Review of Literature

Medicinal plants have been considered as an important therapeutic aid for alleviating humankind. Nearly all cultures from ancient times till today have used plants as source of medicine. In many developing countries traditional medicine is still the mainstay of healthcare and most of the drugs and cures come from plants. Knowldege of plant chemical constituents is desirable, not only for the discovery of therapeutic agensts but also for the synthesis of complex chemical substances. In addition, the knowledge of the chemical constituents of plant would further be valuable in discovering the actual value of folk remedies. Therefore, the study on these phytochemicals in plants particularly of medicinal value is of great importance (Mujab *et al.,* 2003).

Secondary metabolites are the essential components of plants. It is important to quantify the chief active components of plants, which plays a major role in curing the ailments. The quality of secondary metabolites was affected by various environmental factors such as age, season, climatic condition, geographical variations and several other physical conditions influence the synthesis of the active phytochemical compounds in the plants which in turn affect their quality and curability. Hence a systematic study of crude drug by pharmacognostical techniques is important for proper identification and quality evaluation (Meenu *et al.,* 2011).

The medicinal effects of plant materials result from the combinations of secondary metabolites present in the plant such as alkaloids, steroids, tannins, flavonoids, resins, fatty acids, etc. Out of the total number of secondary metabolites reported in the natural products, 33,000 are terpenoids, 16,000 are alkaloids, and 8,182 are flavonoids. These being an essential part of the basic metabolism also have an ecological role and are often involved in plant protection against biotic or abiotic stresses. Some secondary metabolites such as flavonoids are also involved in cell pigmentation in flower and seed, which attract pollinators, seed dispersers and are also involved in plant reproduction. Moreover, plant secondary metabolites have pharmaceutical properties effective for human health (Jain *et al.,* 2019).

Medicinal and aromatic plants from different families have been used to cure various ailments of the people of our country but very little attention has been paid on the medicinal plants of Asteraceae in India. Asteraceae is considered as one of the highly advanced family having about 13 subfamilies, 1,911 genera, and 32,913 species (Hajra *et al.,* 1995; Mabberley, 1997).The genus *Eupatorium* is a flowering plant containing around 1200 species representing the family Asteraceae. Most of them are herbaceous perennials and few are shrubs. The various species from the genus *Eupatorium* are used in folklore medicine in different parts of the world for their medicinal properties (Albuquerque *et al.,* 2010).

Taxonomical position of *Eupatorium*

Class	:	Dicotyledonae
Series	:	Inferae
Order	:	Asterales
Family	:	Asteraceae
Genus	:	*Eupatorium*

***Eupatorium glandulosum* L.**

Synonym

Eupatorium adenophorum, *Ageratina adenophora*

Distribution

It is distributed mainly in the tropical regions of America, Europe, Africa, and Asia. In India it is found in Sikkim, Meghalaya, Tripura, Uttar Pradesh and Tamil Nadu.

Botanical description

E. glandulosum is a perennial, branched, spreading shrub growing upto 2m height, stem and branches densely glandular pubescent, purplish dark; Leaves opposite and decussate, rhomboidal, tapering, crenate, serrate, petioles 3.5-5 cm long; Inflorescence- terminal on the main stem or branches; heads homogamous, white, flat-topped clustered; penduncles 5- 10 mm long, glandular, with a few linear bracteoles 3 mm long; involucral bracts, green, very glandular pubescent outside, in many series; Corolla tube 3 mm long; lobes 5, Stamens 5; ovary inferior, stigma 2-lobed; flowering February to May. The plant has an offensive smell when crushed.

Medicinal uses

Leaves of *E. glandulosum* possess antiseptic, blood coagulant, analgesic and antipyretic, astringent, thermo genic and stimulant in folklore medicine in India (Mandal *et al*., 1981; Ansari *et al*., 1983; Kritikar and Basu, 1987; Rai and Sharma, 1994). The leaf juice has been applied on cut wounds to abate bleeding and also used to cure tooth ache and gum infection (Dahanukar *et al*., 2000; Saha *et al*., 2011).

The tribes of Meghalaya and Nagaland used the leaves to cure stomach ache (Kumar, 2002). Traditional practitioners in Darjeeling used the young leaves and shoots of *E. glandulosum* to treat jaundice, ulcers (Ansari *et al*., 1983) and dysentery (Bantawa and Rai, 2009). The leaves contain a kind of valuable perfumery material which results in economic profit of cosmetic Industry (Vasanthi and Gopalakrishnan, 2013).

Phytochemical studies

Plant part	Phytochemicals	Reference
Aerial parts	β-cymene, α- phellandrene, γ-curcumene, γ-2-carene, camphene and endo bornyl acetate	Paul *et al*., 2002
Leaves	Alkaloids, Tannins, Glycosides, Saponins, terpenes, Carbohydrates, Phenolic compounds, anthroquinone glycosides, Cadinane sesquiterpenes, monoterpenes,diterpenes, coumarins, steroids, propanol, coumaric acid and phenyl proponoids, quercetagetin 7-O-(6”-acetyl-β-d-glucoside), 7-O-glucosides of 6-hydroxykaempferol, quercetin, quercetagetin, 3,5,7-Trihydroxy-6, 4′-dimethoxyflavone and eupalitin 3-O-b- D-galacto pyranoside	Nair *et al.*, 1993; Ding *et al.*, 1992; Voirine, 1995; Mukherjee *et al.*, 2001; Sasikumar *et al.*, 2005; Yan *et al.*, 2006; Tian *et al.*, 2007; He *et al.*, 2008; Li *et al.*, 2008; Zhao *et al.*, 2009; Kurade *et al.*, 2010; Yun *et al.*, 2011; Iyeswarya *et al.*, 2013; Zhang *et al.*,, 2013; Rajeswary and Govindarajan, 2013;

		Vasanthi and Gopalakrishnan, 2013; Samuel *et al.*, 2013 and Desingh *et al.*, 2014
Flowers	Coumarins, steroids, alkaloid, phenyl proponoid, polysaccharide, sesquiterpene lactones, flavonoids, kaempherol diglycoside derivative, quercetin-3-O-beta-d-glucopyronoside, thujene, sabinene, terpinolene, linalool, camphor, borneol,cardinal, selina, amorphenes, p-cymene, bornyl acetate, camphene, amorphene, amorph-4-en-7-ol, 3-acetoxyamorpha-4, 7(11)- dien-8-one and amorph-4, 7(11)-dien-8-one and sesquiterpenoid	Bohlmann and Gupta, 1981; Nair and Sivakumar, 1990; Ding *et al*., 1992; Adhikari and Kraus *et al*., 1994; Weyerstahl *et al.*, 1998; Padalia *et al*., 2009; Padalia *et al*., 2010 and Vasanthi and Gopalakrishnan, 2013
Root	Phenols, coumaric acid, propanol, 7-hydroxy-8, 9-dehydrothymol 9-O-trans-ferulate, 7-hydroxythymol 9-O-trans-ferulate , 7, 8-dihydroxythymol 9-O-trans-ferulate, 7, 8-dihydroxy thymol 9-O-cis-ferulate, methyl (7R)-3-deoxy-4,5-epoxy-D-manno-2-octulosonate 8-O-trans-p-coumarate, methyl (7R)- 3- deoxy-4,5-epoxy-D-manno-2-octulosonate 8-O-cis-p-coumarate, and 3-(2-hydroxyphenyl) propyl methyl malonate	Zhong *et al.*, 2013

Pharmacognostical studies

Activities	**Plant part**	**Reference**
Antipyretic activity	Leaves	Mandal *et al*., 2005
Analgesic activity		
Anticancer activity	Leaves	Adebayo *et al.*, 2010
Anticoccidal activity	Leaves	Yang *et al.*, 2012
Anti inflammatory activity	Leaves	Abena *et al.*, 1993; Moura *et al.*, 2005 and Ashim *et al*., 2011

Anti-acetylcholinestrase activity	Flowers	Vasanthi and Gopalakrishnan, 2013
Antimicrobial activity	Leaves	Ekundayo *et al.*, 1988; Moody *et al.*, 2004; Akinyemi *et al.*,2005; Sasikumar *et al.*, 2005; Chah *et al.*, 2006; Nabin and Geetha, 2009; Kurade *et al.*, 2010; Aditi *et al.*, 2013; Harish Kumar *et al.*, 2014 and Desingh *et al.*, 2014
Antioxidant activity	Leaves	Adebayo *et al.*, 2010; Damodar*et al.*, 2012; Samuel *et al.*, 2013; Vasanthi and Gopalakrishnan, 2013; Shekhar and Anju, 2014; Iyeswarya *et al.*, 2013 and Ralte and lallianrawna, 2014
Therapeutic activity	Leaves	Dadhich and Kanna, 2008; Shaba *et al.*, 2012 and Nonga *et al.*, 2013
Wound healing	Leaves	Durodola, 1977; Almagboul *et al.*, 1985; Oladejo *et al.*, 2003; Mustafa *et al.*, 2005; Chah *et al.*, 2006; Dash and Murthy, 2011; Harish kumar *et al.*, 2014 and Manimaran *et al.*, 2014
Antiproliferative activity	Leaves	Iyeswarya *et al.*, 2013

***Eupatorium odoratum* L.**

Synonym

Chromolaena odorata

Distribution

It is a native of Central and South America which has spread widely in central and western Africa, tropical America, West India, Southeast Asia and western part of Nigeria (Akinmoladun andAkinloye 2007; Ling *et al*., 2007).

Botanical description

It is a perennial shrub that forms dense tangled bushes 1.5-2 m in height; Stem-brittle, pubescent; Root- fibrous root system; Leaves- opposite, acute, 3-nerved, base obtuse or sub truncate; Petiole- long, Capitulate, axillary and terminal clusters; Peduncles- 1-3cm long, bracteate; bracts slender, 10-12mm long; involucres of about 4-5 series of bracts, pale with green nerves; Flower heads are borne in terminal corymbs, flowers are white; Florets about 20-30 or a few more, 10-12mm long; ovary- 4mm long; corolla slender trumpet form; pappus of dull white hairs 5mm long; Achenes glabrous; Seeds are small.

Medicinal uses

The leaves of *E.odoratum* contains antisplasmodic, antiprotozoal, antitrypanosomal, antibacterial, antiviral, anticancer and antihypertensive activities. It has also been reported to possess haemostatic (Akah, 1990), anti-inflammatory, astringent, diuretic and hepatoprotective activity (Alisi *et al*., 2011), anticholesterolemic (Ikewuchi and Ikewuchi, 2011) and hypotensive activity (Watt and Brandwijk, 1962; Feng *et al*, 1964; Weniger and Robinean, 1988; Iwu, 1993; Gonzalez *et al*., 2011 and Ikewuchi *et al*., 2012).

The leaves of *E. odoratum* are used to treat piles (Egunjobi, 1969), malaria (Pisuthanan *et al*., 2005), diarrhea, diabetes (Odugbemi, 2006; Akinmoladun and Akinloye, 2007), burns, wounds and skin infections (Phan *et al*., 2000; Phan *et al*., 2001; Panda and Ghosh, 2010), and inflammation (Habtemariam, 2001; Owoyele *et al*., 2005; Ayyanar and Ignacimuthu, 2009 and Hanh *et al*., 2011),

Traditionally, fresh leaves or decoction have been used for the treatment of leech bite. In the southern part of Nigeria, the leaves are used for wound dressing, skin infection and to stop bleeding (Metwally and Ekejuba, 1981). A decoction of flowers is used as tonic, antipyretic and heart tonic (Bunyapraphatsara and Chokechaijaroenporn, 2000).

Phytochemical studies

Plant Name	Plant part	Phytochemicals	Reference
Eupatorium odoratum	Leaves	Alkaloid, tannins, glycosides, flavonoids, phenols, Terpenoids, Tannin, Saponin, Phlobatannin and Cardiac glycoside	Phan *et al.*, 2001; Afolabi *et al.*, 2007; Debashisha *et al.*, 2010; Srinivasa *et al.*, 2010; Anyasor *et al.*, 2011; Hung *et al.*, 2011; Nayak *et al.*, 2012 and Ikewuchi *et al.*, 2013
	Flower	Tannin, Phytosterols, Triterpinoids, Flavanoids, Coumarins, Quinones, Cardiac Glycosides, Terpenoids, Steroids, Acids, and Phenols	Munmi *et al.*, 2013; Muricken and Joy, 2015

Pharmacognostical studies

Activities	Plant part	Reference
Antiviral activity	Leaves	Irobi, 1997
Immunostimulant activity	Leaves	Lovkov *et al.*, 1999
Antipyretic activity	Leaves	Taiwo *et al.*, 2000
Anti-inflammatory	Leaves	Umukoro and Ashorobi, 2006; Lovkov *et al.*, 1999; Taiwo *et al.*, 2000; Phan *et al.*, 2001; Owoyele *et al.*, 2005 and Akinmoladun and Akinloye 2007
Antiplasmodic activity	Leaves	Taiwo *et al.*, 2000; Phan *et al.*, 2001 and Akinmoladun and Akinloye 2007

Antiprotozoal activity	Leaves	Phan *et al.*, 2001 and Akinmoladun and Akinloye 2007
Analgesic activity	Leaves	Jena and chakraborty, 2010
Anthelmintic activity		Debashisha *et al.*, 2010; Patel *et al.*, 2010 and Velliangiri *et al.*, 2011
Wound healing activity	Leaves	Debashisha *et al.*, 2010; Prabhudutta and Arpita, 2010 and Anyasor *et al.*, 2011
Antioxidant activity	Leaves	Lovkov *et al.*, 1999; Afolabi *et al.*, 2007; Akinmoladun and Akinloye 2007; Alisi *et al.*, 2011; Amatya and Tuladhar, 2011; Anyasor *et al.*, 2011; Venkataraman *et al.*, 2012 and Suresh *et al.*, 2015
Antimicrobial activity	Leaves Flowers	Inya agha *et al.*, 1987; Lovkov *et al.*, 1999; Bounda *et al.*, 2001; Phan *et al.*, 2001; Akinmoladun *et al.*, 2007; Cui *et al.*, 2009; Anyasor *et al.*, 2011; Doss *et al.*, 2011; Nayak *et al.*, 2012; Venkataraman *et al.*, 2012; Menonve *et al.*, 2013; Munmi *et al.*, 2013 and Muricken and Joy, 2015

***Eupatorium triplinerve* Vahl**

Synonym

Ayapana triplinervis

Botanical description

It is a smooth, perennial herb, 30 to 60 cm in height, semi-woody at the base. Leaves are aromatic, smooth, simple, opposite, sub-sessile, tri-nerved, acuminate, glabrous, and lanceolate; Stem reddish brown; Flowers- Headed corymb, numerous, 6 to 13 mm long, bearing about 20 pink flowers, 6 to 7 mm long. Fruit are achenes, narrowly oblong, 5 angled, and about 2 mm long.

Distribution

It is distributed in Tropical regions. It is mainly found in America and Mexico and introduced in many parts of the world.

Medicinal uses

E. triplinerve is widely used in folk medicine as analgesic, anticoagulant, antiparasitic, anthelmintic and sedative. It helps in treating ulcers, haemorrhages, anxiolytic and antidepressive (Yadava and Saini, 1990; Bose *et al*., 2007). Besides, it also used to cure fever with convulsions, burning sensations, indigestion, pneumonia, cough, mucus and tingling sensations within the body (Hossan *et al*., 2009).

Leaves are used as stimulant, tonic in small doses and laxative when taken in large quantity. A hot infusion is used as emetic and diaphoretic. Decoction of the leaves were used as an antiseptic and haemostatic; useful against haemorrhage and to clean foul ulcers. An aqueous extract of the dried leaves is a cardiac stimulant. Fresh leaves are used to cure wounds and stomach ache (Yusuf *et al*., 2009 and Melo *et al*., 2013).

Phytochemical studies

Plant part	**Phytochemicals**	**Reference**
Leaves	Alkaloid, Flavonoids, Saponin, Tannin, Quinon, Steroid, Triterpenoid, Coumarin, Volatile Oil, Carbohydrate, Protein, Amino acid, Glycosides, Phenolic compound, alkanes, carboxylic aldehydes, ketones, aromatic esters, Thymohydroquinone dimethyl ether Caryophyllene, coumarin, volatile oil, Essential oil, ayapanin and ayapin	Chaturvedi and Mulchandani, 1989; Garg and Nakhare, 1993; Mala *et al.*, 1999; Anne and Claude, 2009; Jaripa *et al.*, 2010; Christy and Anusha, 2012; Sugumar *et al.*, 2014; Shirly and Dwi, 2011 and Sugumar and Karthikeyan, 2015
Flower	Borneol, isoeugenol, thymohydroquinone	Garg and Nakhare, 1993

Pharmacognostical studies

Activities	Plant part	Reference
Analgesic Antiparasitic Anticoagulant, CNS Depressant Antiulcerous and Antitussive	Leaves	Leslie, 2006; Mathew *et al.*, 2016
Heptoprotective activity	Leaves	Bose *et al.*, 2007
Antioxidant activity	Leaves	Bose *et al.*, 2007; Anirban *et al.*, 2012; Melo *et al.*, 2013 and Madhubanti *et al.*, 2015
Anthelminitic	Leaves	Leslie, 2006; Subash *et al.*, 2012
Antinociceptive activity	Leaves	Cheriyan *et al.*, 2009 and Parimala *et al.*, 2012
Anti-inflammatory activity	Leaves	Parimala *et al.*, 2012; Binoy *et al.*, 2013 and Sukanlaya *et al.*, 2015
Antimalarial activity	Leaves	Vidushi, 2013
Antimicrobial activity	Leaves	Gupta *et al.*, 2002; Gupta *et al.*, 2004; Sasikumar *et al.*, 2005; Shafiqur and Mohammad, 2008; Jaripa *et al.*, 2010 and Tamyris *et al.*, 2014
Anticancer activity	Leaves	Madhubanti *et al.*, 2015

GCMS analysis

The aqueous and methanol extract of whole plant of *E. odoratum* confirmed 7 and 37 compounds respectively. The major compounds were 2, 4, 6-tris-(1-phenylethyl)-phenol, Neophytadiene, 4-Acetyl-3-Hydroxy-2, 6-Dimethoxy toluene, Methyl commate and (3S)-7-O-Methoxy methyl vestitol (Venkatraman *et al*., 2012).

The GC MS analysis of whole plant of *E. triplinerve* showed eleven differnt compounds namely 2-hydroxy-1,3-propanediyl ester (19.18%), Octadecanoic acid (19.18%), hexadecanoic acid (14.65%), 2,6,10-trimethyl,14-

ethylene-14-pentadecne (9.84%), 2-hydroxy-3-[(9E) -9-octadecenoyloxy] propyl(9E)-9-octadecenoate (8.79%), 1-undecanol (7.82%), 1,14-tetradecanediol (6.78%), Decanoic acid, 8-methyl-, methyl ester (3.86%), Bicyclo [4.1.0] heptane, 7-butyl- (2.38%) and 1-hexyl-1-nitrocyclohexane (2.09%) (Christy and Anusha, 2012).

The flowers of *E. glandulosum* revealed the presence of twenty five compounds contains 91.3% of oils. The essential oil was dominated by sesquiterpene hydrocarbons (36.5) and oxygenated sesquiterpenoids (45.4%). The major compounds are copaen (19.72%), α-bisabolol (9.8%), azulenone (9.5%) and 4, 4- dimethyl-3- (3- methylbut- 3- enylidine) - 2- methylene bicycle (4.1.0) and heptanes (8. 9%) (Vasanthi and Gopalakrishnan, 2013).

HPTLC Analysis

The High performance thin layer chromatography (HPTLC) has become one of the most important tools for the qualitative and quantitative determination of active substances (Attimarad *et al*., 2011). By the fingerprint approach, it is possible to obtain a proper identification of the plant material. It is emerging in the field of pharmaceutical industries, molecular biology, human genetics, clinical chemistry, forensic chemistry, biochemistry, cosmetology, food and drug analysis and environmental analysis. (Morlock *et al*., 2006). Many such reports were available for the evidence of utilization of HPTLC in fingerprinting analysis of drugs of natural origin, and hence, the increasing acceptance of natural products is well suited to provide the core scaffolds for future drugs (Chakraborthy, 2009; Hong *et al*., 2009; Puranik *et al*., 2010; Patel *et al*., 2010).

Table 1

List of plants studied using HPTLC fingerprinting techniques

S.No	Plant Name	Part Used	Compound	References
1	*Coleus forskohlii*	Callus	forskolin	Malathy and Pap, 1999
2	*Podophyllum hexandrum*	Callus	Podophyllotoxin	Ahmad *et al*., 2007
3	*Plumbago indica*	Root	Plumbagin	Unnikrishnan *et al*., 2008
4	*Plumbago zeylanica*			
5	*Nymphaea stellata*	Flower	Quercetin	Rakesh *et al*., 2009
6	*Eruca sativa*	leaf	Gallic Acid, Rutin, Quercetin	Sajeeth *et al*., 2010
7	*Nothapodytes foetida*	Callus	Camptothecin	Ajay *et al*., 2010
8	*Rauwolfia serpentina*	Root	Reserpine	Panwar and Guru, 2011
9	*Azadirachta indica*	leaf	Quercetin-3-O-β-D-Glucoside	Aarti *et al*., 2012; Pratima *et al*., 2014
10	*Pluchea lanceolata*	Callus	Daidzein	Kavi *et al*., 2012
11	*Boerhavia diffusa*	Whole plant	Boeravinone and β-Sitosterol	Aldon *et al*., 2013
12	*Charybdis congesta*	Callus	Scillarin, Scilliroside	Shiva *et al*., 2013
13	*Gymnema sylvestre*	Callus	Gymnemic Acid	Bakrudeen *et al*., 2013
14	*Lagenaria siceraria*	Fruit	Quercetin	Sharada *et al*., 2013
15	*Sesbania sesban*	Stem	Quercetin	Mythili and Ravindhran, 2013
16	*Tylophora indica*	Callus	*Kaempferol*	Pratibha and Abhay, 2013
17	*Artemisia annua*	Whole plant	Artemisinin	Widmer *et al*., 2014
18	*Clausena excavate*	Seeds	Coumarin	Adlis *et al*., 2014

19	*Ocimum sanctum*	Leaves	Eugenol	Nargis and Sharique, 2014
20	*Nyctanthes arbortristis*	Leaf	α-sitosterol, α -amyrin, caffeic acid	Kayalvizhi and Richa Shri, 2014
21	*Toddalia asiatica*	Callus and plant	Nitidine	Chinthala and Ciddi, 2014
22	*Ocimum basilicum*	Aerial parts	Gallic acid, ferrulic acid, quercetin and rutin	Asha *et al*., 2015
23	*Mentha arvensis*			
24	*Hyptis suaveolens*			
25	*Coleus aromaticus*			
26	*Catharanthus roseus*	Callus	Vincristine	Barkat *et al*., 2015
27	*Pterocarpus marsupium*	Callus and plant	Pterostilbene	Patel, 2015
28	*Crataeva nurvala*	Leaves	Quercetin	Dhar *et al*., 2016
29	*Bryophyllum pinnatum*	Leaves	Quercetin	Anjoo and Ajay, 2017
		Stem	stigmasterol	
30	*Guiera senegalensis*	Leaves	Rutin, quercetin, naringenin, and gallic acid	Perwez *et al*., 2017
31	*Holarrhena antidysenterica*	Callus	Conessine	Mahato and Mehta, 2017
32	*Moringa oleifera*	Seeds	Diosgenin	Asha and Balasaheb, 2017
33	*Naringi crenulata*	Callus	Quercetin	Neelam *et al*., 2017
34	*Calamus rotang*	Leaves	Quercetin, rutin	Pallavi and Hemalatha, 2018

RAPD Analysis

The Random Amplified Polymorphic DNA (RAPD) technique is an effective method for analysing genetic variability. It is based on a modified PCR method and uses short oligonucleotide primers of arbitrary sequence to amplify unknown fragments of genomic DNA (Nybom, 2004). It is a reliable method for characterizing variation among species, within a species and among populations. RAPD profile construction has several advantages, such as rapidity of process, low cost and the use of small amounts of plant material.

Molecular markers have been successfully used to study the source of introduction and variability due to new environment. PCR based RAPD has been extensively used to study genetic structure of populations. Among various molecular markers, the RAPD technique is simple, rapid and requires only a few nano grams of DNA, has no requirement of prior information of the DNA sequence and has feasibility of automation with higher frequency of polymorphism, which makes it suitable for routine application for the analysis of genetic diversity (Young *et al*., 2001).

A variety of DNA markers are available that facilitate assessment of genetic variability in plants. The most common markers include Restriction Fragment Length Polymorphism (RFLP), numerous genetic marker assays based on PCR such as Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeats (SSR) and Amplified Fragment Length Polymorphism (AFLP). RAPD technique has been extensively used in plants and animals for evaluation of intra-specific and inter-specific genetic variation and determination of phylogenetic relationship. (Karp *et al*., 1997).

Martins *et al* (2003) analyzed the genetic diversity of *Prunus dulcis* cultivars using RAPD and ISSR markers to study their association with foreign cultivars. Six RAPD and five ISSR primers were used to find their reproducibility and polymorphism. They found that out of 124 PCR fragments. Among them 120 were polymorphic and all the plants could be discriminated and compose a heterozygous group.

The six different genera from Asteraceae family growing wild in Egypt such as *Achilliea fragrantisma, Achilliea santolina, Anacyclus monanthos,* *Artemisia arborescens, Artemisia judaica, Glebionis coronaria, Cotula barbata. Cotola cinerea* and *Matricaria aurea* has been analysed for their phylogenetic relationship using 26 RAPD primers. Among them 15 primers revealed polymorphism (Twab and Zahran, 2010).

RAPD markers are used to assess genetic variation in different population of *Betulaalnoides* (Jie *et al*., 2003), *Changium smyrnioides* (Chengxin *et al*., 2003), *Porphyra* species (Huh *et al*., 2006), *Musa* species (Pankaj *et al*., 2007), *Brassica* species (Sanchita *et al*., 2008), *Trichodesma indicum* (Neelambra *et al*., 2009), *Lycnophora ericoides* (Melo *et al*., 2009), *Cynachum* species (Moon *et al*., 2010), *Dalbergia sissoo* (Ginwal and Maurya, 2010), *Piper* species (Sandeep *et al*., 2010), *Coleus* species (Muthusamy *et al*., 2011), *Suaeda* species (Gurudeeban, 2011), Hibiscus species (Prasad, 2014), *Coleus* sp (Paul *et al*., 2015), *Hawthorn* species (Moghadam *et al*., 2016) and *Ocimum* species (Patel *et al*., 2015).

Hoshi *et al* (2013) analysed three Japanese Aster species such as *Aster ageratoides, Aster iinumae* and *Aster microcephalus* using various RAPD primers. *A. Ageratoides* and *A. Microcephalus* possess high number bands in common whereas less similarity was found in *A.iinumae*. The genetic diversity was studied in fifteen populations of three species of *Anthemis melampodina, Anthemis pseudocotula and Anthemis bornmuelleri* using different RAPD primers. A close genetic relation was observed between *A. melampodina* and *A. Pseudocotula* whereas *A. Bornmuelleri* showed wide variation compare to other two species (Hasan and Qari *et al*., 2017).

Suresha *et al* (2017) analyzed fourteen parental genotypes of *Helianthus annuus* for genetic diversity using 15 RAPD and 44 SSR primers. The genetic similarity index data of SSR and RAPD markers showed very wide range of variation among 14 sunflower genotypes.

Cancer

Cancer is the rapid and uncontrolled formation of abnormal cells, which may mass together to form a tumour cells proliferate throughout the body. Cancer cells usually attack and demolish the normal cells. These cells are born due to imbalance in the body. If the process is not arrested, it may develop and causes death. The ability of the body to control cell multiplicity is achieved by a network of overlapping molecular mechanisms which direct cell proliferation and death. Any alteration in this balance (birth and death of cells), has a potential, if uncorrected, to alter the number of cells in an organ or tissue. Such changes may result in cancer, a disease that is manifested in many forms depending primarily on the organ from which it evolves.

Globally, cancer is one of the leading causes of death. According to American Cancer Society (ACS) in 2009, an estimation of about 1,500,000 new cases and over 500,000 deaths are expected to be recorded in US. South Africa experiences one of the highest incidence rates of cancer in Africa. Every one in four males and six females have the potential of developing cancer. The current statistics by the National Cancer Registry of South Africa indicate that cancers of the bladder, colon, breast, cervix, lungs and melanoma are among the most common (Mqoqi *et al*., 2004).

Table 2

Types of Cancer affecting different parts of the body

S.No	Parts of the body	Type of cancer occur
1	Blood and Lymphatic Systems	Hodgkin’s disease, Leukaemia's Lymphomas, Multiple myeloma
2	Skin	Malignant Melanoma
3	Digestive System	Oesophageal cancer, Stomach cancer, Pancreas cancer, Liver cancer, Colon and Rectal cancer and Anal cancer

4	Urinary system	Kidney cancer, Bladder cancer, Testis cancer, Prostate cancer
5	Body parts affected in women	Breast cancer, Ovarian cancer, Gynecological cancer, Choriocarcinoma
6	Miscellaneous cancers	Brain cancer, Bone cancer, Characinoid cancer, Nasopharyngeal cancer, Retroperitoneal, Soft tissue cancer and Thyroid cancer

Cancer scenario in world

Cancer stands second after cardiovascular disorders in the list of diseases responsible for maximum deaths in the world (Jemal *et al*., 2011). In 2008, about seven million deaths were attributed to cancer alone with 20–26 million new cases (Nath *et al*., 2013). The estimated numbers of new cancer cases worldwide are about 1,685,210 in 2016 (American Cancer Society, 2016). Asia which consists 60% of the total global population bear the burden of about half of the world's cancer cases (Sankaranarayanan *et al*., 2014). About one third of the deaths due to cancer were reported in Asia pacific countries and the death rate was projected to increase to 16 million in 2025 in this region (Shin *et al*., 2012). More than 70% of cancer deaths occurred in low- and middle-income countries. Deaths due to cancer are projected to continuously increase and it has been estimated that there will be 11.5 million deaths in the year 2030 (Russo *et al*., 2006) and 27 million new cancer cases and 17.5 million cancer deaths are projected to occur in the world by 2050 (Douglas, 2015).

Cancer scenario in India

The International Agency for Research on Cancer assessed that 8% of cancer deaths were recorded worldwide and 6% in India (Ferlay *et al*., 2013). It is a major health problem worldwide due to lack of early detection methods (Chanda and Nagani, 2013). According to WHO, India has a cancer mortality rate of 79 per 100,000 deaths and accounts for over 6 % of total deaths (Takiar *et al.*, 2010). The Indian population has been mounting over the last few decades. The approximate cases of cancer deaths was recorded as 0.44 million, 0.51 million, during the year 2011 and 2016 respectively. The estimated number of cancer mortality would increase to 0.70 million by the year 2026. Mortality of cancer in males is higher when compared to females (Souza *et al.*, 2013**).**

Colon Cancer

Colon cancer is a world-wide health problem affecting both men and women. Researchers throughout the world focused mostly on breast and skin cancer. It is the fourth most common cancer in the world with 1.3 million new cases each year. The incidence and mortality rates for colon cancer have been increasing in most of the countries, particularly US, European and part of Asian countries.

The number of deaths estimated for colorectal cancer was 693,333 in 2012. When looking at India and USA, the incidence, mortality, and prevalence rates are all consistently higher in USA while the incidence is higher in males in both the countries. In USA, it was the fourth most common cancer and in India, it is the fifth most common cancer (Ferlay *et al.*, 2013; Bray *et al.*, 2013).

The modern diet and lifestyles, with high meat consumption and excessive alcohol use, along with limited physical activity has led to an increasing mortality rate for colon cancer worldwide. As a result, there is a need to develop novel and environmentally benign drug therapies for colon cancer (Palaniselvam, *et al.*, 2014).

Importance of plants in curing cancer

Cancer is one of the most dangerous diseases in humans and presently there is an extensive scientific discovery of new anti cancer agents developed from natural products (Kasahara and Hemini, 1998). The U.S Natural Cancer Institute recognized the use of plants as anticancer agent in 1950.

Despite the major scientific and technological progress in the treatment and management of cancer, no reliable and definitive cure has been found. This has led to an increase in the dependence of patients on unconventional medical therapies. All over the world, especially in the developing countries the traditional use of plants in the treatment of ailments has been on the increase (Richardson *et al*., 1999).

Several drugs are available in the market to treat various types of cancer. But they are not completely effective and safe. The major problem in the cancer chemotherapy is the prolonged toxicity of the well-established chemical drugs. The plant derived products have been proved effective and safe in the treatment and management of various cancers to some extent. The anticancer drugs were extracted from many plants are found to be potential in curing cancer.

The secondary metabolites derived from plants have biological activities that can be assayed in the laboratory, providing a scientific rationale for the use of the particular plant. In this regard, it has been estimated that about a quarter of all modern drugs were originally derived from plant sources (Kinghorn and Balandrin, 1993; Aung *et al*., 2017).

A recent survey shows that more than 60% of cancer patients use vitamins or herbs as therapy (Madhuri and Pandey, 2008). Globally, the incidence of use of plant-derived products for cancer treatment is 10% to 40%, reaching 50% in Asiatic patients (Tascilar *et al*., 2006; Molassiotis *et al*., 2006). Studies on natural products for cancer prevention had resulted in availability of about 3000 anti-cancer drugs. Most of the pharmaceutical industry of modern era often relies on plants as a source of raw material and essential ingredients of medicine (Sadia *et al*., 2013). Researchers are trying to identify, characterize and provide solid scientific basis for plant based drugs to be used in cancer treatment (Hafidh *et al*., 2013). Several plant-based anticancer agents including taxol, vinblastine, vincristine, camptothecin derivatives, topotecan, irinotecan, and epipodophyllotoxins are in clinical use all over the world (Shruti and Archana, 2015).

Plants from Asteraceae family for cancer treatment

Asteraceae is the largest family of dicotyledons, comprising 950 genera and 20,000 species, of which 697 species occur in India. It is regarded as the most advanced, highly evolved and is considered to occupy the highest position in the plant kingdom and first largest terrestrial plant family.

According to earlier reports, 78 families were documented to fight against different types of cancer. Among them 17 plants from asteraceae, 15 from euphorbiaceae, 12 from apocynaceae and 11 from fabaceae were the most predominant families. Plants from Asteraceae (35%) and Euphorbiaceae (33%) families were used in Kenya and India for the treatment of cancer. The plants from 35 families were used in the *in vitro* studies for anticancer activity. Among them majority of the plants used in the study were from Fabaceae, Asteraceae and Anacardiaceae. Thirty two plants belonging to 23 families were tested in cancer induced animal model for their cancer activity. From the study it was confirmed that leaves are the most active part of most of the plants used, possess anticancer activity (Akash *et al*., 2017). Flavonoids, terpenoids, tannins and saponins were the most frequently reported secondary metabolites in Asteraceae family could be the possible reason for high efficacy of the plants from Asteraceae (Carvalho *et al*., 2013).

Table 3

List of Plants evaluated to treat various types of cancers

S.No	Plant Name	Family	Part used	Type of Cancer	References
1	*Daphne mezereum*	Thymelaeaceae	Leaves	Lymphocytic leukemia	Kupchan and Baxter, 1975
2	*Pfaffia paniculata*	Amaranthaceae	Root	Breast Melanoma	Takemoto *et al*., 1983
3	*Gossypium hirsutum*	Malvaceae	Seed	Centrl nervous system	Coyle *et al*., 1994
4	*Centella asiatica*	Apiaceae	Leaves	Lung	Babu *et al*., 1995
5	*Zingiber officinale*	Zingiberaceae	Rhizome	Skin	Katiyar *et al*., 1996
6	*Camellia sinensis*	Theaceae	Leaves	Prostate	Taylor and Wilt, 1999
7	*Momordica charantia*	Cucurbitaceae	Fruit	Breast	
8	*Salvia miltiorrhiza*	Lamiaceae	Leaves	Breast	Yoon *et al*., 1999
9	*Terminalia chebula*	Combretaceae	Fruits	Breast, Prostate	Saleem *et al*., 2002
10	*Curcuma longa*	Zinziberaceae	Rhizome	Stomach	Agarwal *et al*., 2003
11	*Allium sativum*	Amaryllidaceae	Bulb	Kidney, Stomach	Thomson and Ali, 2003
12	*Annona Muricata*	Annonaceae	Leaves	Lung, breast, Pancreatic, Prostatic	Muriel, 2004
13	*Scutellaria barbata*	Lamiaceae	Leaves	Lung	Yin *et al*., 2004
14	*Calotropis procera*	Ascleipiadaceae	Leaves	Hepatoma, Non hepatoma	Choedan *et al*., 2006
15	*Tinospora cordifolia*	Menispermaceae	Leaves	Ehrlich ascites carcinoma	Jagetia and Rao, 2006
16	*Aspidosperma tomentosum*	Apocynaceae	Aerial part	Breast	Kohn *et al*., 2006

17	*Citrus maxima*	Rutaceae	Fruit	Lung	Lim *et al*., 2006
18	*Oroxylum indicum*	Bignoniaceae	Leaves	Breast	Narisa *et al*., 2006
19	*Cedrus deodara*	Pinaceae	Leaves	Leukemia	Shashi *et al*., 2006
20	*Cirsium japonicum*	Asteraceae	Leaves	Stomach	Liu *et al*., 2007
21	*Cynodon dactylon*	Poaceae	Leaves	Laryngeal	Sultana and Lee, 2007
22	*Annona glabra*	Annonaceae	Leaves	Leukemia	Cochrane *et al*., 2008
23	*Ardisia crenata*	Myrsinaceae	Leaves	Liver	Li *et al*., 2008
24	*Gynostemma pentaphylum*	Cucurbitaceae	Leaves	Lung	Lu *et al*., 2008
25	*Blumea babamitera*	Rutaceae	Leaves	Breast	Norikura *et al*., 2008
26	*Actaea racemosa*	Ranunculaceae	Leaves	Liver	Einbonda *et al*., 2009
27	*Gymnema sylvestre*	Asclepiadaceae	Leaves	Breast	Khanna and Kannabiran, 2009
28	*Rheum officinale*	Polygonaceae	Rhizome	Lung adeno carcinoma, Breast	Li *et al*., 2009
29	*Acorus calamus*	Araceae	Essential oil	Lung, Cervical	Rajkumar *et al*., 2009
30	*Trailliaedoxa gracilis*	Rubiaceae	Whole plant	Small intestine	Bernhards *et al*., 2010
31	*Anemopsis californica*	Saururaceae	Leaves, root and stem	Breast	Catherine *et al*., 2010
32	*Biophytum sensitivum*	Oxalidaceae	Leaves	Lung	Guruvayoorappan and Kuttan, 2007; Bhaskar and Rajalakshmi, 2010
33	*Artocarpus obtusus*	Moraceae	Leaves	Leukemia, Breast cancer	Zeraga *et al*., 2010
34	*Cichorium intybus*	Asteraceae,	Seeds	Prostrate, Breast	Nawab *et al*. 2011
35	*Artemisia vulgaris*	Rubiaceae	Flower		

36	*Smilax glabra*	Smilacaceae	Rhizome	Prostrate, Breast	Nawab *et al*. 2011
37	*Swertia chirayta*	Gentianaceae	Whole plant		
38	*Solanum nigrum*	Solanaceae	Berries	Prostrate, Breast and Cervical	Sanjay *et al*., 2009; Nawab *et al*. 2011
39	*Amoora rohituka*	Meliaceae	Stem bark	Breast	Rabi *et al*., 2002; Chan *et al*., 2011
40	*Bacopa monnieri*	Scrophulariaceae	Leaves	Ehrlich Ascites Carcinoma	Ghosh *et al*., 2011
41	*Abelmoschus moschatus*	Malvaceae	Seed	Lung	Gul *et al*., 2011
42	*Argemone mexicana*	Papavaraceae	Leaves	Cervical Breast cancer	Kiranmayi *et al*., 2011
43	*Podophyllum emodii*	Berberidaceae	Leaves	Lymphomas, bronchial, testicular	Prema *et al*., 2011
44	*Linum usitatissimum*	Linaceae	Leaves	Breast	Sakarkar and Deshmukh, 2011
45	*Blumea balsamifera*	Asteraceae	Leaves	Lung	Saewan *et al*., 2011
46	*Glochidion zeylanicum*	Euphorbiaceae	Roots Root	Liver Prostrate	Reiko*et al*., 2004; Sharma *et al*., 2011
47	*Phyllanthus emblica*	Phyllanthaceae	Fruit	Lung	Sawhney *et al*., 2011
48	*Cola nitida*	Sterculiaceae	Nut	Breast	Susi *et al*., 2011
49	*Nelumbo nucifera*	Nelumbonaceae	Leaves	Breast	Yang *et al*., 2011
50	*Calea pinnatifida*	Asteraceae	Aerial plant	Kidney	Gabriela *et al*., 2012
51	*Alangium salviifolium*	Alangiaceae	Seeds, flowers, roots and leaves	Blood lymphocyte	Ronok *et al*., 2011; Laizuman *et al*., 2012
52	*Boerhaavia diffusa*	Boraginaceae	Roots Leaves	Breast	Manu and Kuttan, 2007; Ahmed*et al*., 2007; Merina *et al*., 2012

53	*Emblica officinalis*	Euphorbiaceae	Fruit	Lymphoma, melanoma	Merina *et al*., 2012
54	*Catharanthus roseus*	Apocynaceae	Flower	Lymphoma, Leukemia, Breast and lung	Michael *et al*., 2012
55	*Erthrophleum suaveolens*	Caesalpiniaceae	Leaves	Prostrate Breast	Fadeyi *et al*., 2013
56	*Berberi saristata*	Berberidaceae	Root	Prostrate, Ovary, Breast and Lung	Gaidhani *et al*., 2013
57	*Withania somnifera*	Solanaceae	Rhizome		
58	*Barleria grandiflora*	Acanthacae	Leaves	Lung	Nishant*et al*., 2014
59	*Moringa oleifera*	Moringaceae	Leaf	Breast	Nair and Varalakshmi 2011; Charoensin, 2014
60	*Artemisia indica*	Asteraceae	Leaves	Breast Prostrate	Bipranash *et al*., 2015
61	*Eupatorium odoratum*				
62	*Maesa macrophylla*	Primulaceae			
63	*Phlogacanthus thyrsiformis*	Acanthaceae			
64	*Momordica dioica*	Cucurbitaceae	Fruit	Breast, lung	Gayathri *et al*., 2016
65	*Cucurbita maxima*	Cucurbitaceae		Liver	Murganatham *et al*., 2016
66	*Saxifraga stolonifera*	Saxifragaceae	Leaf	Tumor cell line	Nagata *et al*., 2016
67	*Plumbago zeylanica*	Plumbaginaceae	Roots	Prostate	Roy *et al*., 2017

Table 4

List of Plants evaluated scientifically to treat colon cancer

S.No	Plant Name	Family	Part used	References
1	*Helianthella quinquenervis*	Asteraceae	Root	Castaneda *et al*., 1996
2	*Adenophyllum aurantium*	Asteraceae	Leaves	Frei *et al*., 1998
3	*Begonia heracleifolia*	Begoniaceae	Rhizome	
4	*Epaltes mexicana*	Asteraceae	Leaves	
5	*Tradescantia zebrina*	Commelinaceae	Aerial part	
6	*Schkuhria schkuhrioides*	Asteraceae	Aerial part	Delgado *et al*., 1998
7	*Camellia sinensis*	Theaceae	Leaves	Taylor and Wilt, 1999
8	*Amoora rohituka*	Meliaceae	Leaves	Mans *et al*., 2000
9	*Amoora rohituka*	Meliaceae	Leaves	
10	*Dysoxylum binectariferum*	Meliaceae	Leaves	
11	*Allium sativum*	Amaryllidaceae	Fruit	Thomson and Ali, 2003
12	*Aronia melanocarpa*	Rosaceae	Leaves	Malik *et al*., 2003
13	*Pentalinon andrieuxii*	Apocynaceae	Root, leaves	Chan *et al*., 2003
14	*Annona Muricata*	Annonaceae	Leaves	Muriel, 2004
15	*Curcuma zedoaria*	Zingiberaceae	Whole plant	Seo *et al*., 2005
16	*Camptotheca acuminate*	Nyssaceae	Leaves	Fuchs *et al*., 2006
17	*Centaurea montana*	Asteraceae	*Seeds*	Shoeb, 2006
18	*Centaurea schischkinii*			
19	*Matricaria chamomilla*	Asteraceae	Whole plant	Srivastava and Gupta, 2007
20	*Citrus aurantifolia*	Rutaceae	Fruit	Patil *et al.*, 2008
21	*Platycodon grandiflorum*	Campanulaceae	Leaves	Lee *et al*., 2008
22	*Taraxacum officinale*	Asteraceae	Leaves	Sigstedt *et al*., 2008

23	*Cinnamomum zeylanicum*	Lauraceae	Bark	Singh *et al.*, 2009
24	*Citrus limon*	Rutaceae	Fruits	Hirata *et al.*, 2009
25	*Pyrus malus*	Rosaceae	Bark, fruit	Madhuri and Pandey, 2009
26	*Tradescantia discolor*	Commelinaceae	Leaves	Mena *et al.*, 2009
27	*Anemopsis californica*	Saururaceae	Leaves, root, stem	Catherine *et al.*, 2010
28	*Aesculus hippocastanum*	Sapindaceae	Leaves	Zhang *et al.*, 2010
29	*Berberis vulgaris*	Berberidaceae	Roots Stem bark	Bono *et al.*, 2010
30	*Curcuma longa*	Zingiberaceae	Rhizome	Park and Conteas, 2010
31	*Plumbago zeylanica*	Plumbaginaceae	Leaves	Checker *et al.*, 2010
32	*Abelmoschus moschatus*	Malvaceae	Seed	Gul *et al.*, 2011
33	*Artemisia Vulgaris*	Asteraceae	Flower	Nawab *et al.* 2011
34	*Cichorium intybus*		Seeds	
35	*Smilax glabra*	Smilacaceae	Rhizome	
36	*Solanum nigrum*	Solanaceae	Berries	
37	*Swertia chirayta*	Gentianaceae	Whole plant	
38	*Coccinia grandis*	Cucurbitaceae	Leaves	Satheesh and Murugan, 2011
39	*Croton macrobotrys*	Euphorbiaceae	Whole plant	Motta *et al.*, 2011
40	*Glochidion zeylanicum*	Euphorbiaceae	Roots	Reiko *et al.*, 2004 and Sharma *et al.*, 2011
41	*Indigofera linnaei*	Fabaceae	Leaves	Kumar *et al.*, 2011
42	*Solanum lycopersicum*	Solanaceae	Fruit	Hahm *et al.*, 2011
43	*Sylibum marianum*	Asteraceae	Leaves, flowers	Ramasamy and Agarwal, 2008 and Colombo *et al.*, 2011
44	*Hedyotis diffusa*	Rubiaceae	Whole plant	Cai *et al.*, 2012
	Moringa oleifera	Moringaceae	Seed	Shaban *et al.*, 2012
45	*Liriodendron tulipifera*	Magnoliaceae	Stem, leaves and roots	Wang *et al.*, 2012
46	*Viscum album*	Santalaceae	Sprouts	Bhouri *et al.*, 2012
47	*Ophiorrhiza rugosa*	Rubiaceae	Leaves and roots	Raveendran *et al.*, 2012

48	*Momordica charantia*	Cucurbitaceae	Leaves, Roots	Weng *et al*., 2013
49	*Lavatera cashmeriana*	Malvaceae	Seeds	Rakashanda *et al*., 2013
50	*Picrorhiza kurroa*	Plantaginaceae	Rhizome	Gaidhani *et al*., 2013
51	*Punica granatum*	Lythraceae	Fruit	Syed *et al*., 2013
52	*Tabernaemontana divaricata*	Apocynaceae	Leaves	Bao *et al*., 2013
53	*Polygonum cuspidatum*	Polygonaceae	Whole plant	Ali *et al*., 2014
54	*Vitis vinifera*	Vitaceae	Fruit	Lim and Park, 2009 and Cheah *et al*., 2014
55	*Eupatorium cannabinum*	Asteraceae	Leaves	Varandas *et al*., 2014
56	*Colchicum autumnale*	Colchicaceae	Seeds	Lin *et al*., 2015
57	*Oldenlandia diffusa*	Rubiaceae	Stem Leaves, Fruit peel	Wozniak *et al*., 2015
58	*Panax ginseng*	Araliaceae	Roots, Leaves	Du *et al*., 2013 and Wang *et al*., 2015
59	*Passiflora caerulea*	Passifloraceae	Flower	Leon *et al*., 2015
60	*Combretum caffrum*	Combretaeae	Bark, kernal and fruit	Lauritano *et al*., 2016
61	*Ginkgo biloba*	Ginkgoaceae	Leaves	Xiong *et al*., 2016
62	*Glycine max*	Fabaceae	Seeds	Srikanth and Chen, 2016
63	*Nigella sativa*	Ranunculaceae	Seed	Tu *et al*., 2016
64	*Pisum sativum*	Fabaceae	Seeds	Runchana and Wanne, 2017
65	*Piper nigrum*	Piperaceae	Fruit	Prashant *et al*., 2017

Tissue culture

Micropropagation is a very advantageous technique for the conservation and amplification of rare or endangered medicinal plants (Debergh, 1992). It provides rapid, year-round production of new plants from minimal tissue samples. This makes the technique preferable for the production of medicinal plants. The literature survey revealed that the selected plants possess various medicinal properties but there is limited information are available regarding callus induction and micro propagation through tissue culture.

In vitro propagation of medicinal plants

In vitro mass multiplication methods were developed for some important medicinal plants like *Podophyllum hexandrum* (Nadeem *et al.*, 2000), *Pinellia ternata* (Satish *et al.*, 2002), *Ceropegia candelabrum* (Beena *et al.*, 2003), *Saussurea obvallata* (Joshi and Dhar., 2003), *Aloe vera* (Zhihua *et al.*, 2004), *Tylophora indica* (Mohammed *et al.*, 2007), *Panax ginseng* (Zhao, 2009), *Holostemma ada-kodien* and *Ipomoea mauritiana* (Geetha *et al.*, 2009), *Chlorophytum borivilianum* (Kumar *et al.*, 2010), *Ochradenus baccatus* (Fahad, 2013), *Iris sanguine* (Wang *et al.*, 2018), *Tylophora indica* (Najar *et al.*, 2018), *Vitex negundo* (Kumar *et al.*, 2018), *Ceropegia juncea* (Binish and Nayagi, 2019), *Dianthus caryophyllus* (Doad *et al.*, 2019) and *Malus domestica* (Jaime *et al*., 2019).

Shoot tip culture

Shoot tip and axillary buds having preformed meristems usually develop axillary shoots on a high cytokinin concentration. The multiplication rates through this technique vary with genotype and the cytokinin requirement has been extremely variable. Kaviani *et al* (2011) reported the micropropagation of *Matthiola incana using* shoot tips on MS medium supplemented with 2 mg/L of Kn.

Kharrazi *et al* (2011) presented an efficient protocol for the micro propagation of *Dianthus caryophyllus* using shoot tip culture. The optimum medium for micropropagation is MS medium with 2mg/l BAP and 0.2 mg/l. Multiple shoot regeneration was also reported in *Dendranthema grandiflora* from axillary buds cultured in modified MS medium with BA (0.1mg/l) and GA3 (0.5 mg/l) (Keresa *et al*., 2012). A high rate of multiple shoot proliferation was achieved in *Dianthus deltoids* using shoot tip and nodal cuttings cultured in MS medium containing 0.1 mg/l of BAP and 0.1 mg/L of NAA (Markovic *et al*., 2013).

Successful shoot tip culture have been developed in *Chlorophytum borivilianum* (Purohit *et al.,* 1994), *Zingiber zerumbet, Scrophularia yoshimurae* (Satish *et al.,* 2002), *Gentiana lutea* (Zeleznik *et al.,* 2002), *Rauwolfia serpentina* (Vandana *et al.,* 2003), *Mentha arvensis* (Chishti and Siddiqui, 2003), *Morinda citrifolia* (Gajakosh *et al.*, 2010), *Saussurea rebaudiana* (Das *et al.,* 2011), *Vinca rosea* (Rukhama *et al.,* 2013), *Plectranthus amboinicus* (Zuraida *et al*., 2015), *Plectranthus bourneae* (Rajaram *et al*., 2015), *Solanum tuberosum* (Hussaini *et al*., 2015), *Phoenix dactylifera* (Khayri and Naik, 2017), *Gerbera jamesonii* (Winarto and Yufdy, 2017), *Lycopersicon esculentum* (Banu *et al*., 2017), *Manihot esculenta* (Carvalho *et al*., 2017), *Achyranthes aspera* (Ishwarya *et al*., 2018), *Colocasia esculenta* (Acedo *et al*., 2018), *Gymnema sylvestre* (Isah, 2019) and *Mucuna pruriens* (Alam and Anis, 2019).

Stem culture

Stem segments are considered as one of the best suited explants for quick response under *in vitro* culture. Multiple shoots were regenerated from internodal explants of *Huernia hystrix* cultured on MS medium supplemented with 5.37 µM NAA and 22.19 µM BA (Amoo *et al*., 2009).

A protocol for micro propagation of *Hemidesmus indicus* using nodal segments have been developed on MS medium supplemented with 0.054 ppm of NAA and 1.5ppm of Kinetin (Patnaik and Debata, 1996). The clonal multiplication was reported in *Syzygium alternifolium* from nodal explants using BAP (4ppm) and NAA (0.5ppm) (Khan *et al*., 1998).

The nodal and shoot tip of *Chrysanthemum morifolium* showed 95% of response for the multiple shoot regeneration on MS medium supplemented with

1.0 mg/l BAP (Karim *et al*., 2002). The nodal segments of *Mucuna pruriens* showed highest efficiency of shoot induction in half strength MS medium supplemented with 5mg/l of BA and 0.5mg/l of NAA (Faisal *et al.,* 2006).

The physiological effect of different plant growth regulators on *in vitro* multiplication of *Cocculus hirsutus* was studied. Multiple shoots were induced from the nodal segments of stem in MS medium containing BAP or Kn alone or in combination. Maximum number of shoots (45 ± 0.69 shoots per explant) was observed on the medium containing BAP (0.5 mg/l) along with additives like adenine sulphate (50.0 mg/l) and glutamine (150 mg/l) (Meena *et al.*, 2012).

The plantlets were micropropagated using node and internodal explants in *Cedrela montana* (Basto *et al*., 2012), *Coccinia grandis* (Patel *et al*., 2015), *Plectranthus bourneae* (Rajaram *et al*., 2015), *Dendrobium jerdonianum* (Mary and Divakar, 2016), *Bambusa vulgaris* (Kaladhar *et al*., 2017), *Lycopersicon esculentum* (Banu *et al*., 2017), *Asparagus densiflorus* (Anna, 2017), *Eucalyptus species* (Trueman *et al*., 2018), *Psychotria ipecacuanha* (Silva, 2018) and *Magnolia sirindhorniae* (Cui *et al.*, 2019) under *in vitro* condition.

Leaf culture

Leaves of medicinal plants are rich in secondary metabolites. Therefore, they are used as explants in tissue culture to increase the secondary metabolites. *Withania somnifera* is a herb having numerous medicinal values, widely used in ayurvedic drug preparations. An efficient protocol for *in vitro* plant regeneration via direct adventitious shoot proliferation from leaf explants of Ashwaganda is developed. MS medium containing 1.5mg/l BAP and 1.5mg/l IAA was found to be the best medium for maximum *in vitro* response. An improved *in vitro* shoot bud elongation and rooting was achieved on MS medium fortified with 0.15mg/l GA3 and 5mg/l IBA respectively (Kumar *et al.*, 2011).

Naing *et al* (2014) developed a protocol for shoot induction directly from leaf segments of the *Chrysanthemum* in MS basal medium supplemented with 1 mg/l of BA + 2 mg/l.

Multiple shoots developed from leaf cultures are reported in *Syzygium alternifolium* (Khan *et al.,* 1999), *Kniphofia leucocephala* (Cartan and Staden, 2003), *Gentiana macrophylla* (Cao *et al.,* 2005), *Chlorophytum arundinaceum* (Lattoo *et al.,* 2006), *Saussurea involucrata* (Binguo *et al.,* 2007), *Catharanthus roseus* (Taha *et al.,* 2008), *Gentiana kurroo* (Fiuk and Rybczynski, 2008), *Abelmoschus esculentus* (Kabir *et al.,* 2008), *Satyrium nepalense* (Mahendran and Bai, 2009), *Oncidiun stramimeum* (Escobar *et al.,* 2008), *Cyrtopodium punctatum* (Dutra *et al.,* 2009), *Picrorhiza kurroa* (Arif, 2010), *Kelussia odoratissima* (Omid *et al*., 2013), *Dendrobium jerdonianum* (Mary and Divakar, 2016), *Gerbera jamesonii* (Winarto and Yufdy, 2017), *Solanum nigrum* (Zou *et al*., 2017), *Lysimachia davurica* (Zhang *et al*., 2017), *Fraxinus nigra* (Lee and Pijut, 2017) and *Malus domestica* (Jaime *et al*., 2019).

Callus culture

Callus is an undifferentiated mass of tissue which appears on explants within a few weeks of transfer to the growth medium with suitable hormones (Bhojwani and Razdan, 1996). *Baliospermum montanum* cultured in MS medium supplemented with 2.0 mg/l of 2, 4-D produced highest percentage of callus (Johnson *et al.,* 2010). The nodal explants of *Aegle marmelos* were cultured on MS medium with 1.5 mg/l of 2, 4-D induced maximum amount of callus (Abirami and Suresh Kumar, 2013).

Micropropagation through callus culture have been carried out in some medicinal plants such as *Eryngium foetidum* (Arockiasami and Ignacimuthu, 1998), *Euphorbia nivulia* (Sunandakumari *et al.,* 2005), *Tylophora indica* (Dennis and Boban, 2005), *Saussurea obvallata* (Dhar and Joshi, 2005), *Cassia angustifolia* (Agrawal and Sardar, 2006), *Bacopa monnieri* (Arun *et al.,* 2013), *Ceropegia pusilla* (Kalimuthu and Prabakaran, 2014), *Oryza sativa* (Din *et al*., 2016), *Chlorophytum borivilianum* (Nakasha *et al*., 2016), *Azadirachta indica* (Gehlot *et al*., 2017), *Brassica napus* (Naz *et al.*, 2018), *Saccharum officinarum* (Thorat *et al*., 2018), W*ithania somnifera* (Gaurav *et al.,* 2018) and *Solanum lycopersicum* (Saeed *et al*., 2019).

Micropropagation of rare medicinal plants

Micropropagation was achieved in rare and endangered medicinal plants such as *Escobaria missouriensis*, *Sclerocactus spinosior* and *Toumeyapa pyracantha* (Philip *et al.,* 1990), *Chlorophytum borivilianum* (Purohit *et al.,* 1994), *Saussurea lappa* (Sudhakar *et al.,* 1997), *Chlorophytum arundinaceum* (Lattoo *et al.,* 2006), *Vanasushava pedata* (Karuppusamy *et al.,* 2006), *Hydrastis canadensis* (He *et al.,* 2007), *Saussurea esthonica* (Agnese *et al.,* 2010), *Semecarpus kathalekanensis* (Hurakadle *et al.*, 2011), *Ceropegia thwaitesii* (Muthukrishnan *et al*., 2012), *Ceropegia elegans* (Krishnareddy and Pullaiah, 2012), *Acorus calamus, Lavandula officinalis, Coleus forskohlii, Elaeocarpus spharicus, Gentiana kurroo, Indigofera tinctoria, Jurinea mollis, Picrorrhiza kurroa, Pyrethrum cinerariaefolium, Psoralea corylifolia, Paris polyphylla, Rheum emodi, Saussurea lappa, Stevia rebaudiana, Salvia sclarea, Swertia cordata, Valeriana wallichii* (Verma *et al.*, 2012), *Dysophylla myosuroides* (Savithramma *et al*., 2012), *Bacopa monnieri* (Sharuti and Narender, 2013), *Ceropegia pusilla* (Kalimuthu and Prabakaran, 2014), *Tylophora indica, Thogalum sp*s (Pan *et al*., 2013), *Thymus broussonetii* (Nordine*et al*., 2014), *Swertia chirayita* (Vikas, 2014**)**, *Begonia homonyma* (Kumari *et al*., 2017) and *Bryonia laciniosa* (Vijayashalini *et al*., 2017).