2. REVIEW OF LITERATURE

2.0 Introduction

The study area Nilgiri Biosphere Nature Park (NBNP) is a nature reserve located in Western Ghats. The park is located at Thuvaipathy village, Anaikatti, 32 km from Coimbatore in Tamilnadu, India. It is an Initiative of the Coimbatore Zoological Park and Conservation Center (CZPCC) which aims to introduce people of all ages to the rich diversity in flora and fauna of the Nilgiri Biosphere Reserve (NBR). Collection of threatened plant species have been recognized by the National Botanical Reserve Institute (NBRI), Lucknow and the Botanic Gardens Conservation International (BGCI), London, all of whom carried out projects in collaboration with NBNP.

NBNP has an aquarium, butterfly garden, amphibian pond, apiary, amphitheater, forest zone, arboretum, zodiac garden, rockery, herbal garden, educational and recreational activity area, nature trails kid's play area, green house (nursery), resting hut and cafetaria. Visitors will be able to experience the various life forms of the region including rare medicinal plants, native vegetation, tiny indigenous life forms, zodiac trees etc. With its first phase covering acres of land. It serves as the migratory step over for butterflies from siruvani and velliangiri hills, an route to the forest of silent valley.

Over 12,000 students from 100+ educational institutions & corporate and 6000+ members of the general public visits the park annually. The park entertains educational institutions and corporate by conducting excursions or tours, encouraging them to make use of available facilities to conduct educational programmes of their own and introduce new members to the biodiversity of the park. The motive of the park is not to be to just take a leisurely day off, but explore and learn more about our ecosystem and the fragility of nature.

2.1 Medicinal plants

Medicinal plants are the important source of Indian traditional medicine such as Ayurveda, Siddha, Unani and Homeopathy etc. They occupied an major position for producing new drugs. There are many research is going on in search for new bioactive principle in medicinal plants (Ahmet *et al*., 2005; Ward, 2008). Medicinal plants have been used for centuries to treat various diseases (Nostro *et al*., 2000; Arokyaraj *et al*, 2008;

Govindarajan *et al*., 2008). Nowadays, medicinal plants have been gaining importance due to their significant properties and their different applicability in various field of research (Hussain *et al*., 2003; Okuda *et al*., 2005; Sharma *et al*.,2009).

Since ancient times, medicinal plants were used as deriving successful drugs and it is important for screening of novel lead compounds (Atanasov *et al*., 2015). They consists of secondary metabolites which is important for a variety of structural arrangement and properties of novel drugs (De Fatima *et al*., 2006). There is a growing interest to reveal the secret of ancient herbal remedies. For this reason, different strategies have been developed (Bibi *et al*., 2011).

Medicinal plants contain chemical substance that creates some physiological function on the human body. Bioactive compounds such as alkaloids, flavonoids, tannins, and phenolic compounds are mostly present in herbal plants. The therapeutic significance of medicinal plants contain complex mixture of phytochemicals. These phytochemicals are serve as effective therapeutic agents for treating ailments (Maregesi *et al*., 2008). Herbal plants have attributed immensely for primary health care system to human being and animals. Instead of using man made synthetic drugs, medicinal plant usage is very effective in treating various diseases (Sandhya *et al*., 2010).

According to the World Health Organization (WHO) over 80% of the Asian and African people were using the medicinal plants for their primary health care problems (Okwu D.E and Okwu M.E, 2004). Medicinal plants are clinically effective and safer alternatives compared to the allopathic antibiotic (Govindasamy and Srinivasan, 2012; Kaur and Mondal, 2014). Phytochemical and pharmacological screening have been analysed in very few plants. It is ascertained that 5000 species have been studied for their therapeutic uses (Payne *et al*., 1991).

2.2 Medicinal properties of Oleaceae

Oleaceae consist of 27 genera and 600 species (Johnson, 1957; Rohwer, 1996; Wallander and albert, 2000; Green, 2004). Taxa of Oleaceae divided into five tribes namely Fontanesieae, Forsythieae, Jasmineae, Myxopyreae and Oleae. Most of the Oleaceae species are fragrant (Wallander and Albert, 2000). Some of the Oleaceae species are used for ornamental purpose. Plant species belonging to the family Oleaceae possesses

traditional uses in curing various diseases (Koca *et al*., 2011). *Olea europaea*, also known as olive tree belongs to the family Oleaceae. It is used to treat sore throat, kidney problems, back ache and eye infection. It also has antimicrobial property. Leaf infusions were used as a gargle to relieve sore throat (Ross, 2005).

Jasminum is one of the genera of Oleaceae. It is a shrub, native to tropical and subtropical regions of Eurasia (Schmidt *et al*., 2002). It is mainly cultivated for fragrance of their flowers. *Jasminum grandiflorum* having various medicinal properties like antiseptic, anthelmintic, ulcerative stomatitis, leprosy, wounds and aromatherapy (Warrier *et al*., 2004). Leaves of *Jasminum multiflorum* possess significant antimicrobial activity (Abdoul-Latif *et al*., 2010; Ankita *et al*., 2014).

Nyctanthes arbor-tristis also known as Night Jasmine. The decoction of leaves were used by Ayurvedic physicians for the treatment of arthritis, malaria, intestinal worms and laxative (Kirtikar and Basu, 1935; Samhita, 1949; Lopra *et al*, 1958; Nadkarni, 1976). *Olea dioica* Roxb. is a tree, having ethnobotanical uses. Fruits were used for skin disease. Bark and fruit paste were used to treat rheumatism (Pullaiah, 2006). *Fraxinus* is the one of the genera of Oleaceae, commonly known as ash tree. It has various pharmacological uses such as anticancer, anti-inflammatory, antioxidant, antimicrobial and neuroprotective properties (Sarfraz *et al*., 2017).

2.3 Ethnobotanical uses of *C. mala-elengi*

Chionanthus mala-elengi (Dennst.) P.S. Green is an endemic tree species of Peninsular India Western Ghats belonging to Oleaceae family. Local name of the species is “kallidala” and “mala-elengi”. It is occasionally near threatened species. Based on the literature, it has been revealed that *C. mala-elengi* was used as an indigenous folk herbal medicine by the local physicians of Malabar area (Kerala). It’s flowering period is December to April. In India, it is distributed in evergreen forest of Karnataka, Kerala and Tamilnadu at an altitude between 150-800 meters (Pius *et al*., 2015; Narayanan *et al*., 2018). Leaves, bark and kernel of the fruits were used for giddiness, liver affection, epilepsy and brain related issues. The whole plant paste is used for wound healing (Manilal and Remesh, 2010; Deepa *et al*., 2016; Kumar *et al*., 2016).

2.4 Significance of Phytochemicals

Plants are important source of drugs. Medicinal plants are capable in producing phytochemicals such as alkaloids, flavonoids, terpenoids, carotenoids, vitamins, lignans, simple phenols, phenolic acids etc. These compounds contain various medicinal properties. Some aromatic herbs possesses antioxidant properties (Liu and Ng, 2000; Sacchetti *et al*., 2005; yu *et al*., 2015).

Alkaloids are mainly found in huge amount in plants. They made an effective physiological impact on human body. Quinine, morphine, ephedrine, nicotine and strychnine are the vital type of alkaloids (Starry, 1998).

Saponins are steroid, triterpenoid, glycosides which contain antitumor, antimutagenic activities and lower the risk of human cancer by preventing cancer cells from growing. It has an antioxidant activity which may lead to reduced risk of heart disease (Rao and Sung, 1995; Osagie and Eka, 1998; Prohp and Onoaybe, 2012).

Flavonoids are polyphenolic structured compound that is derived from plants through polypropanoid pathway. It has an antioxidant property which protect human from toxic effect of free radicals. It also have vasodilator property which is helpful in improving blood circulation in brain and curing Alzheimer disease (Sharma, 2006; Ghasemzadeh. A and Ghasemzadeh. N, 2011).

Photobalamines have a broad range of bioactivities such as wound healing, anti-inflammatory, analgesic and antioxidant properties. It is also present in *Psidium guajava*, *Momordica charantia* and *Prunus persica* (Okwu D.E and Okwu M.E, 2004; Ayinde *et al*., 2007).

Glycosides are phenol, alcohol or sulfur compound. They are attached by a sugar portion or moiety. Numerous plants store chemicals as inactive glycosides which can be activated by hydrolysis enzyme. They have many biological properties such as haemolytic, antimicrobial, antioxidant, molluscicide and ichthyocide (Polt, 1995; Kabera *et al*., 2014).

Steroids are a group of cholesterol derived from low molecular lipophilic compounds. They form diversity in their structure with numerous medicinal applications (Hitchcock *et al*., 1998; Pattenden *et al*., 2004; De Boeck *et al*., 2005; Marion *et al*., 2006; Sultan and Raza, 2015).

Terpenoids are the compounds present in diverse genera of plants. They have various pharmaceutical values. For example Tetracyclic triterpene obtained from Cucurbitaceae species has anticancer, hepatoprotective and anti-inflammatory activities (Jaeger and cuny, 2016).

Tannins contain rich polyphenolic compounds present in plants. Hydrolysable and condensed tannins have the ability to produce strong complexes with certain type of proteins depressing the rate of their digestion. It have been also reported that possesses antioxidant free radical scavenging activity, antimicrobial, gastroprotective, antiulcerogenic activities and anti-fibrotic effects (Feeny, 1970; Barry and Manley, 1986; Reed *et al*., 1990; Haslam, 1996; Makkar and Becker, 1998; Ho *et al*., 2001; Ramirez and Roa Jr., 2003; Amarowicz *et al*., 2004; Chuang, 2011).

Phenolic compounds are well known for their ability to reduce oxidative damage, serves an antioxidant. They can scavenge the free radicals via series of coupled reactions with antioxidant enzyme (Sakat *et al*., 2010). Their localization in lipoprotein domain and cell membrane act as suggesting protective interation with lipid bilayer (Ruiz-Ruiz *et al*., 2017).

2.5 Significance of morphological and microscopical characteristics of plants in their identification

The macroscopic and microscopic description of a medicinal plant is the major step, providing the identity and the degree of purity of them. It should be performed before any tests were undertaken.

Macroscopic and microscopic studies of *Combretum albitum* G. Don. bark showed the presence of 8 to 15 layers of cork consisting of tangentially elongated stored cells and dead tissues of rhytidome. The cells of outer and inner cork were elongated with stone cells (Sreedhar *et al*., 2012).

Cassia fistula is also known as Amaltaas, Garmalo, Purging cassia and golden shower tissue (Kirtikar and Basu, 2005). The leaves were traditionally used as laxative, skin eruption, piles, inflammation and rheumatoid arthritis (Anonymous, 1956). Leaves are arranged in compound paripinnate with 4-8 pairs of leaflet, reticulate venation. As well as microscopical characters revealed the presence of unicellular covering trichomes, upper epidermis, hypodermis and palisade cells (Pandya *et al*., 2012).

Microscopical characters of leaf of *Carica papaya* showed the lamina which was differentiated into upper epidermis, mesophyll and lower epidermis. Vascular bundle was collateral, closed arrangement. Physico chemical parameters values such as total ash (7.4%), acid insoluble ash (13.3%) and Water soluble ash (13.3%) were determined. Stomatal index was calculated to be 31.56±3.41 and the palisade ratio was calculated as 12.65±1.57% (Zunjar *et al*., 2011).

Careya arborea is commonly known as wild guava. It is a medium sized deciduous tree (Nadkarni, 2004). The leaves are simple, glabrous, broadly obovate in shape, acuminate apex with crenate. Microscopical studies of leaf showed the presence of epidermis covered with thick cuticle followed by collenchymatous ground tissue, palisade cells, midrib region contain vascular bundle. Stomatal number of the upper and lower surface is 28 and 188 respectively. The stomatal index of upper and lower surfaces are 8.26 and 31.18 respectively. The stem microscopy of *Careya arborea* showed the presence of cork, cork cambium, cortex, collenchymatous embedded with cortical vascular bundle (Gupta *et al*., 2012).

Ailanthus excelsa bark has brittle, rough surface with horizontal and vertical wrinkles. The microscopical characters showed the presence of periderm, secondary phloem, phellogen and lignified stone cells (Kalaskar *et al*., 2019).

Leaves of *Mangifera indica* (Anacardiaceae) leaves shape are lanceolate to elliptical in shape, 6-16 in long and alternate. It has dark green, glabrous, entire and acute apex. Petiole is 1-4 in long and swollen at base. Microscopical analysis showed the presence of trichome, stomata, mesophyll cells, calcium oxalate prismatic crystals and vascular bundle (Bhuvaneswari and periyanayagam, 2012).

Capsicum frutescens is perennial shrubby plant, native to Tropical America (Cooke, 1967). It is locally known as “Melmukhamenusu”. Leaves are simple, light green, broadly ovate to ovate lanceolate usually wrinkled. Venation is reticulate with 6 to 8 pairs of alternate lateral veins. Vein islets number is 19.66±0.33 and vein termination number is 20.33±0.57. Stomata is anomocytic type (Cooke, 1967; Jain, 1991; Hegde *et al*., 2014).

2.6 Organoleptic characters

Organoleptic evaluation is an important tool in pharmacognostic studies. The study is used to establish the identity and purity to ensure quality of a selected drug. The evaluation is determined to study the various characters of the plant such as shape, size, colour, odour, taste, fracture of stem bark, leaf structure like margin, apex, bark surface, venation and inflorescence etc. (Chanda, 2014).

Psoralea corylifolia Linn. belongs to the family Fabaceae. It is used to treat skin diseases like *Psoriasis leucoderma* and leprosy (Khushboo *et al*., 2010; Chaudhari *et al*., 2014). The stem is grooved, leaves are simple, broadly elliptical and hairy. *P. corylifolia* seed powder blackish brown in colour, characteristic aromatic odour, bitter in taste. Powder microscopic studies showed the presence of oil globules, trichomes, simple fibres, tannin content, stone cells and parenchyma cells (Shresha *et al*., 2018).

Centella asiatica (L.) Urban, a small edible herbaceous medicinal weed belongs to Apiaceae (Arpita *et al*., 2018). It has various pharmacological activities such as wound healing, anti-inflammatory, antiulcer, anticonvulsant and antitumour properties (Orhan, 2012). They are dark green in colour, characteristic odour, sweet taste, cool in nature (Veerabhadraswamy *et al*., 2019).

Capsicum frutescence is a familiar spice. It is commonly known as "African chili". It is a perennial shrubby plant native to Tropical America (Cooke, 1967). The leaves and fruits are used in Ayurveda, Unani and Folk to treat fever, gout dyspepsia, cholera and stomach ache (Kirtikara and Basu, 2001). Leaf powder is dark green in colour with slightly pungent odour and slightly bitter in taste. Ripen fruit powder are orange to red colour with characteristic pungent odour and taste (Hegde *et al*., 2014).

Ankad *et al*., 2015 revealed the organoleptic characters of four species of the family Rubiaceae namely *Spermacoce hispida, S. mauritiana, S. stricta* and *S. ocymoides*. They were green in colour, fine in texture, characteristic odour and pungent taste. Traditionally leaves of this family were used in different systems of medicine (Yadav and Sardesai, 2002).

Terminalia travancorensis is an endemic plant to Western Ghats. It is commonly known as kattu kadukka, pei kadukka (Rao S and Rao M R, 1914). Medicinal properties

of *T. travancorensis* have not yet been explored. It's leaf and bark are dry grey in colour, pleasant smell in odour, acrid in taste and rough in nature (Lakshmi *et al*., 2012).

2.7 Physicochemical analysis

Physicochemical analysis is reliable, accurate and inexpensive for the purpose of quality control (Bignoniya *et al*., 2012). These parameters are also important in detecting the adulteration and inappropriate handling of drugs (Kumar *et al*., 2011). This analysis is useful for the source of information about purity and quality of a drug. Extractive values provide an knowledge about the chemical constituent of the drug. Ash value determines the inorganic composition and other impurities present in the drug (Karthika and manivannan, 2018).

Leucas aspera [willd.] Linn is annual herb. It is distributed in India. Traditionally it is used as an antipyretic and insecticide (Kirtikar and Basu, 1991). Kiruba *et al*., 2016 determined the physicochemical characters of *L. aspera* (Willd.). They reported that the total ash, water insoluble ash, acid insoluble ash and loss on drying values 11.5±0.9%, 7.5±0.6%, 1.8±0.1% and 10.5±1.1% respectively.

Careya arborea (*C. arborea*) Roxb. belongs to the family Lecthyidaceae. It can be identified by large showy flowers and thick dark grey bark. Stem bark of the *C. arborea* is used to treat tumours, bronchitis, skin disease, epileptic fits and astringents (Nadkarni, 2004). Gupta *et al*., 2012, has revealed the physicochemical parameters of leaf. They reported that the total ash content is 6%, water soluble ash is 2.2%, acid insoluble ash is 1.4% and water soluble extractive value is 8.2%.

Hilleria latifolia (Lam.) H. Walt, belongs to the family Phytolaccaceae. It is a perennial herb which can be commonly seen in the forest of Ghana (Woode and Abotsi, 2011). Amponsah *et al*., 2014 investigated the physicochemical parameters of *H. latifolia* leaves. From the result, it was concluded that the total ash value is 16.67±0.29%, water soluble ash value is 16.03±0.4% and moisture content is 80.65±1.19%.

Mangifera indica is belongs to the family Anacardiaceae. It is a major tropical fruits found in world wide. It is also known as the king of the fruits (Dak *et al*., 2007). The leaves were used for the treatment of antibacterial activity (Doughari and Manzara, 2008). Ulcerogenic action and hypoglycemic activity (Aderibibe *et al*., 2001; Severi *et al*., 2009).

Combretum albidum G. Don belongs to the family Combretaceae. It is an effective remedy for severe jaundice. Sreedhar *et al*., 2012, investigated the stem bark of *C. albidum.* They reported that the total ash content is 7.17±0.09%, acid insoluble ash value is 0.16±0.08, moisture content is 10.29±0.13, ethanol extractive value is 6.25% and water soluble extractive value is 10.09%.

2.8 Phytochemical analysis

2.8.1 Qualitative phytochemical analysis

Plants serve a significant role in the prevention and treatment of illness. They are the source of phytocompounds which is pharmacologically important. These phytocompounds are helpful for screening the new lead compounds (Atanasov *et al*., 2015). An important part in the investigation of medicinal plant is identifying the phytochemical constituents present in plant leading to further pharmacological and biological studies (Paz-Alberto and Tamayo-Galvez, 2004; Snelder and Lasco, 2008).

Rajan *et al*., 2011 reported the presence of steroids, alkaloids, phenolic compounds, saponins, tannins, flavonoids, gum and xantho protein in dry *Mangifera indica* seed kernel extracts. Phytochemical screening on ethanolic extract of *Oxalis corniculata* revealed the presence of glycosides, carbohydrates, tannins, phenolic compounds, phytosterols, flavonoids, aminoacids, proteins and volatile oils (Gupta *et al*., 2012).

Preliminary qualitative phytochemical screening test were carried out to identify various phytochemicals present in *Terminalia chebula.* Dried powder of *T. Chebula* leaves were subjected to successive extraction with petroleum ether, chloroform, ethyl acetate, acetone, methanol and water using soxhlet extractor. The results showed the presence of important phytochemical such as alkaloids, phenolics, tannins, flavonoids and saponins etc. (Kathirvel and Sujatha, 2012).

Sahu *et al*., 2012 revealed that the leaves of *Argemone mexicana* were contain flavonoids, tannins, sterols, terpenes and alkaloids in ethanol, methanol and acetone extracts. Rahman and Islam, 2013 made a preliminary evaluation of whole plant ethanolic extract of *Leucas aspera* showed the presence of alkaloids, flavonoids, terpenoids, tannins, phlobatannins, saponins and glycosides. Further antioxidant, antibacterial and cytotoxic effects provided the additional support for the qualitative screening.

Citrullus lanatus seed was subjected to successive solvent extraction using different solvents such as petroleum ether, chloroform, ethyl acetate, ethanol and water in the increasing order of polarity by orbital shaker method. The result revealed the presence of nine phytochemical compounds such as alkaloids, flavonoids, tannins, aminoacids, carbohydrates, cardiac glycosides, terpenoids, oils and fats (Varghese *et al*., 2013).

Phytochemical screening of methanolic extract of *Enhydra fluctuans* (whole plant) showed the presence of flavonoids, triterpenes, carbohydrates, reducing sugars, saponins, phenols, diterpenes, proteins and tannins (Kuri *et al*., 2014). *Olea europaea* leaves and *Eruca sativa* seeds were subjected to qualitative phytochemical screening using standard method. In this experiment methanol was used as a solvent. From the result, it was revealed that flavonoids, saponins, sterols, terpenes and steroids were present in both the extract (Malik, 2015).

Calotropis gigantea and *Curcuma neilgherrensis* materials are subjected to successive extraction using petroleum ether, acetone and methanol by soxhlet apparatus the study records alkaloids, flavonoids, saponins, tannins, carbohydrates and phenols were present in methanol extract (Manjula and Chitra, 2017).

Takaidza *et al*., 2018 reported that eight various *Tulbaghia* species were collected from different indigenous plant nurseries in South Africa. Using acetone as a solvent, preliminary phytochemical screening was done using standard method. The result showed the presence of flavonoids, glycosides, tannins, terpenoids, saponins and steroids.

2.8.2 Quantitative studies

Weli *et al*., 2018 determined the total phenolic content of various solvent extract of *Pteropyrum scoparium.* Maceration method was used for the organic solvent extraction. Solvent used are hydro alcohol, butanol, chloroform and hexane. Total phenolic content of various extract of the plants were determined by the Folin-ciocalteau reagent method. Highest content of phenolics was detected in hydro alcoholic extract (198.4 µg GAE/mg) than the butanol (193.44 µg GAE/mg) and hexane extract (130.34 µg GAE/mg).

Hossain *et al*., 2011 conducted quantitative determination of phytochemicals in four different plant species such as *Ranunculus arvensis*, *Equisetum ravens, Carathamus lanatus* and *Fagonia critica.* Total alkaloid, phenols, flavonoids and saponins were

observed in higher quantity in *E. ravens* (0.399±0.013 mg/100gm), *F. critica* (1.438±0.13 mg/100gm), *R. arvensis* (1.77±0.22 mg/100gm) and *C. lanatus* (2.156±0.016 mg/100gm) respectively. Madike *et al*., 2017 determined the total phenolics and flavonoid contents of leaves, stem and root of *Tulbaghia violacea.*

Elfalleh *et al*., 2012, quantified the total polyphenol, flavonoid, anthocyanin and hydrolysable tannin in various parts of *Punica granatum* such as peel, seeds, leaves and flowers. Solvents used were aqueous and methanol. Total polyphenol was highly present in methanolic peel extract (85.60±4.87 GAE mg/gm), high amount of flavonoid (72.52±5.59 RE mg/gm), anthocyanin (168.91±3.13 (GE mg/gm), hydrolysable tannin (148.24±10.29 TAE mg/gm) were present in methanolic flower extract.

Gurupriya *et al*., 2017 quantified the phytochemicals such as total alkaloid, saponin, phenol and flavonoid in the leaves of *Simarouba glauca*. The leaf extract contains higher content of phenol (102.3 mg/gm) and alkaloid (94.5±0.10 mg/gm), with lesser amount of saponin (61.1±0.08 mg/gm) and flavonoid (86.3±0.29 mg/gm).

2.9 Antioxidant activity

Vegetative parts, flower and fruits of a plant are rich in antioxidant, that is one of the health promoting agents because they inhibit lipid peroxidation, scavenge free radicals (Shon *et al*., 2004). Research suggested that many disease such as cardiovascular disorder, cancer, aging, inflammation and brain dysfunction, is associated with the production of free radicals leading to oxidative stress. Many epidemiological reports have suggested that consumption of vegetable and fruits decrease the risk of cardiovascular diseases and cancers (Kris-Etherton *et al*., 2002). Fruits and vegetables possesses dietary fibres, trace elements, antioxidants and bioactive compounds which are effective remedy to health related problems. Polyphenols are the vital antioxidant abundantly present in the human diet. Antioxidants derived from the plant origin are considered as the effective diet supplements (Hossain *et al*., 2009).

Natural antioxidant are effective in prevention of various pathological disease and also they consider as a lead for the development of new drugs. In addition, they can be used as an alternative remedy to the synthetic food additives like Butylated Hydroxyl Toluene (BHT) or Butylated Hydroxy Anisole (BHA). Several assays have been approached to

determine the activity of antioxidants. These assays helpful to find out the various mechanism of antioxidant defense system reduction of molybdenum or ferric-tripyridyltriazine, Fe^{3+}-TPTZ, 2,2, azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) (Miller *et al*., 1993; Benzie and strain, 1999; Prieto *et al*., 1999).

Many pathological disease have been associated with oxidative stress due to free radicals (Gutteridge and Halliwell, 1993; Buyukokuroglu *et al*., 2001; Dayananda *et al*., 2010). Oxidative stress is caused by resultance of imbalance between the antioxidant defense system and the formation of Reactive Oxygen Species (ROS). They occurred due to the low level of antioxidants and increasing production of reactive species (Halliwell and Whiteman, 2004).

Elevation of serum lipid levels especially, of cholesterol along with production of Reactive Oxygen Species (ROS) are responsible in the development of Coronary Artery Disease (CAD) and atherosclerosis (Ross, 1999). Many reports finding suggested that flavonoid compounds reveal biological activities and scavenging the Reactive oxygen species. It also inhibits oxidation of Low Density Lipoprotein (LDL). Antioxidant compounds such as phenolic acid, polyphenols and flavonoids scavenging free radicals like peroxide, hydroperoxide or lipid peroxyl that leads to pathological disease. Medicinal plants possesses antioxidants such as Vitamin C, Vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been reported as having the potential to reduce human disease risks (Halliwell and Gutteridge, 1984; Halliwell, 1989; Pourmorael *et al*., 2006).

Reactive oxygen species (ROS) can be defined as all highly reactive oxygen containing such as free radicals and the hydroxyl radical. It can be able to react with lipid, nucleic acid, protein, enzyme and their molecule promote the cellular damage. Cell membrane composed of unsaturated lipids which susceptible due to free radical action (Cross *et al*., 1987).

Boehmeria regulosa is a multipurpose tree belongs to Urticaceae family. The plant possess significant antioxidant property which is confirmed by 2,2-Di Phenyl-1-Picryl-hydrazyl-Hydrate (DPPH) Scavenging assay, Ferric Reducing Antioxidant Power (FRAP) assay, 2,2'- Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic acid (ABTS) scavenging activity (Sharma *et al*., 2017).

Oxalis corniculata is a medicinal plant, used for the treatment of inflammatory disorder. Methanol extract of *Oxalis corniculata* possess significant antioxidant activity due to the presence of phenol and flavonoid compounds (Sakat *et al*., 2010). Flavonoids and phenols having the nature of quenching free radicals (Tyler *et al*., 1998; Oksana *et al*., 2012). According to Mak *et al*., 2013, ethanolic extract of *Cassia* had total phenolic, total flavonoid, total flavonol content and exhibit significant activity for inhibition of DPPH.

According to the study results of Das *et al*., 2011 ethanol, n-hexane and ethyl acetates extracts of *Leucas aspera* showed a significant dose dependent inhibition of DPPH. From the study results, it was concluded that the ethanol extract revealed most significant scavenging activity than the other extracts.

Leucas aspera is an annual herb, distributed throughout India. Leaves are used to treat psoriasis, chronic skin eruption and chronic rheumatism (Kirtikar and Basu, 1991). *Mimusops elengi* Linn is also known as bakul belongs to the family Sapotaceae. The tree is distributed in Bangladesh, Pakistan and India. It is used in the treatment of cardiotonic, stomachic, anthelmintic and astringent (Kirtikar and Basu, 1935; Ghani, 2003). *M. elengi* extract's antioxidant activity was evaluated by using DPPH scavenging assay, Reducing power and Total antioxidant capacity. From the study results, it was concluded that the activity was increasing at the increasing concentration (Saha *et al*., 2008). Oleoside dimethyl ester and salidroside are the two phenolic compounds isolated from pulp of *Osmanthus fragrans*. They have been reported to possess antioxidant property (Wang *et al*., 2010). Reports revealed that many phytocompounds are associated with antioxidant property especially poly phenolic and phenolic compounds have also correlated with antioxidant activity (Saleem *et al*., 2001; Kaur and Kapoors, 2002; Devasagayam *et al*., 2004).

Aloe vera have the antioxidant property due to the presence of alkaloids, tannins, flavonoids, sterols and triterpenes. The study revealed that tannin extract of *Aloe vera* possess moderate antioxidant activity, it was determined by DPPH assay (Benzidia *et al*., 2019). *Coscinium fenestratum* (Goetgh) colebr is commonly known as "tree turmeric". It is widely spread in Vietnam, Singapore, Thailand, Srilanka and isolated region of Western Ghats of India. They have antidiabetic, anti inflammatory and antiseptic properties (Newman and Cragg, 2016; Thenmozhi *et al*., 2018). Methanol extract of *C. fenestratum*

was found to be increased with the increase in the concentration which may play a vital indicator for potential antioxidant activity (Karthika *et al*., 2014).

2.10 Antimicrobial activity

Plants are capable of producing antimicrobial substance of natural origins, are available to fight against infections promoted by different pathogenic microorganism (Rahman and Islam, 2013). Traditionally used medicinal plants posssess antioxidant activity that serve as lead for the development of new therapeutic drug (Mendoza *et al*., 1997; Sanches *et al*., 2005). Medicinal plants and their sources provide complex and structurally diverse compounds. Nowadays, researches have aimed on the evaluation of plant, microbial extracts, essential oil, pure secondary metabolites novel synthesized molecule as immense antimicrobial agents (Runyoro *et al*., 2006; Mabona *et al*., 2013; Nazzaro *et al*., 2013). There is an upsurge of interest in agents exhibiting antimicrobial activity whish are supplied to human and animal organism as pharmaceutics and food (Azuma *et al*., 1995).

Recently there has been rising in antibiotic resistant pathogens. It leads to the emergence of novel bacterial strains which are multi resistant (Albinu *et al*., 2004). The high cost new generation antibiotics with limited effective span lead towards morbidity (WHO, 2000). Hence, there is an upsurge to look out for new agents from natural sources with proven antimicrobial activity. Therefore, this led to the search for potential antimicrobial agents of plant origin, with the focus of discovering effective bioactive constituents that can serve as source of novel antimicrobial substance (Moreillon *et al*., 2005). Antibiotics are the substance which is derived from microorganisms in high dilution. They have antagonistic effect to the growth of other microorganisms (Garrod and O'grady, 1971).

Padalia and Chanda, 2015 revealed that acetone extract of *Tagetes erecta* flower showed the maximum zone of inhibion against *Bacillus cereus* and *Klebsiella pneumoniae*. From the study results, they have concluded that flower can be considered as an alternative source of antimicrobial agents against the human pathological strains.

Antimicrobial activity of *Syzygium cumini* leaf extracts in various solvents such as methanol, ethanol, ethyl acetate and petroleum ether extract were tested against agar well diffusion methods. From the result, significant activity was found in petroleum ether and

ethanolic extact with the inhibition zone 8-24 mm than the other solvent extracts. Extract of *S. cumini* revealed significant activity against *Staphylococcus aureus* and *Escherichia coli* (Imran *et al*., 2017).

Lawsonia inermis belongs to the family Lythraceae. It has been used as astringent, antihemorrhagic, antineoplastic, hypotensive and leprosy (Raja sekaran, 2001). Antimicrobial activity of *L. inermis* leaf extract was performed by disc diffusion and well diffusion method. The microbes were used for the study are *Staphylococcus aureus*, *Streptococcus mutans*, *Pseudomonas aeruginosa, Aspergillus niger, A. flavus,* and *Fusarium.* From the study results, it was concluded that the maximum activity was found in methanol extract against all tested human pathogen (Kannahi and vinotha, 2013).

Aqueous and ethanolic extracts of *Launaea procumbens*, *Vitis vinifera*, *Cyperus rotundus* were evaluated for its antimicrobial activity by agar disk diffusion method and agar well diffusion method. From this result it was concluded that, ethanolic extract of all the plants were active against all the tested bacterial strains (Parekh and Chanda, 2006).

Parekh and Chanda, 2007 investigated the antimicrobial activity of 12 plant species belongs to different families using water and methanol were used as solvents. *Bacillus cereus*, *Staphylococcus epidermidis*, *Enterobacter* aerogenes, *Proteus vulgaris* and *Salmonella typhimurium* were used for the study. From the study result, it was concluded that the methanol extract showed some degree of effect than water extract. Among the plant extracts *Bauhinia variegata* showed significant activity.

Antimicrobial activity of leaves of *Parkia biglobosa* (Jacq) Benth and *Parkia bicolor* A. Chev belongs to Mimosaceae were tested using agar cup diffusion and dilution method. The screening was done using hexane, ethyl acetate, ethanol and water extracts against various pathological strains. From the results, it was concluded that *Parkia bicolor* showed more activity than *Parkia biglobosa* (Ajaiyeoba, 2002).

Antimicrobial activity of 18 ethnomedicinal plant extracts were evaluated against 9 bacterial strains and one fungal strains using disc diffusion method at three different concentration 1.25, 2.5 and 5 mg/disc. Among the plant species tested *Acalypha fruticosa*, *Peltophorum pterocarpum, Toddalia asiatica, Cassia auriculata, Punica granatum and*

Syzygium lineare were showed significant activity. Methanol extact of *Peltophorum pterocarpum* and *Punica granatum* showed significant activity against *Candida albicans* (Duraipandiyan *et al*., 2006).

Tridax procumbens L. has been known for its various phytocompounds. It is enriched with bioactive compounds such as tannins, alkaloids, saponins, flavonoids and phenols etc. Antimicrobial activity was determined by using acetone and methanolic extract of plant parts such as root, leaf and flower. From the result, acetone leaf and root extracts revealed significant antibacterial activity than the flower extract. They were used some microbes for testing the antimicrobial activity namely *Staphylococcus aureus*, *Bacillus subtilis, B cereus*, *Escherichia coli* and *Serettia marsescens* (Jhample *et al*., 2015).

2.11 FT-IR analysis

Fourier transform infrared (FT-IR) spectroscopy is an analytical method, used to analyze any sample almost in any state. It is an instrumental technique based on the vibration of a molecule excited by infrared radiation at a particular wavenumber range. This method is non destructive, time consuming, simple to use, precise and mechanically simple. It is also used in quantitative and qualitative analysis. The spectroscopic technique are cost effective, time consuming process (Davis and Mauer, 2010; Smith, 2011).

Bobby *et al*., 2012 revealed the presence of alcohols, phenols, alkanes, carboxylic acids, aromatics, ketones and alkyl halides. FT-IR technique was used to determine the type of organic and inorganic compounds in plants. It is one of the powerful method to identify the chemical bonds or functional group present in the bioactive compounds of plants.

Researchers applied this technique to identify the plant species in *Hypericum* and *Triadenum* (Lu *et al*., 2004; Gorgulu *et al*., 2007). Many Indian scientist have been proved the importance of this technique (Ashok kumar and Ramaswamy, 2014; Naumann *et al*., 2014; Anil kumar *et al*., 2016; Charushila and Swaroopa, 2016). Chandra, 2019 reported that FT-IR spectroscopic analysis of *Nicotiana plumbaginifolia* by revealing the phyto compounds present in different frequencies ranges from 896.90 cm^{-1} to 3352.28 cm^{-1}. It is also confirmed that the plant is rich in amino acid content. This study revealed the presence of different phytocompounds indicates of its potential medicinal value.

Lantana aculeate Linn, belongs to the family Verbenaceae. It has a rich source of antibiotics. It consists of more pharmaceutical properties leads to a new drug (Ebana *et al*., 1991; Rajendiran *et al*., 2014). Narendhran and Sivaraj, 2015, reported that the FT-IR analysis of *L. aculeate* L. shows the various concentration absorption peak with different functional group in the plant extracts. From the result, it was concluded that it possesses phenols, carboxylic acids, alkanes, ketones, alkyl halides and primary amines.

Myristica dactyloides belongs to the family Myristicaceae. The oil is used to treat diarrhoea, nausea, vomiting, chronic bowel complaints and ulcer (Jellin *et al*., 2003). FT-IR study result showed the presence of different functional groups such as carboxylic acid, aromatics, alkanes, alcohols, phenols, aliphatic amines, alkenes and amine group in the *M. dactyloides* fruits (Raju *et al*., 2017).

Embelia ribes BURM. F. is a vulnerable medicinal plant belongs to Myrsinaceae (Ravikumar *et al*., 2000). It is used as Ayurvedic drug (Sivarajan, 1994). Fruit is used to treat fever, inflammatory disease and gastrointestinal ailments (Gupta *et al*., 1977). FT-IR analysis, shows different compounds like amino acids, amides, alkanes, carboxylic acids, alcohols, esters, ethers, aromatics, aliphatic amines, phenolics, aldehydes, ketones, fluorides, halogens, alkyl halides and nitro compounds (Kamble and Gaikwad, 2016).

FT-IR analysis of *Ampelocissus latifolia* tuberous root revealed the presence of alcohols, aromatic compounds, alkanes, aldehydes, ketones, alkenes, amines, amides, nitro compounds, carboxylic acids, ethers, esters and alkyl halide compounds. Presence of these compounds are responsible for its medicinal properties (Theng and Korpenwar, 2015). *Carulluma geniculata* belongs to the family Asclepiadaceae. It is an endemic tree. FT-IR studies were done in their plant which is done by Asha *et al*., 2014. The study revealed the presence of alkene, methylene, isoscyanate, organic nitrates, aliphatic nitro compounds, ammonium ions and aromatic nitro compounds in the plant extract.

2.12 GC-MS analysis

GC-MS technique is used for screening, identifying and quantification of many constituents in the plant samples. The retention time (RT) is used to identify the characterization of a sample Mass spectroscopy (MS) is the detector of GC. The fragmentation pattern for a sample is specific to identify the characteristic of a sample.

Plant sample can be identified by its retention time (GC) and fragmentation pattern (MS) along with the particular information of the sample. GC-MS analysis is one of the confirmation method for examining, the herbal drug (Mythili *et al*., 2013). Nowadays, GC-MS technique have been used for examine the plant samples for it's volatile essential oil, fatty acid, lipids and alkaloids (Milne and Newman, 1995). It is one of the useful tool to determine the fatty acids due to its high speed, resolution and sensitivities (Qureshi *et al*., 2011).

Hossain *et al*., 2011 reported that Gas chromatography-mass spectrometry analysis of various organic extracts of *Merremia borneensis* hexane extract contain 2-hexyl-1-octanol. Ethyl acetate and chloroform extract contains 1,2 dimethoxy-4-(2-propenyl) benzene. Butanol extract contains butanamide and cyclopropane carboxylic acid. These major constituents from various extracts are biologically active molecules. Ethanolic extract of *Peristophe bicalyculata* has seven bioactive compounds. The results showed the presence of Propane,1,1-diethoxy (68.89%), 6Z-nonel-1-ol (24%), 4-methyl-2,4-bis (4'trimethyl silyloxyphenyl pentene-1 (3.56%), Cyclo Octyl alcohol (1.78%), Oxirane, butyl-(0.89%), 2H pyrrole-2 carbonitrile 5-amino-3,4-dihydro- (0.44%) and ethaneperoxic acid, 1-cyano-1-[2-(2-phenyl-1,3-dioxolan-2-y1) ethyl] pentyl ester (0.44%) (Janakiraman *et al*., 2012). De *et al*., 2013 revealed the presence of medicinally active bioactive constituents in the methanolic extract of the plant of *Sphaeranthus amaranthoides* Burm (De *et al*., 2013).

Kanthal *et al*., 2014., analysed the presence of 21 biomolecules in the whole plant methanolic extract of *Lactuca runcinata* DC by GC-MS analysis. The major compounds are E-Ethyl (Z)-3-(4-Acetylphenylthio), cinnamate (33.01%), Lup-20 (29)-ene-3,6-dione,5,12-dihydroxy-(5a,12a) (10.46%), 1(3H)-Isobenzofuronone, 3-ethoxy.

Dryopteris cochleata is the plant species of Dryopteridaceae. Six different phyto compounds were identified in leaves of *D. cochleata* namely 1H-3a, 7-Methanoazulene, Octahydro-1,4,9-9-tetramethyl-, Phenol, 2,6-bis (1,1-dimethyl ethyl)-4 methyl-methyl (carbamate,1,6,10-Dodecatrien-3-ol,3,7,n-trimethyl-[S-(Z)]-,Phytol,1,2 Benzenedicarboxylic acid, butyl octyl ester-oxirane, tetradecyl-. Presence of these compounds may be attributed to their medicinal value (Kathirvel and Sujatha, 2016).

GC-MS chromatogram of the methanolic extract of *Catharanthus roseus* revealed the presence of twelve compounds. Hexadecanoic acid, 9-octade canoic acid, 2-Benzenedi carboxylic acid and tetracontane were majorly present in the tested sample. They were used for antimicrobial, antidiabetic, antioxidant and anticancer activity (Thanwar *et al*., 2017).

GC-MS analysis of ethanolic extract of *Pistia stratiotes* L. showed the presence of seven compounds and *Eichhornia crassipes* (Mart.) Showed the presence of eleven compounds respectively (Tyagi and Agarwal, 2017). Twenty one compounds were present in peeled seed of *Punica granatum* and fourteen constituents were present in *Punica protopica* (AL-Huqail *et al*., 2018).

Ethanol extract of *Silybum marianum* (L) were analysed for phytochemical compounds by GC-MS method. The identified compounds include [+]-2-Bromanone (RT;5.91),9,12,15–Octade Catrienal (RT:6.05), α-Santoline alcohol (RT:6.15), Dodecane (RT:6.41) along with other compounds (Padma *et al*., 2019). The unknown organic compounds in a mixture can be analysed by interpretation and also matching the spectra with reference spectra (Yonzone *et al*., 2012).

2.13 Antidiabetic activity

Diabetes mellitus (DM) is characterized as a metabolic chronic disease which occurs due to defect in insulin secretion or insulin action or both (Amos *et al*., 1997). The effects of DM led to various disease include Cardio Vascular Disease (CVD), Peripheral Vascular Disease (PVD). Coronary Artery Disease (CAD), stroke, neuropathy renal failure, retinopathy amputations and blindness (Bajaj, 1995; David, 1996).

α-amylase and α-glucosidase are intestinal enzyme that breakdown the polysaccharide to monosaccharide. Inhibition of these enzyme led to decrease in blood glucose level. Natural and synthetic glucosidase inhibitors delays the postprandial hyperglycemia in type 2 diabetes. Acarbose and Miglitol are the example of antidiabetic drug (Groop *et al*., 1997; Perfetti *et al*., 1998; De melo *et al*., 2006).

Recently used inhibitors namely acarbose and miglitol that inhibit α glucosidase and α-amylase while voglibose inhibit α-glucosidase. Many of these synthetic hypoglycemic substance have their limitation, produce serious side effects and non specific. The major side effects of are diarrhoea and flatulence (Cheng and Fantus, 2005).

Number of medicines derived from herbal plants have been used for treatment of diabetes (Marles and Farns worth, 1995; Alarcon-Aguillar *et al*., 1998). The action of hypoglycemic activity of these plants are being evaluated. Isolation and identification of chemical substance derived from hypoglycemic potential of herbal plants are being studied (Bailey and Day, 1989; Zaman, 1989; Alarcon-Aguillar *et al*., 1993). The search for novel biologically active compound derived from natural sources like herbal plants and their extracts can lead to potential and specific inhibitors for α-amylase (Tarling *et al*., 2008). Acarbose inhibit both α-amylase and α-glucosidase enzymes (Creutzfeldt, 1999).

Bioactive compounds such as saponin, phenols and flavonoids were studied in various herbal plants such as *Proteus vulgaris, Euphorbia hirta, Cassia glauca.* These plants showed potent α-amylase inhibitors (Cheng and Fantus, 2005). Antioxidant properties of medicinal plants are responsible for disease prevention mechanism in human (Rathi Sre *et al*., 2012). The methanolic extract of root is responsible for hypoglycemic and anti-hyperglycemic activity in alloxan diabetic mice (Syiem *et al*., 2002). Inhibition of α-glcosidase is led to anti hyperglycemic effect of many plant species. (Shim *et al*., 2003; Li *et al*., 2005; Patel and Mishra, 2012).

Aqueous extract of fruit of *Momordiac charantia* has a potential stimulator of insulin released from β cell rich pancreatic islets isolated from obese hyper glycemic mice (Welihinda *et al*., 1982; Kedar and Chakrabarti, 1902). Banerjee *et al*., 2017 conducted *in vitro* α-amylase inhibitory assay and *in vitro* α-glucosie inhibitory assay in *Tinospora sinensis* (Lour.) Merr. it was revealed that the plant possess significant α-amylase and α-glucosidase inhibitory activity with an IC_{50} value 0.75 µg and 0.80 µg dried extract respectively.

In vitro antidiabetic activity of methanol extract of *Caesalpinia digyna* exhibit significant inhibitory activity on α-glucosidase enzyme (IC_{50}-402.23±10.14 µg/ml) and α-amylase (IC_{50}-.686.94±3.98 µg/ml (Narkhede *et al*., 2011). *Phyllanthus amarus* has reported to possess number of *in vitro* and *in vivo* pharmacological activities. It has been also reported that it showed to have α-amylase inhibitory effect (Raphael *et al*., 2002).

Tamilselvi *et al*., 2018 evaluated *in vitro* antidiabetic potential of *Gardenia latifolia* Ait. Ethanolic and Aqueous extract of leaf of *G. latifolia* was tested to check the effect on

inhibition of α-amylase and α-glucosidse enzymes. It was concluded that the ethanol extract displayed inhibitory activity in a dose dependent manner than the aqueous extract. Aqueous extract of *Ximenia americana* extract showed significant α-amylase inhibitory activity (IC_{50}-96.26 µg/ml) (Shettar *et al*., 2017).

Grape seed is rich in oil content and hypoglycemic constituents. Kong *et al*., 2018 study revealed that aqoues extract of grapes seed showed significant inhibition activity against α-amylase (IC_{50}- 66.68±1.1 g/ml) and α-glucosidase (IC_{50}-25.25±0.53 g/ml).

2.14 Anticancer activity

Cancer occur due to uncontrolled growth of tissue of lack of regulation in cell division process. Over 200 types of cells in the body affected with this disease. Around 50% of agents isolated from herbal plants with therapeutic property were under clinical trials for anticancer activity (Cragg and Newman, 2000). Plant extract have more potent cytotoxic activity than their isolate compounds due to the synergistic effect of all the bioactive compounds present in the medicinal plants (Hamburger and Hostettmann, 1991; Maddi *et al*., 2018). Due to cell cycle arrest, number of cells were reduced resulting in the cytotoxic effect determined with MTT and neutral red assays.

MTT assay is based on enzymatic convertion of MTT catalyzed by succinate dehydrogenase in mitochondria while the Neutral red assay is also known as colorimetric assay ensuring the uptake of the dye by functional lysosomes (Repetto *et al*., 2001). Cancer affect severe mortality among human population. Many cancer types were detected and the most common cancers are liver, prostate, colorectal, lung and oesophages type in human being (Rajkumari *et al*., 2019).

MTT reduction in viable cells in culture is a method widely used for evaluating the cytotoxic effect of xenobiotic assessing proliferation rates and examining the cell activity (Mosmann, 1983; Denizot and Lang, 1986). Reduction of MTT inside the viable cells to water-insoluble purple colored formazan by mitochondrial dehydrogenases, Though in different cell lines over 50% of the dye penetrating the cell membrane can be reduced by non mitochondrial, cytosolic, mitochondrial, cytosolic and microsomal enzymes (Gonzalez and Traloff, 2001; Collier and Pritsos, 2003).

Tulasi *et al*., 2018, revealed that the ethanolic extract has potent cytotoxic effect agaist MCF-7 with IC_{50} (101.55±0.94 µg/ml) and IC_{50} (72.06±3.9 µg/ml) in case of *Ficus benghalensis* and *Ficus religiosa* respectively. Antioxidant rich food may reduce breast cancer and mortalities (Fleischaer *et al*., 2003; Cai *et al*., 2004). Antioxidants such as coumarins, lignans, phenolic acids, flavonoids, quinines, tannins and curcuminoids are rich in anticancer property (Cai *et al*., 2004). Anticancer activity of ethanolic kernel extract of *Mangifera indica* L. on breast cancer lines such as (MDA-MB 231 and MCF-7) were tested using MTT assay. From the result, it is concluded that the extract showed significant cytotoxicity towards both cell lines (Abdullah *et al*., 2014).

There are different cytotoxicity assays were available for screening the potent bioactive compounds extract preparation for their anticancer activities. MTT assay is most commonly used assay involving the use of dye stains. The dye is used for cellular processes, providing indirect measure of mitochondria function (Walters *et al*., 2008; Hingorani *et al*., 2011; Yarden and Caldes, 2013).

Babu *et al*., 2019 evaluated cytotoxic activity of ethanolic leaf and fruit extract of *Anona muricata*. The test extracts were tested against $HepG_2$ cell lines by MTT assay. He concluded that the methanolic fruit extract showed significant activity (IC_{50}-12.4 µg/ml) than leaf extract (IC_{50}-82.5 µg/ml). Cytotoxicity of methanolic leaf extract of *Tridax procumbens* against human lung cancer cell line and breast cancer cell lines were evaluated. 250 µg/ml methanolic leaf extract showed high toxicity against lung cancer cells (84±2.8%) than breast cancer cell line (68±3.1%) (Syed *et al*., 2020). Cytotoxic activity has been increased at increasing concentration of crude extract (Priya *et al*., 2011a; Priya *et al*., 2011b).

Thombre *et al*., 2013 assessed the cytotoxic activity of *Vitex negundo* and *Tabernarmontana divaricata* against THP-1 Leucemia cell line using MTT assay. They revealed that the methanolic extract of *Vitex negundo* showed highest inhibition rate than methanolic extract of *T. divoricata*. Garg *et al*., 2016 investigated the *in vitro* anticancer activity of different kind of few indian fruit peels through MTT assay. They reveald among all the fruits *Carica papaya* peel extract showed maximum inhibitory activity with low IC_{50} value of 17.3 µg/ml. Anticancer activity can be confirmed by using various assays

such as XTT, trypan blue dye exclusion followed by *in vivo* studies and clinical trials. These are the worth investigation for analyzing the anticancer studies (Rahmat *et al*., 2002; Li *et al*., 2012).

Many natural products have been evaluated for anticancer activity on different experiment models. Around 30 anticancer agents were available to curing cancer (Ramakrishna *et al*., 1984). Phytochemicals were responsible for anticancer effect. Flavonoids possess antimutagenic, antimalignant effect (Brown, 1980; Hirano *et al*., 1989), Chemopreventive agent (Fotsis *et al*., 1997).

2.15 *In vivo* studies on effect on phytomedicine

2.15.1 Acute toxicity activity

Toxicology is a division of science which deals with toxins, their effects and treatment. Toxicological study helps in the development of novel drugs. The US Food and Drug Administration (FDA) indicates that it is necessary to analyse new molecules for biological activity. Toxicity study is useful to calculate the NO Observed Adverse Effect Level (NOAEL) dose and is useful for clinical experiments studies (Setzer and Kimmel, 2003).

Acute toxicity is defined as the adverse effect occuring immediately or to short time of period exposure to a agents (or) as adverse effect occurring within a short time of administration of a single dose of agents (or) multiple doses administrated within 24 hours. An adverse effect is "any effect" that results in functional impairment and/or biochemical lesions that may affect the function of the whole organism (or) decrease the organ's performance to respond to an additional challenge (Rhodes *et al*., 1993).

Acute toxicity testing is helpful to analyse the effect of a single dose on a specific animal species. The investigational test sample was administered at the dose level of 5, 50, 500 and 2000 mg/kg and the experimental was observed for a particular specified period. The aim of acute toxicity test is to gain information on the biological activity of a chemical and obtain insight into its mechanism of action (Oliver, 1986). Acute toxicity study provides clues on the range of doses that many toxic effect to animal. It could also be used to evaluate the therapeutic index (LD_{50}/ED_{50}) of drug molecule and xenobiotics (Rang *et al*., 2001).

Syzygium guineense belongs to Myrtaceae. The plant species was tested for acute toxicity study in animal models. 80% methanol extract of the leaves was then administrated orally at the dose of 2000 mg/kg and 5000 mg/kg. These doses were selected based on the previous studies (Laha *et al*., 2019).

Clinacanthus nutans Linn. is belongs to Acanthaceae. Methanol extract of *C. nutans* orally administered at a single dose of 5000 mg/kg body weight. It was suggested that, the methanol extract of *C. nutans* is safe to consume. Subchronic oral toxicity has been also studied in *C. nutans.* The test extracts were orally administrated at the single doses of 50, 500 and 2500 mg/kg for 28 days. The extract did not produced any changes in mice general behaviours (Zakaria *et al*., 2016).

Tephrosia purpurea (Linn.) Pers. is belongs to leguminosae. The plant has various pharmacological activities. It is used to treat bronchitis, diuretic, laxative, cough and tumour (Tridevi, 2007). 50% aqueous ethanolic extract of *T. Purpurea* was investigated for oral acute toxicity and subacute toxicity (Hussain *et al*., 2012).

Cassia occidentalis is belongs to the family Leguminosae. It is used to treat laxative, analgesic, febrifuge, diuretic, hepatoprotective, vermifuge (Coimbra, 1994; Di stasi and Hiruma-Lima, 2002). Animals were orally treated with *Cassia occidentalis* at the dose of 0.625, 1.25, 2.5 and 5.0 g/kg. Animals were observed for 14 days. The results suggested that there were no deaths and hazardous signs were noticed (Silva *et al*., 2011).

Pieme *et al*., 2006 investigated the acute and subacute toxicities of hydro-ethanol extract of *Senna alata*. He revealed the medium lethal dose (LD_{50}) was about 18.50 g/kg of body weight. Significant ($P<0.05$) variation of the body weight was noticed after 26 days of treatment. *Allium sativum* Linn belongs to the family Alliaceae. It is traditionally used to treat antiseptic, antihypertensive, anthelmintic and diuretic (Packia Lekshmi *et al*., 2015; Gupta *et al*., 2015). *A. sativum* bulb extract was orally administrated at the dose of 100, 1000, 2500 and 5000 mg/kg. Then, the rats were closely observed for 1 hour. During the study period no mortality was recorded. The tested animals were healthy with no sign of toxicity up to the dose level of 2500 mg/kg. Though at 5000 mg/kg, animals were weak and had ethrema tachy-cardia, However, no death was observed. Thus, LD_{50} was more than 5000 mg/kg (Lawal *et al*., 2016).

Cruz *et al*., 2006, analysed the acute and sub acute toxicity effect of the hydro alcohol seed extract of *Cucurbita maxima* Duch given to mice. It's average lethal dose (LD_{50}) is higher 5000 mg/kg and subacute study revealed increase in body weight. The study recommended that the hydroalcoholic extract of *C. maxima* seed hydro alcoholic extract of *C. maxima* seed at a dose of 5000 mg/kg considered as safe dose, being without acute toxicity.

2.15.2 Wound healing activity

Wound healing is a complicated process which include cellular, physiological and biochemical events led towards re-establishment of structural and functional restoration of injured tissue (Singer and Clark, 1999; Ramsey *et al*., 1999). A wound can be defined as a disruption of tissue integrity that led to damage and is related with loss of function (Shenoy *et al*., 2009). There are three stages in wound healing process, they are inflammation, proliferation and remodeling.

The proliferative phase is indicated by angiogenesis, collagen deposition, epithelialization and wound contraction (Bhaskar and Nithya, 2012). Effective wound management occured by hemeostasis and healing skin wound management prevent infection and further injury. Therefore, animal model experiment studies are helpful to understand basic process of tissue repair and to bring out strategies for the healing process of wound (Shrimanker *et al*., 2013). Initial process of wound healing involves acute inflammatory phases, it is followed by the synthesis of collagen and extra cellular macromolecules, that forms a scar (Chithra *et al*., 1998). Wound healing activity is promoted by free radical scavenging activity of flavonoids. Traditionally used medicines are capable of treating wound and skin related diseases. Plant derived medicine plays a vital role in treating wound, cuts, burns and skin related diseases (Kokane *et al*., 2009; Maver *et al*., 2015).

Flavonoids reduce lipid peroxidation through improving vascularity. Lipid peroxidation has vital process in injuries such as burns, infected wound and skin ulcers. Phytochemicals like flavonoids, glycosides and tannins are involved in promote wound healing mechanism majorly by their astringent and antimicrobial properties (Vinothapooshan and Sundar, 2010; Agrahari *et al*., 2010). The plant compounds are

possess significant compatibility with the human body as they are the part of physiological function of living flora (Kamboj, 2000). Medicinal plants are the most important source of treatment of wounds. Reports revealed that many plants have been experimentally used to cure skin disorders and wound injuries as traditional medicines (Swamy *et al*., 2007; Harish *et al*., 2008).

The flavonoid namely baicalein-7-O-β-D-glucuronide (baiculin) present in *Leucas aspera* was investigated for wound healing activity. From the observation, it is confirmed that wound healing was slow in earlier days which turn to very rapid on the 12^{th} day. The results are proved with the help of histopathological studies (Kalaivanan *et al*., 2013). 2% methanolic and 2% aqueous extract of *Mimosa pudica* is evaluated for its wound healing property. It was determined with the tensile strength of the incision wound of different groups. The animals treated with both test sample showed significantly high ($P<0.001$) tensile strength (Kokane *et al*., 2009).

Petroleum ether extract at a dose 500 mg/kg of *Hyptis suaveolens* was investigated for wound healing activity. From the result, it was found that the tested plant extract possess significant wound healing activity. Wound contraction on 18^{th} day is 97.25±0.17% compared to control (77.446%) (Shenoy *et al*., 2009). Alcoholic stem bark extract of *Annona muricata* was evaluated for wound healing for a 12 days. Wound reduction was seen from 4^{th} day onwards from the day of wounding. The results revealed that wound healing pattern as percentage was observed 19.74, 47.09 and 88.58% on 4^{th}, 7^{th} and 12^{th} day respectively (Paarakh *et al*., 2009).

Aqueous and ethanolic bark extract of *Emblica officinalis* evaluated for wound healing activity in wistar rats. In incision wound model, aqueous (8.3±1.2 mm^2) and ethanol (13.8±4 mm^2) extracts of *E. officinalis* showed significant reduction in wound area (Talekar *et al*., 2012). Methanolic extract of the leaves of *Azadirachta indica* and *Tinospora cordifolia* were evaluated for wound healing activity by excision and incision wound model. In incision wound model, significant increase ($P<0.05$) in tensile strength was observed due to increased proliferation of fibrous cells into myofibroblasts. In the excision wound model, percentage of closure of wound increased significantly ($P<0.01$)

from '0' day till 21st day that was 95.65% in case of *A. indica* whereas 77.02% in *T. cordifolia* (Gupta *et al*., 2007).

Ganju and pathak, 2013, evaluated the wound healing activity of polyherbal formulation by excision wound model. Polyherbal formulation contain ethanolic extract of *Tridax procumbens, Euphorbia hirta, Eclipta alba,* dried rhizome of *Curcuma longa* and gel of *Aloe barbadensis*. 100% wound healing was observed on 12th day of the experiment.

2.15.3 Hepatoprotective activity and histopathological studies

Liver is the important source of protection against damage by ingested agents like xenobiotics. It is a major organ involved in the functions such as metabolism, detoxification and secretary function of the human body. Hepatic injury is a common pathological process in most chronic hepatic ailments and long term liver injury led to hepatic necrosis, fibrosis, liver cirrhosis and hepatocellular carcinoma (Strader *et al*., 2002; Basu *et al*., 2009; Stickel and schuppan, 2007).

Liver disease is a global problem, allopathic drugs used in the treatment of hepatic disease are sometimes inadequate and can cause serious side effects. Hence, it is urge to search for alternative medicine for the treatment of hepatic disease (Ozbek *et al*., 2004). Acute liver failure is caused due to different reasons such as viral hepatitis, toxic liver damage by poisons, drugs and ischemia.

Paracetamol is used as an antipyretic analgesic drug which is available in the market and use of paracetamol can cause excessive production of ROS during formation of N-acetyl-p-benzoquinoneimine (NAPQI) by cytochrome P50 (Dhalin *et al*., 1984). This process has been suggested to involved in the development of oxidative stress and injury in paracetamol-induced hepatotoxicity (James *et al*., 2003).

Many herbal plants and this formulations are used to treat liver disorder in ethnomedicinal practices as well as traditional medicine in India (Subramoniam *et al*., 1998). Traditionally used medicines are the main source of potentially useful novel compounds for the derivation of chemotherapeutic drugs (Sharma and Kumar, 2009).

Recent studies shown that, the plants used in indigenous medicine having antioxidant property (Oja and Ladeji, 2005). Research reports revealed that 160 bioactive compounds derived from 101 plants possesses hepatoprotective activity (Handa, 1986).

Secondary metabolites such as phenols, coumarins, lignins, essential oil. monoterpenes, carotenoids, glycosides, flavonoids, organic acids, lipids, alkaloids and xanthones derivatives are possesses the liver protective activity. About 25 different species of plant extract have been reported to cure liver disorders (Sharma *et al*., 2002). Although massive efforts are needed in the field of modern medicine, for production of a new liver protective drug from natural resource (Chatterjee, 2000).

Number of Indian ethnobotanic tradition suggest a rich source of herbal plants used by the populations for treatment of liver disease. Therefore, there were no adequate scientific investigations on the hepatoprotective activities conferred to these plants. In Indian medicine, about 40 polyherbal commercial formulations are available and prescribed by physicians to cure liver disease. Many of these herbal drugs have also been reported to have extraordinary antioxidant property (Aniya *et al*., 2002; Achuthan *et al*., 2003; Gupta *et al*., 2006).

Ethanolic stem extract of *Calotropis gigantea* at the dose of 2500 and 5000 mg/kg were investigated for hepatoprotective activity. It has been revealed that the plant extract significantly decrease AST, ALT and lipid peroxide level. Thus, it is concluded that *C. gigantea* has prominent hepatoprotective activity (Lodhi *et al*., 2009).

Ethanolic extract of *Pisonia aculeate* was investigated for hepatoprotective activity. It showed treatment of rat at the dose of 250 and 500 mg/kg significantly ($P<0.001$) decrease the serum marker enzymes. Results revealed the hepatoprotective activity of *P. aculeata* against CCl_4- induced hepatotoxicity in rats (Palanivel *et al*., 2008). Ethyl acetate fraction of stem bark of *Ceiba pentandra* was screened for paracetamol induced liver damage in rats. The significant ($P<0.05$) reduction was found in serum enzyme Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline Phosphatase (ALP) and Total bilirubin (Bairwa *et al*., 2010).

The aqueous and methanolic extract of *Capparis dactdua* stem was evaluated for hepatoprotective activity at the dose of 200, 400 mg/kg against CCl_4 induced hepatotoxicity

in rats. It was observed that both the extracts showed significant activity which is comparable with standard drug silymarin (Ali *et al*., 2009). Aqueous and methanolic extract of *Berberis asiatica* roots at the dose of 200 and 300 mg/kg screened for hepatoprotective activity. Hepato toxicity was achieved by administration of 2 ml/kg of CCl_4 in equal proportion of olive oil in wistar albino rats. The result revealed both the extract dosage showed significant ($P<0.001$) hepatoprotective activity by lowering the level of serum marker enzyme (Tiwari and Khosa, 2010).

Histopathology is a scientific evaluation of disease at the tissue and cellular level (Sikandar, 2018). The disease can be detected in the affected tissue portion under the microscope (Subbarayappa, 2001). The pathological sections describes the considerable information of most of the human as well as animal ailments (Culling, 2013). *Moringa oleifera* has hepatoprotective effect. It was confirmed by the histopathological study of liver. Acetaminophen induced rats showed the presence of prominent microvesiculation, moderate infiltration of monocyte and neutrophils with mild scattered focal necrosis. The pathological hallmark of Acetaminophen treatment like bridging necrosis was not found in the liver section obtained from the rats which were pretreated with *M. oleifera* (200 and 800 mg/kg) (Fakurazi *et al*., 2008).

Nelumbo nucifera is a medicinal plant used for treatment of liver disorder in Ayurvedic medicine. The 50% aqueous ethanolic extract of flower of *N. nucifera* (400 mg/kg) exhibited significant hepatoprotective activity. In histopathological study, pathological changes of hepatic lesions caused by hepatotoxicants were cured by treatment with tested extract (Rao *et al*., 2005). Lignan from Fructus *Schisandrae chinensis* (Traditional medicine) (45 mg/kg) with *Astragalus* polysaccharides (150 mg/kg) was evaluated for hepatoprotective activity. The tested sample showed prominent hepatoprotective activity, confirmed by histopathological studies. In normal liver histopathological sections revealed the normal hepatic structure. Liver tissue in rats treated with CCl_4 showed liver injuries that is confirmed by severe hepatocelllar degeneration and necrosis around the central veins. Administration of tested sample improved the hepatocellular injury induced by CCl_4 in rats (Yan *et al*., 2009).