CHAPTER-4

RESULTS

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Plants are used extensively in indigenous Indian medical systems. The Northeastern Region (NER) of India, rich with varied ethnic groupings and socio-cultural complexities, preserves the oldest and most diverse traditions linked with ethno-medicinal plant use. These ethnomedicinal plants in this region is a gift from nature to mankind and can be used as nutritious supplement in a balanced diet and also as an alternative to staple foods in times of food constraints. In addition to providing a healthy diet, these plants are used as a traditional herbal medical base in rural areas to treat a variety of ailments.

4.1. Ethnomedicinal plants used by traditional healers of Goalpara

In this region, traditional herbal practices remain a primary source of healthcare. Despite contemporary medical facilities, people in this part of Assam still rely on locally available plant resources and traditional healers to treat various ailments. Traditional herbal healers referred to as Bej, Oja or Kobiraj generally prescribe and supply remedies to patients. Keeping in view with the objectives, an ethnobotanical survey through semi-structured questionnaires was conducted in tribal population dominated areas of Goalpara District of Assam. The lists of medicinal plants which are used by the traditional healers of Goalpara district of Assam are listed in Table: 4.1.

Based on the integrated assessment of traditional knowledge of plant species gathered, three plant species were selected for the present study. The plants selected for the study were *Zanthoxylum oxyphyllum* Edgew, *Rotheca serrata* (L.) Steane & Mabb. and *Blumea lanceolaria* (Roxb.) Druce. All these three plants are locally used as food as well as medicine for treating various diseases. Owing to the importance of these plant species, the present study aimed to perform in-vitro screening of the plant species on the basis of their ethno medicinal importance.

Table 4.1: Medicinal plants used by the traditional healers of Goalpara, Assam.

Sl. No	Name of the Plant (Botanical Name)	Local Name	Family	Part Used	Diseases cured	Mode of preparation and application	Tribe Used
1.	*Abromaaugusta* L.	Gorokhiakorai	Sterculiaceae	Root	Breast cancer, internal wound healing, jaundice	Paste applied in infection area	Hajong
2.	*Acoruscalamus* L.	Bech Boos (Ass) Bishbifang (Bodo) Rajamuni (Rabha)	Araceae	Rhizome	Epilepsy	Powder	Rabha, Boro
3.	*Acacia arabica* Wild.	TaruaKadam	Mimosaceae	Leaf	Nightfall	Extract	Rabha Tribe
4.	*Achyranthesaspera L*	Ucktishar	Amarantaceae	Root	Bone fracture	Paste (Apply for 1week)	Rabha
5.	*Aeglemarmelas* (L) Corr.	Bel	Acanthaceae	Fresh leaves Bark (Hajong)	Leaves is used for malaria, diabetes Fruit pulp is used to cure diarrhea and also as tonic. Bark diabetes.	Leaves Juice (Fruit Juice or ripen fruit) is eaten daily until recovered.	Rabha, Boro, Hajong
6.	*Adhatodazylanica.* Medic.	BogaBahak	Acanthaceae	Young leaf	Jaundice, Malaria.	Extract	Rabha
7.	*Alstoniascholaris* L R. Br.	Chatian	Apocynaceae	Bark	Asthma, Malaria	Powder & Extract	Rabha, Hajong
8.	*Alocasiamacrorhiza* L.	Man kachu	Araceae	Rhizome	Joint pain	Paste	Rabha
9.	*Allium sativum*L.	Nohoru (Ass), Sambramgufur (Bodo), Raisung(Rabha)	Alliaceae	Bulb	High pressure	Mild eaten roasted	Boro
10.	*Alpiniagalanga* L.	Tora	Zingiberaceae	Rhizome	Mouth ulcer	Extract	Rabha

	Sw.						
11.	*Aloe vera*. Toum. Ex. L.	Sal kuwari	Liliaceae	Leaf	Piles, urinary disorder, burning	Extract, Jelly apply	Rabha
12.	*Amorphophaluspaeoniifolius*	Ol-kochu	Araceae	Stem, tuber	Cancer, pinworm	Eaten raw	Rabha, Garo
13.	*Ageratum conyzoides* L.	Saguntualasi	Asteraceae	Leaf	Cuts wounds	Paste	Hajong
14.	*Aristolochiaindica* L.	Nilakantha	Aristolochiaceae	Leaf, Stem, Root	High blood pressure, Anti venom, Bone fracture	Extract, Paste	Rabha,
15.	*Artocarpuschama*Buch.- Ham.	Dawagos (Ass), Hajong (Bohot)	Moraceae	Bark	Dog bite (Kukurkamra)	Paste	Hajong, Boro
16.	*Asparagus racemosus* Wild	Satmul	Liliaceae	Root	Malaria	Decoction	Boro
17.	*Artemisia vulgaris* L	Nilum (Ass), Nageswar (Hajong)	Asteraceae	Leaf	Menstrual problem	Extract	Hajong
18.	*Averrhoacarambola* L	Kordoi (A)	Rutaceae	Leaf	Bleeding piles	Extract (Taken in empty stomach)	Boro, Rabha
19.	*Azadirichtaindica*Adr. Juss.	Mahaneem (Ass), Khawaneem (Hajong)	Meliaceae	Leaf	Skin diseases	Boiled leaf	Boro, Rabha, Assamese
20.	*BaccuareasapidaMuell*	Leteku	Euphorbiaceae	Fruit	Constipation	Taken orally	Boro
21.	*Basella alba* Linn.	Poi	Basellaceae	Leaf, Fruit, Root	Leaf for constipation and gonorrhea. Decoction of root is given in intestinal	Leaf Juice, Root decoction	Rabha, Boro

					disorders.		
22.	*Bischofiajavanica* Bl.	Urium	Euphorbiaceae	Bark	Burning	Paste	Boro
23.	*Blumealanceolaria* (Roxb.) Druce	Jwglaori (Boro)	Asteraceae	Leaf	Cough, Stomach Trouble	Juice, Decoction	Boro
24.	*Boehananivea* Hook et. Arm.	Remi	Urticaceae	Leaf	Breast Pain and Swelling	Paste	Rabha, Garo
25.	*Boerhaviadiffusa* L.	Punanowa (Ass)	Nyctaginaceae	Root, Leaf	Indigestion, diabetic	Juice	Rabha
26.	*Borreriahispda (L.) Schuman*	Dolicha bon	Rubiaceae	Leaf	Tooth disorder	Extract. Gurgle	Rabha
27.	*Bryophyllumcalycinum Salisb.*	Duportenga	Crassulacaceae	Leaf	Kidney stone	Juice	Rabha
28.	*Cajanuscajan* L.	Rahardal	Fabaceae	Young leaf	Jaundice	-	Rabha
29.	*Calamusrotang* L.	Bet gaj	Arecaceae	Leaf	Antidiabetic	Decoction	Rabha, Hajong
30.	*Calipetriseculenta*	Deki (Hajong)	Arecaceae	Stem	Liver disorder	Juice	Hajong
31.	*Carica papaya* L.	Amita	Caricaeae	Seed Unripe, Leaf	Pin worm, Dengue	Decoction	Rabha, Boro
32.	*Cassia alata* L.	Khorpat	Caesalpiniaceae	Leaf	Skin disease	Extract	Rabha
33.	*Caesalpiniabonduc (L.) Roxb.*	Letaguti	Caesalpiniaceae	Seeds	Stomach ache	Decoction	Rabha
34.	*Catharanthusroseus* (L).G. Don	Nayantara	Apocynaceae	Whole plant	Anti cancerous	-	Rabha
35.	*Centrellaasiatica*(L) Urban	Manimuni, Manikon (Hajong)	Apiaceae	Whole plant	Dysentery	Juice	Rabha, Boro, Hajong, Assmese
36.	*Citrus grandis* (L.)Osb.	Robab-tenga	Rutaceae	Fruit	Gastritis, cardiovascular disease(hypertension)	Juice	Hajong
37.	*Cissampelospariera* L.	Tubukilota	Manispermaceae	Root	Urinary trouble	Extract	Rabha
38.	*Chenopodium album*	JilmilSak	Chenopodiaceae	Tender	Stomach	Vegetables	Rabha,

	L.			shoots	complain		Hajong
39.	*Cissusquadrangularis* L.	Harjoralota	Vitaceae	Stem	Bone fracture	Paste applied in infected area.	Rabha
40.	*Cinnamomumtamala (Buch-Ham.) T. Nees&Eberm.*	Tejpat	Lauraceae	Leaf	Spice and whooping cough	Boiled with water and drunk	Rabha
41.	*Clerodendroncolebrookianum*Walp	Naphaphu	Verbanaceae	Young twig	High blood pressure	Both Extract and decoction	Rabha, Boro
42.	*Clitoriaternatea*L.	Aparajita	Papilonaceae	Roots	Fever, leprosy	Extract, Paste	Rabha, Hajong
43.	*Colocasiaesculenta (L.)*	Kachu	Araceae	Petiole	Petiole is used in cut and itching	Paste	Hajong
44.	*Corchoruscapsularis* L.	Morapat	Tiliaceae	Leaf	Different types of fever.	-	Garo
45.	*Cynodondactylon* Pers.	Dubori bon (Ass), Dubrigangsu (Bodo), Dubla sum (Rabha)	Poaceae extract is used for washing teeth.	Leaf	Fresh Leaf extract is used for washing teeth.	Extract	Rabha, Boro
46.	*Costusspeciosus* (Koenig)Sm	Jamlakhuti (Ass), Hajong (Kengwa)	Costaceae	Stem& root	Jaundice & Snake bite	-	Rabha
47.	*Crinum asiaticum* L.	Gunohoru	Amaryllidaceae	Root	Jaundice	Extract	Rabha
48.	*Crotolariamucronata*. Desv.	Ghantakarna	Papilionaceae	Young leaf	Intestinal worm	Extract	Hajong
49.	*Croton tiglium* L.	Knibih	Euphorbiaceae	Seed	As purgative cathartics	Not Disclosed	Rabha
50.	*Cryptolepisbuchanani.* Roem&Schult.	Anantamul (Kala)	Asclepiadaceae	Root	Bone fracture	Paste	Rabha

51.	*Curcuma zedoaria*(Berg.) Rosc	Keturi	Zingeberaceae	Rhizome	Skin disease	Paste	Rabha, Hajong
52.	*Curcuma longa* L.	Halodhi	Zingeberaceae	Rhizome	Blood purification & pain relief	Juice & Paste	Rabha, Hajong
53.	*Cynodondactylon* (L) Pers.	Dubari ban	Poaceae	Whole plant	Chronic diarrhea	Juice	Rabha
54.	*Datistacannabina* L.	Akalber	Datiscaceae	Root	Gastric	Extract	Rabha
55.	*Dilleniaindica* L.	OuTenga (Ass) Sulta (Hajong)	Dilleniaceae	Fruit	Dandruff and lice	Hair wash with extracted water	Rabha
56.	*Drymariacordata* (L.)Willd.exSchult.	Kurijani (Laijabori)	Caryophyllaceae	Whole plant	Blood dysentery (Tej dysentery)	Paste	Hajong
57.	*Ecliptaprostata L.*	Kehraj	*Euphorbiacea*	Whole plant	Joint pain	Paste	Rabha
58.	*Emblicaofficinalis*Gaertn.	Amlokhi	Euphorbiaceae	Fruit	Jaundice	Extract	Rabha, Boro
59.	*Euphorbia hirta* L.	Posutia	Euphorbiaceae	Whole plant	Asthama& anti cancerous	-	Rabha
60.	*Erythrinaindica* Lim.	Modar	Leguminosae	Root to be grinded and taken along with milk	Helps in Conceiving, provides stamina.	-	Rabha
61.	*Ficushispida* L.	Dimaru	Moraceae	Bark	Excessive menstrual bleeding	Juice	Rabha, Hajong
62.	*Ficusbenghalensis* L.	Borgos (Ass), Dok fang (Rabha)	Moraceae	Prop root	Dental and gum disorder	Paste	Hajong
63.	*Garciniacowa*Roxb.	Kujithekera	Clusiaceae	Fruits	Digestive and anti dysenteric		

					properties		
64.	*Grewiaasiatica* L.	KukurSuta	Liliaceae	Stem bark	Constipation & anti diabetic	Juice	Rabha
65.	*Heydiotispinnifolia*L.	Bon Jaluk	Rubiaceae	Whole Plant	Dysentry	-	Hajong
66.	*Hibiscus rosasinensis* L.	Jaba	Malvaceae	Flower	Excessive menstruation	Extract	Hajong
67.	*Holarrhenaantidysenterica L.*	Dudhkhuri	*Apocynaceae*	Bark	Bone fracture	Paste	Hajong
68.	*Homalomenaaromatica* Schott.	Gandhkachu	Araceae	Rhizome	Chronic pain & diabetes	Juice	Rabha, Hajong
69.	Impatiens sp	Sorial (Hajong)	Balsaminaceae	Leaf	Nose bleeding	-	Hajong
70.	*Ipomeadigitata*L.	Bhuikumura	Convolvulaceae	Root	Sex stimulant	Extract	Rabha
71.	*Ipomearaptans*poir.	Kolmou	Convolvulaceae	Tender shoots	Stomach trouble	Vegetables	Rabha, Boro, Hajong
72.	*Justiciagendarussa* L.	Jatrasita (Ass), Sunsunitita (Hasong)	Acanthaceae	Young leaf	Excessive menstruation	Decoction	Rabha
73.	*Justiciaadhatoda*L.	Bogabahok (Ass) Basikhojola (Bodo) Bokaibaskai(Rabha)	Acanthaceae	Flower	Cough, Diabetic	Juice, Extract	Rabha, Boro, Hajong
74.	*Kaempheriagalanga* L.	Chandra mallika	Zingeberaceae	Rhizome	Cough	Juice	Rabha, Hajong
75.	*Lasiaspinosa* (L.) Thwaites	Songe (Sengmora)	Araceae	Rhizome	Kidney stone/ Gall bladder stone	Decoction	
76.	*Litsaeachinensis L.*	Baghnala	Lauraceae	Bark	Bone fracture	Paste	Rabha, Boro

77.	*Litseasalicifolia*Roxb.	Dighloti	Lauraceae	Leaves	Insecticidal properties	Extract	Rabha
78.	*Litseaglutinosa* L.	Laham	Lauraceae	Bark	Goudh (Muscular swelling)	Paste	Rabha
79.	*Lucas aspera*Spreng.	Durun	Lamiaceae	Whole plant	Jaundice	Juice	Rabha
80.	*Leucasplukenetii* (Roth) Spreng.	Dron (Ass), Khangsingsa (Bodo), Sumkanjai (Rabha)	Lamiaceae	Leaf	Stomach trouble	Juice	Rabha, Boro, Hajong, Assamese
81.	*Menthaspicata* Linn	Podina	Lamiaceae	Leaf	Leaves are used in gastro intestinal disorders, cough, cold, cholera and fever	Juice	Rabha, Boro, Assamese
82.	*Mesuafera* L.	Nahar	Mimosaceae	Flower	Stomachic, bleeding piles	Decoction	Rabha
83.	*Mikaniamicrantha*	Premlata (Hasong)	Asteraceae	Whole plant	Blood coagulation	Juice	
84.	*Mimosa pudica* L.	Nilagi ban	Mimosaceae	Leaf,Root	Used in Fistula	Paste	Rabha
85.	*Momordiacharantia* L.	Titakerala	Cucurbitaceae	Fruit	Diabetes	Eaten as vegetable	Boro
86.	*Murrayakoenigii*Spreng	Narasingha	Rutaceae	Leaf, Root	Stomach Trouble	Juice	Rabha
87.	*Moringaoleifera*Lamk.	Sojina (Ass)	Moringaceae	Leaf	Hypertension	Decoction	Rabha, Boro
88.	*Musa balbisiana* Coll.	Athiyakol (Ass), Thalirathiya (Bodo), Athiyarathe (Rabha)	Musaceae	Rhizome	Pneumonia	Juice	Rabha
89.	*Musa*	Bhimkol (Ass)				Eaten orally	Rabha, Boro,

	*gigantea*Duthie	Thaliraathia (Bodo) Aathiarathe (Rabha)	Musaceae	Fruit	Dysentery		Hajong
90.	*Nigella sativa* L.	Kaljira (Ass)	Asteraceae	Seeds	Diabetic	Decoction	Rabha
91.	*Nymphaea alba* L.	Bogabhet	Nymphaeaceae	Root	Headache	Paste	Rabha
92.	*Nyctanthes arbor tristis*L.	Sewaliphul	Oleaceae	Young leaf	Malaria	Juice	Rabha
93.	*Ocimumgratisimum* L.	Ram tulsi	Lamiaceae	Leaf	Cough	Juice of crushed fresh leaves and mixed with honey	Bodo, Rabha, Hajong
94.	*Ocimum sanctum* Linn.	Tulsi	Lamiaceae	Leaf	Cough, Urinary trouble and diabetes. Also used in liver diseases, gastric troubles and skin diseases.	Juice	Rabha, Boro, hajong, Assamese
95.	*Psidiumguagava*L.	Modhuriam(Ass), Sophari (Bodo), Lamthe (Rabha)	Myrtaceae	Young leaf	Dysentery	Juice	Boro, Rabha
96.	*Padaraeafoetida* L.	Vedailota (Ass), Padurilewa(Boro)	Rubiaceae	Tender leaves	Diarrhea, Stomach Trouble		
97.	*Oroxylumindicum* (L.) Vent.	Bhatghila	Bignoniaceae	Bark	Cough, High fever, pneumonia	Juice	Rabha, Boro
98.	*Piper nigram* L.	Jaluk (Ass), JathwiseAllou (Bodo)	Piperaceae	Fruit	Chest pain	Decoction	Rabha, Hajong
99.	*Plumbagorosea*L.	Agyachit	Plumbaginaceae	Root	Ulcer, Abortion	Decoction	Garo
100.	*Phyllanthusniruri*L.	Poraamlakhi	Euphorbiaceae	Leaf	Ring worm	Paste	Rabha

101.	*Pygmaeopremnaherbacea*Moldenke	Matiajamun.	Verbenaceae	Root	Jaundice	Juice	Rabha
102.	*Punicagranatum* L.	Dalim	Lythraceae	Leaf	(Sinus)	Juice	
103.	*Rouvolfiaserpentina* Benth.	Sarpagandha/ Chandateeta	Apocynaceae	Root	Snake bite, Malaria	Paste apply for snakebite, Decoction	Rabha
104.	*Rothecaserrata* (L) Steane&Mabb.	NangolBhanga	Lamiaceae	Leaf	Stomach trouble, Jaundice, hypertension	Decoction	Rabha, Bodo
105.	*Riccinuscommunis*L.	Era	Euphorbiaceae	Leaves	Rheumatism and muscle pain	Paste	Rabha, Hajong
106	*Scopariadulcis* Linn.	Bondhania (Ass) Dhongfangra khab (Bodo) Sumki sum (Rabha)	Scrophulariaceae	Leaf	Young leaves are boiled and is commonly used as mouth wash.	Decoction	Hajong
107	*Sidaacuta* .Burm.	BogaBorial	Malvaceae	Whole plant	Eye infection	Juice	Rabha
108	*Solanumindicum* Linn.	Tit bhekuri	Solanaceae	Fruits, leaf	Bronchitis, asthma, pneumonia, cough	Directly taken orally	Rabha
109	*Solanumnigrum* L.	Piskati	Solanaceae	Leaf	Antiseptic	Paste	Rabha
110	*Spilanthuspaniculata* Wall.	Suhoni	Asteraceae	Flower	Toothache	Extract	Hajong
111	*Staphniahardendifolia*Wild.	Gandha raj	Menispermaceae	Stem, Tuber, Leaf	Dysentery, Jaundice	Juice	Hajong
112	*Tabernaemontanadivaricata* (L) R. Br.	Kothona	Apocynaceae	Latex, Root,	Intestinal worm, Toothache	Juice, Paste	Rabha

				Seed			
113	*Terminaliaarjuna* W & A	Arjun	Combretaceae	Bark	Chest pain, diabetes, Gastric	Juice (taken orally)	Rabha, Boro, Hajong
114	*Terminaliabelarica* Roxb.	Bhomora	Combretaceae	Fruit	Purgative, acidity	Decoction	Rabha
115	*Terminaliachebula* Retz.	Silikha	Combretaceae	Fruit	Jaundice	Decoction and mixed with kismis and drunk twice a day	Rabha
116	*Thunbergianepalensis*Roxb.	Gurkha kale	Acanthaceae	Root	Jaundice	Juice	
117	*Tinosporacordifolia*(Willd) Hok. F.	Sirdhilota	Menispermaceae	Whole plant	Diabetes	Decoction (2 teaspoons daily)	Boro
118	*Trigonellafoenumgraecum* Linn.	Mithigooti	Leguminosae	Seed	Helps fight Uterus infection, provides stamina.	Seed is grinded and taken along with milk.	
119	*Vitexnegundo* L.	Posotia (Ass), Nisinda(bodo)	Verbenaceae	Leaf	Toothache, earache.	Extract	Rabha
120	*Zanthoxylumoxyphyllum*	Mejenga	Rutaceae	Leaf, Bark	Piles, Stomach trouble, Cancer	Juice and also taken as vegetable	Boro, Rabha, Garo
121	*Zingiberofficinale*	Ada (Ass) Haijeng (Bodo) Sinku (Rabha)	Zingiberaceae	Rhizome	Cold, cough and stomach ache	Chewing	Boro, Rabha

4.2. Collection and identification of plant material

Leaves of *Zanthoxylum oxyphyllum* Edgew, *Rotheca serrata* (L.) Steane & Mabb. and *Blumea lanceolaria* (Roxb). Druce were collected in the month of December, 2019 from Dhupdhara in Goalpara District of Assam. Plant identification was carried out following standard taxonomic procedures. Plants were photographed and collected from Goalpara district of Assam. The dried plant part was then pasted on the herbarium sheet. Identification numbers were collected and a voucher specimen of *Zanthoxylum oxyphyllum* Edgew, *Rotheca serrata* (L.) Steane & Mabb. and *Blumea lanceolaria* (Roxb.) Druce were submitted to Gauhati University Botanical Herbarium and Bodoland University, Botanical Herbarium, with the following accession number given in the table below.

Table: 4.2. Voucher specimen with accession number

Sl. No	Local name	Scientific Name	Family	Accession No
1	Mejenga	*Zanthoxylum oxyphyllum* Edgew	Rutaceae	18924
2	Nangal Bhanga	*Rotheca serrata* (L.) Steane & Mabb.	Lamiaceae	18926
3	Jwglaori	*Blumea lanceolaria* (Roxb.) Druce	Asteraceae	BUBH0000868

The identified plant specimens were confirmed at molecular level through DNA barcoding.

4.3. Description of the selected plants

4.3.1. *Zanthoxylum oxyphyllum* Edgew

Botanical Description

Small deciduous trees called *Zanthoxylum oxyphyllum* Edgew are indigenous to Bhutan, Myanmar, Nepal and India. It is a member of the Rutaceae family and grown primarily in temperate woodlands. They are prickly, dioecious shrubs with thorns, dense foliage and leaves with a strong and acrid flavour (Chayee *et al.*, 1996). Leaves are imparipinnate, flowers are globose, glabrous and dull red in colour. The seeds are

single and brilliantly black. In April and May, flowers begin to bloom and fruiting season lasts from August through September. The young leaves and shoots are cooked and consumed.

Ethnobotanical uses

The young leaves of this plant are preferably consumed used as vegetable by tribal population in North East India and its aqueous extracts are used by tribal people as tonics to treat a variety of ailments. According to reports, leaves can purify the blood, lower the risk of leucoderma and relieve stomach discomfort (Buragohain *et al.*, 2011). Bark of this plant is widely used to treat rheumatism, ulcers, varicose veins and leg pain by traditional healers. Additionally, it can be used to treat inflammation, fevers and hypotension as reported by Arun and Paridhavi, (2012). Fruits have been used as an appetizer, anthelmintic, to treat pain, tumours stomach problems, headaches, body aches, fever, colds and coughs. They have also been used to purify blood.

The bark is considered to stimulate the stomach and the digestive system. Additionally, it is used as a sudorific during fevers (Kanjilal, 1992; Kirtikar and Basu, 1993). Human keratinocyte proliferation is inhibited by the antiproliferative properties in the bark and root extracts of this plant has been demonstrated by Kumar and Muller, (1999). Additionally, it is a digestive, stimulant and has stringent qualities and it has also been used for treatment of dyspepsia and diarrhoea (Medhi *et al.*, 2009).

Systematic position

Kingdom: Plantae

Clade: Angiosperms

Order: Sapindales

Family: Rutaceae

Genus: *Zanthoxylum*

Species: *Zanthoxylum oxyphyllum* Edgew

Local Name: Mejenga

Figure: 4.1. *Zanthoxylum oxyphyllum* Edgew

4.3.2. *Rotheca serrata* (L.) Steane & Mabb.

Botanical Description

R. serrata called Nangol bhanga in Assamese is an important medicinal plant belonging to family Lamiaceae. *Rotheca serrata* (L.) Steane & Mabb. scientifically classified as *Clerodendrum serratum* earlier placed under family Verbanaceae. Phylogenetic analysis of its mitochondrial DNA shifted it to the Lamiaceae family (Steane *et al.* 1997). The plant is widely distributed across the globe ranging through various continents from Asia to Africa (Patel *et al.,* 2014). It mostly grows in tropical and warm temperate regions and is distributed throughout the forests of India and Sri Lanka. It is a shrub 3-8 ft. High, scarcely woody not much branched, stems bluntly quadrangular, young parts usually glabrous. Leaves often some ternate as well as opposite (passing into bracts above), sometimes reaching as much as 11inch. It's flowering and fruiting period occurs during July- November.

Ethnobotanical uses

Traditionally *Rotheca serrata* plant finds wide applicability as ethno medicines among the local people of Assam. The leaf and root is a good source of drugs for diseases including asthma, fever, bronchitis, cholera, dropsy, eye diseases, bodyache, inflammations, tuberculosis, ophthalmia, rheumatism, snakebite, malaria, ulcers and wounds (Vidya *et al.,* 2007). Owing to its biological activities like anti-inflammatory and antipyretic activities, the use of *R. serrata* has been reported for treating diseases

as typhoid, cancer, jaundice and hypertension (Mukesh *et al.* 2012). Saha *et al.* (2012) and Kar *et al.* (2014) informed about the analgesic and anti-diabetic potentials of its leaves.

Systematic position

Kingdom: Plantae

Clade: Angiosperm

Order: Lamiales

Family: Lamiaceae

Genus: *Rotheca*

Species: *Rotheca serrata* (L.) Steane & Mabb.

Local Name: Nangol bhanga

Figure: 4.2. *Rotheca serrata* (L.) Steane & Mabb.

4.3.3. *Blumea lanceolaria* (Roxb.) Druce

Botanical Description

Blumea lanceolaria (Roxb.) Druce locally known as "Jwglaori" in Assam is a tropical perennial herb belonging to the family Asteraceae. It is found in different parts of North-East India. It has serrate elongated leaves; racemose cyme inflorescence and head, and yellow colored flowers and is mostly available during the months from February to April. The plant is utilized in the preparation of traditional medicines (Pandit *et al.,* 1996). The edible leaves possess aromatic smell but are spicy to taste.

Ethnobotanical uses

In rural Assam, leaves are used to alleviate coughs (Saikia *et al.,* 2017). Sawmliana (2003) and Chawngkunga (2005) both acknowledged the plant's effectiveness in the treatment of cancer and ulcers. An oral decoction of the leaves is used in Mizoram, North East India, to cure wounds, diarrhoea and stomach ulcers (Rai and Lalramnghinglova, 2010). The existence of analgesic, antipyretic and anti-inflammatory property is mentioned in several publications from India as being useful in the treatment of bronchitis (Jha and Verma, 1996) and haemorrhoids (Pandit *et al.,* 1996).

Systematic position

Kingdom: Plantae

Clade: Angiosperm

Order: Asterales

Family: Asteraceae

Genus: *Blumea*

Species: *Blumea lanceolaria* (Roxb.) Druce

Local Name: Jwglaori

Figure: 4.3. *Blumea lanceolaria* (Roxb.) Druce

4.4. MOLECULAR STUDIES

Over 80% of the world's population relies on herbal medicine to meet their basic health care needs. However, this widespread popularity is counterbalanced with the lack of relevant studies to authenticate the herbal plants. Herbal plants have a long history of use although there have recently been worries about their validity. Traditionally, plant species identification is primarily dependent on physical characteristics. Identification based on physical characteristics is not always accurate and efficient. Authentication utilising DNA barcoding produced more reliable and accurate results than morphological identification. DNA barcoding technique should be used to identify and verify herbal plants. In plants, rbcL, matK, psbAtrnH, rpoC1 and ITS2 regions are often utilised as DNA barcodes. Among them, ITS2 and rbcL regions have been demonstrated to be more successful and useful in species discrimination among families such as Asteraceae, Rutaceae, Rosaceae and Araliaceae. Based on this, we have selected rbcL and ITS (ITS1 and ITS2) for the amplification of the plant species. Each plant species were analyzed using both rbcL and ITS plant bar-coding markers with an aim of using at least 2 markers for identification. For single plant species, a sample was considered authentic if the listed or given name matched scientific name as

identified by blast hits produced by ITS and rbcL. As long as the species was identified by either of the markers it was recorded as present.

4.4.1. DNA Isolation, PCR amplification and Sequence analysis

The genomic DNA of the plant species was successfully isolated. Amplification of the gDNA with four gene loci- rbcL, ITS1, ITS2 and matK was also carried out. Among them, rbcL, ITS1 and ITS2 showed PCR amplification and hence they had been used in the current study. PCR amplification of each of the plant gDNA with three gene loci- rbcL, ITS1 and ITS2 were shown in Figure. 4.4. with their product length. BLAST analysis was used to compare the obtained sequences to the NCBI sequences. This comparison brought out the genus identification and in some cases species identification. Sequences of rbcL and ITS reference material gene were analyzed for homology using BlastN search program and very closely related

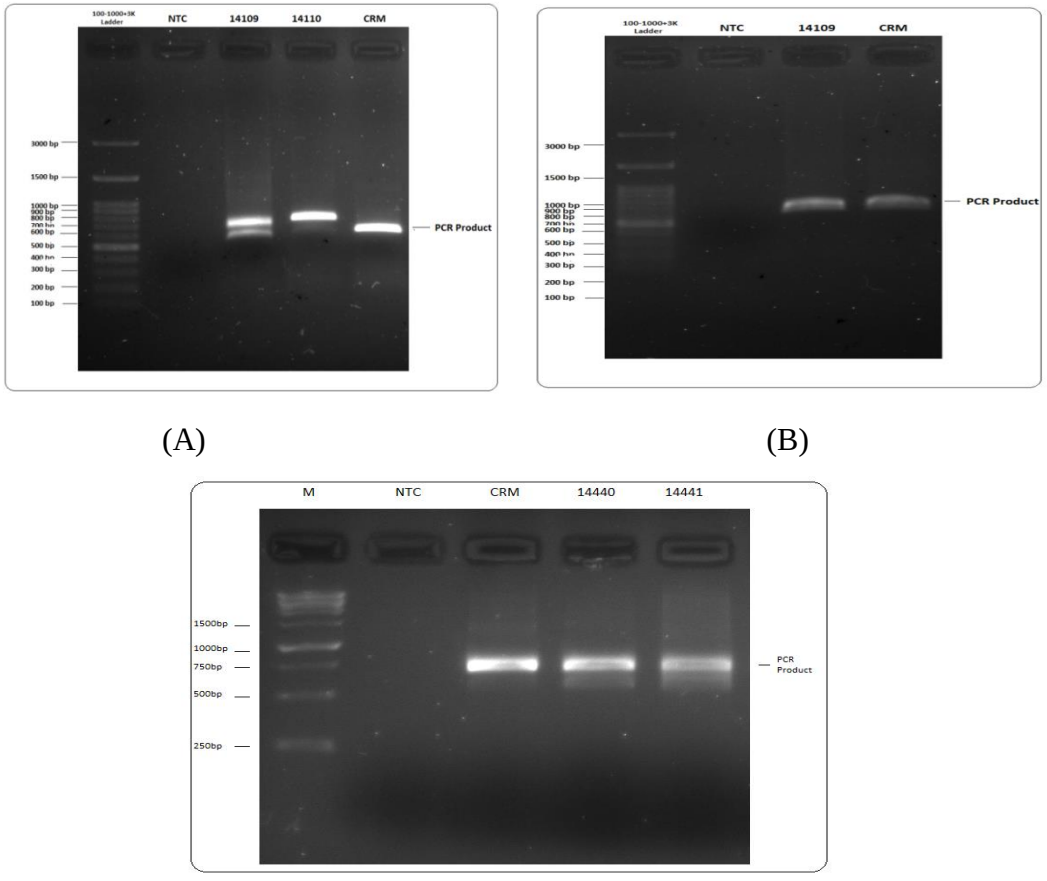

Plate 4.4: 1.5% agarose gels showing amplified products of rbcL (A) for *Z. oxyphyllum*, ITS1 (B) for *R. serrata* and ITS2 (C) for *B. lanceolaria*.

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species showing high level of identity (90-100%) was considered as closest match. Table: 4.3 shows the result of the BLAST analysis of the plant species. It was observed that the rbcL region of *Z. oxyphyllum,* ITS1 of *R. serrata and* the ITS2 region of *B. lanceolaria* showed 100%, 91.25% and 99.50% sequence similarity with the reference sequences respectively. Sequence coverage of all the plant samples is same (100%). From the table, it was also observed that the ITS region has more viability and potentiality in identification process than rbcL as rbcL gene could confirm the identity of only one plant upto the generic level.

Table 4.3: Identification of the plant species using BLASTn.

Sample Species	**Genome region**	**Taxonomic level**	**BLAST similarity**	**Sequence coverage**	**E-Value**	**Similar species with Accession No.**
Z.oxyphyllum	rbcL	Genus	100%	100%	0.0	*Zanthoxylum ailanthoides* (MW478808.1)
R. serrata	ITS1	Genus	91.25%	100%	0.0	*Rotheca incisa* (U77750.1)
B. lanceolaria	ITS2	Species level	99.50%	100%	0.0	*Blumea lanceolaria* (KP052664.1)

4.4.2. Phylogenetic analysis

The phylogenetic relationship of each plant species was constructed using with Kimura 2-parameter model using MEGA 11.00 for all three DNA barcode loci- rbcL, ITS1 and ITS2. From the constructed phylogenetic trees for rbcL, *Z. oxyphyllum* was found to be closely related to *Zanthoxylum ailanthoides* and *Metrodorea nigra* by sharing a common ancestor (Figure: 4.5). Through this study, it was observed that the rbcL region of *Zanthoxylum oxyphyllum* (Isolates 14109) showed 100% sequence similarity with type *Zanthoxylum ailanthoides* (Accession number LC694632.1). The phylogenetic tree confirmed the results and the potent isolates (Isolates number 14109) are shorted out into groups along with their closest relatives retrieved from

NCBI GenBank. The result indicated that all species are closely related to the members of genus *Zanthoxylum sp.*

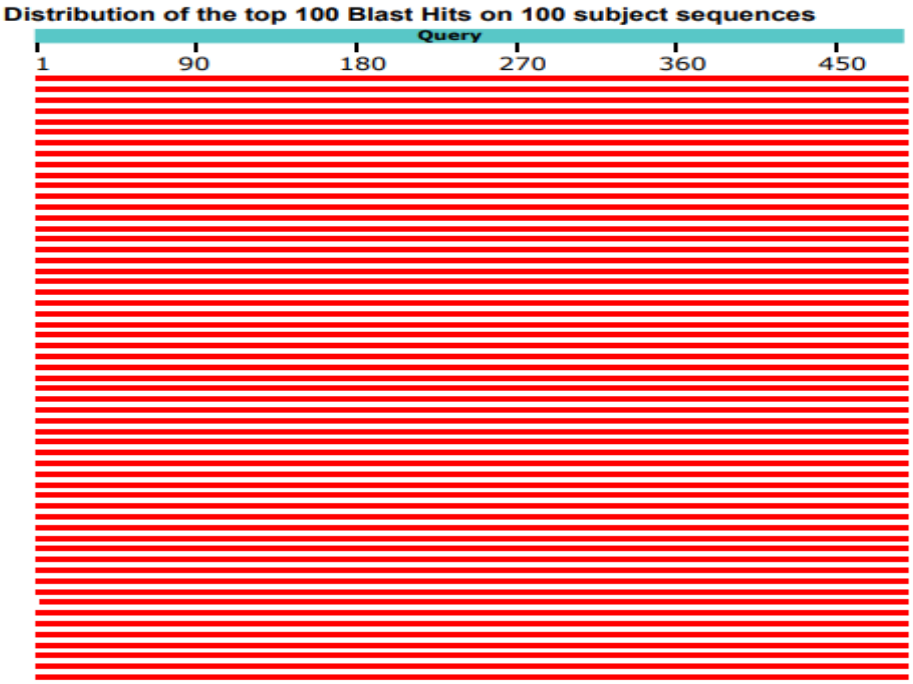


Plate 4.5: Alignment view of *Z. oxyphyllum*. using combination of NCBI GenBank

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Zanthoxylum ailanthoides var. inerme TF<JPN>:AM_CCJ06 chloroplast rbcL gene for ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial cds	Zanthoxylum ailanthoides var. inerme	902	902	100%	0.0	100.00%	647	LC694632.1
Ptelea trifoliata voucher 81176HIM ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	Ptelea trifoliata	896	896	100%	0.0	99.80%	552	MG247115.1
Zanthoxylum myriacanthum isolate SCBGP209_1 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	Zanthoxylum myriacanthum	896	896	100%	0.0	99.80%	562	KP094536.1
Zanthoxylum caribaeum isolate PEEC157 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	Zanthoxylum caribaeum	891	891	99%	0.0	99.79%	582	MG718536.1
Metrodorea nigra isolate PEEC093 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	Metrodorea nigra	891	891	100%	0.0	99.59%	665	MG718478.1
Geijera cauliflora voucher M.J. Bayly 2086 (MEL) ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	Geijera cauliflora	891	891	99%	0.0	99.79%	1272	JN987107.1
Zanthoxylum gilletii voucher PM5537 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	Zanthoxylum gilletii	887	887	100%	0.0	99.39%	550	KC628645.1

Plate 4.6: Sequences of *Z. oxyphyllum* showing significant alignments

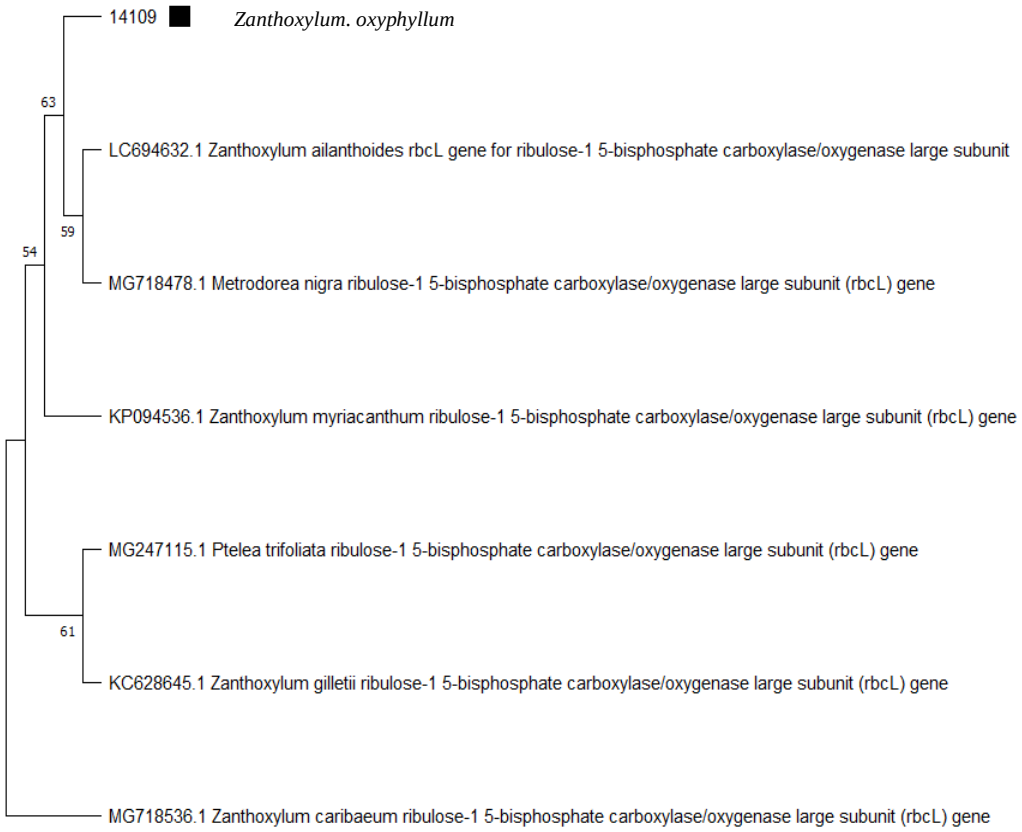


Figure 4.1: Maximum-Likelihood phylogenetic tree of similarity between *Z. oxyphyllum* and the closest species generated by Kimura 2 parameter using rbcL gene. The samples of our study are marked by black squares next to their names.

In case of ITS1 region sequencing of *Rotheca serrata*, the closest species was found to be *Rotheca incisa* with 91.25% similarity. The results showed that the ITS1 marker used and plants studied in this study belonged to the genus Rotheca. *Rotheca incisa* and *Rotheca myricoides* was found to be the most closely related species with a higher extent in comparisons to other species indicating a shared common ancestor which is evident from the highest sequence similarity as seen in BLAST analysis.

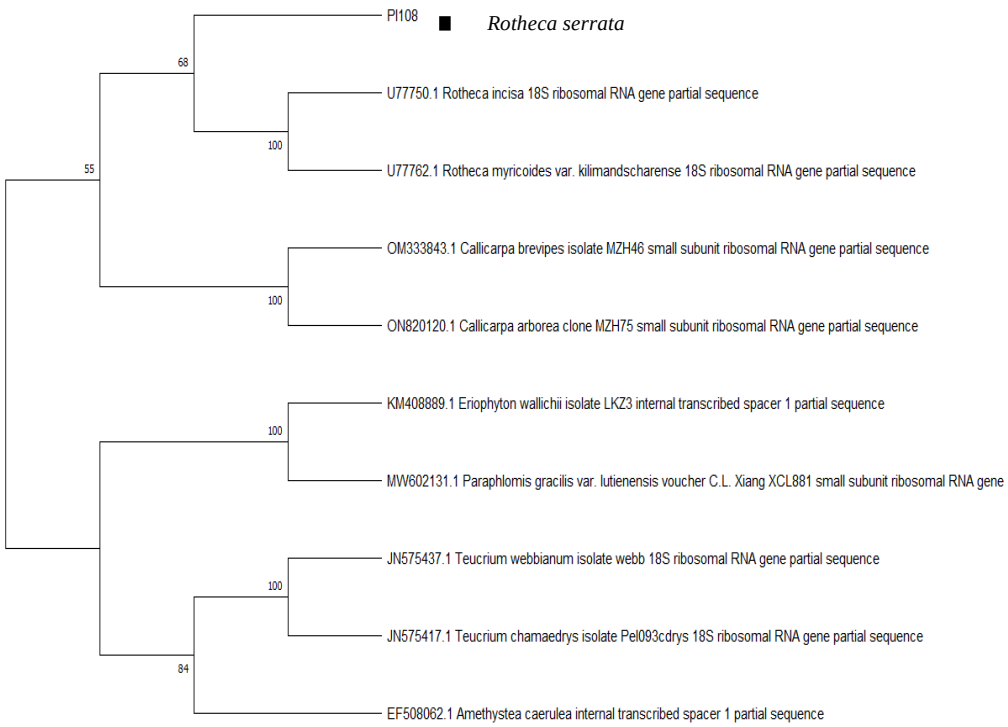


Figure 4.2: Maximum-Likelihood phylogenetic tree of similarity between *Rotheca serrata* and the closest species generated by Kimura 2 parameter using ITS1 gene. The samples of our study are marked by black squares next to their names.

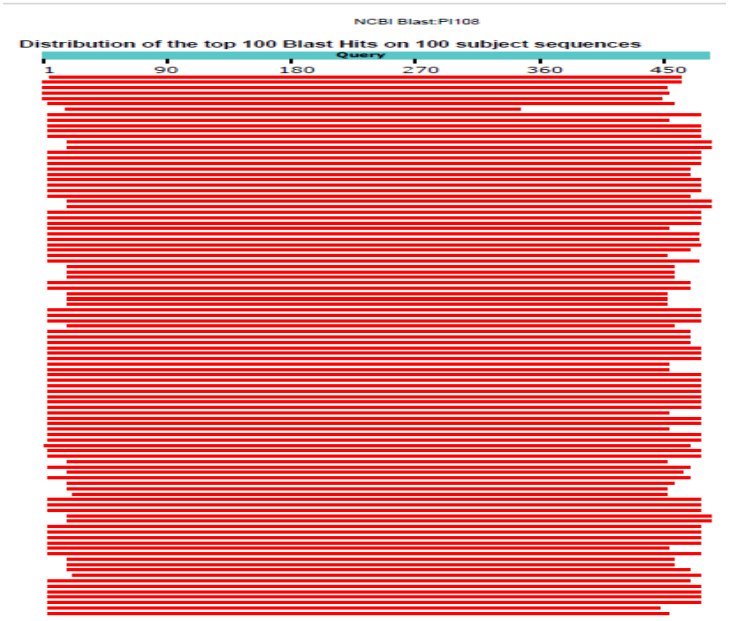


Plate 4.7: Alignment view of *R. serrata* using combination of NCBI GenBank**.**

Descriptions

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Rotheca incisa 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	Rotheca incisa	628	628	94%	3e-175	91.13%	704	U77750.1
Rotheca myricoides var. kilimandscharense 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	Rotheca myricoides var. kilimandscharense	536	536	93%	2e-147	86.64%	696	U77762.1
Callicarpa brevipes isolate MZH46 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	Callicarpa brevipes	457	457	97%	1e-123	84.50%	857	OM333843.1
Eriophyton wallichii isolate LKZ3 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	Eriophyton wallichii	442	442	96%	4e-119	84.24%	596	KM408889.1
Teucrium webbianum isolate webb 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	Teucrium webbianum	442	442	96%	4e-119	84.36%	680	JN575437.1
Teucrium chamaedrys isolate Pel093cdrys 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	Teucrium chamaedrys	440	440	96%	1e-118	84.42%	688	JN575417.1
Amethystea caerulea internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	Amethystea caerulea	440	440	97%	1e-118	83.85%	604	EF508062.1
Paraphlomis gracilis var. lutienensis voucher C.L. Xiang XCL881 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	Paraphlomis gracilis var. lutienensis	436	436	93%	2e-117	84.70%	768	MW602131.1
Callicarpa arborea clone MZH75 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	Callicarpa arborea	429	429	92%	3e-115	83.98%	792	ON820120.1

Plate 4.8: Sequences of *R. serrata* showing significant alignments.

For ITS2 gene, the BLAST result on NCBI showed that the nucleotide sequences obtained were highly similar to those of certain species of Blumea. *Blumea lanceolaria* (Accession number KP052664.1), *Blumea megacephala* (Accession number MW116521.1), *Blumea balsamifera* (Accession number KF443297.1) and *Blumea virens* (Accession number EF210957.1) with similarity ranging from 90.07% to 99.50%. This finding indicates that the isolates (Isolate number 14110) found in the present study are closely related with the members of genus *Blumea* (Figure: 4.7). The ITS2 region of our sample specimen *Blumea lanceolaria* showed the highest percent identity i.e., 99.50% sequence similarity with the reference sequences of type *Blumea lanceolaria* **(**KP052664.1**)** species in NCBI and interestingly falls in one group. From this result, it indicates that this taxonomically identified plant *Blumea lanceolaria* was confirmed the molecular identification at the species level through DNA barcoding.

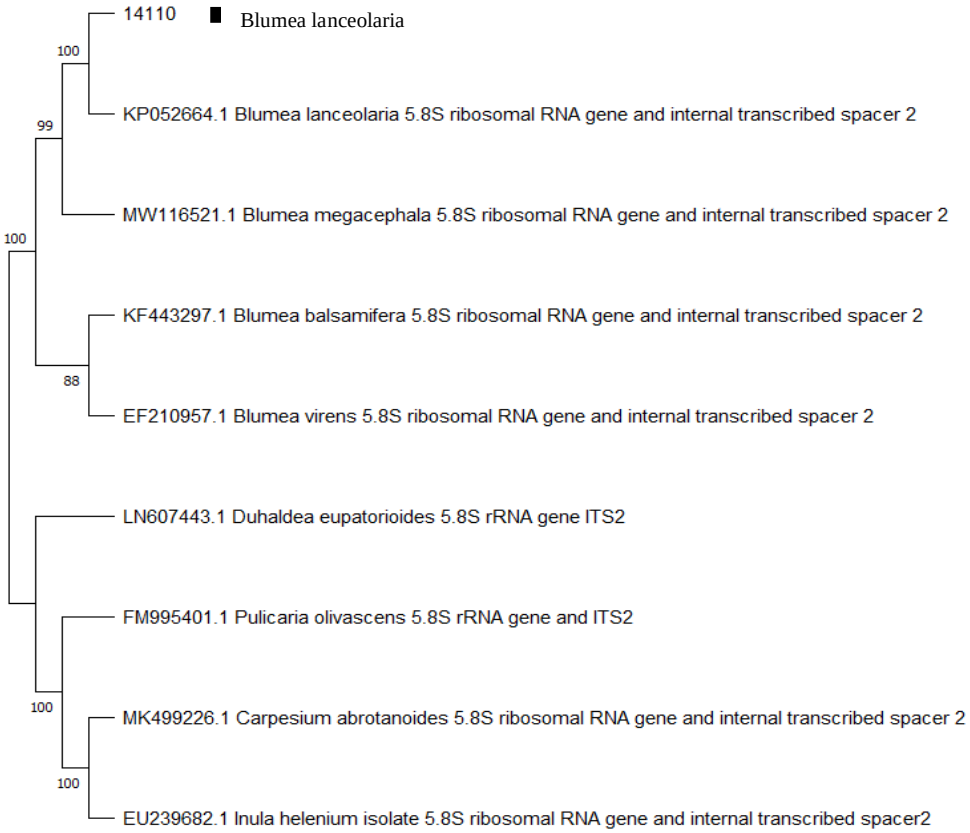


Figure 4.3. Maximum-Likelihood phylogenetic tree of similarity between *Blumea lanceolaria* and the closest species generated by Kimura 2 parameter using ITS2 gene. The samples of our study are marked by black squares next to their names.

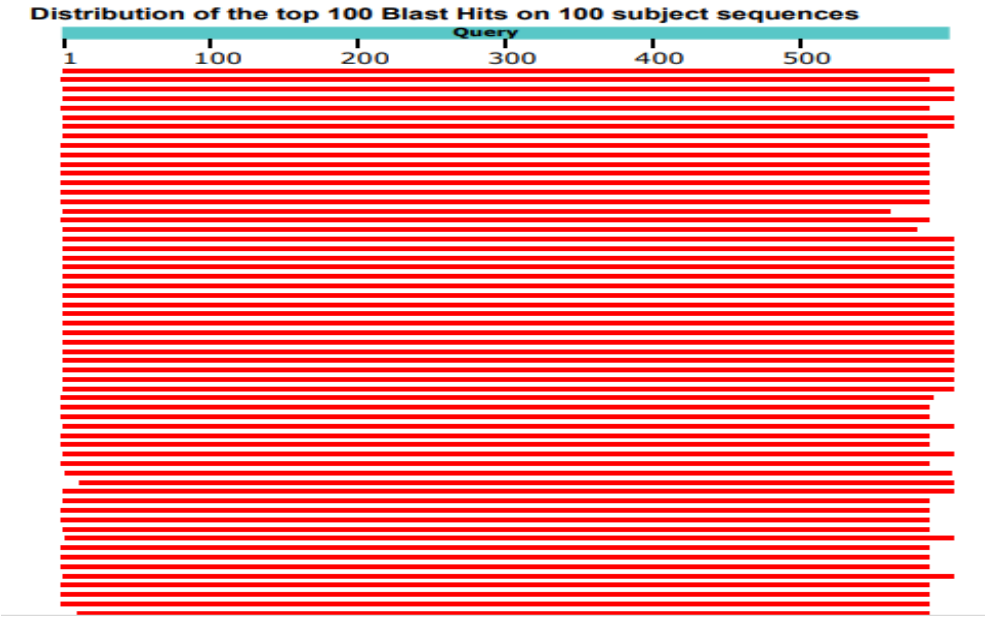


Plate 4.9: Alignment view of *B. lanceolaria* using combination of NCBI GenBank.

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Blumea lanceolaria voucher Yuxinpang CATAS00170 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	Blumea lanceolaria	1088	1088	100%	0.0	99.50%	722	KP052664.1
Blumea megacephala voucher KUN:Nie1986 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	Blumea megacephala	1007	1007	100%	0.0	96.99%	715	MW116521.1
Blumea balsamifera voucher ZY130605 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	Blumea balsamifera	793	793	100%	0.0	90.55%	752	KF443297.1
Blumea virens voucher Pornpongrungrueng 378 (AAU) internal transcribed spacer 1, partial sequence; and 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence	Blumea virens	756	756	97%	0.0	90.07%	637	EF210957.1
Duhaldea eupatorioides genomic DNA sequence contains ITS1, 5.8S rRNA gene, ITS2, specimen voucher Pornpongrungrueng 488 (AAU)	Duhaldea eupatorioides	676	676	100%	0.0	87.19%	710	LN607443.1
Pulicaria olivascens 18S rRNA gene (partial), ITS1, 5.8S rRNA gene and ITS2, specimen voucher B:Rechinger 32023	Pulicaria olivascens	656	656	100%	0.0	86.67%	797	FM995401.1
Carpesium abrotanoides voucher Y.L. Xu 319-1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	Carpesium abrotanoides	649	649	100%	0.0	86.40%	660	MK499226.1
Inula helenium isolate I1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	Inula helenium	649	649	100%	0.0	86.47%	716	EU239682.1

Plate 4.10: Sequences of *B. lanceolaria* showing significant alignments

4.5. NUTRITIONAL VALUES

From ancient times, medicinal plants with multifunctional properties have been used in medicine, functional foods and nutraceutical food components. The proximate composition of any food reflects its nutritive value. As ethnomedicinal plants are also known for their food value among the tribals of this region, nutritional study of the three ethnomedicinal plants were performed to evaluate their potent contribution in field of nutritional.

4.5.1. PROXIMATE COMPOSITION

The proximate composition of leaves of *Zanthoxylum oxyphyllum*, *Rotheca serrata* and *Blumea lanceolaria* are presented in Table: 4.4. Results showed that the moisture content value of the leaves ranged from 44.97 to 59.4 g/100g. The highest moisture content value was obtained from *B. lanceolaria* (59.4±0.50 g/100g) while the least value was obtained in *R. serrata* (44.97±0.06 g/100g). Of the three leaves, *R. serrata* had the highest content of total ash (12.02±0.06 g/100g), crude fibre content

(17.43±0.05 g/100g), carbohydrate content (23.22±0.45 g/100g) and energy content (189.1±1.04 kcal/100g) but low moisture content. Whereas *Z. oxyphyllum* had the highest protein content (24.30±0.34 g/100g) and lowest content of fat (2.00 ±0.10 g/100g), ash (10.37±0.41 g/100g), fibre (14.43±0.52 g/100g) and carbohydrate content (10.98±0.12 g/100g). *Blumea lanceolaria* demonstrated the highest moisture and fat content (59.4±0.50 and 4±0.10 g/100g respectively) but lowest protein content (10.75±0.11 g/100g).

Table 4.4: Proximate composition analysis of studied plants (g/100g) dry weight

Sl.No	Parameters	*Z. oxyphyllum*	*R. serrata*	*B. lanceolaria*
1.	Moisture	50.94±1.59	44.97±0.06	59.4±0.50
2.	Protein	24.30±0.34	15.73 ±0.05	10.75±0.11
3.	Fat	2.00 ±0.10	3.3 ±0.26	4±0.10
4.	Ash	10.37±0.41	12.02 ±0.06	10.43±0.40
5.	Fibre	14.43±0.52	17.43 ±0.05	15.46±0.35
6.	Carbohydrate	10.98±0.12	23.22±0.45	15.61±0.32
7.	Energy (kcal/100g)	160.01±0.09	189.1 ±1.04	140.33±1.15

Note: Values are in triplicate mean ±SD

4.5.2. MINERAL COMPOSITION

Mineral composition of leaves of *Z. oxyphyllum*, *R. serrata* and *B. lanceolaria* were analyzed for 10 mineral elements of which two were heavy metal. The result of mineral composition were calculated in mg/100g dry matter and compared to the daily recommended dietary allowance (RDA) (DRI, 2004) for both male and female human adults aged 19-30 years, recommended by the Food and Nutrition Board, Institute of Medicine, National Academics, US.

Results showed that *Z. oxyphyllum* was highest in Potassium content compared to the other two plant leaves. The highest amount of mineral element Potassium and Calcium found in *Z. oxyphyllum* and *B. lanceolaria* respectively. Macro minerals in *Z. oxyphyllum* including Potassium, Calcium, Sodium and Magnesium can fulfill about RDA of 0.17% to 4.13% of daily RDA requirement in both human male and female. On the contrary, micro nutrients such as Manganese, Zinc, Iron and Copper can mitigate nearly about daily RDA mineral requirement of 0.15% to 82% in male and 0.20% to 112.75% in female. Heavy metals such as Nickel and Chromium can fulfil

5% and 33.33% of the daily RDA in both human male and female. In *R. serrata*, macro minerals including Potassium, Calcium, Sodium and Magnesium can fulfill around 0.19% to 6.27% RDA in both human male and female. On the other hand, micro nutrients such as Manganese, Zinc, Iron and Copper can meet RDA of around 0.39 % to 42.87 % in male and 0.51% to 27.50% in female. Macro minerals in *B. lanceolaria* including Potassium, Calcium, Sodium and Magnesium can fulfill approximately RDA of about 0.19% to 6.63% of daily RDA requirement in both male and female. Among micro nutrients Manganese, Zinc, Iron and Copper can meet RDA of around 0.34% to 174.44% in male and 0.44% to 174.44% in female. Heavy metals such as Nickel can fulfil the RDA requirement of 47% in both male and female. Chromium was absent in both *R. serrata* and *B. lanceolaria*.

Table 4.5. Recommended intake of essential minerals per day compared with *Z. oxyphyllum*.

Sl. No	**Minerals**	***Z. oxyphyllum* (mg/100g)**	**Intake recommended for 25 year old adults (mg per day)**		**% fulfilment of recommended intake**	
			Male	Female	Male	Female
1.	Sodium	2.57 ± 0.02	1500	1500	0.17	0.17
2.	Potassium	104.7 ± 0.20	4700	4700	2.22	2.22
3.	Magnesium	4.39 ± 0.09	400	310	1.09	1.41
4.	Calcium	41.38 ± 0.01	1000	1000	4.13	4.13
5.	Manganese	0.66 ± 0.01	420	320	0.15	0.20
6.	Zinc	9.02 ± 0.02	11	8	82	112.75
7.	Iron	3.08 ± 0.02	8	18	38.50	17.11
8.	Copper	0.06 ± 0.02	0.9	0.9	6.66	6.66
9.	Nickel	0.05 ± 0.03	1.0	1.0	5	5
10.	Chromium	0.01 ± 0.005	0.03	0.03	33.33	33.33

Note: Values are in triplicate mean ±SD

Table 4.6. Recommended intake of essential minerals per day compared with *R. serrata*.

Sl. No	**Minerals**	***R. serrata* (mg/100g)**	**Intake recommended for 25 year old adults (mg per day)**		**% fulfilment of recommended intake**	
			Male	Female	Male	Female
1.	Sodium	2.90 ± 0.1	1500	1500	0.19	0.19
2.	Potassium	47.76 ± 0.04	4700	4700	1.01	1.01
3.	Magnesium	6.53 ± 0.32	400	310	1.63	2.10

4.	Calcium	62.73 ± 0.20	1000	1000	6.27	6.27
5.	Manganese	1.65 ± 0.05	420	320	0.39	0.51
6.	Zinc	2.20 ± 0.02	11	8	20	27.50
7.	Iron	3.43 ± 0.40	8	18	42.87	19.05
8.	Copper	0.09 ± 0.01	0.9	0.9	10	10
9.	Nickel	0.01± 0.02	1.0	1.0	1	1
10.	Chromium	-	0.03	0.03	-	-

Note: Values are in triplicate mean ±SD

Table 4.7. Recommended intake of essential minerals per day compared with *B. lanceolaria.*

Sl. No	**Minerals**	***B. lanceolaria* (mg/100g)**	**Intake recommended for 25 year old adults (mg per day)**		**% fulfilment of recommended intake**	
			Male	Female	Male	Female
1.	Sodium	2.92 ± 0.05	1500	1500	0.19	0.19
2.	Potassium	44.80 ± 0.18	4700	4700	0.95	0.95
3.	Magnesium	5.30 ± 0.04	400	310	1.32	1.70
4.	Calcium	66.37 ± 0.06	1000	1000	6.63	6.63
5.	Manganese	1.43 ± 0.04	420	320	0.34	0.44
6.	Zinc	5.17 ± 0.03	11	8	47	64.62
7.	Iron	1.39 ± 0.02	8	18	17.37	7.72
8.	Copper	1.57 ± 0.05	0.9	0.9	174.44	174.44
9.	Nickel	0.47 ± 0.03	1.0	1.0	47	47
10.	Chromium	-	0.03	0.03	-	-

Note: Values are in triplicate mean ±SD

4.5.3. AMINO ACID COMPOSITION

Food plants are a rich source of amino acids (AAs) and each AA's specific content is crucial for good nutrition. Therefore, investigating the nutritional significance of food plants is essential.The amino acid composition analysis of *Z. oxyphyllum* is shown in Table: 4.8. The UPLC chromatogram of amino acid is shown in Figure: 4.9. Most of the essential amino acids were found to be present in varied amount. Phenyalanine, leucine and lysine constituted the main essential amino acids in the sample. Of the non essential amino acid arginine, proline and tyrosine was the main. Among the essential amino acid, histidine and among the non essential amino acid cysteine was not detected in the sample. Phenylalanine was the most abundant among the essential and arginine

among the non essential amino acid recorded in *Z. oxyphyllum.* Non essential amino acids amounted to a total of 59.56% of the total amino acid. Essential amino acids constitute 40.37% of the total detected amino acids. The amino acid scores of *Z. oxyphyllum* is presented in Table: 4.9. The maximum amino acid score was found for phenylalanine and tyrosine with 325%. Methionine was found to be the limiting amino acid in the sample with an amino acid score of 42%. Adequate amounts of both essential and non essential amino acids were found in the ratio of 1.40.

Table 4.8. Amino acid profile of *Z. oxyphyllum* (in mg/100g).

Essential Amino Acid	**Amino acid composition**	**% of total Amino acid**	**Non-Essential Amino Acid**	**Amino acid composition**	**% of total Amino acid**
Threonine	2.90 ± 0.02	5.12	Arginine	7.47 ± 0.03	13.20
Valine	2.57 ± 0.02	4.54	Asparagine	2.37 ± 0.02	4.19
Methionine	0.59 ± 0.01	1.04	Serine	3.02 ± 0.01	5.34
Isoleucine	1.28 ± 0.005	2.26	Glutamine	1.07 ± 0.01	1.89
Leucine	4.51 ± 0.03	7.97	Proline	6.63 ± 0.02	11.72
Phenylalanine	7.08 ± 0.02	12.51	Glycine	5.37 ± 0.03	9.49
Histidine	ND	ND	Cysteine	ND	ND
Lysine	3.92 ±0.06	6.93	Alanine	3.28 ± 0.02	5.80
			Tyrosine	4.49 ± 0.01	7.93
Total EAA	**22.85±0.16**	**40.37**	**Total NEAA**	**33.70±0.15**	**59.56**

Note: Values are expressed as mean ± S.D of triplicates measurement. ND: not detected

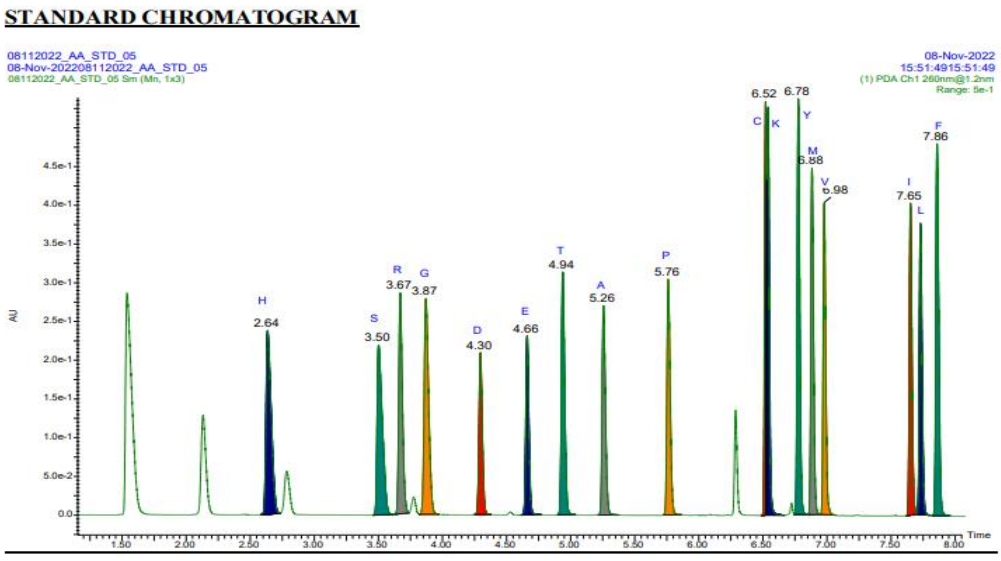


Figure 4.4: UPLC chromatogram of amino acid (Standard)

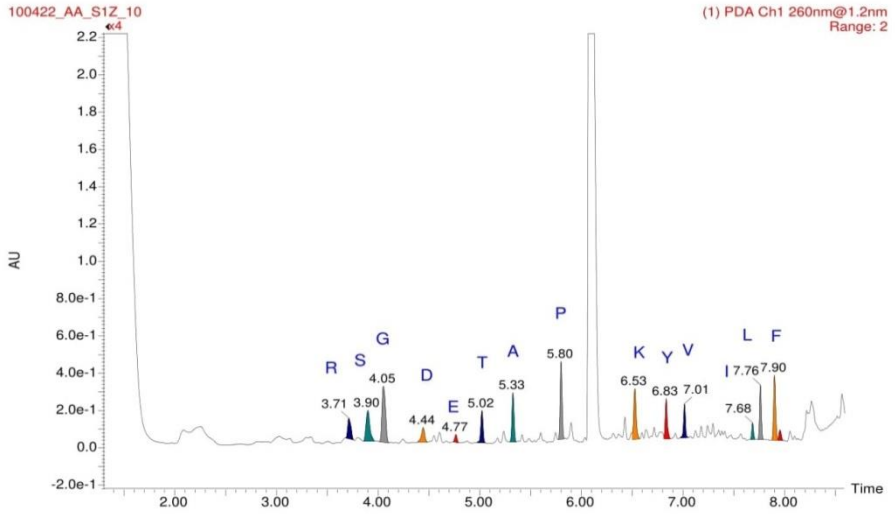


Figure 4.5: UPLC chromatogram of amino acid in *Z. oxyphyllum*

Table 4.9: Amino acid score of *Z. oxyphyllum* based on FAO/WHO/UNU (1985) consultation pattern of requirement for a 2- 5 year preschool child.

Amino acids	FAO/WHO/UNU (2007) (mg/g protein)	Chemical score (%)
Histidine	19	ND
Isoleucine	28	81
Leucine	66	121
Lysine	58	119
Methionine	25	42
Phenylalanine +	63	325

tyrosine		
Threonine	34	151
Tryptophan	11	-
Valine	35	130
Amino Acid Score		42
Limiting amino acid		**Methionine**

The UPLC chromatogram of amino acid for *R. serrata* is shown in Figure: 4.6. Table: 4.10 shows the result of amino acid analysis of *R. serrata*.

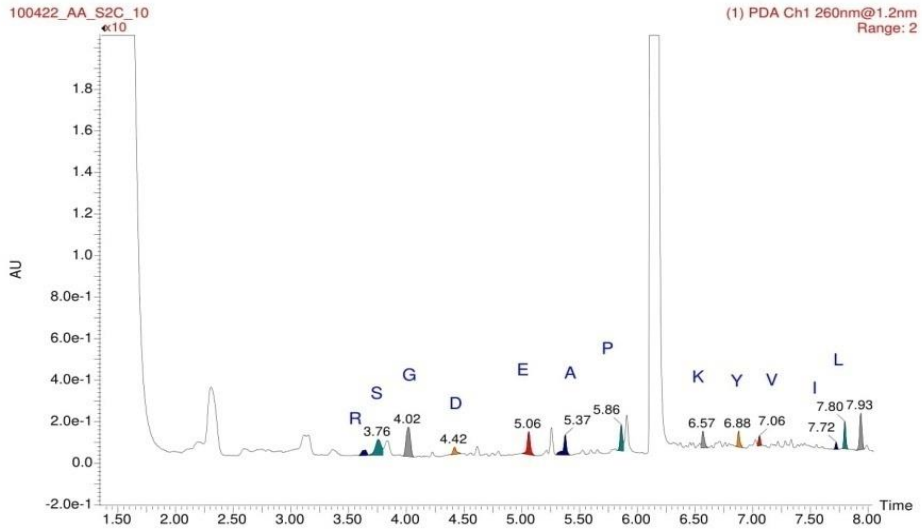


Figure 4.6. UPLC chromatogram of amino acid in *R. serrata*

Phenylalanine constituted the main essential amino acid followed by leucine in the sample. Threonine, methionine and histidine were not detected in *R. serrata*. Among the non essential amino acid, cysteine was not detected. Glutamine constituted the main non essential amino acid in the *R. serrata*. Non essential amino acids amounted to a total of 64.44% of the total amino acid. Essential amino acids constitute 35.5% of the total detected amino acids. The ratio between non essential and essential amino acid was found to be 1.81. Table: 4.11 represent the amino acid scoring pattern of *R. serrata*. The maximum amino acid score is detected in phenylalanine and tyrosine. The amino acid score was 57% and valine was the limiting amino acid in *R. serrata*.

Table 4.10: Amino acid profile of *R. serrata* (in mg/100g).

Essential Amino Acid	Amino acid composition	% of total Amino acid	Non-Essential Amino Acid	Amino acid composition	% of total Amino acid
Threonine	ND	ND	Arginine	0.30 ± 0.01	3.18
Valine	0.19 ± 0.01	2.01	Asparagine	0.28 ± 0.01	2.96
Methionine	ND	ND	Serine	0.83 ± 0.02	8.80
Isoleucine	0.17 ± 0.005	1.80	Glutamine	1.50 ± 0.01	15.90
Leucine	0.84 ± 0.001	8.90	Proline	0.91 ± 0.01	9.65
Phenylalanine	1.76 ± 0.03	18.66	Glycine	1.09 ± 0.01	11.55
Histidine	ND	ND	Cysteine	ND	ND
Lysine	0.39 ± 0.02	4.13	Alanine	0.53 ± 0.05	5.62
			Tyrosine	0.64 ± 0.02	6.78
Total EAA	**3.35±0.06**	**35.5**	**Total NEAA**	**6.08 ± 0.14**	**64.44**

Note: Values are expressed as mean ± S.D of triplicates measurement. ND: not detected

Table 4.11. Amino acid score of *R. serrata* based on FAO/WHO/UNU (1985) consultation pattern of requirement for a 2- 5 year preschool child.

Amino acids	FAO/WHO/UNU (2007) (mg/g protein)	Chemical score (%)
Histidine	19	ND
Isoleucine	28	64
Leucine	66	135
Lysine	58	71
Methionine	25	ND
Phenylalanine + tyrosine	63	404
Threonine	34	ND
Tryptophan	11	-
Valine	35	57
Amino Acid Score		57
Limiting amino acid		**Valine**

Table 4.12. Amino acid profile of *B. lanceolaria* (in mg/100g).

Essential Amino Acid	Amino acid composition	% of total Amino acid	Non-Essential Amino Acid	Amino acid composition	% of total Amino acid
Threonine	0.64 ± 0.01	3.16	Arginine	1.21 ± 0.02	5.99
Valine	0.66 ± 0.02	3.26	Asparagine	2.21 ± 0.03	10.94

Methionine	0.70 ± 0.01	3.46	Serine	0.28 ± 0.005	1.38
Isoleucine	0.47 ± 0.02	2.32	Glutamine	1.83 ± 0.02	9.06
Leucine	1.12 ± 0.02	5.54	Proline	0.70 ± 0.01	3.46
Phenylalanine	5.77 ± 0.03	28.57	Glycine	0.68 ± 0.02	3.36
Histidine	0.28 ± 0.005	1.38	Cysteine	ND	ND
Lysine	0.90 ± 0.01	4.45	Alanine	0.89 ± 0.01	4.40
			Tyrosine	1.85 ± 0.03	9.16
Total EAA	**10.54±0.12**	**52.14**	**Total NEAA**	**9.65±0.14**	**47.75**

Note: Values are expressed as mean ± S.D of triplicates measurement. ND: not detected

All essential amino acids were present in *B. lanceolaria* including threonine, histidine and methionine (Table 4.12). Phenylalanine and asparigine were the most abundant among the essential and non essential amino acids respectively. Cysteine was not detected in *B. lanceolaria*. Non essential amino acids amounted to a total of 47.75% of the total amino acid. Essential amino acids constituted 52.14**%** of the total investigated amino acids. The ratio between non essential to essential amino acid was 1.09. The UPLC chromatogram of amino acid for *B. lanceolaria* is shown in Figure: 4.7. Table: 4.13 represent the amino acid scoring pattern of *B. lanceolaria*. The ratio between the non essential and essential amino acid was 1.09. The highest amino acid score was attained by Phenylalanine + tyrosine with 599%. Leucine with an amino acid score of 64% was found to be the limiting amino acid in *B. lanceolaria*.

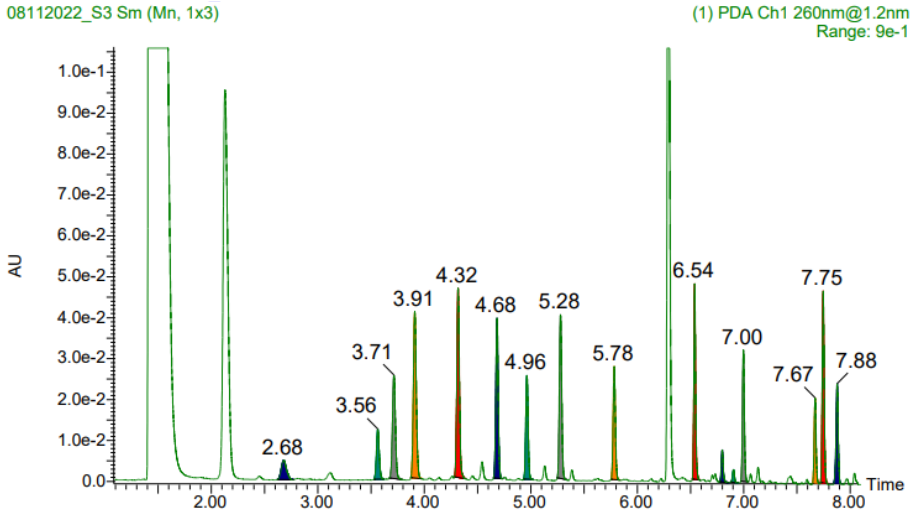


Figure 4.7. UPLC chromatogram of amino acid in *B. lanceolaria.*

Table 4.13. Amino acid score of *B. lanceolaria* based on FAO/WHO/UNU (1985) consultation pattern of requirement for a 2- 5 year preschool child.

Amino acids	FAO/WHO/UNU (2007) (mg/g protein)	Chemical score (%)
Histidine	19	73
Isoleucine	28	83
Leucine	66	64
Lysine	58	77
Methionine	25	138
Phenylalanine + tyrosine	63	599
Threonine	34	93
Tryptophan	11	-
Valine	35	93
Amino Acid Score		64
Limiting amino acid		**Leucine**

4.5.4. FATTY ACID ANALYSIS

Fatty acids are extremely important in assessing food nutrition. Fatty acids are significant bioactive molecules that play key functions in complex metabolic pathways. Fatty acids play an important role in pharmacology and illness diagnosis. Fatty acids occur naturally and exist as mixtures of saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) and

they are integral to human diet so it is important to know the properties of fatty acids in any food for nutritional and/or therapeutic standpoints.

GC-MS method is a simple, fast and reliable method developed to identify fatty acid. Fatty acids are specifically identified by this method; it is a useful for food industries, pharma industries and research purposes. Names of fatty acids, chemical formula and molecular weight were obtained from Gas chromatography mass spectrometry (GC-MS) analysis as shown in Tables. Figure: 4.8 shows the GC-MS chromatogram of *Z. oxyphyllum*. In the sample, saturated and unsaturated fatty acids were found. 7 fatty acids were identified in *Z. oxyphyllum*, and in particular could be grouped as 6 saturated and 1 unsaturated. Of the 9 fatty acids in *R. serrata,* 1 unsaturated and 8 saturated fatty acid was observed and in *B. lanceolaria*, 8 fatty acids were found, among them 1 was monounsaturated (Oleic acid) and 7 were saturated fatty acid.

Through the GC-MS analysis of fatty acids, it was proven that ethnomedicinal plants are important source of fatty acids. In all the plants, same types of 7 fatty acids were present.

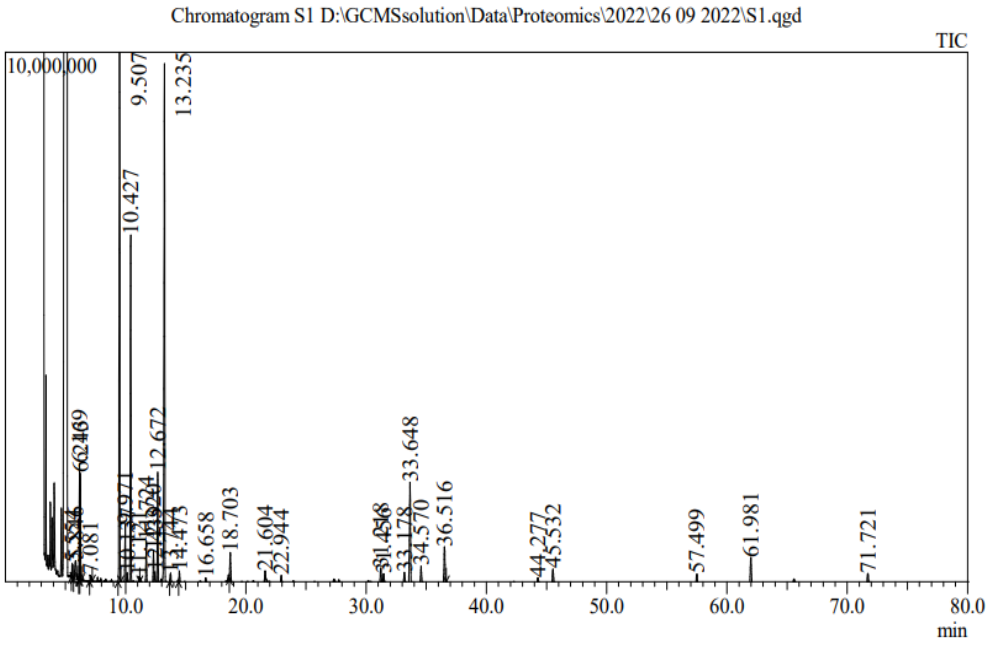


Figure 4.8. GCMS chromatogram of fatty acids in *Z. oxyphyllum*

Table 4.14. Fatty acid identified in *Z. oxyphyllum*.

Sl. No	Name	Chemical Formula	Molecular Weight	Type of Fatty acid
1.	Methoprene acid	$C_{19}H_{36}O_3Si$	340	Saturated
2.	Succinic acid	$C_{10}H_{22}O_4Si_2$	262	Saturated
3.	Mesaconic acid	$C_{11}H_{22}O_4Si_2$	274	Unsaturated
4.	Palmitic acid	$C_{19}H_{40}O_2Si$	328	Saturated
5.	Stearic acid	$C_{21}H_{44}O_2Si$	356	Saturated
6.	Caproic acid	$C_9H_{20}O_2Si$	188	Saturated
7.	Octanoic acid	$C_{11}H_{24}O_2Si$	216	Saturated

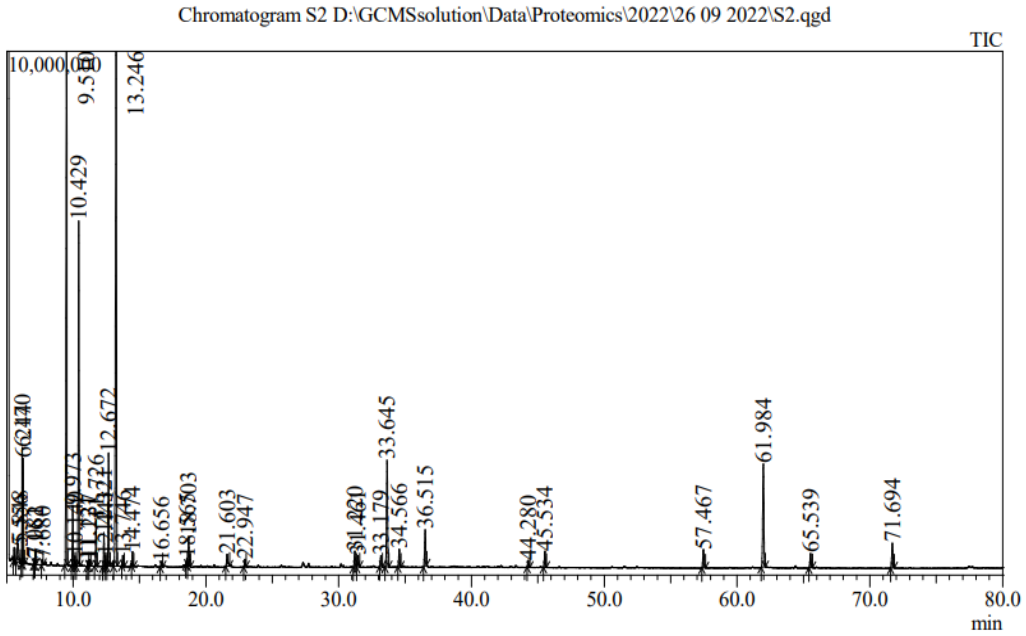


Figure 4.9: GCMS chromatogram of fatty acids in *R. serrata*

Table 4.15. Fatty acid identified in *R. serrata.*

Sl. No	Name	Chemical Formula	Molecular Weight	Type of Fatty acid
1.	Methoprene acid	$C_{19}H_{36}O_3Si$	340	Saturated
2.	Succinic acid	$C_{10}H_{22}O_4Si_2$	262	Saturated
3.	2-Deoxytetronic acid	$C_{13}H_{32}O_4Si_3$	336	-
4.	Mesaconic acid	$C_{11}H_{22}O_4Si_2$	274	Unsaturated
5.	Palmitic acid	$C_{19}H_{40}O_2Si$	328	Saturated
6.	Stearic acid	$C_{21}H_{44}O_2Si$	356	Saturated
7.	Caproic acid	$C_9H_{20}O_2Si$	188	Saturated
8.	Octanoic acid	$C_{11}H_{24}O_2Si$	216	Saturated
9.	Decanoic acid	$C_{13}H_{28}O_2Si$	244	Saturated

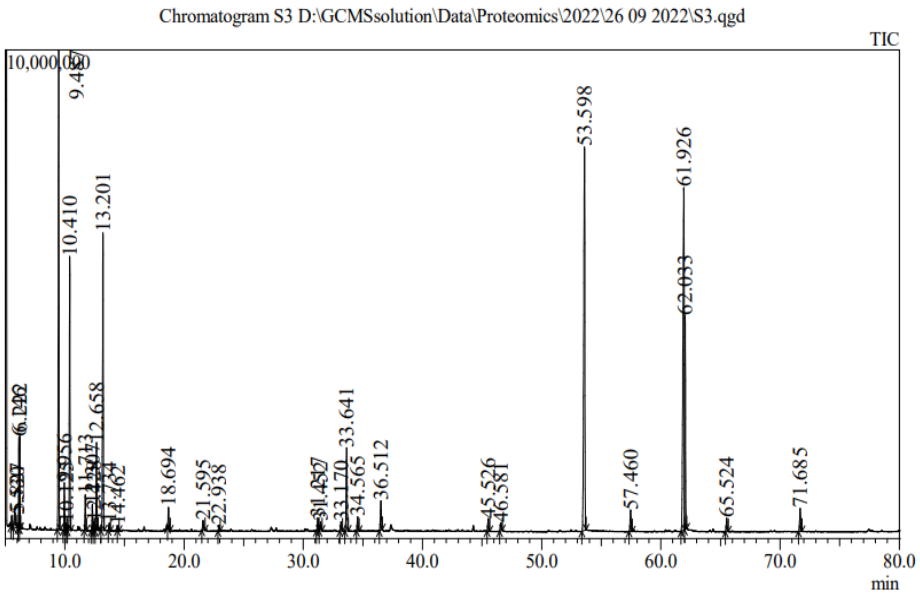


Figure 4.10: GCMS chromatogram of fatty acids in *B. lanceolaria*

Table 4.16. Fatty acid identified in *B. lanceolaria.*

Sl. No	Name	Chemical Formula	Molecular Weight	Type of Fatty acid
1.	Succinic acid	$C_{10}H_{22}O_4Si_2$	262	Saturated
2.	Methoprene acid	$C_{19}H_{36}O_3Si$	340	Saturated
3.	Oleic acid	$C_{21}H_{42}O_2Si$	354	MUFA
4.	Palmitic acid	$C_{19}H_{40}O_2Si$	328	Saturated
5.	Stearic acid	$C_{21}H_{44}O_2Si$	356	Saturated
6.	Caproic acid	$C_9H_{20}O_2Si$	188	Saturated
7.	Octanoic acid	$C_{11}H_{24}O_2Si$	216	Saturated
8.	Decanoic acid	$C_{13}H_{28}O_2Si$	244	Saturated

4.5.5. ANTI-NUTRITIONAL STUDIES

Three anti nutritional factors including oxalate, alkaloids and tannins were determined. Result of the anti-nutrient components found in the present study is shown in Table: 4.17. The concentrations of tannin was calculated from the respective equations Y=0.074x-0.517, R^2=0.901 obtained from the respective standard graphs and the result was expressed in mg/100g dry weight (DW) of sample. All of the antinutrients evaluated in the plant samples were confirmed to be at permissible levels. Lower levels of antinutrients may be a factor in the plant's safe ingestion as food.

Table 4.17. Anti-nutritional contents of plant species (in mg/100g dry weight).

Antinutritional	***Z. oxyphyllum***	***R. serrata***	***B. lanceolaria***
Oxalate	7.99±0.02	7.96±0.13	11.04 ±0.06
Alkaloids	7.83± 0.03	7.56±0.04	10.65 ±0.30
Tannins	8.35±0.04	12.51±0.13	9.37 ±0.23

Note: Values are in triplicate mean ±SD

4.6. PHYTOCHEMICAL STUDIES

Studies on phytochemical analysis of various solvent extracts of leaves of three selected ethnomedicinal plant species viz. *Zanthoxylum oxyphyllum* Edgew, *Rotheca serrata* (L.) Steane & Mabb and *Blumea lanceolaria* (Roxb.) Druce were done in the present study. Four freshly prepared solvent extracts of each sample (*Z. oxyphyllum*, *R. serrata* and *B.lanceolaria*) were subjected to preliminary phytochemical screening to identify the presence of secondary metabolites such as alkaloids, tannins, phenolics, flavonoids, steroids, fixed oil and fats etc. Hexane, chloroform, methanol and water extracts of each sample were prepared in this research work for both qualitative and quantitative analysis of the phytochemicals. Results are presented in tables and figures in the following sections.

4.6.1. Extractive Value:

Various solvents viz., hexane, chloroform, methanol and water were used to achieve extraction of active substances with diversity in their polarity. In this manner four different extracts were obtained. After extraction of 30g of dried plant material, the highest yield of crude extract was obtained using polar solvents. Among the solvent extracts used, the yield % was generally observed to be high in the methanol extracts of all the plant species and the lowest extraction yield was obtained in aqueous extract of *R. serrata* leaves. Extracts prepared with hexane presented the lowest extract yield values as compared to other solvents. The methanol extract of *Z. oxyphyllum* and *B. lanceolaria* leaves showed very good extractive values (i.e., 18 % and 18.2% respectively) indicating that the extraction efficiency favors highly polar solvents. These high extractive values of methanol extracts can be attributed to their capabilities

to dissolve polar along with non-polar molecules (Mujtaba *et al.*, 2016). The chloroform and aqueous extracts of *B. lanceolaria* showed approximately the same extract percentage value. At a fixed temperature, the extract yields of all the plants species were increased by the varying polarity of solvents except aqueous extract. As per previous reports of Dhanani *et al.* (2017) and Madhiha *et al.* (2017), the effect of solvent polarity on the percentage yields of the crude extracts is mentioned to depend on various factors such as plant species, varying nature of the components present, solvent polarity and extraction time.

Table 4.18. Yield percentage (%) of the plant species extracted using different solvents.

Extracts	**Yield percentage (%)**		
	Z. oxyphyllum	***R. serrata***	***B. lanceolaria***
AE	11.2	5.4	12.2
HE	6	4	9
CE	14.2	9.2	12
ME	18	11.4	18.2

Note: AE- aqueous extract, HE- hexane extract, CE- Chloroform extract, ME- methanol extract

4.6.2. QUALITATIVE PHYTOCHEMICAL SCREENING

The preliminary phytochemical analysis of leaf extracts of aqueous, hexane, chloroform and methanol showed the presence of a number of different phytochemical constituents in *Zanthoxylum oxyphyllum* Edgew, *Rotheca serrata* (L.) Steane & Mabb. and *Blumea lanceolaria* (Roxb.) Druce. Studies on phytochemicals carried out by several researchers also showed the presence of several phytochemicals in different parts of medicinal plants extracted by different solvent (Usman *et al.*, 2020; Olivia *et al.*, 2021). In this study, the phytochemical profile from preliminary investigation revealed that the extracts of the studied plant species are enriched with a variety of essential phytochemicals including alkaloids, phenolics, tannins, flavonoid, glycosides, steroids, phytosterols, terpenoids, oil and fats as depicted in the Tables below. Among the solvents used, the aqueous and methanolic extracts showed the presence of

maximum number of phytochemicals in all the plant species studied as compared to other solvents used in the study.

Table 4.19. Phytochemicals in *Zanthoxylum oxyphyllum* extracts

Phytochemicals	AE	HE	CE	ME
Alkaloids	+	+	-	+
Phenols	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	-	-	-
Terpenoids	+	+	+	+
Steroids	+	+	+	+
Glycosides	-	+	+	+
Tannins	+	-	+	-
Phytosterols	+	+	+	+
Oil and Fats	-	+	+	+

Note: "+" indicates present, "-" indicates absent

AE: Aqueous extract, HE: Hexane extract, CE: Chloroform extract, ME: Methanol extract

Table 4.20. Phytochemicals present in *Rotheca serrata* extracts

Phytochemicals	AE	HE	CE	ME
Alkaloids	+	-	+	+
Phenols	+	+	+	+
Flavonoids	+	+	+	+
Saponins	-	-	-	-
Terpenoids	+	+	-	+
Steroids	+	+	+	+
Glycosides	+	+	-	-
Tannins	+	-	+	-
Phytosterols	+	+	-	+
Oil and Fats	-	-	+	+

Note: "+" indicates present, "-" indicates absent

AE: Aqueous extract, HE: Hexane extract, CE: Chloroform extract, ME: Methanol extract

Table 4.21. Phytochemicals present in *Blumea lanceolaria* extracts

Phytochemicals	AE	HE	CE	ME
Alkaloids	+	+	-	+
Phenols	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	-	-	-
Terpenoids	+	+	+	+
Steroids	+	+	+	+
Glycosides	+	+	-	+
Tannins	+	-	+	+

Phytosterols	+	+	+	+
Oil and Fats	-	+	+	+

Note: "+" indicates present, "-" indicates absent

AE: Aqueous extract, HE: Hexane extract, CE: Chloroform extract, ME: Methanol extract

4.6.3. QUANTITATIVE PHYTOCHEMICAL ANALYSIS

4.6.3.1. Total phenolic content (TPC)

The TPC of the various leaf extracts are expressed in terms of GAE and presented in Table: 4.22. The TPCs were calculated using the following linear regression equation obtained from the standard plot of gallic acid: y= 0.004x + 0.059, r2 = 0.984, where y is absorbance and x is the amount of gallic acid in µg (Figure 4.11). The screening of the hexane, chloroform, methanol and aqueous extracts of studied plants revealed that there was a wide variation in the amount of total phenolics ranging from 12.11 ± 0.01 to 94.36±0.11mg GAE/g dry extract. The highest amount of phenolic content was found in the ZOAE (94.36±0.1 mg GAE/g dry material). Another three extracts i.e. HE, CE and ME of this plant were also found to contain a very good amount of phenolic compounds (15.29 ± 0.50, 26.90 ± 0.71 and 92.10 ± 0.17 mg GAE/g dry extract respectively). An appreciable amount of phenolic content was also present in the aqueous of RSAE and BLAE (74.78±0.10 and 71.11 ± 0.49 mg GAE/g dry extract respectively). The RSME contain significant amount of phenolic compounds (91.46 ±0.15 mg GAE/g dry extract). The lowest amount of phenolic content was observed in BLHE (12.11 ± 0.01 mg GAE/g dry extract).

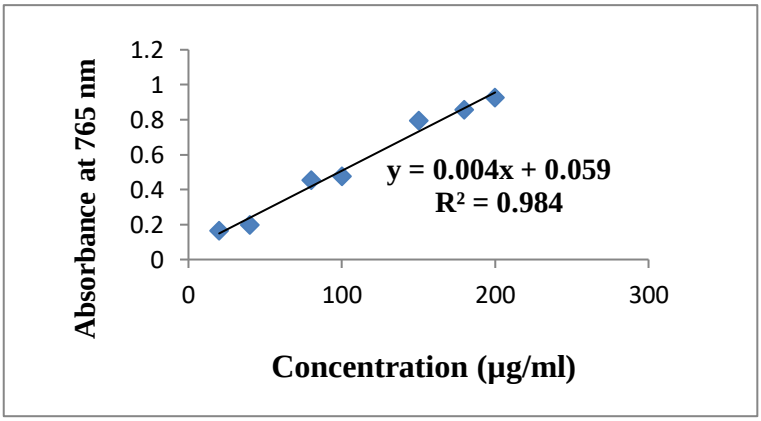


Figure 4.11: Standard gallic acid calibration curve for total phenolic content

In comparison between the quantative values of phytochemicals of three plants different content was found. In the extracts obtained using polar solvents (water and methanolic) order of concentrations for phenolic compounds is: *Z. oxyphyllum* > *R. serrata* > *B. lanceolaria*. But in extracts obtained from non polar solvents the order range of phenolics content is varied. i.e., in CE, the phenolic content of three plants species is in the order of concentrations of *R. serrata* >*Z. oxyphyllum* >*B. lanceolaria*. And in non polar solvents like HE the phenolic content is in the order of *R. serrata* > *Z. oxyphyllum* > *B. lanceolaria.*

Table 4.22. Total phenolic content of plant extracts

Crude Extract	Total Phenolic Content (mg of GAE/g dry extract)		
	Z. oxyphyllum	***R. serrata***	***B. lanceolaria***
AE	94.36±0.11	74.78±0.10	71.11 ± 0.49
HE	15.29 ± 0.50	32.44±0.40	12.11 ± 0.01
CE	26.90 ± 0.71	58.78±0.11	23.48±0.45
ME	92.10±0.17	91.46 ±0.15	73.6± 0.61

Note: AE: Aqueous extract, HE: Hexane extract, CE: Chloroform extract, ME: Methanol Extract

Values are the mean of triplicate experiments and represented as mean ± SD. The TPC values were expressed in mg gallic acid equivalent per gram.

4.6.2.2. Total flavonoid content (TPC)

Flavonoids are important components of human and animal diet because of their common presence in plants. Due to the different biological activities of plant secondary metabolites, their regular consumption may have serious consequences for health, both positive and negative (Stobiecki and Kachlicki, 2006). Flavonoids contents in various extracts of our studied plants were determined using spectrophotometric method with aluminum chloride. The TFCs of the various crude extracts are expressed in terms of quercetin (QE) presented in Table: 4.23. The TFCs were calculated using the following linear regression equation obtained from the standard plot of quercetin: y= 0.004x + 0.714, r2 = 0.917, where y is absorbance and x is the amount of quercetin in µg (Figure 4.12). The flavonoid content of the extracts in terms of quercetin equivalent was ranging between 16.21

± 0.24 QE mg/g dry extract. High concentration of flavonoids was measured in BLAE (71.19 ± 0.24 QE mg/g dry extract).

ZOME and RSME were found to contain a good amount of flavonoids (58.62 ± 0.97 and 32.20± 0.98 QEmg/g dry extract respectively). The lowest flavonoid concentration was measured in RSHE. The other extracts of all plant species also contain an appreciable amount of flavonoids and showed approximately equal values.

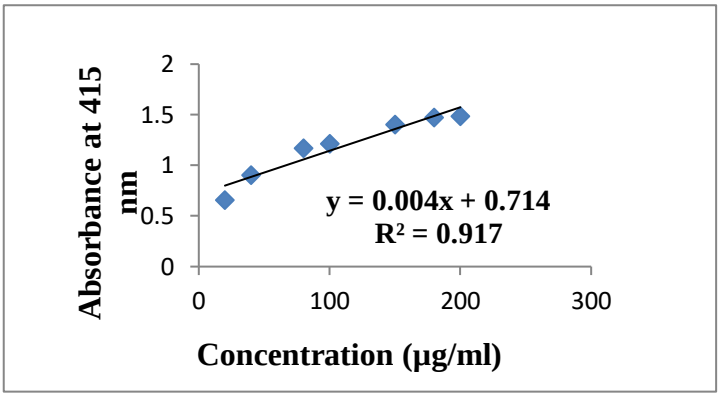


Figure 4.12: Standard quercitin calibration curve for total flavonoid content

Table 4.23. Total flavonoid content of plant extracts

Crude Extract	Total Flavonoid Content (mg of QE/g dry extract)		
	Z. oxyphyllum	***R. serrata***	***B. lanceolaria***
AE	53.33±0.20	21.20±0.30	71.19 ±0.24
HE	35.09±0.10	16.21 ±0.03	22.20 ± 1.01
CE	20.03±0.05	21.5±0.10	24.39 ± 0.95
ME	58.62 ± 0.97	32.20 ± 0.98	70.73 ±0.23

Note: AE- Aqueous extract, HE- Hexane extract, CE- Chloroform extract, ME- Methanol extract Values are the mean of triplicate experiments and represented as mean ± SD. TFC values are expressed in mg quercetin equivalent per gram**.**

Analyzing the results of phenolic and flavonoid content in all extracts, it was noticed that the highest concentration of total phenolic and flavonoid content in the extracts were obtained using solvents of high polarity.

4.7. ANTIOXIDANT ACTIVITY STUDIES

Various antioxidant assays like DPPH, H_2O_2 assay and Ferric Reducing Power assay were used to evaluate the antioxidant potential of various solvent extracts. Ascorbic acid was used as positive control for all the assays. IC_{50} value was calculated to evaluate the antioxidant effectiveness for DPPH radical scavenging assay and H_2O_2 assay. In Ferric Reducing Power Assay, the reducing powers of all the crude extracts were determined from the linear regression equation obtained from the standard (ascorbic acid) calibration curve (Figure: 4.13).

The selection of extraction solvent is critical for the determination of antioxidant activity of plant extracts since the polarity of any solvent has an on the impact the recovery of natural antioxidants from herbals (Goulas *et al.,* 2012). The antioxidant activity of solvent extracts substantially is reliant on the choice of extraction solvent used, according to Arakaki *et al.* (2016). Mekonnen *et al.* (2018) explored the antioxidant activities of chloroform and methanol leaf extracts of *Euclea schimperi*, and discovered that polar molecules are more responsible for antioxidant activity. Therefore, the antioxidant activity of *Z. oxyphyllum*, *R. serrata* and *B. lanceolaria* leaf extracts were evaluated using four solvents, namely aqueous, hexane, chloroform and methanol extracts. With regard to solvent extracts used, polar solvent such as AE and ME were discovered to be more proficient for the recovery of antioxidant compounds from plant samples.

4.7.1. DPPH Radical Scavenging Assay

In the present study, antioxidant activity as determined by DPPH assay was found to be maximum in BLAE with IC_{50} value (113.32 ± 0.32 µg/ml) which is very close to the IC_{50} value of standard ascorbic acid (112.79 ± 0.02 µg/ml). Minimum activity was observed in BLHE (245.67 ± 0.05µg/ml) in DPPH assay. IC_{50} values represent the concentration of antioxidants that cause 50% neutralization of DPPH radicals. IC_{50} values were calculated from the plot of inhibition percentage against concentrations. In this assay, a concentration dependent scavenging activity was observed in each plant extracts.

In *B.lanceolaria* and *Z.oxyphyllum*, the order of IC_{50} value in decreasing order was HE>CE>ME>AE. However, in *R. serrata*, methanol extracts performed better showing higher scavenging activity than the other extracts. IC_{50} value determined were in the decreasing order of HE> CE>AE> ME>.

Table 4.24. IC_{50} values (µg/mL) of *Z. oxyphyllum*, *R. serrata* and *B. lanceolaria* in DPPH assay

Crude Extracts	***Z. oxyphyllum***	***R. serrata***	***B. lanceolaria***
AE	116.82 ± 0.02	168.10 ± 0.01	113.32 ± 0.32
HE	239.06 ± 0.05	226.95 ± 0.99	245.67 ± 0.05
CE	223.68 ± 0.02	205.01 ± 0.02	172.61 ± 0.02
ME	153.28 ± 0.03	156.39 ± 0.02	145.4 ± 0.67
Ascorbic Acid (Standard)	112.79 ± 0.02		

Note: AE- Aqueous extract, HE- Hexane extract, CE- Chloroform extract, ME- Methanol extract

Values are expressed as mean ± S.D. of triplicate measurements.

4.7.2. H_2O_2 Radical Scavenging Assay

The H_2O_2 assay percentage inhibition of plant extracts against the concentration was calculated using linear regression equations. IC_{50} values in various extracts of plants in H_2O_2 assays are summarized in Table: 4.25. Extracts were capable of scavenging H_2O_2 in a concentration dependent manner in this study. Through antioxidant activity quantified by this assay, the plant extracts with low IC_{50} value was BLME (139.51 ± 0.01µg/ml) which indicates the present of highest antioxidant activity among the extracts of analyzed plant samples. The RSME is found to have the lowest antioxidant activity with IC_{50} value of 248.12 ± 0.04µg/ml. In ZOME showed the highest antioxidant activity in comparison to other solvent extracts which was in the order of ME>AE>CE>HE. All the extracts showed lower antioxidant activity in comparison to the standard ascorbic acid.

Table 4.25. IC_{50} values (µg/ml) of *Z. oxyphyllum*, *R. serrata* and *B. lanceolaria* in H_2O_2 assay

Crude Extracts	***Z. oxyphyllum***	***R. serrata***	***B. lanceolaria***
AE	164.54 ± 0.04	202.36 ± 2.83	144.4 ± 0.01
HE	225.27 ± 0.55	151.64 ± 0.02	227.61 ± 0.02
CE	192.82 ± 0.02	178.80 ± 0.04	198.49 ± 0.43
ME	158.34 ± 0.44	248.12 ± 0.04	139.51 ± 0.01
AA (Standard)	120.5 ± 0.43		

Note: AE- Aqueous extract, HE- Hexane extract, CE- Chloroform extract, ME- Methanol extract

Values are expressed as mean ± S.D. of triplicate measurements

4.7.3. Ferric Reducing Power Assay

As can be observed in Ferric Reducing Power Assay, an antioxidant activity was varied between samples. With the increase of antioxidant compounds in tested medicinal plants, there was an observed increase in reducing power. Each plant extracts displayed a dose-dependent reducing power (shown as absorbance at 700 nm) within range of 36.95 ± 0.04 mg of ascorbic acid/g dry extract to 67.45 ± 0.47 mg of ascorbic acid/g dry extract. Reducing power of ZOAE was found to be highest antioxidant activity quantified by this method (67.45 ± 0.47 mg of ascorbic acid/g dry extract). While that of RSHE was found to be the lowest (36.95 ± 0.04 mg of ascorbic acid/g dry extract). Extraction efficiency of components with antioxidative properties of *Z. oxyphyllum* was lowering in the following order: AE > ME > CE > HE. But the values obtained in *B. lanceolaria* exhibited that the ME with the highest reducing power quantified by this method followed by AE, CE and HE. In *R. serrata*, the CE showed the highest reducing power as compared to ME, AE and HE.

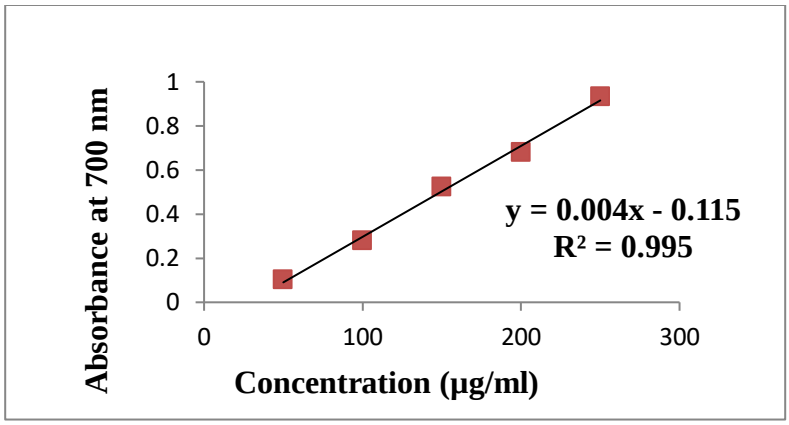


Figure 4.13. Standard graph (Ascorbic acid) in Ferric Reducing Power Assay

Table 4.26. Antioxidant activity of *Z. oxyphyllum*, *R. serrata* and *B. lanceolaria* in Ferric Reducing Power Assay

Crude extract	*Z. oxyphyllum* (mg of Ascorbic Acid/g dry extract)	*R. serrata* (mg of Ascorbic Acid/g dry extract)	*B. lanceolaria* (mg of Ascorbic Acid/g dry extract)
AE	67.45 ± 0.47	40.36 ± 0.15	41.22 ± 0.02
HE	44.72 ± 0.02	36.95 ± 0.04	37.94 ± 0.05
CE	45.16 ± 0.13	54.20 ± 0.07	39.15 ± 0.13
ME	55.23 ± 0.02	47.5 ± 0.20	56.96 ± 0.05

Note: AE- Aqueous extract, HE- Hexane extract, CE- Chloroform extract, ME- Methanol extract

Values are expressed as mean ± S.D. of triplicate measurements.

4.8. GAS CHROMATOGRAPHY MASS SPECTROMETRY (GC-MS) STUDIES:

The analytical GC-MS technique was used for the identification and quantification of the constituents present in the plant samples. The present investigation revealed the presence of many bioactive compounds from the various crude extract of the leaves of *Z. oxyphyllum*, *R. serrata* and *B.lanceolaria*. The identification of the bioactive compounds was assured by observing the molecular formula, retention time and peak area of the data. Identified bioactive compounds have been associated with several biological properties such as antioxidant, anti-inflammatory, antimicrobial, anticancer and hypocholesterolemic activities as reported by Moreno-Rojas *et al.* (2021).

In *Z. oxyphyllum* leaf extract, total 61 bioactive compounds were detected of which 17 in HE, 16 in CE, 18 in ME and 10 bioactive compounds in AE. Among the identified bioactive compounds in various extract of *Z. oxyphyllum*, n-Hexadecanoic acid is the common bioactive compounds present in HE, CE and ME. The GCMS chromatograms are shown in figures 4.14, 4.15, 4.16, 4.17 and the list of ioactive compounds are shown in Tables: 4.27., 4.28., 4.29., 4.30. Most of the bioactive compounds identified are similar in extracts of our investigated sample extracts. Based on the peak area percentage, the most dominant of all the identified compounds in HE were n-Hexadecanoic acid (29.78%) and 2- Dodecanone (16.24 %). The CE contained n-Hexadecanoic acid (17.40 %) and Triacontane, 1-Bromo (5.64%) as major bioactive compounds. In ME, Methyl 9-Eicosenoate (8.04%) and 1-[3,3-Dimethyl-2-(3-Methyl-Buta-1,3-Dienyl)-Cyclopent (5.93%) is identified as major compound and in AE, 5-methyl-Z-5-Docosene is major compound identified. In addition, out of 61 bioactive compounds identified, only 49 bioactive compounds are reported with pharmacological activities.

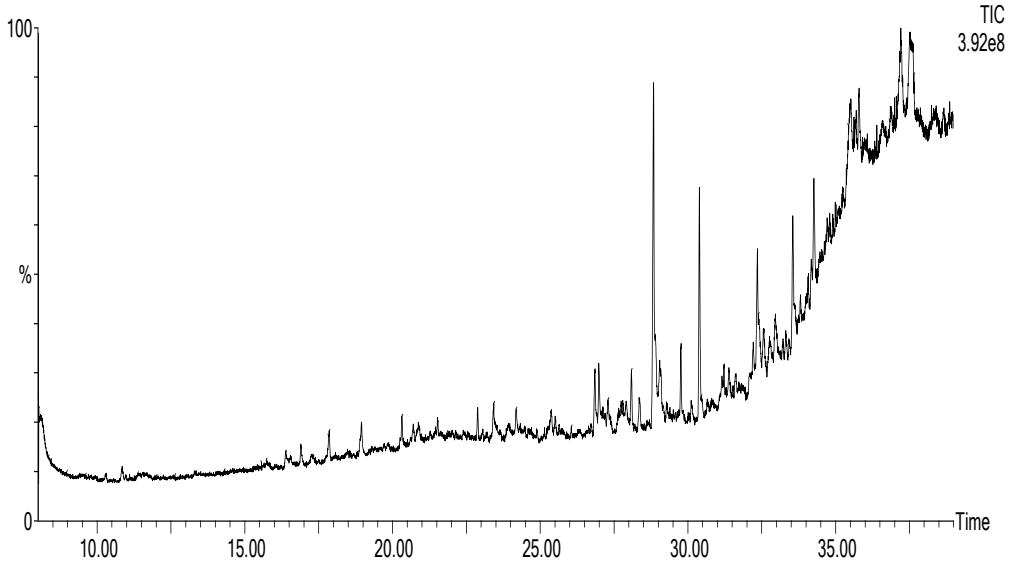


Figure 4.14: GC-MS chromatogram of aqueous extract of *Z. oxyphyllum*

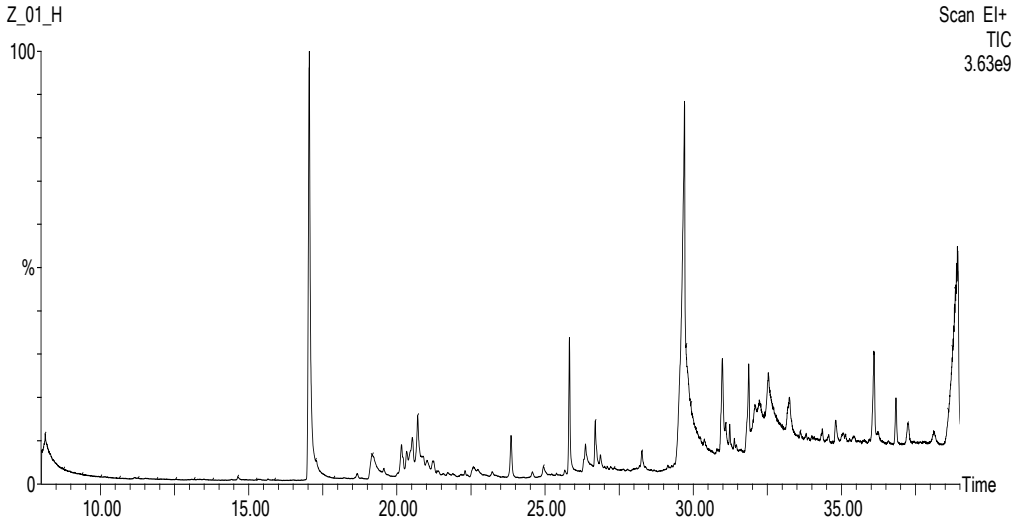


Figure 4.15: GC-MS chromatogram of hexane extract of *Z. oxyphyllum*

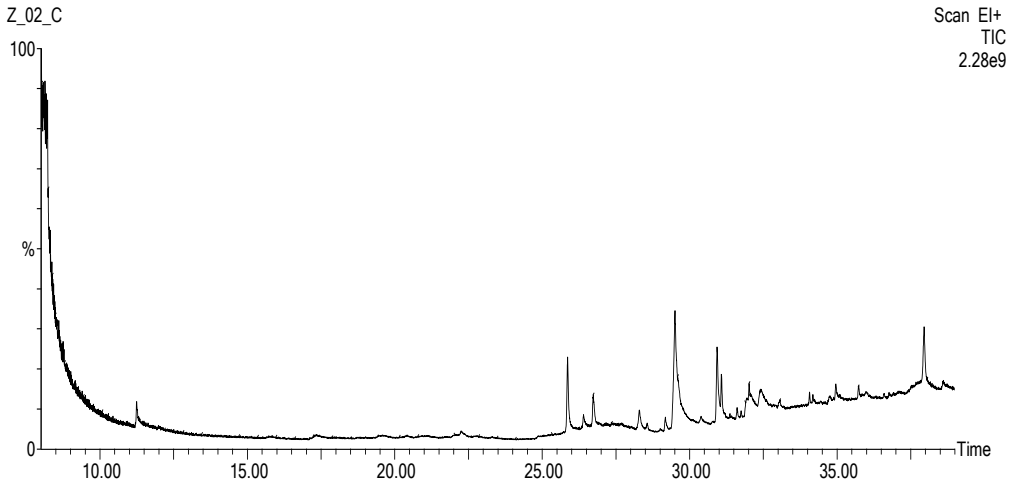


Figure 4.16: GC-MS chromatogram of chloroform extract of *Z. oxyphyllum*

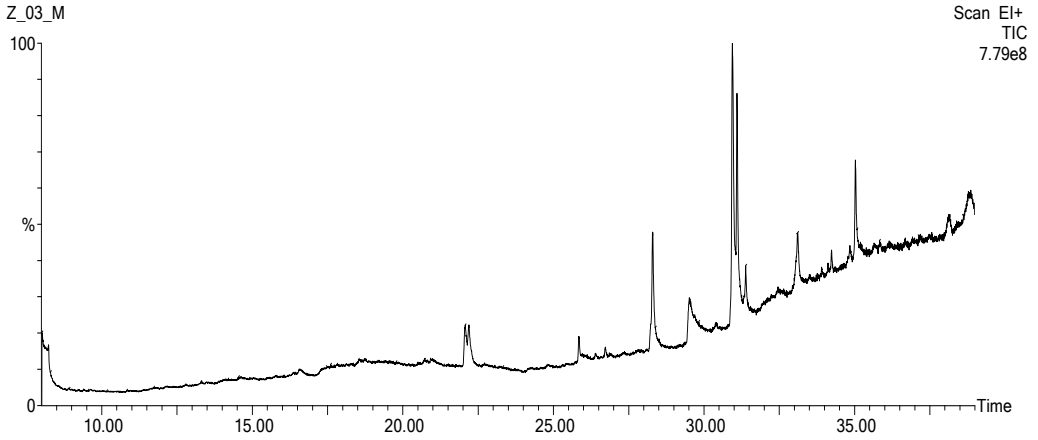


Figure 4.17: GC-MS chromatogram of methanol extract of *Z. oxyphyllum*

Table 4.27. Bioactive compounds identified in aqueous extracts of *Zanthoxylum oxyphyllum*

Sl. No	RT	Bioactive compounds	Peak Area (%)	Biological Activities	References
1.	17.85	5-methyl-Z-5-Docosene	10.56	Antibacterial, antidiabetic and antitumour activities	Ralte*et al.*, 2022
2.	18.94	Dodecane, 1-Fluoro-	0.78	Antioxidant, antimicrobial activities	Khan *et al.*, 2016; Shedzad*et al.*, 2018
3.	20.31	Z,z,6, 28-heptatriactontadien-2-one	0.61	Vasodilatory properties	Mallikadevi*et al.*, 2012
4.	26.85	Hexacosyl acetate	0.88	α-amylase inhibitory activity	Keerthana*et al.*, 2013
5.	26.98	Chloroacetic acid, tetradecyl ester	0.98	Antioxidant properties	Shyam*et al.*, 2013
6.	28.83	Dodecanoic acid	4.49	Antibacterial, antioxidant and anti-apoptotic activity	Nachiyar*et al.*, 2020
7.	29.76	Didodecyl phthalate	0.60	Vasodilator, Antihypertensive, angiotensin AT2 receptor antagonist, Uric acid excretion stimulant and diuretic	Paulsamy*et al.*, 2012
8.	33.54	Neophytadiene	1.69	Analgesic, antipyretic, anti-inflammatory, antimicrobial and antioxidant compound	Raman *et al.*, 2012
9.	30.38	Furanodienone	2.22	Anticancer activity	Ahmed *et al.*, 2022
10.	32.34	Tetradecanoic acid	2.37	Antioxidant, antimicrobial, lubricant, anticancerous, cosmetics	Bano and Deora, 2020

Table 4.28. Bioactive compounds identified in hexane extract of *Zanthoxylum oxyphyllum*

Sl. No	RT	Bioactive compounds	Peak Area (%)	Biological Activities	References
1.	17.04	2- Dodecanone	16.24	Potent insecticidal, repellent activities and antimicrobial activities	Wang, 2019
2.	20.70	2-Tridecanone	1.74	Antioxidant activities	Rubilar*et al.*, 2023
3.	22.59	1-hexadecanol, 2-methyl-	0.57	Anticancer, anti-inflammatory and	Shalaby*et al.*, 2021

				antimicrobial, antioxidant activities	
4.	23.85	2-Pentacosanone	1.25	Not reported	-
5.	25.82	Z, z- 6, 28-Heptatriactontadien-2-one	3.16	Vasodilatory properties	Mallikadevi*et al.*, 2012
6.	26.36	3,7,11,15-tetramethyl-2-hexadecen-1-ol	1.30	Antimicrobial, anti-inflammatory, anticancer and diuretic activities	Rajalakshmi and Mohan, 2016
7.	28.26	6-octen-1-ol, 3,7-dimethyl,propanoate	0.85	Flavouring agent	Muthuswami and Fathimath, 2020
8.	29.70	N- Hexadecanoic acid	29.78	Antimicrobial, antioxidant, antiatherosclerotic, antiandrogenic, anticancer and antitumor activities	Nabi*et al.*, 2022
9.	30.37	Pentadecanoic acid	1.13	Anti-cancer effects of pentadecanoic acid in human breast carcinoma MCF-7/stem-like cells (SC)	Cho *et al.*, 2020
10.	30.97	Phytol	3.37	Cytotoxicity activity against breast cancer cell lines (MCF7)	Satyal*et al.*, 2012
11.	31.09	Hexanedioic acid, bis (2-ethylhexyl) ester	0.90	Cosmetics	www.chemicalsubstances.gc.ca).
12.	32.22	L-(+)-ascorbic acid 2,6-dihexadecanoate	2.15	Anticancer, antiviral, anti tumor and antihypersensitive activity	Singh *et al.*, 2022
13.	32.52	Tetratriacontane	4.90	Antibacterial activity	Garaniya and Bapodra, 2014
14.	33.23	1-heptatriacotanol	1.86	Antioxidant and anticancer activities	Hadi*et al.*, 2016; Junwei*et al.*, 2018
15.	36.09	Hentriacontane	3.03	Antibacterial activity	Olubunmi*et al.*, 2011
16.	36.83	1,2- Benzenedicarboxylic acid, disooctyl ester	1.14	Antioxidant property, antibacterial properties	Li *et al.*, 2012; Padmashree*et al.*, 2018
17.	37.24	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	0.86	Cytotoxic activity against HepG 2 and MCF-7 cancer cell lines.	Krishnan *et al.*, 2014

Table 4.29. Bioactive compounds identified in chloroform extract of *Zanthoxylum oxyphyllum*

Sl. No	RT	Bioactive compounds	Peak Area (%)	Biological Activities	References
1.	8.08	D-limonene	0.83	Chemopreventive activity, potent anticancer agent against human bladder cancer, inducing significant apoptosis with an increase in the expression of caspase-3	Ye *et al.*, 2020
2.	11.25	GeranylTiglate	1.18	Reduce inflammation and oxidative stress, reduce the production of nitric oxide linked with hypertension and cancer	
3.	22.24	3-Eicosyne	0.86	Antimicrobial	Ram *et al.*, 2018
4.	25.86	Z,Z-6,28-Heptatriactontadien-2-One	4.20	Vasodilatory properties	Mallikadevi*et al.*, 2012
5.	26.40	Butanoic Acid, 3-Methyl-, 3,7-Dimethyl-6-Octenyl Ester	0.84	Antimicrobial activity	Umarani and Nethaji, 2021
6.	26.73	Phytol	2.03	Cytotoxicity activity against breast cancer cell lines (MCF7)	Satyal*et al.*, 2012
7.	29.17	Methyl 11-methyl-dodecanoate	0.67	Anti-fungal and antioxidant activities	Nithyadevi and Sivakumar, 2015
8.	29.50	N-Hexadecanoic Acid	17.40	Antimicrobial, antioxidant,antiatherosclerotic, antiandrogenic, anticancer and antitumor activities	Nabi*et al.*, 2022
9.	30.38	L-(+)-ascorbic acid 2,6 dihexadecanoate	0.50	Anticancer, antiviral, anti tumor and antihypersensitive activity	Singh *et al.*, 2022

10.	30.92	6-Octadecenoic Acid, Methyl Ester	3.54	Analgesic, anti-inflammatory and antipyretic activity	Jaddoa*et al.*, 2016
11.	32.01	Cholestan-3-ol, 2-Methylene-	4.19	Antimicrobial, anticancer, diuretic, anti-asthma and anti-arthritic	Jegadeeswari*et al.*, 2012
12.	32.41	Dichloroacetic acid, Tridec-2-Ynyl Ester	5.32	Cosmetic treatments	Tanti*et al.* (2019)
13.	33.06	Methyl 9,10-Methylene-Octadecanoate	0.79	Not reported	-
14.	35.97	Trichloroacetic acid, tridec-2-ynyl ester	0.67	-	-
15.	37.77	Dotriacontane	1.66	Antioxidant, antimicrobial, antitumor and antiprotozoal activities as well as chemopreventive value	Gallo and Sarachine, 2009
16.	37.95	Triacontane, 1-Bromo	5.64	-	-

Table 4.30. Bioactive compounds identified in methanol extract of *Zanthoxylum oxyphyllum*

Sl. No	RT	Bioactive compounds	Peak Area (%)	Biological Activities	References
1.	14.21	Ethyl 9,12,15 Octadecatrienoate	0.80	Antimicrobial activity	Hameed*et al.*, 2015
2.	14.62	17-Octadecynoic Acid, Methyl Ester	1.24	-	-
3.	16.58	Methyl 9,10-Methylene-Octadecanoat	2.14	-	-
4.	17.60	Phenol, 2,4-Bis (1,1-Dimethylethyl)	2.81	Antimicrobial, antioxidant, antifungal and antitumor.	George *et al.*, 2018
5.	18.72	1-[3,3-Dimethyl-2-(3-Methyl-Buta-1,3-Dienyl)-Cyclopent	5.93	-	-

6.	20.92	Methyl 8,11,14,17-Eicosatetraenoate	2.58	Anti-bacterial, anti-oxidant, anti-fungal and anti-tumor	Somashekar*et al.*, 2023
7.	28.28	I-Propyl 14-Methyl-Pentadecanoate	4.24	Not reported	-
8.	29.51	N-Hexadecanoic Acid	4.15	Antimicrobial, antioxidant,antiatherosclerotic, antiandrogenic, anticancer and antitumor activities	Nabi*et al.*, 2022
9.	30.94	Methyl 9-Eicosenoate	8.04	Not reported	-
10.	31.09	11-Octadecenoic Acid, Methyl Ester	4.74	Antioxidant, anticancer and antiviral activity	Alam*et al.*, 2021
11.	31.38	Butyl 9,12,15-Octadecatrienoate	0.84	Anti-inflammatory, hypocholesterolemic, cancer preventive and hepatoprotective	Sudha*et al.*, 2017
12.	33.10	Cholesta-22,24-Dien-5-Ol, 4,4-Dimethyl-	2.66	Antibacterial and trypanocidal activity	Tyagia and Agarwal, 2017
13.	34.23	13-Docosenoic Acid, Methyl Ester	0.55	Anticancer activity	Paudel and Pant, 2017
14.	34.83	2,2-Dibromocholestanone	0.77	Not reported	
15.	35.02	Methyl 11-Docosenoate	2.69	Anti-fungal and antioxidant activities	Nithyadevi and Sivakumar, 2015
16.	35.18	Ergost-25-Ene-3,5,6,12-Tetrol	0.39	Antibacterial and inhibitory activity	Akbar *et al.*, 2020
17.	38.12	Methyl 2-Hydroxy-Octadeca-9,12,15-Trienoate	0.70	Not reported	-
18.	38.85	Methyl 9,10-Methylene-Octadecanoate	1.02	Not reported	-

In comparison, the GCMS profile of *R. serrata* identified total of 53 bioactive compounds which represents the medicinal quality of the plant sample. Among the identified bioactive compounds, highest in HE i.e., 15 bioactive compounds followed by CE and ME (14 bioactive compounds) and 10 bioactive compounds in AE. The compounds identified in the leaf extract by GC-MS analysis are listed in Table: 4.31., 4.32., 4.33., 4.34 and the GCMS chromatograms are shown in figures 4.18, 4.19, 4.20, 4.21. Of the compounds identified in HE, hentriacontane was the most abundant with the highest peak area (11.97 %) followed by chloroacetic acid, tetra decylester (8.22%). In CE the major bioactive compound identified was 1, 2-Benzenedicarboxylic acid, diisooctyl ester (69.89%) and n-Hexadecanoic acid (4.18%). The most prevailing bioactive compounds detected in ME were Trans-13-octadecenoicacid, methyl ester (15.45%). In AE, hentriacontane (13.23%) is identified as major compound. A total of 43 bioactive compounds were reported having various biological activities which add to the therapeutic values of this plant species.

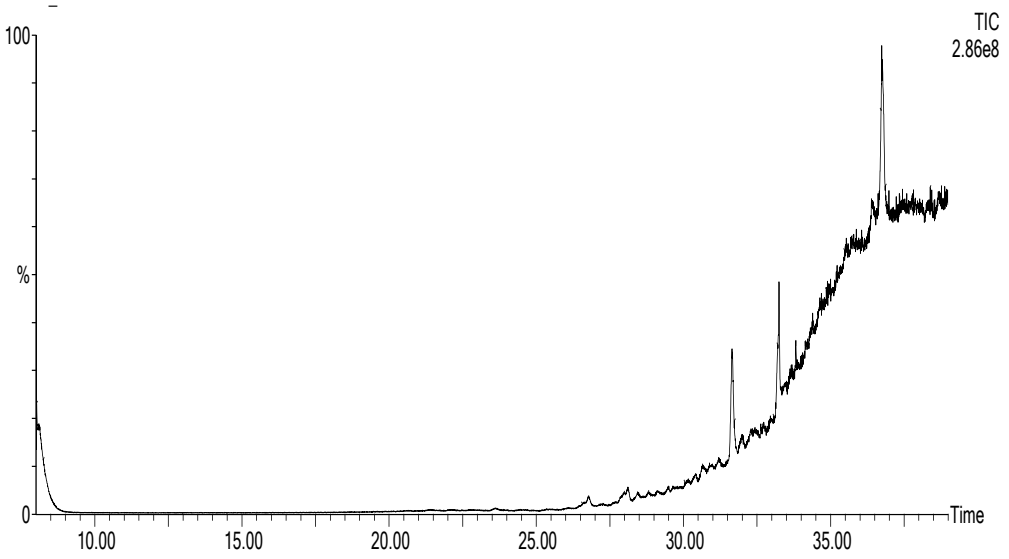


Figure 4.18: GC-MS chromatogram of aqueous extract of *R. serrata*

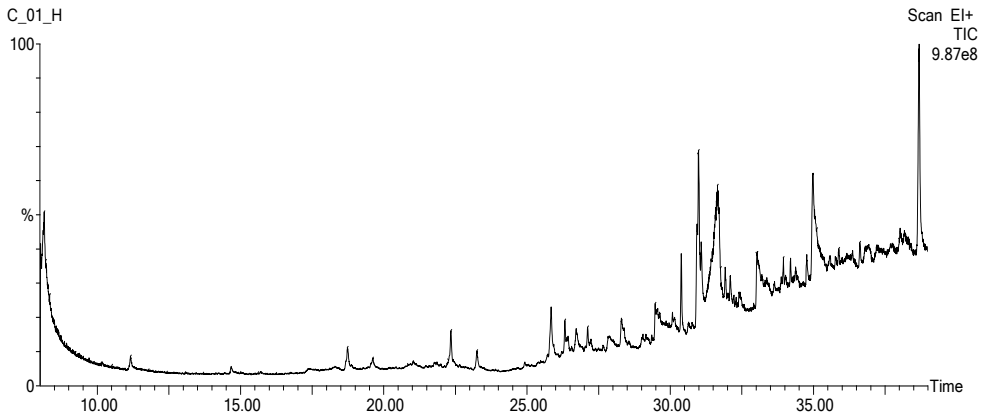


Figure 4.19: GC-MS chromatogram of hexane extract of *R. serrata*

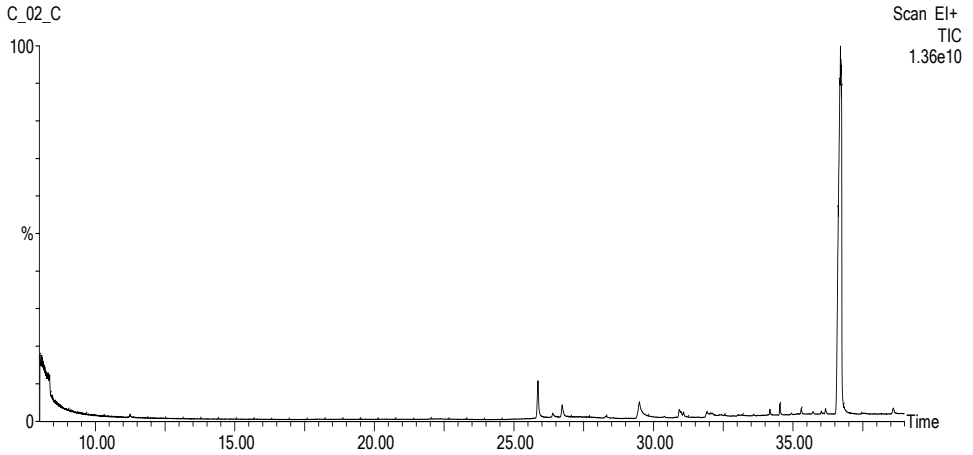


Figure 4.20: GC-MS chromatogram of chloroform extract of *R. serrata*

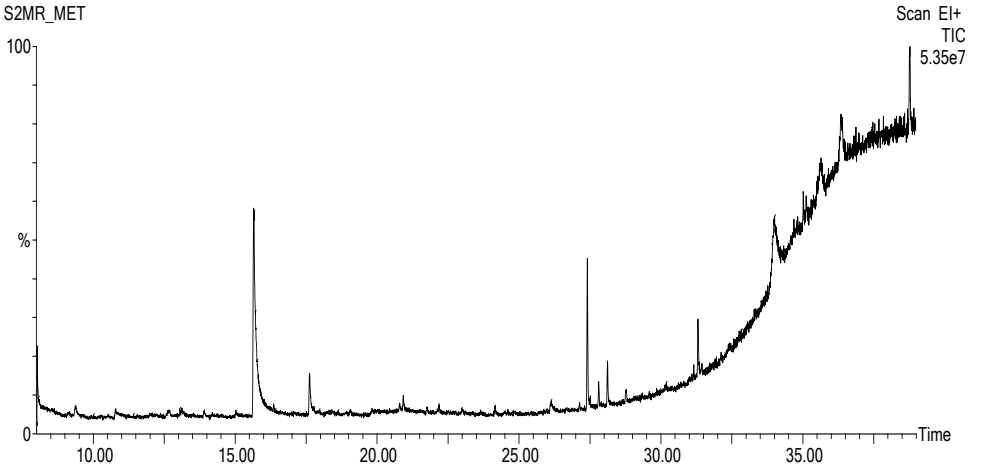


Figure 4.21: GC-MS chromatogram of methanol extract of *R. serrata*

Table 4.31: Bioactive compounds identified in aqueous extracts of *Rotheca serrata*

Sl. No	RT	Bioactive compounds	Peak Area (%)	Biological Activities	References
1.	23.65	Hentriacontane	13.23	Anti-inflammatory, antioxidant, and anti-tumor activities	Kim *et al.*, 2011
2.	26.78	5-Methyl-Z-5-Docosene	0.66	No activity reported	
3.	28.11	Carbonic Acid, Eicosyl Vinyl Ester	0.68	Antioxidant, Antibacterial	Paul *et al.*, 2019
4.	28.44	1,1-Dichloropentane	0.37	Antibacterial activity	Bhakyashree and Krishnan, 2018
5.	29.48	Hexacosyl Acetate	1.17	Antidiabetic activity	Muhammad *et al.*, 2021
6.	30.16	1-Decanol, 2-Hexyl-	0.33	Anti Cancer activity (Skin cancer)	Ahmed *et al.*, 2018
7.	31.64	Hexanedioic Acid, bis (2-Ethylhexyl) Ester	4.99	Anti fungal	Xue-Na *et al.*, 2012
8.	33.24	Neophytadiene	3.86	Analgesic, antipyretic, anti-inflammatory, antimicrobial and antioxidant compound	Raman *et al.*, 2012
9.	33.81	4-Tert-Octylphenol	0.65	Antimicrobial, antioxidant, antibacterial	Ismail *et al.*, 2020
10.	36.75	Cyclotrisiloxane, hexamethy	11.60	Antioxidant, antidiabetic activity	Ismail *et al.*, 2019

Table 4.32: Bioactive compounds identified in hexane extract of *Rotheca serrata*

Sl. No	RT	Bioactive compounds	Peak Area (%)	Biological Activities	References
1.	21.02	Cyclohexanol, 5-Methyl-2-(1-Methylethyl)-, (1.Alpha.,	0.50	-	-
2.	22.34	Triacontane	1.17	Antibacterial, antidiabetic and antitumor activities	Mammen*et al.*, 2010
3.	25.84	Phytol	2.29	Cytotoxicity activity against	Satyal*et al.*, 2012

				breast cancer cell lines (MCF7)	
4.	26.71	Z,Z-6,28-Heptatriactontadien-2-One	1.01	Vasodilatory properties	Mallikadevi*et al.*, 2012
5.	28.29	Nonadecanoic Acid, 18-Oxo-, Methyl Ester	1.98	Not reported	
6.	29.62	Phthalic Acid, Butyl Dodecyl Ester	1.93	Antifouling	Ranganathan, 2014
7.	30.38	I-Propyl 11,12-Methylene-Octadecanoate	1.59	Antimicrobial activity	Prarthana*et al.*, 2017
8.	30.98	Phthalic acid, butyl nonyl ester	6.09	Antifouling	Ranganathan, 2014
9.	31.64	Hentriacontane	11.97	Anti-inflammatory, antioxidant and anti-tumor activities.	Olubunmi*et al.*, 2009
10.	33.03	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	2.90	antimicrobial activity of this compound	Tyagia and Agarwal, 2017
11.	34.98	Chloroacetic acid, tetra decylester	8.22	Antioxidant properties	Shyam*et al.*, 2013
12.	36.93	1,2-Benzenedicarboxylic Acid, Butyl Cyclohexyl Ester	0.88	Antimicrobial and antifouling activity	Paulsamy*et al.*, 2015
13.	37.69	Docosanedioic Acid, Dimethyl Ester	0.69	Antifungal activity	Prakash*et al.*, 2019
14.	38.17	Octacosanoic Acid, 27-Oxo, Methyl Ester	0.78	-	-
15.	38.68	Squalene	6.32	Anticancerous, gastro-preventive, hepatoprotective and pesticidal properties	Ukiva*et al.*, 2002; Ganesh and Mohankumar, 2017

Table 4.33: Bioactive compounds identified in chloroform extract of *Rotheca serrata*

Sl. No	**RT**	**Bioactive compounds**	**Peak Area (%)**	**Biological Activities**	**References**
1.	8.36	Butanoic Acid, 3-Methyl-, 3,7-Dimethyl-6-Octenyl Este	0.27	Antimicrobial activity	Umarani and Nethaji, 2021
2.	25.86	3,7,11,15-tetramethyl-2-hexadecen-1-ol	3.55	Antimicrobial, anti-inflammatory, anticancer and diuretic activities	Rajalakshmi and Mohan, 2016

3.	26.39	6-Octen-1-ol, 3,7-Dimethyl-, Propanoate	0.46	Flavouring agent	Muthuswami and Fathimath, 2020
4.	26.72	Z,Z-6,28-Heptatriactontadien-2-One	1.44	Vasodilatory properties	Mallikadevi*et al.*, 2012
5.	28.29	Sulfurous Acid, 2-Ethylhexyl Octadecyl Ester	0.33	-	-
6.	29.49	N-hexadecanoic acid	4.18	Antimicrobial, antioxidant,antiatherosclerotic, antiandrogenic, anticancer and antitumor activities	Nabi*et al.*, 2022
7.	30.92	1-heptacosanol	0.76	Nematicidal, anticancer, antioxidant and antimicrobial activities	Raman *et al.*, 2012
8.	31.90	Methyl 6,9-octadecedienoate	0.89	Leprosy, antioxidant activities	Kumar *et al.*, 2014; Berdeaux*et al.*, 1998
9.	32.11	L-(+)-Ascorbic Acid 2,6-Dihexadecanoate	0.66	Anticancer, antiviral, anti tumor and anti hypersensitive activity	Singh *et al.*, 2022
10.	34.16	1,2-Benzenedicarboxylic Acid, Mono(2-Ethylhexyl)	0.40	Cytotoxic activity against HepG 2 and MCF-7 cancer cell	Krishnan *et al.*, 2014
11.	34.52	Oxalic acid, 2-ethylhexyl pentadecyl ester	0.63	-	-
12.	35.29	I-Propyl 14-Methyl-Pentadecanoate	0.37	Not reported	
13.	36.69	1,2-Benzenedicarboxylic acid, diisooctyl ester	69.89	Antioxidant property	Li *et al.*, 2012
14.	38.59	Oxalic Acid, 2-Ethylhexyl Octadecyl Ester	0.59	-	-

Table 4.34: Bioactive compounds identified in methanol extract of *Rotheca serrata*

Sl. No	RT	Bioactive compounds	Peak Area(%)	Biological Activities	References
1.	8.02	3-methyl-2-(2-oxopropyl) furan	5.03	Antioxidant, antimicrobial, anti inflammatory, antibacterial and antipyretic activity	Nithyadevi and Sivakumar, 2015
2.	8.62	1-nonylcycloheptane	1.23	Antimicrobial activity	ElZanaty*et al.*,

					2022
3.	20.92	Cyclopenta(C) Pyran-7-carboxaldehyde, 4-methyl	0.60		
4.	22.07	2-Dodecanone	1.91	Insecticidal and repellent activities and antimicrobial	Wang, 2019
5.	25.84	3,7,11,15-tetramethyl-2-hexadecen-1-ol	3.55	Antimicrobial, anti-inflammatory, anticancer and diuretic activities	Rajalakshmi and Mohan, 2016
6.	26.13	1-phenyl-5-methyl heptanes	0.41	-	-
7.	27.40	Z,Z-6,28-Heptatriactontadien-2-One	2.75	Vasodilatory properties	Mallikadevi*et al.*, 2012
8.	28.29	Hexadecanoicacid, methylester	1.98	Antimicrobial, antioxidant, hemolytic, 5-alpha reductase inhibitor cancer enzyme inhibitors in pharmaceuticals	Ouyang*et al.*, 2012
9.	29.51	N-tetracosanol-1	7.88	Antibacterial activity, nematicidal, anticancer, antioxidant and Antimicrobial activity	Kuppuswamy*et al.*, 2013
10.	30.94	9-octadecenoicacid, methyl ester	3.37	Anticancer and antioxidant, antimicrobial activity	Asghar *et al.*, 2011
11.	31.38	Trans-13-octadecenoicacid, methyl ester	15.45	Anti-inflammatory, antiandrogenic, anticancerous, dermatitigenic, hypocholesterolemic, anemiagenic and insectifuge properties	Krishnamoorthy*et al.*, 2014
12.	33.10	D-limonene	1.68	Anticancer agent against human bladder cancer, inducing significant apoptosis	Ye *et al.*, 2020
13.	35.67	Arsenous acid, tris (trimethylsilyl) ester	1.11	Antiviral, antithyroid and anticatract activity	Alexander and Rosy, 2022
14.	36.44	Cyclotrisiloxane, hexamethyl	0.34	Antimicrobial agents, antibacterial activity and antioxidant activity	Abdel-Karim*et al.*, 2019

On the other hand, *B. lanceolaria* accounts a total of 49 bioactive compounds of which ME have the maximum number of bioactive compounds present followed by HE and CE. The AE showed 10 bioactive compounds. The most abundant bioactive compounds identified in HE is chloroacetic acid, tetra decylester (18.83%) and Z, z-6, 28-heptatriactontadien-2-one (9.07%) in the chloroform extract. Chloroacetic acid, tetradecyl ester (18.15%) in ME. 4-Methoxy-2, 4-bis (p-hyroxyphenyl) pent-1-ene (13.77%) as the major bioactive compound in an AE. The compounds identified are presented in Table: 4.35., 4.36., 4.37., 4.38 and GCMS chromatograms are shown in figure 4.22, 4.23, 4.24, 4.25.

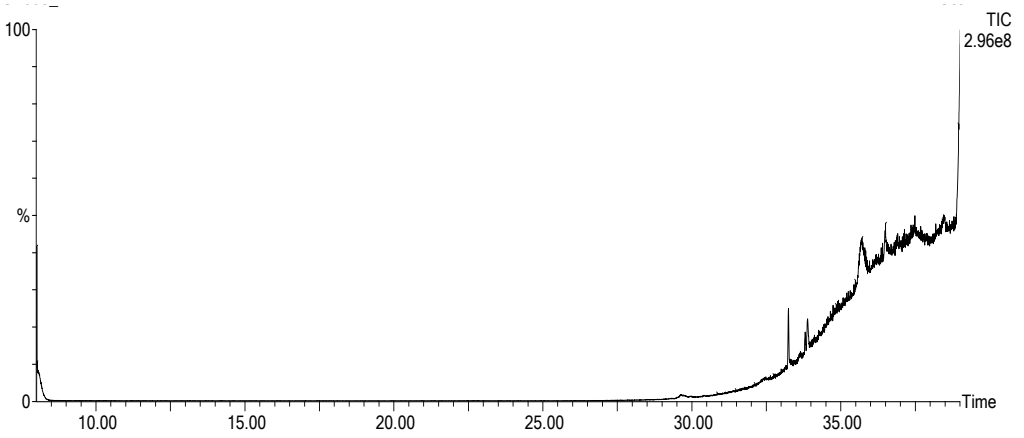


Figure 4.22: GC-MS chromatogram of aqueous extract of *B. lanceolaria.*

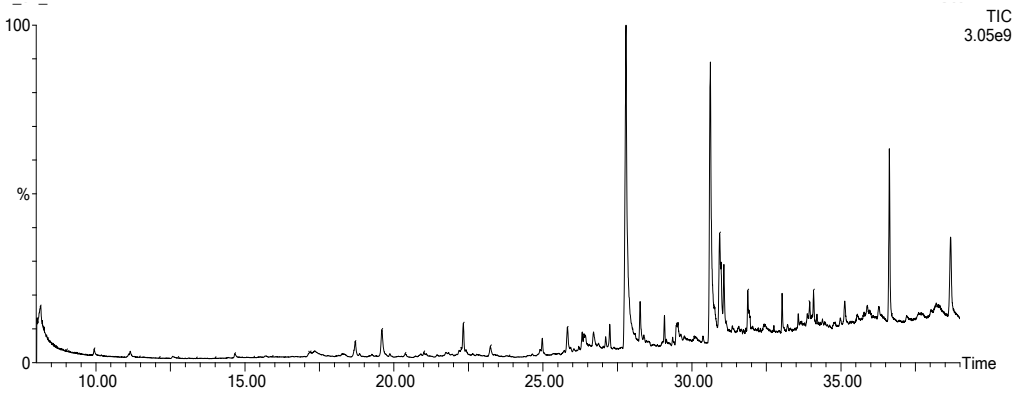


Figure 4.23: GC-MS chromatogram of hexane extract of *B. lanceolaria.*

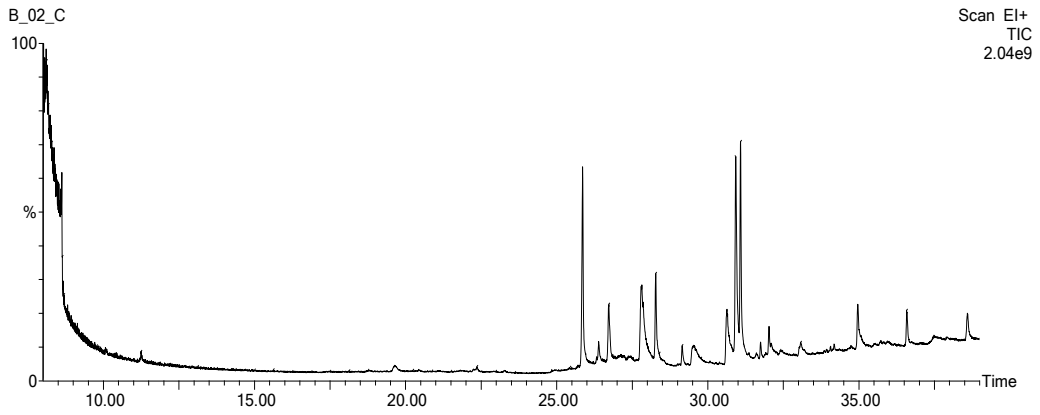


Figure 4.24: GC-MS chromatogram of chloroform extract of *B. lanceolaria.*

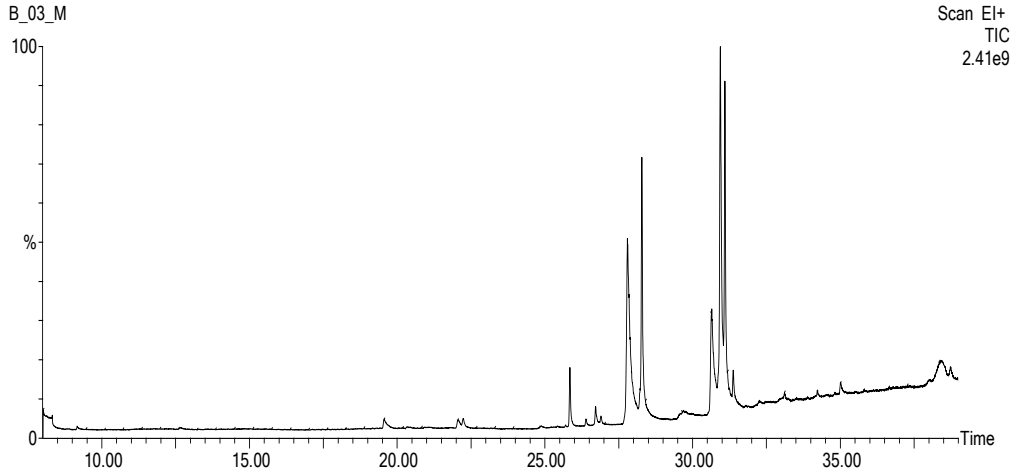


Figure 4.25: GC-MS chromatogram of methanol extract of *B. lanceolaria.*

Table: 4.35. Bioactive compounds identified in aqueous extracts of *Blumea lanceolaria*

Sl. No	RT	Bioactive compounds	Peak Area (%)	Biological Activities	References
1.	29.61	2-Propenoic acid, 6- methyl heptyl ester	0.66	Antioxidant and antidiabetic	Tuluckue*t al.,* 2012
2.	32.48	Cyclotrisiloxane, hexamethyl	0.33	Antimicrobial, antioxidant, antibacterial	Ismail *et al.,* 2020
3.	33.88	3,7,11,15-Tetramethyl-2-Hexadecen-1-ol	3.30	Antimicrobial, anti-inflammatory, anticancer and diuretic activities	Rajalakshmi and Mohan, 2016
4.	35.70	4-Methoxy-2, 4-bis(p-hyroxyphenyl) pent-1-ene	13.77	Anticancer activity	Huang *et al.,* 2021
5.	36.39	1-Pentene, 4,4-dimethyl-1,3-diphenyl-1-(trimethylsilyloxy)	0.36	Not reported	
6.	36.49	1,1,1,3,5,5,5-Heptamethyltrisiloxane	3.60	Antimicrobial activity	Mereen and Daiz, 2020
7.	37.48	Trisiloxane, 1,1,1,5,5,5-hexamethyl-3,3-bis[(trimethylsily)oxy]	1.52	Antioxidant activity	Khan et al., 2016
8.	38.44	Ethanedioic acid, dibutyl ester	4.06	Not reported	
9.	33.24	Neophytadiene	3.83	Anti- inflammatory, antimicrobial, analgesic, antipyretic, antioxidant activity	Raman *et al.,* 2012
10.	36.48	2- Ethylhexanol ethylene glycol acetal	3.60	Not reported	

Table: 4.36. Bioactive compounds identified in hexane extract of *Blumea lanceolaria*

Sl. No	RT	Bioactive compounds	Peak Area (%)	Biological Activities	References
1.	22.32	N-Tetracosanol-1	1.43	Antibacterial activity, nematicidal, anticancer, antioxidant and antimicrobial activity	Kuppuswamy*et al.*, 2013
2.	26.70	Behenic alcohol	0.71	Anti-viral agent (Herpes simples virus)	Shettar*et al.*, 2017
3.	27.78	Chloroacetic acid, tetradecyl ester	18.83	Antioxidant properties	Shyam*et al.*, 2013
4.	29.48	Hexacosanol, Acetate	0.63	Not activity reported	-
5.	30.61	1-heptacosanol	12.93	Nematicidal, anticancer, antioxidant and antimicrobial activities.	Raman *et al.*, 2012
6.	30.92	Phytol	5.06	Cytotoxicity activity against breast cancer cell lines (MCF7)	Satyal*et al.*, 2012
7.	31.87	Cis-1-Chloro-9-Octadecene	1.70	Not reported	
8.	35.88	1,2-Benzenedicarboxylic Acid, Mono (2-Ethylhexyl) Ester	1.06	Cytotoxic activity against HepG 2 and MCF-7 cancer cell lines.	Krishnan *et al.*2014
9.	36.62	1,2-benzenedicarboxylic acid, diisooctyl ester	5.05	Antioxidant property	Li *et al.*, 2012
10.	35.96	Methyl 11-Methyl-Dodecanoate	0.55	Anti-fungal and antioxidant activities	Nithyadevi and Sivakumar, 2015
11.	38.67	Squalene	3.58	Anticancerous, gastro-preventive, hepatoprotective and pesticidal properties	Ukiva*et al.*, 2002; Ganesh and Mohankumar, 2017

Table: 4.37. Bioactive compounds identified in chloroform extract of *Blumea lanceolaria*

Sl. No	RT	Bioactive compounds	Peak Area (%)	Biological Activities	References
1.	8.629	2h-Benzocyclohepten-2-One, Decahydro-9a-Methyl-trans	2.53	Anti-angiogenic effects and anti-tumor efficacy	Alamery and Algaraawi, 2020
2.	25.85	Z, z-6, 28-heptatriactontadien-2-one	9.07	Vasodilatory properties	Mallikadevi*et al.*, 2012
3.	26.38	3-Eicosyne	1.07	Antimicrobial	Ram *et al.*, 2018
4.	26.72	3,7,11,15-tetramethyl-2-hexadecen-1-ol	3.47	Antimicrobial, anti-inflammatory, anticancer and diuretic activities	Rajalakshmi and Mohan, 2016
5.	27.11	Butanoic acid, 3-Methyl-, 3,7-Dimethyl-6-Octenyl Ester	0.52	Antimicrobial activity	Umarani and Nethaji, 2021
6.	27.81	1-heptacosanol	9.46	Nematicidal, anticancer, antioxidant and antimicrobial activities.	Raman *et al.*, 2012
7.	28.27	Hexadecanoic acid, methyl ester	4.09	Antimicrobial, antioxidant, hemolytic, 5-alpha reductase inhibitor cancer enzyme inhibitors in pharmaceuticals	Ouyang*et al.*, 2012
8.	29.52	N-pentadecanol	4.04	Antioxidant activity, antibacterial activity	Geetha*et al.*, 2015; Chatterjee*et al.*, 2017
9.	30.63	N-Tetracosanol-1	4.44	Antibacterial activity, nematicidal, anticancer, antioxidant and antimicrobial activity	Kuppuswamy*et al.*, 2013
10.	31.08	Methyl 6,9- octadecadienoate	6.26	Leprosy, antioxidant activities	Kumar *et al.*, 2014; Berdeaux*et al.*1998
11.	32.02	Di-N-Octyl Phthalate	1.22	Antibacterial and cytostatic activity, cytostatic activity against breast cancer cell	Boudjelala*et al.*, 2011; Amalarasi and Jothi, 2019
12.	34.95	1-Octacosanol	3.50	Insecticidal activity	Zavala-Sanchez, 2020
13.	38.58	Methyl 9-Methyltetradecanoate	1.79	Antimicrobial activities	Chandrasekaran*et al.*, 2008

Table: 4.38. Bioactive compounds identified in methanol extract of *Blumea lanceolaria*

Sl. No	RT	Bioactive compounds	Peak Area (%)	Biological Activities	References
1.	25.84	3,7,11,15- tetramethyl-2-hexadecen-1-ol	2.54	Antimicrobial, anti-inflammatory, anticancer and diuretic activities	Rajalakshmi and Mohan, 2016
2.	26.71	Z, z-6, 28-heptatriactontadien-2-one	1.08	Vasodilatory properties	Mallikadevi*et al.*, 2012
3.	27.79	Chloroacetic acid, tetradecyl ester	18.15	Antioxidant properties	Shyam*et al.*, 2013
4.	28.28	Hexadecanoic acid, methyl ester	12.36	Antimicrobial, antioxidant, hemolytic, 5-alpha reeducates inhibitor cancer enzyme inhibitors in pharmaceuticals	Ouyang*et al.*, 2012
5.	29.68	2h-Benzocyclohepten-2-One, Decahydro-9a-Methyl	0.88	Anti-angiogenic effects and anti-tumor efficacy	Alamery and Algaraawi, 2020
6.	30.09	9,12-Octadecadienoic Acid (Z,Z)-, Methyl Ester	8.72	Anti-inflammatory, antihistaminic, anti-arthritic and hepatoprotective	Henry *et al.*, 2002; Mensah-Agyei*et al.*, 2020
7.	30.64	N- tetracosanol-1	7.88	Antibacterial activity, nematicidal, anticancer, antioxidant and antimicrobial activity	Kuppuswamy*et al.*, 2013
8.	30.93	Trans-13-octadecenoic acid, methyl ester	15.45	Anti-inflammatory, antiandrogenic, anticancerous, dermatitigenic, hypocholesterolemic, anemiagenic and insectifuge properties	Krishnamoorthy*et al.*, 2014
9.	31.37	Oleyl Alcohol	1.22	Anti-tumor or anti-cancer properties	Orienti*et al.*, 2007
10.	32.25	N-Tetracosanol-1	0.30	Antibacterial activity, nematicidal, anticancer, antioxidant and antimicrobial activity	Kuppuswamy*et al.*, 2013
11.	33.04	Methyl 9-Cis,11-Trans-Octadecadienoate	0.37	Antibacterial activity	Lalthanpuii and Lalchhandama, 2019

12.	33.11	Pentadecanoic Acid, 14-Methyl-, Methyl Ester	0.66	Antifungal and antimicrobial activity	Akpuaka*et al.*, 2013
13.	34.81	Methyl 11-Methyl-Dodecanoate	0.17	Anti-fungal and antioxidant activities (Nithyadevi and Sivakumar, 2015).	Nithyadevi and Sivakumar, 2015
14.	38.03	Bis (2-Ethylhexyl) Phthalate	0.51	Anti mutagenic activity, anti antibacterial and larvicidal	Javed*et al.*, 2022
15.	38.40	1-Heptacosanol	5.98	Nematicidal, anticancer, antioxidant and antimicrobial activities.	Raman *et al.*, 2012

4.9. FOURIER TRANSFORM INFRARED SPECTROSCOPY (FT-IR) STUDY

FT-IR technique is useful for revealing different types of organic and inorganic compounds present in plants. It defines the molecule and determines its structure (Eberhardt *et al.,* 2007). It is a high-resolution analytical instrument that can be used to classify the chemical constituents and elucidate their structural compounds. FT-IR is a quick and non-destructive investigation to fingerprint herbal extracts or powders. In the present study, the FT-IR analysis was carried out in dried leaf samples of *Z. oxyphyllum*, *R. serrata* and *B. lanceolaria* to know the functional groups of their compounds. From the FT-IR spectra we can clearly see that each band represents characteristic absorption peaks for functional groups present in the samples. Screening of functional groups represents the presence of phenolic group, alkanes, aliphatic amines, alcohols, alkyl halide and aromatic amines. These functional groups confirm the presence of secondary metabolites from the screening test. Also the presence of functional groups indicates medicinal properties of analyzed samples through we have attempted to justify the ethnomedicinal claim of selected plants used by the people of Goalpara.

In this study, the FT-IR spectrum was used to identify the functional group of the active components based on the peak value in infrared region. Results of FT-IR peak values and functional groups used in medicinal plants are represented in tables. FTIR spectrums of the plant materials are presented in figures. All the three samples under this study showed almost the same wave numbers which clearly indicate the presence of similar functional groups. Seven different functional groups were observed in between peak ranges from 497.98-3342.17 cm^{-1} in crude leaf powder of each sample.

Table: 4.39 shows the functional groups, peak values and types of bonds present in *Zanthoxylum oxyphyllum.* The FTIR spectrum for *Zanthoxylum oxyphyllum* is presented in Figure: 4.26. The Infra red spectroscopic (IR) analysis of *Z. oxyphyllum* reveals the presence of different functional group in the peak ranges from 3267 to 1012 cm^{-1} wave number. C-H for alkyne at band 3267cm^{-1}, the band at 2918.56 cm^{-1} and 2850.21cm^{-1} corresponds to C-H bond which reveals the presence of aliphatic compound, alkanes and alkyne. The band at 1731.30 cm^{-1} can be attributed to

C=O which indicates the existence of an aldehyde compound group. C=C in aldehyde compound at the band 1601.35 cm^{-1} whereas C-N in aliphatic amines, protein at the band 1243.13 cm^{-1} and C-N for amines at 1012 cm^{-1}.

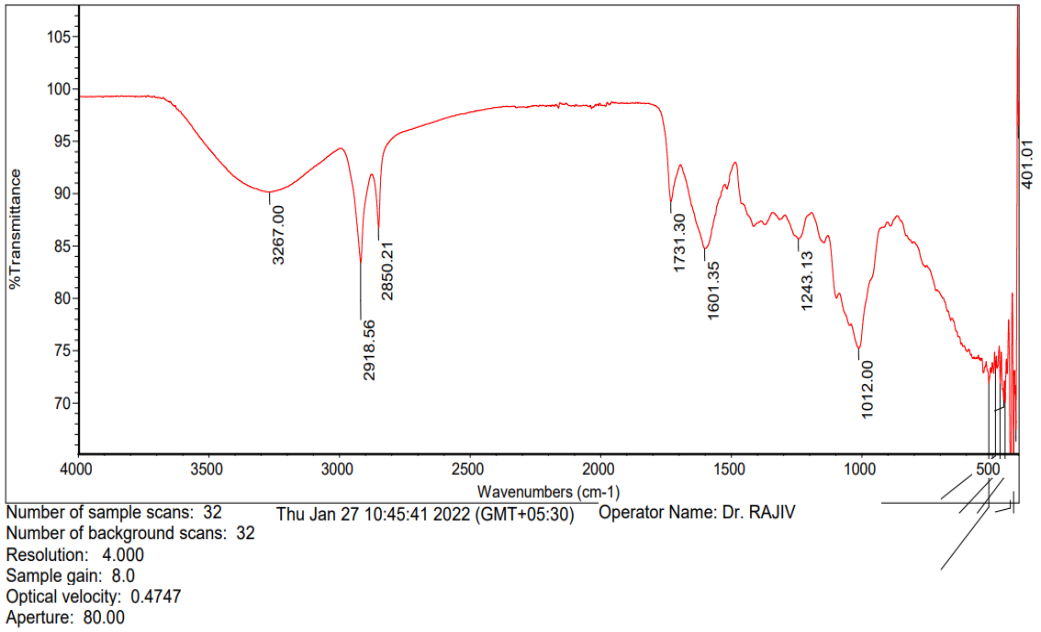


Figure 4.26: FTIR spectrum of *Zanthoxylum oxyphyllum* leaves

Table 4.39: FTIR peak values and functional groups in *Zanthoxylum oxyphyllum* leaf

Sl. No.	Wave Number cm^{-1} (Test Sample)	Frequency range cm^{1} (Reference number)	Chemical Bond	Functional Group
1	3267	3333-3267	C-H	Alkyne
2	2918.56	3000-2850	C-H	Aliphatic compound
3	2850.21	3000-2850	C-H	Aliphatic compound
4	1731.30	1740-1720	C=O	Aldehyde compound
5	1601.35	1680-1600	C=C	Aldehyde compound
6	1243.13	1250-1080	C-N	Aliphatic amines
7	1012	1400-1000	C-N	Amine

In *Rotheca serrata* seven different functional groups was observed in between intensities from 506.24 to 3274.42 cm^{-1} by FT-IR analysis (Table: 4.40). FT-IR result revealed the presence of C-H bond corresponded to alkyne by the peak at 3274.42 cm^{-1}. The frequency peak at 2918.08 and 2850.04 cm^{-1} refers to stretching of C-H aliphatic group. Aldehyde compound (C=C) group was observed at 1601.07 cm^{-1}. Frequency peak recorded at 1247.38 cm^{-1} ascertains the presence of aliphatic amines (C-N), Sulfoxide (S=O) at the peak 1008.68 cm^{-1}, 506.24 cm^{-1} denote C-Br and this indicates the presence of alkyl halides.

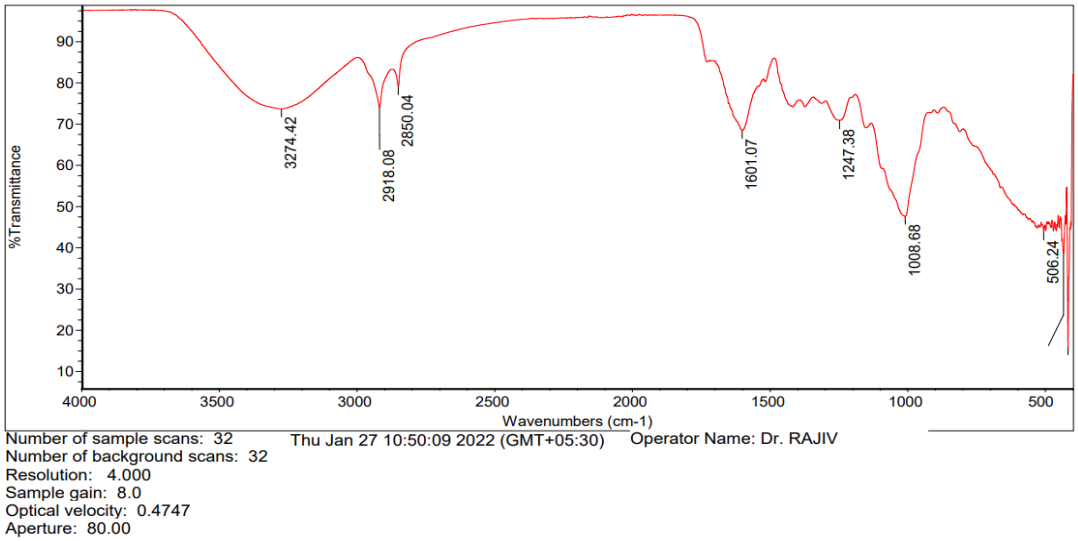


Figure 4.27: FTIR spectrum of *Rotheca serrata* leaves.

Table 4.40: FTIR peak values and functional groups in *Rotheca serrata* leaf

Sl. No.	Wave Number cm^{-1} (Test Sample)	Frequency range cm1 (Reference number)	Chemical Bond	Functional Group
1	3274.42	3333-3267	C-H	Alkyne
2	2918.08	3000-2850	C-H	Aliphatic compound
3	2850.04	3000-2850	C-H	Aliphatic compound
4	1601.07	1680-1600	C=C	Aldehyde compound
5	1247.38	1250-1080	C-N	Aliphatic amines
6	1008.68	1400-1000	S=O	Sulfoxide
7	506.24	600-500	C-Br	Alkyl halide

Seven functional groups were observed in *B. lanceolaria* crude sample. In *B. lanceolaria* through FT-IR analysis, major peaks observed were at 3342.17 cm^{-1} that is

assigned as the 0-H group, this indicates the presence of alcohol. The absorption peak at 2923.28 cm^{-1} and 2852.51 cm^{-1} indicates the presence of C-H aliphatic compound. C=O aldehyde compound was detected at 1737.31 cm^{-1}. Alkanes were observed at absorption peak 1455.76 cm^{-1} (C-H) and aromatic amine (C-N) at 1260.77 cm^{-1} peak. Alkyl halide (C-I) was observed at 497.98 cm^{-1} by FT-IR analysis.

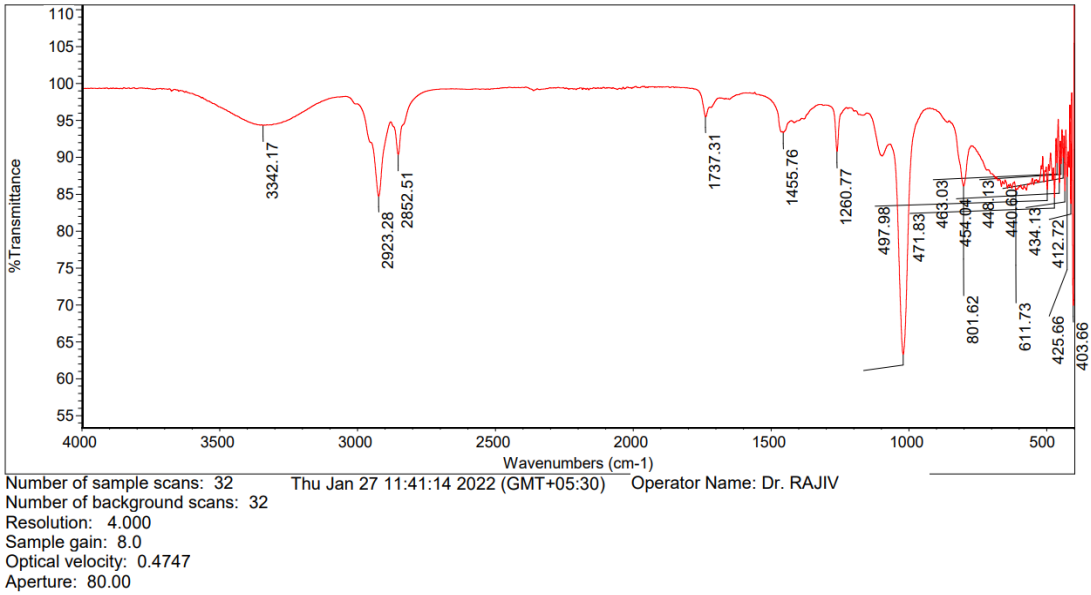


Figure 4.28: FTIR spectrum of *B. lanceolaria* leaves

Table 4.41: FTIR peak values and functional groups in *Blumea lanceolaria* leaf

Sl. No.	Wave Number cm^{-1} (Test Sample)	Frequency range cm^{1} (Reference number)	Chemical Bond	Functional Group
1	3342.17	3650-3000	O-H	Aromatic compound
2	2923.28	3000-2850	C-H	Aliphatic compound
3	2852.51	3000-2850	C-H	Aliphatic compound
4	1737.31	1740-1720	C=O	Aldehyde compound
5	1455.76	1432-1621	C-H	Alkanes
6	1260.77	1270-1150	C-N	Aromatic amine
7	497.98	500	C-I	Alkyl halide

4.10. CYTOXICITY STUDIES

In the current study, the cytotoxic activity of ME of investigated plants viz., *Z. oxyphyllum*, *R. serrata* and *B. lanceolaria* were determined using MTT assay in two

different human cancer cell line, MCF-7 for breast cancer cell and HeLa cell line for human cervical. Doxorubicin was used as a standard reference on the mentioned cancer cell lines. Result shows that the cytotoxicity of each plant extracts was increased as the concentration of them was increased. The IC_{50} observed on MCF-7 and HeLa cell line was tabulated in Table 4.42.

Table 4.42. Cytotoxicity of the plant extracts against MCF-7 and HeLa cell.

Sample	Cytotoxicity activity (IC_{50} in µg/mL)	
	MCF-7 cell	HeLa cell
Z. oxyphyllum	77.17±0.24	81.94±0.14
R. serrata	54.68±0.28	85.06±0.08
B. lanceolaria	75.78±0.29	57.34±0.49
Doxorubicin	0.14±0.12	2.38±3.5

Note: Values are expressed as mean ± SD of triplicate measurements

The cell viability was determined by calculating the percentage of viable cells using the MTT assay and the inhibitory effects of plant extracts on cell proliferation were also assessed. In MCF-7 cell line, the cytotoxicity activity of plants extract showed increase in cell death with the increase in concentration of plant extracts. From the results obtained, the three samples showed increased anticancer activity with respect to increase in its concentrations. The ME of all three samples showed IC_{50} values lower than 100µg/mL on MCF-7 cell lines. This indicates that the extracts of investigated samples had cytotoxic effects on the MCF-7 cancer cell lines. Among the investigated samples, the ME of *R. serrata* had strong cytotoxic activity with low IC_{50} value (54.53µg/mL). The cytotoxicity of leaf extracts by gradual decrease of cell viability at a concentration ranges from 5-100µg/ml. The lowest percentage of cell viability was observed at 100µg/ml for all the samples ranging from 36.84 % to 45.22 %.

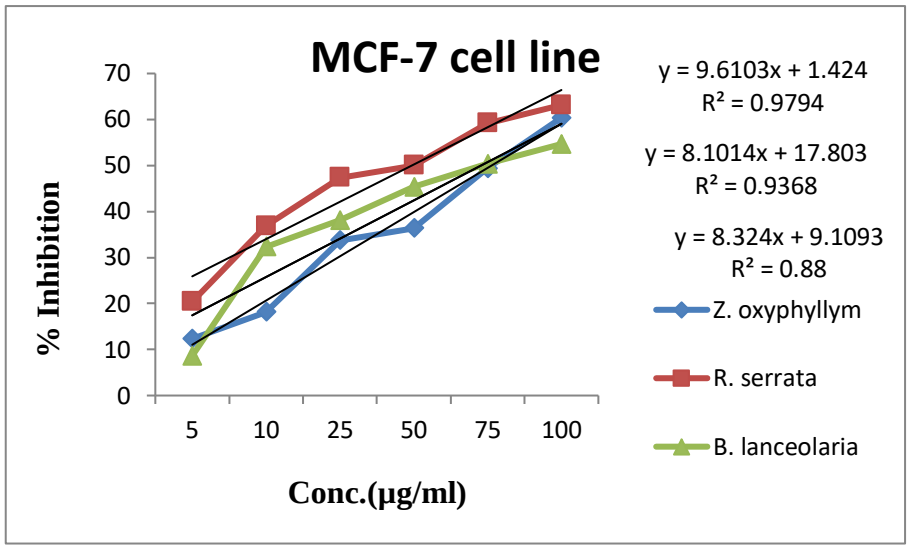


Figure 4.29: % of inhibition of the plant extracts against MCF-7 cell line**.**

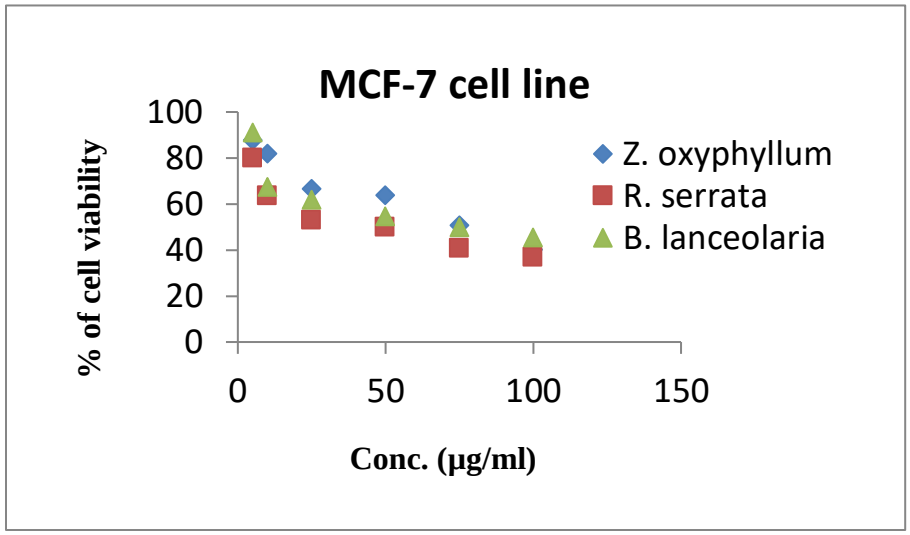


Figure 4.30: % Cell viability of promising extracts against MCF-7 cancer cells.

In HeLa cell line, the ME of *Z. oxyphyllum* and *B. lanceolaria* showed more toxicity with low IC_{50} values. Each extract was administered at different concentrations which were progressively increased over a period of 24hrs. Findings indicate that the ME derived from *B. lanceolaria* exhibited a dose-dependent inhibition of HeLa cell growth. The strong cytotoxicity activity exhibited by *B. lanceolaria* with low IC_{50} value (57.14µg/mL). A growth inhibition that varied in magnitude based on the dosage was detected within a concentration range of 5 to 100µg/ml. In addition, *R. serrata* had a modest level of cytotoxic acidity against HeLa cells. The percentage of cell viability was observed lowest at 100µg/ml for all the samples ranging from

32.21% to 44.13%. Cytotoxic effects against the cervical cell line HeLa were seen in the ME of *Z. oxyphyllum* and *B. lanceolaria* as evidenced by morphological observations. The extracts exhibited observable cellular morphological distortions following the treatment in HeLa cells in a time-dependent manner at 24, 48 and 72 hrs.

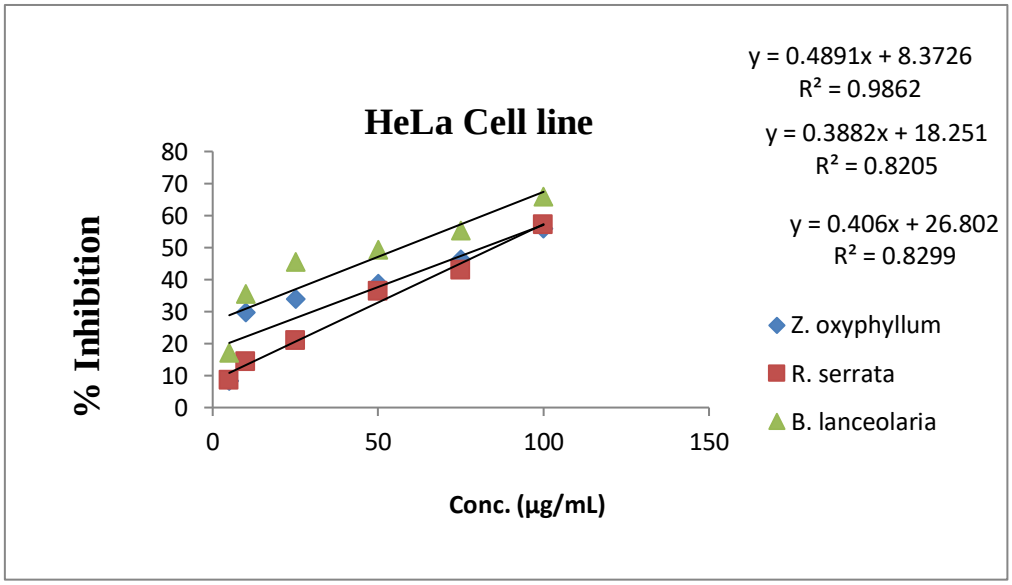


Figure 4.31: % inhibition of the plant extract against HeLa cell line

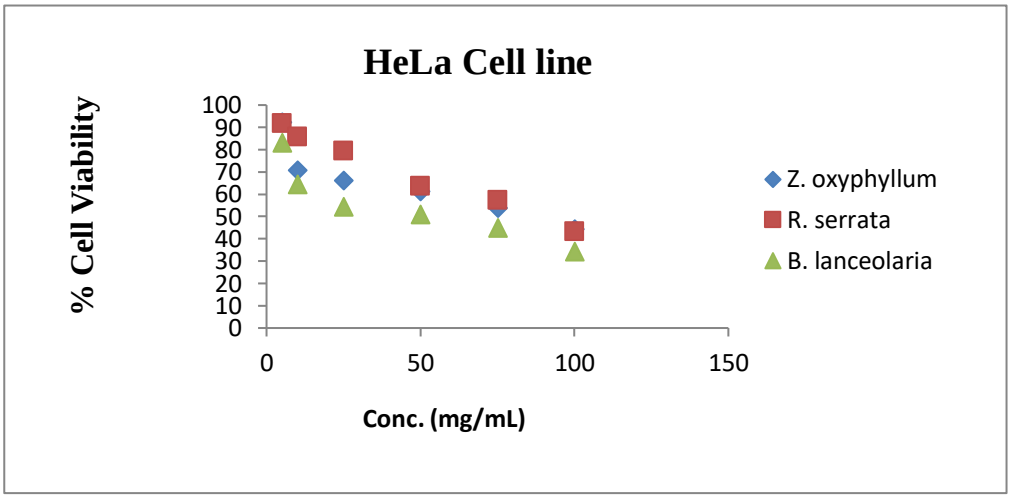


Figure 4.32: % Cell viability of promising extracts against HeLa cancer cell

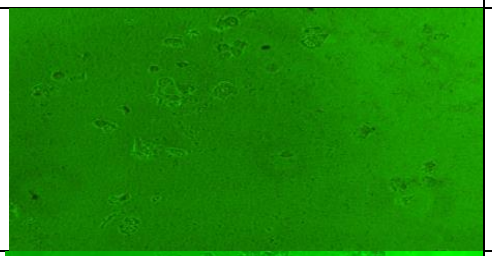
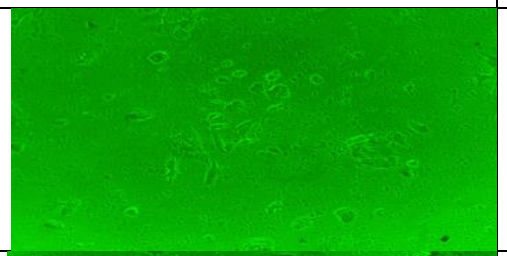
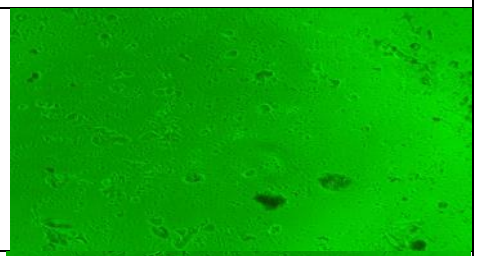
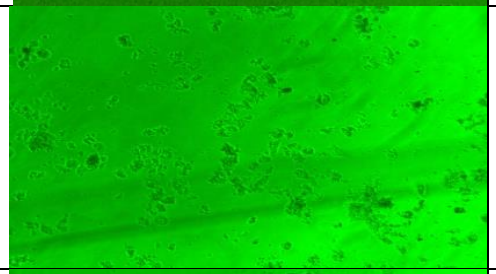
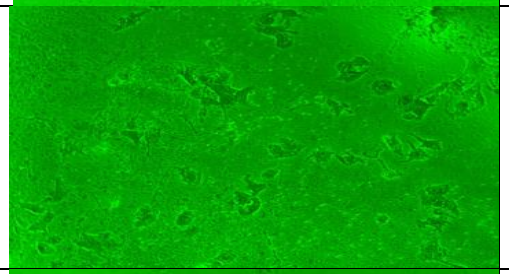
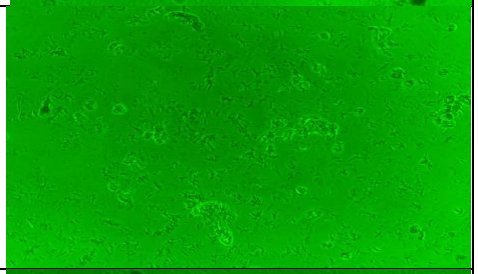
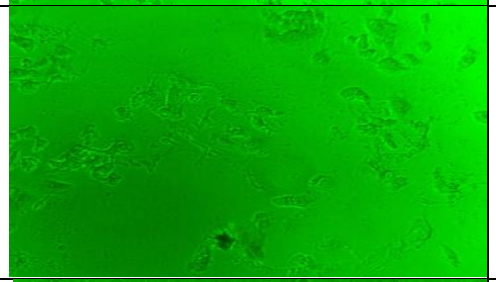
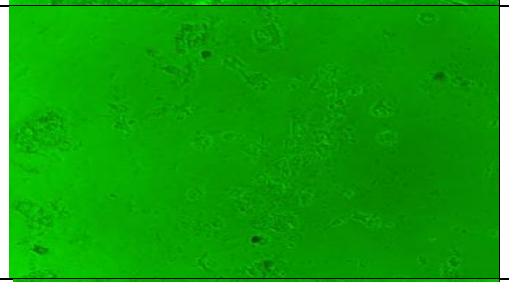
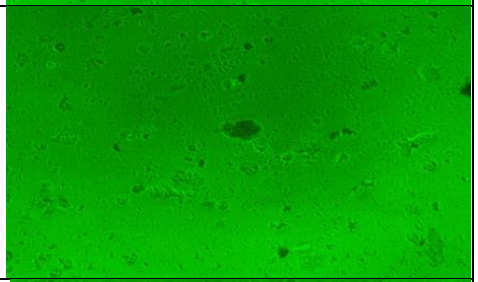
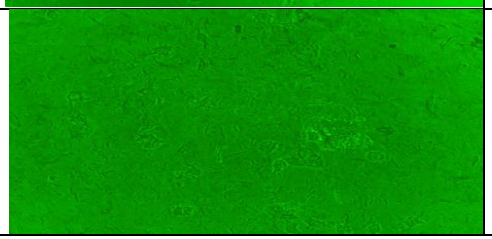
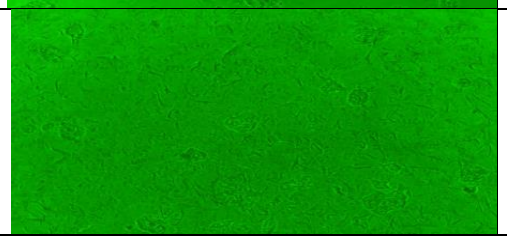
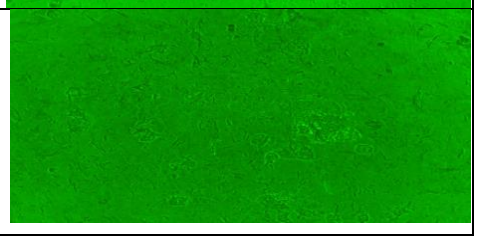
Samples	MCF-7		
	24hrs	**48 hrs**	**72hrs**
Control			
Z.oxyphyllum			
R. serrata			
B.lanceolaria			

Plate 4.11: Morphological changes of MCF-7 cells over treatment of the plant extracts.

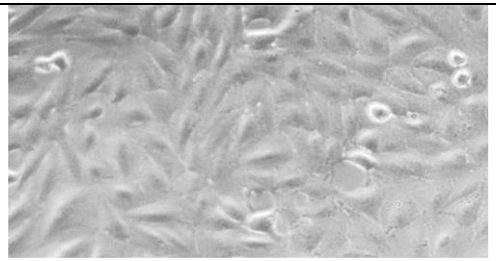
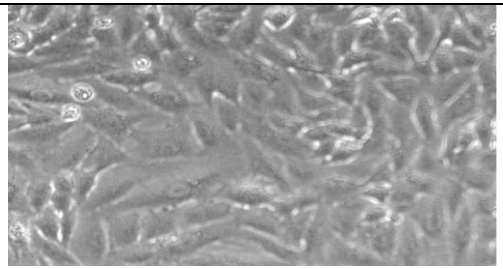
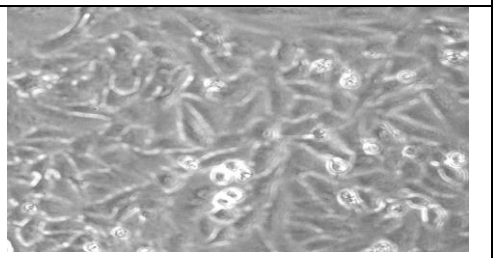
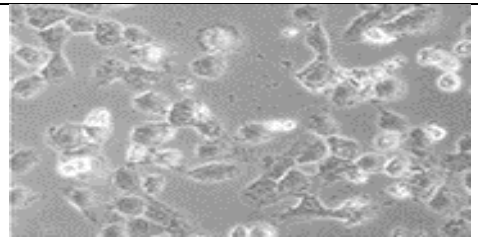
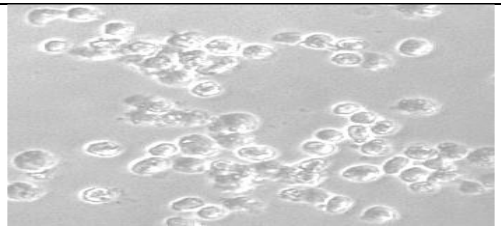
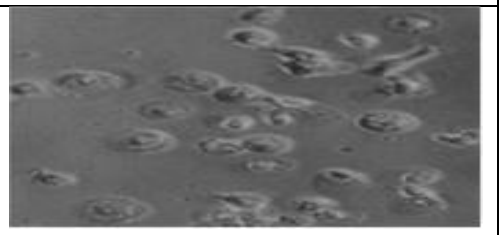
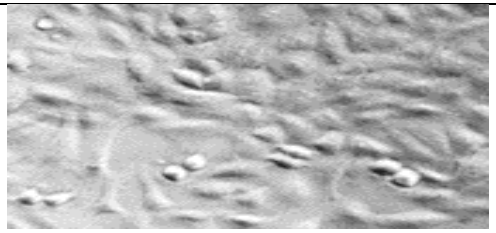
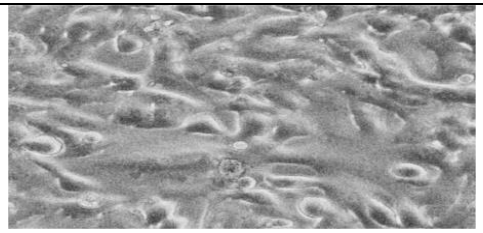
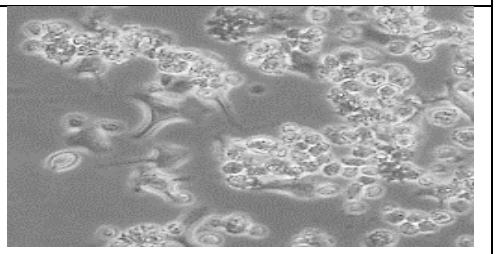
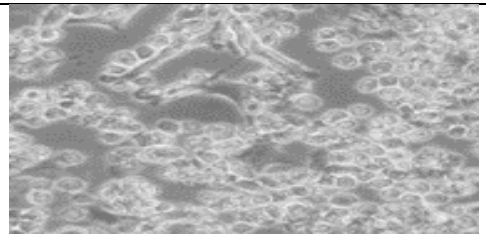
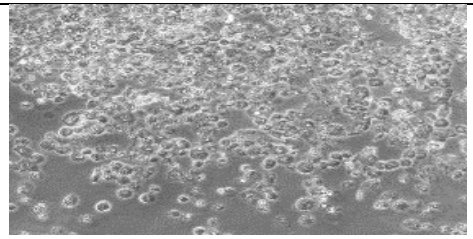
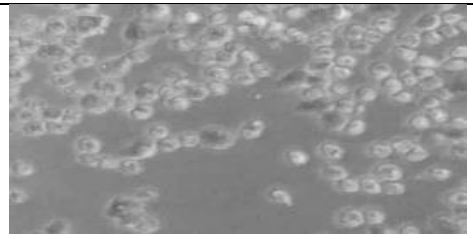
Samples	HeLa Cells		
	24 hrs	48 hrs	72 hrs
Control			
Z. oxyphyllum			
R. serrata			
B. lanceolaria			

Plate: 4.12. Morphological changes of HeLa cells over treatment of the plant extracts

4.10.2. AO/Et-Br dual staining and Flow cytometric analysis

AO/Et-Br staining method was used in conjunction with flow cytometric analysis to estimate the early apoptosis, late apoptosis, necrosis and live cell ratios of the IC_{50} values of plant extracts'. Staining with AO/Et-Br demonstrated the stage of cell death morphology, which appeared in different colours. Viable cells (V) were stained green with intact nucleus structure; early apoptotic cells (E) were marked yellow with condensed nuclear structure, cell shrinkage and the production of apoptotic bodies and late apoptotic cells (L) were stained reddish orange with patches of condensed chromatin in the nucleus. Control cells had an evenly positioned circular nucleus in the centre and fluoresced greenish. Conversely, necrotic cells (N) increased in volume and showed uneven stained with uniform red color fluorescence at their periphery. The outcome demonstrates that the extracts from the samples induce apoptosis in a dose-dependent manner.

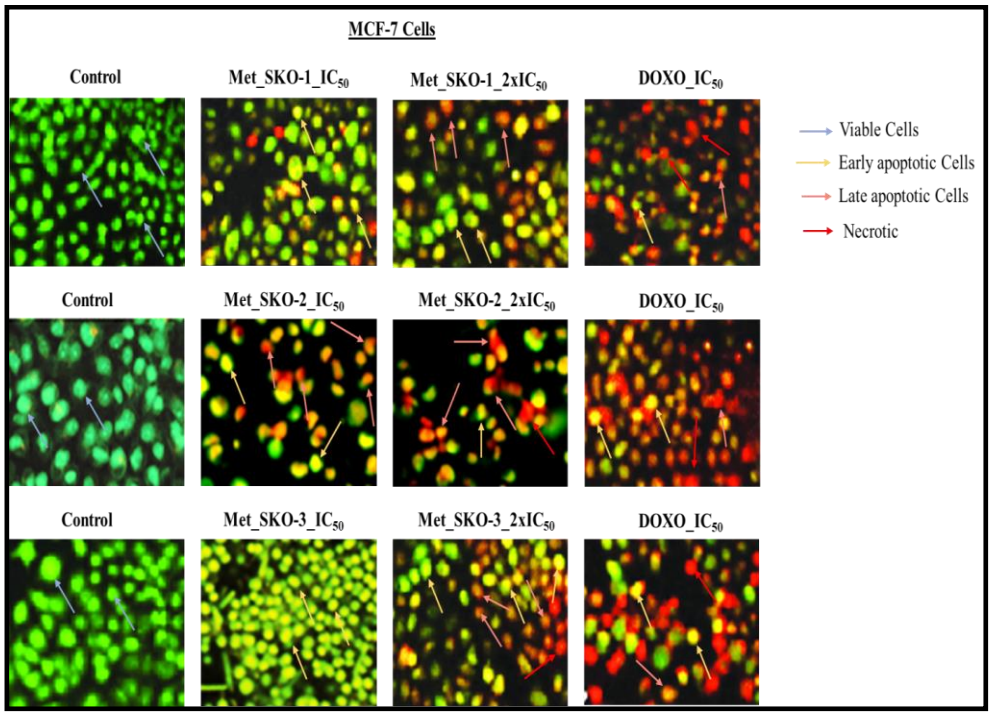


Plate 4.13: Apoptotic Cell death analysis of MCF-7 cells over the treatment of Met_SKO-1(*Z.oxyphyllum*), Met_SKO-2 (*R. serrata*) and Met_SKO-3 (*B. lanceolaria.*) at their IC_{50} and $2xIC_{50}$ using AO/Et-Br staining method. Doxorubicin was used as a positive control.

Table 4.43. Quantitative apoptotic analysis against MCF-7 Cells using AO/Et-Br dual staining.

Z.oxyphyllum	**Control**	**IC_{50}**	**$2xIC_{50}$**	**$DOXO\_IC_{50}$**
% Viable Cells	99.3 ± 8.6	46.3± 3.9	21.3± 3.6	16.8± 1.2
% Early Apoptotic cells	0.1± 0.0	39.2± 2.4	29.8± 3.4	23.6± 2.8
% Late Apoptotic cells	0±0.0	9.8± 1.1	38.5± 4.9	45.7± 5.3
% Necrotic Cells	0.6± 0.0	4.7± 0.62	10.4± 0.8	13.9± 1.1
R. serrata				
% Viable Cells	99.5± 9.5	24.3± 2.8	18.5± 1.9	16.8 ± 1.2
% Early Apoptotic cells	0.2± 0.0	48.2± 5.1	26.9± 2.7	23.6 ± 2.8
% Late Apoptotic cells	0± 0.0	22.1± 2.3	47.7± 5.3	45.7 ± 5.3
% Necrotic Cells	0.3± 0.0	5.4± 0.6	6.9± 0.7	13.9 ± 1.1
B. lanceolaria				
% Viable Cells	98.7± 9.3	47.3± 5.6	22.4± 2.9	16.8 ± 1.2
% Early Apoptotic cells	0.2± 0.0	41.5± 6.2	40.5± 4.7	23.6 ± 2.8
% Late Apoptotic cells	0± 0.0	6.1± 0.8	27.4± 3.1	45.7 ± 5.3
% Necrotic Cells	1.1± 0.0	5.1± 0.4	9.7± 0.6	13.9 ± 1.1

Note: Data presented as the mean ± SD of three experiment

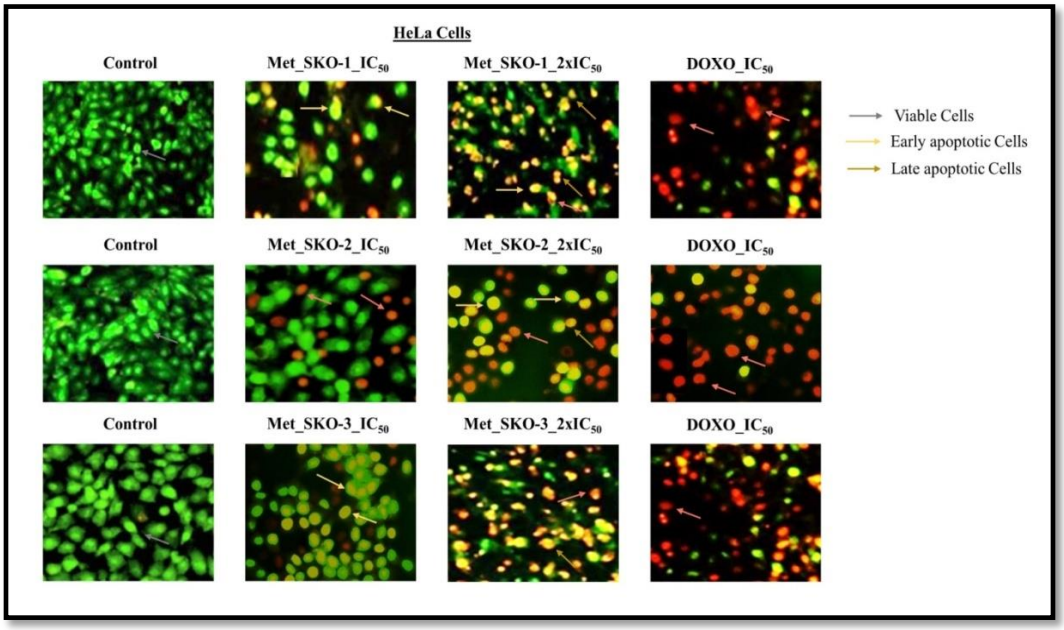


Plate 4.14: Apoptotic Cell death analysis of HeLa cells over the treatment of Met_SKO-1(*Z.oxyphyllum*), Met_SKO-2 (*R. serrata*) and Met_SKO-3 (*B. lanceolaria.*) at their IC_{50} and $2xIC_{50}$ using AO/Et-Br staining method. Doxorubicin was used as a positive control.

Table 4.44. Quantitative apoptotic analysis against HeLa Cells using AO/Et-Br dual staining

Z.oxyphyllum	**Control**	**IC_{50}**	**$2xIC_{50}$**	**$DOXO\_IC_{50}$**
% Viable Cells	99.1 ± 6.5	43.1± 3.1	29.4± 1.6	15.7± 1.9
% Early Apoptotic cells	0.1± 0.01	34.3± 2.6	26.6± 1.3	19.1± 1.6
% Late Apoptotic cells	0± 0.00	16.4± 1.2	34.7± 2.7	46.5± 3.5
% Necrotic Cells	0.8± 0.09	6.2± 0.5	9.3± 0.8	18.7± 1.3
R. Serrata				
% Viable Cells	99.1 ± 6.5	56.8± 3.9	41.9± 5.7	15.7± 1.9
% Early Apoptotic cells	0.1 ± 0.01	19.5± 1.5	14.4± 1.2	19.1± 1.6
% Late Apoptotic cells	0 ± 0.00	11.3± 1.3	27.8± 1.9	46.5± 3.5
% Necrotic Cells	0.8 ± 0.09	12.4± 1.8	15.9± 1.1	18.7± 1.3
B. lanceolaria				
% Viable Cells	99.1 ± 6.5	34.6± 2.8	13.5± 1.1	15.7± 1.9
% Early Apoptotic cells	0.1 ± 0.01	37.4± 2.1	22.8± 1.6	19.1± 1.6
% Late Apoptotic cells	0 ± 0.00	21.7± 1.3	54.6± 2.7	46.5± 3.5
% Necrotic Cells	0.8 ± 0.09	6.3± 0.5	9.1± 0.8	18.7± 1.3

Note: Data presented as the mean ± SD of three experiments.

FLOW CYTOMETRY ANALYSIS

Using flow cytometry and annexin V/PI studies, apoptotic cells were found in a time-dependent fashion. The distribution of MCF-7 and HeLa cells over four quadrants is shown in Plate 4.15 and Plate 4.16. Necrosis is represented by Q1 (An-, PI+), late apoptosis by Q2 (An+, PI+), viable cells are represented by Q3 (An-, PI-) and early apoptosis is represented by Q4 (An+, PI-). The ability of all plant extracts to induce cell death in MCF-7 and HeLa cells at an early stage of apoptosis was more visible in IC_{50} but in $2xIC_{50}$ it became more visible at the late stage of apoptosis. This was demonstrated by comparing the results obtained using flow cytometry analysis and quantitative fluorescence staining.

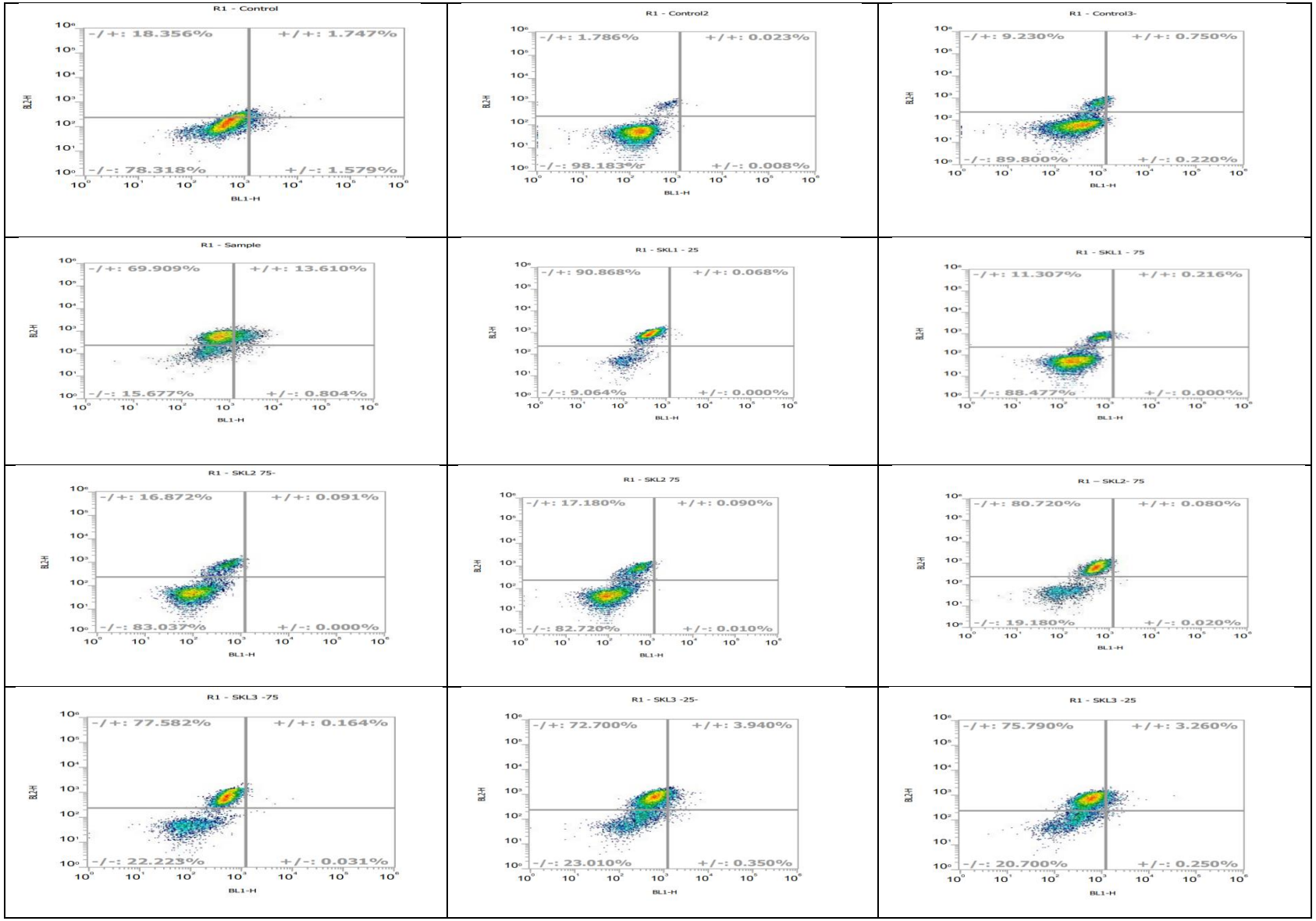


Plate 4.15. Flow cytometry result of the apoptotic activities of the samples against MCF-7 cancer cell

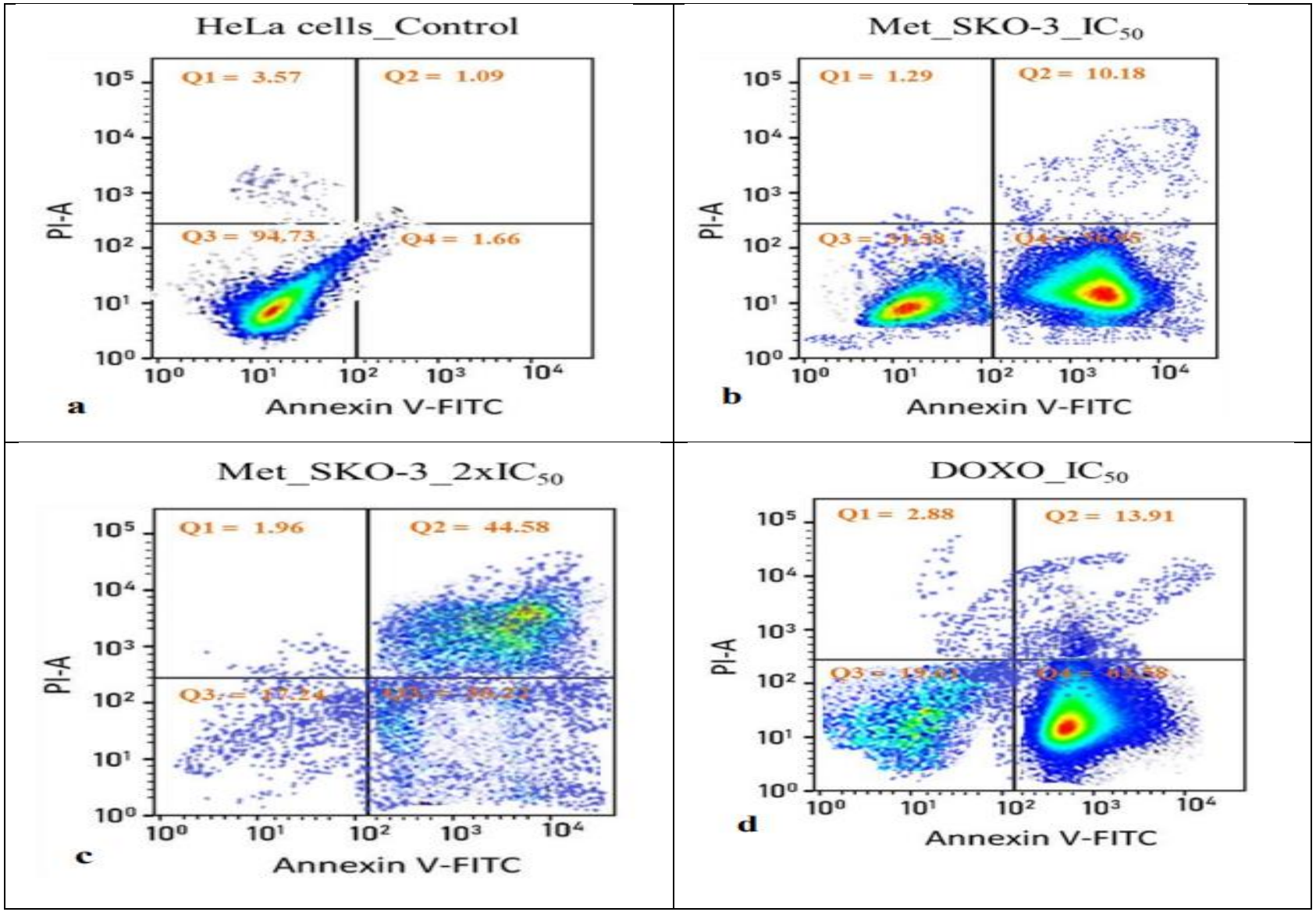


Plate 4.16. Flow cytometry result of theapoptotic activity of *B. lanceolaria* against HeLa cancer cell

Table 4.45: Quantitative cell distribution of MCF-7 cells over the treatment of plant extracts in various apoptotic stages

	Control	**SK01 (IC_{50})**	**SK02(IC_{50})**	**SK03 (IC_{50})**
Q1= Necrotic cells (%)	9.78±8.2	57.35±41.23	38.24±36.78	75.35±2.46
Q2= Late Apoptotic Cells (%)	0.83±0.8	4.62±7.70	0.08±0.005	2.45±2.01
Q3= Viable Cells (%)	88.79±9.9	37.73±44.06	61.64±36.77	21.97±1.17
Q4= Early apoptotic cells (%)	0.59±0.8	0.80±0.00	0.015±0.01	0.21±0.16

Note: Data presented as the mean ± SD of three experiments
SK01 (*Z. oxyphyllum*), SK02 (*R. serrata*), SK03 (*B. lanceolaria*)

Table 4.46: Quantitative cell distribution of HeLa cells over the treatment of *B. lanceolaria* in various apoptotic stages

	Control	**SK03 (IC_{50})**	**SK03 (2× IC_{50})**	**DoXo (IC_{50})**
Q1= Necrotic cells (%)	3.57±0.27	1.29±0.09	1.96±0.08	2.88±0.09
Q2= Late Apoptotic Cells (%)	1.09±0.09	10.18±1.23	44.58±3.56	13.91±1.62
Q3= Viable Cells (%)	93.68±7.54	31.58±2.54	17.24±1.84	19.63±2.18
Q4= Early apoptotic cells (%)	1.66±0.08	56.95±6.03	36.22±4.25	63.58±8.46

Note: Data presented as the mean ± SD of three experiments.
SK03 (*B. lanceolaria*)