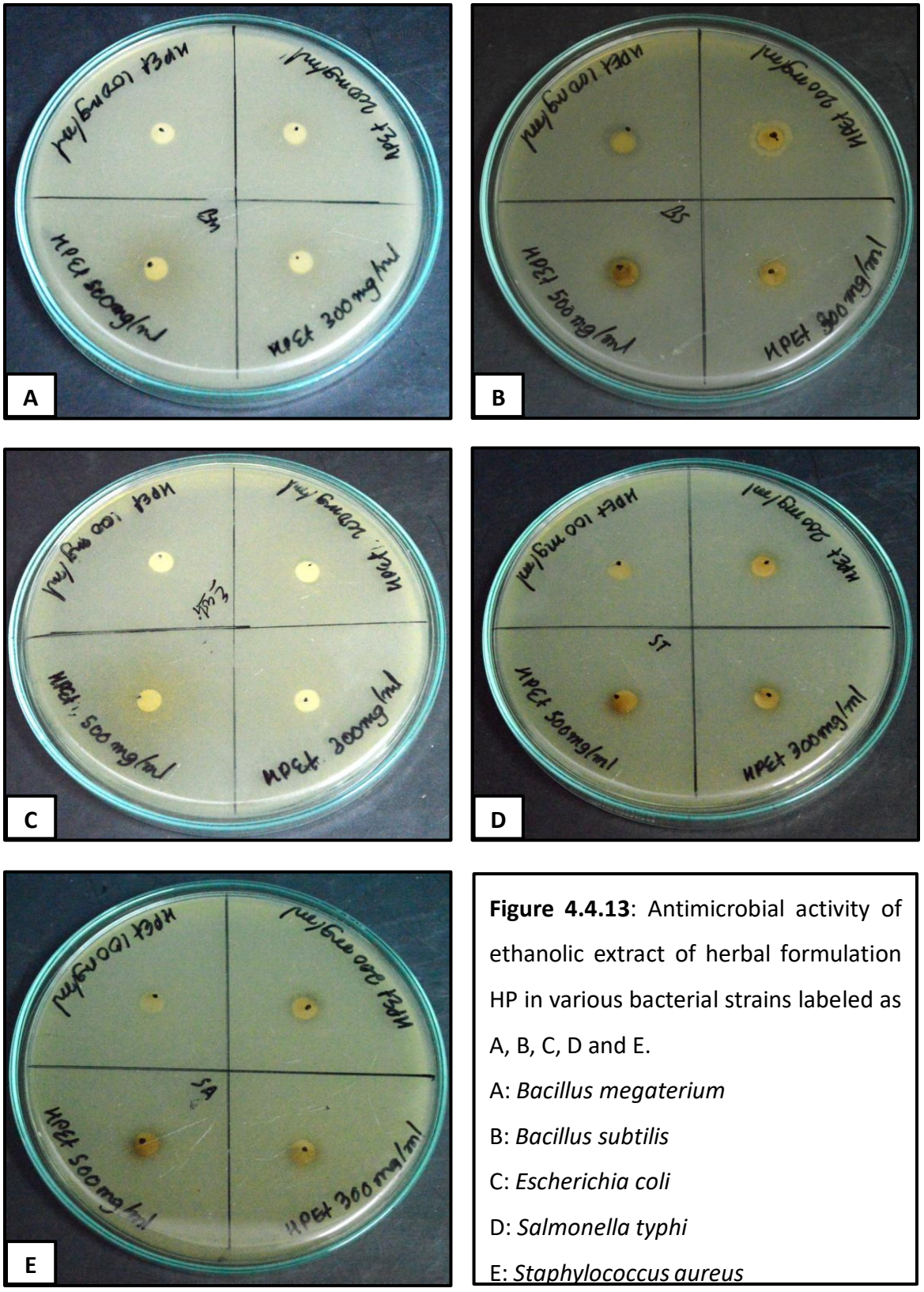


Figure 4.4.13: Antimicrobial activity of ethanolic extract of herbal formulation HP in various bacterial strains labeled as A, B, C, D and E.

A: *Bacillus megaterium*

B: *Bacillus subtilis*

C: *Escherichia coli*

D: *Salmonella typhi*

E: *Staphylococcus aureus*

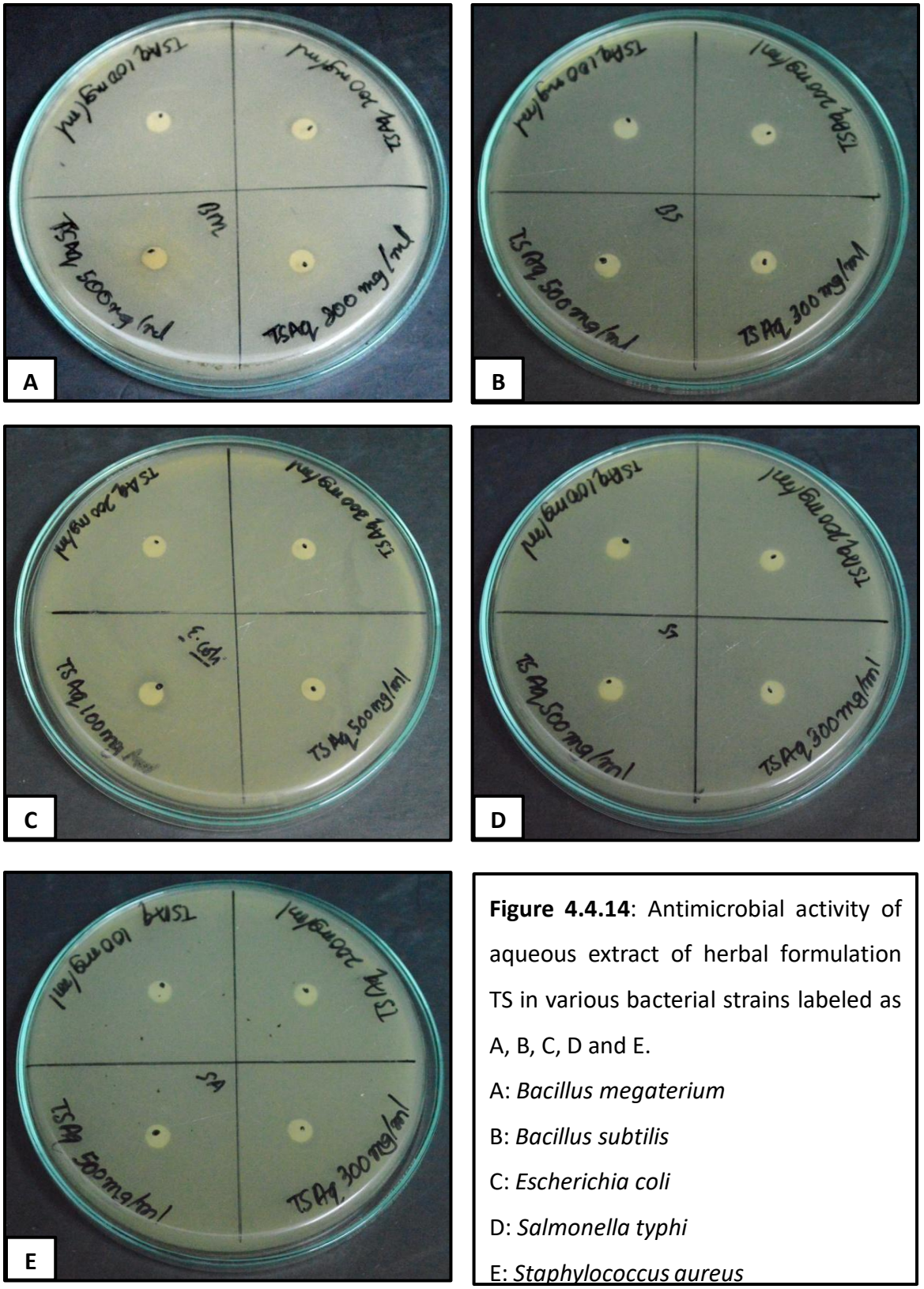


Figure 4.4.14: Antimicrobial activity of aqueous extract of herbal formulation TS in various bacterial strains labeled as A, B, C, D and E.

A: *Bacillus megaterium*

B: *Bacillus subtilis*

C: *Escherichia coli*

D: *Salmonella typhi*

E: *Staphylococcus aureus*

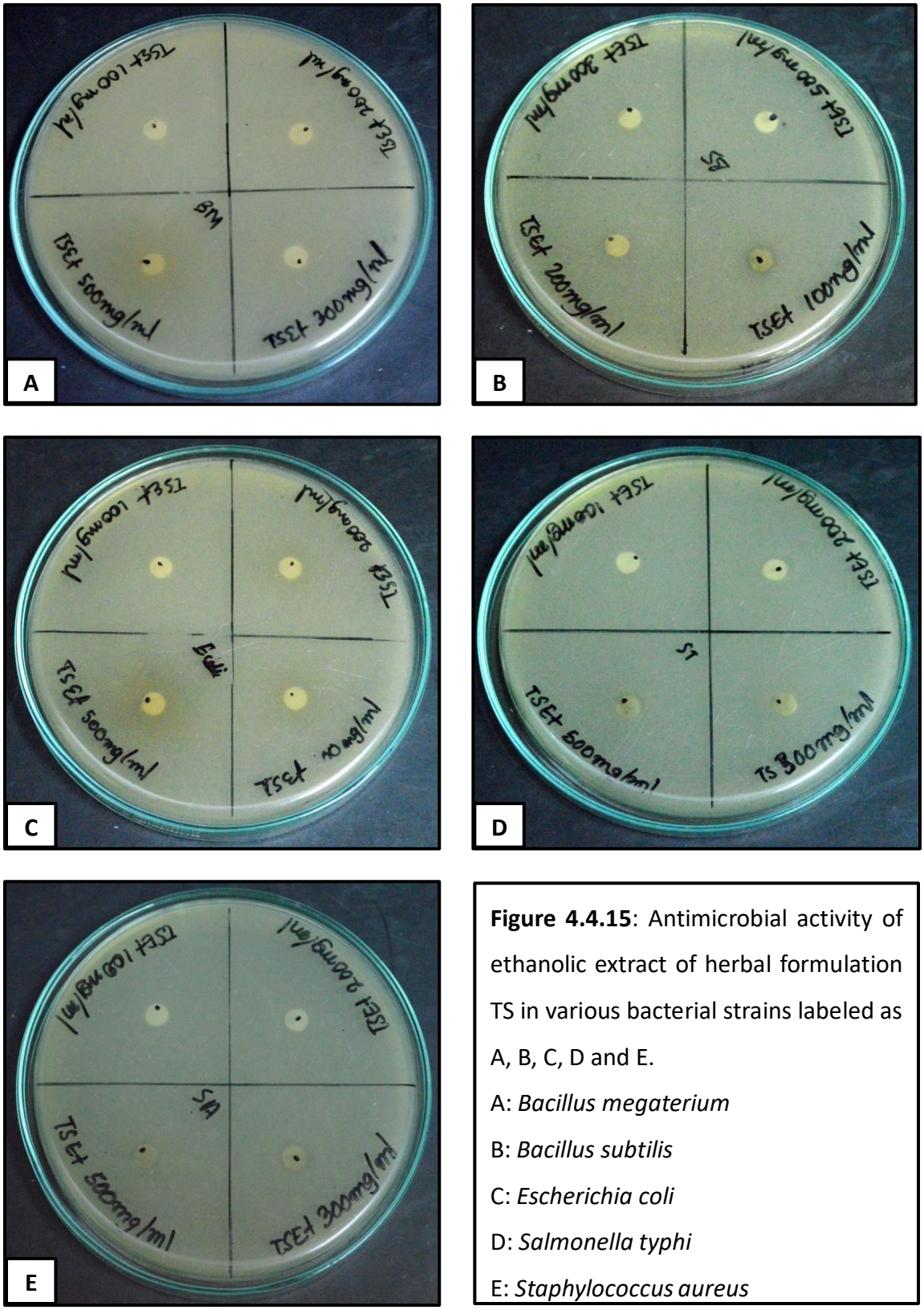


Figure 4.4.15: Antimicrobial activity of ethanolic extract of herbal formulation TS in various bacterial strains labeled as A, B, C, D and E.

A: *Bacillus megaterium*

B: *Bacillus subtilis*

C: *Escherichia coli*

D: *Salmonella typhi*

E: *Staphylococcus aureus*

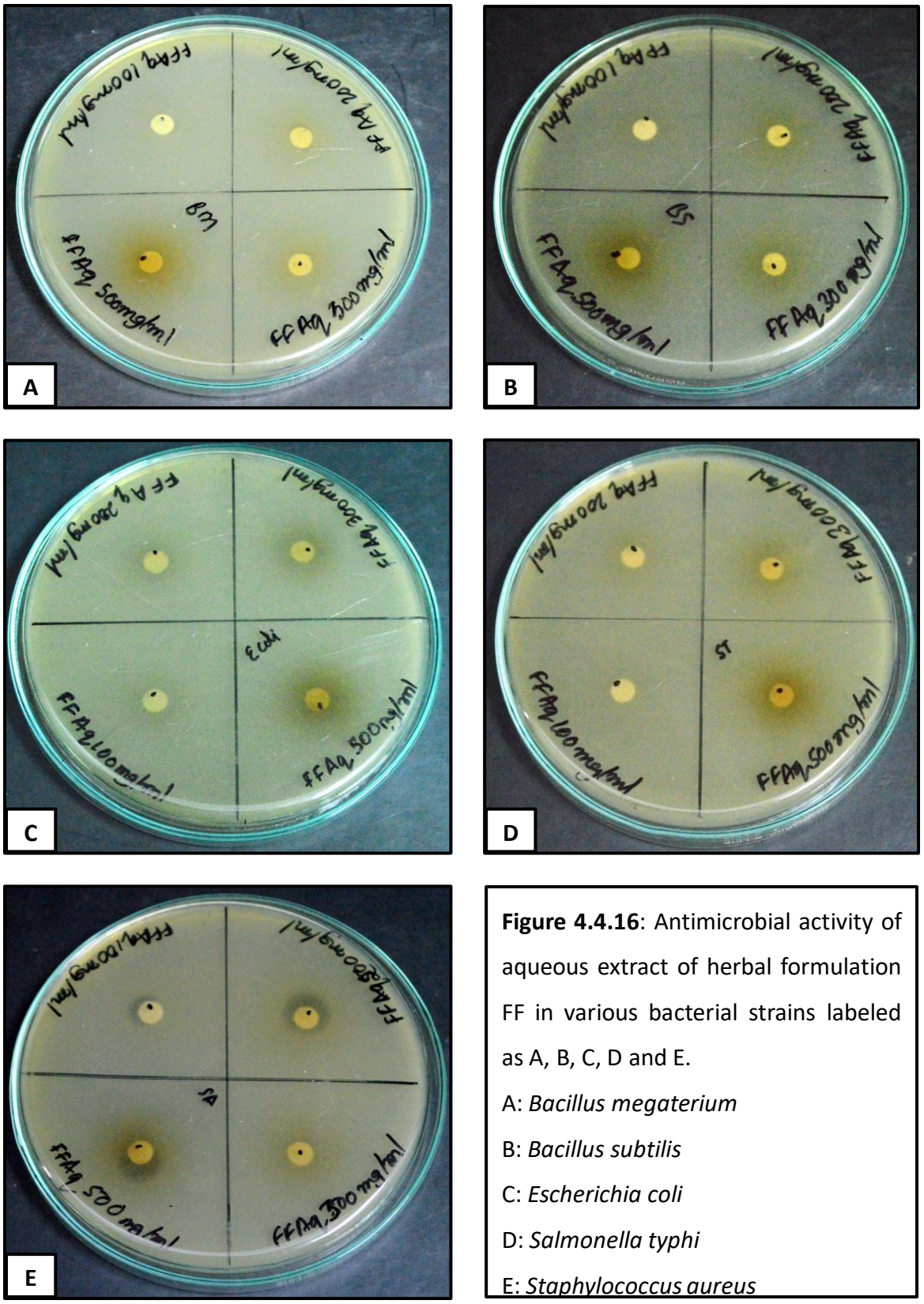


Figure 4.4.16: Antimicrobial activity of aqueous extract of herbal formulation FF in various bacterial strains labeled as A, B, C, D and E.

A: *Bacillus megaterium*

B: *Bacillus subtilis*

C: *Escherichia coli*

D: *Salmonella typhi*

E: *Staphylococcus aureus*

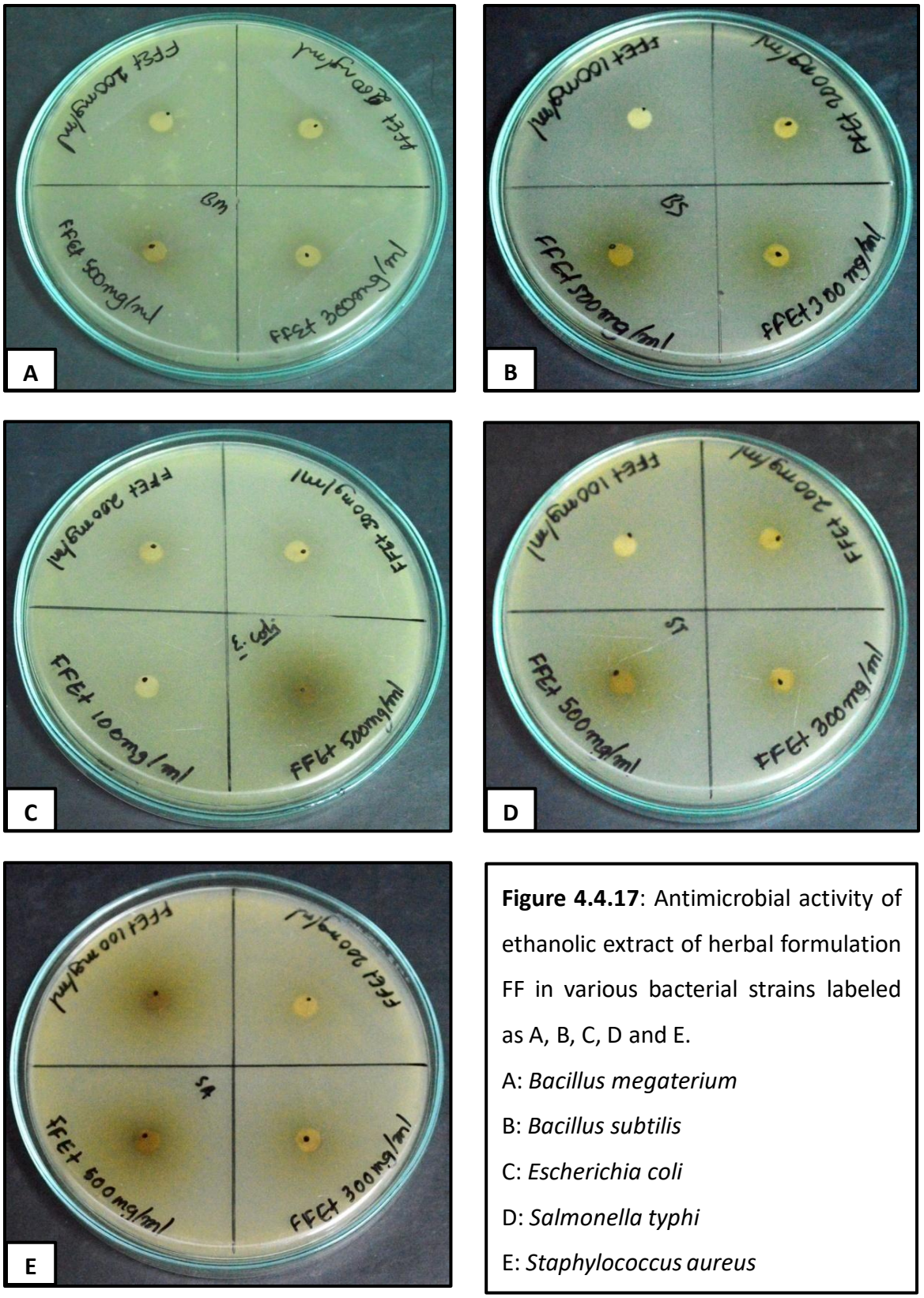


Figure 4.4.17: Antimicrobial activity of ethanolic extract of herbal formulation FF in various bacterial strains labeled as A, B, C, D and E.

A: *Bacillus megaterium*

B: *Bacillus subtilis*

C: *Escherichia coli*

D: *Salmonella typhi*

E: *Staphylococcus aureus*

Table 4.4: Antimicrobial activity of ethanol and aqueous extracts of the herbal formulations against the some bacterial isolates with disc diffusion method

Sample	Concentration (mg/ml)	Inhibition zone diameter (cm)				
		Escherichia coli	*Salmonella typhi*	*Bacillus megaterium*	*Bacillus subtilis*	*Staphylococcus aureus*
Streptomycin (standard)	25	$1.17\pm0.15_{fgh}$	$2.07\pm0.06_{c}$	$2.47\pm0.15_{d}$	$1.97\pm0.15_{d}$	$1.50\pm0.10_{e}$
	50	$2.10\pm0.10_{c}$	$2.23\pm0.06_{bc}$	$2.77\pm0.06_{c}$	$2.33\pm0.06_{c}$	$1.87\pm0.06_{c}$
	100	$2.60\pm0.10_{b}$	$2.50\pm0.10_{b}$	$3.03\pm0.06_{b}$	$2.53\pm0.06_{b}$	$2.20\pm0.10_{b}$
	500	$3.67\pm0.06_{a}$	$3.00\pm0.10_{a}$	$3.27\pm0.0.15_{a}$	$2.80\pm0.10_{a}$	$2.70\pm0.10_{a}$
HPEt	100 200 300 500	NI	NI	NI	NI	NI
HPAq	100	$0.57\pm0.06_{pq}$	NI	NI	$0.63\pm0.06_{jk}$	NI
	200	$0.70\pm0.10_{nop}$			$0.83\pm0.06_{i}$	
	300	$0.80\pm0.10_{lmno}$			$0.87\pm0.06_{i}$	
	500	$0.93\pm0.06_{jkl}$			$1.07\pm0.12_{h}$	
AREt	100	$1.1\pm0.06_{hij}$	NI	NI	NI	$0.63\pm0.06_{lmno}$
	200	$1.3\pm0.06_{f}$				$0.77\pm0.06_{ijkl}$
	300	$1.5\pm0.06_{e}$				$0.87\pm0.06_{hi}$
	500	$1.7\pm0.15_{d}$		$0.77\pm0.06_{ijk}$		$1.07\pm0.06_{g}$
ARAq	100 200 300 500	NI	NI	NI	NI	NI
BPEt	100 200 300 500	NI	NI	NI	NI	NI
BPAq	100 200 300 500	NI	NI	NI	NI	NI
ASEt	100 200 300 500	NI	NI	NI	NI	NI
ASAq	100	NI	NI	NI	$0.57\pm0.06_{k}$	$0.57\pm0.06_{no}$
	200				$0.80\pm0.10_{i}$	$0.80\pm0.10_{hijk}$
	300				$0.87\pm0.15_{i}$	$0.87\pm0.15_{hi}$
	500				$1.03\pm0.06_{h}$	$1.03\pm0.06_{g}$

Sample	Concentration (mg/ml)	Inhibition zone diameter (cm)				
		Escherichia coli	*Salmonella typhi*	*Bacillus megaterium*	*Bacillus subtilis*	*Staphylococcus aureus*
TSEt	100	$0.47\pm0.06_{q}$	NI	NI	NI	NI
	200	$0.67\pm0.06_{op}$				
	300	$0.80\pm0.10_{lmno}$				
	500	$0.90\pm0.06_{q}$				
TSAq	100	NI	NI	NI	NI	NI
	200					
	300					
	500					
GSEt	100	$0.60\pm0.10_{pq}$	NI	$0.67\pm0.06_{kl}$	$0.57\pm0.06_{k}$	$0.70\pm0.06_{jklmn}$
	200	$0.87\pm0.06_{klm}$		$0.83\pm0.06_{hi}$	$0.77\pm0.06_{i}$	$0.87\pm0.10_{hi}$
	300	$1.10\pm0.10_{ghi}$		$0.93\pm0.06_{gh}$	$0.87\pm0.06_{i}$	$1.03\pm0.06_{g}$
	500	$1.23\pm0.06_{fg}$		$1.30\pm0.10_{f}$	$1.37\pm0.06_{f}$	$1.27\pm0.06_{f}$
GSAq	100	$0.60\pm0.10_{pq}$	NI	$0.53\pm0.06_{l}$	NI	$0.53\pm0.06_{o}$
	200	$0.80\pm0.10_{lmno}$		$0.63\pm0.06_{kl}$		$0.60\pm0.10_{mno}$
	300	$0.87\pm0.06_{klm}$		$0.73\pm0.06_{ijk}$		$0.73\pm0.06_{ijklm}$
	500	$1.30\pm0.10_{f}$		$0.83\pm0.06_{ijk}$		$0.93\pm0.06_{gh}$
FPEt	100	$0.57\pm0.06_{pq}$	$0.73\pm0.06_{g}$	$0.63\pm0.06_{kl}$	$0.73\pm0.06_{ij}$	$0.70\pm0.06_{jklmn}$
	200	$0.77\pm0.12_{mno}$	$0.87\pm0.06_{fg}$	$0.70\pm0.10_{ik}$	$0.80\pm0.10_{i}$	$0.87\pm0.06_{hi}$
	300	$0.90\pm0.10_{klm}$	$0.63\pm0.46_{g}$	$1.27\pm0.06_{f}$	$0.87\pm0.06_{i}$	$1.07\pm0.10_{g}$
	500	$1.23\pm0.06_{fg}$	$1.30\pm0.10_{e}$	$1.53\pm0.06_{e}$	$1.27\pm0.06_{fg}$	$1.30\pm0.06_{f}$
FPAq	100	$0.80\pm0.10_{lmno}$	$0.87\pm0.06_{fg}$	$0.83\pm0.06_{hi}$	$0.83\pm0.06_{i}$	$0.67\pm0.10_{klmno}$
	200	$0.93\pm0.06_{jkl}$	$1.07\pm0.06_{ef}$	$1.03\pm0.06_{g}$	$1.03\pm0.06_{h}$	$0.83\pm0.06_{hij}$
	300	$1.23\pm0.12_{fg}$	$1.30\pm0.10_{e}$	$1.27\pm0.06_{f}$	$1.23\pm0.06_{g}$	$1.20\pm0.06_{f}$
	500	$1.63\pm0.12_{d}$	$1.70\pm0.10_{d}$	$1.60\pm0.10_{e}$	$1.70\pm0.10_{e}$	$1.67\pm0.06_{d}$
FFEt	100	NI	NI	NI	NI	NI
	200					
	300					
	500	$0.97\pm0.06_{ijk}$				
FFAq	100	$0.83\pm0.06_{klmn}$	NI	NI	NI	NI
	200	$0.93\pm0.06_{jkl}$				
	300	$1.23\pm0.06_{fg}$				
	500	$1.60\pm0.10_{de}$				

HPEt and HPAq: Ethanolic extract and aqueous extract of HP (formulation for the treatment of heart palpitation); AREt and ARAq : Ethanolic and aqueous extract of AR(formulation for the treatment of arthritis); BPEt and BPAq: Ethanolic and aqueous extract of BP (formulation for the treatment of high blood pressure); ASEt and ASAq: Ethanolic and aqueous extract of AS (formulation for the treatment of asthma); TSEt and TSAq: Ethanolic and aqueous extract of TS (formulation for the treatment of tonsillitis); GSEt and GSAq: Ethanolic and aqueous extract of GS (formulation for the treatment of GS); FPET and FPAq: Ethanolic and aqueous extract of FP (formulation for the treatment of food poisoning); FFEt and FFAq: Ethanolic and aqueous extract of FF (*Fraxinus floribunda* for the treatment of diabetes); NI: No inhibition.

from Human Cervix Carcinoma. Results of the cytotoxicity evaluation are presented in figure 4.5.1 and 4.5.2. Cell viability percentage was determined and presented in figure 4.5.1 where it was observed that all the extracts showed anti-proliferative activity in different concentration. Highest cell viability percentage was shown by HP extract at 250 µg/ml concentration (61.04%). Extracts of AS and GS at 250 µg/ml concentration and TS at both 200 and 250 µg/ml concentration showed cell viability percentage above 30%. In case of AR, BP, GS, FP, FF and TS, cytotoxic activity was observed only at concentration of 150µg/ml and above. Atjanasuppat *et al*. (2009) categorized the anti-proliferative activities of the extracts into four groups: ≤ 20 µg/ml, active; >20–100 µg/ml, moderately active; >100–1000 µg/ml, weakly active; and >1000 µg/ml, inactive. Based on the report, IC_{50} values of cell viability of the herbal formulations (figure 4.11.2) fall under the range of extracts having anti-proliferative activity but none on active category. Extract of AR showed highest cytotoxic activity with lowest IC_{50} value (173.44±9.82 µg/ml) which falls under weakly active category along with rest of the formulations falling under the same category too. The overall result from MTT assay showed that the IC_{50} values of cell viability against human liver cancer cell line (WRL-68) ranged from 100 to 1000µg/ml and were categorized as weakly active.

4.6 *IN VITRO* ANTIDIABETIC ACTIVITY

In vitro antidiabetic activity of all the formulations was determined by α-glucosidase inhibiting activity of the extracts. All the formulations exhibited antidiabetic activity but not by all the solvent extracts. A variation in anti-diabetic activity was observed according to the polarity of solvents in which they were extracted. The percentage inhibition of the enzyme by the extracts was calculated and IC_{50} values were obtained and presented in figure 4.6.1 to 4.6.8. From the figures it was revealed that acetone extract exhibited highest antidiabetic activity in case of HP (0.02±0.001 mg/ml), AR (0.013±0.001 mg/ml), FP (0.05±0.002 mg/ml) and TS (0.5±0.06 mg/ml) while in case of BP (0.34±0.09 mg/ml), GS (0.02±0.002 mg/ml) and AS (0.10±0.02 mg/ml), ethanol extract showed the highest activity. Only in case of FF (*Fraxinus floribunda*) which is also used for treating diabetes in traditional system, aqueous extract exhibited highest antidiabetic activity (0.01 mg/ml). It was also observed that the non polar solvent extracts showed less

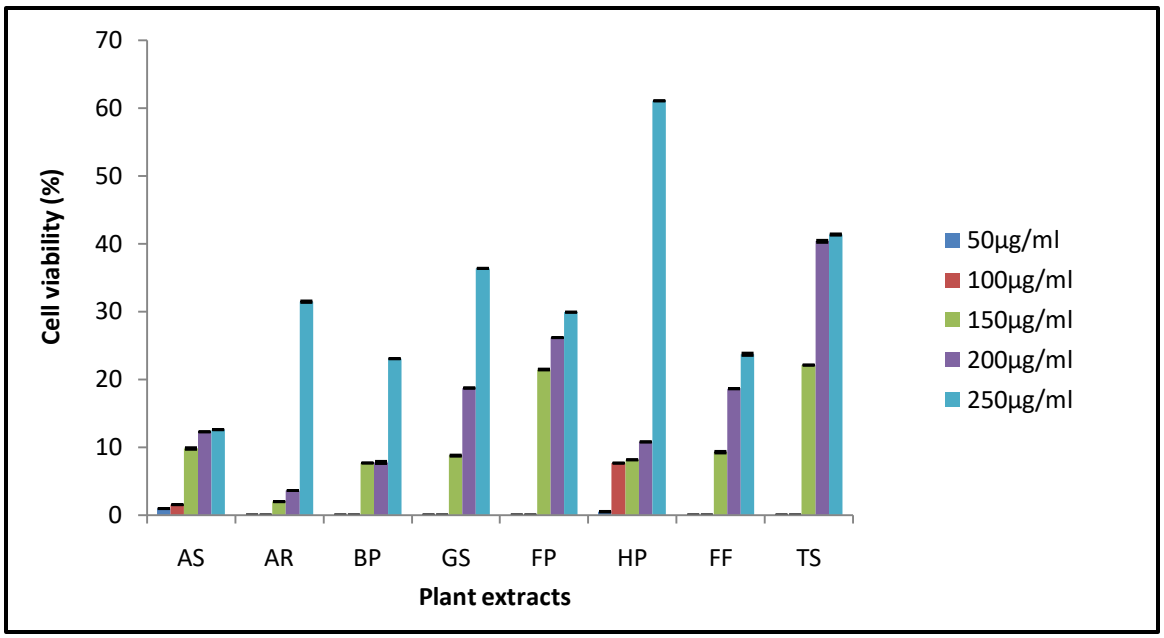


Figure 4.5.1: Anticancer activity of herbal formulation extracts against WRL-68 cell line
AS: Asthma; **AR**: Arthritis; **BP**: High blood pressure; **GS**: Gastritis; **FP**: Food poisoning; **HP**: heart palpitaion; **FF**: *Fraxinus floribunda* for diabetes; **TS**: Tonsilitis

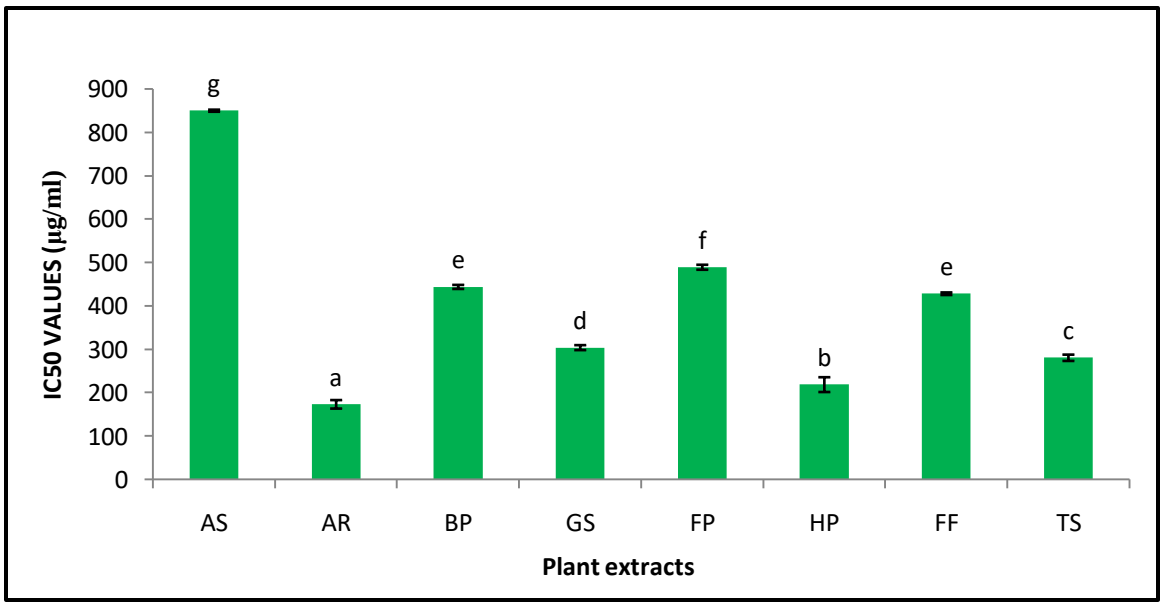


Figure 4.5.2: IC50 values of MTT assay of herbal formulation extracts against WRL-68 cell line
AS: Asthma; **AR**: Arthritis; **BP**: High blood pressure; **GS**: Gastritis; **FP**: Food poisoning; **HP**: heart palpitaion; **FF**: *Fraxinus floribunda* for diabetes; **TS**: Tonsilitis

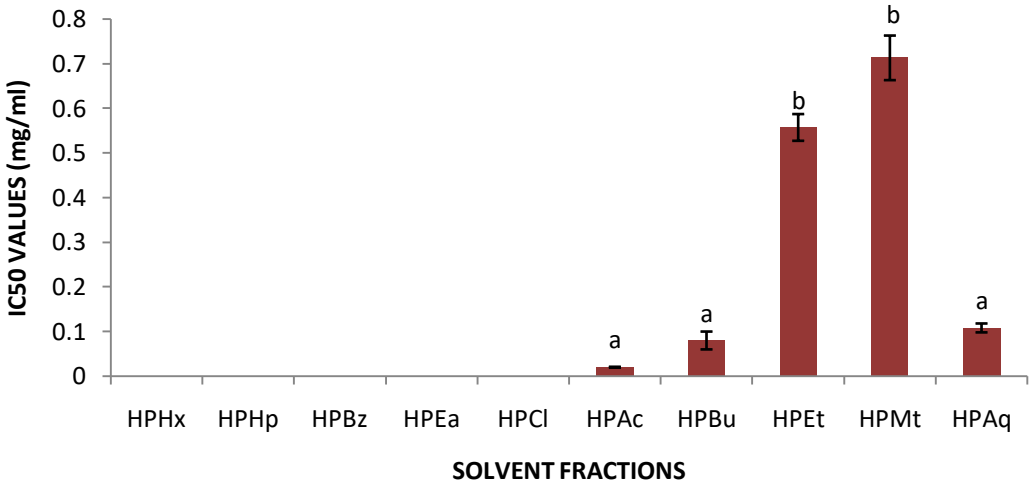


Figure 4.6.1: Alpha glucosidase inhibiting activity of herbal formulation HP for heart palpitation

HPHx: Hexane extract; HPHp: Heptane extract; HPBz: Benzene extract; HPEa: Ethyl acetate extract; HPCl: Chloroform extract; HPAc: Acetone extract; HPBu: Butanol extract; HPEt: Ethanol extract; HPMt: Methanol extract; HPAq: Aqueous extract

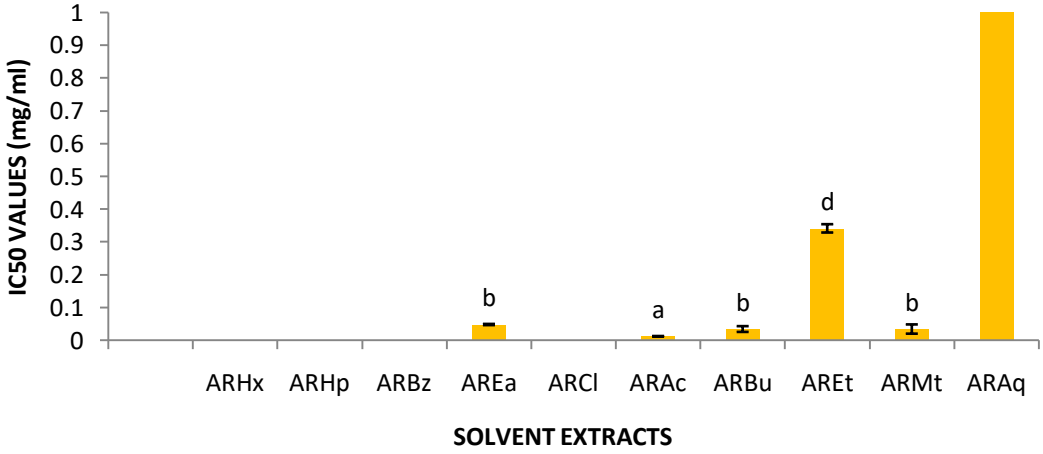


Figure 4.6.2: Alpha glucosidase inhibiting activity of herbal formulation AR for arthritis

ARHx: Hexane extract; ARHp: Heptane extract; ARBz: Benzene extract; AREa: Ethyl acetate extract; ARCl: Chloroform extract; ARAc: Acetone extract; ARBu: Butanol extract; AREt: Ethanol extract; ARMt: Methanol extract; ARAq: Aqueous extract

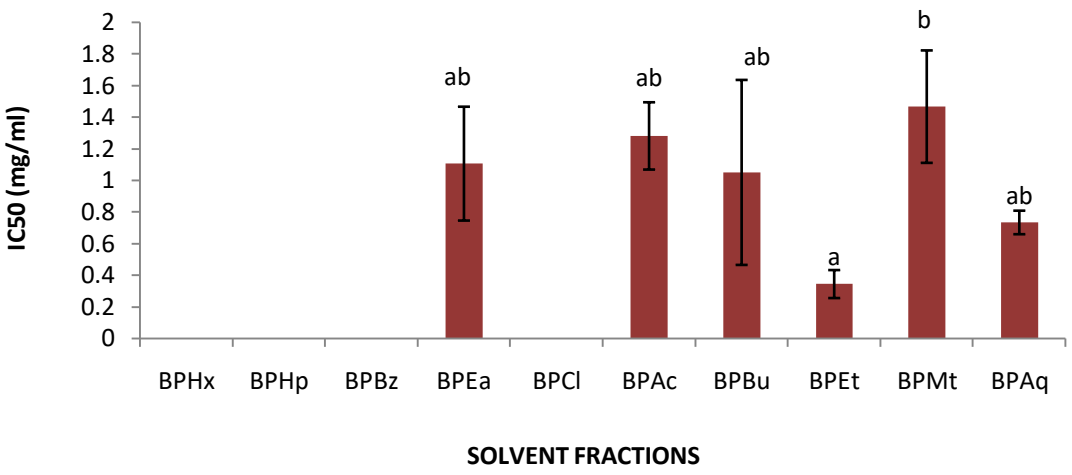


Figure 4.6.3: Alpha glucosidase inhibiting activity of herbal formulation BP for high blood pressure
BPHx: Hexane extract; BPHp: Heptane extract; BPBz: Benzene extract; BPEa: Ethyl acetate extract; BPCl: Chloroform extract; BPAc: Acetone extract; BPBu: Butanol extract; BPEt: Ethanol extract; BPMt: Methanol extract; BPAq: Aqueous extract

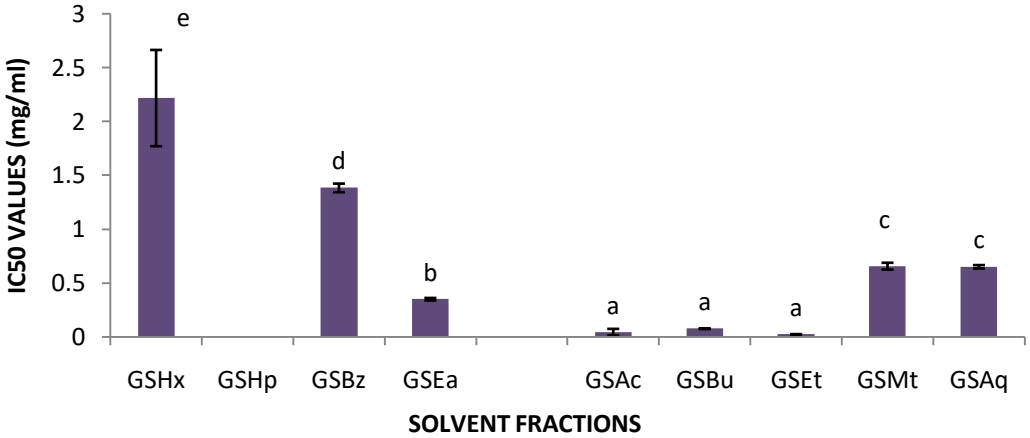


Figure 4.6.4: Alpha glucosidase inhibiting activity of herbal formulation GS for gastritis.
GSHx: Hexane extract; GSHp: Heptane extract; GSBz: Benzene extract; GSEa: Ethyl acetate extract; GSAc: Acetone extract; GSBu: Butanol extract; GSEt: Ethanol extract; GSMt: Methanol extract; GSAq: Aqueous extract

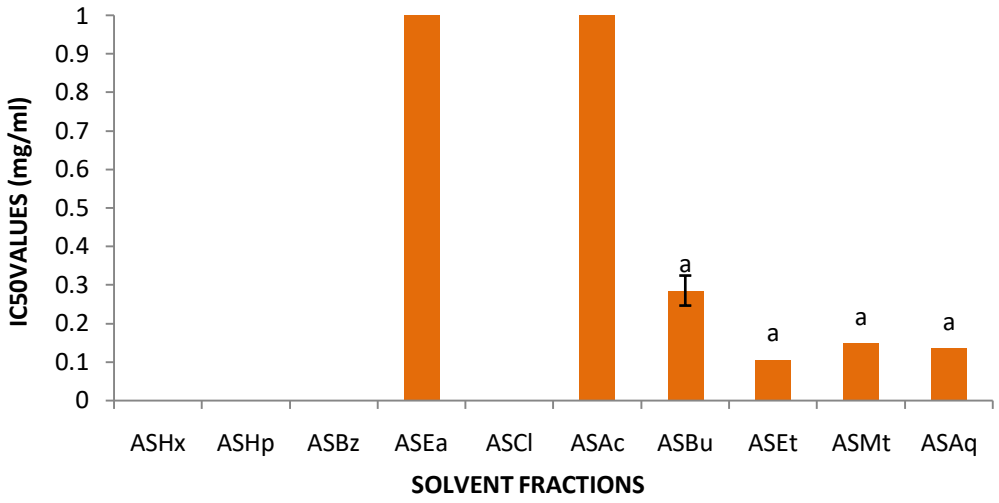


Figure 4.6.5: Alpha glucosidase inhibiting activity of herbal formulation AS for asthma
ASHx: Hexane extract; ASHp: Heptane extract; ASBz: Benzene extract; ASEa: Ethyl acetate extract; ASCl: Chloroform extract; ASAc: Acetone extract; ASBu: Butanol extract; ASEt: Ethanol extract; ASMt: Methanol extract; ASAq: Aqueous extract

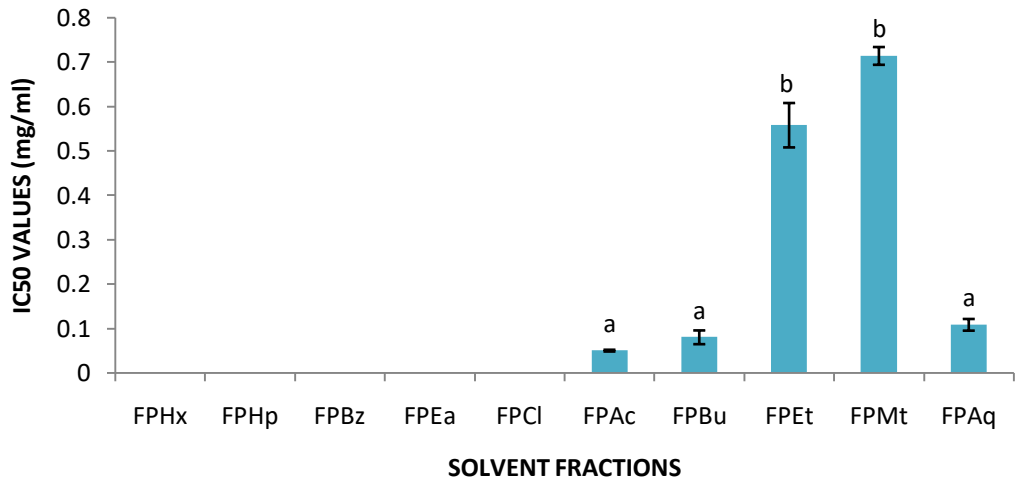


Figure 4.6.6: Alpha glucosidase inhibiting activity of herbal formulation FP for food poisoning
FPHx: Hexane extract; FPHp: Heptane extract; FPBz: Benzene extract; FPEa: Ethyl acetate extract; FPCl: Chloroform extract; FPAc: Acetone extract; FPBu: Butanol extract; FPEt: Ethanol extract; FPMt: Methanol extract; FPAq: Aqueous extract

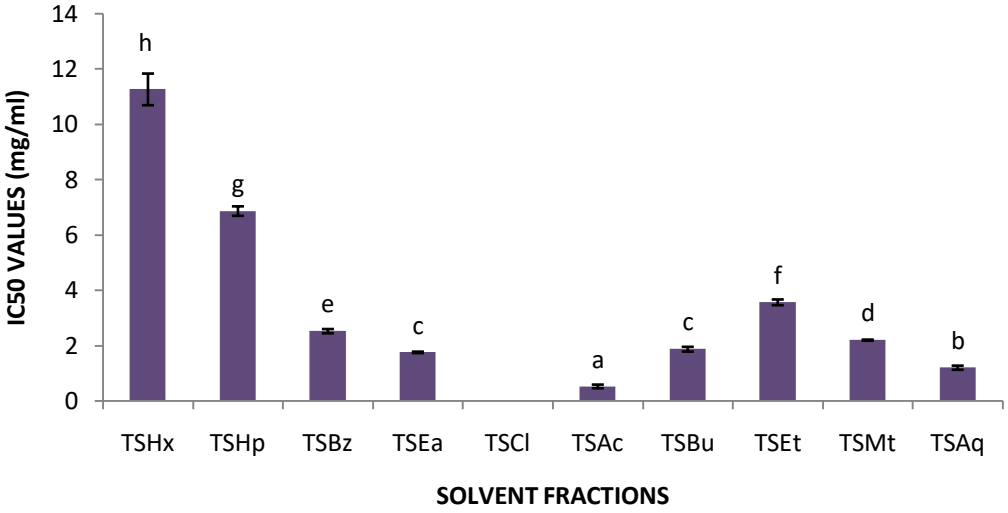


Figure 4.6.7: Alpha glucosidase inhibiting activity of herbal formulation TS for tonsilitis
TSHx: Hexane extract; TSHp: Heptane extract; TSBz: Benzene extract; TSEa: Ethyl acetate extract; TSCl: Chloroform extract; TSAc: Acetone extract; TSBu: Butanol extract; TSEt: Ethanol extract; TSMt: Methanol extract; TSAq: Aqueous extract

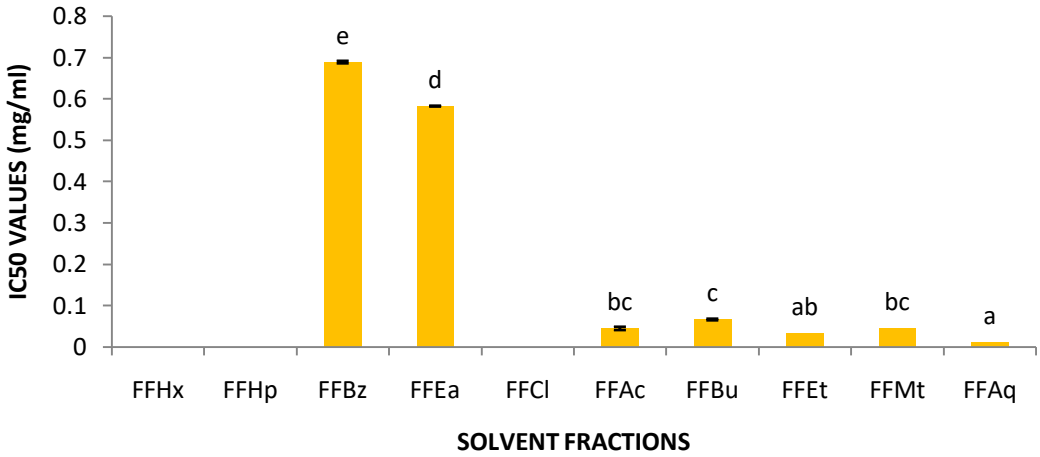


Figure 4.6.8: Alpha glucosidase inhibiting activity of herbal formulation FF (bark of *Fraxinus floribunda*) for diabetes
FFHx: Hexane extract; FFHp: Heptane extract; FFBz: Benzene extract; FFEa: Ethyl acetate extract; FFCl: Chloroform extract; FFAc: Acetone extract; FFBu: Butanol extract; FFEt: Ethanol extract; FFMt: Methanol extract; FFAq: Aqueous extract

antidiabetic activity and chloroform extracts of all the formulation showed no inhibition of the enzymes. Overall, the lowest IC_{50} value was exhibited by aqueous extract of FF (0.011±0.002 mg/ml).

4.7 FREE RADICAL SCAVENGING ACTIVITY

Free radicals such as superoxide, hydroxyl, peroxyl etc are derived from oxygen generated in living organism during metabolic processes (Fang *et al*., 2002 and Valko *et al*., 2007) and are known as reactive oxygen species (ROS). Another group of radicals are nitric oxide and nitrogen dioxide which are derived from nitrogen also known as reactive nitrogen species (RNS). Both ROS and RNS are normal products of metabolic processes and function as defence for body from infectious agents and also play roles in some cellular signalling system in low concentration. However over production of ROS and RNS are harmful and can damage and decrease the function of cellular lipid, proteins, and DNA in biological process causing oxidative stress or nitrosative stress. In this study we have evaluated free radical scavenging activity along with metal chelating potential and ferric reducing antioxidant power of the eight herbal formulations. The figures and tables are presented in figure 4.7.1 to 4.7.6 and table 4.7.1 and 4.7.6.

DPPH scavenging assay is a simple and efficient way to find out the free radical scavenging potential of any sample and it is presented in figure and table 4.7.1. All the extracts of herbal formulation showed DPPH scavenging activity except for heptanes extract of BP and also heptane and chloroform extract of TS. The results are given in IC_{50} values and the lowest value showed the highest activity. From the figure and table, it was found that the lowest IC_{50} value was found in the aqueous extract of FF (0.01±0.002 mg/ml). In all the formulations, the lowest IC_{50} values were exhibited by different solvent extracts.

$ABTS^{+}$ scavenging activity is another simple method to find out the free radical scavenging activity and it is presented in figure and table 4.7.2. In this study, ethyl acetate extract of GS showed lowest IC_{50} value (0.005±0.001 mg/ml). There are other extracts also which showed potential ABTS scavenging activity such as FPBu (0.008±0.001 mg/ml), FFAq (0.01±0.001 mg/ml), FFAc (0.011±0.001 mg/ml), FPMt (0.012±0.001 mg/ml), FPAc (0.011±0.001 mg/ml), FPEt

(0.017±0.001 mg/ml) andFPMt (0.012±0.001 mg/ml). From figure 4.7.2, it was observed that among all the herbal formulations, HP, BP, GS and FP were potential ABTS scavengers.

Superoxide scavenging activity of the herbal formulation is presented in figure and table 4.7.3. The results revealed that all the formulations were able to scavenge superoxide radical. In case of HP (0.369±0.151 mg/ml), AR (0.149±0.136 mg/ml), AS (0.275±0.039 mg/ml), TS (10.54±0.17 mg/ml) and FF (0.02±0.001 mg/ml), aqueous extract showed lowest IC_{50} values and highest scavenging activity. In case of AR, butanol extract exhibited lowest IC_{50} value (0.327±0.083 mg/ml) and in case of GS, heptane extract showed highest activity (0.238±0.79 mg/ml) while for GS, ethyl acetate extract showed lowest IC_{50} value (0.034±0.001 mg/ml). Among all, the highest superoxide scavenging activity was shown by FFAQ.

Nitric oxide scavenging activity is presented in figure and table 4.7.4. It was seen that some of the extracts could not scavenge nitric oxide except for FP and FF in which all the solvents extracts showed nitric oxide scavenging activity. Among all the other extracts, extracts of FF showed the most potent nitric oxide scavenging activity with low values. The lowest IC_{50} value was revealed by the aqueous extract of FF (0.98±0.003 mg/ml).

Chelation of ferrous ions by the extracts of herbal formulations was determined and presented in figure and table 4.7.5. The results showed the metal chelating activity was exhibited by only some selective extracts of the formulations except for AS and FF which showed metal chelating activity by the solvent extracts. In case of HP and AR, heptane extract showed the highest metal chelating activity as 0.328±0.036mg/ml and 0.83±0.031mg/ml respectively. Methanol extract showed the highest activity in case of BP (0.852±0.483 mg/ml) and FP (1.049±0.104 mg/ml). Benzene extract was showed strong chelating activity by AS (0.904±0.168 mg/ml) and TS (1.279±0.143 mg/ml). Among all the formulations, aqueous extract of FF was the strongest metal chelator (0.65±0.007 mg/ml).

Ferric reducing antioxidant power or electron donating ability of extracts of herbal formulations was determined and presented in figure and table 4.7.6. The results revealed that some of the extracts showed remarkable reducing power such as

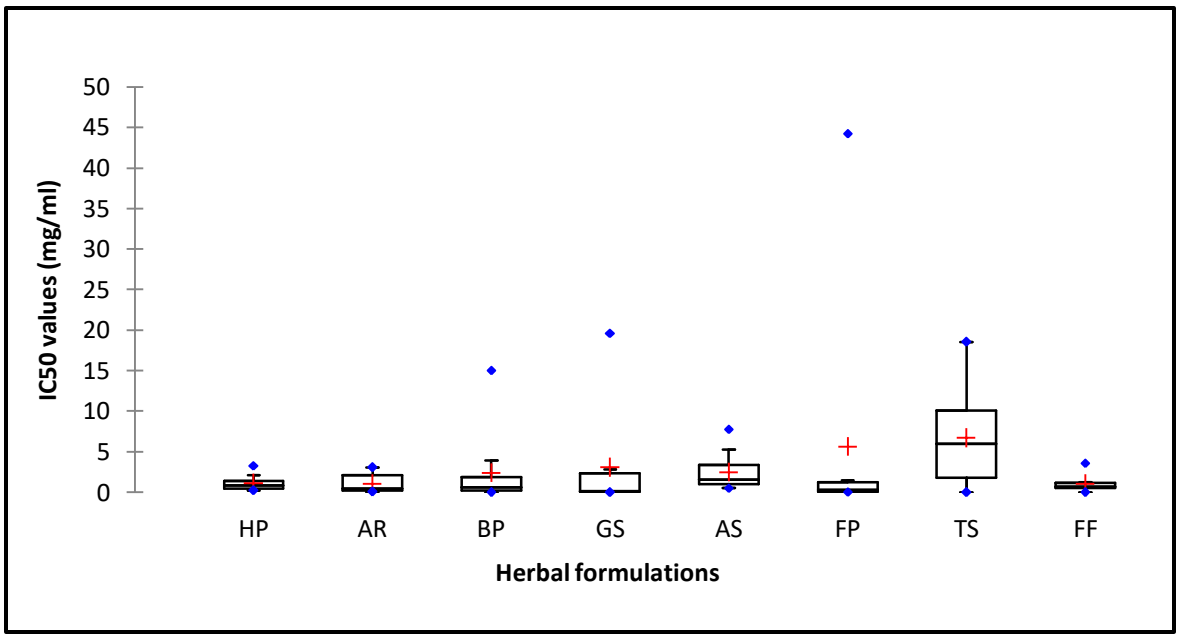


Figure 4.7.1: DPPH˙ scavenging activity of herbal formulations (IC50 mg/ml)
HP: Heart palpitation; **AR**: Arthritis; **BP**: High blood pressure; **GS**: Gastritis; **AS**: Asthma; **FP**: Food poisoning; **TS**: Tonsilitis; **FF**: *Fraxinus floribunda* (Diabetes)

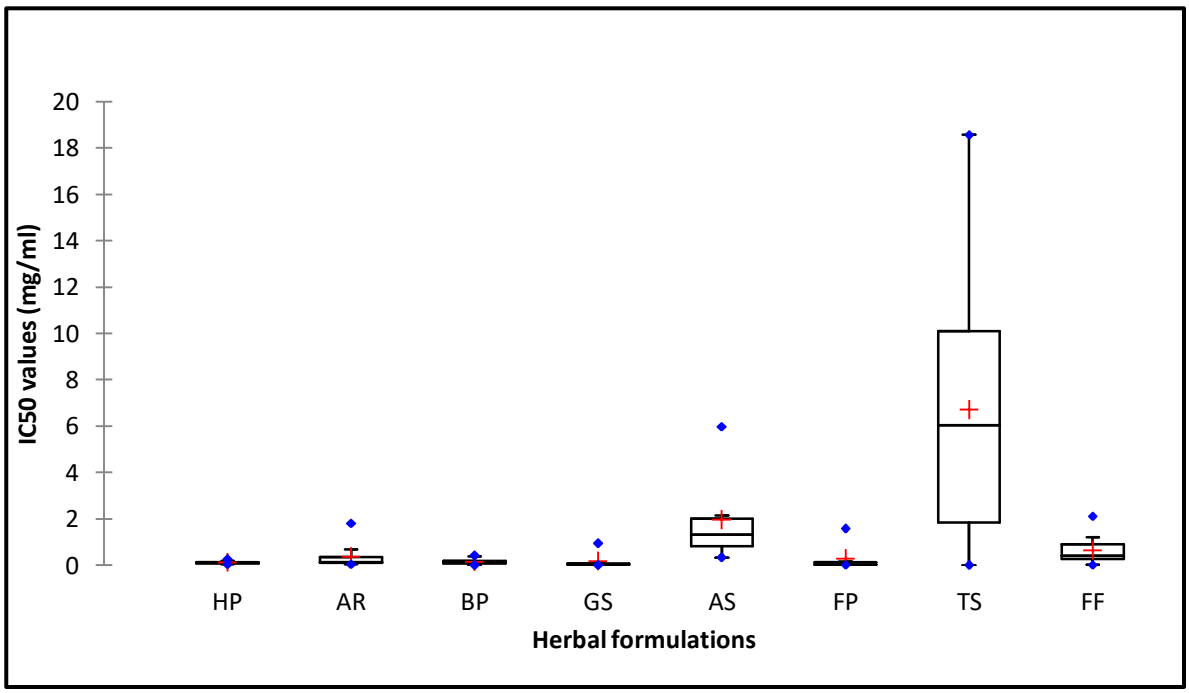


Figure 4.7.2: ABTS˙ scavenging activity of herbal formulations (IC50 mg/ml)
HP: Heart palpitation; **AR**: Arthritis; **BP**: High blood pressure; **GS**: Gastritis; **AS**: Asthma; **FP**: Food poisoning; **TS**: Tonsilitis; **FF**: *Fraxinus floribunda* (Diabetes)

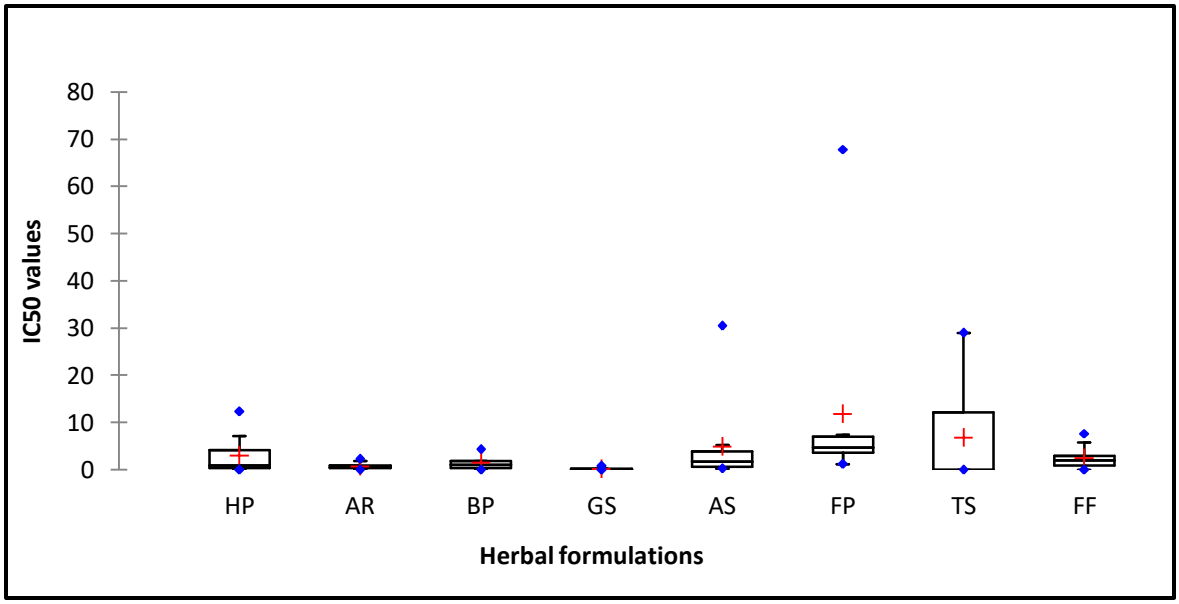


Figure 4.7.3: Superoxide scavenging activity of herbal formulations (IC50 mg/ml)
HP: Heart palpitation; **AR**: Arthritis; **BP**: High blood pressure; **GS**: Gastritis; **AS**: Asthma; **FP**: Food poisoning; **TS**: Tonsilitis; **FF**: *Fraxinus floribunda* (Diabetes)

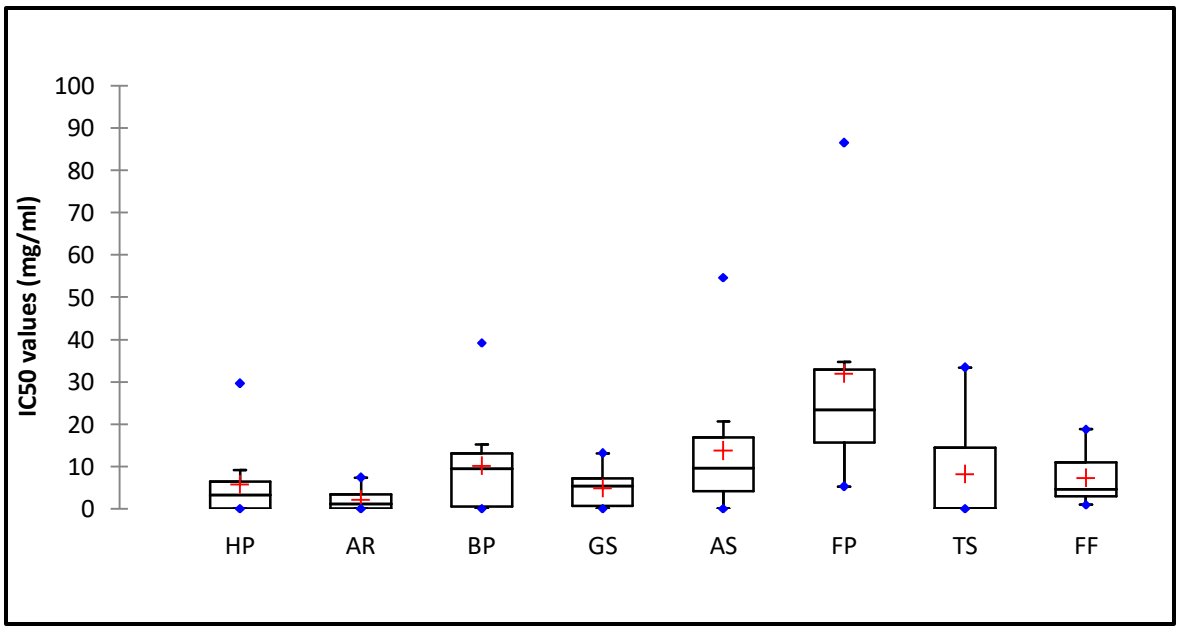


Figure 4.7.4: Nitric oxide scavenging activity of herbal formulations (IC50 mg/ml)
HP: Heart palpitation; **AR**: Arthritis; **BP**: High blood pressure; **GS**: Gastritis; **AS**: Asthma; **FP**: Food poisoning; **TS**: Tonsilitis; **FF**: *Fraxinus floribunda* (Diabetes)

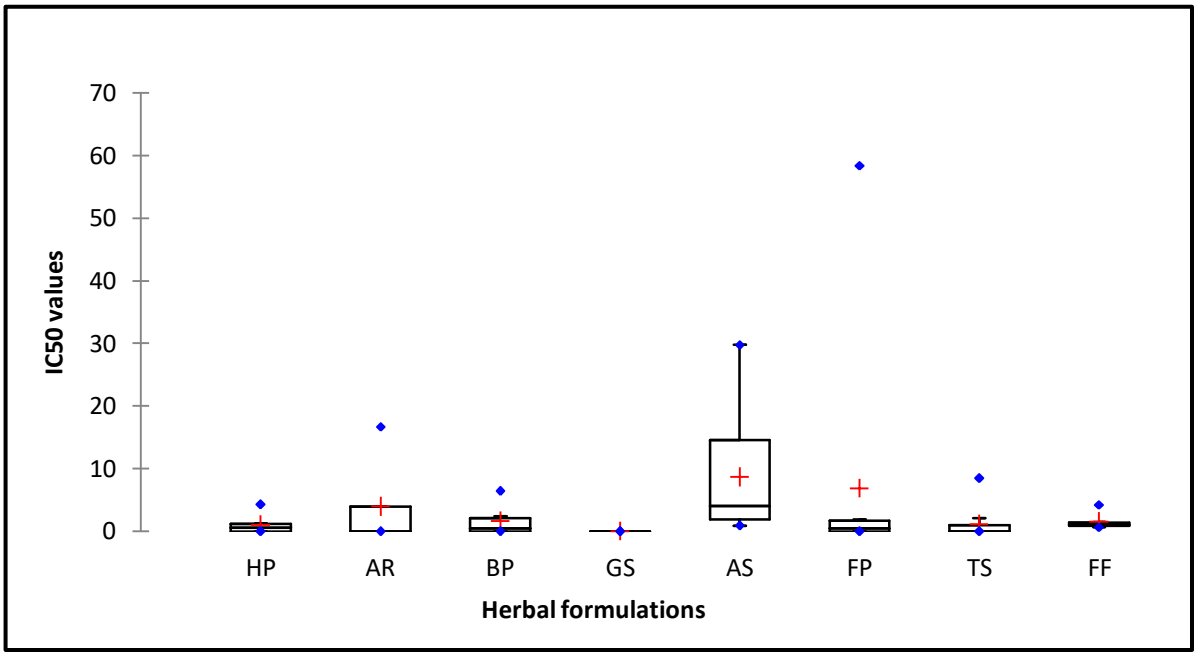


Figure 4.7.5: Metal chelating activity of herbal formulations (IC50 mg/ml)
HP: Heart palpitation; **AR**: Arthritis; **BP**: High blood pressure; **GS**: Gastritis; **AS**: Asthma; **FP**: Food poisoning; **TS**: Tonsilitis; **FF**: *Fraxinus floribunda* (Diabetes)

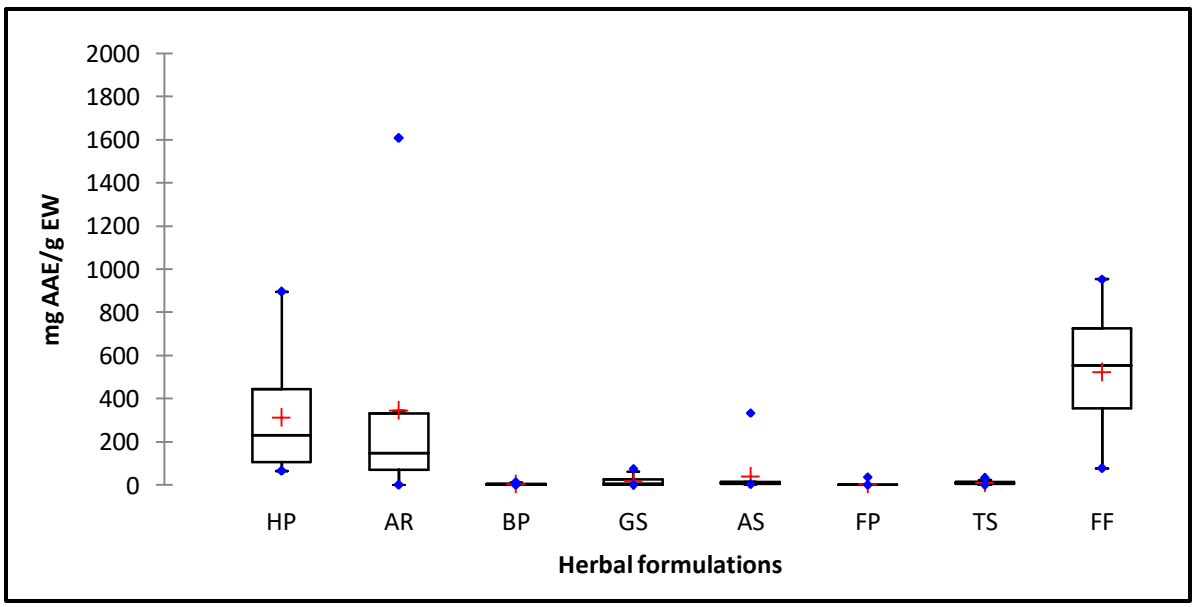


Figure 4.7.6: Ferric reducing antioxidant power of herbal formulations (mg AAE/gEW) [AAE: Ascorbic Acid Equivalent]
HP: Heart palpitation; **AR**: Arthritis; **BP**: High blood pressure; **GS**: Gastritis; **AS**: Asthma; **FP**: Food poisoning; **TS**: Tonsilitis; **FF**: *Fraxinus floribunda* (Diabetes)

HPAc (896.655±0.029mg AAE/g EW) and FFAq (954.12±0.88mg AAE/g EW). Other solvent extracts of HP and FF also showed potential reducing power as compared to the formulations.

4.8 ANTI-HYPERTENSIVE ACTIVITY

Anti-hypertensive activity of the extracts of herbal formulations was determined by the inhibiting activity of angiotensin converting enzyme. It was found that only two formulations were able to inhibit the enzyme and the result is presented in figure 4.8. *Fraxinus floribunda* was used for the treatment of diabetes and BP is the formulation used for the treatment of high blood pressure. Antihypertensive activity was exhibited only by the acetone and aqueous extracts of FF as well as benzene and ethanol extracts of BP. Overall, the lowest IC_{50}value was shown by the benzene extract of BP (55.25±5.59 mg/ml). Other extracts showed IC_{50} values in ascending order as 64.41 mg/ml (FFAq), 110.845 mg/ml (FFAc) and265.21±4.79 mg/ml (BPEt).

4.9 CORRELATION

4.9.1 Pearson correlation

Analysis of relation between phytochemical content, free radical scavenging activity and α-glucosidase inhibiting activity was determined by Pearson corelation coefficient. Table 4.9.1 represents the Pearson correlation analysis of the above assays in the extracts of the studied herbal formulations. The result suggested that DPPH has significant positive correlation ($p< 0.01$) with ABTS, superoxide and nitric oxide scavenging activity which means the IC_{50} value of DPPH will increase with the increase of IC_{50} values in ABTS, SO and NO scavenging assays. DPPH showed significant negative correlation with TPC, TTC ($p<0.01$) and FRAP ($p<0.05$) which means that the increase in phenol, tannin content and reducing power will increase the DPPH scavenging activity of the studied herbal formulations. ABTS also showed significant positive correlation ($p<0.01$) with SO and significant negative correlation ($p<0.01$) with TPC and TTC. Superoxide and nitric oxide scavenging activity were also positively correlated with each other. Nitric oxide scavenging activity showed significant negative correlation with FRAP

Table 4.7.1: DPPH˙ scavenging activity of herbal formulations (IC_{50} mg/ml): **NA**: Not applicable

Solvents used	HP	AR	BP	GS	FP	AS	TS	FF
Hexane	2.156±0.148	3.087±0.218	3.946±0.094	6.9±0.023	8.995±0.27	3.921±0.332	10.115±1.594	1.23±0.002
Heptane	0.653±0.003	2.501±0.025	NA	19.636±0.021	44.221±0.109	5.258±0.049	NA	2.35±0.012
Benzene	1.094±0.256	2.615±0.065	15.029±1.278	2.827±0.055	1.507±0.01	1.870±0.295	11.991±0.074	0.98±0.003
Ethyl acetate	0.465±0.1667	0.328±0.026	0.433±0.139	0.018±0.002	0.217±0.008	0.514±0.027	1.555±1.128	0.67±0.005
Chloroform	1.511±0.057	0.986±0.019	2.173±0.044	0.903±0.016	0.406±0.004	0.777±1.33	NA	3.61±0.001
Acetone	0.256±0.034	0.085±0.005	0.846±0.285	0.026±0.002	0.046±0.001	0.607±0.017	18.573±2.312	0.06±0.001
Butanol	0.612±0.023	0.145±0.009	1.009±0.256	0.049±0.006	0.171±0.044	1.047±0.089	10.038±2.474	0.56±0.06
Ethanol	3.288±0.594	0.331±0.048	0.207±0.009	0.09±0.006	0.069±0.006	1.041±0.067	5.083±1.355	0.77±0.01
Methanol	1.243±0.148	0.231±0.009	0.267±0.072	0.098±0.013	0.075±0.157	1.424±0.039	2.712±0.543	0.54±0.05
Water	0.412±0.015	0.588±0.069	0.203±0.185	0.247±0.027	0.491±0.157	1.721±0.191	6.978±1.189	**0.01±0.002**

Table 4.7.2: ABTS˙ scavenging activity of herbal formulations (IC50 mg/ml): **NA**: Not applicable

Solvents used	HP	AR	BP	GS	FP	AS	TS	FF
Hexane	0.171±0.003	1.813±0.329	0.411±0.041	0.634±0.097	0.793±0.049	2.15±0.002	8.12±0.61	0.98±0.001
Heptane	0.089±0.001	0.684±.009	NA	NA	1.573±0.295	4.76±0.007	NA	1.21±0.12
Benzene	0.091±0.012	0.372±0.079	0.091±0.006	0.942±0.055	0.143±0.039	1.56±0.001	9.31±0.82	0.65±0.004
Ethyl acetate	0.062±0.001	0.079±0.029	0.182±0.035	**0.005±0.001**	0.019±0.002	0.33±0.003	0.87±0.06	0.45±0.002
Chloroform	0.065±0.003	0.277±0.032	0.101±0.009	0.101±0.03	0.055±0.006	5.98±0.01	NA	2.11±0.023
Acetone	0.037±0.004	0.043±0.01	0.368±0.011	0.013±0.001	0.011±0.001	0.34±0.003	15.45±0.34	0.02±0.008
Butanol	0.106±0.012	0.084±0.038	0.195±0.023	0.015±0.004	0.008±0.001	0.98±0.008	7.89±0.48	0.34±0.006
Ethanol	0.249±0.002	0.127±0.019	0.038±0.006	0.024±0.004	0.017±0.001	0.76±0.007	3.78±0.05	0.32±0.002
Methanol	0.094±0.006	0.115±0.005	0.072±0.016	0.025±0.004	0.012±0.001	1.12±0.001	1.34±0.01	0.24±0.004
Water	0.134±0.012	0.138±0.016	0.079±0.005	0.044±0.002	0.043±0.007	1.54±0.034	4.99±0.09	0.01±0.001

HP: Heart palpitation; **AR**: Arthritis; **BP**: High blood pressure; **GS**: Gastritis; **FP**: Food poisoning; **AS**: Asthma; **TS**: Tonsilitis; **FF**: *Fraxinus floribunda* (Diabetes)

Table 4.7.3: Superoxide˙ scavenging activity of herbal formulations (IC_{50} mg/ml): **NA**: Not applicable

Solvents used	HP	AR	BP	GS	FP	AS	TS	FF
Hexane	NA	2.352±0.056	1.954±0.224	NA	15.66±0.67	1.228±0.176	NA	3.23±0.01
Heptane	NA	NA	NA	NA	67.83±1.34	0.559±0.003	NA	5.79±0.007
Benzene	7.147±0.073	0.461±0.039	0.238±0.79	0.722±0.051	7.44±0.45	5.284±0.078	16.45±0.21	2.13±0.003
Ethyl acetate	1.197±0.276	0.613±0.197	1.26±0.277	0.034±0.001	3.24±0.02	0.632±0.216	12.66±0.45	1.65±0.007
Chloroform	3.379±0.079	1.883±0.038	0.901±0.138	0.842±0.167	5.99±0.04	30.503±0.196	NA	7.67±0.04
Acetone	0.782±0.311	0.904±0.022	4.337±0.064	0.119±0.016	1.23±0.006	0.903±0.094	NA	0.67±0.03
Butanol	0.833±0.178	0.327±0.083	4.256±2.074	0.239±0.0565	5.12±0.02	2.339±0.042	NA	1.95±0.09
Ethanol	4.482±0.511	1.005±0.034	0.719±0.066	0.259±0.04	3.45±0.03	3.192±1.136	28.98±0.66	2.12±0.06
Methanol	12.331±0.158	0.402±0.108	1.465±0.625	0.296±0.059	4.23±0.07	4.198±0.774	NA	0.26±0.002
Water	0.369±0.151	0.149±0.136	NA	0.134±0.002	4.34±0.012	0.275±0.039	10.54±0.17	0.02±0.001

Table 4.7.4: Nitric oxide˙ scavenging activity of herbal formulations (IC_{50} mg/ml): **NA**: Not applicable

Solvents used	HP	AR	BP	GS	FP	AS	TS	FF
Hexane	2.990±0.145	NA	NA	NA	23.45±0.67	54.669±0.077	NA	18.79±0.21
Heptane	NA	NA	NA	NA	77.34±1.01	3.381±0.009	NA	12.03±0.05
Benzene	NA	2.139±0.021	13.077±4.964	7.242±0.385	15.66±0.32	20.651±0.154	19.563±1.519	7.82±0.05
Ethyl acetate	3.516±0.379	NA	7.387±0.251	4.59±2.059	34.68±0.12	2.097±0.129	14.746±0.445	5.34±0.13
Chloroform	NA	NA	NA	NA	86.54±0.45	NA	NA	15.61±0.56
Acetone	5.046±0.476	2.479±0.018	39.236±2.159	6.942±0.926	5.24±0.22	6.770±0.413	NA	2.32±0.01
Butanol	9.224±0.010	5.381±0.439	15.221±1.901	8.592±1.987	15.67±0.87	11.776±0.925	NA	3.56±0.04
Ethanol	6.832±0.0568	7.399±0.377	2.265±0.029	6.119±0.068	10.11±0.09	10.817±0.531	33.398±1.228	3.91±0.02
Methanol	NA	3.699±0.423	11.504±0.684	2.498±0.667	27.86±0.36	18.656±0.457	NA	2.78±0.007
Water	29.608±0.416	NA	13.032±1.048	13.1±0.584	23.41±0.67	8.332±0.884	13.633±0.271	**0.98±0.003**

HP: Heart palpitation; **AR**: Arthritis; **BP**: High blood pressure; **GS**: Gastritis; **FP**: Food poisoning; **AS**: Asthma; **TS**: Tonsilitis; **FF**: *Fraxinus floribunda* (Diabetes)

Table 4.7.5: Metal chelating activity of herbal formulations (IC_{50} mg/ml): **NA**: Not applicable

Solvents used	HP	AR	BP	GS	FP	AS	TS	FF
Hexane	0.976±0.084	2.312±0.069	2.45±0.131	NA	NA	2.598±0.275	NA	2.67±0.004
Heptane	0.328±0.036	0.83±0.031	5.751±0.312	NA	NA	1.385±0.008	2.143±0.054	1.46±0.006
Benzene		4.067±0.094	NA	NA	NA	0.904±0.168	1.279±0.143	1.21±0.12
Ethyl acetate	NA	NA	NA	NA	NA	29.791±0.461	8.479±1.097	0.98±0.09
Chloroform	1.272±0.061	NA	NA	NA	NA	3.151±0.181	NA	4.21±0.03
Acetone	4.278±0.193	NA	NA	NA	58.427±o.66	16.214±1.826	NA	0.74±0.001
Butanol	NA	NA	NA	NA	1.886±0.245	1.729±0.079	NA	1.07±0.004
Ethanol	NA	NA	1.23±0.015	NA	6.223±1.628	16.361±1.135	NA	1.22±0.002
Methanol	NA	2.889±0.408	0.852±0.483	NA	1.049±0.104	4.867±0.776	NA	0.89±0.003
Water	3.10±0.946	1.047±0.414	6.424±0.259	NA	1.051±0.322	9.690±1.938	NA	**0.65±0.007**

Table 4.7.6: Free radical antioxidant power (FRAP) of the eight herbal formulations (mg AAE/g EW): **NA**: Not applicable

FRAP	HP	AR	BP	GS	FP	AS	TS	FF
Hexane	102.555±0.031	19.585±0.365	NA	60.778±1.104	NA	14.136±0.226	15.146±0.228	98.12±1.23
Heptane	316.949±0.006	NA	NA	76.51±0.011	NA	11.283±0.006	8.185±0.104	324.66±3.21
Benzene	189.339±0.088	NA	2.088±0.031	6.007±0.012b	6.981±0.014	9.409±0.064	24.327±0.482	452.11±3.56
Ethyl acetate	487.822±0.084	2.636±0.011	8.212±0.016	0.659±0.001	0.585±0.004	333.73±0.127	4.066±0.034	476.34±0.98
Chloroform	95.185±0.011	3.444±0.063	NA	6.541±0.046	3.205±0.244	15.857±0.106	NA	77.21±1.98
Acetone	896.655±0.029	0.667±0.001	12.762±0.029	34.133±0.015	0.638±0.006	4.021±0.119	11.548±0.153	778.43±2.76
Butanol	273.469±0.038	1.284±0.006	8.835±0.142	0.437±0.011	0.371±1.058	4.960±0.050	33.901±0.265	632.42±1.23
Ethanol	65.711±0.12	2.725±0.152	2.645±0.013	0.328±0.012	1.185±0.008	6.314±0.117	NA	734.12±0.97
Methanol	121.847±0.482	6.773±0.071	4.101±0.018	0.535±0.017	NA	7.951±0.129	6.426±0.038	698.23±1.56
Water	579.099±0.028	5.710±0.002	3.456±0.021	5.359±0.779	NA	5.293±0.025	8.585±0.011	**954.12±0.88**

HP: Heart palpitation; **AR**: Arthritis; **BP**: High blood pressure; **GS**: Gastritis; **FP**: Food poisoning; **AS**: Asthma; **TS**: Tonsilitis; **FF**: *Fraxinus floribunda* (Diabetes)

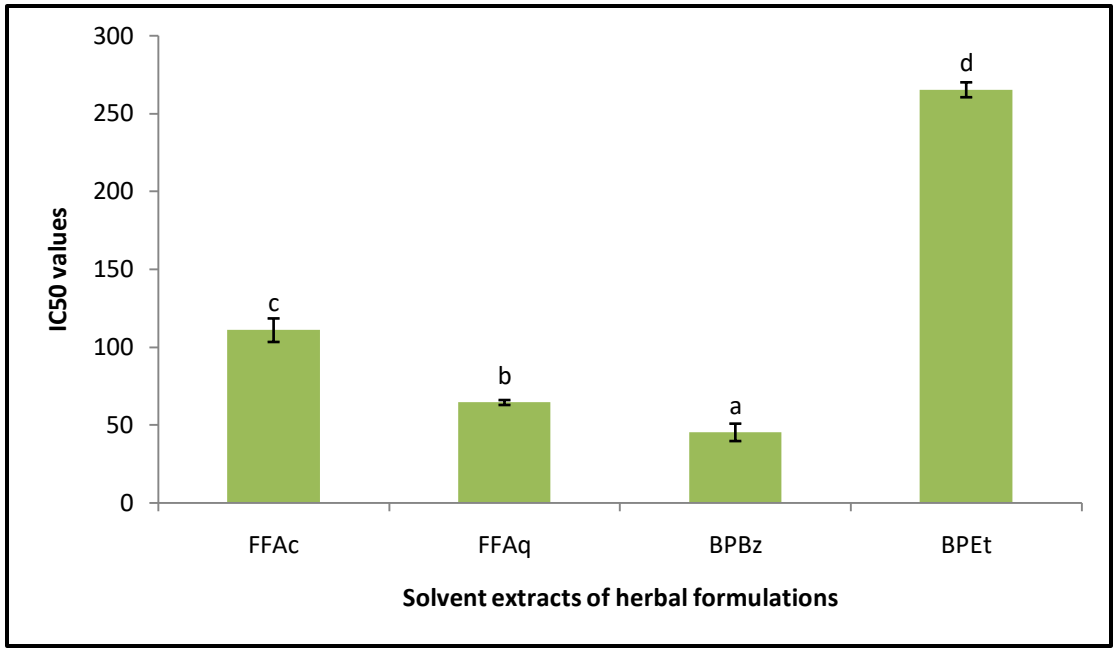


Figure 4.8: Angiotensin converting enzyme inhibiting activity of extracts of herbal formulations. FFAc: acetone extract of *F. floribunda*; FFAq: aqueous extract of F. floribunda; BPBz: benzene extract of herbal formulation BP used for the treatment of high blood pressure; BPEt: ethanol extract of herbal formulation BP. Values with different letters (a, b, c, d,) are significantly ($p < 0.05$) different from each other by Duncan's multiple range test (DMRT);

($p<0.05$), TPC ($p<0.01$) and TTC ($p<0.01$) which indicates the influence of phenols and tannin on nitric oxide scavenging activity of extracts of herbal formulations. Total steroid content showed significant positive correlation ($p<0.01$) with total flavonoids content.

4.9.2 Principal Component Analysis

Principal component analysis (PCA) explains the differences among samples and obtaining information among variables influencing the quality attributes of samples (Samec *et al*., 2016). To obtain the inter-relationship among different variables, PCA was applied on original variables of antioxidants (DPPH, ABTS, SO, NO) and responsible phytochemicals (TPC, TFC, TOPC) to reduce and optimize in a much smaller number of principal components (underlying variables). The first (PC1) and second (PC2) principal component accounted for 26.29 % and 14.63 % of the total variance respectively.

The factor loadings and squared cosine of antioxidant, antidiabetic and phytochemical variables for the first two components of ten different solvent extracts of eight formulations were shown in figure 4.9. As the bioactivity and phytochemical density extracts are dependent on hydropathic property of extracted solvents for analyzing their inter-relationship, the data dimensionality was reduced from 13 partially co-related variables to underlying principal components with almost 59.08 % loss of variation. When the bioactivity and phytochemical were analyzed together in different solvent extracts, free radical scavenging activities like DPPH, SO, reducing power (FRAP) and phytochemicals like TPC, TFC and TTC were heavily loaded in PC1 and bears active association among them. PCA plot (figure 4.9.2) indicates that these components are far from ordinates along x-axis (F1) and antioxidants and phytochemicals are clustered in two opposite directions indicating better extraction of bioactive phenolic phytochemicals is associated with lowering of IC_{50} values (better antioxidant activity) for free radical scavengers like DPPH, superoxide and $ABTS^{+}$. On the other hand, antidiabetic activity (alpha glucosidase assay) is associated with scavengers of nitric oxide as they are heavily loaded on PC2 and diverged in two different directions along y-axis; indicating that nitric oxide accumulation might be related with complex metabolic disorders like

Table 4.9.1: Pearson correlation matrix of phytochemical content, antioxidant and antidiabetic activity of solvent extracts of eight studied herbal formulations

	DPPH	ABTS	SO	NO	MC	FRAP	TPC	TFC	TOPC	TAC	TTC	TSC
ABTS	0.489**											
SO	0.848**	0.412**										
NO	0.499**	0.139	0.596**									
MC	-0.175	-0.122	-0.133	-0.192								
FRAP	-0.252*	-0.205	-0.238	-0.288*	-0.149							
TPC	-0.374**	-0.316**	-0.370**	-0.378**	-0.212	0.483**						
TFC	-0.129	-0.229	-0.182	-0.101	-0.121	0.596**	0.557**					
TOPC	-0.120	-0.226	-0.140	0.003	-0.205	0.132	0.473**	0.552**				
TAC	0.262	0.444**	0.337	-0.118	0.486*	0.093	-0.203	0.052	-0.201			
TTC	-0.307**	-0.254*	-0.286*	-0.333*	-0.110	0.391**	0.604**	0.482**	0.339**	-0.034		
TSC	0.195	0.311**	-0.006	-0.175	0.021	0.089	0.099	0.340**	0.048	0.245	0.087	
AGI	0.266	0.264	0.176	0.075	0.183	0-.212	-0.329*	-0.239	-0.302*	0.450*	-0315*	0.030

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

ABTS: ABTS+ scavenging activity; **AGI**: Alpha glucosidase activity; **DPPH**: DPPH scavenging activity; **FRAP**: Ferric reducing antioxidant potential; **MC**: Metal chelating activity; **NO**: Nitric oxide scavenging activity; **SO**: Superoxide scavenging activity; **TAC**: Total alkaloid content; **TFC**; Total flavonoid content: **TOPC**: Total orthodihydric phenol content; **TPC**: Total phenol content; **TSC**: Total steroid content; **TTC**: Total tannin content

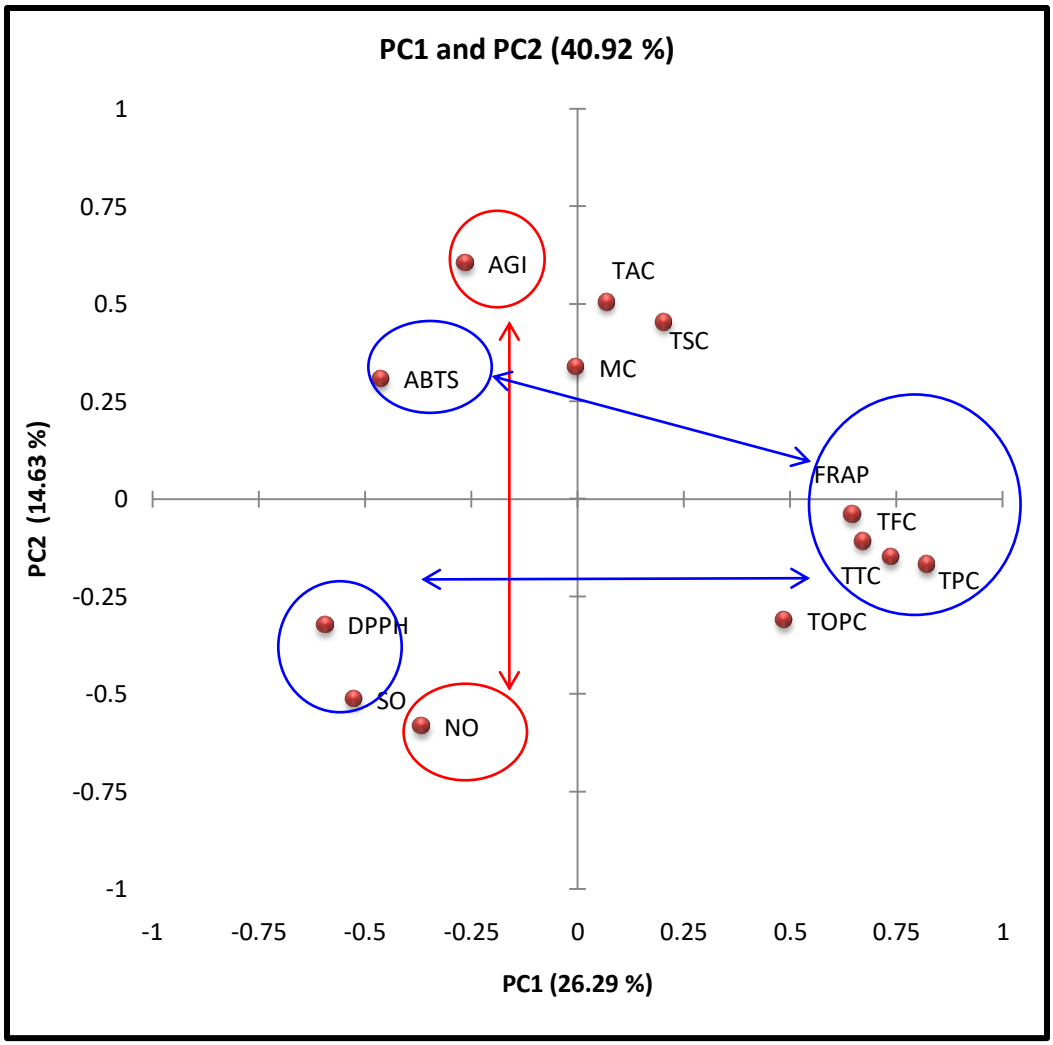


Figure 4.9 : Principal component analysis of phytochemical content, antioxidant activity and antidiabetic activity of the studied eight herbal formulations.

ABTS: ABTS+ scavenging activity; **AGI**: Alpha glucosidase activity; **DPPH**: DPPH scavenging activity; **FRAP**: Ferric reducing antioxidant potential; **MC**: Metal chelating activity; **NO**: Nitric oxide scavenging activity; **SO**: Superoxide scavenging activity; **TAC**: Total alkaloid content; **TFC**; Total flavonoid content: **TOPC**: Total orthodihydric phenol content; **TPC**: Total phenol content; **TSC**: Total steroid content; **TTC**: Total tannin content

Table 4.9.2: Principal components loadings of variables for the ten solvent extracts of eight herbal formulations

Variable	Factor loading		Squared cosine	
	PC1	PC2	PC1	PC2
DPPH	-0.594	-0.322	0.353	0.104
ABTS	-0.463	0.308	0.214	0.095
SO	-0.525	-0.513	0.276	0.263
NO	-0.367	-0.582	0.135	0.339
MC	-0.003	-0.339	0.000	0.115
FRAP	0.646	-0.039	0.418	0.002
TPC	0.813	-0.169	0.675	0.028
TFC	0.671	-0.108	0.451	0.012
TOPC	0.487	-0.311	0.236	0.097
TAC	0.068	0.505	0.005	0.255
TTC	0.737	-0.149	0.544	0.022
TSC	0.204	0.454	0.042	0.206
AGI	-0.264	0.604	0.069	0.365
% of variance				

PC: Principal component; **DPPH**: DPPH scavenging assay**; ABTS**: ABTS scavenging activity; **SO**: Superoxide scavenging activity; **NO**: Nitric oxide scavenging activity; **MC**: Metal chelating activity; **FRAP**: Ferric reducing antioxidant power; **TPC**: Total phenol content; **TFC**: Total flavonoid content; **TOPC**: Total orthodihydric phenol content; **TAC**: Total alkaloid content; **TTC**: Total tannin content; **TSC**: Total steroid content; **AGI**: Alpha glucosidase inhibiting activity.

diabetes. As a whole, the inter-relationship among phenolic components and pharmacological properties are important to cluster the variables and PCA analysis is required for better understanding of how these variables (bioactive phytochemicals) contribute to pharmacological properties of different solvent extracts obtained from herbal formulation.

4.10 INFLUENCE OF EXTRACTION METHODS ON THE BIOACTIVITY OF *FRAXINUS FLORIBUNDA*

From the studies above, it was found that out of all the herbal formulations, one formulation that stood out in terms of antioxidant potentiality, phytochemical content and antidiabetic activity was aqueous extract of *Fraxinus floribunda*. It also showed cytotoxic activity as well as antimicrobial activity against some pathogenic bacteria. Thus this formulation was selected among all other formulations for some pharmacological activities in animal model.

The bark of *Fraxinus floribunda* (BOFF) is traditionally used in the form of decoction to reduce blood sugar in diabetic patients. During survey, it was found that BOFF is boiled in water and the decoction is consumed orally for treatment. The bark material is used until the colour of the water becomes faint which mean it is successively boiled until the colour as well as the bitterness remains.

Thus in this study we have extracted BOFF in four different methods viz. autoclave boiling under pressure (AB), in a soxhlet (S), boiling at normal pressure through reflux similar to traditional method (NB) and in cold condition under -4ºC (CP). This was done to observe the influence of variation in extraction methods in their bioactivity. We also carried out the process for thrice successively and labeled as 1^{st} and 3^{rd} boiled so as to observe the retention of colour of the bark as well as bioactivity in the different methods.

4.10.1 Antioxidant activity

The aqueous extracts of BOFF showed potential antioxidant activity and phytochemical content. However our focus was to observe the variation in bioactivity of BOFF due to the change in extraction methods. The box plots (figure

4.10.1.a to figure 4.10.1.f) clearly illustrate the changes in the bioactivity as well as retention of this activity in BOFF extracted through various methods after their successive extraction. From the box plots we have observed that soxhletion process could extract a concentrated amount of bioactive components at the 1st stage of boiling which then rapidly decreases in the 2nd and the 3rd stages due to which the IC_{50} values in DPPH scavenging activity ranges from low (2.17 mg/ml) in the 1st stage to high (486.61 mg/ml) in the 3rd stage (figure 4.10.1.a). Similar results were found in $ABTS^+$ (figure 4.10.1.b), superoxide (figure 4.10.1.c), nitric oxide scavenging activity (figure 4.10.1.d) along with metal chelating activity (figure 4.10.1.e) and reducing power (figure 4.10.1.f). In case of normal boiling, autoclave boiling and cold percolation, the bioactivity has gradually decreased from 1st to 3rd stage showing more retention and gradual extraction of bioactive compounds from the extracts. Among all the processes, autoclave boiling showed highest antioxidant capacity with on the basis of DPPH, $ABTS^+$ and NO scavenging activity. It has also showed higher retention of bioactivity until 3rd stage of extraction.

4.10.2 Phytochemical content

We also carried out the quantitative estimation of phytochemicals such as total phenol, total flavonoid and total orthodihydric phenol content from the aqueous extract of BOFF and illustrated the results in figure 4.10.2.a to figure 4.10.2.c. The total phenol content in extracts ranged from 1.706 to 26.979 (mg GAE /g FWT) and the highest phenol content was found in the extract obtained through pressure boiling at first stage. Similarly the flavonoid and orthodihydric phenol content ranged from 0.0461 to 5.9236 (mg QE /gm FWT) and 0.0632 (mg CE/gm FWT) respectively. In the present experiment it was observed that the potential antioxidant activity of the sample was retained till 2nd boiling stage. From the third stage the antioxidant potentiality was found to be very less particularly in case of soxhletion.

4.10.3 *In vitro* antidiabetic activity

In vitro antidiabetic activity was determined with the inhibiting activity of α-glucosidase enzyme. The result of inhibiting activity the enzyme by BOFF is presented in figure 4.10.3. Since the sample was extracted through four different methods to observe the variation in the bioactivity, it was found that extraction

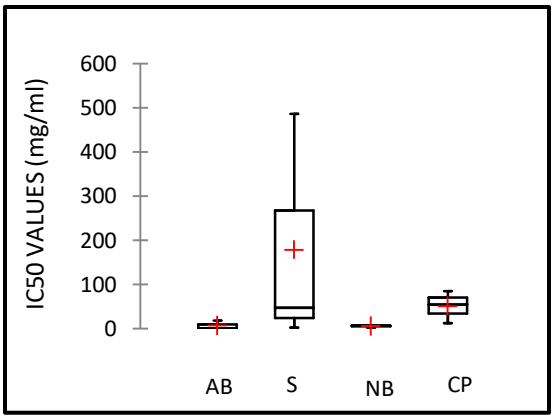


Figure 4.10.1.a: DPPH radical scavenging activity of process variation extracts of BOFF

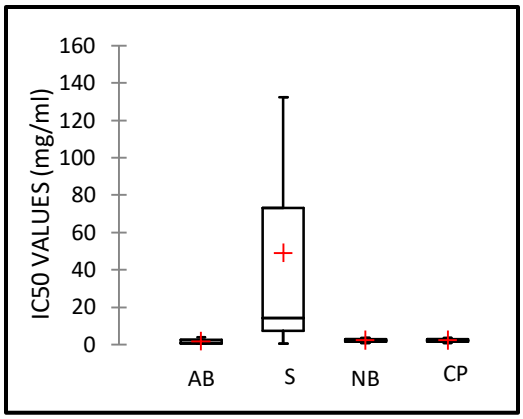


Figure 4.10.1.b: ABTS+ radical scavenging activity process variation extracts of BOFF

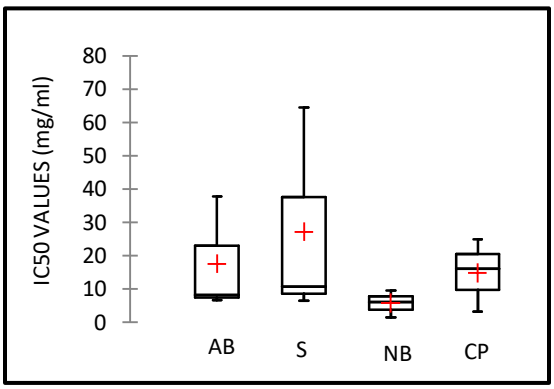


Figure 4.10.1.c: Superoxide scavenging activity process variation extracts of BOFF

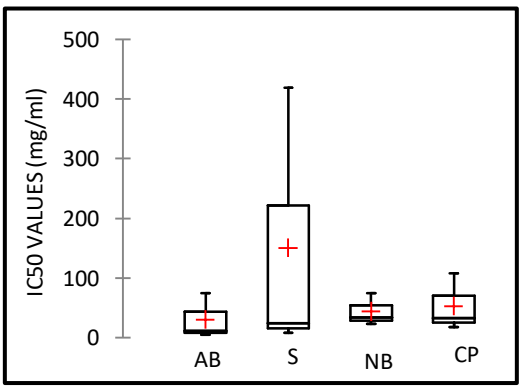


Figure 4.10.1.d: Nitric oxide scavenging activity process variation extracts of BOFF

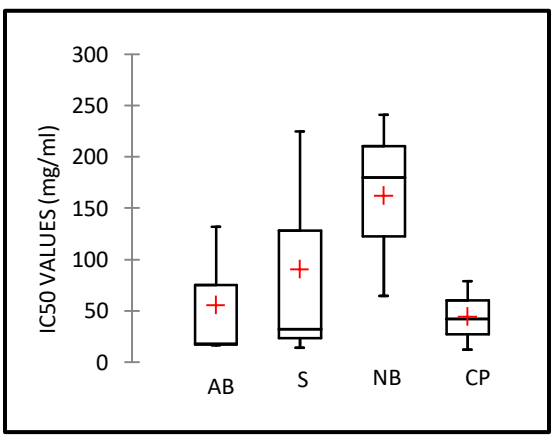


Figure 4.10.1.e: Metal chelating activity process variation extracts of BOFF

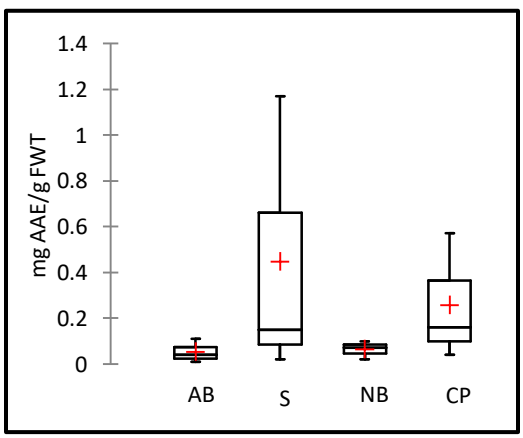


Figure 4.10.1.f: Ferric reducing antioxidant power process variation extracts of BOFF

AB: Autoclave boiling; **S**: Soxhletion; **NB**: Normal boiling; **CP**: Cold percolation; **BOFF**: Bark of *Fraxinus floribunda*

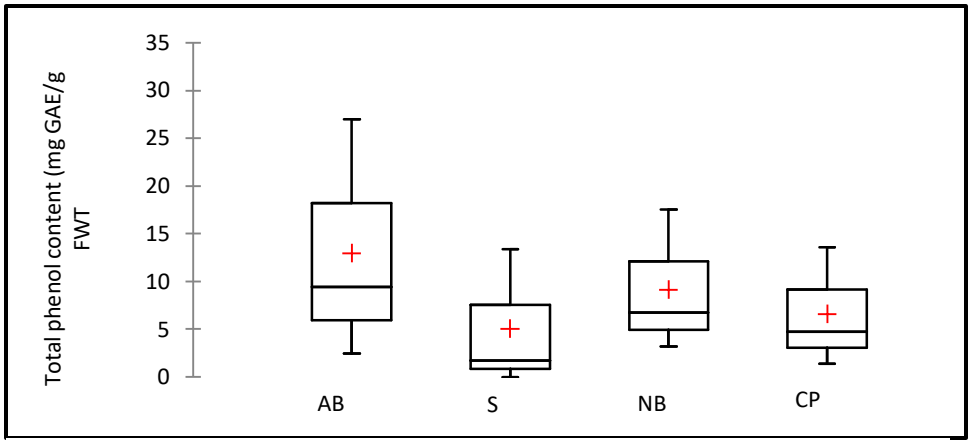


Figure 4.10.2.a: Total phenol content process variation extracts of BOFF

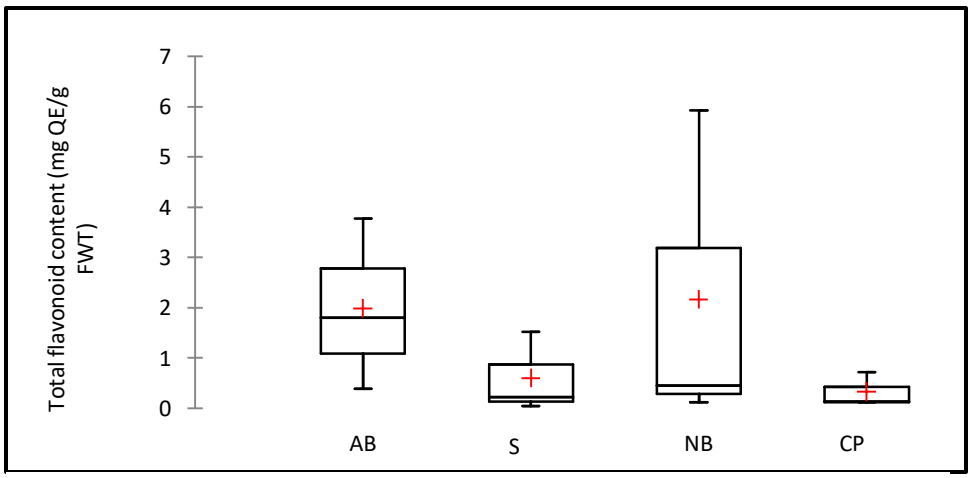


Figure 4.10.2.b: Total flavonoid content process variation extracts of BOFF

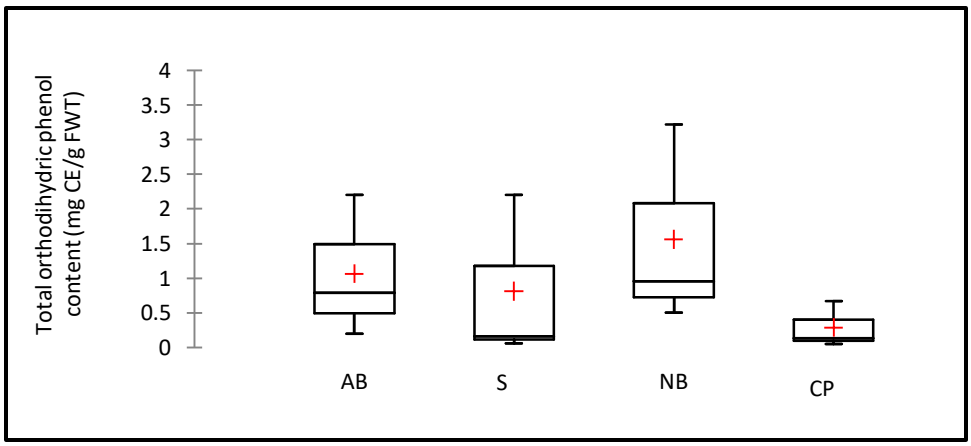


Figure 4.10.2.c: Total orthodihydric phenol content process variation extracts of BOFF

AB: Autoclave boiling; **S**: Soxhletion; **NB**: Normal boiling; **CP**: Cold percolation; **BOFF**: Bark of *Fraxinus floribunda*

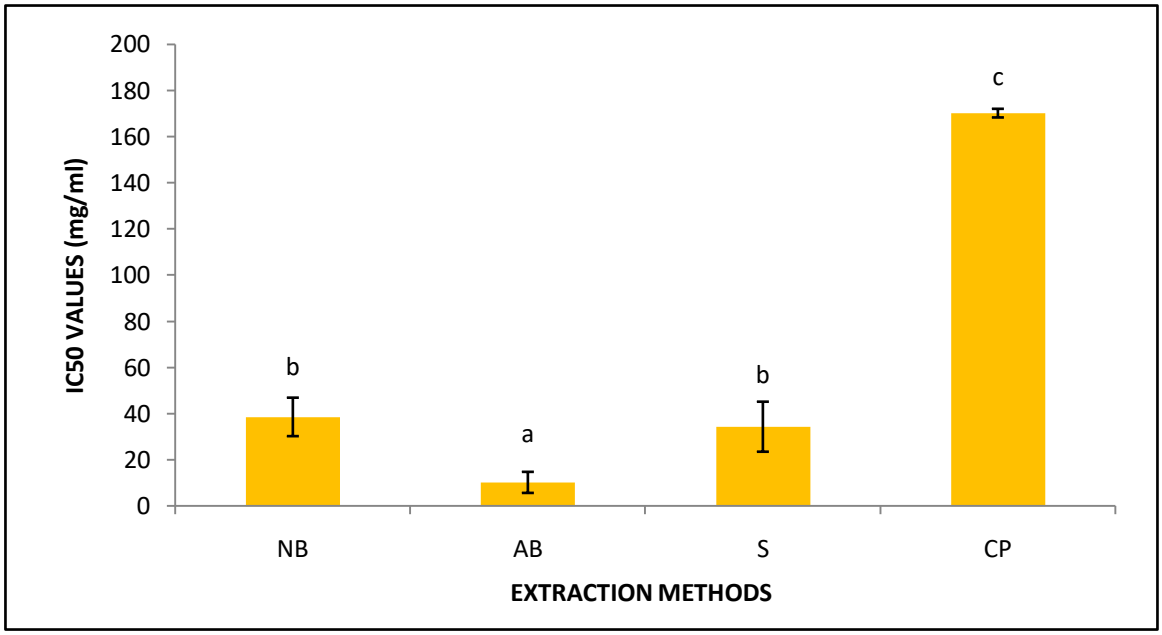


Figure 4.10.3: α-glucosidase inhibiting activity of aqueous extracts of *F. floribunda*. **NB**: normal boiling; **AB**: autoclave boiling; **S**: soxhletion; **CP**: cold percolation. Values with different letters (a, b, c) are significantly ($p < 0.05$) different from each other by DMRT

process has a great influence on the bioactivity of plant extracts. The extract obtained through pressure boiling (PB) has showed the highest activity with lowest IC_{50} values (10.25±4.56 mg/ml FWT). While the cold percolation extract (CP) exhibited the lowest antidiabetic activity.

4.11 PHARMACOLOGICAL ASSAYS

On the basis of *in vitro* studies, BOFF showed best antioxidant and antidiabetic activity in the extract obtained through pressure boiling. Thus the same extract was used to carry out *in vivo* pharmacological activities.

4.11.1 Anti-inflammatory activity

The process of Carrageenan-induced mice paw edema has two phases. During early hyperemia, which occurs 0-2 hours after the carrageenan injection, there is an increase in vascular permeability due to the release of histamine, serotonin and bradykinin. The inflammatory edema reaches its highest level after 2 hr and then will start to decline. In our study, paw edema was increased and reached a maximum at 2 hr after carrageenan injection. Treatment with BOFF extract at the 100 mg/kg b.w. significantly ($p < 0.05$) reduced paw edema formation (0.833 ± 0.01 ml) after 2 hr as shown in Table 4.11.1. Percentage inhibition of paw oedema was 55% by BOFF which was close to the standard formulation aspirin (59%) which is illustrated in figure 4.11.1.a. The experimental procedures are along with animal model is given in figure 4.11.1.b.

4.11.2 Hepatoprotective activity

To evaluate the hepatoprotective activity of BOFF, liver damage and recovery from damage was assessed on 10^{th} day by measuring serum marker enzymes to evaluate the biochemical changes in liver. Hepatotoxicity by CCl_4 occurs due to enzymatic activation to release free radical state. CCl_4 is bio-transformed in liver by Cyp-P_{450} enzyme to form CCl_3 active free radical with oxygen, which then covalently binds with cellular macromolecules and creates loss of cellular integrity and hepatic damage. CCl_4 causes a range of histological changes to the liver including inflammation and cellular swelling. Therefore, antioxidant and anti-inflammatory efficacy are regarded as some important parameters indicating

Table 4.11.1: Effect of aqueous extract of BOFF on anti – inflammatory activity on carrageenan induced rat oedema with time and percentage of inhibition

	Dose	30 mins	60 mins	120 mins	180 mins
NaCl Control Gr		1.59 ± 0.07^{b}	1.84 ± 0.05^{c}	1.9 ± 0.04^{b}	1.86 ± 0.06^{b}
Standard Gr (Aspirin)	100 mg/kg b.w.	1.37 ± 0.02^{a}	1.28 ± 0.06^{a}	1.19 ± 0.05^{a}	0.77 ± 0.04^{a}
% Inhibition		12.9 %	30.6 %	37.3 %	59 %
Fraxinus floribunda	100 mg/kg b.w.	1.5 ± 0.02^{b}	1.43 ± 0.02^{b}	1.25 ± 0.04^{a}	0.833 ± 0.01^{a}
% Inhibition		5.2 %	22.1 %	34.1 %	55 %

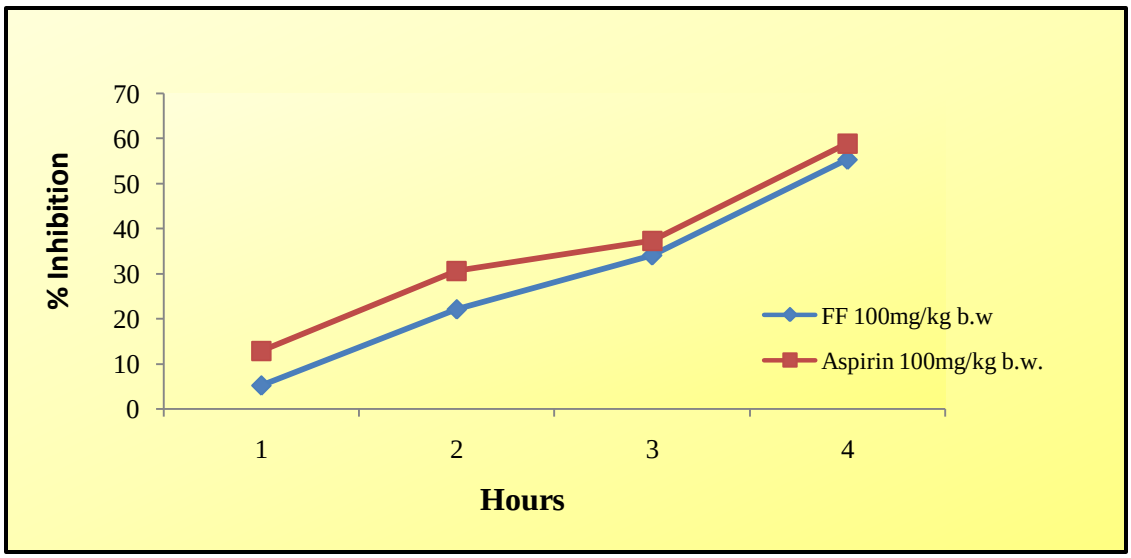


Figure 4.11.1.a: Percentage inhibition of paw oedema by BOFF extract compared with aspirin

Values are expressed as mean ± S.E.M (n=6). *p* values were analyzed using Tukey's test. $p < 0.05$ were considered to be significant when treated groups were compared with the control group. 'a' is more significant than 'b' and 'c'

BOFF: Bark of *Fraxinus floribunda*

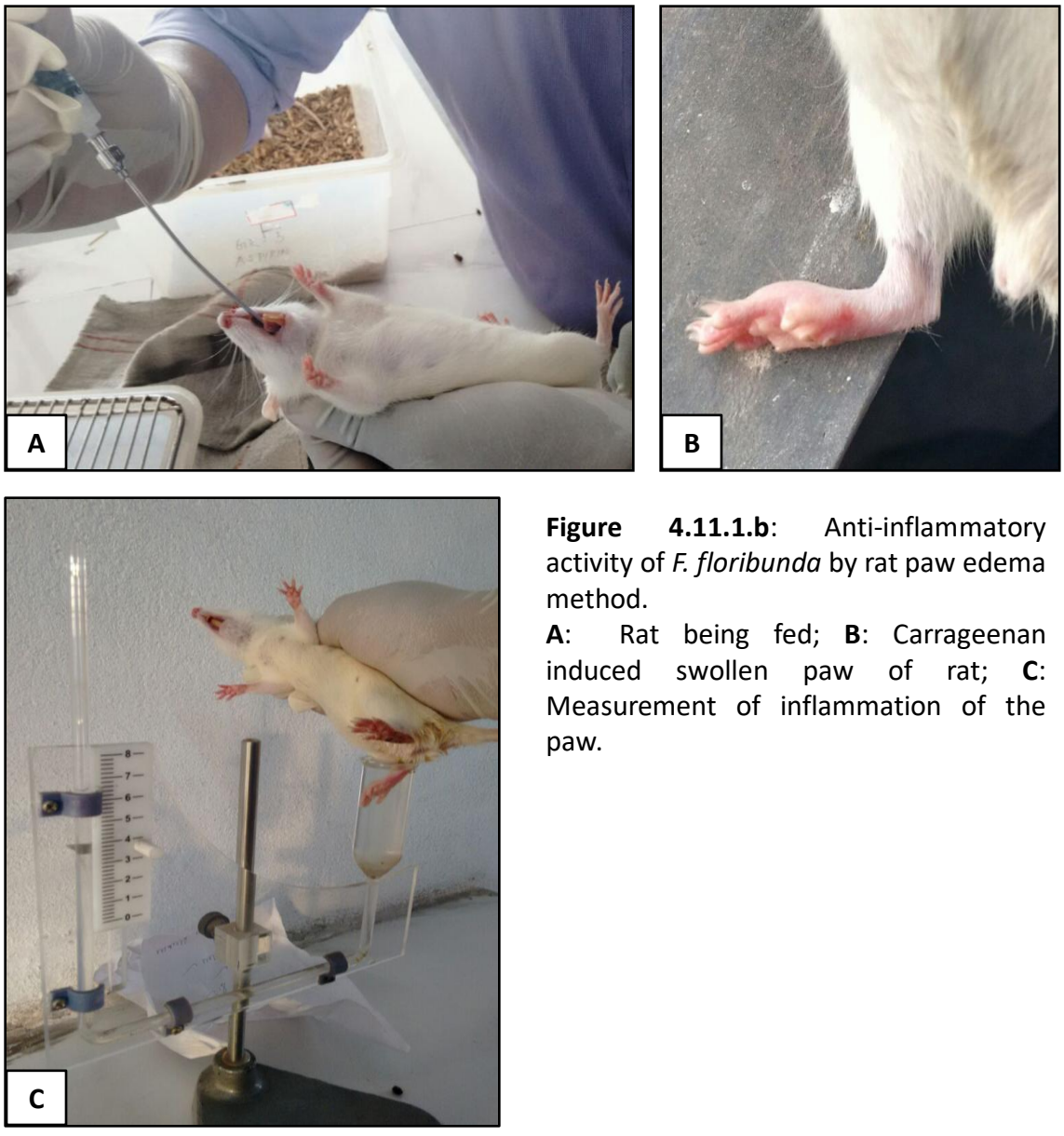


Figure 4.11.1.b: Anti-inflammatory activity of *F. floribunda* by rat paw edema method.
A: Rat being fed; **B**: Carrageenan induced swollen paw of rat; **C**: Measurement of inflammation of the paw.

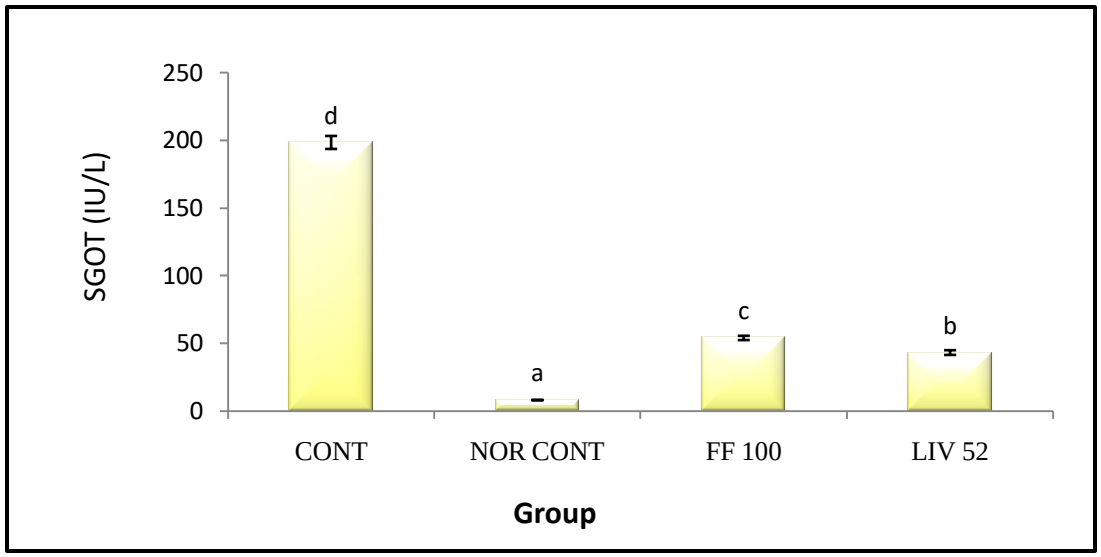


Figure 4.11.2.a: Effect of BOFF extracts on SGOT level in hepatotoxic rats

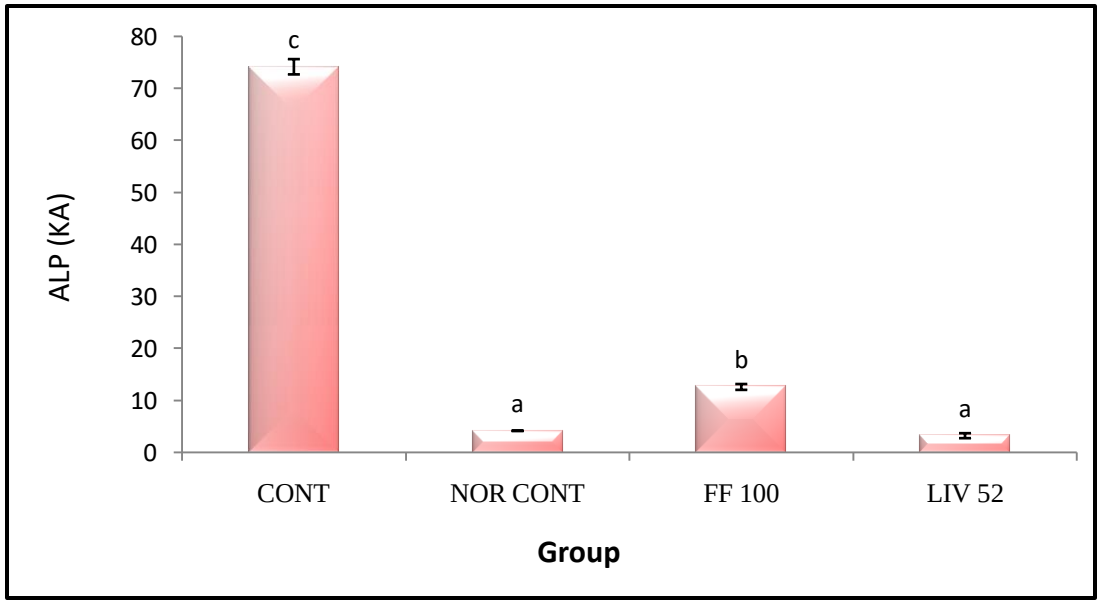


Figure 4.11.2.b: Effect of BOFF extracts on ALP level in hepatotoxic rats

CONT: Control group fed with CCl4; **NOR CONT**: Normal control fed with water only; **FF100**: Rats fed with *F.floribunda* extract 100mg/ml **LIV52**: Stadard; **BOFF**: Bark of *Fraxinus floribunda*; **SGOT**: Serum Glutamic Oxaloacetic Transaminase; **ALP**: Alkaline phosphatase. Values are expressed as mean ± S.E.M. *p* values were analyzed using Tukey's test. $p < 0.05$ were considered to be significant when treated groups were compared with the control group.

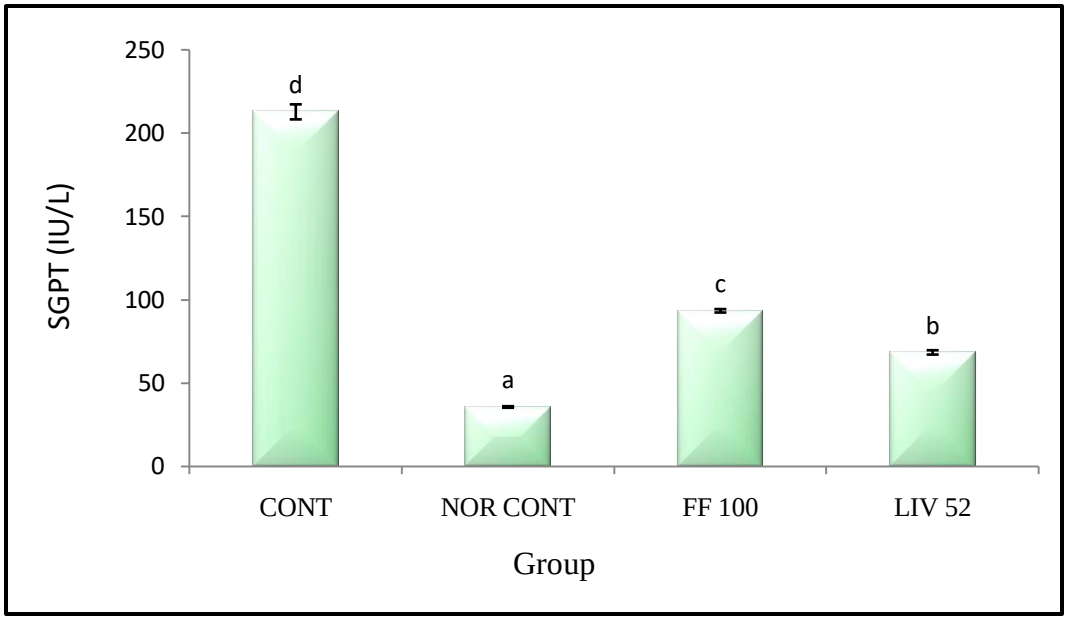


Figure 4.11.2.c: Effect of BOFF extracts on SGPT level in hepatotoxic rats

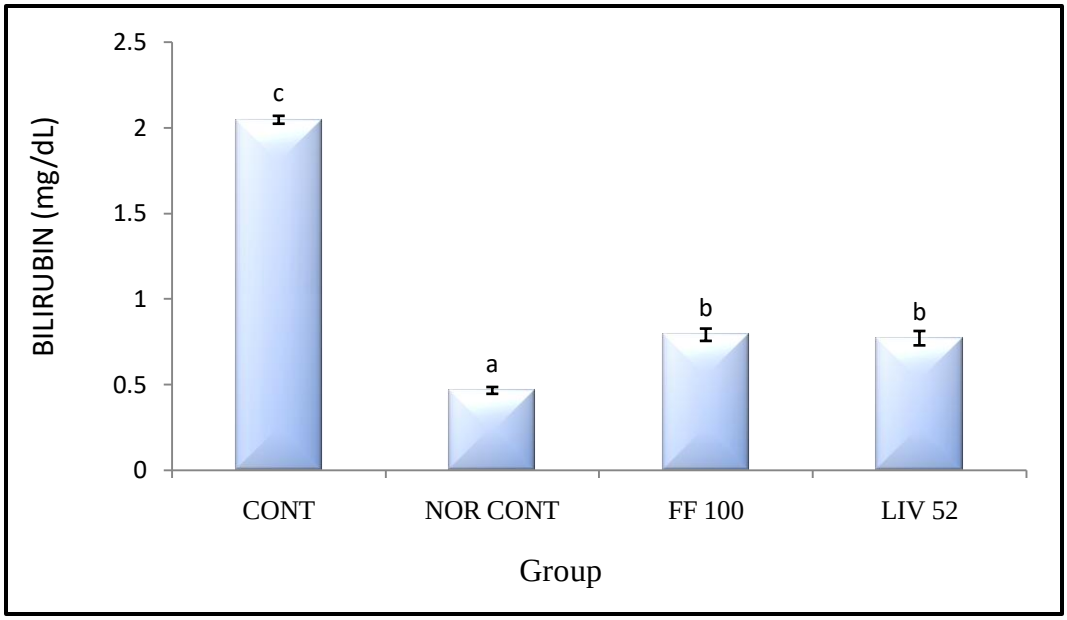


Figure 4.11.2.d: Effect of BOFF extracts on Bilirubin level in hepatotoxic rats

CONT: Control group fed with CCl4; **NOR CONT**: Normal control fed with water only; **FF100**: Rats fed with *F.floribunda* extract 100mg/ml **LIV52**: Stadard; **BOFF**: Bark of *Fraxinus floribunda*; **SGPT: Serum Glutamic Pyruvic Transaminase**. Values are expressed as mean ± S.E.M. *p* values were analyzed using Tukey's test. $p < 0.05$ were considered to be significant when treated groups were compared with the control group.

possible mechanism of hepatoprotection. The increased level of SGOT, ALP, SGPT, and bilirubin is conventional indicator of liver injury (Grigonisa *et al*., 2005; Michieles *et al*., 2012; Lee *et al*., 2007). In this study, it was found that administration of CCl_4 elevated the levels of serum marker enzymes SGOT, ALP, SGPT and serum bilirubin in CONT Gr as represented in figure 4.11.2.a to figure 4.11.1.d respectively. The experimental results of pretreatment with BOFF through autoclave boiling and Liv-52 treated groups showed to lower the levels of SGPT, SGOT, ALP and bilirubin as compared to CCl_4-treated group indication the inhibition of CCL_4 induced elevation of SGOT, SGPT, ALP and bilirubin levels in hepatic rats.

4.11.3 *In vivo* antidiabetic activity

4.11.3.a Acute toxicity study

The acute toxicity study of aqueous extract of FF bark was evaluated as per the CPCSEA guideline no 420 (fixed dose method). The aqueous extracts were orally fed to the rats at the dose level of 5, 50, 300, and 1000 mg/kg, respectively. The test showed no mortality even at maximum dose of 1000 mg/kg body weight (b.w.). Hence, 200 mg/kg and 400 mg/kg doses were selected for further study.

4.11.3.b Oral glucose tolerance test (OGTT)

Results obtained from OGTT is given in table 4.11.3.a where it can be observed that there is significant increase of blood glucose concentration ($p<0.05$) in diabetic control group compared to the normal control at the end of 12^{th} day experiment duration. The elevated glucose level was significantly lowered ($p<0.05$) by the aqueous extracts of bark of FF while comparing with the diabetic control. The significant reduction in plasma glucose level in glucose loaded rats by 200 and 400 mg/kg extract of FF bark was observed and it came down to normal level after 90 min.

4.11.3.c Effect on fasting blood glucose

Administration of streptozotocin induced hyperglycemia in rats with significant ($p<0.05$) elevation of blood glucose as compared to normal control to the level greater than 250 mg/dl (table 4.11.3.b). However it was significantly ($p<0.05$)

lowered by the oral administration of glibenclamide (0.05 mg/kg) and aqueous extract of FF bark (200 and 400 mg/kg). As expected, 400 mg/kg of extract showed more significant antidiabetic property. The glucose level in streptozotocin induced diabetic rats lowered to normal after day 14 in case of glibenclamide and 400 mg/kg extract while the 200 mg/kg extract could lower the glucose level to normal after day 28.

4.11.3.d Body weight

Effect of standard drug and bark extract of FF on the body weight of diabetes induced rats is shown in table 4.11.3.c. In normal control rats, it was observed that bodyweight continuously increased. Diabetic control rats showed significant decrease ($p<0.05$) in bodyweight compared to normal control. The diabetic rats administered with glibenclamide and extract of FF bark (200 and 400 mg/kg) showed significant increase ($p<0.05$) in bodyweight when compared to diabetic control.

4.11.3.e Effect of extracts on lipid profile

Lipid profile of the streptozotocin induced diabetic rats is presented in table 4.11.3.d. It can be observed that TGL, TCL and LDL level were significantly increased ($p<0.05$) in diabetic rats as while HDL level was significantly decreased as compared to normal control. The bark extracts of FF (200 and 400 mg/kg) significantly decreased the serum TGL, TCL and LDL and increased the HDL when compared with the diabetic control. As expected, standard glibenclamide administered rats significantly prevented the increase of TGL, TCL and LDL and decrease of HDL compared to diabetic control. The extracts were able to restore the lipid profile of diabetic rats to almost normal level.

4.11.3.f Histopathology

The effect of bark extracts of FF on the histological architecture of liver is given in figure 4.11.3.e. Liver sections of normal group showed normal hepatic structure in the figure. Normal hepatic cells were observed distinctively forming a network around central veins with peripheral portal areas in the surrounding. However liver sections of diabetes induced rats showed hepatocellular injury with

Table 4.11.3.a: Effect of different extracts on oral glucose tolerance test

Group	Plasma glucose concentration (mg/dl)		
	0 min	30 min	90 min
Normal control	73.15±3.24	76.42±2.73	74.83±4.51
Glucose control	76.61±6.17	221.28±5.38#	155.19±3.64#
Glucose + Glibenclamide (0.5 mg/kg)	78.34±4.42	90.71±3.25*	75.52±5.18*
Extract (200 mg/kg)	75.58±3.71	128.21±4.86*	96.17±4.43*
Extract (400 mg/kg)	74.83±5.36	108.47±3.18*	78.63±3.29*

Values are expressed as mean ± SEM (Number of animals, n=6); significantly different at #P<0.05 when compared with normal control group, *P<0.05 when compared with diabetic control group

Table 4.11.3.b: Effect of different extracts on fasting plasma glucose level in rats

Group	Fasting plasma glucose concentration (mg/dl)			
	Day 0	Day 7th	Day 14th	Day 28th
Normal Control	78.62±2.15	75.39±5.12	80.17±3.41	74.69±5.28
Diabetic control (Streptozotocin)	149.29±3.62#	208.34±2.57#	249.48±5.62#	287.11±2.71#
Diabetic + Standard Glibenclamide (0.50 mg/kg)	134.43±2.83	112.65±4.32*	90.29±2.39*	75.38±4.68*
Diabetic + Extract (200 mg/kg)	129.51±4.34	132.74±3.51*	113.68±4.73*	92.76±2.15*
Diabetic + Extract (400 mg/kg)	132.24±3.05	118.43±4.26*	97.36±2.86*	78.42±3.52*

Values are expressed as mean ± SEM (Number of animals, n=6); significantly different at #P<0.05 when compared with normal control group, *P<0.05 when compared with diabetic control group

Table 4.11.3.c: Effect of extracts on changes in body weight in rats

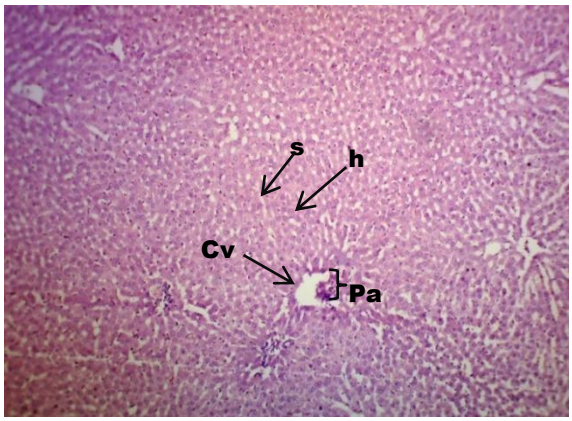
Group	Change in Body weight (gm)		
	Before Induction	After Induction	After Treatment
Normal control	182.32 ±2.14	171.49±3.28	177.83±2.63
Diabetic control (Streptozotocin)	185.21±1.98	139.67±2.68#	112.38±3.86#
Diabetic + Standard Glibenclamide (0.50 mg/kg)	168.19±3.05	132.72±3.14	175.31±4.56*
Diabetic + Extract (200 mg/kg)	176.15±3.53	142.59±2.43	151.84±2.49*
Diabetic + Extract (400 mg/kg)	173.64±2.79	146.12±1.54	169.76±2.18*

Values are expressed as mean ± SEM (Number of animals, n=6); significantly different at #P<0.05 when compared with normal control group, *P<0.05 when compared with diabetic control group

Table 4.11.3.d: Determination of biochemical parameters after treatment with different extracts

Group	Lipid Profile (mg/dl)			
	TGL	TCL	HDL	LDL
Normal control	79.24±3.28	76.65±4.36	72.84±2.68	56.28±4.53
Diabetic control (Streptozotocin)	186.37±3.69#	198.41±2.72#	31.56±4.25#	165.69±5.74#
Diabetic + Standard Glibenclamide (0.50 mg/kg)	78.53±4.17*	72.84±4.69*	76.29±6.43*	63.82±2.28*
Diabetic + Extract (200 mg/kg)	96.52±2.69*	101.62±2.81*	51.35±3.79*	85.26±4.15*
Diabetic + Extract (400 mg/kg)	83.41±2.47*	79.95±5.24*	70.16±4.41*	58.64±3.26*

Values are expressed as mean ± SEM (Number of animals, n=6); significantly different at #P<0.05 when compared with normal control group, *P<0.05 when compared with diabetic control group



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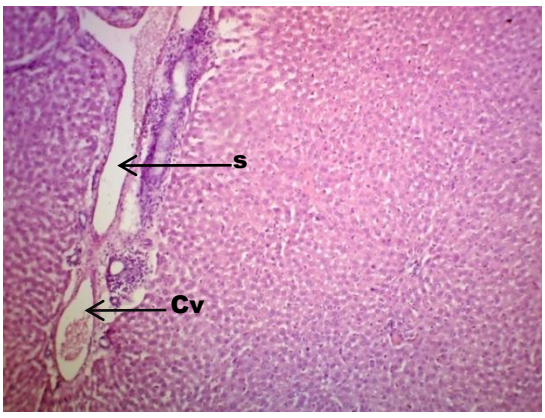

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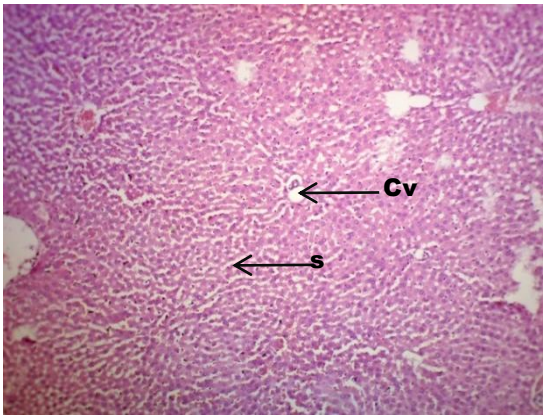
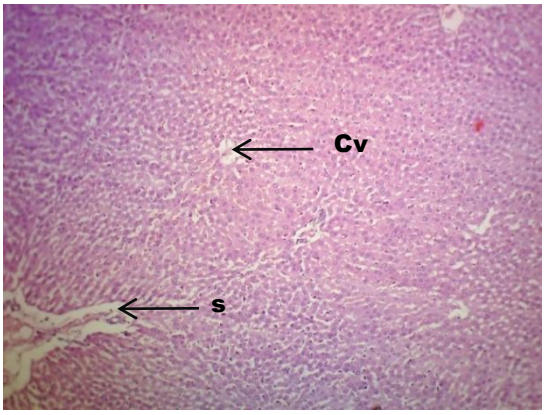
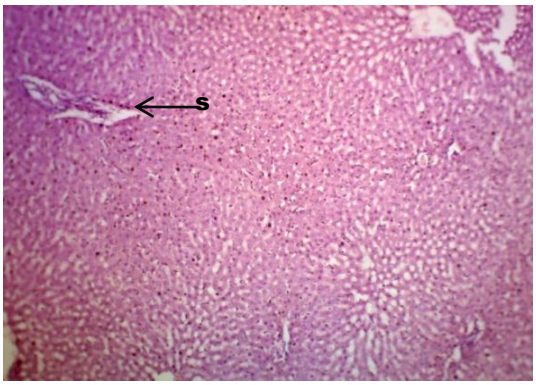


C



D



E

Figure 4.11.3.e: Liver histopathology of Streptozotocin induced rats and effects of bark of *Fraxinus floribunda* (FF) on it. **A**: Normal control, **B**: Diabetic control, **C**: Diabetic + glibenclamide, **D**: Diabetic + 200 mg/kg extract, E: 400 mg/kg extract. s:sinusoids; h: hepatocytes; Cv: central vein; Pa: portal area.

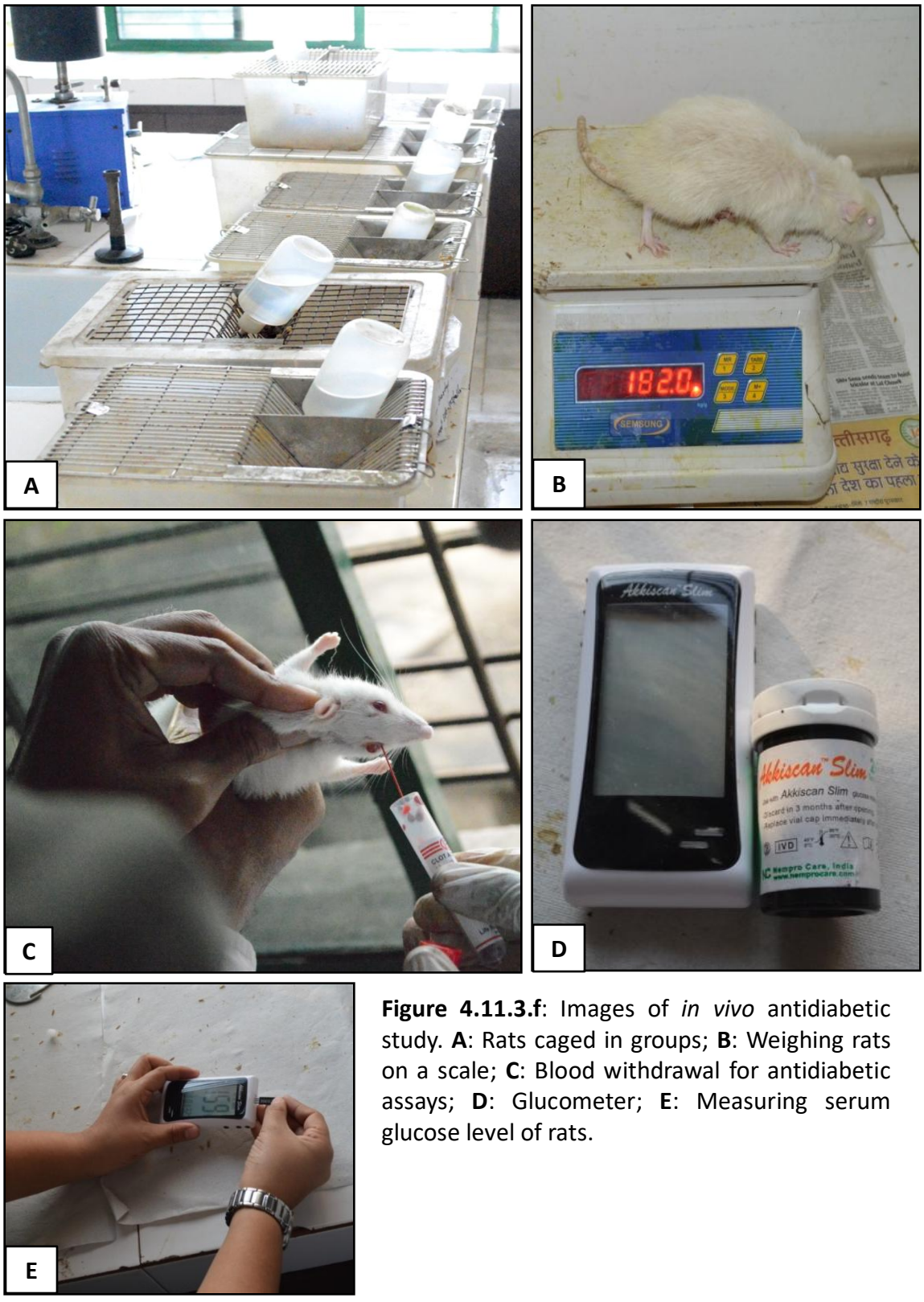


Figure 4.11.3.f: Images of *in vivo* antidiabetic study. **A**: Rats caged in groups; **B**: Weighing rats on a scale; **C**: Blood withdrawal for antidiabetic assays; **D**: Glucometer; **E**: Measuring serum glucose level of rats.

the loss of normal architecture of the liver as compared to normal group (Diagram A). Inflammation and vacuolization of cytoplasm was observed. There was dilation in central vein along with dilation and congestion of blood sinusoids. There was also an enlargement of the space between the hepatocytes and sinusoidal dilation. This hepatic injury was observed to be almost recovered to normal by the extracts (200 and 400 mg/kg) of FF bark (Diagram D and E). The sinusoids were restored with the reduction of enlargement, inflammation of central veins were also reduced. Administration of glibenclamide on diabetic rats was able to repair the hepatic injury to almost like normal control (Diagram C). The various experimental methods performed for antidiabetic activity are given in the images presented in figure 4.11.3.f.

4.12 BIOASSAY GUIDED PARTIAL PURIFICATION OF BARK OF *FRAXINUS FLORIBUNDA*

4.12.1 Column and Thin layer chromatography

Medicinal plants have played a vital role in primary healthcare as well as have contributed in the development of several novel drugs. *Fraxinus floribunda* is a tree which is usually found in the Eastern Himalayas and has been used as a medicinal plant in some parts of Sikkim. This is very popularly used in traditional system of medicine for various ailments but the literature review has revealed no any extensive work on it. There are some evidences of the leaves of *F. floribunda* possessing free radical scavenging activity (Lingadurai *et al*., 2009), anti-nociceptive as well as anti-inflammatory activity (Lingadurai *et al*., 2007). From the study we have performed, BOFF showed a significant free radical scavenging activity, phytochemical content, antidiabetic activity as well as *in vivo* anti-inflammatory, antidiabetic, hepatoprotective activities. *F. floribunda* is a tall tree, thus the bark can be easily taken out in large quantity and at the same time it was found to have high medicinal properties which validate its selection for bio-assay guided purification to phytochemicals. Phytoconstituents which were present in BOFF were extracted through pressure boiling method in distilled water. The aqueous extract was then separated successively in six solvents in increasing polarity (hexane < diethyl ether < ethyl acetate < chloroform < butanol < water). All six extracts obtained after successive separation were screened for the phytochemical content (TPC and TFC)

and antioxidant activity (DPPH and $ABTS^+$ scavenging assays). The entire steps of purification are summarized in a flowchart given in figure 4.12.1.a.

From the results of preliminary screening of the various solvent extracts, butanol extract showed the highest DPPH and $ABTS^+$ scavenging activity (figure 4.12.1.b) as well as total phenol (figure 4.12.1.c) and flavonoid content (4.12.1.d) amongst all other solvent extracts. On the basis of these activities, butanol extract of BOFF was run through silica gel column chromatography. A total of 596 fractions (10 ml each) were collected and subjected for screening with DPPH and total flavonoid content assays (figure 4.12.1.e). The fractions which exhibited high antioxidant activity were subjected to TLC and the TLC profiles of these fractions are presented in figure 4.12.1.f). On the basis of TLC profiles the fractions with merged into 6 sub-fractions (F-1, F-2, F-3, F-4, F-5, F-6). These fractions were further subjected to *in vitro* antidiabetic assay on the basis of α-glucosidase inhibiting activity (figure 4.12.1.g). Among all the fractions, F-1 showed highest antidiabetic activity and thus this fraction was selected for GC-MS analysis to find out a elucidated data of phytochemicals present in the extract which could help in explaining the structure of the compounds which might be responsible for the bioactivity of the extract.

4.12.2 GC-MS analysis

GC-MS technique was performed on the F-1 and the chromatogram is shown in figure 4.12.2.a. A total of ten compounds were identified through NIST library matching and the chromatogram of each of the ten compounds is presented in figure 4.12.2.b to figure 4.12.2.k. From table 4.12.2, it is clear that 2(1H)-Quinolinone, hydrazone was the most abundant compound in the extract with the amount 25.7%. It is a heterocyclic aromatic organic compound which was found to have many pharmacological activities such as anti cancer, anti-arteriosclerosis, antimicrobial, anti-malarial and antidiabetic. Flavone was present in 2.2 % which has antibacterial, anti-inflammatory and antidiabetic activity. Coumarin, 6-amino-3-phenyl was present in 1.9% with anti-inflammatory, antibacterial, antineoplastic. Some fatty acids (Heptadecanoic acid, 10-Octadecenoic acid, methyl ester) were also present in 5% which has anticancer and hypoglycemic activity. Acacetin was also present in the extract which is a flavones having antimicrobial as well as anti –tumour effect.

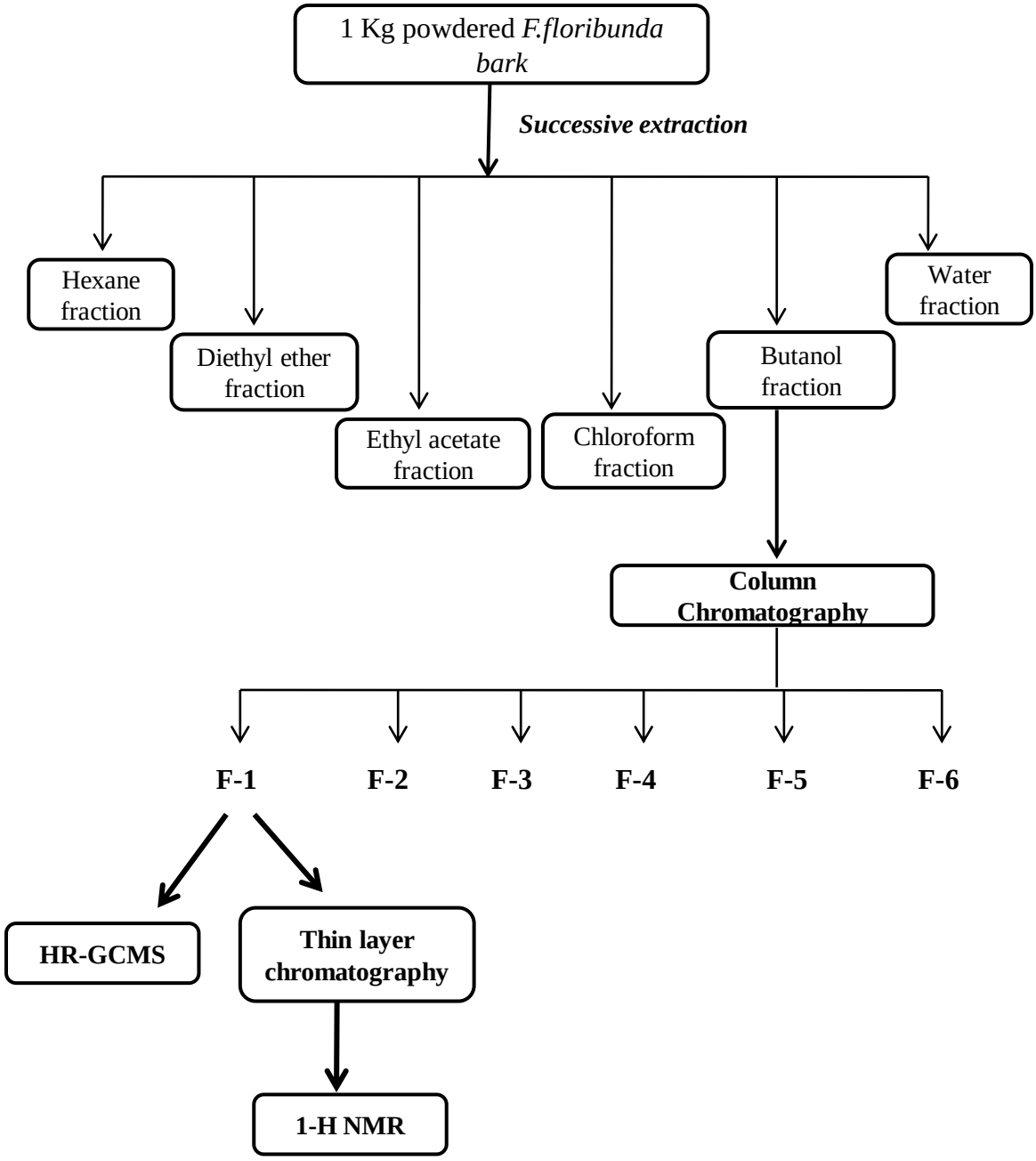


Figure 4.12.1.a: Schemes of fraction for bioassay guided purification of *Fraxinus floribunda bark*. F= fractions obtained from column chromatography.

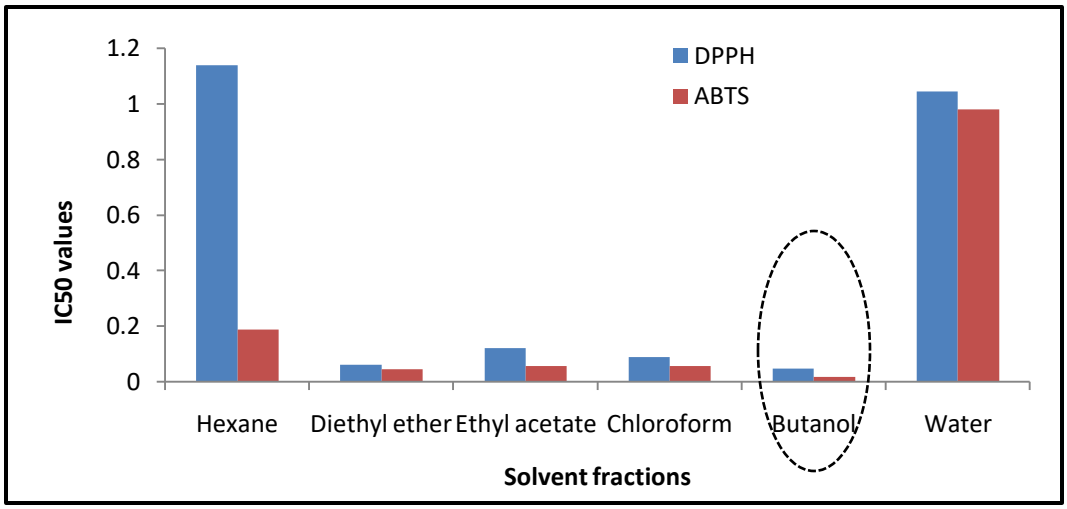


Figure 4.12.1.b: DPPH and ABTS radical scavenging activities of fractions obtained from successive extraction

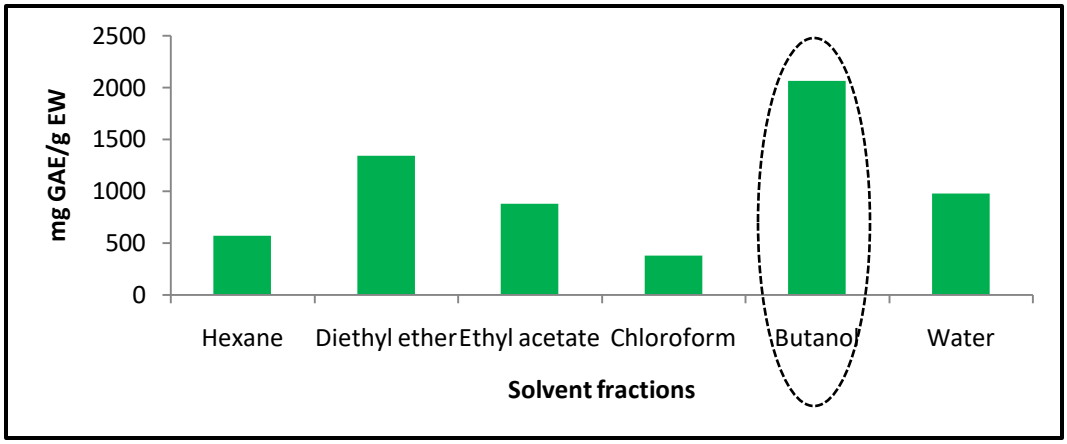


Figure 4.12.1.c: Total phenol content of fractions obtained from successive extraction

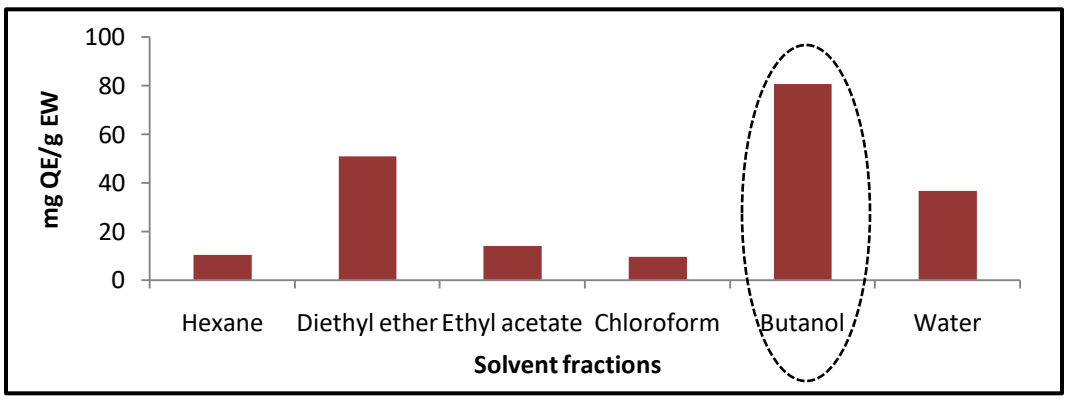


Figure 4.12.1.d: Total flavonoid content of fractions obtained from successive extraction

NB: Bar within the ellipse indicates highest DPPH˙ and ABTS⁺ activity scavenging activity and total phenol as well as total flavonoid content

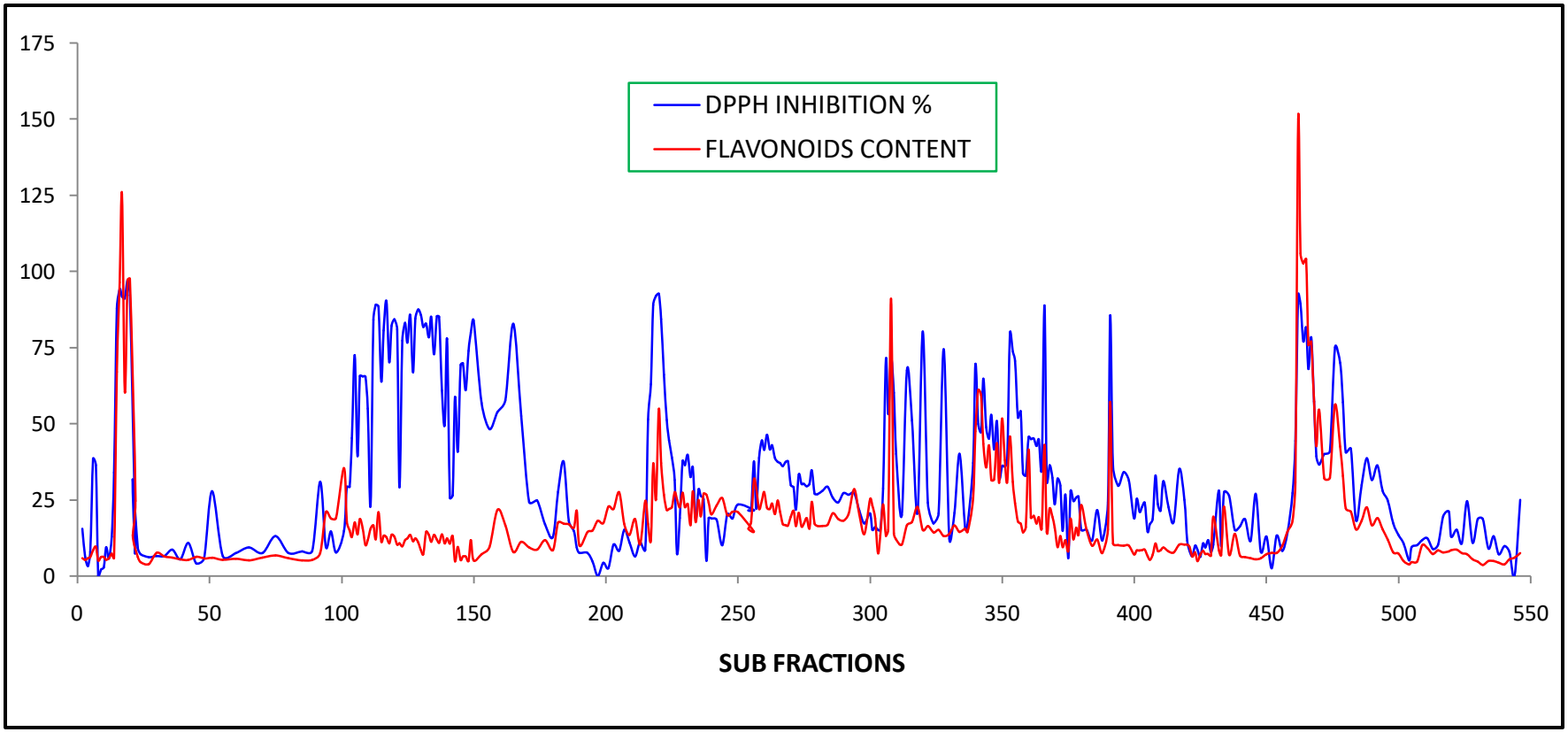


Figure 4.12.e: DPPH$^{\cdot}$ scavenging activity and total flavonoid content of fractions of *F. floribunda* obtained from column chromatography

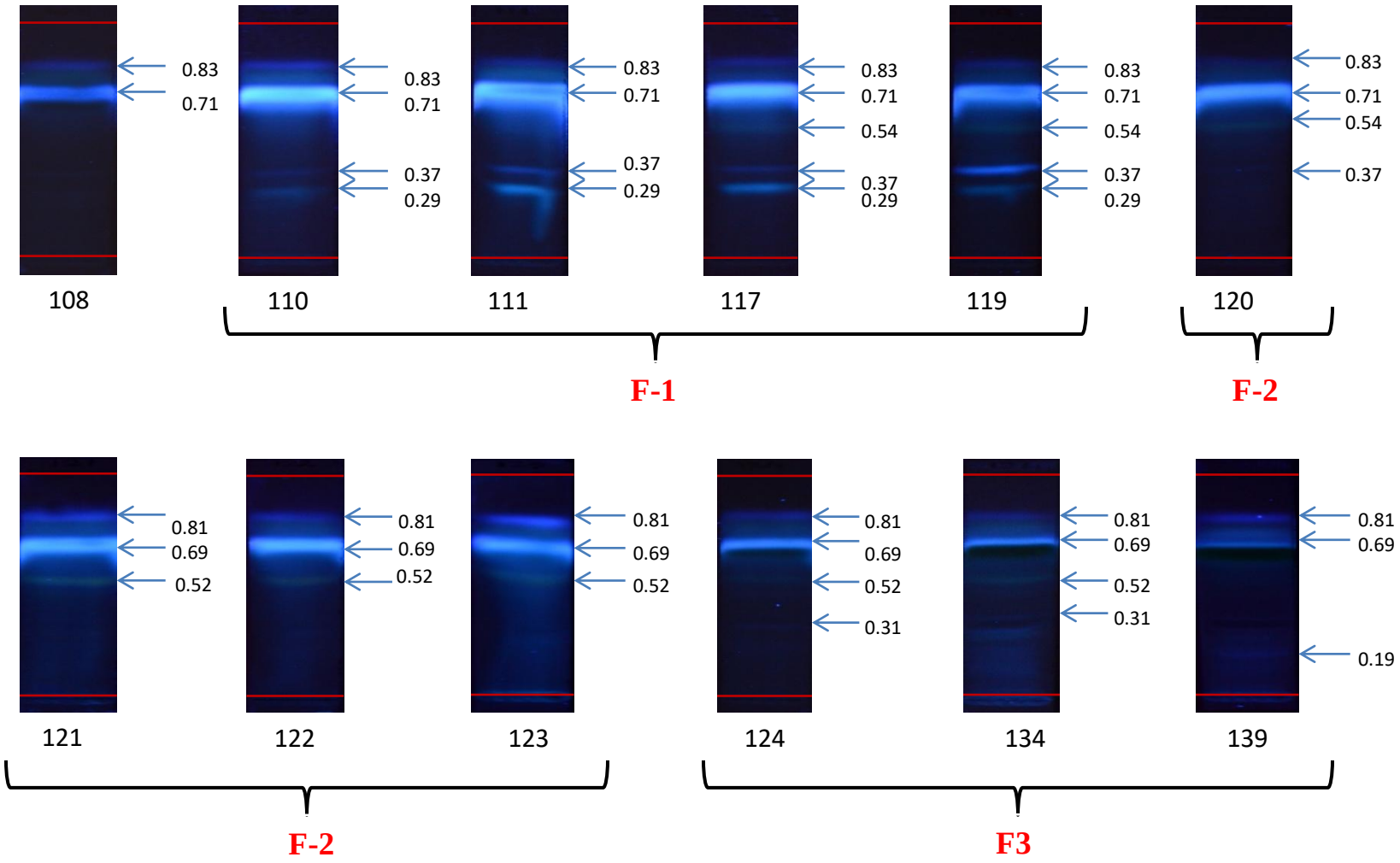


Figure 4.12.1.f: TLC profiling of bioactive sub-fractions of *F. floribunda* bark obtained from column chromatograph (contd.)

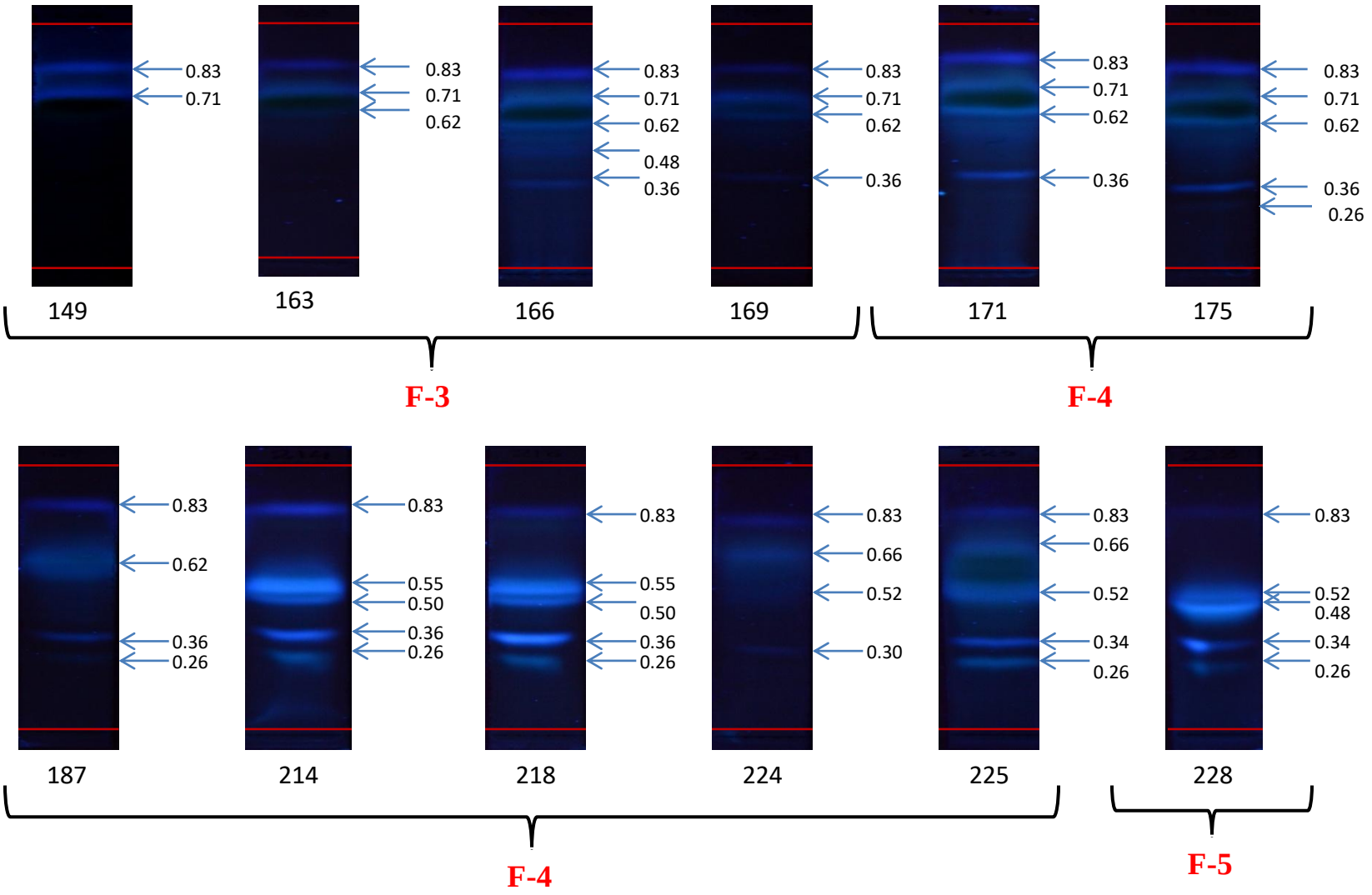


Figure 4.12.1.f: TLC profiling of bioactive sub-fractions of *F. floribunda bark* obtained from column chromatograph (contd.)

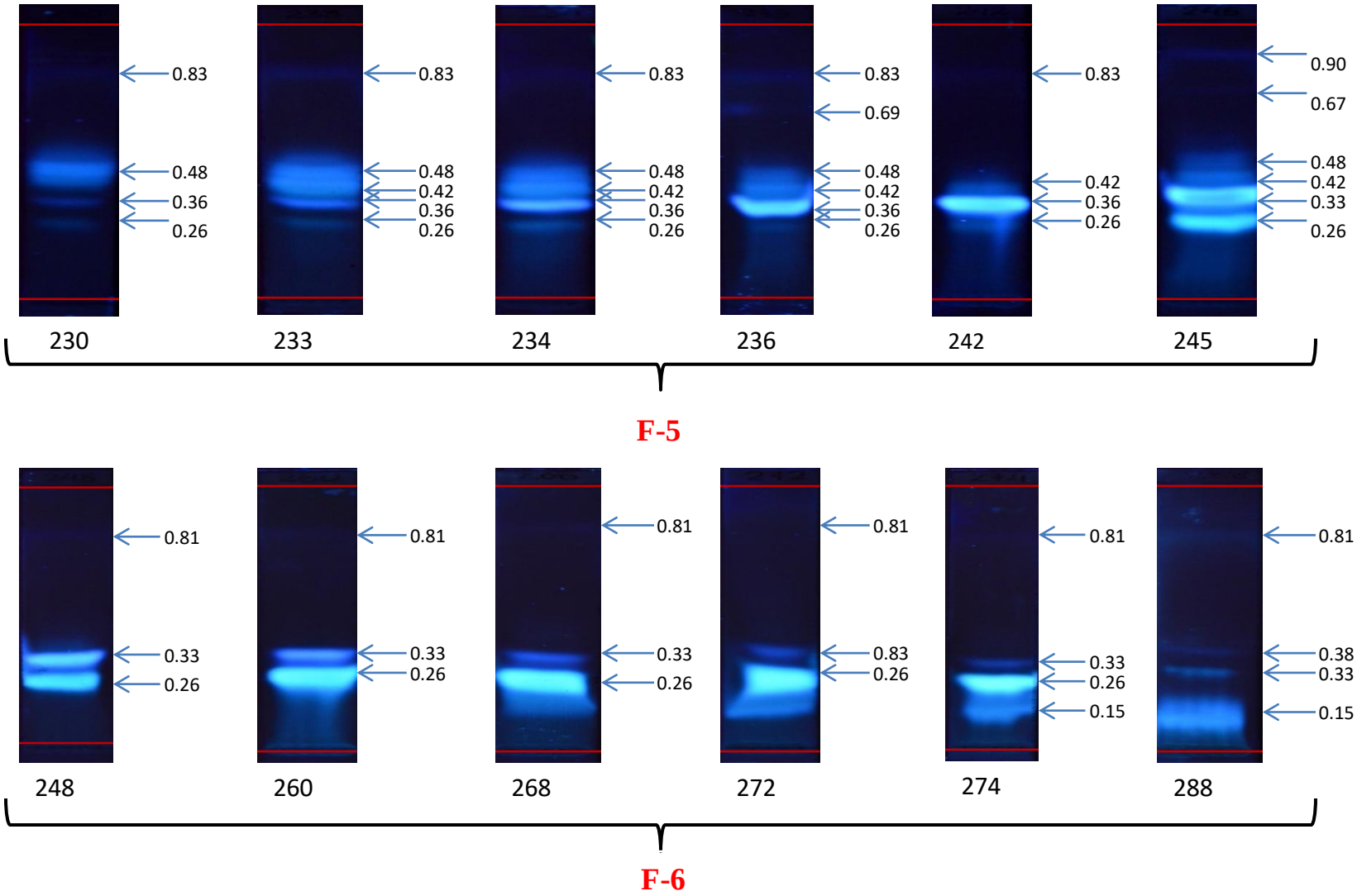


Figure 4.12.1.f: TLC profiling of bioactive sub-fractions of *F. floribunda bark* obtained from first column chromatograph (contd.)

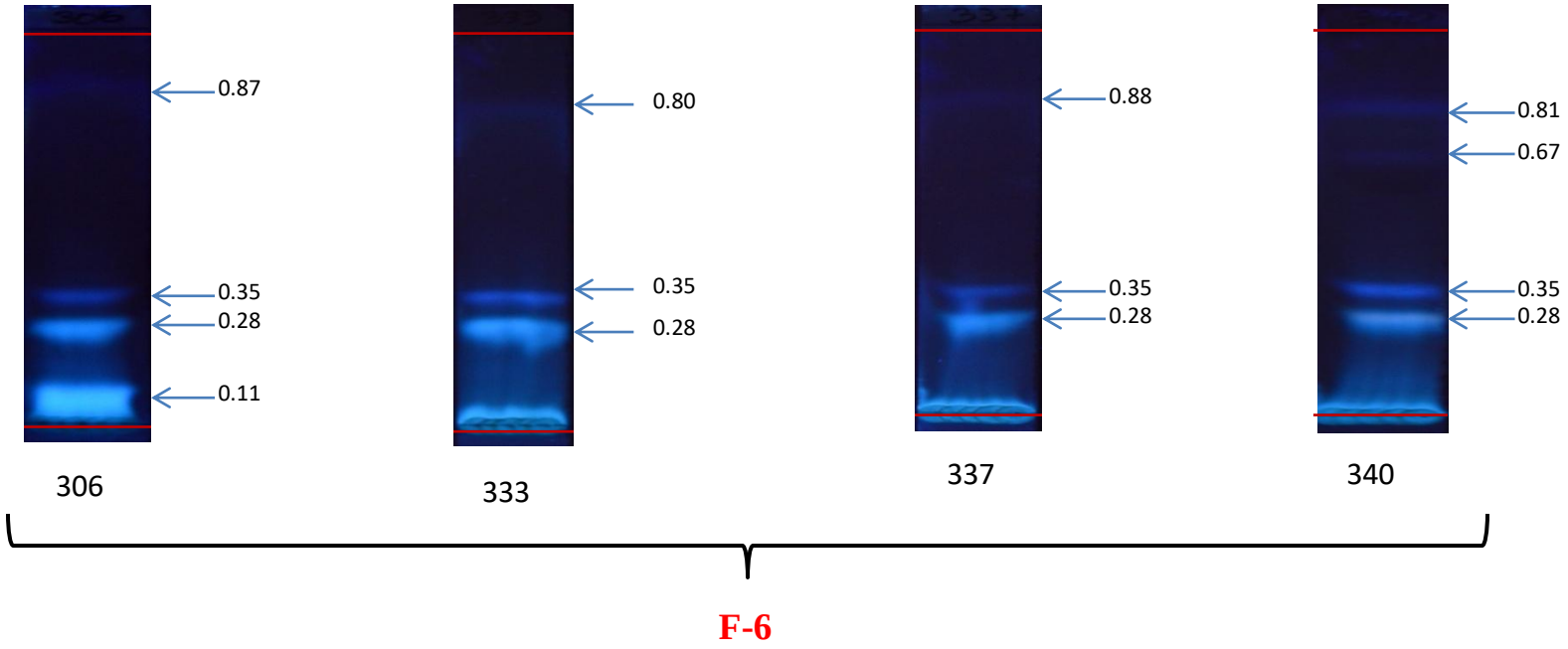


Figure 4.12.1.f: TLC profiling of bioactive sub-fractions of *F. floribunda bark* obtained from first column chromatograph .

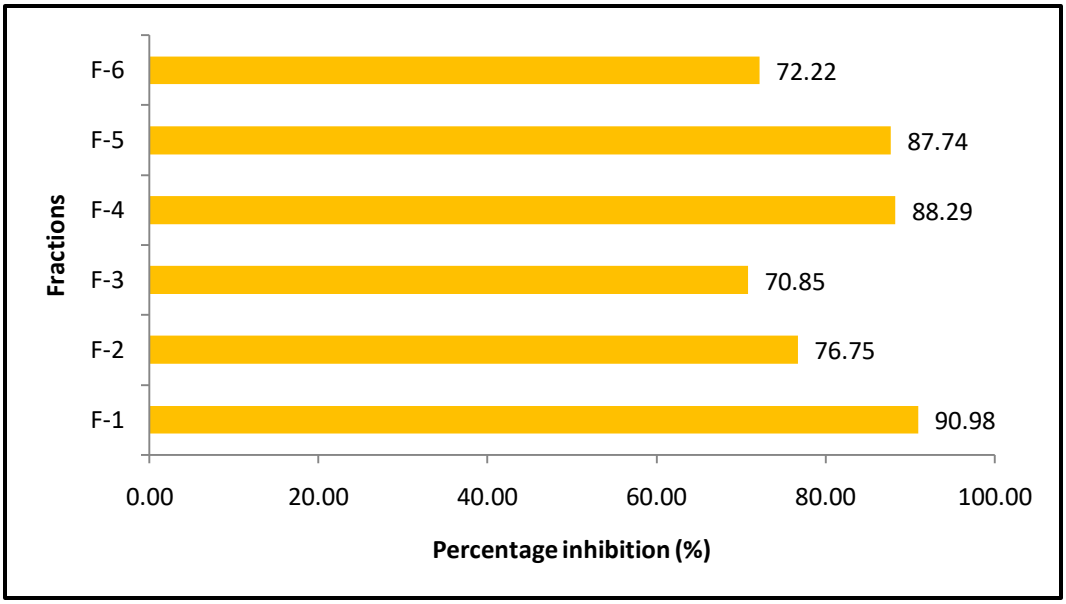


Figure 4.12.1.g: Alpha glucosidase inhibiting activity of merged fractions from TLC profiling

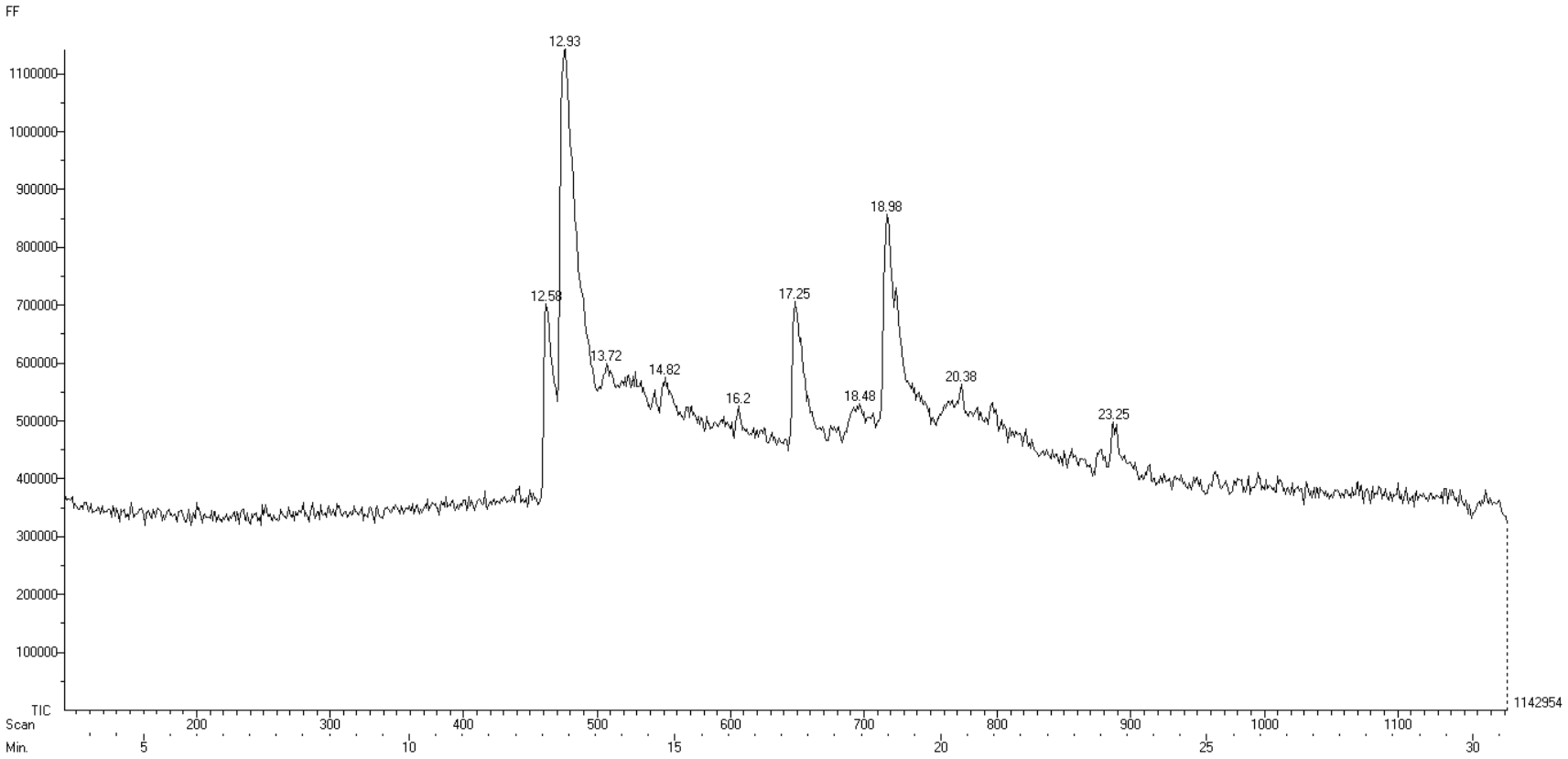


Figure 4.12.2.a: HR- GCMS scanning chromatogram of fraction FF-1 of *F. floribunda*

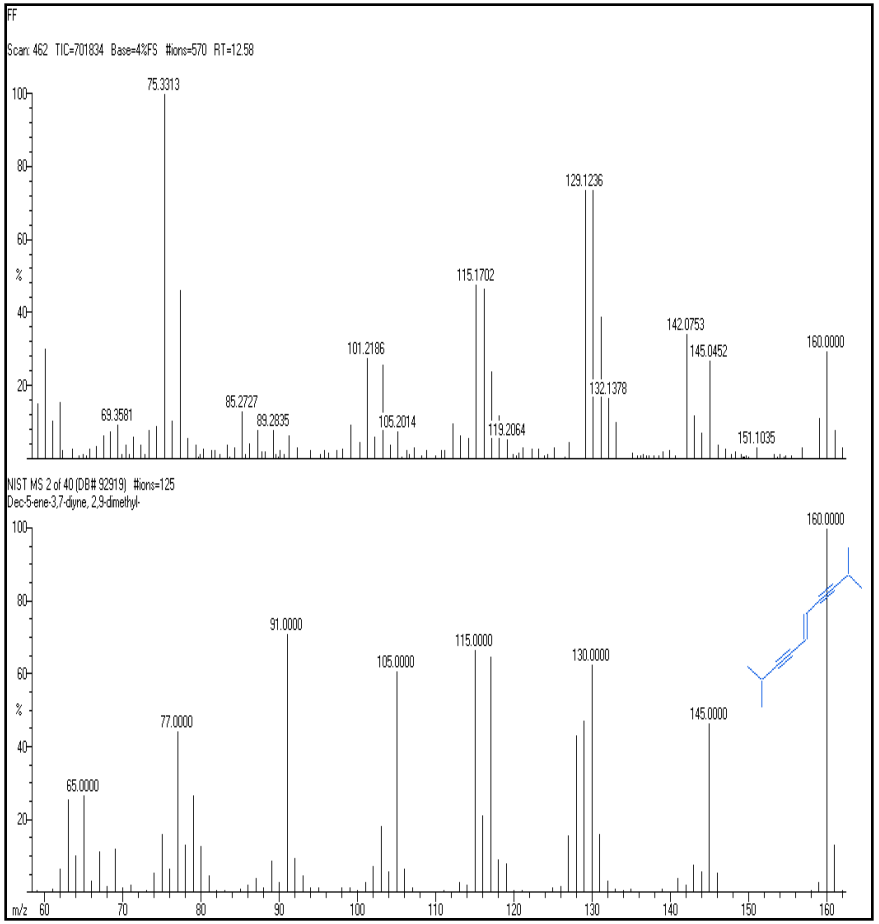


Figure 4.12.2.b: Structure and MS spectrum of Dec-5-ene-3,7-diyne-2,9-dimethyl compared with NIST Library Spectral Database

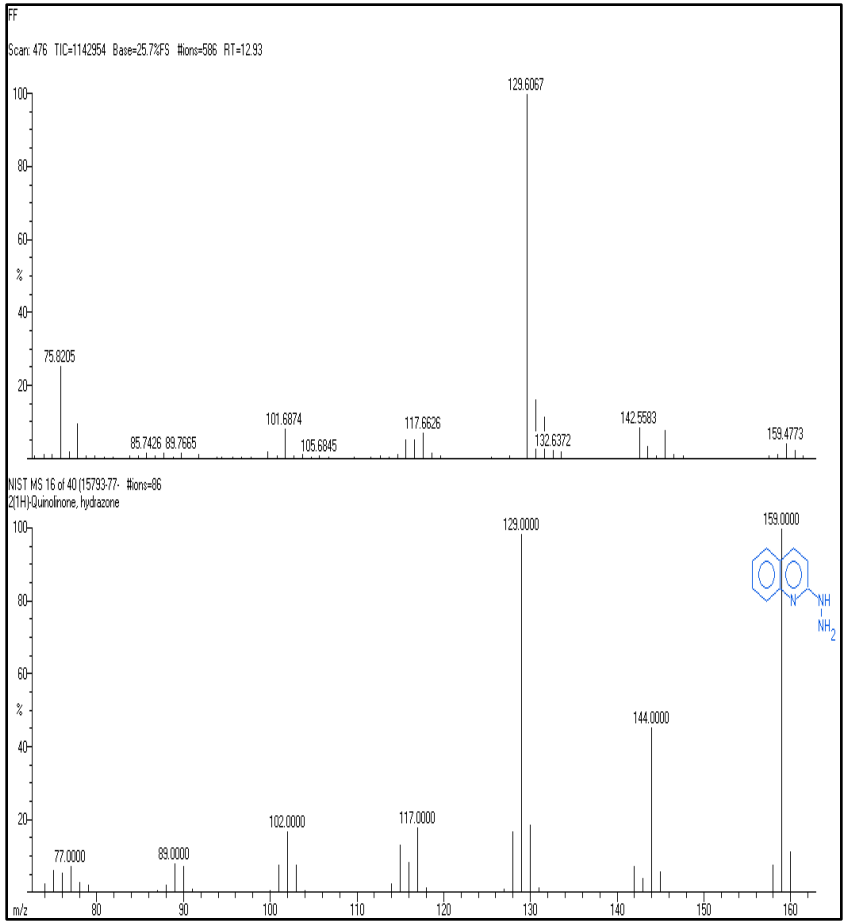


Figure 4.12.2.c: Structure and MS spectrum of 2(1H)-Quinolinone, hydrazone compared with NIST Library Spectral Database

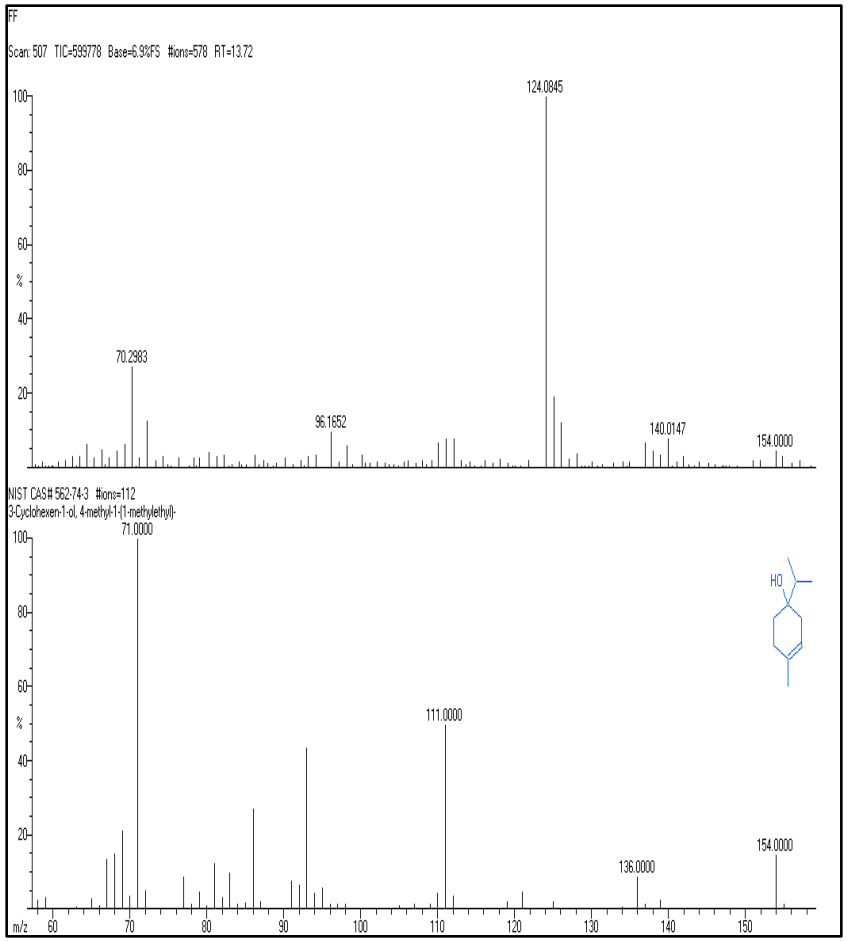


Figure 4.12.2.d: Structure and MS spectrum of 3-cyclohexen-1-ol,4-methyl-1-(1-methylethyl) compared with NIST Library Spectral Database

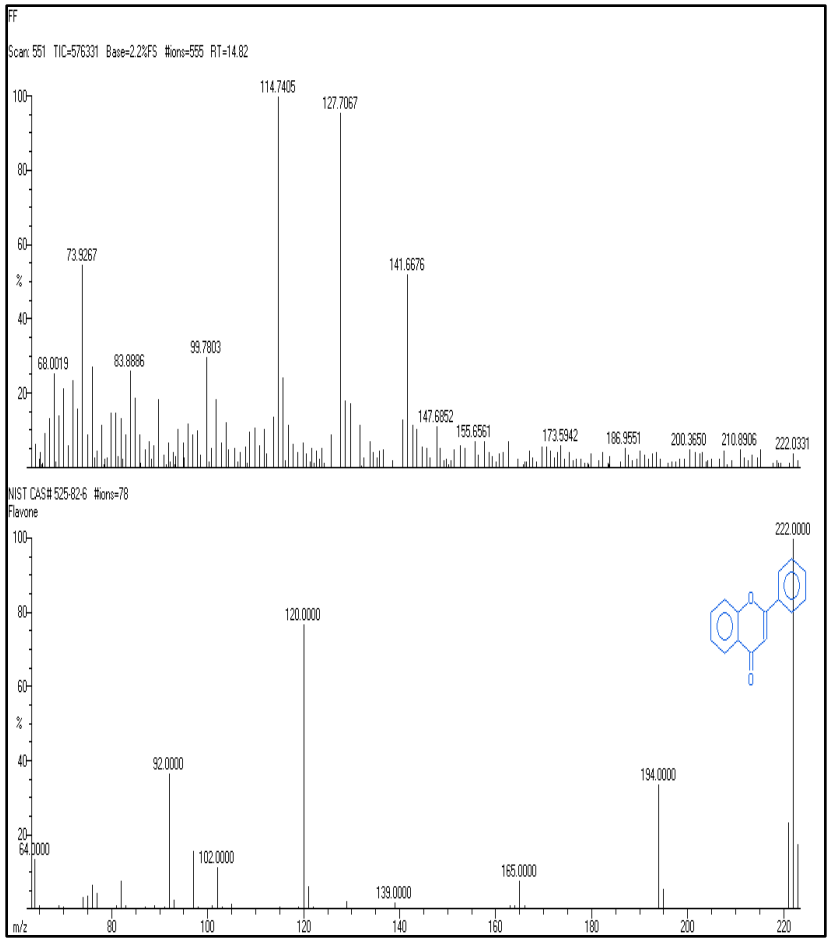


Figure 4.12.2.e: Structure and MS spectrum of Flavone compared with NIST Library Spectral Database

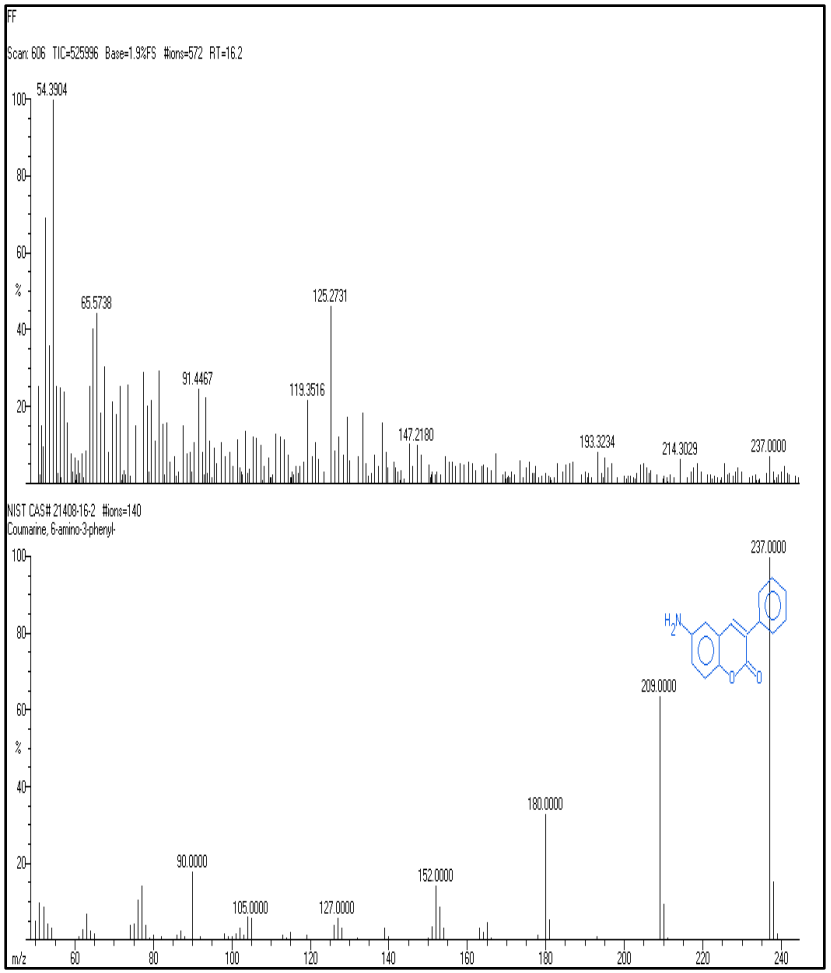


Figure 4.12.2.f: Structure and MS spectrum of Coumarin,6-amino-3-phenyl compared with NIST Library Spectral Database

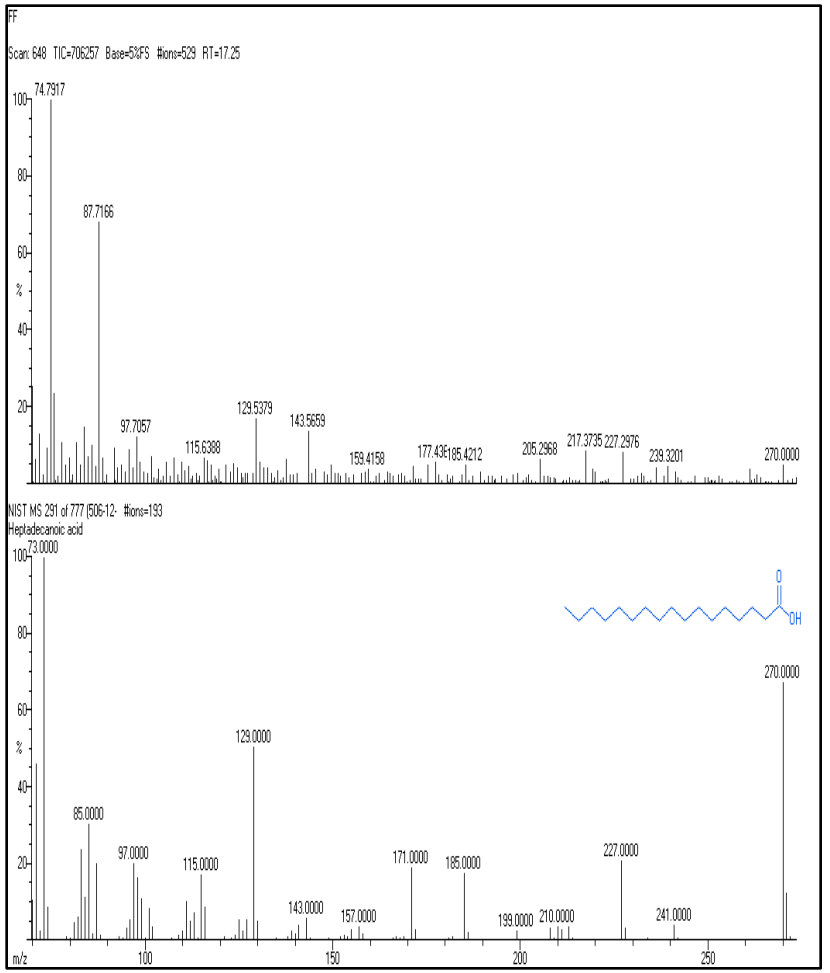


Figure 4.12.2.g: Structure and MS spectrum of Heptadecanoic acid compared with NIST Library Spectral Database

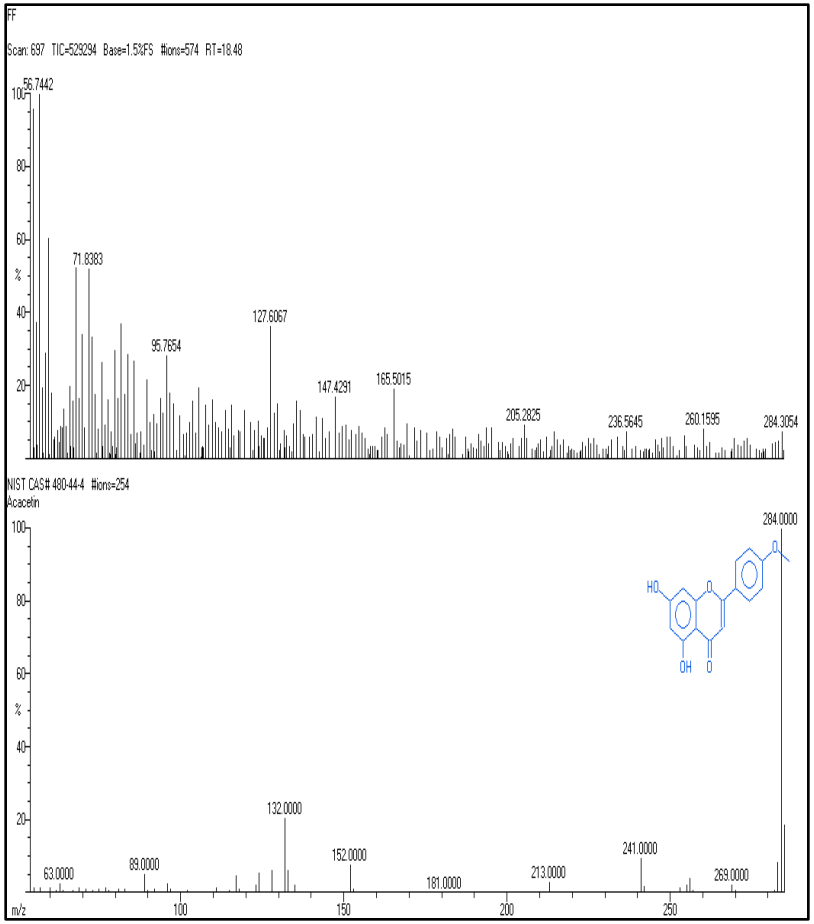


Figure 4.12.2.h: Structure and MS spectrum of Acacetin compared with NIST Library Spectral Database

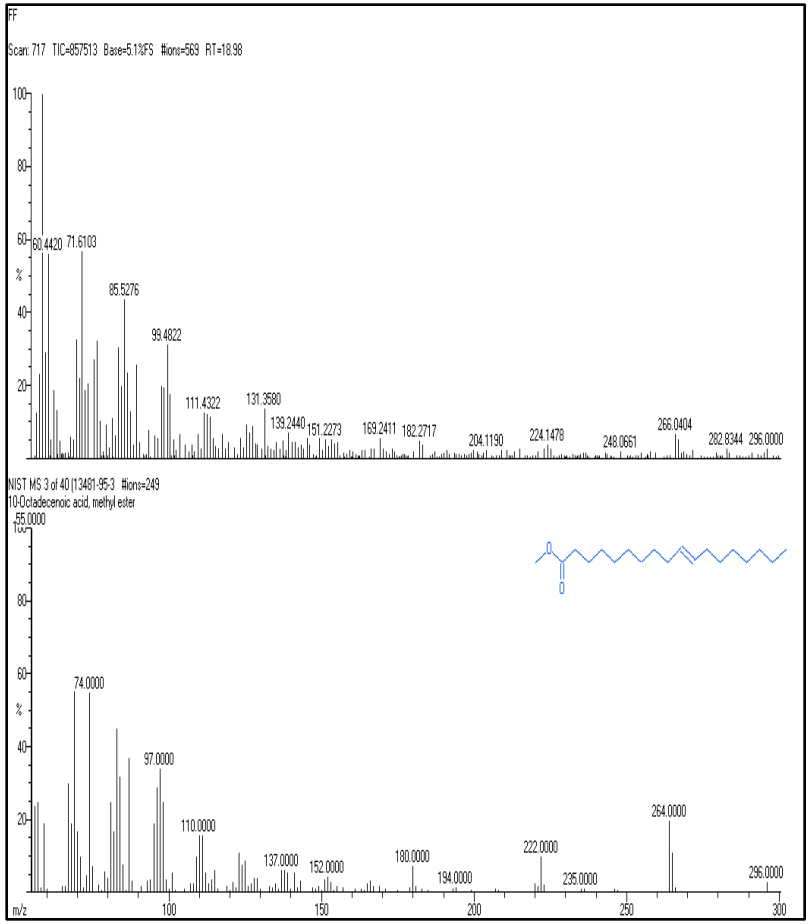


Figure 5.12.2.i: Structure and MS spectrum of 10-Octadecenoic acid, methyl ester compared with NIST Library Spectral Database

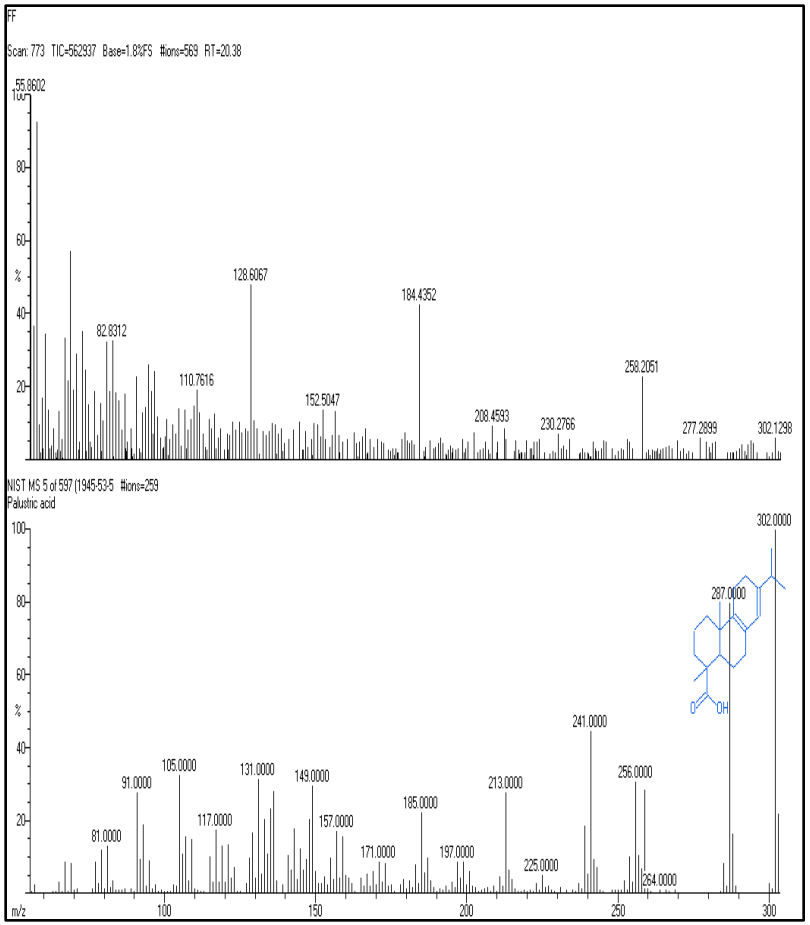


Figure 4.12.2.j: Structure and MS spectrum of Palustric acid compared with NIST Library Spectral Database

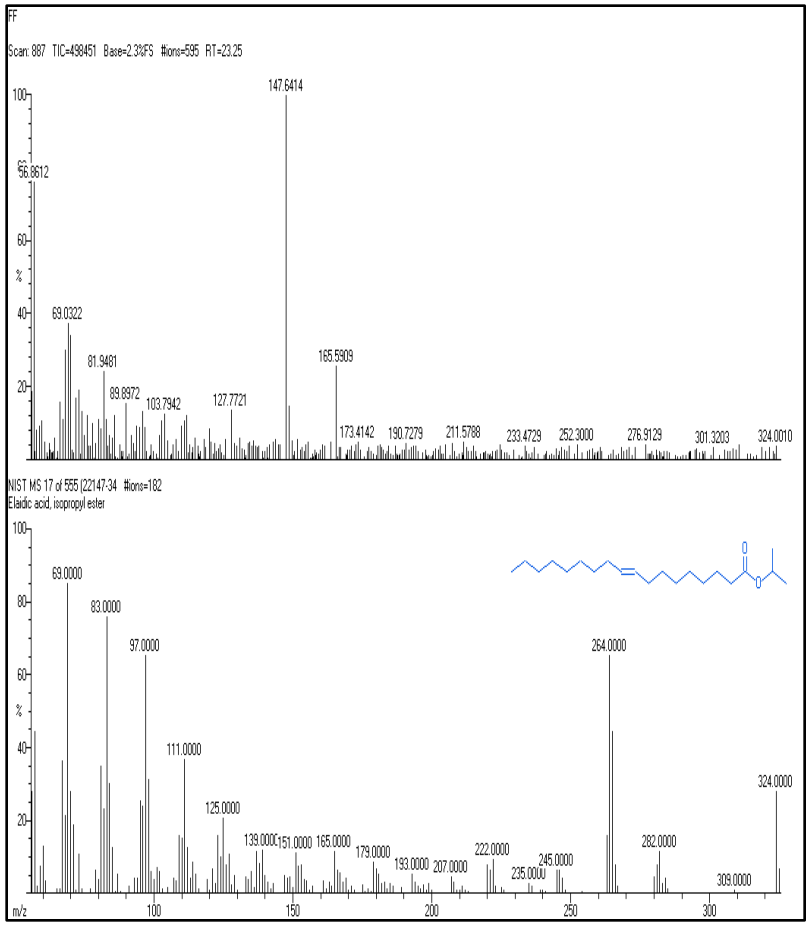


Figure 4.12.2.k: Structure and MS spectrum of Elaidic acid isopropyl ester compared with NIST Library Spectral Database

Table 4.12.2: List of phytochemicals present in Fraction-1 and their activities

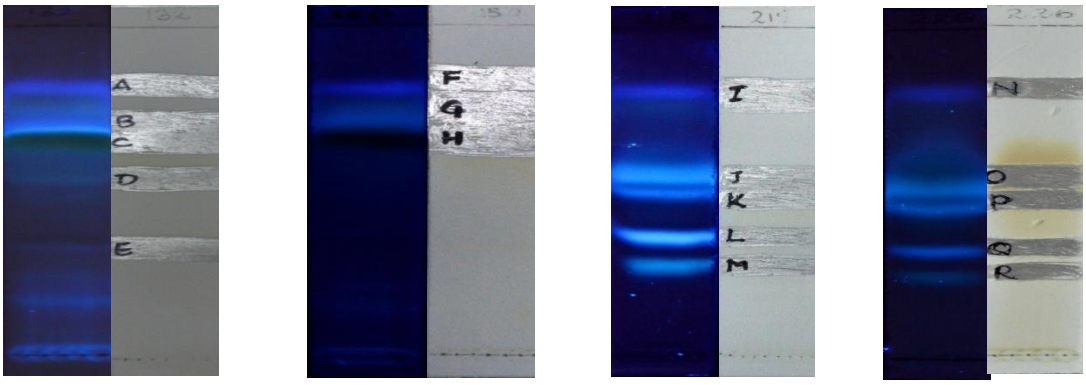
Sl. No.	*Compound name*	*RT*	*Molecular formula*	*Molecular weight*	*Percentage amount*	*Compound nature*	*Activities*
1	Dec-5-ene-3,7-diyne-2,9-dimethyl	12.58	$C_{12}H_6$	160.25 g/mol	4%	-	-
2	2(1H)-Quinolinone, hydrazone	12.93	$C_9H_9N_3$	159.19 g/mol	25.7 %	Hydrazone derivative (Heterocyclic aromatic organic compound)	Anti-inflammatory (Almasirad *et al*., 2005), anti-arteriosclerosis (Uno *et al.* (1995), anti-arterostenotic agent (Koga *et al.*, (1998), antimicrobial (Khodair *et al*., 1998), antidiabetic (Smalley *et al*., 2006).
3	3-cyclohexen-1-ol,4-methyl-1-(1-methylethyl)	13.72	$C_{12}H_{20}O_2$	196.29 g/mol	6.9%	Terpene	
4	Flavone	14.82	$C_{15}H_{10}O_2$	222.24 g/mol	2.2%	Flavonoid	Antimicrobial (Goker *et al*., (2005), anti-inflammatory (Moroney and Somanchi, 1999), antidiabetic (Shin *et al*., 1999), antioxidant (Chen *et al*., 2004).
5	Coumarine, 6-amino-3-phenyl	16.2	$C_{15}H_{11}NO_2$	237.25 g/mol	1.9%	Coumarin	Antibacterial Canning *et al*., 2013), anti-inflammatory (Witaicenis *et al*., 2014), anti-neoplastic activity (Nasr *et al*., 2014: Bronikowska *et al*., 2014).
6	Heptadecanoic acid	17.25	$C_{17}H_{34}O_2$	270.5 g/mol	5%	Saturated fatty acid	Hypoglycemic (Forouhi *et al*., 2014), anti-cardiovasular (Khaw *et al*., 2012), anticancer (Xu *et al*., 2019).
7	Acacetin	18.48	$C_{16}H_{12}O_5$	284.26 g/mol	1.5%	Flavonoid	Anti-tumour (Kim *et* al., 2013), antimicrobial (Bi *et al*., 2016).
8	10-Octadecenoic acid, methyl ester	18.98	C19H36O2	296.5 g/mol	5.1%	Long chain fatty acid	Enhance immunity (Yamada *et al*., 2009),
9	Palustric acid	20.38	$C_{20}H_{30}O_2$	302.5 g/mol	1.8%	Resin	-
10	Elaidic acid isopropyl ester	23.25	$C_{21}H_{32}O_2$	322.5 g/mol	2.3%	Unsaturated fatty acid	

4.12.3 NMR ANALYSIS

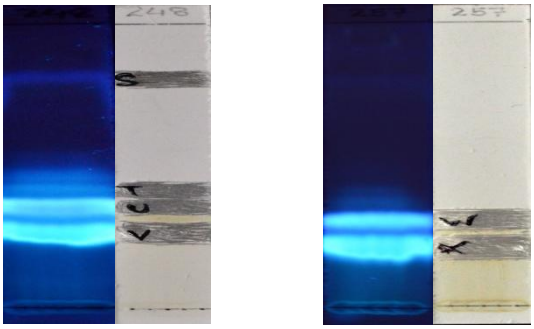
The extract of *F. floribunda* bark obtained from column chromatography which was subjected to GC-MS analysis was further subjected to ^{1}H NMR analysis to find out the functional group present in the crude extract. All the six fractions merged from TLC were again run through a readymade TLC plate in the solvent system, ethyl acetate:methanol:water (100:13.5:10). Each bands formed on the TLC plates were scratched and filtered to determine antidiabetic activity (figure 4.12.3.a and figure 4.12.3.b). F-1 showed a strong inhibition of alpha glucosidase enzyme by all the five bands observed on the plate. The same extract was run in high quantity on larger TLC plates and two bands were scratched, filtered and dried (figure 4.12.3.c and figure 4.12.3.d). The most abundant compound in the extract obtained through GC-MS analysis was 2-hydrazinoquinoline. Thus commercially available 2-hydrazinoquinoline from Sigma was used to compare it with the extract of the plant. Two TLC purified extracts and standard, 2-hydrazinoquinoline was subjected to 1H NMR analysis.

The result of NMR spectroscopy of the two bands obtained from TLC plates of extracts and NMR spectra of the standard (2-hydrazinoquinoline) are presented in figure 4.12.3.e, 4.12.3.f and 4.12.3.g. The estimation of ^{1}H NMR of the same was done and the possible spectra and the functional group with the chemical structure are presented in figure 4.12.3.h, 4.12.3.i and 4.12.3.j. On comparing the three spectra (figure 4.12.3.e, 4.12.3.f and 4.12.3.g), it is apparent that the down field region is identical in all the spectra but the sharp peak at around δ (2.0) is new in the spectra of extracts in both the cases. In order to understand the origin of new peak, we stimulated the NMR spectra of the protonated isomers as well as that for 2-hydrazinoquinoline. In figure 4.12.3.h, the simulated spectra of 2-hydrazinoquinolinone indicated the presence of −NH and $-NH_2$ peaks at 4.0 and 2.0 ppm (δ) respectively which might have totally exchanged in methanol-d4. However in figure 4.12.3.i and 4.12.3.j, it was observed that the spectra of first and second band, the peak at 2.0 ppm (δ) is clearly visible. Comparing with the simulated spectra, this peak can be assigned as $-NH_2-$ and absence of any peak at 4.0 ppm (δ) indicates that there is no protonation at terminal NH_2. However $-NH_2-$ proton may have stabilized by bridge formation with the imine atom.

One more conspicuous thing is the appearance of a sharp peak at 9.0 ppm (δ) in figure 4.12.3.e and 4.12.3.f. This might have originated due to some rearrangement or degradation of the reference compound in which proton at 9.0 ppm have originated. From the 1-H NMR spectra analysis, it can be concluded that similar peaks were observed in the standard 2-hydrazinoquinoline and the TLC purified extracts which indicates that the extract of *F.floribunda* bark contain 2-hydrazinoquinoline.



F-1 F-2 F-3 F-4



F-5 F-6

Figure 4.12.3.a: TLC profiling of the merged fractions and the bands scratched out to obtain the extract

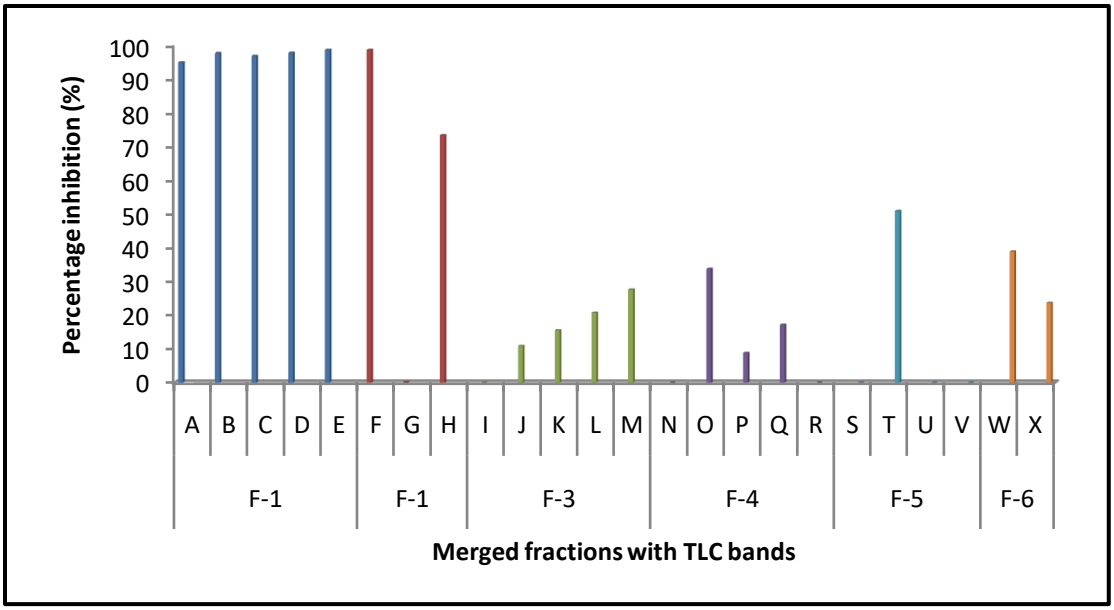


Figure 4.12.3.b: Alpha glucosidase inhibiting activity of each scratched bands from the TLC profiling of the merged fractions

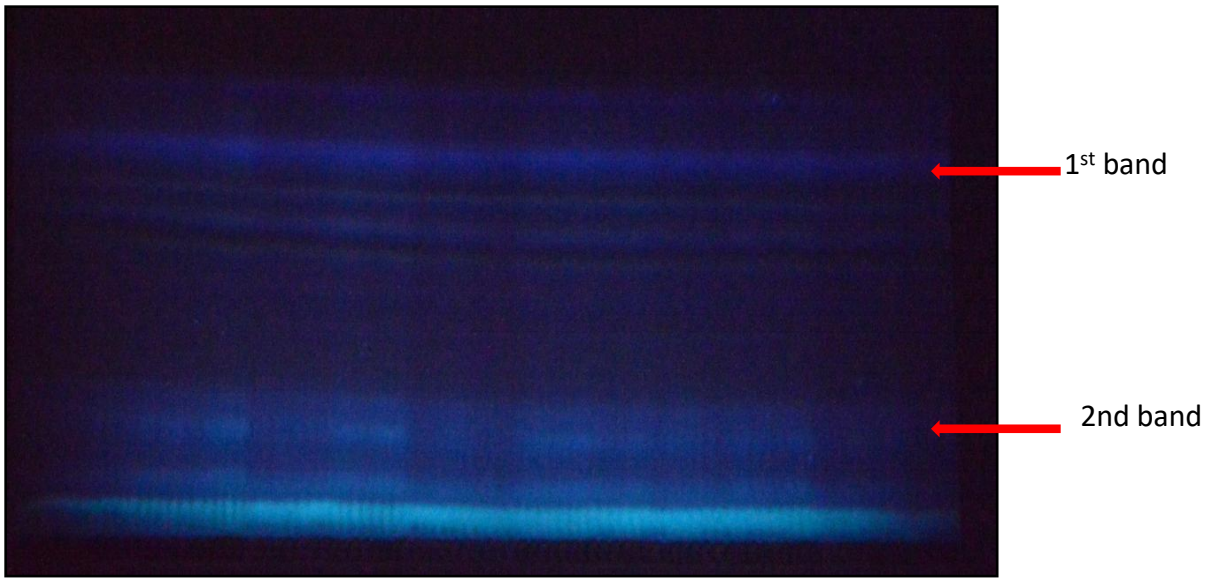


Figure 4.12.3.c: TLC profiling of Fraction-1 to obtain extract for NMR analysis (UV-365 nm)

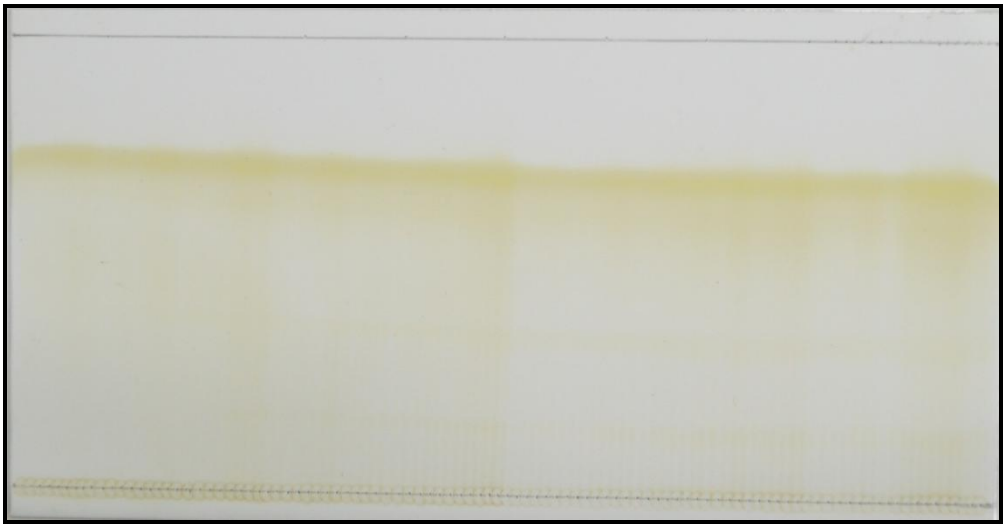


Figure 4.12.3.d: TLC profiling of Fraction-1 to obtain extract for NMR analysis (visible light)

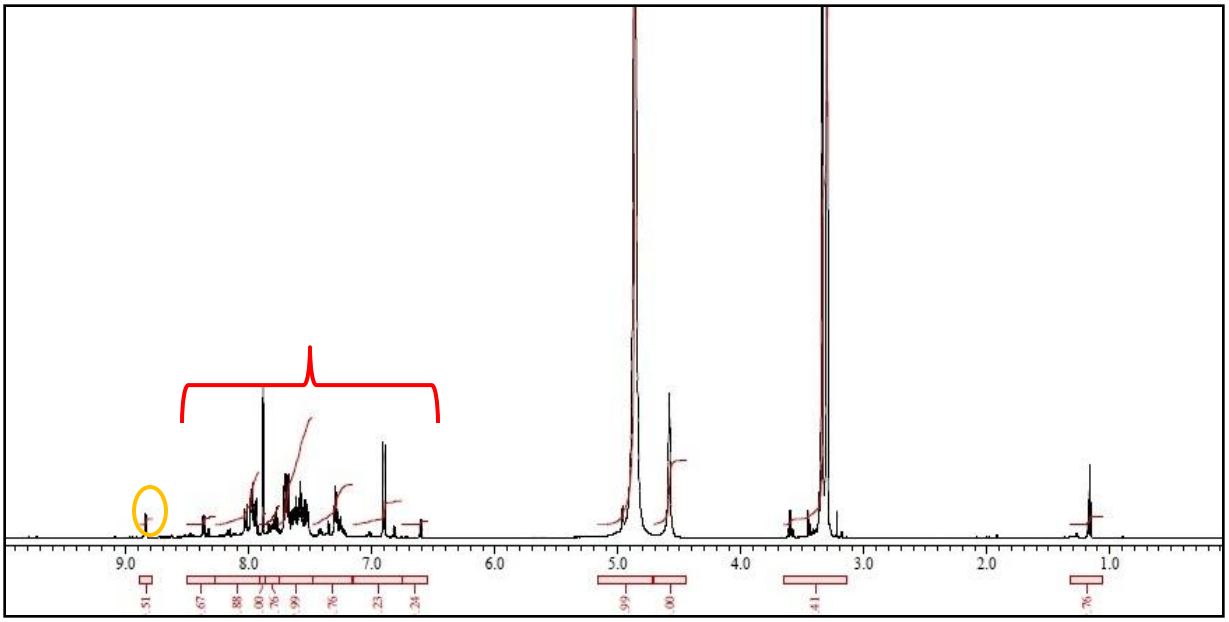


Figure 4.12.3.e: 1-H NMR spectra of commercially available 2-hydrazinoquinoline purchased from Sigma dissolved in methanol

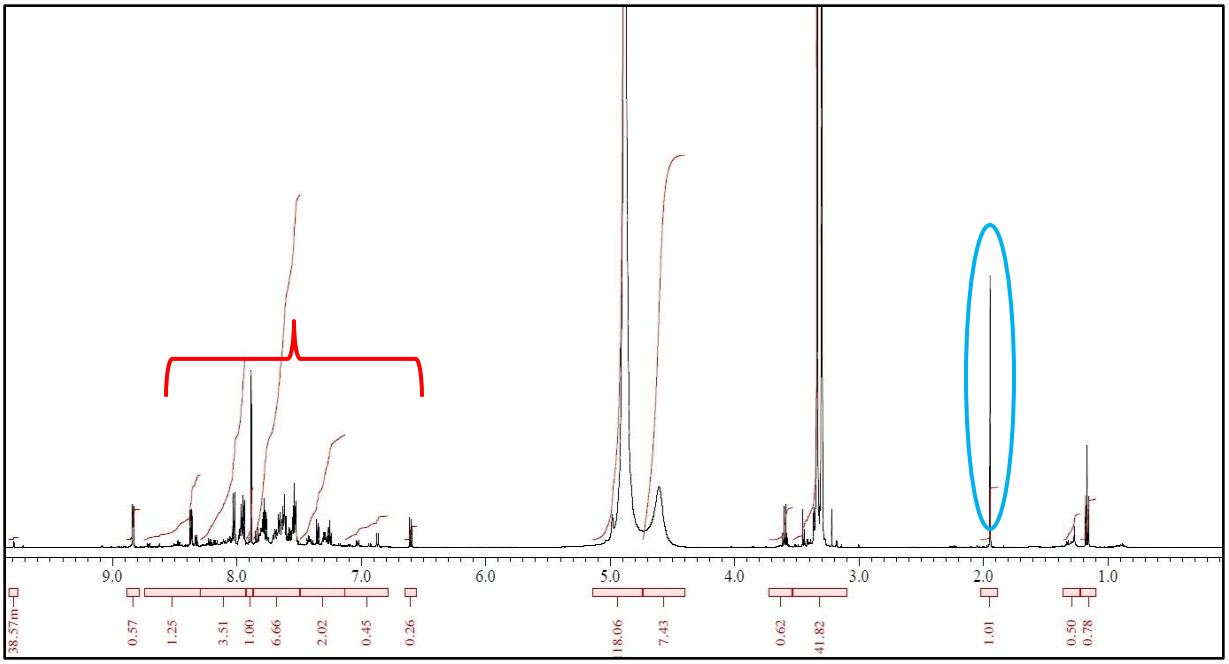


Figure 4.12.3.f: 1-H NMR spectra of column and TLC purified extract (FIRST BAND) of *Fraxinus floribunda* bark dissolved in methanol

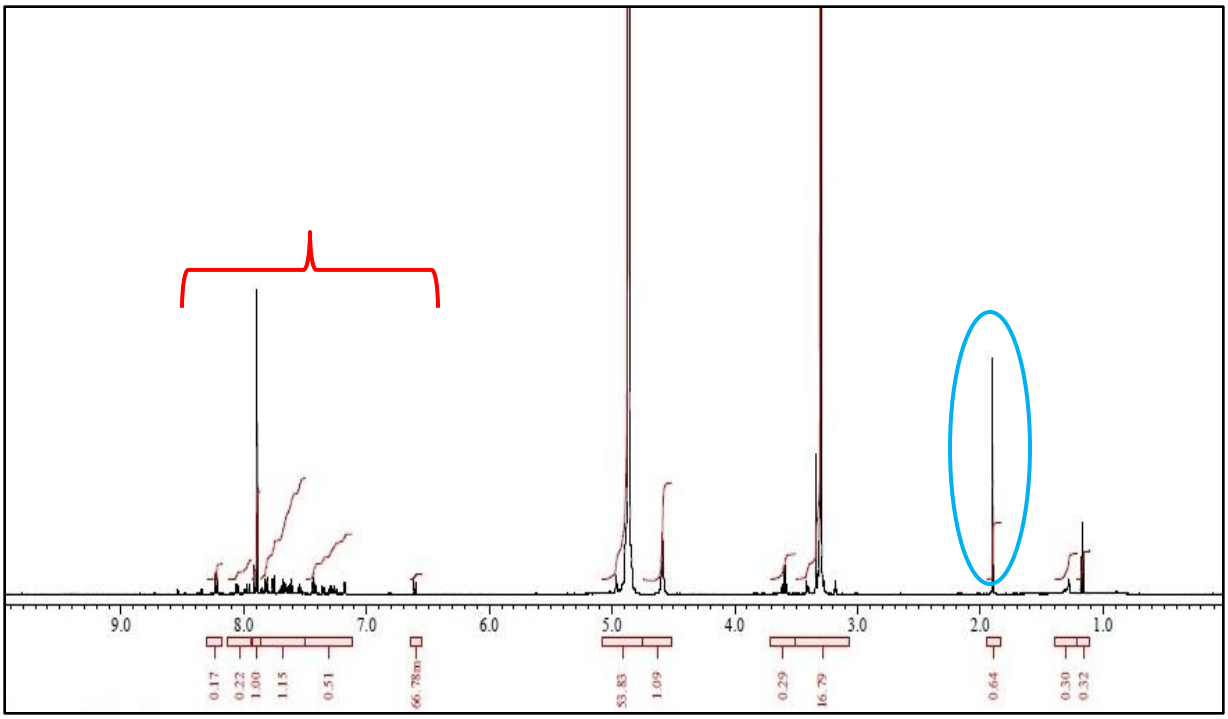
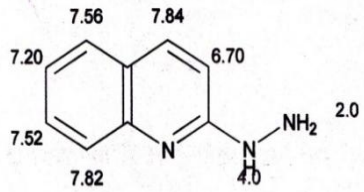
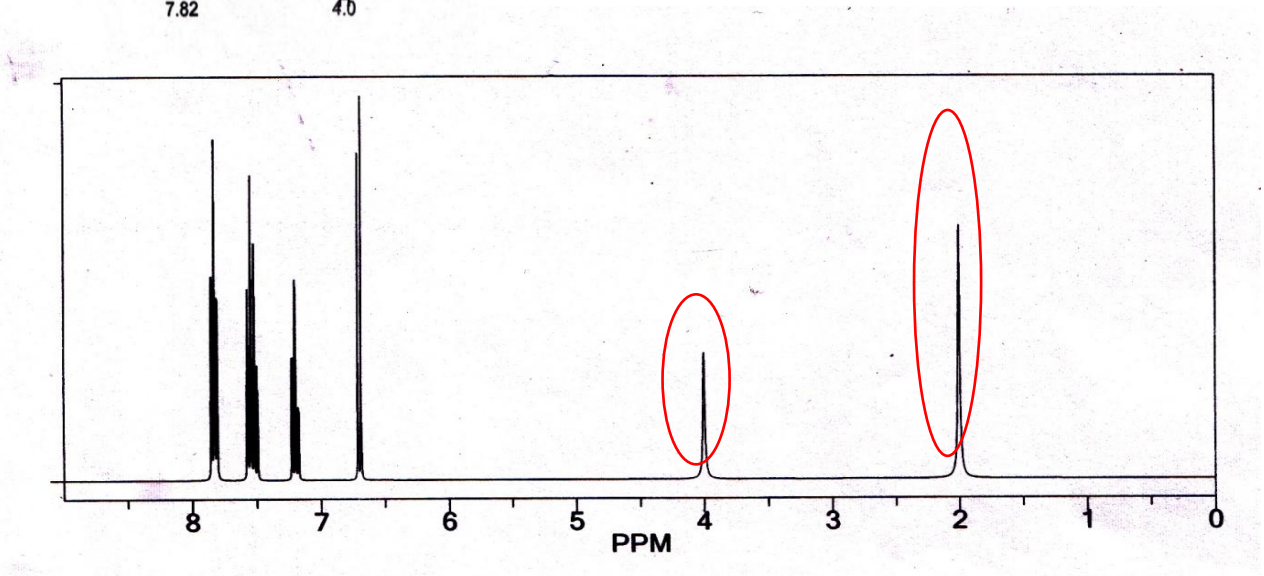


Figure 4.12.3.g: 1-H NMR spectra of column and TLC purified extract (SECOND BAND) of *Fraxinus floribunda* bark dissolved in methanol

ChemNMR H-1 Estimation



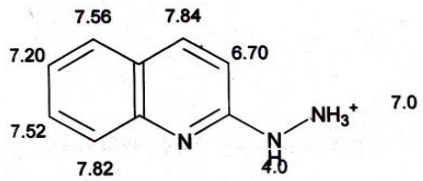


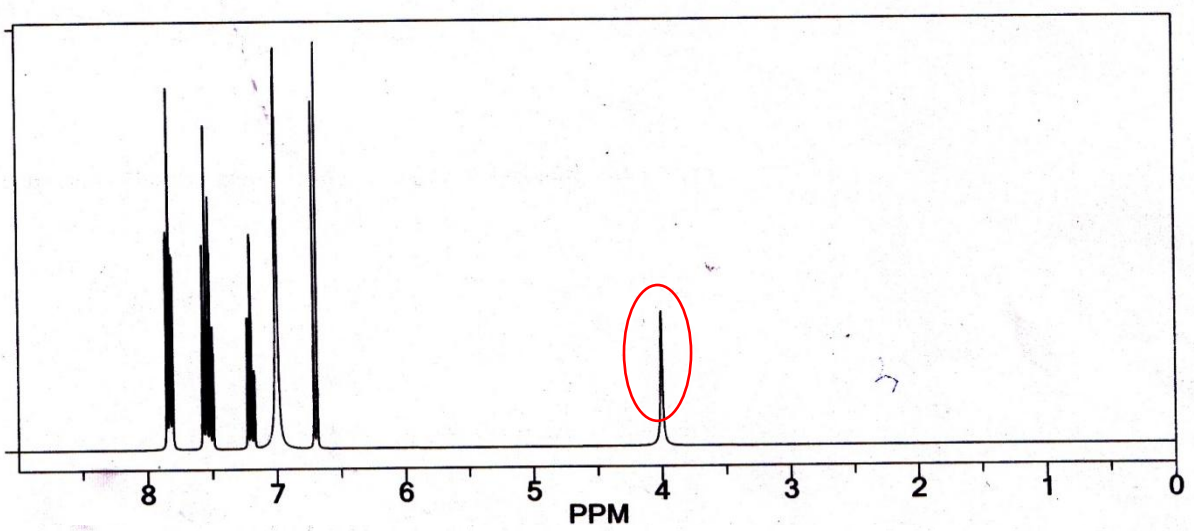
Protocol of the H-1 NMR Prediction:

Node	Shift	Base + Inc.	Comment (ppm rel. to TMS)
CH	6.70	7.26	quinoline
		-0.56	1 -N from 2-naphthalene
CH	7.84	8.00	quinoline
		-0.16	1 -N from 2-naphthalene
CH	7.56	7.68	quinoline
		-0.12	1 -N from 2-naphthalene
CH	7.20	7.43	quinoline
		-0.23	1 -N from 2-naphthalene
CH	7.52	7.61	quinoline
		-0.09	1 -N from 2-naphthalene
CH	7.82	8.05	quinoline
		-0.23	1 -N from 2-naphthalene
NH	4.0	4.00	aromatic C-NH
NH2	2.0	2.00	amine

Figure 4.12.3.h: Possible NMR spectrum and the functional group with the structure of 2-hydrazinoquinoline and the prediction of the 1-H NMR

ChemNMR H-1 Estimation



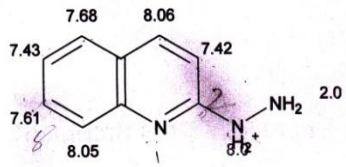


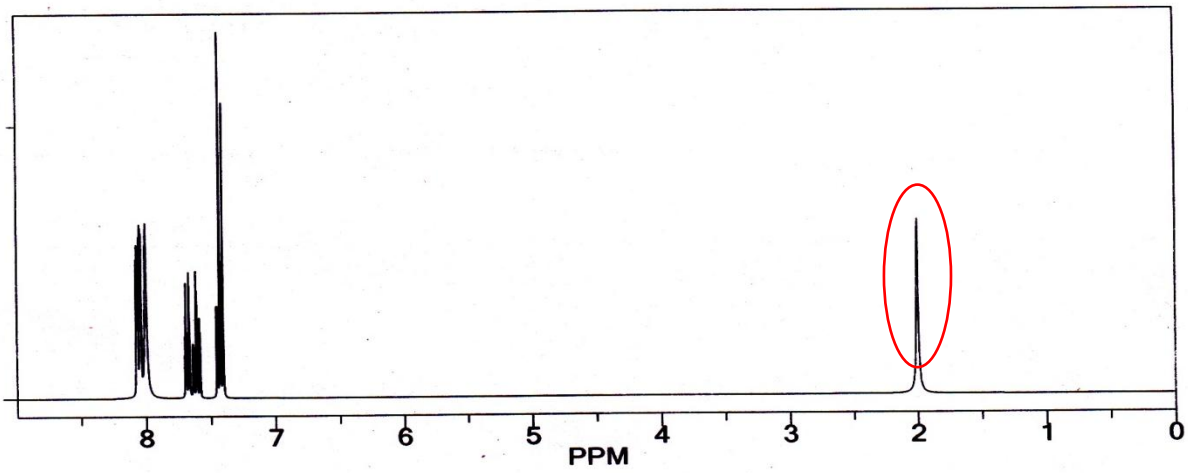
Protocol of the H-1 NMR Prediction:

Node	Shift	Base + Inc.	Comment (ppm rel. to TMS)
CH	6.70	7.26	quinoline
		-0.56	1 -N from 2-naphthalene
CH	7.84	8.00	quinoline
		-0.16	1 -N from 2-naphthalene
CH	7.56	7.68	quinoline
		-0.12	1 -N from 2-naphthalene
CH	7.20	7.43	quinoline
		-0.23	1 -N from 2-naphthalene
CH	7.52	7.61	quinoline
		-0.09	1 -N from 2-naphthalene
CH	7.82	8.05	quinoline
		-0.23	1 -N from 2-naphthalene
NH	4.0	4.00	aromatic C-NH
NH3+	7.0	7.00	ammonium

Figure 4.12.3.i: Possible NMR spectrum and the functional group with the structure of the first band of the extract of *Fraxinus floribunda* bark and the prediction of 1-H NMR

ChemNMR H-1 Estimation





Protocol of the H-1 NMR Prediction:

Node	Shift	Base + Inc.	Comment (ppm rel. to TMS)
CH	7.42	7.26	quinoline
		?	1 unknown substituent(s) from 2-naphthalene
		?	1 unknown substituent(s) from 2-pyridine, in DMSO
		0.16	1 -Np from 1-benzene
CH	8.06	8.00	quinoline
		?	1 unknown substituent(s) from 2-naphthalene
		?	1 unknown substituent(s) from 2-pyridine, in DMSO
		0.06	1 -Np from 1-benzene
CH	7.68	7.68	quinoline
		?	1 unknown substituent(s) from 2-naphthalene
CH	7.43	7.43	quinoline
		?	1 unknown substituent(s) from 2-naphthalene
CH	7.61	7.61	quinoline
		?	1 unknown substituent(s) from 2-naphthalene
CH	8.05	8.05	quinoline
		?	1 unknown substituent(s) from 2-naphthalene
NH2+	8.0	8.00	aromatic C-NH+
NH2	2.0	2.00	amine

Figure 4.12.3.j: Possible NMR spectrum and the functional group with the structure of the second band of the extract of *Fraxinus floribunda* bark and the prediction of 1-H NMR