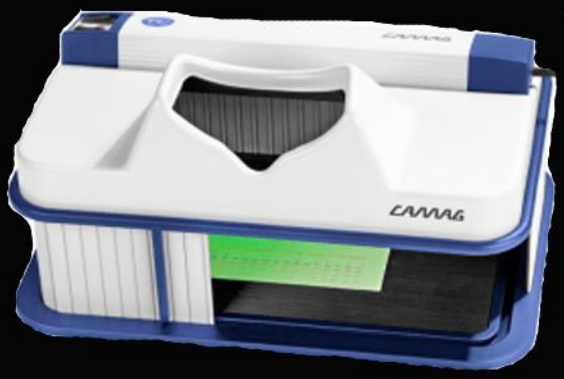
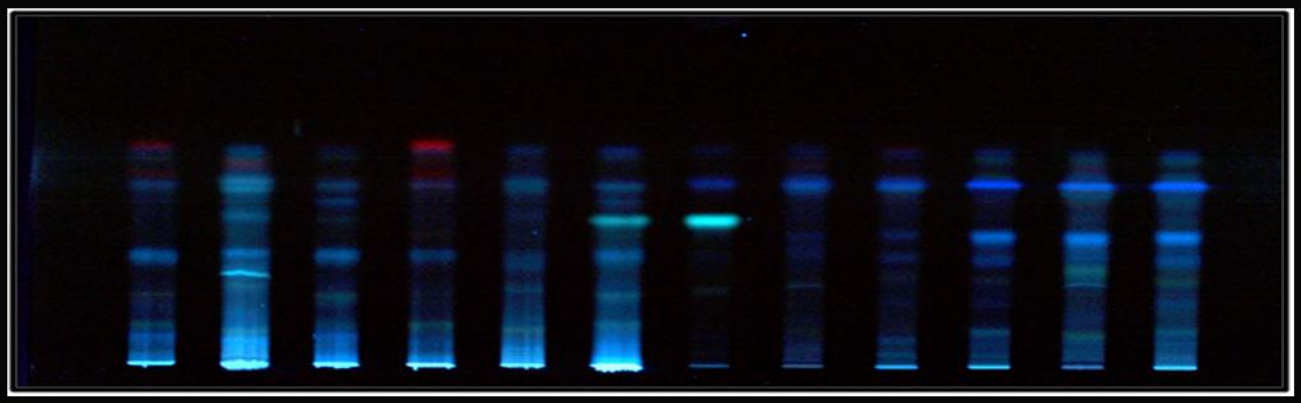


CHAPTER 5
PHYTOCHEMISTRY



5.1. INTRODUCTION

Traditional systems of medicine are having a vogue for centuries all over the world. According to Ekor (2014), 80% of the world population still depending on herbal products for their primary health care. However, after industrialization, there was a boon for synthetic drugs and rapid expansion of allopathic medicinal system took place. In the current scenario due to high toxicity of market drugs as well as gradual increase in the resistance power of pathogens in many diseases (e.g. malaria, cancer, tuberculosis etc.) and lack of medicines for many chronic ailments have led to re-emergence of the herbal medicine, with lots of possible treatments for many health problems (Cui and Su, 2009; Chaudhary and Singh, 2011). Consequently, the use of plant-based medicines has been increasing all over the world. According to an estimate 20,000 plant species out of 2,50,000 species are in use as medicines all over the world (Ramawat and Mérillon, 2008).

असंबाधंबध्यतोमानवानांयस्याउद्वतःप्रवतःसमंबहु।

नानावीर्याओषधीर्याबिभर्तिपृथिवीनःप्रथतांराध्यतांनः॥

Mother earth extends unimpeded freedom (both outer and inner) to human beings through her mountains, slopes and plains. She bears many plants and medicinal herbs of various potencies to make us healthy (Prakash, 1992).

Since the beginning of mankind, people were relied on the different plants for food (fruits, flowers, leaves, vegetables, tubers, rhizomes), shelter (trunk, wood, branches), clothes (leaves and barks), poison for hunting, hallucinogenic agents and stimulant beverages. People use various flowers, frankincense and fruits in different rituals and social ceremonies (Fulekar, 2010). Every civilization accrues widespread knowledge of diverse biological flora and fauna and their medicinal potentials which were carried forward from many generations and still many people are using it as a folk medicine or as a part of food on the daily basis (Feleszko and Jaworska, 2013). Over the past several decades, scientific literature and media articles shows an increased interest in natural products isolation, identification and their pharmacological applications by the general public (Folashade et al., 2012; Krause and Tobin, 2013). A majority of the world still relies heavily on herbal remedies for their primary healthcare (Cooper and Nicola, 2014).

5.2. PHYTOCHEMISTRY

The word phytochemistry derives from the Greek word *phyton* which means plant and *Chemeia* means chemistry. **Phytochemistry** is the branch of chemistry/botany deals with study of natural products including primary (carbohydrates, fats, vitamins, proteins etc) as well as secondary metabolites (alkaloids, phenolics and terpenes) (Schmidt and Cheng, 2017). The **primary metabolites** of plants are the substance which takes parts in promotion of growth, metabolism and reproduction of the plant. Other than primary metabolites, there are other metabolites present in plants which do not have direct significant role in growth and metabolism called as **secondary metabolites**. However they perform chemical defense mechanism, act as allelochemics, promote pollination, aroma, flavor, color, protect from biotic and abiotic stress, as well as of medicinal important (Seigler, 2012; Siddiqui et al., 2017). They also exhibit a number of protective functions, increases immunity and medicinally significant for human beings. Due to enormous medicinal applications of secondary metabolites scientist are working globally for developing the techniques for extraction, isolation and structural elucidation of secondary metabolites, using various chromatographic and spectroscopic techniques.

5.2.1. Classification of Phytochemicals

Phytochemicals are broadly classified in three classes namely **Alkaloids, Phenolics and Terpenoids.**

5.2.1.1. Alkaloids

Alkaloid represent group of secondary chemical constituents comprising basically of nitrogen as a heteroatom synthesized from amino acid building blocks with various radicals replacing one or more of the hydrogen atoms in the peptide ring. The compounds have basic properties and are alkaline in reaction, turning red litmus paper blue. The degree of basicity varies considerably with the nature of nitrogen, whether the nitrogen is primary, secondary, tertiary or/quaternary. Generally, alkaloids are intensely bitter in taste. They possess wide range of pharmacological activities *viz.* antibacterial, antimalarial, anticancer, analgesic, antiarrhythmic and antiasthmatic (Fattorusso and Taglialatela-Scafati, 2008).

Biosynthetic classification of alkaloids

- **True alkaloids:** Alkaloids which are originated from amino acids as biosynthetic products and nitrogen atom present as a heteroatom in the chemical moiety e.g. Nicotine and Coniine.
- **Proto alkaloids**: Alkaloids contains Nitrogen but not as a heteroatom but originate from amino acids e.g. Mescaline and Epinephrine.
- **Pseudo alkaloids** are the compounds which are not originate from amino acids e.g. Caffeine and Theophylline.

Alkaloids are further classified based on their chemical nature

a) Aporphine (Dicentrine is an anticancer compound isolated from *Lindera* sp.)

b) Isoquinoline (Berberine extracted from *Berberis vulgaris* to treat burns and trachoma).

c) Indole (β-Carboline was reported from *Banisteriopsis caapi* used as convulsive, anxiogenic and memory enhancing effects).

d) Imidazole (Jaborandi leaves contains pilocarpine which is used in ophthalmic work).

e) Pyrrolizidine (Retronecine, a pyrrolizidine alkaloid found in the Common groundsel (*Senecio vulgaris*) and comfrey (*Symphytum* spp.) used as anticancer and antivenom).

f) Pyridine (Nicotine is found in the leaves of *Nicotiana rustica* used as additive and as an insecticide).

g) Purine (Caffeine and Theobromine from *Camellia* species used as improve mental alertness).

h) Piperidine (Carpaine is extracted from papaya leaves used to increase platelet count).

i) Quinoline (Quinine isolated from *Cinchona* sp. used as antimalaria).

j) Quinolizidine (Sparteine extracted from *Lupinus mutabilis* used as a class-1a antiarrhythmic agent).

k) Tropane (Hyoscine, also known as scopolamine isolated from *Datura* sp. used to treat motion sickness and postoperative nausea and vomiting).

5.2.1.2. Phenolics

Phenolics are the chemical components that occur ubiquitously as natural color pigments responsible for the color of fruits. The biosynthesis of phenolics takes place either via acetate-malonate or shikimic acid pathway. The most significant role of phenolics in plant is defense against pathogens and herbivore predators by having puckered taste and to attract insects for pollination. They have significant roles in biological, electrochemical systems and natural dyes. Phenolics have potential against oxidative damages occur during metabolic processes in the organism. Because of their antioxidant activities, they are widely used as a natural antioxidant (Cheynier et al., 2013; Ramawat and Mérillon, 2013). The classification of phenolics along with their example is given in Table 5.1.

Table 5.1: Classification of phenolics

Groups	Types	Examples
Phenolic acid	Simple phenolics (C_6)	pyrocatechol, resorcinol, hydroquinone, phloroglucinol, pyrogallol
	Simple phenolic acids and derivative (C_6-C_1)	p-hydroxybenzoic acid, gallic acid, protocatecheuic acid, salicylic acid
	Acetophenones and phenylacetic acids (C_6-C_2)	acetovanillone, paeonol, 2-hydroxyl-phenyl acetic acid, 4-hydroxyl-phenyl acetic acid
Cinnamic acid	Cinnamic acid and derivative (C_6-C_3)	cinnamic acid, p- coumaric acid, caffeic acid, ferulic acid
	Coumarins, isocoumarins and chromones (C_6-C_3)	umbelliferone, bergenin
	Lignan (C_6-C_3)	p-coumaryl alcohol, coniferyl alcohol, sinapyl alcohol
Flavonoids	Chalcones (C_6-C_3-C_6)	xanthohumol, cardamonin
	Aurones (C_6-C_3-C_6)	aureusidin, sulfuretin
	Flavones (C_6-C_3-C_6)	kaemferol, quercetin, myricetin
	Flavanones (C_6-C_3-C_6)	naringenin
	Flavanonols (C_6-C_3-C_6)	taxifolin

Groups	Types	Examples
	Leucoanthocyanins (C_6-C_3-C_6)	teucocyanidin, leucodelphinidin
	Anthocyanidins (C_6-C_3-C_6)	pelargonidin, cyanidin, peonidin, delphinidin, petunidin, malvidin
	Anthocyanins (C_6-C_3-C_6)	petanin
Others	benzophenone, xanthone (C_6-C_1-C_6)	benzophenones, xanthones
	Stilbenes (C_6-C_2-C_6)	resveratrol, pinosylvin
	Benzoquinones (C_6), anthraquinones (C_6-C_1-C_6), Naphthaquinones (C_{10})	2,6-dimethoxybenzoquinone, ubiquinone, juglone, emodin
	Betacyanins (C_{15})	betanidin, indicaxanthin
Polymers	Biflavonyls (C_{30})	ginkgetin
	Lignin (C_n)	polymer of p-coumaryl alcohol, coniferyl alcohol, sinapyl alcohol
	Tannin (C_n)	Condensed tannins: polymer of flavan-3-ol Gallotannins: polymer of 10-12 gallic acid Ellagitannins: polymer of pentagalloyl glucose Complex tannins: polymer of catechin unit bound glycosidically to either a gallotannin or an ellagitannin unit
	Phlobaphenes (C_n)	polymers of flavan-4-ols, apiferol and luteoferol

5.2.1.3. Terpenoids

The term 'terpene' came from turpentine, which was a volatile liquid isolated from pine trees. "**Terpenoids** are the hydrocarbons of plant origin of the general formula $(C_5H_8)_n$ as well as their oxygenated, hydrogenated and dehydrogenated derivatives" (Yadav et al., 2014). The classification of terpene is given in Table 5.2.

Table 5.2: Classification of terpenes

SN	Number of carbon atoms	Class
1.	10	Monoterpenoids ($C_{10}H_{16}$) **e.g.** Geraniol, Limonine, Carene
2.	15	Sesquiterpenoinds ($C_{15}H_{24}$) **e.g.** Farnesol, Bisabolene, Selinene, Santonin
3.	20	Diterpenoids ($C_{20}H_{32}$) **e.g.** Phytol, Abeitic Acid, Gibberellic acid
4.	25	Sesterpenoids ($C_{25}H_{40}$) **e.g.** Haslene, Ceroplastol
5.	30	Triterpenoids ($C_{30}H_{48}$) **e.g.** Squalene, Cholesterol, Estrone
6.	40	Tetraterpenoids ($C_{40}H_{64}$) **e.g.** Lycopene, Luteine

5.2.2. Phytochemicals of Orchids

Orchids are rich in secondary metabolites as it is reflected by their vast medicinal properties. In search of different phytochemicals groups, many scientists explored different parts of numbers of orchids for secondary metabolite screening (Da Silva, 2013; Seigler, 2012; Sut et al., 2017; Teoh, 2016a, b). Orchids are enriched with various phytochemicals such as alkaloids, bibenzyl derivatives, flavonoids, phenanthrenes, terpenoids as mentioned in Table 5.3.

Table 5.3: Orchids and their phytochemicals

SN	Plant name	Compounds	Reference
		ALKALOIDS	
1	*Dendrobium loddigesii* Rolfe	Shihunidine, Shihunine	Lam et al., 2015; Li et al., 1991; Rungwichaniwat et al., 2014
2.	*D. macraei* Lindl.	Jebantine	Esha et al., 2016; Singh and Duggal, 2009
3.	*D. moniliforme* (L.) Sw.	Dendrobine	Bi et al., 2004; Chen and Chen, 1935; Chen et al., 2001
4.	*D. moschatum* (Buch.-Ham.) Sw.	Rotundatin, moscatin	De, 2015; Shailajan et al., 2015
5.	*D. nobile* Lindl.	Dendrobine	Tang and Eisenbrand, 1992; Wang et al., 2014
6.	*Gastrodia elata* Blume	N^6-(4-hydroxybenzyl) adenine riboside	Lei et al., 2011; Shi et al., 2014; Tang et al., 2017
		PHENANTHRENE (Group of Alkaloid)	
7.	*Aerides crispum* Lindl. & *A. affine* Wall. ex Lindl.	Aeridin	Anuradha and Rao, 1998; Singh, 2011
8.	*Bulbophyllum gymnops* Hook.f.	Gymopusin	Kalaiarasan and John, 2011; Singh, 2011
9.	*Cypripedium calceolus* var. *pubescens* (Willd.) Correll	Cypripedin	De, 2015; Singh and Duggal, 2009
10.	*Eulophia nuda* Lindl.	Nudol	Bhandari et al., 1985; Kshirsagar et al., 2010

SN	Plant name	Compounds	Reference
TERPENOID			
12.	*Agrostophyllum brevipes* King & Pantl., *A. callosum* Rchb.f.	Agrostophyllinol, Agrostophyllinone, Isoagrostophyllol	(Majumder et al., 1996, 2003, 1999)
15.	*Dendrobium moniliforme* (L.) Sw., *D. nobile* Lindl.	Dendromoniliside A, B & C, Dendroside A, D, E, F & G, Dendronobiloside A	Pridgeon et al., 2014; Singh et al., 2012; Zhou et al., 2011
17.	*Ephemerantha lonchophylla* (Hook.f.) P.F.Hunt & Summerh.	Lonchophylloid A & B	Ma et al., 1998; Pridgeon et al., 2014; Teoh, 2016a
18.	*Nidema boothii* (Lindl.) Schltr.	Nidemin	Hernández-Romero et al., 2004; Singh et al., 2012
GLYCOSIDE			
19.	*Anoectochilus formosanus* Hayata	Kinsenoside	Chung et al., 2017; Shiau et al., 2002; Wang et al., 2002
20.	*Vanda roxburghii* R.Br.	Melianin	Ahmed et al., 2002; De, 2015
GLUCOSIDE (Glycoside derived from Glucose)			
21.	*Orchis latifolia* L.	Loroglossin	Singh, 2011; Singh and Duggal, 2009
FLAVONOID (Group of Phenolic compounds)			
22.	*Anoectochilus roxburghii* (Wall.) Lindl.	Kaempferol-3-O-β-D-glucopyranoside, Kaempferol-7-β-D-glucopyranoside, Isorhamnetin-3-β-D-rutinoside, 8-C-phydroxybenzylquercetin, Quercetin-7-	He et al., 2006; Liu et al., 2014; Ye et al., 2017

SN	Plant name	Compounds	Reference
		O- β-D-[6"-O-(transferuloyl)]-glucopyranoside, 5-Hydroxy-3',4',7-trimethoxyflavonol-3-β-D-rutinoside, Isorhamnetin-3-O β-D-glucopyranoside, Isorhamnetin-7-O-β-D-glucopyranoside	
23.	*Cypripedium macranthos* Sw.	Chrysin	Ángeles Ramírez-Cisneros et al., 2016; Shimura et al., 2007
24.	*Dendrobium densiflorum* Lindl.	Homoeriodictyol	Fan et al., 2001; Luo et al., 2008
25.	*Spiranthes australis* (R.Br.) Lindl.	(2S)-5,2',6'-trihydroxy-6-lavandulyl-4"-(γ,γ-dimethylallyl)-2",2"-dimethylpyrano-[5",6",7,8]-flavanone	Peng et al., 2007
BIBENZYL DERIVATIVE (Group of Phenolic compounds)			
26.	*Bulbophyllum kwangtungense* Schltr.	Cumulatin, Densiflorol A	Teoh, 2016a; Wu et al., 2006
27.	*Cymbidium goeringii* (Rchb.f.) Rchb.f. & *Scaphyglottis livida* (Lindl.) Schltr.	Gigantol	Déciga-Campos et al., 2007; Won et al., 2006
28.	*Dendrobium amoenum* Wall. ex Lindl.	Isoamoenylin	Venkateswarlu et al., 2002
29.	*D. chrysotoxum* Lindl.	Erianin	Gong et al., 2004
30.	*D. loddigesii* Rolfe	Moscatilin, Moscatin diacetate	Chen et al., 1994; Ho and Chen, 2003; Miyazawa et al., 1999
31.	*D. moniliforme* (L.) Sw.	Alkyl ferulates	Agrawal, 2017; Lo et al., 2004

SN	Plant name	Compounds	Reference
32.	*D. nobile* Lindl.	Nobilin D & E, Moscatilin	Zhang et al., 2007
33.	*Ephemerantha lonchophylla* (Hook.f.) P.F.Hunt & Summerh.	3-Methylgigantol	Chen et al., 2000
34.	*Nidema boothii* (Lindl.) Schltr.	Aloifol	Hernández-Romero et al., 2004
STILBENOID (Group of Phenolic compounds)			
35.	*Arundina graminifolia* (D.Don) Hochr.	Arundinan	Nugroho et al., 2016; Sugathan et al., 2017
36.	*Agrostophyllum callosum* Rchb.f.	Orchinol, 6-methoxycoelonin, imbricatin, oxoflaccidin, flaccidin, isooxoflaccidin, callosinin, callosuminin, flaccidinin, callosin, agrostophyllin, callosumin, callosumidin.	Majumder et al., 1996

5.2.3. Ethnopharmacological Properties of Orchids

Orchids are the group of bizarre plants which are albeit most economical and beautiful ornamental plants, shows many ethnopharmacological properties as well as recent medicinal applications. However, the scantiness of scientific evaluation and documentation as well as gradual decrease in conventional uses of orchid diminishes its uses as a medicinal alternative. Therefore, it is essential to comprehension the ethnopharmacological relevance of orchids to enhance further advancement in this direction. The enrichment of many phytochemicals in orchids directly reflects in its diverse medicinal properties reported conventionally (Pant, 2013; Singh, 2011; Wiart, 2007). Different parts of orchids associated with various medicinal properties were described in Table 5.4.

Table 5.4: Ethnobotanical importance of Orchids (The species with bold letter are found in Gujarat region.)

SN	Botanical name	Part used	Uses	Reference
1.	***Acampe papilliosa* Lindl.**	R	Treat rheumatism	Akhter et al., 2017; Hoque et al., 2016; Hossain, 2009; Teoh, 2016a
2.	*Aerides multiflora* Roxb.	L, B & R	Antibacterial, leaf paste to treat cuts & wounds.	Ghanaksh and Kaushik, 1993; Singh, 2011; Teoh, 2016a
3.	*A. odoratum* Lour.	L		Ghanaksh and Kaushik, 1993; Singh, 2011; Teoh, 2016a
4.	*Anoectochilus roxburghii* (Wall.) Lindl.	WP	Treatment of tuberculosis	Ye et al., 2017
5.	*Arundina graminifolia* (D.Don) Hochr.	R	Analgesic	Singh and Duggal, 2009
6.	*Brachycorythis obcordata* (Lindl.) Summerh	R	Tonic	Balami, 2004
7.	*Bulbophyllum careyanum* (Hook.) Sprengel	L & Pb	Leaf powder for abortion; pseudobulb to cure burns	Subedi et al., 2013
8.	*B. leopardinum* (Wall.) Lindl.	WP	To cure burns	Subedi et al., 2013
9.	*B. odoratissimum* (Sm.) Lindl.	WP	Cure tuberculosis, cancer & fracture	Sharangi and Datta, 2015; Wiart, 2012
10.	*B. umbellatum* Lindl.	WP	Nootropics	Panda et al., 2015
11.	*Calanthe plantaginea* Lindl.	RZ	Tonic & aphrodisiac	Subedi et al., 2013; Teoh, 2016a
12.	*C. puberula* Lindl.	RZ	Tonic	Subedi et al., 2013; Teoh, 2016a
13.	*C. sylvatica* (Thou) Lindl.	Fl	Juice to stop nose bleeding	Mythili, 2015

SN	Botanical name	Part used	Uses	Reference
14.	*C. tricarinata* Lindl.	L & Pb	Aphrodisiac, leaf paste to treat sores & eczema.	Bhat et al., 2013; Joshi et al., 2009
15.	*Cephalanthera longifolia* K. Fritsch	R	Promoting lactation	Faizul Haq, 2012
16.	*Coelogyne corymbosa* Lindl.	Pb	Cure wound & headache	Hossain, 2011
17.	*C. cristata* Lindl.	L & Pb	Aphrodisiac, to cure boils, constipation & wound	Pramanick, 2016
18.	*C. flaccida* Lindl	Pb	Antipyretic & analgesic	Kaur and Bhutani, 2014
19.	*C. fuscescens* Lindl.	Pb	Cure abdominal pain & burns	Koirala et al., 2013
20.	*C. nitida* (Wall. ex Lindl) D. Don	Pb	Antipyretic & analgesic	Pant, 2013
21.	*C. ovalis* Lindl.	Pb	Aphrodisiac	De et al., 2014
22.	*C. prolifera* Lindl.	Pb	Antipyretic, analgesic, cure burns, boils & backache	Singh et al., 2012; Teoh, 2016a
23.	*C. stricta* (D. Don) Schltr.	Pb	Antipyretic & analgesic	Moin et al., 2012
24.	*Conchidium muscicola* Lindl.	WP	To treat cardiac, respiratory & nervous disorder	Teoh, 2016a
25.	*Crepidium acuminatum* (D. Don) Szlach	RZ, R & Ps	Tonic, root powder for burns; bulbs for bronchitis, tuberculosis, fever & weakness	Arora et al., 2017
26.	*Cymbidium aloifolium* (L.) Sw.	RZ, R & Ps	Tonic, paste for dislocated bones & fracture; bulbs as demulcent	Shubha and Chowdappa, 2016

SN	Botanical name	Part used	Uses	Reference
27.	*C. devonianum* Lindl. ex Paxton	WP	Root paste to treat boils; decoction for cough & cold	Pant, 2013
28.	*C. elegans* Lindl.	L, Ps & R	To treat deep wound, coagulator	Pant, 2013; Subedi et al., 2013
29.	*C. iridioides* D. Don	L, Ps & R	Tonic, coagulator	Pant and Swar, 2011
30.	*Cypripedium cordigerum* D. Don	R	Tonic, treatment of mental disorders	Khan et al., 2013; Rana et al., 2013
31.	*C. elegans* Reichenb .f. Nep	R	To treat hysteria, rheumatism, madness, spasm & epilepsy	Hossain, 2011
32.	*C. himalaicum* (Rolfe) Kranzl	WP	To treat urine blocks, stone, heart disease, chest disorder & cough	Pant, 2013; Teoh, 2016a
33.	*Dactylorhiza hatagirea* (D. Don) Soo	T	Aphrodisiac, tonic, wound healing, control bleeding, burns & bronchitis	Pant and Rinchen, 2012; Selvam, 2012
34.	*Dendrobium amoenum* Wall. ex Lindl.	P	To treat burns & dislocated bones	Pant, 2013
35.	*D. crepidatum* Griff.	P	Anticancer, nerve tonic	Li et al., 2013; Prasad and Koch, 2016
36.	*D. densiflorum* Lindl.	Pb	To treat boils, pimples, other skin eruption, relieve throat inflammation, fatigue, reduce peripheral vascular obstruction & enhance body immunity	Luo et al., 2008; Pradhan et al., 2013
37.	*D. eriaeflorum* Griff.	Pb	Tonic, treat fractured & dislocated bones	Pant, 2013
38.	*D. fimbriatum* Hook.	WP	In liver upset & nootropic	Shailajan et al., 2017

SN	Botanical name	Part used	Uses	Reference
39.	*D. heterocarpum* Wall. ex Lindl.	Pb	To treat fractured & dislocated bones	Subedi et al., 2013; Teoh, 2016a
40.	*D. longicornu* Lindl.	WP	Antipyretics, antioxidant, antitumor & antimutagenic	Li et al., 2009; Paudel et al., 2017
41.	*D. macraei* (Lindl.) Seidenf.	WP	Astringent, aphrodisiac, brain tonic, expectorant, treat asthma & bronchitis	Vatsa and Bora, 2016
42.	*D. monticola* P.F. Hunt & Summerh.	WP	Treat boils, pimples & other skin eruptions	Vaidya et al., 2000
43.	*D. moschatum* Lindl.	Pb	To treat fractured & dislocated bones	Teoh, 2016a
44.	*D. nobile* Lindl.	S	Tonic, antipyretic	Bulpitt et al., 2007
45.	*D. transparens* Wall. ex Lindl.	Pb	For fractured & dislocated bones	Subedi et al., 2013; Teoh, 2016a
46.	*Dienia cylindrostachya* Lindl	Pb	Tonic	Teoh, 2016a
47.	*Epipactis helleborine* (L.) Crantz.	L & RZ	Aphrodisiac, to treat insanity, gouts, headache & stomach ache	Hossain, 2011
48.	*Eria spiccata* (D. Don) Hand. Mazz.	S	For stomach ache, applied externally to reduce headache	Nongdam, 2014
49.	*Eulophia dabia* (D. Don) Hochr.	RZ	Appetizer, aphrodisiac, tonic, to treat cough & cold	Patil and Mahajan, 2013
50.	*E. nuda* Landl.	T	Appetizer, to treat tuberculosis glands in neck, tumors & bronchitis	Patil and Mahajan, 2013
51.	*Flickingeria fugax* (Rchb. f.) Seidenf.	WP	Tonic, debility stimulant	Subedi et al., 2013

SN	Botanical name	Part used	Uses	Reference
52.	*Goodyera repens* (L.) R. Br.	T	Externally applied in syphilis & as a blood purifier	Pant, 2013
53.	*Gymnadenia orchidis* Lindl.	R & Pb	For cuts, wounds, liver, urinary & gastric disorders, diabetes	Show et al., 2017; Teoh, 2016a
54.	***Habenaria commelinifolia* (Roxb.) Wall. ex Lindl.**	WP	Demulcent, cure chronic diarrhoea & bilious fevers	Teoh, 2016a
55.	*H. intermedia* D. Don	T	Tonic, to cure hyperdipsia, fever, cough, asthma, leprosy & skin diseases	Chauhan et al., 2007; Khare, 2007
56.	***H. marginata* Colebr.**	T & WP	Tonic, boiled plant extract to treat flatulence & wound	Teoh, 2016a
57.	*H. pectinata* (Sm.) D.Don	T	To treat snake bites & arthritis	Chauhan, 1999; Singh and Duggal, 2009
58.	*Herminium lanceum* (Thunb. ex Sw.) Vuijk	WP	Cure urinary disorders, diabetes, fever & bleeding	Singh and Babbar, 2016
59.	*H. monorchis* (Linn.) R.Br.	R	Tonic	Pant, 2013
60.	*Liparis nervosa* (Thunb) Lindl.	T	Detoxicating & hemostatic functions.	Huang et al., 2013
61.	*Luisia trichorhiza (*Hook.) Bl.	T	Paste is applied externally to cure muscular pain, jaundice & diarrhoea	Dash et al., 2008
62.	*L. zeylanica* Lindl.	L	Juice to treat chronic wounds, boils & burns	Sohag and Hoque, 2017; Vaidya et al., 2000

SN	Botanical name	Part used	Uses	Reference
63.	*Malaxis acuiminata* D.Don	B	Tonic, used to treat bronchitis	Joshi et al., 2009
64.	*M. muscifera* (Lindl.) Kuntze	S base	Tonic	Dhyani et al., 2010
65.	*Neottianthe calcicola* (W.W.Sm.) Soo.	RZ	Tonic	Pant, 2013
66.	***Nervilia aragoana* Gaudich**	WP	Used in uropathy, haemoptysis, asthma, vomiting, diarrhoea & mental instability	Dilipkuma and Janardhana, 2013; Thomas et al., 2013
67.	*Oberonia caulescens* Lindl.	T	Used in liver ailments	Pant, 2013; Teoh, 2016a
68.	*Otochilus albus* Lindl.	WP	Tonic	Subedi et al., 2013
69.	*O. lancifolius* Griff.	Pb	Treat fractured & dislocated bones	Pant, 2013
70.	*O. porrectus* Lindl.	WP	Tonic & to treat sinusitis rheumatism	Teoh, 2016a
71.	*Papilionanthe teres* (Roxb.) Schltr.	WP	To treat dislocated bones, cough & cold	Mazumder et al., 2010; Medhi and Chakrabarti, 2009
72.	*Phaius tankervilliae* (Banks) Blume.	Pb	Cure swellings of hands and legs, skin ailments, freckles, small blisters & rashes	Sultana et al., 2012; Thokchom et al., 2017
73.	*Pholidota articulata* Lindl.	R, Fr, Pb & WP	Tonic, root powder for cancer, juice for skin ulcers, eruptions & bone healing	Jalal et al., 2009; Singh, 2017
74.	*P. imbricate* (Roxb.) Lindl.	B & Pb	Juice to relieve naval, abdominal & rheumatic pain.	Subedi et al., 2013; Teoh, 2016a
75.	*P. pallida* Lindl.	R & Pb	Juice to relieve naval, abdominal & rheumatic pain.	Prasathkumar and Ramesh, 2016

SN	Botanical name	Part used	Uses	Reference
76.	*Platanthera edgeworthii* (Hook. f. ex Collett) R. K.Gupta.	R & L	Blood purifier	Teoh, 2016a
77.	*P. sikkimensis* (Hook. f.) Kraenzlin.	B & Pb	Juice to relieve naval, abdominal & rheumatic pain.	Pant, 2013
78.	*Pleione humilis* (Sm.) D. Don	Pb	Tonic, paste to cure cut & wounds	Tamang, 2017; Teoh, 2016a
79.	*P. maculate* (Lindl.) Lindl.	RZ	For liver & stomach ailments	Bhatnagar and Ghosal, 2018
80.	*P. praecox* (Sm.) D. Don	PB	Tonic, to cure cut & wounds	Teoh, 2016a
81.	***Rhynchostylis retusa* (L.) Bl.**	WP	For rheumatism, root juice for cuts & wounds	Bhatnagar et al., 2017; Bhattacharjee and Islam, 2015
82.	*Satyrium nepalense* D.Don	T, R & WP	Tonic, used in diarrhoea, malaria; juice applied externally in cut & wounds	Jalal et al., 2008; Mishra and Sarla, 2012
83.	*Smitinandia micrantha* (Lindl.) Holttum	WP	Root powder as a tonic & stem has antibacterial property	Pant, 2013
84.	*Spiranthes sinensis* (Pers.) Ames	T	Treat hemoptysis, epistaxis, headache, chronic dysentery & meningitis	Acharya and Rokaya, 2010
85.	*Thunia alba* (Lindl.) Rchb. F.	WP	Paste for dislocated bones	Pant, 2013; Teoh, 2016a
86.	*Trudelia cristata* (Lindl.) Senghas	R & L	Root paste for cuts, wounds, boils & dislocated bones	Pant, 2013; Teoh, 2016a
87.	*Vanda cristata* Wall. ex Lindl.	S & L	Tonic	Hossain, 2011

SN	Botanical name	Part used	Uses	Reference
88.	***V. tessellata* (Roxb.) Rchb. f.**	R & L	Treat rheumatism, nervous problems, bronchitis, dyspepsia & fever	Chowdhury et al., 2014; Suresh et al., 2000
89.	***V. testacea* (Lindl.) Rchb.f.**	L	Antiviral, anticancer & to treat earache	Sebastinraj et al., 2014; Teoh, 2016a
90.	***Zeuxine strateumatica* (L.) Schltr.**	R & T	Tonic	Subedi et al., 2013; Teoh, 2016a

B – Bulb; **Fl** – Flower, **Fr** – Fruit; **L** – Leaves; **Pb** – Pseudobulb; **R** – Roots; **WP** – Whole Plant, **RZ** – Rhizome, T- Tubers

Few popular formulations prepared from various species of orchids in different civilization are given in Table 5.5. Most of them were used as aphrodisiac, antipyretic and for curative property.

Table 5.5: Popular orchid preparations (The species with bold letter are found in Gujarat region.)

SN	Product	Species	Uses
1.	Salep (Kull and Arditti, 2013; Schiff, 2017)	Dried tubers of *Orchis mascula, O. latifolia, Cymbidium aloifolium,* ***Zeuxine strateumatica*** and some species of ***Dendrobium, Eulophia*** **and** ***Habenaria***	Demulcent, cure chronic diarrhoea & bilious fevers
2.	Vanilla (Menon and Nayeem, 2013; Shanmugavalli et al., 2009)	Pods of *Vanilla planifolia.* Other species are *V. pompona*, and *V. tahitensis*	Antispasmodic, anti-inflammatory, analgesic; to treat dysmenorrhea, fever, hysteria, dyspepsia, prevents dental caries, alleviates tooth ache & ulcers.

SN	Product	Species	Uses
3.	Ashtavargha (Dhyani et al., 2010; Hamid et al., 2012; Singh, 2011)	*Malaxis muscifera*, *M. acuminata*, *Habenaria intermedia* and *H. edgeworthii*	Antioxidant, blood purifier, rejuvenating tonic, a mild laxative, an adaptogen & anti-aging
4.	Shi-Hu (Meng et al., 2013; Teoh, 2016a)	Derived from different species of *Dendrobium*	Gastroprokinetic agent, aphrodisiac, anti-inflammatory, analgesic, antipyretic & anticancer
5.	Tian-Ma (Chik et al., 2013; Hong, 2015; Zeng and Zhao, 2017)	Tubers of *Gastrodia elata*	Anti-delirium & anti-convulsive effects
6.	Bai-Ji (Chik et al., 2013; Hong, 2015; Zeng and Zhao, 2017)	Tubers of *Bletilla striata*	Treat tuberculosis, gastric, duodenal ulcers, bleeding, cracked skin on the feet & hands

In search on the phytochemistry aspects of orchids, very sparse literatures were found all over the world as well as in India. On the contrary there were no phytochemical report on orchids from Gujarat. Inspired by the review, this chapter was an approach to evaluate the nature of different phytochemical groups present in ethnobotanically important orchids using modern technique *i.e.* HPTLC.

5.3. MATERIALS AND METHOD

5.3.1. Plant material

Four different species of orchids namely *Nervilia concolor*, *N. plicata*, *Eulophia ochreata* and *E. herbacea* were collected from various regions of Gujarat, India. The samples were processed for preliminary phytochemical analysis using HPTLC technique.

5.3.2. Preparation of plant extracts

The foremost step in analysis of phytochemicals is preparation of extract (Azwanida, 2015; Pandey et al., 2014). Extract preparation is governed by different extraction techniques, chemical nature of phytochemicals, sample particle size as well as presence of interfering substances (Doughari, 2012). Tubers of the above stated orchids were washed under running tap water and dried at room temperature for 24 hours followed by complete drying in oven at 38 °C. The dried tubers were powdered by an electrical blender. 250 mg of respective plant powder were weighed and extracted in 5 mL of methanol. For each plant sample, three different extracts were prepared to extract different phytochemicals based on pH *i.e.* methanolic extract, acidic extract (prepared by adding 2 drops of Hydrochloric acid in the methanolic extract) and basic extract (prepared by adding 1 mL of Ammonia in the methanolic extract). Samples were vortexed, sonicated for 30 min and filtered through Whatmann filter paper no 1. All the filtrates were used for further analysis.

5.3.3. Chemicals

Anisaldehyde, vanillin, formic acid, bismuth subnitrate, potassium hydroxide, sodium hydroxide, diethyl amine, 2-aminoethyl diphenyl borate, ferric chloride and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Aldrich (India). Conductivity water was used for all the analysis. Methanol, chloroform, n-hexane, ethyl acetate, toluene, tetrahydrofuran, n-butanol, sulfuric acid, hydrochloric acid, nitric acid and ammonia were purchased from SD-fine Chemical Limited, Mumbai, India. Whatmann qualitative filter paper of grade 1 was purchased from Merck India. Thin layer chromatographic (TLC) precoated silica gel 60 F_{254} plates with 0.2 mm thickness aluminum sheet support were procured from Merck, India. All the chemicals used in the experiments were of analytical grade.

5.3.4. HPTLC conditions

High performance thin layer chromatography (HPTLC) is sophisticated, robust, automated and most improved form of TLC. It is a powerful analytical method which can able to perform both qualitative as well as quantitative analytical measurement. The accuracy, reproducibility and documentation make the technique more appreciated, effective and one of the most widely using chromatographic technique.

A CAMAG HPTLC system equipped with Linomat 4 (CAMAG Linomat 4 is used for spraying the samples on TLC plates in the form of bands either by automatic or manual injector syringe with the help of nitrogen gas) Automatic TLC sampler 4 (ATS 4) (CAMAG, Muttenz, Switzerland), CAMAG TLC plate scanner 4 (scanner based on densitometric evaluation of TLC/HPTLC chromatograms and other planer objects) and winCATS planar chromatography manager software version 1.4.10 (software which organize the workflow of HPTLC, control every system of CAMAG HPTLC and manage all data) was used for the analysis. Samples were applied using 100 µL CAMAG Linomat syringe (Switzerland). Chromatographic separations were achieved on 20 × 10 cm TLC plates precoated with silica gel 60 F_{254} (Merck, India) of 0.2 mm thickness with aluminum sheet support. Samples were applied as bands of 6 mm wide at 10 mm from bottom, 15 mm from the sides and 8 mm space between two bands. Samples were applied on TLC plates at the rate of 160 nL/s. 20 cm × 10 cm, twin-trough glass chamber was used for the development of TLC plates lined with Whatmann filter paper 1 on one of the inner walls (at room temperature (25 ± 2) °C and relative humidity (60 ± 5%)). The respective mobile phase was poured on the filter paper so that the saturation of mobile phase achieves rapidly. The chamber was tilted at an angle of 45 degree to equally distribute the mobile phase in both the troughs. The chamber was saturated for 15 mins. Ascending development of the plate in the pre-saturated chamber was performed up to a height of 70 mm from the base of the plate. After development, the plate was dried and evaluated by CAMAG densitometric scanner linked to winCATS software IV. The plate was scanned in absorbance mode at 254 nm, 366 nm and 580 nm before and after derivatization with following conditions: slit width 6 mm × 0.45 mm, scanning speed 20 mm/s and data resolution 100 µm/step. Photo documentation was carried out using Reprostar 3 system (CAMAG, Muttenz, Switzerland) at 254 nm, 366 nm and 580 nm.

5.3.5. HPTLC Fingerprint Profiling

Every plant parts have range of phytochemical constituents and are prone to variation with respect to their environment, stress factors, their life span, seasons etc. Therefore, it is very crucial to obtain reliable fingerprints that abiding separation of as many phytoconstituents of the plant drug as possible. Phytochemical fingerprint information provides a potential data for the identification and authentication of plant sample, adulterants as well as impurities profiling and maintaining the quality and consistency of the drug. Several analytical techniques have been used for development of fingerprint profiling of the herbal drugs. These fingerprint profiling studies deals with the advanced extraction methods as well as new and modified analytical techniques. The studies significantly help in qualitative and quantitative evaluation of plant drug and serve as a rapid and unambiguous tool in the herbal research thereby allowing the manufacturers to set quality standards, specifications and seek marketing approval from regulatory authorities. Among all analytical techniques used for fingerprint profiling of the plant samples, HPTLC technique was used in current studies (Attimarad et al., 2011).

The plant extracts were prepared using different extraction techniques as mentioned in section 5.3.2. and applied on TLC plates. Various solvent systems were used to optimize the fingerprinting method. The optimized solvent system was **toluene: ethyl acetate: glacial acetic acid (16: 4: 0.5 v/v/v)**. The developed plates were dried and scanned at 254 nm, 366 nm and 580 nm as mentioned in section 5.3.4. The plate was sprayed using **10% methanolic sulfuric acid** as a derivatizing reagent for detection of other functional groups.

5.3.5.1. Preparation of 10% Methanolic Sulfuric Acid

180 mL of methanol was placed in a 200 mL glass bottle and the temperature was maintained at 0 °C using ice bath. 20 mL of concentrated Sulfuric Acid was added slowly to the ice-cold methanol. The resultant solution was stirred well and allowed to attend the room temperature.

5.3.6. Preliminary Phytochemical analysis using HPTLC

Various groups of phytochemicals were analyzed using HPTLC technique. Different plant extracts were accomplished by the development of TLC using optimized solvent system followed by its derivatization using selective derivatizing reagents for

detection and confirmation of various phytochemical groups present (Wagner and Bladt, 1996).

5.3.6.1. Detection of Alkaloids

The sample extracts were prepared as mentioned in section 5.3.2. The extracts were applied on TLC plate and developed using **toluene: ethyl acetate: diethylamine (7: 2: 1 v/v/v)** followed by drying and scanning at 254 nm, 366 nm and 580 nm as mentioned in section 5.3.4. The plate was further derivatized with **Dragendorff reagent** and again scanned at 366 nm and 580 nm.

5.3.6.1.1. Preparation of Dragendorff reagent

Solution A: 0.17 g of bismuth sub nitrate was dissolved in 10 mL distilled water along with 2 mL glacial acetic acid. Solution B: 4 g of potassium iodide was added with 10 mL of glacial acetic acid and diluted up to 20 mL with water. Solution A and B were mixed together and diluted up to 100 mL.

5.3.6.2. Detection of Glycosides

The prepared plant extracts as mentioned in section 5.3.2. were applied on TLC plate and the plate was developed using **ethyl acetate: methanol: water (20: 2.8: 2 v/v/v)**. The developed plate was dried and scanned at 254 nm, 366 nm and 580 nm as mentioned in section 5.3.4. The plate was derivatized with **alcoholic KOH reagent** and again scanned at 366 nm and 580 nm.

5.3.6.2.1. Preparation of Alcoholic KOH Reagent

5 g of potassium hydroxide (KOH) was dissolved in 100 mL of methanol in calibrated volumetric flask. The flask was stoppered tightly and stored for further use.

5.3.6.3. Detection of Saponins

The extracts prepared as mentioned in section 5.3.2. were applied on TLC plate and developed using **chloroform: glacial acetic acid: methanol: water (6.4: 3.2: 1.2: 0.8 v/v/v/v)** followed by drying and scanning at 254 nm, 366 nm and 580 nm as mentioned in section 5.3.4. The plate was derivatized with **ASR** and again scanned at 366 nm and 580 nm**.**

5.3.6.3.1. Preparation of Anisaldehyde Sulfuric Acid reagent (ASR)

0.5 mL of anisaldehyde was mixed with 10 mL glacial acetic acid, followed by 85 mL methanol and 5 mL concentrated sulfuric acid in the sequence order.

5.3.6.4. Detection of Steroids

The prepared samples according to section 5.3.2. were applied on TLC plate and developed using **n-butanol: methanol: water (3: 1: 1 v/v/v)**. The developed plate was dried and scanned at 254 nm, 366 nm and 580 nm as mentioned in section 5.3.4. The plate was derivatized with **ASR** and again scanned at 366 nm and 580 nm**.**

5.3.6.5. Detection of Sterols

Extracts prepared as mentioned in section 5.3.2. were applied on TLC plate and was developed using **chloroform: ethyl acetate (4: 6 v/v)** followed by drying and scanning at 254 nm, 366 nm and 580 nm as mentioned in section 5.3.4. The plate was derivatized with **ASR** as mentioned in section 5.3.5.2.3.1. and scanned at 366 nm and 580 nm**.**

5.3.6.6. Detection of Flavonoids

The prepared sample extracts as cited in section 5.3.2. were applied on TLC plate and developed using **ethyl acetate: formic acid: glacial acetic acid: water (10: 0.5: 0.5: 1.3 v/v/v/v)**. The developed plate was dried and scanned at 254 nm, 366 nm and 580 nm as mentioned in section 5.3.4. The plate was further derivatized with **NP reagent** and scanned at 366 nm and 580 nm**.**

5.3.6.6.1. Preparation of Natural Product reagent (NP reagent)

1 g of 2-aminoethyl diphenyl borate was dissolved in 100 mL of methanol.

5.3.6.7. Detection of Terpenoids

The samples as mentioned in section 5.3.2. were applied on TLC plate and was developed using **n-hexane: ethyl acetate (1: 1 v/v)** followed by drying and scanning at 254 nm, 366 nm and 580 nm as mentioned in section 5.3.4. The plate was derivatized with **ASR** as mentioned in section 5.3.5.2.3.1. and again scanned at 366 nm and 580 nm**.**

5.3.6.8. Detection of Essential Oils

The prepared samples as cited in section 5.3.2. were applied on TLC plate and developed using **toluene: ethyl acetate (9.3: 0.7 v/v)**. The developed plate was dried and scanned at 254 nm, 366 nm and 580 nm as mentioned in section 5.3.4. After scanning this plate was derivatized with **VSR** and again scanned at 366 nm and 580 nm**.**

5.3.6.8.1. Preparation of Vanillin Sulfuric Acid reagent (VSR)

1 g of vanillin was dissolved in 100 mL of ethanol (96%) followed by careful addition of 2 mL sulfuric acid.

5.3.6.9. Detection of Tannins

The extracts as mentioned in section 5.3.2. were applied on TLC plates. The plate was developed using **toluene: ethyl acetate: formic acid (6: 4: 0.3 v/v/v)** followed by drying and scanning at 254 nm, 366 nm and 580 nm as mentioned in section 5.3.4. After scanning plate was heated up to 102 °C and then derivatized using **neutral $FeCl_3$ reagent** and scanned at 366 nm and 580 nm**.**

5.3.6.9.1. Preparation of Neutral Ferric Chloride ($FeCl_3$) reagent

10 g of Ferric chloride ($FeCl_3$) was dissolved in 100 mL water followed by drop wise addition of ammonium hydroxide (NH_4OH) until slight precipitate persists on shaking. The whole mixture was filtered and filtrate was used as neutral $FeCl_3$ reagent.

5.3.6.10. Detection of Phenolics

The sample extracts as mentioned in section 5.3.2. were applied on TLC plate and developed using **tetrahydrofuran: toluene: formic acid: water (16: 8: 2: 1 v/v/v/v)** followed by drying and scanning at 254 nm, 366 nm and 580 nm as mentioned in section 5.3.4. The plate was further derivatized with **VSR** as mentioned in section 5.3.5.2.8.1. and again, scanned at 366 nm and 580 nm**.**

5.3.7. Antioxidant activity

All metabolisms in aerobic life are associated with crucial oxidation reactions which metabolize fats, proteins and carbohydrates to produce energy. However, during these metabolic processes some side products are also formed one of them is free radicals. Free radicals are chemical species that are capable of independent existence, possessing

one or more unpaired electrons and are highly unstable. Oxygen-centered free radicals are also known as reactive oxygen species (ROS), include superoxide ($O_2 \cdot^-$), hydroxyl (HO·), peroxyl (ROO·), alkoxyl (RO·) and nitric oxide (NO·). ROS are either radicals that contain at least one unpaired electron or reactive non-radical compounds, capable of oxidizing biomolecules. Free radicals lead to many types of diseases like cancer, cardiovascular diseases, cataracts, asthma, hepatitis, liver injury and immunodeficiency diseases etc.

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Antioxidant compounds can scavenge free radicals and increase cells life. Many reports suggested that plant contains many nutrients as well as secondary metabolites which play a role of antioxidant. Many scientists all over the globe are in search of various food supplements which are having very good antioxidant potential. Several analytical methods were recently developed for measuring the total antioxidant capacity of food and beverages. These assays differ in the mechanism for generation of different radical species and/or target molecules and in the way end-products are measured. Methods like ABTS, TEAC, DPPH, Folin total phenols assay, FRAP and CUPRAC are decolorization assays.

DPPH is a free radical, stable at room temperature, is reduced in the presence of an antioxidant molecule present in plant sample. The whole mechanism is based on electron-transfer reaction which results into discoloration of violet color on TLC plate. The use of DPPH assay provides an easy and rapid way to evaluate antioxidants of plants on TLC as well as in assay. Therefore, HPTLC technique was furthermore used to check the antioxidant potential of four species of orchids using DPPH solution as a derivatizing reagent.

5.3.7.3.1. Antioxidant activity using DPPH

The prepared sample extracts as cited in section 5.3.2. were applied on TLC plate and developed using **tetrahydrofuran: toluene: formic acid: water (16: 8: 2: 1 v/v/v/v)** as mentioned in section 5.3.5.2.10. The developed plate was dried and scanned at 254 nm, 366 nm and 580 nm as mentioned in section 5.3.4. The plate was derivatized with **DPPH solution** and placed in dark for 10 mins. Later the plate was scanned at 366 nm and 580 nm**.**

5.4. RESULTS AND DISCUSSION

5.4.1. HPTLC Fingerprint Profiling

The HPTLC fingerprint profiling of samples depicts that **toluene: ethyl acetate: glacial acetic acid (16: 4: 0.5 v/v/v)** was the best solvent system for better resolution of all the extracts. All tracks showed well resolved 3 – 5 bands at 254 nm as shown in Figure 5.1 (a). The bands at Rf 0.35 are highly abundant in *Eulophia* as compared to *Nervilia*. The band at Rf 0.25 in *E. herbacea* (T-10 – 12) shows a black zone at 254 nm (Figure 5.1 (a)) and after derivatization it appears as dark blue color at 366 nm (Figure 5.1 (c)) and brown color at 580 nm (Figure 5.1 (e)), that could be used for distinguishing both the species of genus *Eulophia.* The acidic extract of both the species of *Nervilia* in track 2 and 5 show two new band at Rf 0.2 and 0.45 due to hydrolysis of some glycosidic linkage (Figure 5.1 (a)). Figure 5.1 (b) shows appearance of 5 – 9 well resolved bands in all tracks. The methanolic extract of *N. plicata* and *N. concolor* shows peculiar red band at Rf 0.25 (T-1 and 4) and 0.4 (T-2 and 4), which are distinguishable phytochemicals for the genus (Figure 5.1 (b)). Furthermore, the blue zone in *Eulophia ochreata* (T-7 – 9) at Rf 0.45 can be used to differentiate the *Eulophia ochreata* with that of *E. herbacea* species (Figure 5.1 (b)). When the plate was derivatized using 10% methanolic sulfuric acid reagent, 7 – 8 bands were well separated at 366 nm (Figure 5.1 (c)). A new dark blue zone (Figure 5.1 (c)) and reddish-brown zone (Figure 5.1 (e)) appear at Rf 0.35 in all the tracks but its abundance is very high in *E. herbacea* as compared to others. In all the tracks, the compound at Rf 0.45, shows white fluorescent band at 366 nm (Figure 5.1 (c)) and gives purple coloration at 580 nm (Figure 5.1 (e)) after derivatization. All the acidic extracts (T-2, 5, 8 and 11) show a new white fluorescent band (Figure 5.1 (c)) and orange band (Figure 5.1 (e)) at Rf 0.8 in good amount. The scan at 580 nm doesn't show any significant result as shown in Figure 5.1 (d). All the band patterns in Figure 5.1 (e) are similar to that of Figure 5.1 (c). From the fingerprint point of view, all the four species shows a significant difference in the phytochemical abundance as well as their selectiveness in different species. Hence fingerprinting is an important tool for differentiating *Nervilia* and *Eulophia* at generic as well as at species level.

Track (T) details

T-1: *Nervilia concolor* (MeOH)	T-7: *Eulophia ochreata* (MeOH)
T-2: *N. concolor* (MeOH+HCl)	T-8: *E. ochreata* (MeOH+HCl)
T-3: *N. concolor* (MeOH+NH_3)	T-9: *E. ochreata* (MeOH+NH_3)
T-4: *N. plicata* (MeOH)	T-10: *E. herbacea* (MeOH)
T-5: *N. plicata* (MeOH+HCl)	T-11: *E. herbacea* (MeOH+HCl)
T-6: *N. plicata* (MeOH+NH_3)	T-12: *E. herbacea* (MeOH+NH_3)

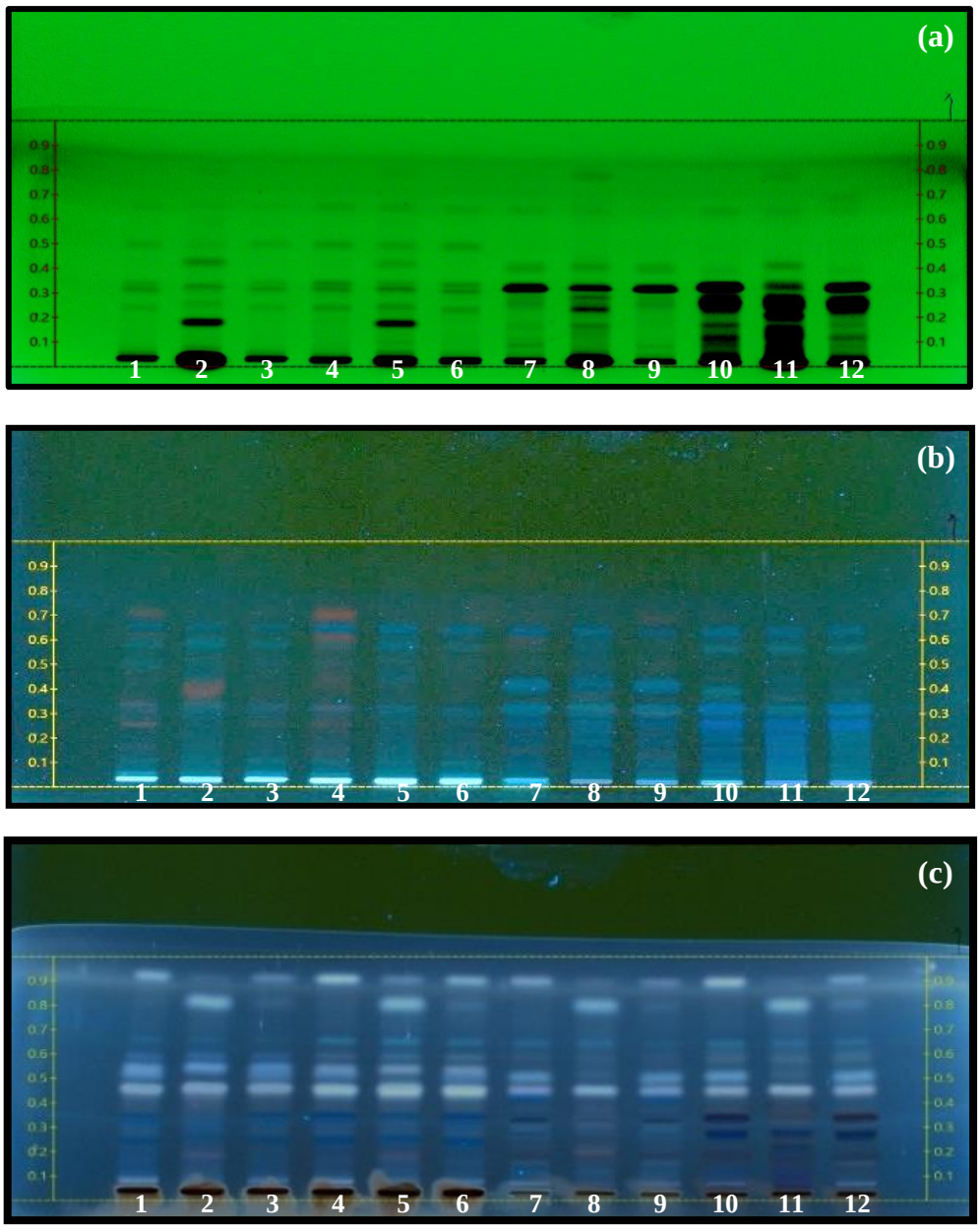


figure cont.

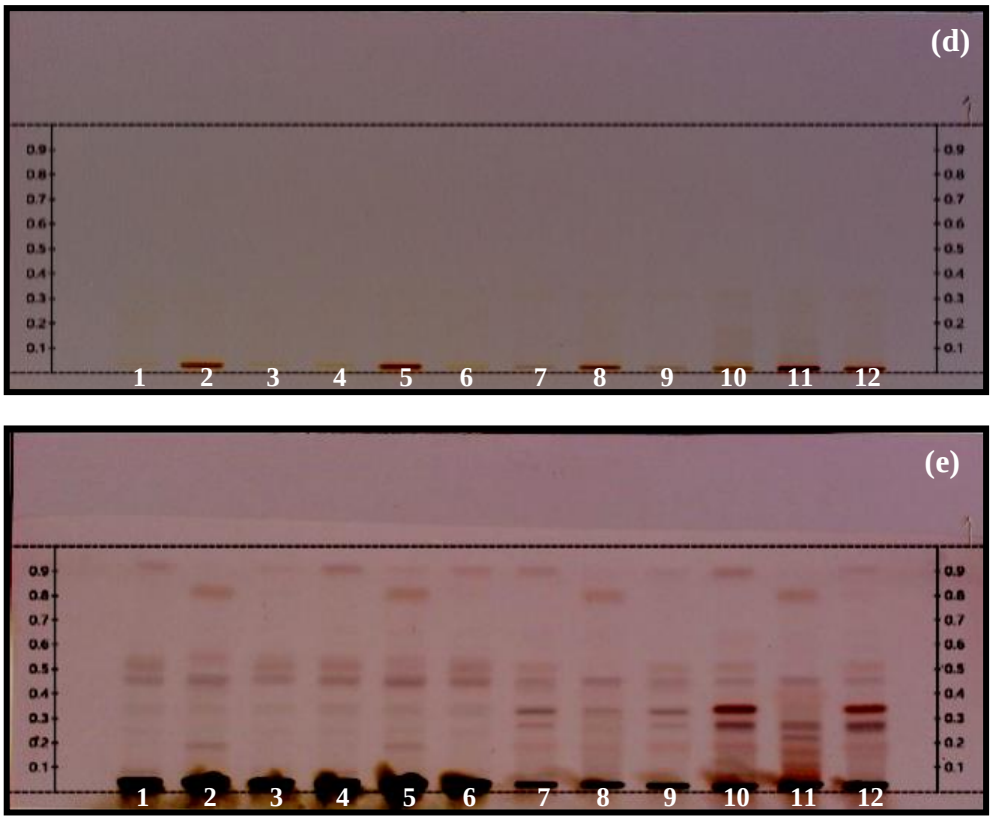


Figure 5.1: HPTLC plate photo of fingerprint profiling (a) at 254 nm (b) 366 nm (c) 366 nm after derivatization (d) 580 nm (e) 580 nm after derivatization

Table 5.6: Percent area and retention time of peaks in fingerprint profiling at 580 nm

Peaks at 580 nm	Rf	Area (%)											
		T-1	T-2	T-3	T-4	T-5	T-6	T-7	T-8	T-9	T-10	T-11	T-12
1	0.005	20.25	25.73	23.06	**22.08**	19.57	17.49	6.68	17.02	4.53	7.49	12.46	8.83
2	0.034	5.64	-	-	3.31	-	1.18	2.07	-	-	1.09	-	-
3	0.063	-	-	-	-	-	-	-	-	-	-	1.39	-
4	0.103	-	-	-	-	-	-	-	-	-	-	2.07	-
5	0.144	-	10.25	-	-	8.28	-	2.80	-	5.63	1.74	-	2.27
6	0.166	-	-	-	-	-	-	-	-	-	-	12.59	-
7	0.187	3.72	1.58	2.98	2.85	2.23	4.10	-	7.57	-	-	-	-
8	0.221	-	-	-	-	-	-	12.29	-	14.94	25.28	21.58	32.85
9	0.306	13.37	-	9.92	9.15	8.62	12.44	**31.68**	17.76	**39.33**	**39.16**	7.93	**37.10**
10	0.398	-	**26.21**	21.72	20.81	**27.52**	**23.74**	16.39	21.62	19.61	5.05	15.39	6.53
11	0.463	**34.79**	10.63	**27.92**	20.27	-	23.44	10.22	-	15.41	5.03	-	6.08
12	0.792	-	25.59	-	-	24.59	-	-	**36.02**	-	-	**26.59**	-
13	0.895	22.23	-	14.41	21.53	9.19	17.61	17.87	-	-	15.15	-	6.33

5.4.2. Preliminary Phytochemical analysis using HPTLC

5.4.2.1. Alkaloids

Dragendorff reagent visualizes alkaloids by forming an orange-brown colored complex. In Figure 5.2 (d) a brown band at Rf 0.5 (Figure 5.2 (d)) appear after derivatization confirms the presence of alkaloids in *N. plicata* (T-6) and *E. ochreata* (T-7). Even though the extraction method of alkaloids for both the species are different (as mentioned in section 5.3.2.) then also the nature of alkaloids is similar because of appearance at same Rf 0.7. The amount of alkaloidal group is less due to which percentage area could not be calculated.

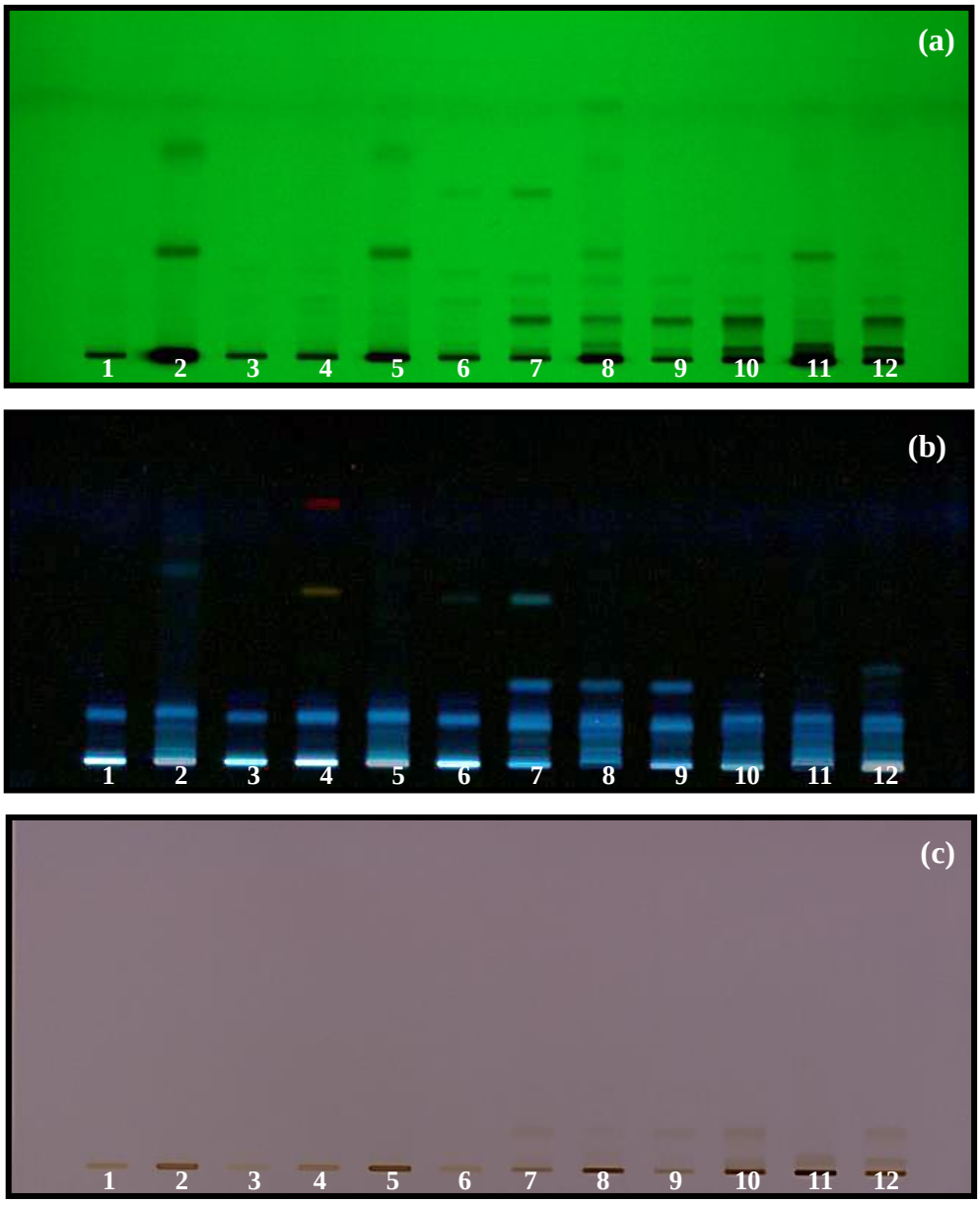


figure cont.

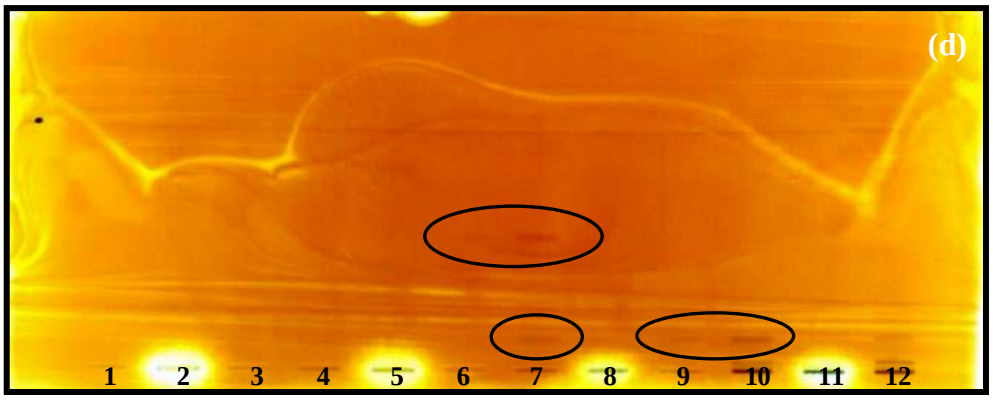


Figure 5.2: HPTLC plate photo of Alkaloidal group (a) at 254 nm (b) 366 nm (c) 580 nm (d) 580 nm after derivatization

5.4.2.2. Glycosides

Glycosides when treated with strong alkali, they get hydrolyzed into glycone and aglycone portion. The aglycone portion like anthraquinones, anthrones and coumarins show red, yellow and blue color respectively at 366 nm (Figure 5.3 (b)), which get intensified after derivatization (Figure 5.3 (c)). The acidic fraction of genus *Nervilia* (T-2 and 5) show a new hydrolyzed band at Rf 0.7 (Figure 5.3 (a)) which is not present in *Eulophia*. The bands between Rf 0.75 to 0.81 are in good concentration (50 – 60%) in *Eulophia* than that of *Nervilia* (30 – 47%) (Table 5.7; Figure 5.3 (a)). The same bands were appearing as brown color at 580 nm (Figure 5.3 (e)) after derivatization. The Figure 5.3 (b) and (c) shows pink color band in track 1 and 4, at Rf 0.6, 0.7 and 1.0 confirms the probability of anthraquinone glycosides. The result confirms that all fractions contain various types of glycosides in all the sample extracts.

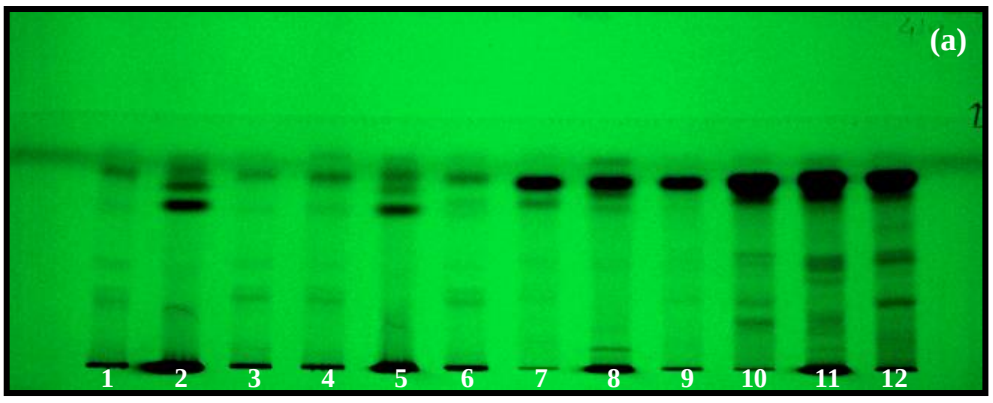


figure cont.

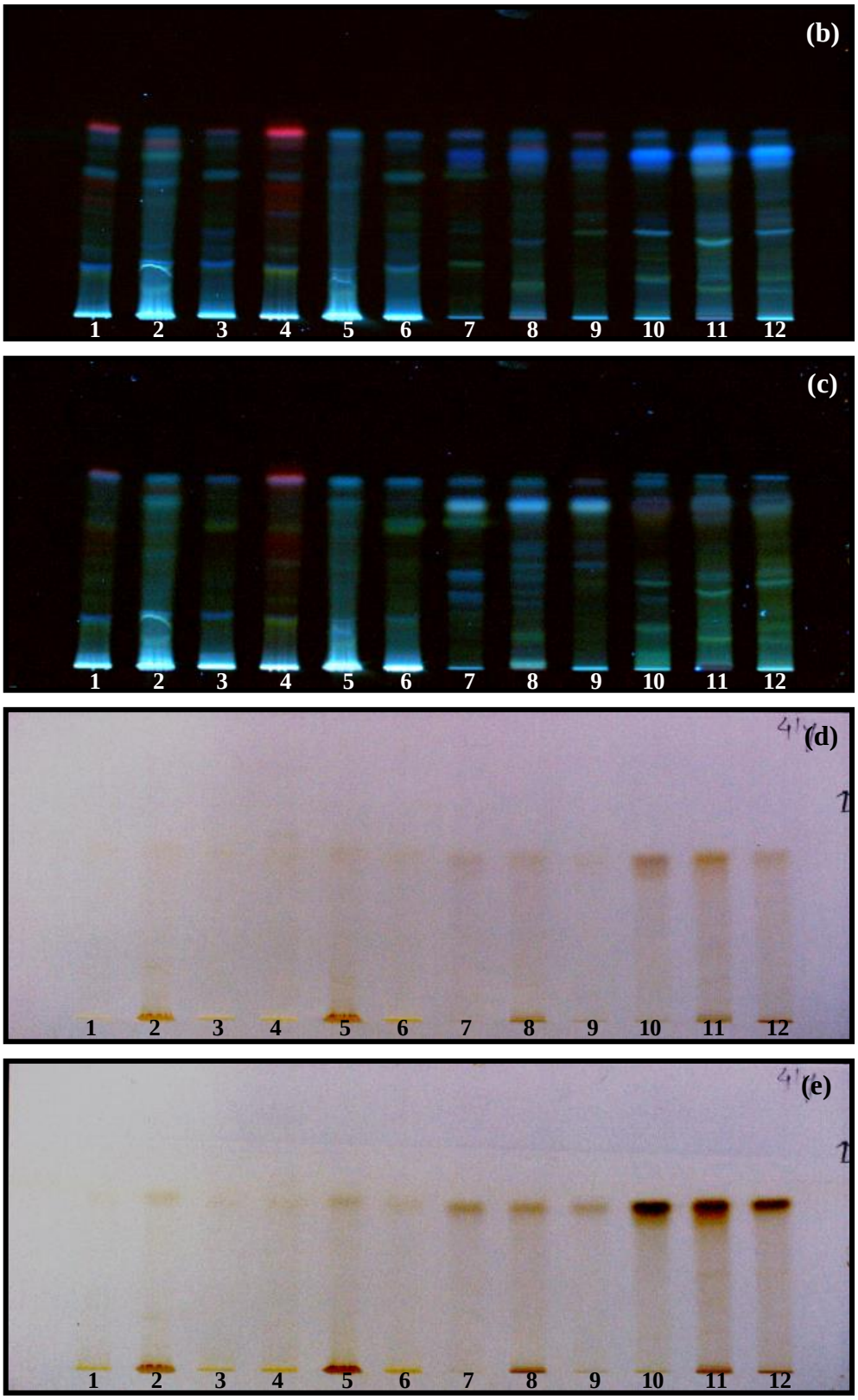


Figure 5.3: HPTLC plate photo of Glycosides group (a) at 254 nm (b) 366 nm (c) 366 nm after derivatization (d) 580 nm (e) 580 nm after derivatization

Table 5.7: Percent area and retention time of peaks in glycoside analysis at 254 nm

Peaks at 254 nm	Rf	Area (%)											
		T-1	T-2	T-3	T-4	T-5	T-6	T-7	T-8	T-9	T-10	T-11	T-12
1	0.01	13.4	**23.4**	20.26	13.48	25.3	18.96	6.42	14.22	11.97	6.48	8.34	9.26
2	0.09	6.01	-	3.02	4.2	-	-	-	4.32	-	-	2.91	-
3	0.1	-	-	5.16	-	-	-	3.61	-	3.52	-	-	-
4	0.15	-	-	-	-	-	-	-	-	-	-	2.82	7.76
5	0.2	-	-	-	-	8.73	-	-	2.66	-	9.48	7.48	2.7
6	0.28	10.6	7.59	10.35	8.78	-	7.21	2.83	-	5	8.03	-	7.56
7	0.32	3.98	-	3.22	3.61	-	3.45	1.96	-	-	-	-	-
8	0.39	-	4.07	-	-	3.92	-	-	-	-	-	5.15	-
9	0.42	6.85	-	5.1	3.52		3.1	5.55	5.07	4.5	6.02	-	-
10	0.48	-	2.33	-	-	1.88	1.96	-	-	2.48	4.5	12.41	13.89
11	0.59	-	-	-	-	-	-	-	-	4.2	-	-	7.63
12	0.67	10	23.32	6.54	12.95	22.89	16.44	21.06	13.38	8.41	-	-	-
13	0.75	-	16.42	-	-	-	-	**57.5**	**48.23**	**58.77**	**60.1**	**60.4**	-
14	0.81	**47.25**	21.8	**44.68**	**37.72**	**36.55**	**37.27**	-	-	-	-	-	**50.72**
15	0.87	-	-	-	14.25	-	10.2	-	11.29	-	4.82	-	-
16	1.04	1.91	1.07	1.67	1.5	0.73	1.42	1.06	0.83	1.17	0.58	0.49	0.46

5.4.2.3. Saponins

Saponins are phytochemicals having triterpenoidal glycosides. Saponins when treated with ASR, the aglycone portion get derivatized and give reddish brown color at 366 nm (Figure 5.4 (c)). The same bands appear as greenish black color under 580 nm (Figure 5.4 (e)). In acidic extract (T-2, 5, 8 and 11) the saponin molecules get hydrolyzed and show two different bands at Rf 0.2 and 0.5 (Figure 5.4 (c) and (e)). The nature of these bands corresponds to flavonoid glycosides and corresponding chalcones which show yellow to brown colored zone. All fractions contain various types of saponins between Rf 0.1 to 0.5. The amount of saponin is very high in *Nervilia* (T-1 – 6) (~ 50%) as compared to *Eulophia* (T-7 – 12) (~ 30%) (Table 5.8; Figure 5.4 (c) and (e)). The unknown compound showing blue fluorescent band at Rf 0.85 (T-1 – 6) and Rf 0.7 and 0.8 (T-7 – 12) could be used for differentiating both the genus (Figure 5.4 (b) and (c)). The compounds showing dark blue zone at Rf 0.5 (T-7 – 12) are highly abundant in *E. herbacea* as compared to *E. ochreata* (Figure 5.4 (c)). The series of prominent blue fluorescent bands from Rf 0.3 to 0.9 represent phenolic carboxylic acids and coumarins (Figure 5.4 (c)). In Figure 5.4 (c) brown colored bands at Rf 0.4 (all tracks) could be of rutin and blue fluorescent bands at Rf 0.8 (T-10 – 12) may be of caffeic acid (Wagner and Baldt 1995).

Furthermore, the presence of saponin in the genus *Nervilia* was confirmed by Wei et al., (2012, 2013) by isolating seven new cycloartane glycosides, named as nervisides A – H from water extract of aerial parts of *N. fordii*.

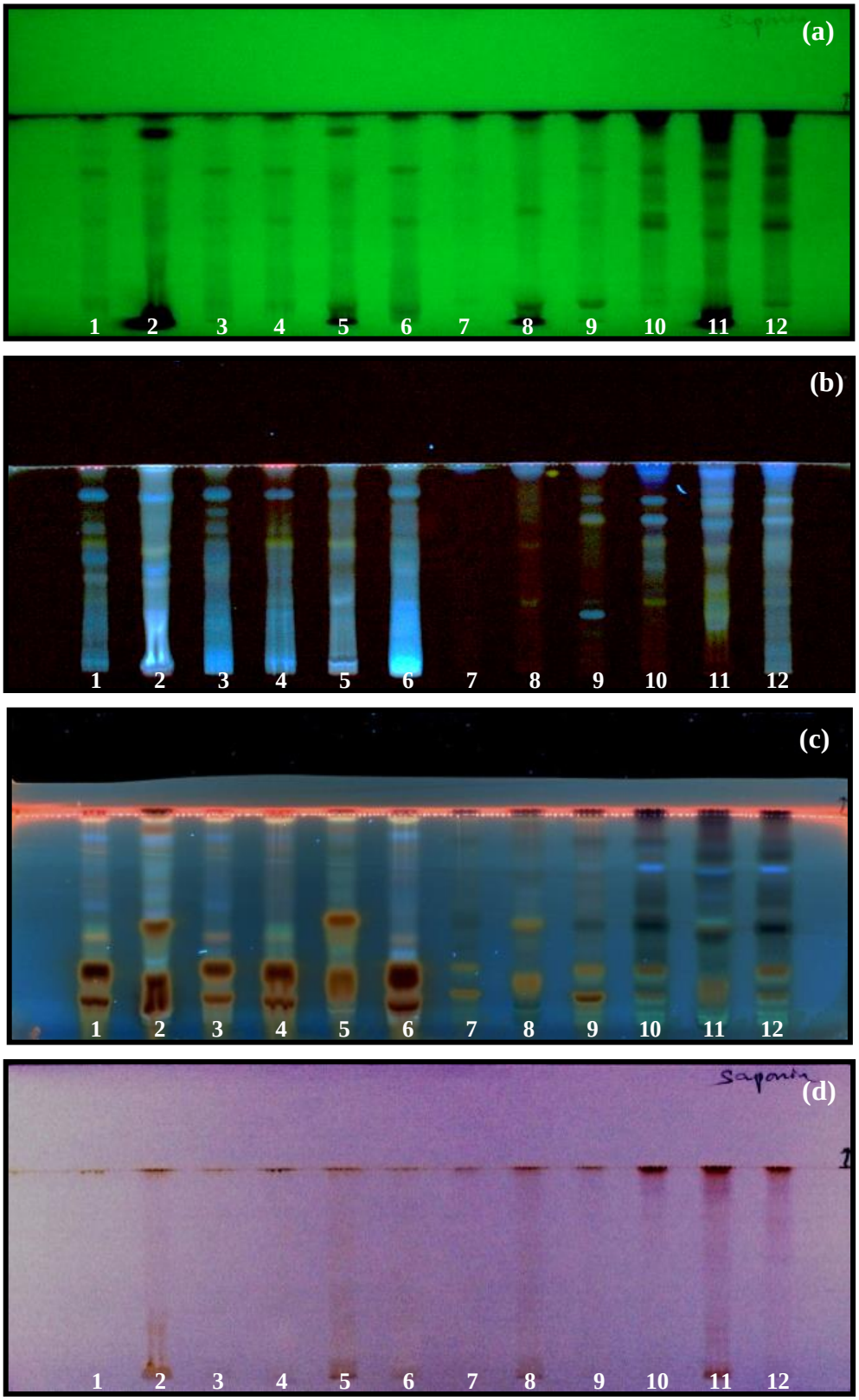


figure cont.

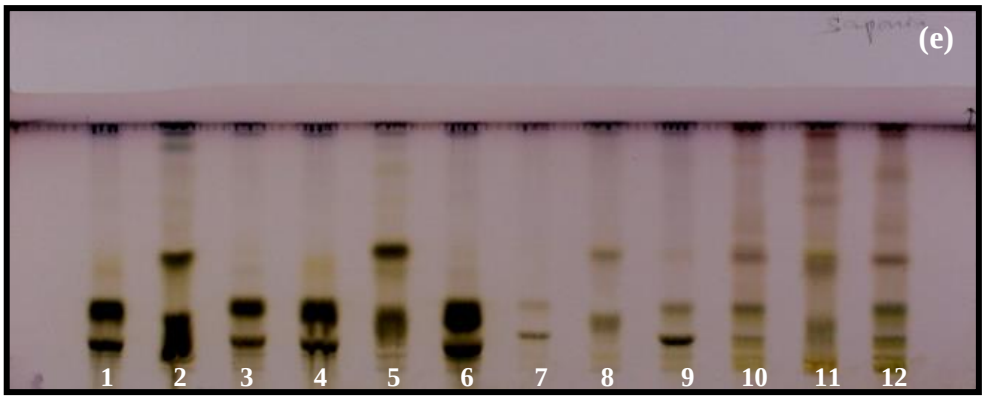


Figure 5.4: HPTLC plate photo of Saponin group (a) at 254 nm (b) 366 nm (c) 366 nm after derivatization (d) 580 nm (e) 580 nm after derivatization

Table 5.8: Percent area and retention time of peaks in saponin analysis at 580 nm

Peaks at 580 nm	Rf	Area (%)											
		T-1	T-2	T-3	T-4	T-5	T-6	T-7	T-8	T-9	T-10	T-11	T-12
1	0.05	-	-	-	-	-	-	-	-	1.07	4.30	-	9.64
2	0.08	-	**31.72**	-	-	1.57	-	-	1.16	-	5.14	5.13	-
3	0.1	27.99	-	-	26.61	-	26.89	-	-	4.32	-	-	5.88
4	0.12	-	-	28.73	-	-	-	-	-	**57.67**	-	-	-
5	0.15	-	-	-	-	-	-	-	-	-	-	-	-
6	0.17	-	-	-	-	-	-	39.78	-	-	18.59	-	19.27
7	0.2	-	**23.86**	-	-	-	-	-	-	-	-	-	-
8	0.22	**51.94**	-	-	-	**47.34**	-	-	**56.81**	-	-	35.94	-
9	0.25	-	-	-	**55.19**	-	**58.87**	-	-	-	-	-	-
10	0.27	-	-	**51.05**	-	-	-	-	-	-	-	-	-
11	0.29	-	-	-	-	-	-	14.9	-	28.66	**37.22**	-	**33.44**
12	0.38	-	-	-	-	-	-	-	2.43	-	-	-	-
13	0.42	1.12	-	0.88	0.62	5.00	0.42	-	-	-	-	-	-
14	0.47	-	20.89	-	-	-	-	-	-	-	-	**44.18**	-
15	0.5	-	-	-	-	30.12	-	-	30.91	4.31	25.27	-	18.49
16	0.6	-	0.29	-	-	-	-	-	-	-	-	1.24	-
17	0.63	-	-	-	-	-	-	-	-	-	5.95	-	5.13
18	0.7	-	0.43	-	-	-	-	-	-	-	-	-	-
19	0.74	-	-	-	-	-	-	-	-	-	-	6.21	-
20	0.84	-	0.67	-	-	0.70	-	-	-	-	-	5.43	6.51
21	0.93	-	4.63	-	-	0.95	-	-	-	-	-	-	-
22	1.01	18.95	17.51	19.33	17.58	-	-	-	-	0.63	-	1.88	0.87
23	1.04	-	-	-	-	14.33	13.82	45.33	8.69	3.34	2.53	-	0.78

5.4.2.4 Steroids

Steroidal moieties are terpenoid lipids characterized by the sterane or steroid nucleus having a carbon skeleton with four fused rings. The nature of steroids varies by

their functional groups attached to parent ring and its oxidation state. After derivatization with ASR it forms different colored complex *viz.* red, blue, purple, brown, orange and violet at 580 nm. The analysis showed that *E. herbacea* (T-10 – 12) is rich in steroids which were confirmed by 2 blue bands at 366 nm (Figure 5.5 (c)) and 2 brown bands at 580 nm (Figure 5.5 (e)) at Rf 0.6 and 0.8. The concentration of unknown compound between Rf 0.84 – 0.86 and Rf near to 0.6 is ~ 30 – 50% and 10 – 20% respectively, which is exclusively seen in *E. herbacea* (Figure 5.5 (c)) and it could be used for differentiating both the species of *Eulophia*. The brown colored zones (Figure 5.5 (c)) between Rf 0.4 to 0.45 are prominent in all tracks with high concentration in *Nervilia* (30 – 60%) than that of *Eulophia* (7 – 36%) (Table 5.9).

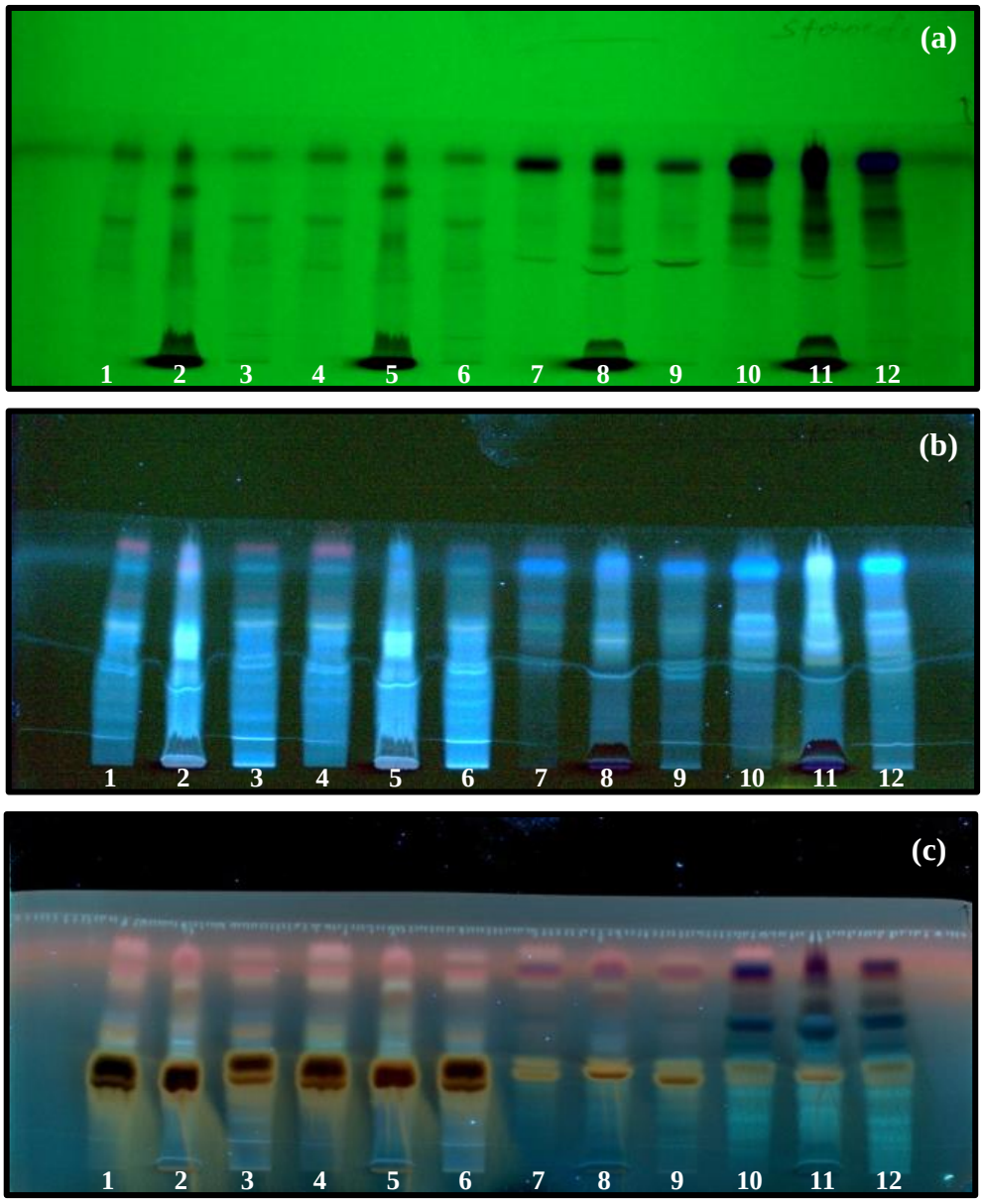


figure cont.

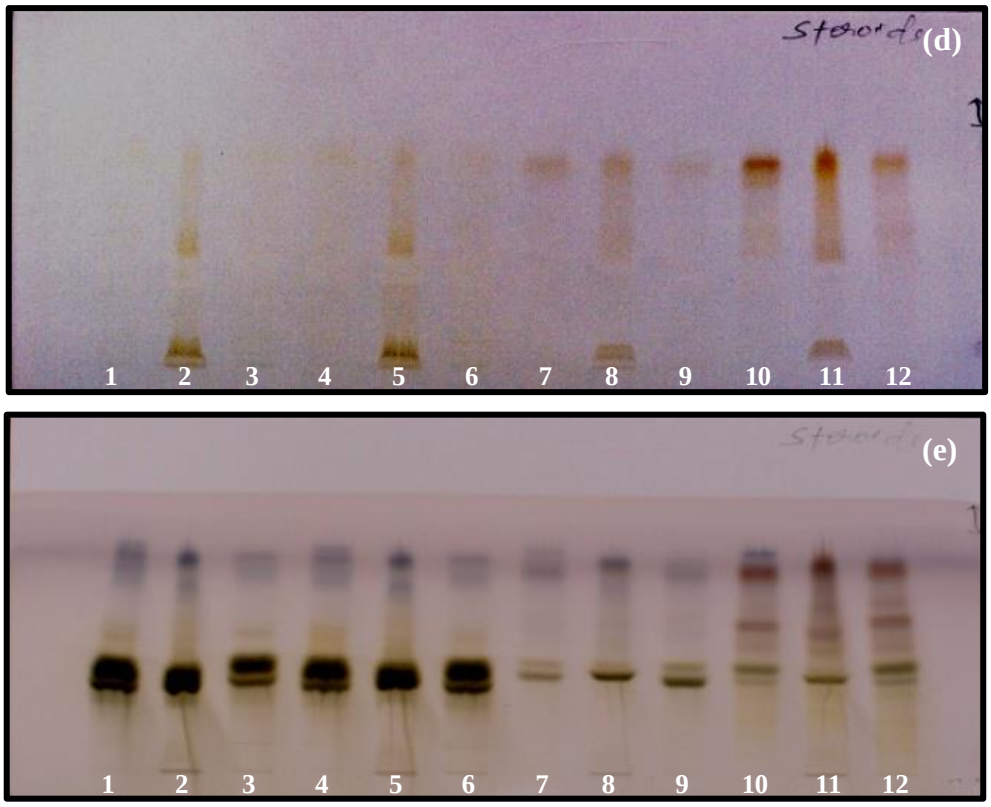


Figure 5.5: HPTLC plate photo of Steroid group (a) at 254 nm (b) 366 nm (c) 366 nm after derivatization (d) 580 nm (e) 580 nm after derivatization

Table 5.9: Percent area and retention time of peaks in steroidal analysis at 580 nm

Peaks at 580 nm	Rf	Area (%)											
		T-1	T-2	T-3	T-4	T-5	T-6	T-7	T-8	T-9	T-10	T-11	T-12
1	0.01	1.41	3.21	1.85	1.18	2.84	2.48	3.25	3.02	2.22	0.96	2.54	3.2
2	0.05	-	1.35	-	-	-	-	-	-	-	-	-	-
3	0.08	-	4.16	-	-	4.25	-	-	3.34	-	-	4.08	-
4	0.13	2.13	-	2.21	-	-	2.09	-	-	-	-	-	-
5	0.22	-	-	-	-	-	-	-	-	-	-	-	2.52
6	0.27	-	-	-	-	-	-	-	-	-	-	4.11	-
7	0.34	-	-	-	-	-	-	-	-	-	-	-	5.26
8	0.36	33.86	-	-	29.1	-	30.43	-	-	-	-	-	-
9	0.38	-	-	31.2	-	30.36	-	-	-	36.52	8.92	17.69	-
10	0.4	-	**64.76**	-	-	**36.06**	-	22.73	32.92	-	-	-	7.86
11	0.42	**42.88**	-	-	**46.38**	-	**43.89**	-	-	-	-	-	-
12	0.45	-	-	**43.33**	-	-	-	8.44	-	11.07	13.79	-	17.53
13	0.53	-	-	-	-	-	-	-	-	-	7.03	7.53	7.47
14	0.55	-	2.33	-	-	-	-	-	5.69	-	-	-	-
15	0.57	4.46	-	3.42	-	-	3.71	-	-	3.62	-	15.31	-
16	0.6	-	-	-	2.29	-	-	-	-	-	20.55	-	-
17	0.62	-	-	-	-	-	-	-	7.45	-	-	-	-
18	0.64	-	-	-	-	-	-	10.53	-	4.85	-	-	16.26

Peaks at 580 nm	Rf	Area (%)											
		T-1	T-2	T-3	T-4	T-5	T-6	T-7	T-8	T-9	T-10	T-11	T-12
19	0.69	-	-	-	-	-	-	-	-	-	-	12.39	-
20	0.71	-	-	-	-	-	-	-	-	-	-	-	9.7
21	0.78	-	7.84	-	-	9.84	17.39	-	-	-	-	-	-
22	0.84	-	-	-	-	-	-	**55.05**	-	**41.71**	**48.75**	-	-
23	0.86	-	-	-	-	-	-	-	**47.58**	-	-	**36.36**	**30.22**
24	0.88	15.26	16.37	18	21.05	16.65	-	-	-	-	-	-	-

5.4.2.5 Sterols

Steroids having alcohol (OH) as one of the functional group is called as sterols. After treatment with ASR, sterols form pinkish to orange colored complex at 366 nm as shown in Figure 5.6 (c) and the same bands give violet coloration in 580 nm as shown in Figure 5.6 (e). The abundance of these compounds is very high in the genus *Nervilia* than that of *Eulophia*. Among them, in acidic fraction (T-2 and 5), three new hydrolyzed bands appear at Rf 0.39, 0.51 and 0.61 with highest abundance of ~ 23 – 24%, ~ 4 – 6% and 26 – 30% (Table 5.10) respectively (Figure 5.6 (c) and (e)). In *E. herbacea*, some compounds significantly show distinct colors at Rf 0.55 (orange), 0.6 (maroon), 0.65 (brown) and 0.7 (orange) (Figure 5.6 (e)). The pink band at Rf 0.27 – 0.31 and 0.61 in every track (Figure 5.6 (c)) is possibly due to presence stigmasterol and β-sitosterol respectively (Reich and Schibli 2006).

Rao et al. (2013) and Tatiya et al. (2015), provided evidence for the presence of sterols in genus *Eulophia* by reporting β-sitosterol, which was isolated from the petroleum ether extract of tubers of *Eulophia campestris* Wall. (HPLC) and *E. herbacea* (GC-MS). The result signifies that all the analyzed species contain sterols.

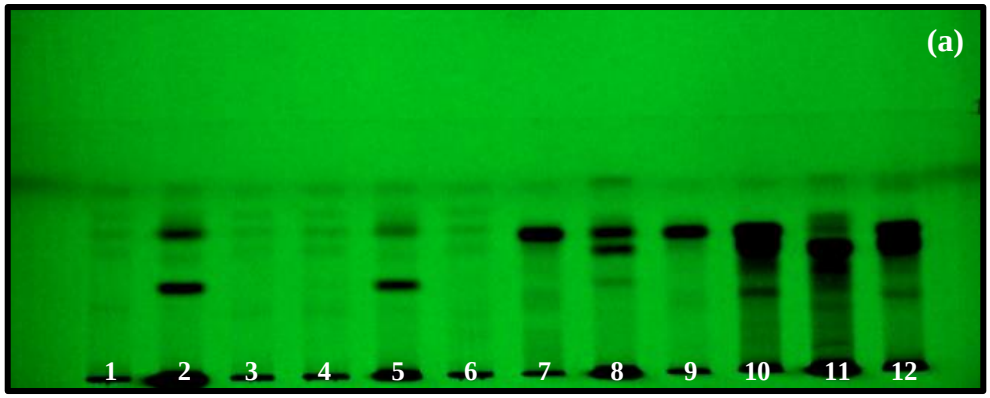


figure cont.

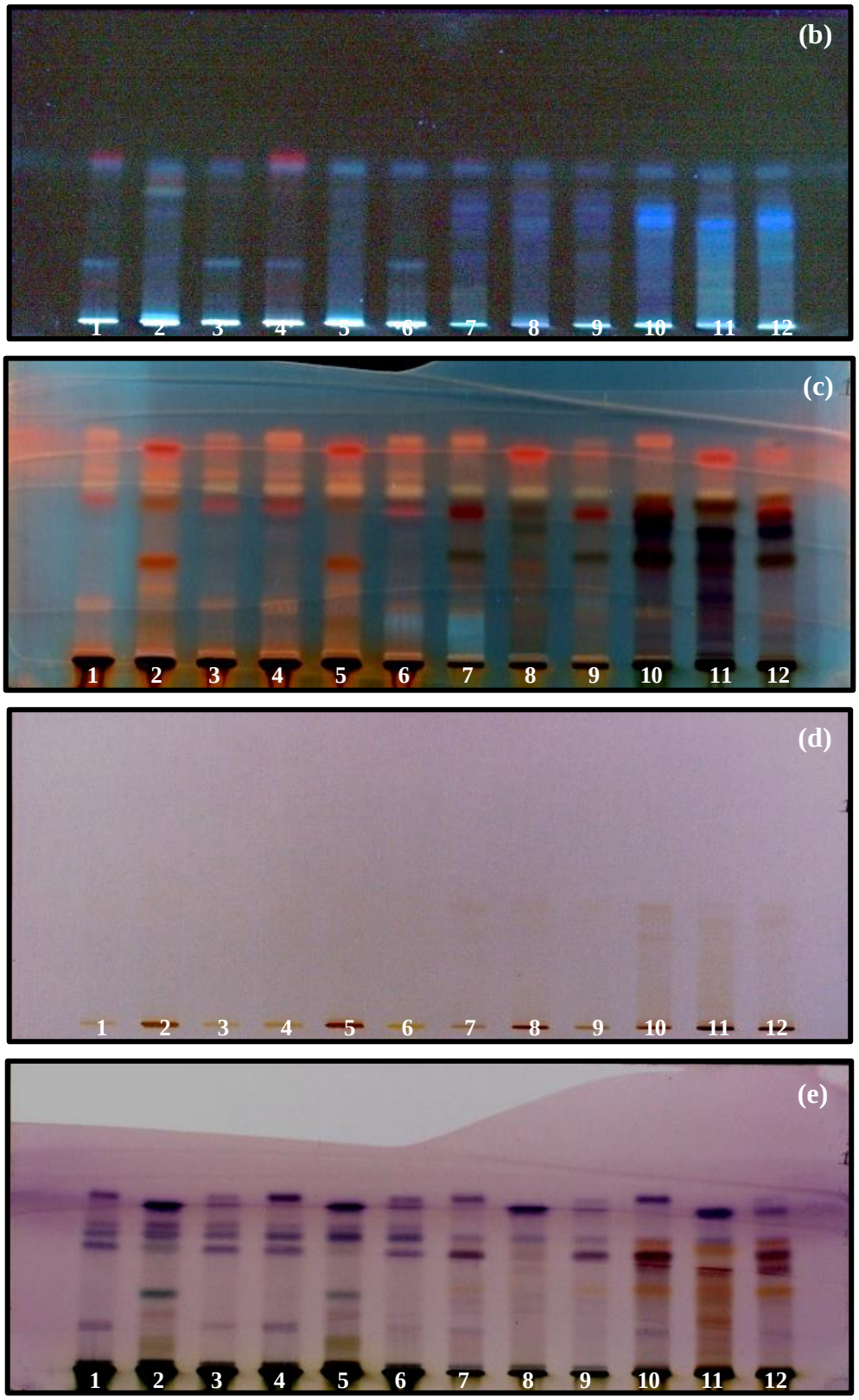


Figure 5.6: HPTLC plate photo of Sterols group (a) 254 nm (b) 366 nm (c) 366 nm after derivatization (d) 580 nm (e) 580 nm after derivatization

Table 5.10: Percent area and retention time of peaks in sterols analysis at 254 nm

Peaks at 254 nm	Rf	Area (%)											
		T-1	T-2	T-3	T-4	T-5	T-6	T-7	T-8	T-9	T-10	T-11	T-12
1	0.03	33.82	26.43	34.65	32.71	30.94	35.23	20.02	24.33	23.88	14.94	18	20.61
2	0.08	-	-	8.46	-	-	-	-	-	-	-	-	-
3	0.15	-	-	-	-	-	-	-	-	1.92	3.15	5.91	-
4	0.2	-	-	-	-	-	8.18	5.69	-	-	-	-	-
5	0.23	-	-	-	-	-	-	-	-	-	-	4.54	-
6	0.25	-	-	-	4.79	-	-	-	-	-	4.56	-	-
7	0.27	4.32	-	-	-	-	-	-	-	-	-	-	-
8	0.29	-	-	5.32	7.99	4.34	4.03	-	2.83	-	-	-	-
9	0.31	7.64	2.44	-	-	-	-	6.56	-	6.54	-	8.49	-
10	0.34	-	-	-	-	-	3.15	5.8	-	-	-	-	12.33
11	0.36	-	-	-	-	-	-	-	-	-	15.96	-	-
12	0.39	-	24.1	-	5.96	23.1	-	-	-	-	-	-	-
13	0.41	-	-	-	-	-	-	-	11.69	-	-	-	-
14	0.44	-	-	-	-	-	-	-	-	2.82	-	-	-
15	0.46	-	-	-	-	-	-	-	-	-	-	23.98	-
16	0.48	-	-	-	-	-	-	8.59	-	-	13.13	-	-
17	0.51	-	5.67	-	-	4.56	-	-	-	-	-	-	-
18	0.53	9.65	-	9.38	10.76	-	11.62	-	21.54	-	-	-	-
19	0.55	-	-	-	-	-	-	-	-	-	**22.05**	**21.63**	**41.03**
20	0.61	10.62	**30.02**	10.56	11.19	**26.04**	12.45	**44.5**	**27.45**	**53.8**	21.51	11.91	20.01
21	0.67	8.99	-	9.92	8.33	-	8.63	-	-	-	-	-	-
22	0.78	**23.7**	10.64	**20.41**	**17.56**	10.41	**15.82**	8.29	11.72	10.37	3.67	-	-
23	0.8	-	-	-	-	-	-	-	-	-	-	5.29	6.02
24	0.86	-	-	-	-	-	-	-	-	-	0.79	-	-
25	1.05	1.25	0.71	1.29	0.72	0.61	0.9	0.55	0.43	0.66	0.24	0.25	-

5.4.2.6. Flavonoids

Flavonoids are the subclass of polyphenolics which are present in most of the plants. It shows dark yellow, green or blue fluorescence under UV which get intensify after derivatization. The analysis showed probable presence of chlorogenic acid as justified by the typical blue and intense blue fluorescent band at Rf 0.6 (T-6 and 7) at 254 nm and 366 nm respectively. Derivatization with NP reagent increased the intensity of bands (Figure 5.7 (c)). All the observations confirm the presence of flavonoidal group in *N. plicata* and *E. ochreata* (Figure 5.7 (c)). Similar compounds were also present in Track 2 and 3 (green color band). Other unknown compound appearing as blue color at Rf 0.45 in all the samples of *Nervilia* makes them distinctly different from *Eulophia* (Figure 5.7 (b) and (c)). The distinct blue fluorescent zone at Rf 0.43 was unique in *E. herbacea* (T-

10 – 12) which is useful for distinguishing it from *E. ochreata* (T-7 – 9) (Figure 5.7 (b) and (c)).

142 species from 75 genera of the Orchidaceae comprised of flavone C-glycosides (53%) and flavonols (37%) as the most common constituents according to a survey conducted by Williams (1979). He also infered that these compounds are not uniformly distributed and strongly depends on plant geography. The presence of flavonoids was further confirmed by Tian et al. (2009). They isolated five new 7-O-methylkaempferol and quercetin glycosides from whole plant of *N. fordii* with seven known flavonoids and one known coumarin. Furthermore three new flavanol glysosides namely nervilifordizins A – C were isolated from *N. fordii* by Zhang et al. (2012).

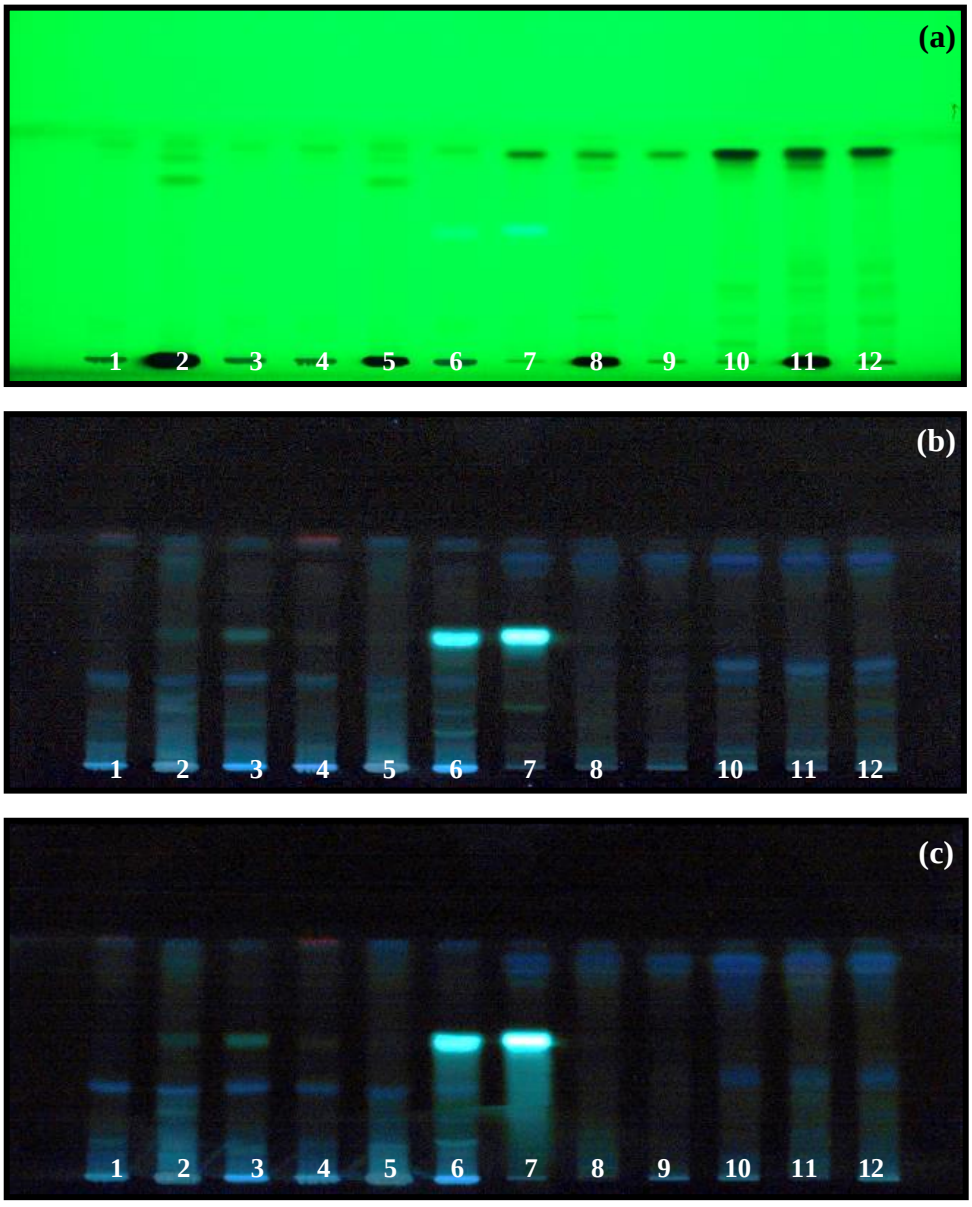


figure cont.

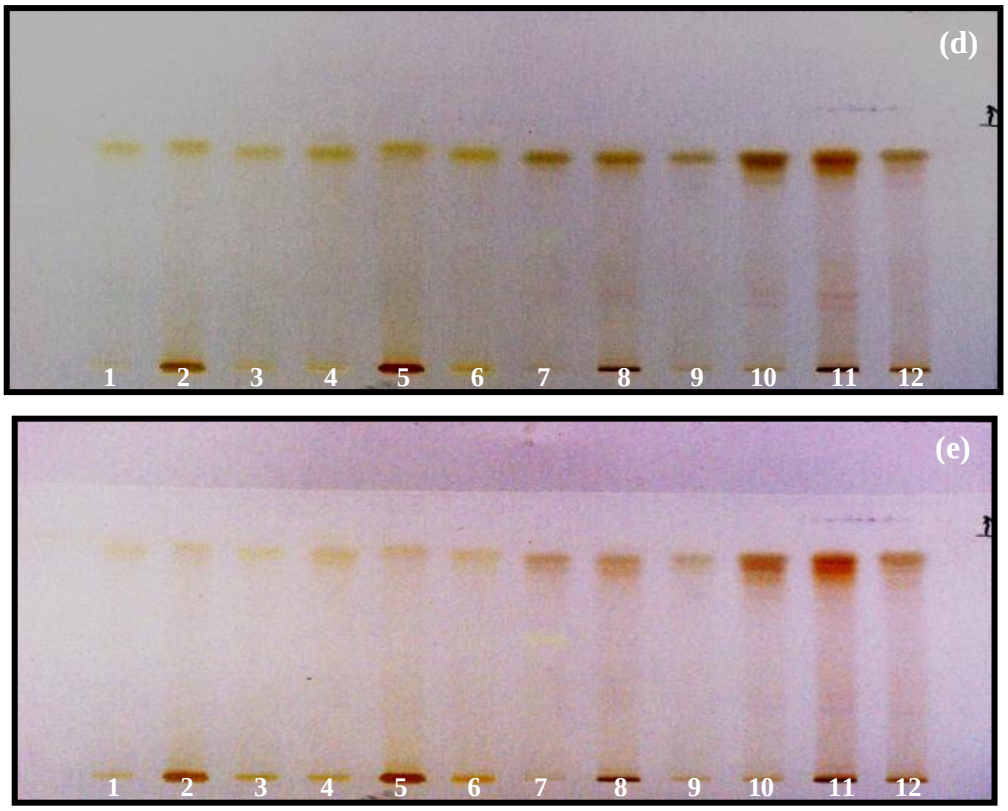


Figure 5.7: HPTLC plate photo of Flavonoid group (a) at 254 nm (b) 366 nm (c) 366 nm after derivatization (d) 580 nm (e) 580 nm after derivatization

Table 5.11: Percent area and retention time of peaks in flavonoid analysis at 254 nm

Peaks at 254 nm	Rf	Area (%)											
		T-1	T-2	T-3	T-4	T-5	T-6	T-7	T-8	T-9	T-10	T-11	T-12
1	0.01	19.68	17.8	28.42	19.89	-	25.03	-	19.69	16.18	7.96	-	-
2	0.04	-	13.76	-	-	-	-	-	-	-	-	8.91	9.37
3	0.1	-	-	-	-	-	-	-	-	-	7.22	4.73	2.74
4	0.15	13.15	3.41	-	-	-	-	-	-	-	-	-	1.71
5	0.17	-	-	12.61	11.51	-	11.35	3.39	-	5.71	-	5.36	-
6	0.22	-	-	-	-	-	-	1.79	5.72	-	6.69	2.57	7.88
7	0.24	-	1.15	-	-	-	-	-	-	-	-	-	-
8	0.33	-	-	-	-	1.62	-	-	2.47	-	-	7.75	-
9	0.35	3.63	-	2.71	2.76	-	1.77	2.52	-	2.71	9.74	-	10.28
10	0.4	-	1.56	-	-	2.18	-	-	-	-	-	5.56	-
11	0.43	-	-	-	-	-	-	-	3.61	3.22	4.55	9.68	9.02
12	0.49	-	-	-	-	-	-	-	-	-	3.42	-	3.74
13	0.55	-	-	-	-	-	6.14	11.09	-	-	-	-	-
14	0.68	3.6	-	2.84	-	-	-	-	-	-	-	-	4.05
15	0.77	7.1	**24.82**	6.34	7.7	23.5	5.68	-	12.62	-	-	-	-
16	0.85	-	15.11	-	-	11.95	-	-	13.07	-	-	-	-
17	0.88	-	-	-	-	-	-	**62.4**	-	-	-	-	-

Peaks at 254 nm	Rf	Area (%)											
		T-1	T-2	T-3	T-4	T-5	T-6	T-7	T-8	T-9	T-10	T-11	T-12
18	0.91	**35.91**	22.4	**34.6**	**42.96**	**19.97**	**39.03**	-	**33.82**	**72.18**	**54.92**	**50.88**	**46.64**
19	0.96	16.94	-	12.48	15.18	7.29	10.99	9.1	9	-	5.5	4.37	4.55

5.4.2.7. Terpenoids

Terpenoids are also called as isoprenoids having plant hydrocarbon backbone in simple or derived forms. The aglycon portion after derivatization with ASR reagent leads to formation of pinkish to orange colored complex at 366 nm and same bands gave blue and violet color at 580 nm which signifies the presence of terpenoidal moiety in the sample. All the significant details are discussed in section 5.4.2.3, 5.4.2.4, 5.4.2.5 and 5.6.2.8. From Figure 5.8 (c) and (e) it was confirmed that all 4 species of orchids are rich in terpenoids.

The presence of terpenoids in the genus *Eulophia* was supported by Schuster et al. (2017) by the isolation of terpenoids (namely 1-4, 6, 16, 18, 4-methoxy-9,10-dihydro-2,7-phenanthrenediol, 1,5-dimethoxy-2,7-phenanthrenediol, 1,5,7-trimethoxy-2,6-phenanthrenediol and 1-(4-hydroxybenzyl)-4,8-dimethoxy-2,7-phenanthrenediol) from 50% ethanolic extract of *E. macrobulbon* roots.

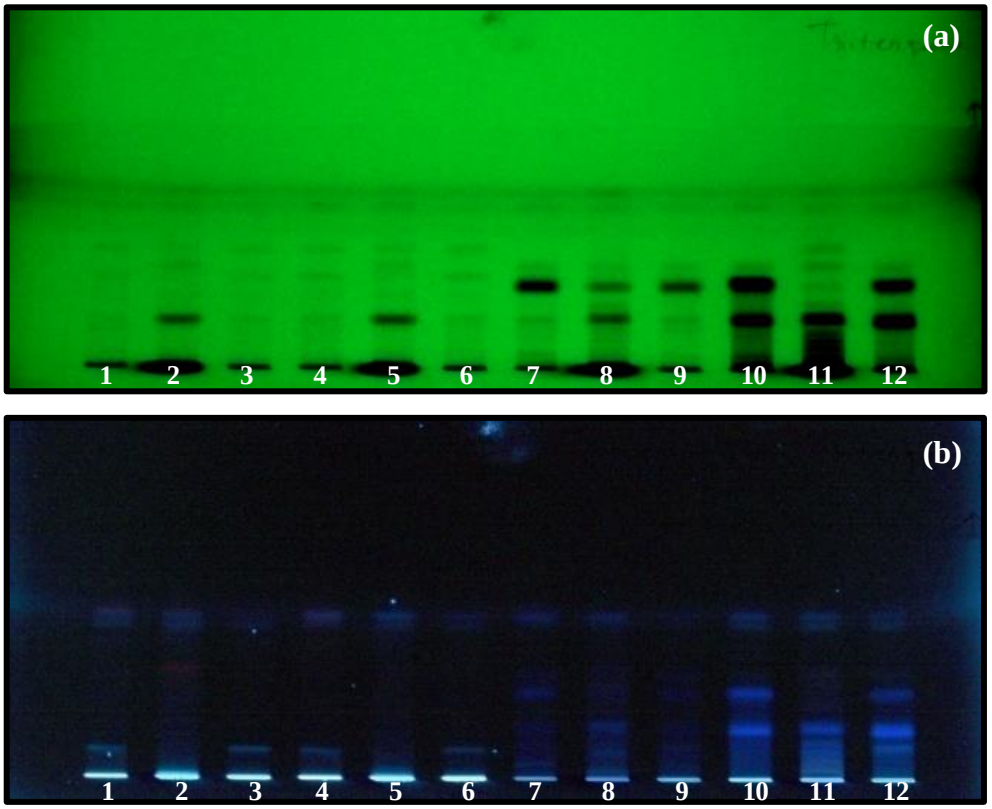


figure cont.

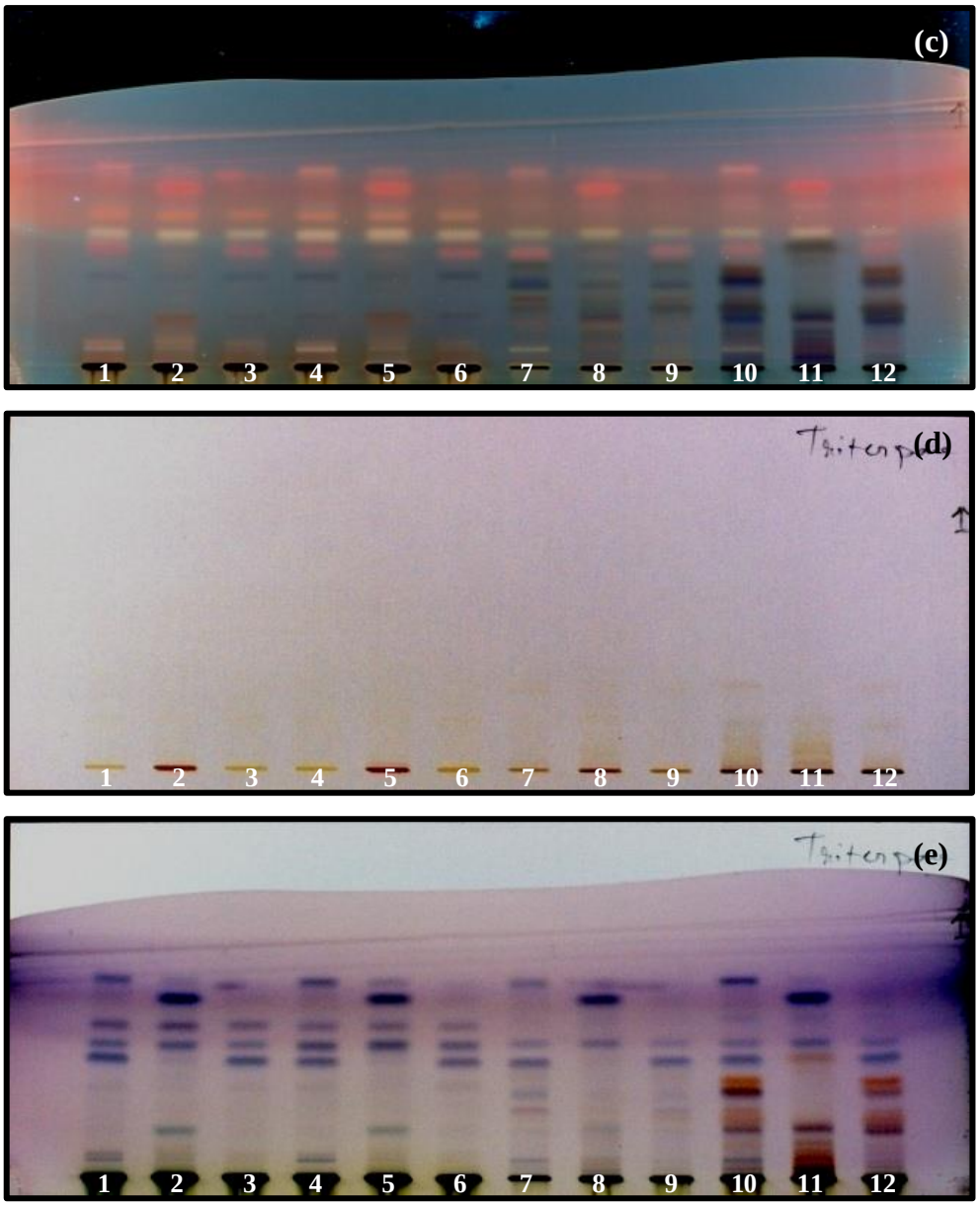


Figure 5.8: HPTLC plate photo of Terpenoid group (a) at 254 nm (b) 366 nm (c) 366 nm after derivatization (d) 580 nm (e) 580 nm after derivatization

5.4.2.8. Essential Oil

Essential oil belongs to the class of monoterpene to sesquiterpene. After derivatization with VSR, it gives yellow to brown colored derivative at UV 366 nm and blue to violet color at 580 nm (Figure 5.9 (c) and (e)). The blue – purple bands between Rf 0.1 to 0.4 shows the presence of terpene alcohols (Figure 5.9 (e)). Purple band at Rf 0.31 could be due to presence of linalool which is selectively present (~ 15 – 20%) in Genus *Nervilia* (T-1 – 6) (Table 5.12; Figure 5.9 (e)). The fluorescent yellow (Figure 5.9 (c)) and violet – blue color (Figure 5.9 (e)) at Rf 0.23 present in all the tracks (13 – 21%)

might be owing to borneol. The band at Rf 0.15 gets hydrolyzed in all acidic extracts (T-2, 5, 8 and 11) and disappear from the respective tracks. A new hydrolyzed compound appearing at Rf 0.7 is in good amount (~ 40 – 52%) (Table 5.12) and it shows yellow fluorescent bands at 366 nm (Figure 5.9 (c)) and intense violet at 580 nm (Figure 5.9 (e)). The band shows the probability of presence of terpene esters. The bands in all the tracks confirm that all the orchid samples are rich in essential oil compounds.

There is no report on essential oil from the orchid tubers. Ono and Miyazawa (1999) investigated essential oil content in flower of *Neofinetia falcata* using capillary GC and GCMS. According to their findings, essential oil contains 66.2% of terpenes.

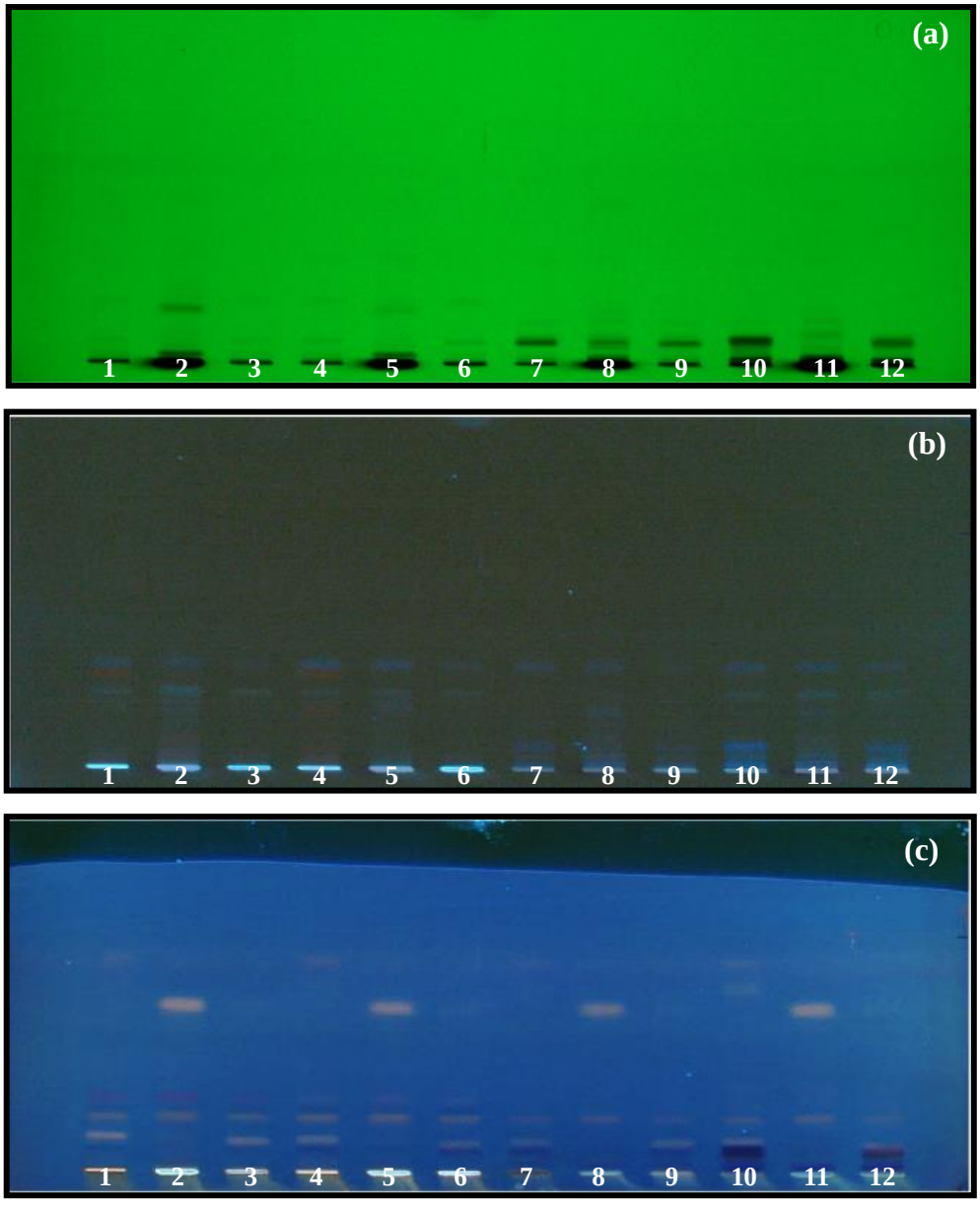


figure cont.

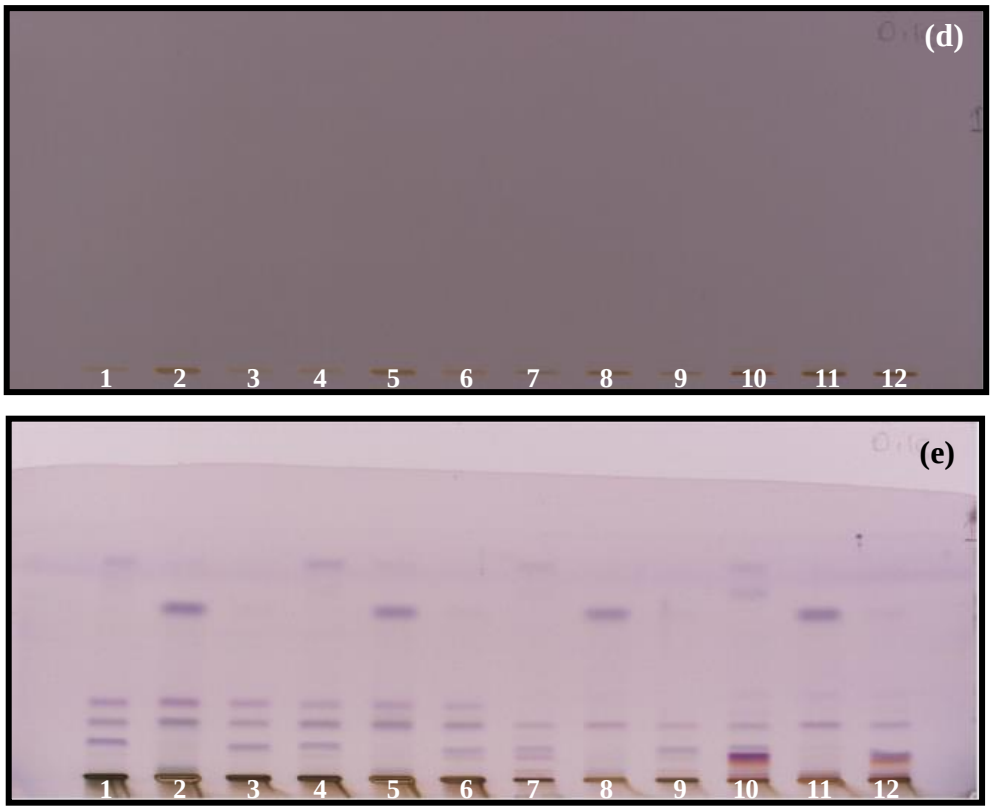


Figure 5.9: HPTLC plate of Essential Oil group (a) at 254 nm (b) 366 nm (c) 366 nm after derivatization (d) 580 nm (e) 580 nm after derivatization

Table 5.12: Percent area and retention time of peaks in essential oil analysis at 580 nm

Peaks at 580 nm	Rf	Area (%)											
		T-1	T-2	T-3	T-4	T-5	T-6	T-7	T-8	T-9	T-10	T-11	T-12
1	0.05	-	1.08	-	1.25	0.44	-	1.31	-	-	-	-	-
2	0.1	-	-	-	-	-	-	13.14	-	-	**37.38**	-	**30.43**
3	0.15	22.25	1.33	23.38	14.81	0.87	17.66	15.18	-	24	7.17	1.1	-
4	0.23	19.19	20.74	19.93	21.25	21.51	**23.79**	13.58	15.03	13.81	9.75	15.62	13.37
5	0.31	19.23	19.51	20.70	13.85	12.48	15.98	-	-	-	2.63	-	-
6	0.36	-	-	-	-	-	-	-	3.3	-	3.91	6.11	5.04
7	0.48	-	-	-	-	-	-	-	4.7	-	-	-	-
8	0.56	3.78	2.22	-	5.97	-	6.35	9.43	5.29	6.88	-	-	8.22
9	0.61	3.48	2	-	5.71	-	-	-	-	-	5.9	-	-
10	0.7	-	**41.15**	10.92	-	**46.58**	13.09	-	**51.58**	24.33	-	**52.56**	13.73
11	0.78	-	-	-	7.60	-	-	12.12	-	-	15.96	-	-
12	0.89	**32.07**	11.97	**23.69**	**29.56**	17.13	23.14	**35.24**	20.1	**30.99**	17.3	22.74	29.21
13	1	-	-	1.39	-	0.99	-	-	-	-	-	1.87	-

5.4.2.9. Tannins

Tannins are subclass of polyphenolic compounds having high molecular weight. Tannins present in the plant samples react with ferric ion of ferric chloride giving bluish

– black color complex. The formation of dark bluish band at Rf 0.4 in track 10 – 12 as shown in Figure 5.10 (e) confirms the presence of tannins in *E. herbacea*. These data are very much useful in distinguishing the sample of *E. herbacea* with that of other species of the same genus.

The presence of tannins in genus *Eulophia* was confirmed by Nagulwar et al. (2017) using preliminary phytochemical screening. Tannins have also been reported from *Cymbidium aloifolium* (Radhika et al., 2013), *Vanda tessellata* (Bhattacharjee et al., 2015) and *Rhynchostylis retusa* (Bhattacharjee and Islam, 2015) using simple phytochemical analysis.

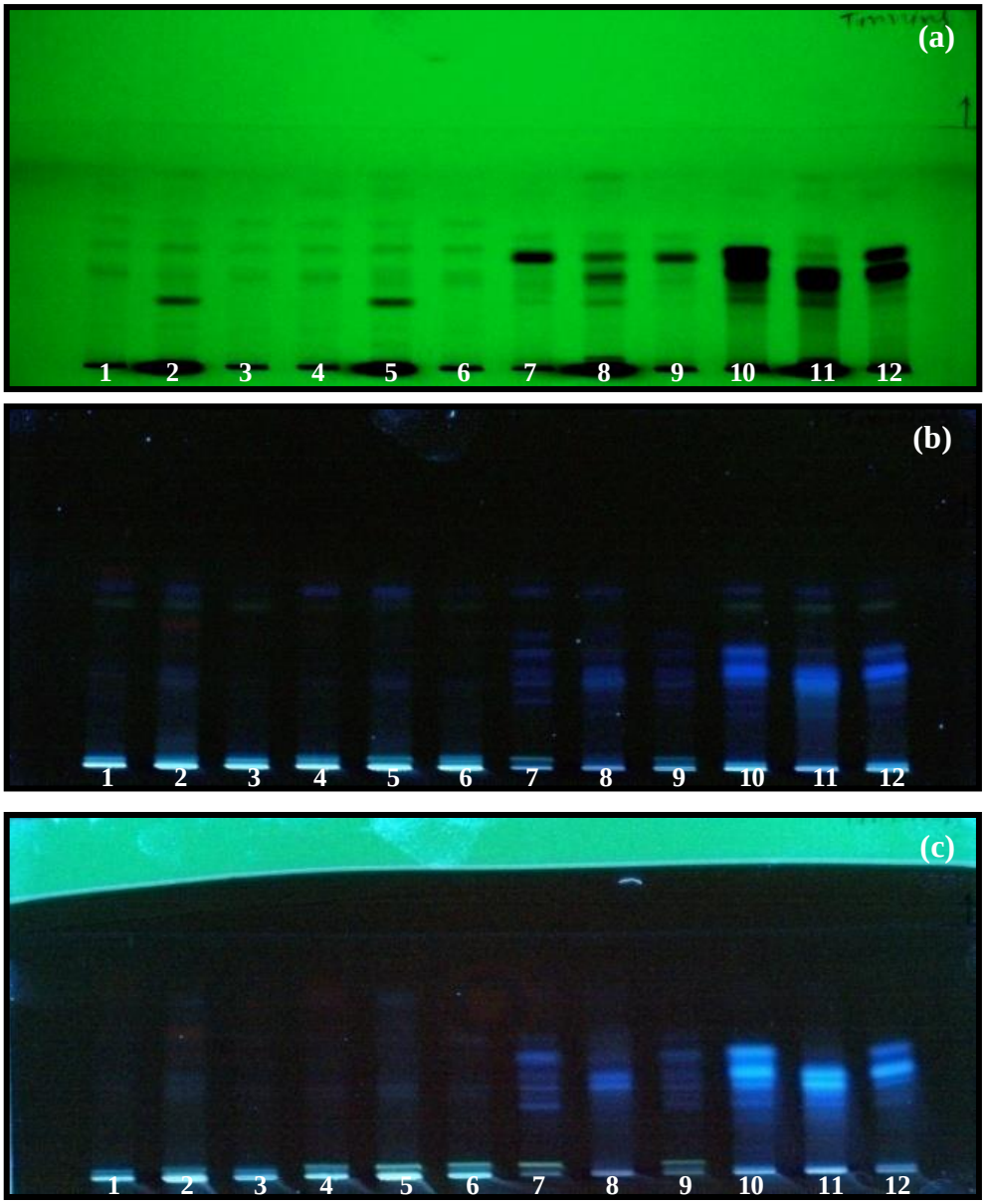


figure cont.

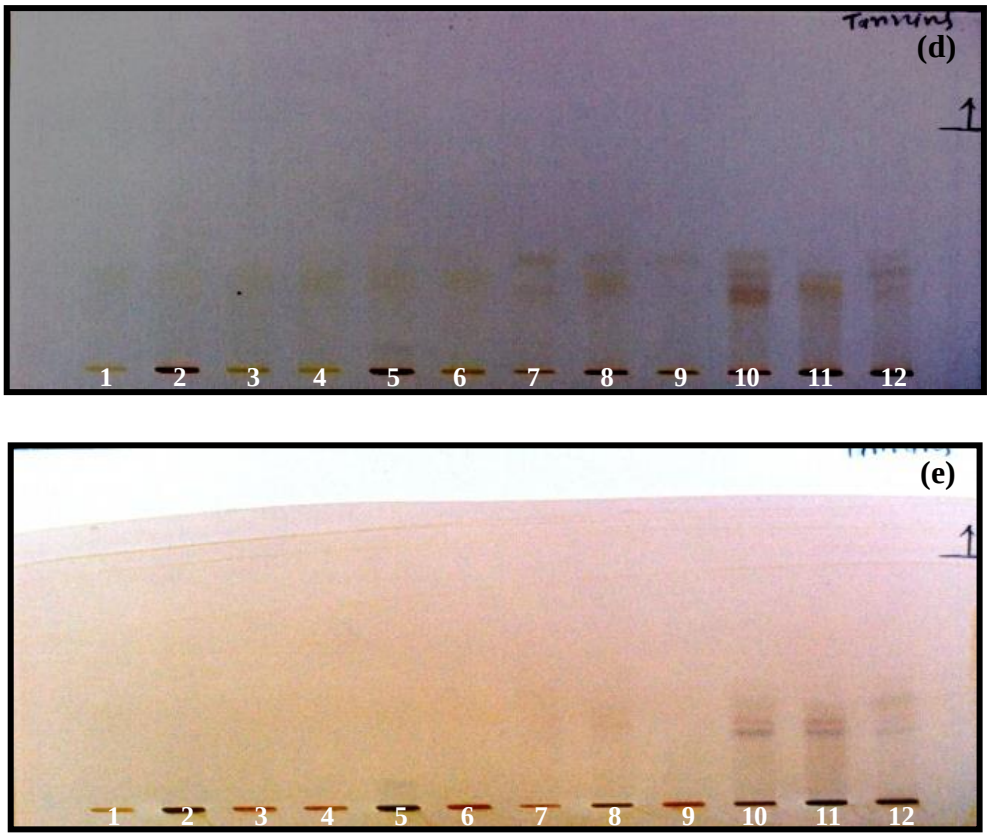


Figure 5.10: HPTLC plate photo of Tannin group (a) 254 nm (b) 366 nm (c) 366 nm after derivatization (d) 580 nm (e) 580 nm after derivatization

5.4.2.10. Phenolics

Phenolic compounds have phenolic group as parent moiety in simple or polymeric form. They generally show, white to blue fluorescence in UV 366 nm as shown in Figure 5.11 (b). After derivatization with VSR, these bands appear as blue color in UV 366 nm and purple in 580 nm as seen in Figure 5.11 (c) and (e) respectively. In acidic extract of *Nervilia* (T-2 and 5), a new hydrolyzed band appear at Rf 0.73 as brown color at 366 nm (Figure 5.11 (c)) and green color at 580 nm (Figure 5.11 (e)) having concentration of 6 – 11%. The orange band at Rf 0.3 (T-1, 3, 4 and 6) and white band at Rf 0.85 (every track) shows the possibility of rutin and caffeic acid respectively as seen in Figure 5.11 (c). The prominent blue fluorescent band at Rf 0.63 (T-6 and 7) in Figure 5.11 (b) and (c) may be because of chlorogenic acid. At Rf 0.64, an unknown phenolic compound shows white zone (Figure 5.11 (c)) and violet zone (Figure 5.11 E) having abundance of 13 – 17% in *Nervilia* and 7 – 11% in *Eulophia* (Table 5.13). In all the *Eulophia* species many reddish – brown color bands appear from Rf 0.5 to Rf 0.78 are might be because of different derivatives of procyanidins. Among them, one of the

unknown compound having a prominent red band at Rf 0.78 is in good amount in *E. herbacea* (30 – 35%) as compared to *E. ochreata* (25 – 29%) (Table 5.13). Hence from the HPTLC data, it was confirmed that all orchids samples are rich in phenolics.

The identification of different phenolics namely protocatechuic acid, syringic acid, ferulic acid, sinapic acid, p-coumaric acid, benzoic acid and ellagic acid were done by Minh et al. (2016) in *Phalaenopsis* orchid using HPLC in the ethanolic extracts of leaf and root samples.

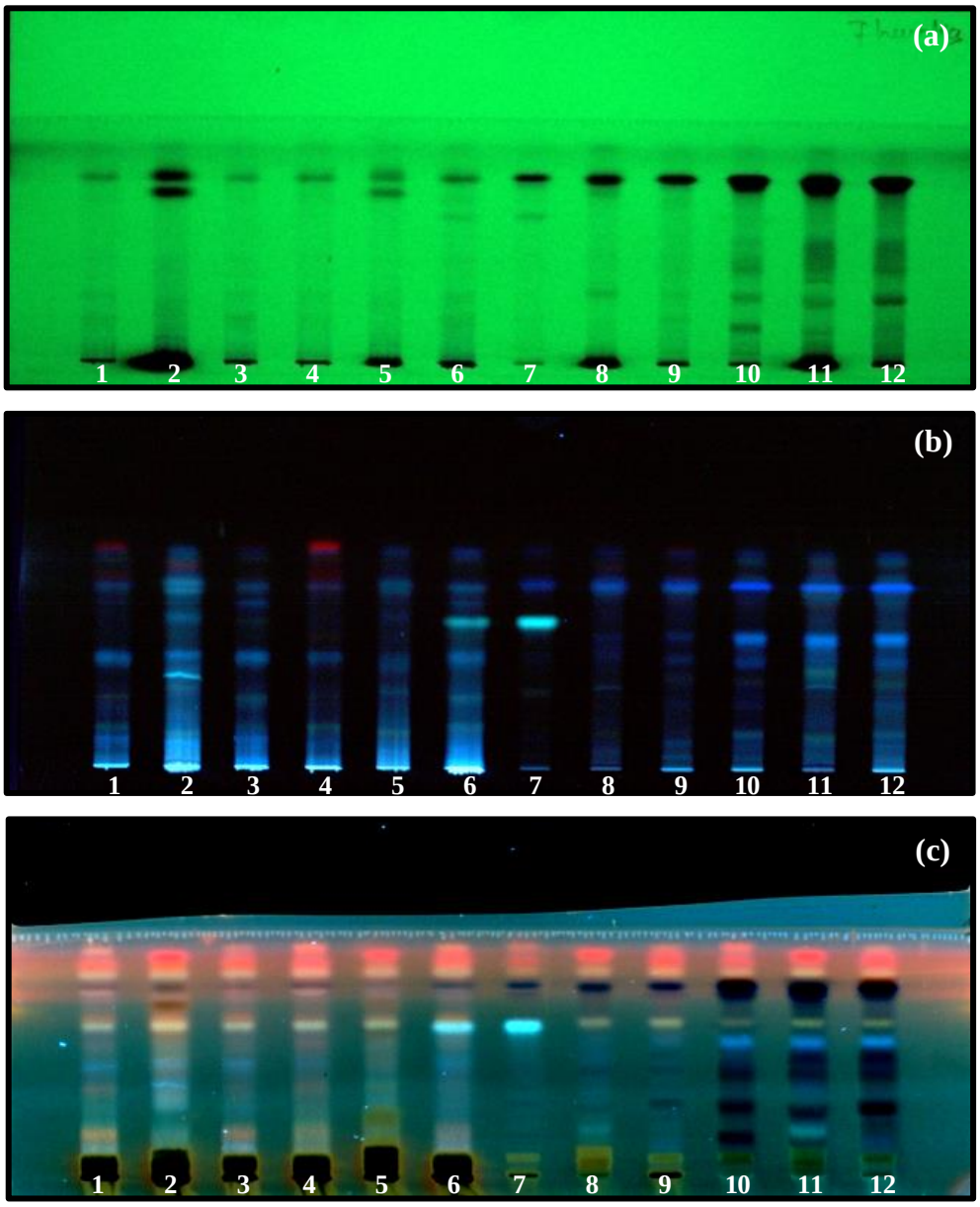


figure cont.

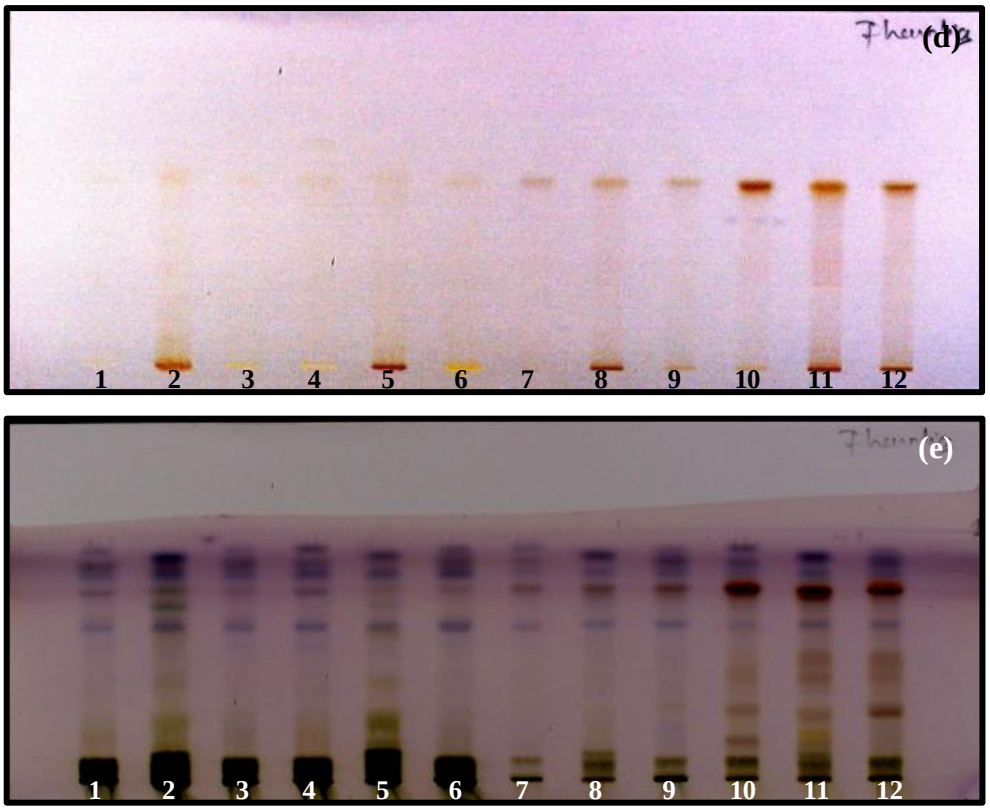


Figure 5.11: HPTLC plate photo of Phenolic group (a) at 254 nm (b) 366 nm (c) 366 nm after derivatization (d) 580 nm (e) 580 nm after derivatization

Table 5.13: Percent area and retention time of peaks in phenolic analysis at 580 nm

Peaks at 580 nm	Rf	Area (%)											
		T-1	T-2	T-3	T-4	T-5	T-6	T-7	T-8	T-9	T-10	T-11	T-12
1	0.01	1.02	0.74	1.26	0.88	0.86	1.74	7.63	2.07	4.36	2.96	0.97	0.77
2	0.07	20.21	6.96	19.71	18.86	-	16.85	-	7.5	6.04	7.32	2.75	6.66
3	0.11	-	6.12	-	-	24.11	-	5.34	3.34	-	-	1.3	-
4	0.17	-	-	-	-	0.26	-	-	-	-	3.31	0.28	-
5	0.19	1.13	-	-	-	-	-	-	-	-	-	-	-
6	0.21	-	-	1.16	-	-	-	-	-	-	-	-	-
7	0.25	-	1.65	-	-	6.3	-	-	-	-	-	2.27	7
8	0.29	-	-	-	-	-	-	-	-	-	2.94	-	-
9	0.35	-	-	-	-	-	-	-	0.71	-	-	0.56	-
10	0.38	1.37	-	-	0.65	-	-	-	-	-	-	-	-
11	0.41	-	2.88	-	-	2.01	-	-	-	-	-	4.5	-
12	0.44	1.94	-	1.07	-	-	-	-	-	-	3.63	-	3.22
13	0.49	-	-	-	-	-	-	-	-	-	-	6.95	6.84
14	0.57	4.96	-	4.1	4.71	-	-	-	-	-	-	-	-
15	0.64	13.25	17.57	11.43	10.86	13.52	16.1	10.07	11.91	10.82	7.79	10.06	8.72
16	0.73	-	10.51	-	-	5.91	-	-	-	-	-	-	-
17	0.78	18.41	10.88	15.18	16.4	-	12.86	25.94	27.4	29.03	**35.06**	**34.55**	30.85

Peaks at 580 nm	Rf	Area (%)											
		T-1	T-2	T-3	T-4	T-5	T-6	T-7	T-8	T-9	T-10	T-11	T-12
18	0.85	-	-	-	**16.19**	**23.64**	18.83	-	15.56	-	-	11.13	-
19	0.89	**37.7**	-	**46.1**	15.33	-	**33.63**	**33.04**	-	**49.75**	23.2	-	**35.94**
20	0.92	-	**42.68**	-	-	23.39	-	-	-	-	-	24.67	-
21	0.95	-	-	-	16.11	-	-	17.98	**31.51**	-	13.78	-	-

All the four species of orchids (*Nervilia concolor, N, plicata, Eulophia ochreata* and *E. herbacea*) are rich in almost all phytochemicals groups. The enriched phytochemical groups in different extracts were summarized in Table 5.14.

Table 5.14: Groups of phytochemicals present in the selected species of orchid

SN	Group of Phytoconstituents	Observations											
		N. concolor			*N. plicata*			*E. ochreata*			*E. herbacea*		
		T-1	T-2	T-3	T-4	T-5	T-6	T-7	T-8	T-9	T-10	T-11	T-12
1.	Alkaloids	-	-	-	-	-	+	+	-	-	-	-	-
2.	Essential Oils	+	+	+	+	+	+	+	+	+	+	+	+
3.	Flavonoids	-	+	+	-	-	+	+	-	-	-	-	-
4.	Glycosides	+	+	+	+	+	+	+	+	+	+	+	+
5.	Phenolics	+	+	+	+	+	+	+	+	+	+	+	+
6.	Saponins	+	+	+	+	+	+	+	+	+	+	+	+
7.	Steroids	-	-	-	-	-	-	-	-	-	+	+	+
8.	Sterols	+	+	+	+	+	+	+	+	+	+	+	+
9.	Tannins	-	-	-	-	-	-	-	-	-	+	+	+
10.	Terpenoids	+	+	+	+	+	+	+	+	+	+	+	+

"+" Present; "-" Absent

The primary screening of phytochemicals using HPTLC revealed that all four species of orchids are rich in essential oils, glycosides, phenolics, saponins, steroids and triterpenes. Alkaloidal group was present only in *N. plicata* and *E. ochreata*. Among all the orchid species, *E. herbacea* is enriched with tannins. *N. concolor*, *N. plicata* and *E. ochreata* showed the presence of flavonoidal group.

Haridas et al. (2014) confirmed the presence of alkaloids, flavonoids, phenol, tannins, glycosides, steroids, terpenoids and triterpenoids from *N. plicata* bulb through various basic phytochemical tests. However, in present studies tannins were not detected in *N. plicata* even using HPTLC technique. The probable reason could be ecological condition. Huang et al (2015) found flavonoids and terpenoids in leaf and corms of *N. fordii*.

The methanolic extract of *E. herbacea* tubers showed the presence of essential oils, glycosides, phenolics, saponins, steroids, sterols, tannins and terpenoids in current analysis. Tatiya et al. (2012) obtained similar results using preliminary phytochemical analysis. However, glycosides and steroids were absent in their analysis. Similar results were obtained by Kurapa et al. (2012) and Nagulwar et al. (2017) from the tuber of *E. nuda* using phytochemical screening method. Koprade and Magdum (2015), revealed that the tuber of *E. ochreata* contains alkaloids, saponin glycosides, tannins, flavonoids, steroids and triterpenoids which are also matching with our observations. The medicinal importance of *E. nuda* was shown by presence of alkaloids, flavonoids, saponins, cardiac glycosides, terpenoids and steroids. Ola (2017) reported glycoside, alkaloid, tannins, phlobatannins and flavonoids from the methanolic extract of tubers of *E. gracilis* which are in good agreement with our studies.

5.4.3. Antioxidant compounds

The scavenging ability of DPPH is due to presence of various phytochemicals which have antioxidant properties in plant sample like phenolics and flavonoids. This scavenging property was studied based on discoloration of violet color of DPPH reagent. the extent of discoloration will reflect the antioxidant properties.

The result suggested that an unknown compound in all the tracks at Rf 0.81 is having highest antioxidant property. The abundance of the compound is very high in *Eulophia* genus (48 – 60%) compared to *Nervilia* (21 – 44%) (Table 5.15). The base of every track at Rf 0.01 also have mixture of compounds which show an efficient antioxidant scavenging potential. At Rf between 0.01 to 0.3 in all the acidic treated samples (T-2, 5, 8 and 11), many unknown compounds were formed after acidic hydrolysis which also impart in the activity. Furthermore, at Rf 0.6 (T-6 and 7), a blue fluorescent zone at 366 nm (Figure 5.12 (b)) and dark orange color zone at 580 nm (Figure 5.12 (c)) also show good antioxidant potential. Hence concludes that all 4 orchid species are having efficient potential to scavenge the free radical which directly proportional to good antioxidant activity.

Few more scientists carried out antioxidant studies using DPPH assay in orchids. The evidence of better antioxidant activity of *Nervilia aragoana* (syn. *N. concolor*) was provided by Reddy et al. (2010), during the comparison between *N. aragoana* and

Atlantia monophyla. The scavenging properties of phytochemicals present in the *Eulophia* were confirmed by Kshirsagar et al. (2010) using the DPPH assay in the bioassay guided fractionation in different fractions of bulb of *Eulophia ochreata*. The scavenging potential of methanolic extract of *E. ochreata* tubers showed by Narkhede et al. (2012) are matching with our observations. More evidences of the same was provided by Narkhede et al. (2016) during the comparative studies of antioxidant studies in 14 *Eulophia* species. Antioxidant potential of different Eulophia species were supported by Kumar et al. (2013) Chinsamy et al. (2014) and Saidu (2015). The DPPH scavenging potential of *E. herbacea* tubers in our studies was supported by Tatiya et al. (2018) during their investigation of antioxidant activity in *E. herbacea*.

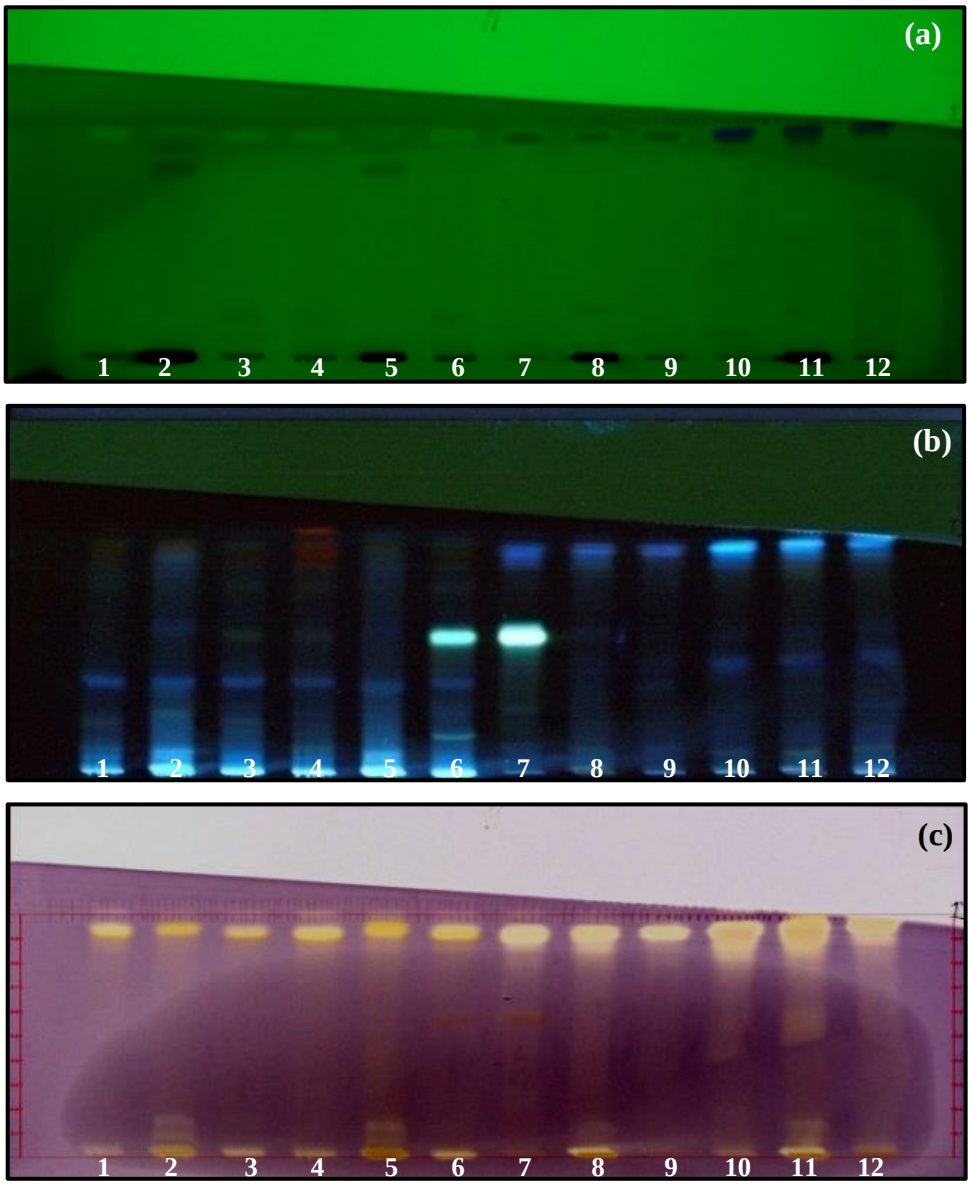


Figure 5.12: HPTLC plate photo of Phenolic group after derivatization (a) at 254 nm (b) 366 nm (c) 580 nm

Table 5.15: Percent area and retention time of peaks in antioxidant study at 580 nm

Peaks at 580 nm	Rf	Area (%)											
		T-1	**T-2**	**T-3**	**T-4**	**T-5**	**T-6**	**T-7**	**T-8**	**T-9**	**T-10**	**T-11**	**T-12**
1	0.01	13.4	23.4	20.26	13.48	25.3	18.96	6.42	14.22	11.97	6.48	7.91	9.26
2	0.08	6.01	-	3.02	4.2	-	-	-	4.32	-	-	2.91	-
3	0.1	-	-	5.16	-	-	-	3.61	-	3.52	-	-	-
4	0.15	-	-	-	-	-	-	-	-	-	-	2.82	7.76
5	0.19	-	-	-	-	8.73	-	-	2.66	-	9.48	7.48	2.7
6	0.25	-	7.59	-	-	-	-	-	-	-	-	-	-
7	0.28	10.6	-	10.35	8.78	-	7.21	2.83	-	5	8.03	-	7.56
8	0.32	3.98	-	3.22	3.61	-	3.45	1.96	-	-	-	-	-
9	0.39	-	4.07	-	-	3.92	-	-	-	-	-	5.15	-
10	0.42	6.85	-	5.1	3.52	-	3.1	-	5.07	-	-	-	-
11	0.45	-	-	-	-	-	-	5.55	-	4.5	6.02	12.41	13.89
12	0.48	-	2.33	-	-	1.88	1.96	-	-	2.48	4.5	-	-
13	0.59	-	-	-	-	-	-	-	-	4.2	-	-	7.63
14	0.67	10	23.32	6.54	12.95	22.89	16.44	21.06	13.38	8.41	-	-	-
15	0.75	-	16.42	-	-	-	-	-	-	-	-	-	-
16	0.81	**47.25**	**21.8**	**44.68**	**37.72**	**36.55**	**37.27**	**57.5**	**48.23**	**58.77**	**60.1**	**60.4**	**50.72**
17	0.87	-	-	-	14.25	-	10.2	-	11.29	-	4.82	-	-
18	1.04	1.91	1.07	1.67	1.5	0.73	1.42	1.06	0.83	1.17	0.58	0.49	0.46

5.5. CONCLUSION

This study provides useful information regarding the ethnobotanically important species and their phytochemical distinction. From HPTLC analysis it is concluded that *Nervilia concolor, N. plicata, Eulophia ochreata* and *E. herbacea* comprises of variety of bioactive compounds and they also possess very good antioxidant potential. Based on the HPTLC fingerprint, it was found that both the species of genus *Nervilia* were differentiated by presence of red band at Rf 0.25 (T-1 and 4) and 0.4 (T-2 and 4) while, the blue band in *Eulophia ochreata* (T-7 – 9) at Rf 0.45 was the prominent for differentiating this species. The occurrence of tannins in *E. herbacea* is also a useful information to distinguish it from remaining speices. The presence of many other bands at different Rf value were useful in differentiating the species among genera as well as the between two genera.

The work provides important data with respect to the identification and standardization of phytochemical groups in *Nervilia concolor, N. plicata, Eulophia ochreata* and *E. herbacea.* The developed HPTLC methods will be helpful for quality control, standardization of herbal formulations, identification of adulterant and biomarkers in orchids. All the phytochemicals observed during the analysis must be further validated using standards with the help of various chromatographic techniques.

5.6. REFERENCES

Acharya, K.P., Rokaya, M.B., 2010. Medicinal Orchids of Nepal: Are They Well Protected? Our Nat. 8, 82 – 91. https://doi.org/10.3126/on.v8i1.4315

Agrawal, D.C., 2017. Biotechnology of Medicinal Plants and Fungi in Taiwan: Production of Bioactive Secondary Metabolites in In Vitro Culture Systems: in Medicinal Plants and Fungi: Recent Advances in Research and Development, Medicinal and Aromatic Plants of the World. Springer Singapore Publisher, 459 – 463.

Ahmed, F., Sayeed, A., Islam, A., Salam, S.M.A., Sadik, G., Sattar, M.A., G.R.M. Astaq Mohal Khan, 2002. Antimicrobial Activity of Extracts and a Glycoside from *Vanda roxburghii* Br. Pakistan J. Biol. Sci. 5, 189 – 191.

Akhter, M., Hoque, M.M., Rahman, M., Huda, M.K., 2017. Ethnobotanical investigation of some orchids used by five communities of Cox's Bazar and Chittagong hill tracts districts of Bangladesh. J. Med. Plant Stud. 5, 265 – 268.

Ángeles Ramírez-Cisneros, M., Ríos Gómez, R., Rios, M.Y., 2016. HPLC quantification method for chrysin and tectochrysin in *Flourensia* extracts. Planta Med. 81, 1 – 138. https://doi.org/10.1055/s-0036-1596747

Anuradha, V., Rao, N.S.P., 1998. Aeridin: A phenanthropyran from *Aerides crispum*. Phytochemistry 48(1), 185 – 186.

Arora, M., Kaur, G., Kahlon, P.S., Mahajan, A., Sembi, J.K., 2017. Pharmacognostic Evaluation & Antimicrobial Activity of Endangered Ethnomedicinal Plant *Crepidium acuminatum* (D. Don) Szlach. Pharmacogn. J. 9, 6s, 53 – 63.

Attimarad, M., Mueen A.K.K., Aldhubaib, B.E., Harsha, S., 2011. High-performance thin layer chromatography: A powerful analytical technique in pharmaceutical drug discovery. Pharm. Methods 2(2), 71 – 75. https://doi.org/10.4103/2229-4708.84436

Azwanida, N., 2015. A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation. Med. Aromat. Plants 4, 3 – 8. https://doi.org/10.4172/2167-0412.1000196

Balami, N.P., 2004. Ethnomedicinal uses of plants among the newar community of pharping village of kathmandu district, Nepal. Tribhuvan Univ. J. 24(1), 1 – 10. https://doi.org/10.3126/tuj.v24i1.251

Bhandari, S.R., Kapadi, A.H., Mujumder, P.L., Joardar, M., Shoolery, J.N., 1985. Nudol, a phenanthrene of the orchids *Eulophia nuda*, *Eria carinata* and *Eria stricta*. Phytochemistry 24, 801 – 804.

Bhat, J.A., Kumar, M., Bussmann, R.W., 2013. Ecological status and traditional knowledge of medicinal plants in Kedarnath Wildlife Sanctuary of Garhwal Himalaya , India. J. Ethnobiol. Ethnomed. 9, 1 – 18.

Bhatnagar, M., Ghosal, S., 2018. Antibacterial and antimycobacterial activity of medicinal orchid of Arunachal Pradesh. Int. J. Pharm. Sci. Res. 9, 712 – 717. https://doi.org/10.13040/IJPSR.0975-8232.9(2).712-17

Bhatnagar, M., Sarkar, N., Gandharv, N., Apang, O., Singh, S., Ghosal, S., 2017. Evaluation of antimycobacterial, leishmanicidal and antibacterial activity of three medicinal orchids of Arunachal Pradesh, India. BMC Complement. Altern. Med. 17, 1 – 10. https://doi.org/10.1186/s12906-017-1884-z

Bhattacharjee, B., Islam, S., 2015. Assessment of Antibacterial and Antifungal Activities of the Extracts of *Rhynchostylis retusa* Blume-a Medicinal Orchid. World J. Pharm. Pharm. Sci. 4, 74 – 87.

Bhattacharjee, B., Islam, T., Rahman, Z., Islam, S.M.S., 2015. Antimicrobial activity and phytochemical screening of whole plant extracts of *Vanda tessellata* (ROXB.) Hook. ex. G. Don. World J. Pharm. Pharm. Sci. 4, 72 – 83.

Bi, Z.M., Wang, Z.T., Xu, L.S., 2004. Chemical Constituents of *Dendrobium moniliforme*. Acta Bot. Sin. 46, 124–126.

Bulpitt, C.J., Li, Y., Bulpitt, P.F., Wang, J., 2007. The use of orchids in Chinese medicine. J. R. Soc. Med. 100, 558 – 563. https://doi.org/10.1258/jrsm.100.12.558

Chaudhary, A., Singh, N., 2011. Contribution of world health organization in the global acceptance of ayurveda. J. Ayurveda Integr. Med. 2, 179 – 186. https://doi.org/10.4103/0975-9476.90769

Chauhan, N.S., 1999. Medicinal and Aromatic Plants of Himachal Pradesh. Indus Publishing Company, 1 – 632.

Chauhan, R.S., Nautiyal, M.C., Prasad, P., 2007. *Habenaria intermedia* D.Don - an Endangered Medicinal Orchid. McAllen Int. Orchid Soc. Journal, 8(10), 15 – 20.

Chen, C.C., Huang, Y.L., Teng, C.M., 2000. Antiplatelet aggregation principles from *Ephemerantha lonchophylla*. Planta Med. 66(4), 372 – 374. https://doi.org/10.1055/s-2000-8553

Chen, C.C., Wu, L.G., Feng-Nien, K., Teng, C.M., 1994. Antiplatelet aggregation principles of *Dendrobium ioddigesii*. J. Nat. Prod. 57, 1271 – 1274. https://doi.org/10.1021/np50111a014

Chen, K.K., Chen, L.A., 1935. The alkaloid of Chin-Shih-Hu. J. Biol. Chem. 111, 653 – 658.

Chen, Y.L., Zhang, M., Hua, Y.F., He, G.Q., 2001. Studies on polysaccharide alkaloids and minerals from *Dendrobium moniliforme* (L.) Sw. China J. Chinese Mater. medica (Zhongguo Zhong yao za zhi) 26, 4, 709 – 710.

Cheynier, V., Comte, G., Davies, K.M., Lattanzio, V., Martens, S., 2013. Plant phenolics: Recent advances on their biosynthesis, genetics, andecophysiology. Plant Physiol. Biochem. 72, 1 – 20. https://doi.org/10.1016/j.plaphy.2013.05.009

Chik, S.C.C., Or, T.C.T., Luo, D., Yang, C.L.H., Lau, A.S.Y., 2013. Pharmacological effects of active compounds on neurodegenerative disease with gastrodia and uncaria decoction, a commonly used poststroke decoction. Sci. World J. 2013, 1 – 22. https://doi.org/10.1155/2013/896873

Chinsamy, M., Finnie, J.F., Van Staden, J., 2014. Anti-inflammatory, antioxidant, anti-cholinesterase activity and mutagenicity of South African medicinal orchids. South African J. Bot. 91, 88 – 98. https://doi.org/10.1016/j.sajb.2013.12.004

Chowdhury, M.A., Rahman, M.M., Chowdhury, H.R.H., Uddin, M.J., Sayeed, A.A., Hossain, M.A., 2014. Antinociceptive and cytotoxic activities of an epiphytic medicinal orchid: *Vanda tessellata* Roxb. BMC Complement. Altern. Med. 14, 1 – 7. https://doi.org/10.1186/1472-6882-14-464

Chung, H.H., Shi, S.K., Huang, B., Chen, J.T., 2017. Enhanced Agronomic Traits and Medicinal Constituents of Autotetraploids in *Anoectochilus formosanus* Hayata, a Top-Grade Medicinal Orchid. Molecules 22(1907), 1 – 13. https://doi.org/10.3390/molecules22111907

Cooper, R., Nicola, G., 2014. Natural Products Chemistry: Sources, Separations and Structures. CRC Press, 1 – 206.

Cui, L., Su, X., 2009. Discovery, mechanisms of action and combination therapy of artemisinin. Expert. Rev. Anti-Infect. Ther. 7, 999 – 1013. https://doi.org/10.1053/j.seminhematol.2008.12.005.Pathobiology

Da Silva, J.A.T., 2013. Orchids: Advances in tissue culture, genetics, phytochemistry and transgenic biotechnology. Floric. Ornam. Biotechnol. 7, 1 – 52.

Dash, P.K., Sahoo, S., Bal, S., 2008. Ethnobotanical Studies on Orchids of Niyamgiri Hill Ranges, Orissa, India. Ethnobot. Leafl. 12, 70 – 78.

De, L.C., 2015. Commercial Orchids. De Gruyter GmbH & Co KG Press, 1 – 322.

De, L.C., Pathak, P., Rao, A.N., Rajeevan, P.K., 2014. Medicinal and Aromatic Orchids, in: Commercial Orchids. De Gruyter GmbH & Co KG Press, 243 – 249.

Déciga-Campos, M., Palacios-Espinosa, J.F., Reyes-Ramírez, A., Mata, R., 2007. Antinociceptive and anti-inflammatory effects of compounds isolated from *Scaphyglottis livida* and *Maxillaria densa*. J. Ethnopharmacol. 114, 161 – 168.

Dhyani, A., Nautiyal, B.P., Nautiyal, M.C., 2010. Importance of Astavarga plants in traditional systems of medicine in Garhwal, Indian Himalaya. Int. J. Biodivers. Sci. Ecosyst. Serv. Manag. 6, 13 – 19. https://doi.org/10.1080/21513732.2010.521490

Dilipkuma, E.K., Janardhana, G.R., 2013. Antidiabetic and regenerative effects of alcoholic corm extract of *Nervilia aragoana* Gaud. in streptozotocin-nicotinamide induced NIDDM rats. Int. J. Phytomedicine 5, 207 – 210.

Doughari, J.H., 2012. Phytochemical constituents of some selected medicinal plants, in: Phytochemicals - A Global Perspective of Their Role in Nutrition and Health, Intech Press, 1 – 33.

Ekor, M., 2014. The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. Front. Pharmacol. 4, 1 – 10.

Esha, V., Shilpa, C., Bharat, P., Neeru, 2016. Physico-chemical and phytochemical evaluation of *Dendrobium macraei* Lindl. Int. J. Pharmacogn. Phytochem. Res. 8, 1801 – 1811.

Faizul Haq, 2012. The Ethno Botanical Uses of Medicinal Plants of Allai Valley, Western Himalaya Pakistan. Int. J. Plant Res. 2, 21 – 34. https://doi.org/10.5923/j.

Fan, C., Wang, W., Wang, Y., Qin, G., Weimin Zhao, 2001. Chemical constituents from *Dendrobium densiflorum*. Phytochemistry 57, 1255 – 1258.

Fattorusso, E., Taglialatela-Scafati, O., 2008. Modern Alkaloids. Structure, Isolation, Synthesis and Biology. Wiley-VCH Verlag GmbH & Co. KGaA Press, 1 – 665. https://doi.org/10.1021/np3001688

Feleszko, W., Jaworska, J., 2013. Probiotics and Prebiotics in immune Modulation, in: Bioactive Food as Dietary Interventions for Arthritis and Related Inflammatory Diseases (Watson, R.R., Preedy, V.R.,), Academic Press, 357 – 370. https://doi.org/10.1016/B978-0-12-397156-2.00026-0

Folashade, O., Omoregie, H., Ochogu, P., 2012. Standardization of herbal medicines -A review. Int. J. Biodivers. Conserv. 4, 101 – 112. https://doi.org/10.5897/IJBC11.163

Fulekar, M.H., 2010. Environmental Biotechnology. CRC Press, 1 – 620.

Ghanaksh, A., Kaushik, P., 1993. Antibacterial Effect of *Aerides multiflora* Roxb. A Study in Vitro. J. Orchid Soc. India 13, 65 – 68.

Gong, Y.Q., Fan, Y., Wu, D.Z., Yang, H., Hu, Z.B., Wang, Z.T., 2004. In vivo and in vitro evaluation of erianin, a novel anti-angiogenic agent. Eur. J. Cancer 40, 1554 – 1565. https://doi.org/10.1016/j.ejca.2004.01.041

Hamid, K., Urmi, K.F., Kabir, M.S., Ullah, M.O., Choudhuri, M.S.K., 2012. Toxicological studies of Astavarga Kvatha Curna, an ayurvedic formulation, on liver function parameters of rat plasma. Biol. Med. 4, 27 – 31.

Haridas, R., Thangapandian, V., Thomas, B., 2014. Phytochemical evaluation of a medicinal orchid: *Nervilia plicata* (Andrews) Schltr. Int. J. Pharm. Dev. Technol. 4, 204 – 209.

He, C.N., Wang, C.L., Guo, S.X., Yang, J.S., Xiao, P.G., 2006. A novel flavonoid glucoside from *Anoectochilus roxburghii* (Wall.) Lindl. J. Integr. Plant Biol. 48, 359 – 363. https://doi.org/10.1111/j.1744-7909.2006.00179.x

Hernández-Romero, Y., Rojas, J.I., Castillo, R., Rojas, A., Mata, R., 2004. Spasmolytic Effects, Mode of Action and Structure-Activity Relationships of Stilbenoids from *Nidema boothii*. J. Nat. Prod. 67, 160 – 167. https://doi.org/10.1021/np030303h

Ho, C.K., Chen, C.C., 2003. Moscatilin from the orchid *Dendrobrium loddigesii* is a potential anticancer agent. Cancer Invest. 21, 729 – 736.

Hong, H.A.I., 2015. Principles of Chinese Medicine: A Modern Interpretation. Imperial College Press, 1 – 284.

Hoque, M.M., Khaleda, L., Al-forkan, M., 2016. Evaluation of pharmaceutical properties on microbial activities of some important medicinal orchids of Bangladesh. J. Pharcognosy Phytochem. 5, 265 – 269.

Hossain, M.M., 2009. Traditional therapeutic uses of some indigenous orchids of Bangladesh. Med. Arom. Plant Sci. Biotechnol. 3, 100 – 106.

Hossain, M.M.M., 2011. Therapeutic orchids: Traditional uses and recent advances - An overview. Fitoterapia 82, 102 – 140. https://doi.org/10.1016/j.fitote.2010.09.007

Huang, Q., Liang, L., He R., Ma, X., Zhan, R., Chen, W., 2015. The first insight into transcriptome profile of herbaceous plant *Nervilia fordii* based on RNA-seq. Plant Omics Journal 8(6), 493 – 499.

Huang, S., Zhou, X.L., Wang, C.J., Wang, Y.S., Xiao, F., Shan, L.H., Guo, Z.Y., Weng, J., 2013. Pyrrolizidine alkaloids from *Liparis nervosa* with inhibitory activities against LPS-induced NO production in RAW264.7 macrophages. Phytochemistry 93, 154 – 161. https://doi.org/10.1016/j.phytochem.2013.03.009

Jalal, J.S., Kumar, P., Pangtey, Y.P.S., 2008. Ethnomedicinal Orchids of Uttarakhand,

Western Himalaya. Ethnobot. Leafl. 12, 1227 – 1230.

Jalal, J.S., Tewari, L.M., Pangtey, Y.P.S., 2009. *Pholidata articulata* Lindl., An Orchid Used in Bone Jointing in Kumaun Region, Western Himalaya. Ethnobot. Leafl. 13, 1047 – 1050.

Joshi, G.C., Tewari, L.M., Lohani, N., Upreti, K., Jalal, J.S., Tewari, G., 2009. Diversity of Orchids in Uttarakhand and their Conservation Strategy with Special Reference to their Medicinal Importance. Rep. Opin. 1, 47 – 52.

Kalaiarasan, A., John, S.A., 2011. GC-MS Analysis of *Bulbophyllum Kaitense* Rechib . Pseudobulbs Estern Ghats of India. Int. J. Chem. Appl. 3, 215 – 220.

Kaur, S., Bhutani, K.K., 2014. In vitro conservation and asymbiotic propagation of *Coelogyne flaccida* (Lindl.): A threatened orchid. Plant Biosyst. 148, 935 – 944.

Khan, S.M., Page, S., Ahmad, H., Shaheen, H., Ullah, Z., Ahmad, M., Harper, D.M., 2013. Medicinal flora and ethnoecological knowledge in the Naran Valley, Western Himalaya, Pakistan. J. Ethnobiol. Ethnomed. 9, 1 – 13. https://doi.org/10.1186/1746-4269-9-4

Khare, C.P., 2007. *Habenaria intermedia* D. Don., in: Indian Medicinal Plants: An Illustrated Dictionary. Springer, New York, 301. https://doi.org/10.1007/978-0-387-70638-2_725

Koirala, D., Pradhan, S., Pant, B., 2013. Asymbiotic Seed Germination and Plantlet Development of *Coelogyne fuscescens* Lindl., A Medicinal Orchid of Nepal. Sci. World 11, 97 – 100. https://doi.org/10.3126/sw.v11i11.8561

Koparde, A.A., Magdum, C.S., 2015. Preliminary phytochemical and pharmacognostic investigation of *Eulophia ochreata* Lindl. tubers (Orchidaceae). World J. Pharm. Res., 4(10), 1063 – 1073.

Krause, J., Tobin, G., 2013. Discovery , Development , and Regulation of Natural Products. Intech 3 – 36. https://doi.org/10.5772/56424

Kshirsagar, R., Kanekar, Y., Jagtap, S., Upadhyay, S., Rao, R., Bhujbal, S., Kedia, J., 2010. Phenanthrenes of *Eulophia ochreata* Lindl. Int. J. Green Pharm. 4, 147.

Kumar, V., Lemos, M., Sharma, M., Shriram, V., 2013. Antioxidant and DNA damage protecting activities of *Eulophia nuda* Lindl. Free Radicals and Antioxidants, 3, 55 – 60.

Kurapa, S.R., Upadhyay, R., Trivedi, U.S., Tiwari, S., 2012. Qualitative phytochemical analysis of *Eulophia nuda* Lind an endangered terrestrial orchid. Int. J. Pharma Bio Sci. 1(5), 456 – 462.

Lam, Y., Ng, T.B., Yao, R.M., Shi, J., Xu, K., Sze, S.C.W., Zhang, K.Y., 2015. Evaluation of chemical constituents and important mechanism of pharmacological biology in *Dendrobium* plants. Evidence-based Complement. Altern. Med. 2015, 1 – 25. https://doi.org/10.1155/2015/841752

Lei, Y., Wang, L., Cheng, M., Xiao, H., 2011. Identification of major metabolites in rat urine and plasma of N6-(4-hydroxybenzyl) adenine riboside by LC/MS/MS. Biomed. Chromatogr. 25, 344 – 352. https://doi.org/10.1002/bmc.1452

Li, C.B., Wang, C., Fan, W.W., Dong, F.W., Xu, F.Q., Wan, Q.L., Luo, H.R., Liu, Y.Q., Hu, J.M., Zhou, J., 2013. Chemical components of *Dendrobium crepidatum* and their neurite outgrowth enhancing activities. Nat. Products Bioprospect. 3, 70 – 73. https://doi.org/10.1007/s13659-012-0103-3

Li, J.T., Yin, B.L., Liu, Y., Wang, L.Q., Chen, Y.G., 2009. Mono-aromatic constituents of *Dendrobium longicornu*. Chem. Nat. Compd. 45, 234 – 236. https://doi.org/10.1007/s10600-009-9293-2

Li, M.F., Hirata, Y., Xu, G.J., Niwa, M., Wu, H.M., 1991. Studies on the chemical constituents of *Dendrobium loddigesii* Rolfe. Yao xue xue bao (Acta Pharm. Sin. 26, 307 – 310.

Liu, Q., Ha, W., Liu, Z., Xu, J., Tian, Y., Zhou, X., Mu, X., 2014. 3-hydroxybutanolide derivatives and flavonoid glucosides from *Anoectochilus roxburghii*. Phytochem. Lett. 8, 109 – 115. https://doi.org/10.1016/j.phytol.2014.02.013

Lo, S.F., Mulabagal, V., Chen, C.L., Kuo, C.L., Tsay, H.S., 2004. Bioguided fractionation and isolation of free radical scavenging components from in vitro propagated chinese medicinal plants *Dendrobium tosaense* Makino and

Dendrobium moniliforme SW. J. Agric. Food Chem. 52, 6916 – 6919. https://doi.org/10.1021/jf040017r

Luo, J.P., Wang, Y., Zha, X.Q., Huang, L., 2008. Micropropagation of *Dendrobium densiflorum* Lindl. ex Wall. through protocorm-like bodies: Effects of plant growth regulators and lanthanoids. Plant Cell Tissue Organ Cult. 93, 333 – 340. https://doi.org/10.1007/s11240-008-9381-1

Ma, G.X., Wang, T.S., Yin, L., Pan, Y., Guo, Y.L., LeBlanc, G.A., Reinecke, M.G., Watson, W.H., Krawiec, M., 1998. Two pimarane diterpenoids from *Ephemerantha lonchophylla* and their evaluation as modulators of the multidrug resistance phenotype. J. Nat. Prod. 61, 112 – 115. https://doi.org/10.1021/np970065o

Majumder, P., Banerjee, S., Sen, S., 1996. Three stilbenoids from the orchid *Agrostophyllum callosum*. Phytochemistry 42, 847 – 852. https://doi.org/10.1016/0031-9422(95)00954-X

Majumder, P.L., Majumder, S., Sen, S., 2003. Triterpenoids from the orchids *Agrostophyllum brevipes* and *Agrostophyllum callosum*. Phytochemistry 62, 591 – 596. https://doi.org/10.1016/S0031-9422(02)00487-9

Majumder, P.L., Sen, S., Banerjee, S., 1999. Agrostophyllol and Isoagrostophyllol, Two Novel Diastereomeric 9,10-Dihydrophenanthropyran Derivatives from the Orchid *Agrostophyilum callosum*. Tetrahedron 55, 6691 – 6702.

Mazumder, P.B., Sharma, G.D., Dutta Choudhury, M., Nath, D., Das Talukdar, a, Mazumder, B., 2010. In Vitro Propagation and Phytochemical Screening of *Papilionanthe teres* (Roxb.) Schltr. Assam Univ. J. Sci. Technol. 5, 37 – 42.

Medhi, R.P., Chakrabarti, S., 2009. Traditional knowledge of NE people on conservation of wild orchids. Indian J. Tradit. Knowl. 8, 11 – 16.

Meng, L.Z., Lv, G.P., Hu, D.J., Cheong, K.L., Xie, J., Zhao, J., Li, S.P., 2013. Effects of polysaccharides from different species of *Dendrobium* (Shihu) on macrophage function. Molecules 18, 5779 – 5791. https://doi.org/10.3390/molecules18055779

Menon, S., Nayeem, N., 2013. *Vanilla planifolia*: A review of a plant commonly used as

flavouring agent. Int. J. Pharm. Sci. Rev. Res. 20, 225 – 228.

Minh, T., Khang, D., Tuyen, P., Minh, L., Anh, L., Quan, N., Ha, P., Quan, N., Toan, N., Elzaawely, A., Xuan, T., 2016. Phenolic Compounds and Antioxidant Activity of *Phalaenopsis* Orchid Hybrids. Antioxidants 5, 1 – 12. https://doi.org/10.3390/antiox5030031

Mishra, A.P., Sarla, S., 2012. Satyrium nepalense: a rare medicinal orchid of Western Himalaya (India); phytochemical screening, antimicrobial evaluation and conservation studies. Indones. J. Pharm. 23, 162 – 170.

Miyazawa, M., Shimamura, H., Nakamura, S.I., Sugiura, W., Kosaka, H., Kameoka, H., 1999. Moscatilin from *Dendrobium nobile*, a naturally occurring bibenzyl compound with potential antimutagenic activity. J. Agric. Food Chem. 47, 2163 – 2167. https://doi.org/10.1021/jf970930a

Moin, S., Shibu, S.B., Wesley, S.P., Devi, C.B., 2012. Bioactive potential of Coelogyne stricta (D.Don) Schltr: An ornamental and medicinally important orchid. J. Pharm. Res. 5, 2191 – 2196.

Mythili, K., 2015. Development standardization and preclinical evaluation of a new herbal formulation. Sri Ramachandra University. Ph.D. Thesis

Nagulwar, V.P., Nandgave, M., Mahajan, M.S., Deshpande, S.A., 2017. Phytochemical screening and evaluation of pharmacological activities of *Eulophia nuda* Lind. tuber extracts, International Journal of Pharmaceutical Sciences and Research 8(8), 3516 – 3523.

Narkhede, A., Mahajan, M., Singh, E., Harsulkar, A., Jagtap, S., 2016. antioxidant activity of fourteen *Eulophia* species traditionally known as Amarkand. Int. J. Pharm. Pharm. Sci. 8(9), 313 – 316

Narkhede, A., Nirmal, P., Tupe, R., Kulkarni, O., Harsulkar, A., Jagtap S., 2012. In vitro Antioxidant, Antiglycation and a- amylase inhibitory potential of *Eulophia ochreata* L. J. Pharm. Res., 5(5), 2532 – 2537.

Nongdam, P., 2014. Ethno-medicinal Uses of some Orchids of Nagaland, North-east India. Res. J. Med. Plants 8, 126 – 139.

Nugroho, L.H., Pratiwi, R., Susandarini, R., Wardoyo, E.R.P., Megawati, O., Handayani, S., 2016. Isolation of bioactive compounds from two orchid species and preliminary test of their cytotoxicity against T47D breast cancer cells. Int. J. Pharmacogn. Phytochem. Res. 8, 150 – 155.

Ola, O.S., 2017. Preliminary proximate analysis, chemical composition and phytoconstituents of *Eulophia gracilis* Orchid. Int. J. Sci.: Basic Appl. Res. 36(8), 215 – 222.

Ono, T., Miyazawa, M., 1999. Headspace constituents of flowers of *Neofinetia falcata*. Nat. Prod. Lett. 13, 53 – 57. https://doi.org/10.1080/10575639908048491

Panda, S.P., Sharief, M.U., Hameed, S.S., Pramanik, A., 2015. Traditional phytotheraphic record of orchids of Odisha and their conservation strategies. Ann. Plant Sci. 4, 1204 – 1207.

Pandey, A., Tripathi, S., Pandey, C.A., 2014. Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. J. Pharmacogn. Phytochem. 115, 115 – 119.

Pant, B., 2013. Medicinal orchids and their uses: Tissue culture a potential alternative for conservation. African J. Plant Sci. 7, 448 – 467. https://doi.org/10.5897/AJPS2013.1031

Pant, B., Swar, S., 2011. Micropropagation of *Cymbidium iridioides*. Nepal J. Sci. Technol. 12, 91 – 96. https://doi.org/10.3126/njst.v12i0.6485

Pant, S., Rinchen, T., 2012. *Dactylorhiza hatagirea*: A high value medicinal orchid. J. Med. Plants Res. 6, 3522 – 3524. https://doi.org/10.5897/JMPR12.097

Patil, M.C., Mahajan, R.T., 2013. Ethnobotanical potential of *Eulophia* species for their possible biological activity. Int. J. Pharm. Sci. Rev. Res. 21, 297 – 307.

Paudel, M.R., Chand, M.B., Pant, B., Pant, B., 2017. Cytotoxic Activity of Antioxidant-Riched *Dendrobium longicornu*. Pharmacogn. J. 9, 499 – 503. https://doi.org/10.5530/pj.2017.4.81

Peng, J., Xu, Q., Xu, Y., Qi, Y., Han, X., Xu, L., 2007. A new anticancer

dihydroflavanoid from the root of *Spiranthes australis* (R. Brown) Lindl. Nat. Prod. Res. 21, 641 – 645. https://doi.org/10.1080/14786410701371165

Pradhan, S., Paudel, Y.P., Pant, B., 2013. Efficient regeneration of plants from shoot tip explants of *Dendrobium densiflorum* Lindl., a medicinal orchid. African J. Biotechnol. 12, 1378 – 1383. https://doi.org/10.5897/AJB12.2731

Prakash, S., 1992. Atharva-Veda Saṃhita. Veda Pratishthana, 1 – 1271.

Pramanick, D.D., 2016. Pharmacognostic studies on the pseudobulb of *Coelogyne cristata* Lindl . (Orchidaceae) -An epiphytic orchid of ethno-Medicinal importance. J. Pharmacogn. Phytochem. 5, 120 – 123.

Prasad, R., Koch, B., 2016. In vitro Anticancer Activities of Ethanolic Extracts of *Dendrobium crepidatum* and *Dendrobium chrysanthum* against T-cell lymphoma. J. Cytol. Histol. 7, 1 – 8. https://doi.org/10.4172/2157-7099.1000432

Prasathkumar, M., Ramesh, T., 2016. Conservation of *Pholidota pallida* Lindl. medicinal orchid – in vitro. Int. J. Res. Instinct 3, 678 – 688.

Pridgeon, A.M., Cribb, P., Rasmussen, F.N., Chase, M.W., 2014. Genera Orchidacearum: Epidendroideae, Genera Orchidacearum. Oxford University Press, 1 – 672.

Radhika, B., Murthy, J.V.V.S.N., Nirmala Grace, D., 2013. Preliminary Phytochemical Analysis and Antibacterial Activity Against Clinical Pathogens of Medicinally Important Orchid *Cymbidium aloifolium* (L.) Sw. Int. J. Pharm. Sci. Res. 4, 3925 – 3931. https://doi.org/10.13040/IJPSR.0975-8232.4(10)

Ramawat, K.G., Mérillon, J.M., 2013. Natural Products: Phytochemistry, Botany and Metabolism of Alkaloids, Phenolics and Terpenes, Springer Berlin Heidelberg Press, 1 – 4242. https://doi.org/10.1007/978-3-642-22144-6

Rana, C.S., Tiwari, J.K., Dangwal, L.R., Gairola, S., 2013. F,aith herbal healer knowledge document of Nanda Devi Biosphere Reserve, Uttarakhand, India. Indian J. Tradit. Knowl. 12, 308 – 314.

Rao, N., Mittal, S., Menghani, E., 2013. Antioxidant Potential and Validation of Bioactive B -Sitosterol in *Eulophia campestris* Wall., Adv. Biores., 4(1), 136 – 142.

Reddy, K.H., Sharma, P.V.G.K., Reddy, O.V.S., 2010. A comparative in vitro study on antifungal and antioxidant activities of *Nervilia aragoana* and *Atlantia monophyla*. Pharm. Biol., 48(5): 595 – 602.

Reich, E., Schibli, A., 2007. High-performance Thin-layer Chromatography for the Analysis of Medicinal Plants. Thieme Publishers, 1 – 264.

Rungwichaniwat, P., Sritularak, B., Likhitwitayawuid, K., 2014. Chemical Constituents of *Dendrobium williamsonii*. Pharmacogn. J. 6, 36 – 41. https://doi.org/10.5530/pj.2014.3.6

Saidu, M.B. 2015. Pharmacognostic and antioxidant studies of the pseudobulbs of *Eulophia guineensis* Lindl (Orchidaceae), Ahmadu Bello University, Zaria, Nigeria, Ph.D. Thesis.

Schiff, J.L., 2017. Rare and Exotic Orchids: Their Nature and Cultural Significance. Springer International Publishing Press, 1 – 186.

Schmidt, B.M., Cheng, D.M.K., 2017. Ethnobotany: A Phytochemical Perspective. John Wiley & Sons Publishers, 1 – 376.

Schuster, R., Zeindl, L., Holzer, L., Khumpirapang, N., Okonogi, S., Viernstein, H., Mueller, M., 2017. *Eulophia macrobulbon* - an orchid with significant anti-inflammatory and antioxidant effect and anticancerogenic potential exerted by its root extract, Phytomedicine 15(24), 157 – 165.

Sebastinraj, J., Britto, S.J., Kumar, D.V., Robinson, J.P., Thangavel, P., 2014. Rapid Propagation of *Vanda testacea* (Lindl .) Rchb . F . – A Highly Medicinal Value Epiphytic Orchid of India. World J. Agric. Sci. 10, 223 – 230. https://doi.org/10.5829/idosi.wjas.2014.10.5.1827

Seigler, D.S., 2012. Plant Secondary Metabolism. Springer US Publisher, 1 – 759.

Selvam, A.B.D., 2012. Orchidaseae; *Dactylorhiza hatagirea* (D. Don) Soo, in:

Pharmacognosy of Negative Listed Plants. Botanical Survey of India, Ministry of Environment and Forests, 59 – 68.

Shailajan, S., Kumaria, S., Gurjar, D., Joshi, M., Paul, P., Khongthaw, N., 2015. Variation in the marker content of five different *Dendrobium* species: Comparative evaluation using validated HPTLC technique. J. Appl. Pharm. Sci. 5, 32 – 38. https://doi.org/10.7324/JAPS.2015.501006

Shailajan, S., Pednekar, S., Menon, S., Gurjari, D., 2017. Evidence based validation of *Dendrobium fimbriatum* as a hepatoprotectant: An orchid from Northeast India. Nat. Prod. Chem. Res. 5(5), 75. https://doi.org/10.4172/2329-6836-C1-017

Shanmugavalli, N., Umashankar, V., Raheem, 2009. Anitmicrobial activity of *Vanilla planifolia*. Indian J. Sci. Technol. 2, 37 – 40.

Sharangi, A.B., Datta, S., 2015. Value Addition of Horticultural Crops: Recent Trends and Future Directions. Springer India, 1 – 342.

Shi, Y., Dong, J.W., Tang, L.N., Kang, R.X., Shi, J.G., Zhang, J.J., 2014. N6-(3-methoxyl-4-hydroxybenzyl) adenine riboside induces sedative and hypnotic effects via GAD enzyme activation in mice. Pharmacol. Biochem. Behav. 126, 146 – 151.

Shiau, Y.J., Sagare, A.P., Chen, U.C., Yang, S.R., Tsay, H.S., 2002. Conservation of *Anoectochilus formosanus* Hayata by artificial cross-pollination and in vitro culture of seeds. Bot. Bull. Acad. Sin. 43, 123 – 130.

Shimura, H., Matsuura, M., Takada, N., Koda, Y., 2007. An antifungal compound involved in symbiotic germination of *Cypripedium macranthos* var. *rebunense* (Orchidaceae). Phytochemistry 68, 1442 – 1447. https://doi.org/10.1016/j.phytochem.2007.03.006

Show, S., Ghosal, C., Chattopadhyay, B., 2017. Root Extracts (*Gymnadenia orchidis* Lindl) Facilitated Rapid Synthesis of Fluorescent Silver Nanoparticles (Ag-NPs) for Various Biological Applications. J. Biomater. Nanobiotechnol. 8, 109 – 124.

Shubha, J., Chowdappa, S., 2016. Phytochemical analysis and Antibacterial activity of *Cymbidium aloifolium* L. a medicinal orchid from Western Ghats of Karnataka, India. Int. J. Adv. Sci. Res. Publ. 2, 19 – 23.

Siddiqui, M.W., Prasad, K., Bansal, V., 2017. Plant Secondary Metabolites, Three-Volume Set. CRC Press, 1 – 908.

Singh, A., 2011. Herbalism, Phytochemistry and Ethnopharmacology. CRC Press, 1 – 440.

Singh, A., Duggal, S., 2009. Medicinal Orchids - An Overview. Ethnobot. Leafl. 13, 399 – 412.

Singh, D.K., Babbar, S.B., 2016. In vitro propagation and chemical profiling of *Herminium lanceum* (Thunb. ex Sw.) Vuijk, a medicinally important orchid, for therapeutically important phenolic acids. Plant Biotechnol. 33, 153 – 160.

Singh, S., Singh, A.K., Kumar, S., Kumar, M., Pandey, P.K., Singh, M.C.K., 2012. Medicinal properties and uses of orchids : a concise review. Elixir Appl. Bot. 52, 11627 – 11634.

Singh, V., 2017. Medicinal plants and bone healing. Natl. J. Maxillofac. Surg. 8, 4 – 11. https://doi.org/10.4103/0975-5950.208972

Sohag, S.I., Hoque, M.M., 2017. Phytochemical screening and antioxidant activity of rare medicinal orchid *Luisia zeylanica* Lindl. J. Pharmacogn. Phytochem. 6, 688 – 692.

Subedi, A., Kunwar, B., Choi, Y., Dai, Y., van Andel, T., Chaudhary, R.P., de Boer, H.J., Gravendeel, B., 2013. Collection and trade of wild-harvested orchids in Nepal. J. Ethnobiol. Ethnomed. 9(64), 1 – 10. https://doi.org/10.1186/1746-4269-9-64

Sugathan, S., Pradeep, N.S., Abdulhameed, S., 2017. Bioresources and Bioprocess in Biotechnology: Volume 2 : Exploring Potential Biomolecules. Springer Singapore, 1 – 438.

Sultana, N., Jahan, T.A., Barai, T.K., Akhter, M.S., Ara, N., 2012. Tissue culture propagation of tropical orchid (*Phaius tankervilliae*) plant. J. Innov. Dev. Strateg. 6, 81 – 85.

Suresh, K.P.K., Subramoniam, A., Pushpangadan, P., 2000. Aphrodisiac activity of *Vanda tessellata* (Roxb.) Hook. ex Don extract in male mice. Indian J. Pharmacol.

32, 300 – 304.

Sut, S., Maggi, F., Dall'Acqua, S., 2017. Bioactive Secondary Metabolites from Orchids (Orchidaceae). Chem. Biodivers. 14, 1 – 30.

Tamang, R., 2017. Micropropagation of some orchids of Darjeeling and Sikkim Himalayas. University of North Bengal, Ph.D. Thesis.

Tang, C., Wang, J., Yu, J., Wang, L., Cheng, M., Cui, W., Zhao, J., Xiao, H., 2017. Identification, characterization and in vitro neuroprotection of N6-(4-hydroxybenzyl) adenine riboside and its metabolites. Phytochem. Lett. 20, 146 – 150. https://doi.org/10.1016/j.phytol.2017.04.035

Tang, W., Eisenbrand, G., 1992. *Dendrobium nobile* Lindl., in: Chinese Drugs of Plant Origin: Chemistry, Pharmacology, and Use in Traditional and Modern Medicine. Springer Berlin Heidelberg Press, 451 – 454. https://doi.org/10.1007/978-3-642-73739-8_57

Tatiya, A., Kalaskar, M., Patil, Y., Surana, S., 2018. Chemical analysis , nutritional content and antioxidant property of *Eulophia herbacea* Lindl. tubers : a medicinally versatile Indian tribal nutritional food supplement. Indian J. Tradional Knowl. 17, 141 – 147.

Tatiya, A., Surana, S., Bhavsar, S., Patil, D., Patil, Y., 2012. Pharmacognostic and preliminary phytochemical investigation of *Eulophia herbacea* Lindl. tubers (Orchidaceae). Asian Pacific J. Trop. Dis. 50 – 55.

Teoh, E.S., 2016a. Medicinal Orchids of Asia, Medicinal Orchids of Asia. Springer International Publishing, 1 – 733. https://doi.org/10.1007/978-3-319-24274-3

Teoh, E.S., 2016b. Secondary Metabolites of Plants, in: Medicinal Orchids of Asia. Springer International Publishing, Cham, 59 – 73. https://doi.org/10.1007/978-3-319-24274-3_5

Thokchom, R., Maitra, S., Sharma, S., 2017. In vitro Mass Propagation of Endangered Terrestrial Orchid *Phaius tankervilliae* (L'Her.) Blume through Green Seed Pod Culture. Int. J. Curr. Microbiol. Appl. Sci. 6, 722 – 728. https://doi.org/10.20546/ijcmas.2017.605.081

Thomas, E., Aneesh, T.P., Thomas, D.G., Anandan, R., 2013. GC-MS analysis of phytochemical compounds present in the rhizomes of *Nervilia aragoana* Gaud. Asian J. Pharm. Clin. Res. 6, 68 – 74.

Tian, L., Pei, Y., Zhang, Y., Wang, Y., Yang, C., 2009. 7-O-Methylkaempferol and -quercetin Glycosides from the whole plant of *Nervilia fordii*. J. Nat. Prod. 72, 1057 – 1060.

Vaidya, B., Shrestha, M., Joshee, N., 2000. Report on Nepalese Orchid Species with Medicinal Properties, in: Proceedings of Nepal-Japan Joint Symposium. 146 – 152.

Vatsa, E., Bora, S.K., 2016. Memory Enhancing Activity of *Dendrobium macraei* Lindl. in Swiss Albino Mice. Br. J. Pharm. Res. 13, 1 – 11. https://doi.org/10.9734/BJPR/2016/28090

Venkateswarlu, S., Raju, M.S.S., Subbaraju, G. V, 2002. Synthesis and biological activity of isoamoenylin, a metabolite of *Dendrobium amoenum*. Biosci. Biotechnol. Biochem. 66, 2236 – 2238. https://doi.org/10.1271/bbb.66.2236

Wagner, H., Bladt, S., 1996. Plant Drug Analysis: A Thin Layer Chromatography Atlas. Hendrickson Publishers, 1 – 322.

Wang, S.Y., Kuo, Y.H., Chang, H.N., Kang, P.L., Tsay, H.S., Lin, K.F., Yang, N.S., Shyur, L.F., 2002. Profiling and characterization antioxidant activities in *Anoectochilus formosanus* Hayata. J. Agric. Food Chem. 50, 1859 – 1865. https://doi.org/10.1021/jf0113575

Wang, Y., Avula, B., Abe, N., Wei, F., Wang, M., Ma, S., Ali, Z., Elsohly, M., Khan, I., 2014. Structural characterization of dendrobine-type sesquiterpene alkaloids from the stems of *Dendrobium* nobile using LC-MS/MS. Planta Med. 80, 662 – 670.

Wei, L., Chen, J., Ye, W., Yao, X., Zhou, G., 2012. Three new cycloartane glycosides from *Nervilia fordii*. J. Asian Nat. Prod. Res. 14, 521 – 527.

Wei, L., Yuan, H., Chen, J., Wang, Q., Wang, H., Ye, W., 2013. Five New Cycloartane-Type Triterpenoid Saponins from *Nervilia fordii*. Helv. Chim. Acta 96, 150 – 157.

Wiart, C., 2012. Medicinal Plants of China, Korea, and Japan: Bioresources for

Tomorrow's Drugs and Cosmetics. CRC Press, 1 – 454.

Williams, C.A., 1979. The leaf flavonoids of the Orchidaceae. Phytochemistry 18, 803 – 813.

Won, J.H., Kim, J.Y., Yun, K.J., Lee, J.H., Back, N.I., Chung, H.G., Chung, S.A., Jeong, T.S., Choi, M.S., Lee, K.T., 2006. Gigantol isolated from the whole plants of *Cymbidium goeringii* inhibits the LPS-induced iNOS and COX-2 expression via NF-kappaB inactivation in RAW 264.7 macrophages cells. Planta Med. 72, 1181 – 1187. https://doi.org/10.1055/s-2006-947201

Wu, B., He, S., Pan, Y., 2006. New dihydrodibenzoxepins from *Bulbophyllum kwangtungense*. Planta Med. 72, 1244 – 1247.

Yadav, N., Yadav, R., Goyal, A., 2014. Chemistry of terpenoids. Int. J. Pharm. Sci. Rev. Res. 27, 272 – 278.

Ye, S., Shao, Q., Zhang, A., 2017. *Anoectochilus roxburghii*: A review of its phytochemistry, pharmacology, and clinical applications. J. Ethnopharmacol. 209, 184 – 202. https://doi.org/10.1016/j.jep.2017.07.032

Zeng, B.Y., Zhao, K., 2017. Neurobiology of Chinese Herb Medicine, International Review of Neurobiology. Elsevier Science, 1 – 330.

Zhang, L., Zhao, Z., Lin, C., Zhu, C., Gao, L., 2012. Three new flavonol glycosides from *Nervilia fordii*. Phytochem. Lett. 5, 104 – 107.

Zhang, X., Xu, J.K., Wang, J., Wang, N.L., Kurihara, H., Kitanaka, S., Yao, X.S., 2007. Bioactive bibenzyl derivatives and fluorenones from *Dendrobium nobile*. J. Nat. Prod. 70, 24 – 28. https://doi.org/10.1021/np060449r

Zhou, J., Xie, G., Yan, X., 2011. Encyclopedia of Traditional Chinese Medicines - Molecular Structures, Pharmacological Activities, Natural Sources and Applications: Vol. 6: Indexes. Springer Berlin Heidelberg, 1 – 730.