Review of Literature

2. REVIEW OF LITERATURE

2.0 Introduction

The study area "Velliangiri hills" in Coimbatore District ,Tamil Nadu, India are the major hills range of Western Ghats come under Boluvampatti reserve forests . It occupies the southernmost part and the "spur" of Nilgiri Biosphere Reserve which lies between the 6˚40' to 7˚10' E longitude, 10˚55' to 11˚10' N latitude and forms a part of western boundary of Coimbatore District, Tamil Nadu bordering the Palghat District of the State of Kerala.

The study area includes different types of forest which includes southern tropical thorn forests (Scrub jungles), tropical dry deciduous forests, tropical wet evergreen forests, temperate forests (sholas) and southern montane humid grass lands. The soil type are loamy, acidic, red and ferruginous with the average rainfall in the hills is ca.3500mm at the foot hills and ca.4500mm at the peak year.

Velliangiri hills is enriched with biodiversity, many siddhars and folks depends on bioresources for the preparation of drugs. The existing local markets at every mountain range are living evidence for the exploitation of flora.

The Malasars, Mudugars and Irulars, are located in different areas of Velliangiri hills. The tribes are mainly dependent on the hills for their food and medicine.

The Velliangiri hills of India are also known for their rich anthropogenic diversity. The aboriginals residing in the Velliangiri hills have abundant ethnobotanical knowledge by their long association with their diverse, local flora. These aboriginals can identify plants, many ethno taxa and even gained an ecological knowledge of them. This techniques has provided clues to identity several new species while working with the aboriginals in the Velliangiri hills.

2.1 Ethanobotanical uses of selected medicinal plants

Natural products derived from plants has been very useful to human kind. The phytochemicals in plants have revolutionized pharmaceutical industry. The bioactive ingredients has stimulated scientific interest in research to produce plant based drugs (Moghadamtousi *et al*., 2015)

Annona senegalensis has been in traditional use for many applications. The leaves treat yellow fever, small pox (Dambatta & Aliyu., 2011) etc. The root helps in diagnosing gastritis, snake bite, male infertility and various infectious diseases. The extract of this plant has wide usage as antidotes and anti-diabetic drugs. In Guinea, *A.senegalensis* has been employed in treating malaria (Traore *et al*., 2013) and the bark used to treat open sores (National Research Council Custard Apples, 2008).

Annona squamosa L. belongs to the family Annonaceae. It is also known as custard apple and has wide usage among west Indies and India. According to the folkloric report this plant has immense properties such as insecticidal, anti-tumor, anti-diabetic, anti-oxidant, anti-lipidimic and anti-inflammatory agent due to the presence of cyclic peptides (Gajalakshmi *et al*., 2011).

Annona reticulate commonly known as bullock's heart in English has immense pharmacological effects such as anti-oxidant, anti-cancer, analgesic and CNS depressant, antimalarial, antihelminthic etc. Analysis were performed on the seed, root and leaves and evidenced that the biochemical compound causes cell death in cancer cells (Chavan *et al.*, 2014).

Annona muricata L. commonly known as soursoup, graviola, guanabana has wide usage among tribes. Indigenous communities in Africa and South America use this plant as their traditional medicine. Phytochemical analysis of this plants exhibits the presence of annonaceous acetogenins from leaves, barks, seeds, roots and fruits. This plant has highly ethanopharmacological activities as anticancer,anticonvulsant, anti-arthritic, antiparasitic, antimalarial and antidiabetic effects (Patel, 2017 & Patel, 2016).

Spermacoce latifolia has a biochemical compound and this leads to isolation of a new ursane triterpene acid, a new oleanane triterpene acid, 3β, 6β, 23-trihydroxy-urs-12,20(30)-dien-28-oic acid and 3β, 6β, 29-trihydroxy-olean-12-en-28-oic acid together with seven known triterpenic acids . These compounds exhibits anticancer activity against MCF-7, NCI-H460 and HepG-2 human cell lines with IC50 values from 9.53-80.20 mM (Luo *et al*., 2015)

The whole plant of *Spermacoce ocymoides* extracted with n-hexane, ethyl acetate and ethanol subjected to anti-inflammatory analysis using carrageenan-

induced paw edema at the dose of (100 and 200 mg/kg b.w.p.o.) Among the three, ethanolic extract of *Spermacoce ocymoides* achieved high significant anti-inflammatory effect than n-hexane and ethyl acetate extract. Indomethacin (10mg/kg b.w.p.o) was used as a control.

The ethanolic seed extract of *S. hispida* was administered at a dose of 200mg/kg to triton WR-1339 induced hyperlipidaemic rats. Under observation, it clearly indicated that there is a significant reduction in the levels of cholesterol, phospholipids, triglycerides, LDL, HDL and evenly showed an increase in the level of HDL in serum. (Sivaelango et al., 2012).

GC-MS analysis of various extracts *of Spermacoce articularis* L.f. such as Ethanol, Acetone, Chloroform and Ethyl acetate were screened for phytochemical constituents. It clearly indicated the presence of carbohydrates, saponins, phenols, phytosteroids, tannins, flavonoids and catechin. Twenty five biochemical constituents were identified in that 2-Benzylidene-3-oxo4-(octlsulfany1)-2, 3- dihydrothiophene-1 dioxide (27.71), Tridecanoic acid [CAS] (17.46), 3, 7-Dimethyl-3-hydroxyl-4-isopropenyl-6-octadiene (6.73), Octadecanoic acid (CAS) (5.60), Methyl-threo-9,10-dichloro-octacecanoate (4.05) were the major constituents (Soosairaj *et al*., 2013).

The Plant *Rauvolfia tetraphylla* L. commonly known as devil-pepper plays a significant role in etahnopharmacological industry. It is prevalent among south indian tribes. Recent study confirms the presence of alkaloids isolated from this plant. The presence of alkaloids possess significant antimicrobial, anti-inflammatory, anticancer, anti hypertensive effects. (Iqbal *et al.*, 2013)

Rauvolfia serpentina Benth has more ethanopharmacological properties. The serpasil a crystalline alkaloid has been obtained from this plant. Intravenous injection of serpasil in a single dose of 0.5 to 1.0 mgm/kgm was given. A gradual persistent blood pressure and respiratory depression were observed in barbitalized dogs.

Rauvolfia serpentine has been in wide use from vedic period. From ancient times it has been in use for the treatment of snake bite, insect stings, hypertension, insomnia, psychological disorders, epilepsy, gastricdisorders etc. It is rich in phytochemical constituents such as alkaloids, carbohydrates, flavonoids, glycosides,

Phlobatannins, phenols, resins, saponins, sterols, tannins and terpenes etc. The main alkaloid of this plant is reserpine. It exhibits anti hypertensive property by depleting catecholamine. (Kaur., 2018)

Methanolic extract from the leaves of *Rauvolfia serpentine* were subjected to phytochemical analysis. Using chromatography and spectroscopic techniques 3, 5, 7, 4'- tetrahydroxy flavone i.e., Kaempherol was identified. The anti-oxidant potential of the drug was confirmed by DPPH method which showed immense ethanopharmacological benefits. (Gupta & Gupta., 2015)

2.2 Significance of Morphological and Anatomical characters of plants in their identification

The macroscopic and microscopic character of *Malva parviflora L.* Leaves shows that the stem shows distinguishing characters with parenchyma, cork cells, irregular shaped starch granules and fusiform fibres and pits. Root microscopic characters shows the presence of starch granules with lignified xylem fibres, where as fruit microscopy shows the presence of epicarp, thin walled parenchyma and collenchyma, mesocarp and endocarp with micro rosette crystals.

Macroscopic studies shows reticulate renin lobe form venation in leaves, where as the macroscopic structure of root shows taproot surface glabrous, and stem 1-10 dm tall simple to branch with schizocarp fruit type (Akbar *et al*., 2014).

According to the recent report, the leaf of *Punica granatum L*. is a plant with decidious shiny leaves which grows at a height of 1.8-4.6m. The plants has immense medicinal usage. The pharmacognostic study shows the presence of simple opposite leaf arrangement with calcium Oxalate crystals in transverse section. Chemomicroscopic characters include starch and calcium oxalate crystals. The macroscopic and microscopic character of the plant helped in the preparation of a monograph of the plant (Bapodara *et al*., 2011).

Microscopic and Macroscopic studies of *Cassia occidentalis* shows the presence of dorsiventral leaf with flat lamina. The surface of the leaf displays unicellular trichomes, 124.0 - 333.3 μm in length. The stomata number and index number for the upper surface of the leaf is 104 -130 -156 and 13 -14 -16% and the stomata number and index for the lower surface is 247 -273 -312 and 26-27%

respectively. The palisade ratio are of 6-8-11 in the palisade cells. The powder microscopy of this leaf shows the presence of broken trichromes, wavy epidermal cells and anomocytic stomata (Amponsah, *et al*., 2016).

Stellaria media Linn. (Caryophyllaceae) has immense potential therapeutic usage for inflammation, asthma, mental tension etc. Microscopic and macroscopic studies indicates the character of leaves with the presence of palisade cells, epidermis, trichomes and vascular bundles. The stem shows the presence of epidermis, vascular bundles, epidermis, cortex etc. Total ash values are 11.24,acid insoluble ash value-2.04, ethanol soluble extract value-6.3 and water soluble extract value-37.7 % w/w respectively. This study is considered to be essential for the standardization of the drug studied (Arora & Sharma., 2012).

According to the recent study (Kumar *et al*., 2011) *Callistemon lanceolatus* known as bottle brush, is a shrub that grows up to a height of 7.5m. The leaves are lanceolate with clear veins oilglands, and midrib. Microscopic character indicates that leaf surface have anomocytic stomata with vein, veinlets. The leaves have upper epidemis layer followed by cuticle and vascular bundles. The mesophyll of the leaves shows differentiation as palisade and spongy parenchyma. Stomatal number, stomatal index, veinlet terminations were measured in leaf constants.

2.3 Importance of Organoleptic Characters

According to the recent report (Radhika., 2017) The organoleptic character of *Plumeria pudica* shows the presence of stomata in upper and lower epidermis of leaves. Leaves of this plant are different from other plant genus with fiddle or a spatula. Fresh leaves are green in colour with the characteristic bitter taste.

Powder analysis exhibits that the Plumeria powder contains countless raphide shaped calcium oxalate crystals, presence of xylem, multicellular long and covering trichromes with less starch grains, but shows the presence of phloem fibres.

Fagonia schweinfurthii Hadidi is an herb that grows in between rocks with traditional, medicinal uses. The whole plant powdered shows potential antibacterial activity. The plant has opposite entire leaves with spiny stipules, The stem is yellowish green with characteristic odour. The organoleptic evaluation reveals bitter taste and acrid. (Naik., 2010)

The fruits of *Tribulus terrestris* and *Pedalium murex* are used for treating various urinary disorder like calculus formation. The T.S of *Tribulus terrestris* fruit is pentagonal, mesocarp wide and transversed with vascular bundles and stone cells.

Pedalium murex fruit is 4 angled indehiscent mucronate spine, 4 sharp spreading spines, pedicel short curved, mucilaginous, T.S of fruit is Quadrangular, Mesocarp shows oxlate crystals, oil globule with 2 seeded fruits (Jayanthy *et al.,* 2013).

2.4 Phytochemical Analysis

Phytochemicals are biochemical compounds from plant source that has immense benefits to humans. Recent study emphasizes that 4000 phytochemical constituents have been studied for various physiological, protective function and chemical characteristics. About 150 phytochemicals have been analysed for further studies. (American Cancer Society, 2000).

Medicinal plants enriched with biochemical constituents act as a source of pharmacological effects against various ailments and diseases. The active phytochemical constituents act as anti-infectious, anti-oxidant and anti-cancer agents.

Bioactive constituents from plants prevents diabetes, blood pressure and muscular degeneration. In turn, phytochemicals are categorized by function, even as single compound has the property of both anti-oxidant and antibacterial agents. Phytochemicals extracted from plants are classified as primary or secondary constituents based on their role in plant metabolism.

Primary compounds include sugars, proteins and chlorophyll's etc. Secondary compounds include alkaloids, terpenes and phenols.

Terpenoids exhibit anti-inflammatory, anti-cancer, anti-malarial, anti-viral and anti-bacterial activities. Alkaloids are used as anaesthetic agents in medical field. Medicinal plant properties depends on chemical constituents such as alkaloids, terpenoids, phenols, tannins, sugar, proteins saponins, etc. The medicinal property of the plant depends on its solubility nature in various solvents (O'hara *et al.,* 1998; Monisha *et al.,* 2017; Nisa *et al*., 2013) Alkaloids derived from plants has various

biological activities such as anti-inflammatory, anti-microbial, Cytotoxicity and pharmacological effects (Minakshi *et al.*, 2016).

The phytochemical compounds with numerous ethanopharmacological properties enhance the scope for pharmacologist in active drug discovery (Wadood *et al.*, 2013).

Alkaloids and Flavonoids have active defensive mechanism against different pathogens (Hafiza, 2000). The terpenoids group exhibit various activities such as anti-viral, anti-bacterial, anti-malarial and anti-cancer activities (Mahato & Sen 1997).

Saponins with anti-coagulating and anti-ulceric activites are also used to stop bleeding and treating wounds (Okwu & Josiah., 2006).

Preliminary phytochemical analysis of *Hypochaeris radicata* L. confirmed the presence of alkaloids, flavonoids, saponins, terpenoids, glycosides, phenols and resins. These secondary metabolites has biological and therapeutic properties (Vishnu *et al.*, 2013).

Phytochemical analysis of *Taraxacum officinale* leaves revealed the presence of alkaloids, flavonoids, steroids, saponins, tannins and triterpenoid. It has more medicinal properties such as anti-inflammatory, analgesic and anti-diabetic activity. According to recent report (Kubmarawa., 2007 ; Mensah *et al*., 2008) alkaloids were present in 12 leafy vegetables studied . Ayitey and Addae., 1997 studied that bitter leaf contains alkaloids with the potential of reducing headaches associated with hypertension.

Nigerian soft wood has more phytochemical constituents. The qualitative and quantitative analysis determines that tannin was prevalent in Nigerian woods. *Cordia millenii* and *Sterculia oblonga* has high alkaloid content, where as *Barteria nigritiana* and *Moringa oleifera* exhibits more alkaloid content. The plant *Combretodendron macrocarpum* and *Glyphaea brevis* shows high oxalate content (Ezeonu & Ejikeme., 2016).

Ephedra intermedia plant has numerous phytochemical constituents. It shows the presence of sugars, cardiac glycosides, phenolic compounds, flavanoids and

alkaloids. On quantitative estimation it indicates the potent anti-oxidant effect (Gul *etal.*, 2017).

2.5 Antioxidant Activity

Free radicals are atoms or groups of atoms which are highly unstable and reactive. The most common free radicals has significant biological activity. It includes hydrogen peroxide, oxygen, hydroxyl radical, carboxy radical and superoxide radical. (Cayuela., 1995 and Schöneich 1999). This free radical when it associates with human body can result in oxidative damage, membrane damage, aging, heart ailments and cancer (Finkel & Holbrook 2000 and Olson & Kobayashi 1992).

Ethanopharmacognosy has gained more importance in the medicinal field due to its wide applications in treating all diseases. Natural antioxidants efficiently improves the nutrients, drugs, food, nutrients, and also enhance the activity of anti-inflammatory, antiallergic and antitumour capacities (Moure *et al*., 2001).

Natural antioxidants are usually derived from polyphenols, flavonoids and proanthocyanins. (Pellegrini *et al*., 2000 ; Pietta *et al.*, 2000). The plants with phenolic compounds has high antioxidant potential source (Akyuz *et al*., 2014). The isolation and purification procedures for antioxidant compound, though it is expensive, it requires broad research findings due to its high usage on human kind (Amarowicz *et al*., 2004).

Free radicals are involved in the pathophysiology of many chronic ailments such as inflammation, hypertension, cancer, and cardiac diseases (Florence, 1995).

Antioxidant compound potentially capable of scavenging these free radicals provide great scope for such oxidative imbalances. Synthetic antioxidants such as Butylated hydroxyl Toluene (BHT) and Butylated Hydroxyanisole (BHA) were in high use for mitigating oxidative stress related pathologies. But its toxicity and carcinogenicity necessitated the endeavour to opt for natural antioxidants (Ito *et al*., 1986).

Many research has been initiated on natural antioxidants of plant based origin as therapeutic agents for pathologies and diseases prevention (Greenwell & Rahman

2015). According to WHO, around 80% of the world population depends on herbal health care system. The traditional and folk system of medicinal plants has become necessary for two reasons (i) the plant based formulations and extracts improves human health. (ii) the purified compounds helps to formulate novel and readily available drugs.

Artemisia absinthium an important medicinal herb, has been evaluated for its antioxidant and cytotoxic properties against different cancer cell lines. The quantitative estimation of this plant revealed that the polar extracts of methanol and hydro-alcoholic are rich in phenolic and flavonoid content which is highly responsible for antioxidant activity (Parasuraman & Maithili , 2014).

Numerous assay such as DPPH, ABTS, and lipid peroxidation radicals has been approached for evaluating antioxidant potential (Gul *et al*., 2013). These assay has different mechanisms for scavenging activities. (Singh *et al.,* 2009 ; Gil *et al*., 2000).

Evolvulus alsinoides occurs throughout the region from India to West Cameroon and spread in tropical Africa and worldwide. It has wide pharmacological activity such as antibacterial, antidiabetic and anti-inflammatory potency. (Gomathi, *et al*., 2012). It involves in treating central nervous system depression, and a potential antioxidant. According to the recent study it indicated that *Evolvulus alsinoides* has antioxidant activity and it increase with increasing concentration.

Oxidation in natural food system is widely prevented by antioxidant. Natural antioxidants are safe and economically recoomendable. Many studies have been reported that black and green tea act as a potential antioxidant (Nedamani *et al*., 2015). Natural antioxidant has dominant role as bioactive nutraceuticals, bio-pharmaceuticals, food additives on routine basis. Apart from various applications it is intensively performed for anti-aging process (Kusuma *et al*., 2014).

Melastoma malabathricum possess antioxidant activity due to the presence of flavonoids, phenolics, alkaloids, tannin, aminoacids and volatile constituents. The results of this plant extract shows that high percentage of antioxidant at 1minute time immersion (Tangjitman *et al*., 2015).

Nutraceutical values in fruits has increased consumption of fruits both nationally and internationally (Jacques *et al*., 2009). Fruits includes many bioactive and antioxidant compound. The bioactive compound constitute heterogenous compounds such as glucosinolates, carotenoids or polyphenols including flavonoids and anthocyanins (Horst & Lajolo, 2012).

According to Chu *et al.,* 2000 it is observed that evaluation of antioxidant activity in fruits, vegetables and other plant cannot be estimated accurately by single method due to the complexity of phytochemicals (Du *et al.* 2009). Therefore it is necessary to characterise the antioxidant potential by its interaction against species responsible for oxidative damage (Yamaguchi *et al.,* 1999).

It is reported that *Helicteres isora* L. fruit and *Ceiba pentandra* L. *Gaertn.* Seed has potential scavenging activity, From the study it is concluded that *C. pentandra* seeds has superior antioxidant activity and radical free scavenging activities over the other extract. It is used in medicinal practice due to its wide nutraceutical and pharmacological activities.

Pomegranate juice has gained popularity due to its biological contribution (Lansky *et al.,* 1998). Hence the anti-oxidant and anti-tumoral activity of pomegranate barks tannins have been reported. (Schubert *et al.,* 1999). The juice is enriched with many phytochemical compounds such as anthocyanins, and the 3-glucosides and 3, 5-diglucosides of delphinidin, cyanidin, and pelargonidin have been reported (Du *et al.,* 1975).

Pomegranate juice also contains 1 g/L citric acid and only 7 mg/L ascorbic acid (El-Nemr *et al.,* 1990). The leaf, bark (Tanaka *et al*., 1986) and the fruit husk of pomegranate (Mayer *et al.,* 1977) are rich in ellagitannins and gallotannins. It shows high antioxidant activity higher than red wine and a green tea infusion. This difference is due to the presence of tannins but anthocyanins and ellagic acid contribute to the total antioxidant capacity.

2.6 Antimicrobial activity

According to a recent study by (Graybill, 1988; Ng, 1994; Gonzalez *et al*., 1996) multi-drug resistant microbial strains have been increasing continuously. This has led to the discriminate use of broad spectrum antibiotics. To add to it, synthetic

drugs are prone to side effects and very expensive for the treatment of various diseases. Therefore there is an immense need to search for new infection-fighting strategies to control microbes (Sieradzki *et al*, 1999).

From time immemorial traditional medicine has been inherited to many generations but it is in danger of being lost. Synthetic drugs replaced many herbal formulations. But however due to its efficicacy and economically affordability the search of potentially important pharmaceutical substances from plants is essential (Diehl *et al*., 2004 and Hammond *et al*., 1997).

Many plants possess antimicrobial activity (Martinez *et al*., 1996) African traditional medicinal plays vital role in many parts of Africa. In this community, traditional herbal healers takes advantage of the biodiversity of plant species to cure various ailments (Gelfand *et al*., 1985).

The resistance of antibiotics has threatened the human community and cause high mortality each year. Inappropriate use of antibiotics leads to multi drug resistance and cause treatment failure.

Curcuma xanthorrhiza, Ocimum sanctum, Senna alata, Kaempferia pandurata, Zingiber officinale, Moringa oleifera, Tamarindus indica, Pangium edule, were studied for antibacterial activities *Invitro* by the agar microdilution method against three resistant bacteria.

Itelima *et al.*, 2017 selected 14 plants such as *Allium sativum, Bryophyllum pinnatum, Citrus sinensis, Euphorbia hirta, Psidium guajava, Spermacoce verticilata, Aloe vera, Cassia ocidentalis, Mangifera indica, Myristica fragrans, Ocimum gratissimum, Piper guineese, Vernonia amygdalina* and *Zingiber officinale* were checked against 3 antibiotics drugs namely ampicillin, ciprofloxacin and streptomycin on *Escherichia coli* O157:H7 strain isolated from human clinical sample. Agar well diffusion method were employed to study the antimicrobial activity.

The results revealed that the ethanolic and water extracts of *Psidium guajava* showed maximum inhibitory effect with growth inhibition zone of 29.9 and 26.0 mm respectively at 500mg/ml. followed by *P. guagava, A. sativum, Z. officinale, V. amygdalina* and *M. indica* with inhibition zone of 21.2, 20.8, 20.3 and 19.9 mm respectively at 500 mg/ml.

Paenibacillus larvae spore forming gram positive bacteria is causative agent for American foulbrood disease in bees. The antibacterial activity was checked on this bacteria using extracts of the following plants such as *Achillea millefolium* (yarrow)*Thymus vulgaris* (thyme), *Urtica dioica* (nettle) and *Ocimum basilicum* (basil). The extracts were tested to determine the Minimal inhibitory Concentration (MIC) *Utrica dioica* (neetle) plant exhibited highest antimicrobial activity with less polyphenolic content where as *Ocimum basilicum* (Basil) exhibited both high level phenolic content and anti-microbial activity. *Thymus vulgaris* (thyme) shows least anti-microbial effect even with high phenolic content (Marghitas *et al*., 2011).

Crassula ovta has been in immense use among communities of Khoi of South Africa and in Chinese culture. It has been used in treating diarrhoea and disinfecting wounds. Aqueous and methanolic extraction of this plant were used to get better results. The strains used were the causative agent for diarrohea and wounds. Both the extracts were checked for anti-microbial activity. From the above results, it was concluded that the aqueous plant extract shows maximum inhibition than other extract (Muiruri & Mwangi., 2016).

Aloe vera L. has been known for its various phytochemical constituents. It is enriched with secondary metabolites such as tannin, saponin, flavonoids and terpenoids. The *Aloe vera* leaves has been screened for anti-microbial activity selected against human clinical pathogens by agar diffusion method. The anti-microbial activity was checked using different solvents Petroleum extract of *Aloe vera* L. showed maximum activity than chloroform extract. The strains such as *Aspergillus niger, Aspergillus fumigates* and *Neurospora crassa* were used for antifungal activity. The maximum anti fungal effect were seen in petroleum ether and ethanol extracts than chloroform (Kedarnath *et al*., 2012).

The plant extract of *Xanthium strumarium* (leaf and root) and *Mentha piperita* (stem) has wide antimicrobial activity against *Staphylococcus aureus*. *Ricinus communis* (leaves, stem, roots), *Ipomea aquatica* (stem) has wide antimicrobial activity against *E.coli*. but *Oldenlandia corymbosa* has very less anti-microbial activity against *Staphylococcus aureus* and *Escherichia coli*. The study clearly provided evidence for the presence of various medicinally important bioconstituents (Minakshi *et al*., 2016).

Biochemical constituents of *Zingiber officinale*, *Allium sativum*, *Allium cepa*, *Emblica officinalis*, and *Benincasa hispida* indicated the presence of alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins and reducing sugars this biochemical compounds exhibits anti-microbial effect against various pathogens. (Jadon & Dixit., 2014).

Allium sativum (Garlic) among five plants proved to be more effective against *E. coli*, *B. subtilis* and *S. aureus* 100 ul/well where as *Emblica officinalis* (Amla) showed less or no activity against the above pathogens.

2.7 *Invitro* Anti-inflammatory activity

Inflammation is the succession of changes occurring in living tissue. The inflammatory response towards tissue injury includes an array of enzyme, mediator release, fluid extravasations, cell migration and repair which activated in host defense conditions. Erythrocyte membrane or HRBC (Human Red Blood cell Membrane) is analogous to lysosomal membrane .

The stabilization of HRBC (Human RedBlood cell Membrane) by induced membrane lysis include as an *Invitro* measure of anti-inflammatory activity of the plant drug. The hydro alcoholic and ethanolic extract of the *Vitex leucoxylon* Linn leaves were screened for anti-inflammatory activity by Human Red blood cell membrane stabilization (HRBC). The membrane stabilization exhibited biphasic effects. Prednisolone taken as control and the acivities were decreased with time. The results revealed that this leaves has significant anti-inflammatory activity and provides evidence to use as drug against many diseases (Althaf Faimum *et al*., 2013).

The anti-inflammatory activity of *Symplocos cochinchinensis* Lour ssp laurina (Symplococaceae) by human red blood cell membrane stabilization method revealed that the methanol extract exhibited potential membrane stabilizing action on Human Red blood cell membrane by comparing with the standard diclofenac (50mg/kg) (Vadivu & Lakshmi, 2008).

The ethanol and ethyl acetate extract of *Lantana camara*, Linn was investigated for anti-inflammatory activity using cell membrane lysing technique with the standard drug ibuprofen. The ethyl acetate extract exhibited the presence of flavonoids, saponins etc. From the results it was concluded that the extracts exhibited

protection against the erythrocyte membrane effectively. In that the highest protection was given by ethyl acetate which needs further investigations for analysis. (Oyedapo *et al*., 2010).

Amaranthus gangeticus of the family Amaranthaceae, is widely used in eastern zone of India. Qualitative screening and membrane stabilization property were checked in alcoholic and aqueous extract of this plant. In this membrane stabilization study 50 microgram/ml sodium diclofenec used as reference drug. The study clearly indicated that this plant possess significant membrane stabilization property (De, Sarkar & Mukhophadhyay, 2017).

Solanum aethiopicum prevalent is in Asia and tropical Africa commonly known as African garden Egg. Its methanolic extract potentially inhibited lysis at a percentage of 40.8, 53.3 and 50.8 respectively at a dosage of 400,600 and 800 μg/ml. The inhibition concentration increased with increase in extract concentration and was comparable with Indomethacin (Anosike *et al*., 2012).

The hydroalcoholic extract and ethanloic extract of the V*itex leucoxylon Linn* leaves were analysed for anti-inflammatory activity by Human red blood cell membrane stabilization method on analysis, all fractions showed biphasic effect on membrane stabilization. Prednisolone served as a control.

The results showed that *V.leucoxylon* possess anti-inflammatory activities mediated by prostaglandin synthesis inhibition. It also clearly indicated the importance of leaves in the treatment of anti-inflammatory disorders (Althaf Faimum *et al*., 2013).

The leaf extract of *Gendarussa vulgaris* was screened for anti-inflammatory activity by human red blood cell membrane stabilization. The result indicated that *G.vulgaris* at various concentrations had significant anti- inflammatory activity. The alcoholic leaf extract of *Gendarussa vulgaris* at a concentration of 300 mg/mL exhibited potential anti-inflammatory effect using standard drug diclofenac sodium. This potential activity was attributed due to the inhibition of any inflammatory mediators or glycosides present in the extracts. (Saleem *et al*., 2011).

The methanolic extract of *Millettia pachycarpa* leaves was investigated for the anti-inflammatory activity by Human Red Blood membrane stabilization method.

It is commonly used in chinese traditional medicine. It showed good response by inhibiting haemolysis (34.30%) at the highest concentration. The inhibition was considered to be due to the presence of alkaloids, flavonoids and steroids which is screened during phytochemical investigation (Chowdhury *et al.*, 2013).

Gardenia coronaria has wide pharmaceutical properties used in the treatment of various diseases such as rheumatic pain, bronchitis etc. The leaves has been screened for Anti-inflammatory properties by membrane stabilization method. The methanolic leaf extract of *Gardenia coronaria showed potential anti-inflammatory property* at a concentration of *300 µ*g/mL The leaf extract prevented lysis at 33% but not greater than Aspirin as standard control (Chowdhury *et al*., 2014).

2.8 Techniques employed in identification of phytochemicals

Numerous techniques have been employed to estimate the bio active substance such as alkaloids, terpenoids, flavonoids, steroids, tannins, etc. The most popular techniques includes chromatography and spectroscopic techniques.

TLC: (Thin layer chromatography)

Thin layer chromatography is a techniques used to separate many compounds at different ranges. It is utilized for quantitative and qualitative work (Egon Stahl, 1965). It is a simple quick and inexpensive technique that has many advantages for multiple detection and specific derivatization on the same plate.

Thin Layer chromatography of *Guiera senegalensis* extracts showed the presence of various phytochemical components.The ethanol extract of *G. senegalensis* in solvent system hexane and methanol 4 : 1 (V/V) showed 5 detected Rf values and hexane extract showed 6 spots rf values and in water 5 spots were detected.

Samples of *Potentilla* sps were chromatographed on silica gels using the mobile phase: chloroform- diethyl ether-methanol-formic acid (30:10:1:0.2 v/v/v/v); They were derivatized and visualized in UV 366 nm. Then *Potentilla* sps finger print were processed.

From the analysis the separation of closely related five triterpenic acids were presented. The analysed *β*-sitosterol, stigmasterol phytosterols didn't interfere.4 triterpenic acids such as ursolic/oleanolic acid, pomolic acid and two optical isomers-

euscaphic and tormentic acid) were separated without prechromatographic derivatization. The oleanolic acid and ursolic acid were separated by prechromatographic derivatization method.(Świeboda *et al.,* 2014).

TLC (Thin layer chromatographic) studies of *Moringa olifera* root extract were carried out by using different solvents with different polarities such as hexane, chloroform, ethyl acetate, acetone and methanol extracts. It indicated the presence of alkaloids, saponins, glycosides, phenolic compounds, tannins, phytosterols, carbohydrates, proteins, amino acids, flavonoids, quinones and terpenoids . Different types of results were obtained in different solvents extracts.

2.9 GC-MS Analysis

The analysis of compounds directly in traditional plants was carried out by Gas Chromatography – Mass Spectrometry (GC-MS). It is a valuable method to identify the non-polar compound and volatile essential oil, lipids, fatty acids and alkaloids (Betz *et al.,* 1997). Hydrocarbon with long chains, alcohols, acids, esters have been identified by using this method. (Balamurugan *et al*., 2012).

GC-MS chromatogram of the ethanolic extract of *Ludwigia perennis* revealed the presence of twenty nine compounds. In that bioactive compounds,13 - Docosenamide (Z) has anti-microbial activity; (4, 3', 4' - Tris (methoxy carbonyl methoxy)-6, 6' - dimethy lbiphenyl-3- yloxy) acetic acid methyl ester has anti-oxidant and antimicrobial activity; Ergost-7-en-3-ol, (3á) has anti-tumor, immunomodulatory activity, inhibitory hemolytic activity, anti-inflammatory and anti-viral activity (Sharmila *et a l*., 2017).

In Clerodendrum Phlomidis leaf extract seven different compounds were identified by GC-MS analysis namely Isopropyl Linoneate, Hexadecanoic Acid, 2-Hydroxyl-1-[Hydroxymethyl] Ethyl Ester, 9- Octadecenoic Acid [Z]-, 2-Hydroxy-1-[Hydroxymethyl] Ethyl Ester, 1,11- Tridecadiene, Hexadecane, Benzene 1- methyl-4-nitroso, 1 [2Acetoxyethyl] 3,6 diazahomo adamantan-9-one oxime. (Balaji *et al*., 2014).

Zayed and Samling (2016) analysed the presence of 30 compounds in the leaf *Leucaena leucocephala* by GC-MS analysis. The major compounds are Squalene

(41.02%), Phytol (33.80%), and 3, 7, 11-Tridecatrienenitrile, 4, 8, 12-trimethyl (25.64%).

The plant *Cucumis callosus* has 32 compounds identified by GC-MS analysis Doxorubicin brings potential changes in biochemical parameters and anti-oxidants in the heart (Varadharajan, *et al*., 2016).

The methanol extract of *Justicia adhatoda* were analysed for phytochemical compounds by GC-MS method. The isolated compounds includes Amrinone (RT: 15.88) n- Hexadecanoic acid (RT: 16.33) Phytol (RT: 17.81) 9, 12, 15- Octa deca trienoic acid, (Z, Z, Z) - (RT: 18.04) along with other constituents (Jayapriya & Shoba, 2015).

Patel *et al.,* (2017) reported that the methanolic extracts of *Terminalia coriacea* leaves using GC-MS method reveals the presence of 14 bioactive volatile compounds. The biochemical constituents are 1H-inden-1-one, 2, 3-dihydro - 3, 3, 5, 6, - tetramethyl; levoglucosan; neophytadiene; phytol; hexadecanoic acid; n-hexadecanoic acid; stigmasterol; β-sitosterol; raffinose; 1, 2-benzenedi carboxylic acid; undecanoic acid; (2 propyl-1,3-dioxolan-2-yl) acetic acid; 2, 2 dimethyl propane, and octadecatrienoic acid (Thanwar *et al ., 2017).* The bioactive constituents were reported by GC-MS method in of *Catharanthus roseus* leaves. The identified major compounds are Mono-inosital, Hexadecanoic acid, methyl ester, Hexadecanoic acid, 9-octadecanoic acid(Z)-methyl ester, Heptadecene-(8)-Carbonic acid-(1), Octanoic acid, Eicosanoic acid, methyl ester, Icosanoic acid, 1, 2-Benzenedicarboxylic acid, Squalene, Desmethoxy-vidoline respectively.

2.10 Significance of Heavy Metal Analysis in Medicinal Plants

Heavy metals are elemental property of metals with an atomic Number less than 20. Soil has metals which are natural components in soil (Lasat, 1999) The contaminants of the soil includes Cd, Cr, Cu, Hg, PB and Zn Which plays a potential role in soil pollution.

The metals which are necessary for plant growth such as Zn, Mn, Ni and Co are micronutrients and some metals such as Cd, Pb, Hg (Gaur and Adholeya, 2004) have unknown biological function.

Toxic heavy metal pollution causes many hazards to human biological systems and are non-biodegradable. They even cause many disorder and life threat in very low concentration (Pehlivan *et al.*, 2009). It even has negative impacts on soil microflora (Roy *et al*., 2005). Since this toxic metals cannot be chemically degraded it is a great challenege to be removed physically or to be transformed in to non-toxic compounds.

Heavy metals accumulation in soil contributes to contamination of the ecosystems. Besides from nature, humans have a major part in contaminating the soil with toxic metals. These contaminants migrate in to non-contaminated areas as dust or leachates in the form of soil and cause major hazards in soil pollution.

Many technologies have been in access to prove the presence or mobility of metals in soil, water and waste water, but phytoremediation has become effective and appropriate tool to remove or inactivate the toxic metals. This technology proves to be cost effective and eco-friendly (Shtangeeva *et al*., 2004).

As plants are widely used in traditional medicine the knowledge of heavy metal content in plant is essential to prevent toxicity in humanbeings (Chuparina and Aisueva, 2011).

Nickel is an essential elements in regulating lipid contents in tissues and to form red blood cells. The high level concentration of nickel becomes toxic and cause severe diseases like heart failure, skin problems, vision impairment etc.

Heavy metal analysis shows that the plant *Sarcocephalus latifolius* harvested in farm land shows high concentration of nickel (Khan *et al*., 2008).

According to WHO The permissible limit of nickel is 1.5 ppm,where as the nickel concentration in this plant was 7.69 ppm which is even hazardous to the environment. The possibility of nickel concentration is due to natural and anthropogenic sources (Rathor *et al*., 2014).

Heavy metal absorbtion is time dependent and accumulate in plant parts (Khan *et al*., 2007). Prolonged exposure to metals in certain plants become hazardous to humans.

The international agency for research on cancer (IARC) identified toxic metals such as arsenic, antimony, beryllium, cadmium, chromium, cobalt, lead, nickel, and vanadium as highly carcinogenic. These toxic metal results in DNA damage and thus stimulate mutagenic activity.

So the researchers has emphasized on monitoring metal toxicity in plants to inhibit carcinogenic effects in humans (Liang *et al.*, 2004; Arceusz *et al.*, 2010).

According to the report, grasses are metal tolerant plants (Rosselli *et al.,* 2003) and has high metal accumulation (Pichtel and Salt 1998) hence they are harmful to health. *Vetiveria zizanioides*, a grass species potentially used in treating cardiovascular diseases (Rajurkar and Damame 1997). The plant collected from metal contaminated sites may result in adverse toxic effects on the consumer. Heavy metal accumulation alter the chemical composition in plants and thus affect the quality of the plant species (Lizhong and Cullen1995).

Clematis gouriana used in treating skin ailments takes up very high Cd levels. Regularly consuming trace amount of Cd can influence the physiology, reproductive and health of the organisms (Meena *et al.,* 2010).

Woodfordia floribunda has been in wide usage in pharmaceutical industry for its active bioactive content. This plant also accumulates high level of Pb beyond permissile limit. High level of Pb content damage majority of the organs like Kidneys,circulatory system and central nervous system which is highly fatal to human beings.(Lansdown and Yule 1986).

Cleistanthus collinus has therapeutic properties like anti-leshmanial, anticancer, anti-HIV (Karayil *et al.,* 2014). Besides its therapeutic potential, it contains numerous lethal compounds and minerals which result in many medical issues.

The most popular culinary and medicinal herb basil has been in use from ancient time. It has wide pharmacological properties. The leaves and flowers have been in use for the treatment of headaches, cough, kidney malfunctions and anti-pasmodic properties (Hassanpouraghdam *et al.,* 2010).

The heavy metal analysis has been carried out in different species such as sweet basil (*Ocimum basilicum*) black basil (*Ocimum sanctum*) and dwarf basil (*Ocimum minumum*). Among three species, sweet basil showed highest heavy metal content. Accumulation of metals are relatively high in flowers and leaf,stem comparatively very less in root. Their contents are found in decreasing order: leaf > flower > stem > root (Dunbabin *et al.*, 1992).

The contamination of heavy metals in basil leaves are expected due to contaminated soil, ground water and dust. These sources contribute for heavy metal contamination. The medicinal plants grown in polluted site are highly dangerous and hazards to all living organisms (Reid Hayes, 2003).

2.11 Application of Medicinal Plants in Green Synthesis of Silver Nanoparticles

The synthesis of nanoparticles using plants has wide applications. The synthesis of nanoparticles has been initiated by adding 1mM silver nitrate to the wheat grass extract. The formation of nanoparticles is indicated by the absorption at 420nm. The study revealed that synthesized nanoparticles has potential antimicrobial activity against gram positive and gram negative bacteria and also exhibits effects on seed germination and formation of nodules (Nandedkar Pushpa and Khandare Sopan A, 2018).

Gloriosa superba (family Liliaceae) proves to be rich in alkaloids. It is an endemic shrub found in Talakona forest of Andhrapradesh. The aqueous leaf extract of *Gloriosa superba* has the potential to synthesis silver nanoparticles. This silver nanoparticles inhibited the growth of four different human cancer cell lines *(*SKOV3, DU145, A549, PC-3), at a minium IC of C50 79.45 is ± 5.26,61.80 ± 4.27, 94.74 ± 9.26, and 90.10 ± 8.24 μg/mL. The potential of anticancer activities exhibited by plants is due to the presence of alkaloid and other secondary metabolite of the plants (Bethu, 2017).

The synthesis of silver nanoparticles were characterized by UV-Visible, particle size analyser (DLS), Scanning electron microscopy (SEM), transmission electron microscopy (TEM) and energy-dispersive spectroscopy (EDS). FTIR analysis were carried out to confirm the compound responsibe for the capping agents in leaf extracts of three plants such as *Musa balbisiana* (banana), *Azadirachta indica*

(neem) and *Ocimum tenuiflorum* (black tulsi). This synthesis of silver nanoparticles exhibited significant antimicrobial activities against *E. coli* and *Bacillus sps* compare to raw plant extract (Banerjee *et al*., 2014).

The synthesis of silver nanoparticles from the peel of *Raphanus sativus L.* extract showed potential reducing and stabilizing agent. The formation of silver nanoparticles were indicated by the color change of mixture from red to dark-brown. The silver nanoparticles were characterized by using UV-visible spectroscopy, scanning Electron Microscopy (SEM), X-ray diffraction (XRD) and dynamic light scattering (DLS). The silver nanoparticles showed surface plasmon resonance at 430nm (Fadel & Al-Mashhedy, 2017).

The aqueous fruit extract of *Garcinia mangostana* shows the potential of synthesizing silver nanoparticle which were confirmed by UV-Visible spectrophotometer at 430nm. These bioctive silver nanoparticles were tested for antibacterial activities against *Escherchia coli, Pseudomonas auroginosa,* and *Staphylococcus aureus.* The results showed significant inhibitory activity on the selected bacteria. The synthesized nanoparticles were also tested for antioxidant activity by DPPH assay. The biosynthesized silver nano particles showed significantly higher antioxidant activity compared to *Garcinia mangostana* extract. (Rajakannu *et al*., 2015).

2.12 UV- VIS & FTIR analysis

Phytochemical characterization of plants is significant for therapeutic action. The chemical composition of plants depends on species, subspecies, geographical location, harvest time, plant parts used and isolation method using this background the screening of plant extracts for anti-microbial and antioxidant activity is essential (Botanical Survey of India 2002).

Fourier Transform Infrared (FTIR) spectroscopy reveals the amount of composition and structural information in plants and seems to be time saving to characterize and identify the functional groups. In this technique molecules shows absorbtion in different frequency and most of the molecules shows its absorbtion in the range of 4000 - 400cm-1 which helps to study these compounds (Srinivasan *et al.*, 2011).

UV-Visible spectroscopy compute the functional groups present in the plant, by comparing with the standards (Vijayarekha & Sengottaiyan, 2015). The light in UV-VIS spectroscopy is in visible ranges. The absorbtion range is indicated by its colour or chemicals and electronic transitions of the molecules occur in the UV-VIS ranges of the spectrum.

Caralluma nilagiriana species was reported (Renuka et al., 2016) to have anti-pyretic, antihelminthic and analgesic activity. The bioactive compounds has been identified using UV–VIS FTIR spectrum profile. Based on the analysis the functional groups were identified based on the peak values. FTIR analysis confirmed the presence of alcohol, phenol, terpene group. The UV-VIS spectrum were done at the range of 190-900nm wave length. The absorption bands at 227.6, 401.6 and 664.6 nm exhibits flavonoids content. The precise position and relative intensities give valuable information on the nature of the compounds (Saxena& Saxena 2012 ; Sahu & Saxena 2013).

Characterization of bioactive constituents in *Meizotropis pellita* were analysed using UV-VIS and FTIR spectroscopy method. The methanolic extract of the plants when analysed through UV-VIS that showed peaks at 660, 340, 270, 235 and 210 nm with the absorption 0.085, 1.250, 2.605, 4.455 and 3.639. The FTIR spectra exhibits the presence of alcohol, aldehydes, carboxylic acid and halogen compounds (Rani *et al.*, 2016).

The extracts of *Micrococca mercurialis* (acetone, petroleum ether, chloroform, ethanol) were subjected to UV-VIS and FTIR spectrum analysis for the presence of biochemical constituents. The UV-VIS spectrum showed the peaks at 214, 446 and 472 nm with the absorption of 0.599, 0.655, and 0.550 respectively, revealing the presence of alcohols, phenols, alkanes, alkynes, alkyl halids, aldehyde, aromatics, nitrocompounds and amines (Dhivya & Kalaichelvi, 2017).

2.13 Studies on the effect of Phytomedicines in animal models

2.13.1 Acute toxicity

Toxicology is an art of medicine which includes observational data gathering and data utilized to predict the effect of plant drugs on humans and animals. Some

traditional medicines has been categorized as harmful and some as safe effective drugs (WHO Guidelines 1992).

All living things are exposed frequently to xenobiotics such as manmade chemicals viz. drugs, pesticides, pollutant pyrolysis in cooked foods, secondary plant metabolite like alkaloids, toxins released by moulds, plants and animals.

In a biological system poisons are released as a deleterious response as a result of injuring the system or producing death. Toxicologist usually divide the exposure of animals in to four categories which includes acute, subacute, sub chronic and chronic.

In India Ayurveda has been in use since time immemorial. It is considered as safe and effective drugs. Polyherbal formulations were in indigenous use to counteract the toxic effect of drugs extracted from plants. (Rajakaruna *et al.,* 2002).

According to organization of (OECD) Economic Co-operation and Development The acute toxicity is the effect that occurs after oral administration of a single or multiple dose. This advanced effect of acute toxicity occurs within 24hrs.Phytocompounds with toxic effects leads to injury or death of living tissues.

Toxicologist proves that toxicity arises from potent toxic compounds and non-toxic compounds also shows toxicity even at a lower dose by producing adverse effects on interaction with animals and plants. Hence it clearly indicates that not all medicinal plants are safe to consume, therefore proper investigation has to be done for its safety, efficacy and properties.

Euphorbia hirta is well known for its pharmacological activities and studied for acute toxicity effect (Bown, 1995). It is often been used traditionally for respiratory disorders, jaundice, gonorrhea, digestive problems and tumours.

Senna alata Linn. belongs to the family Fabaceae known for its immense medicinal activities (Lim 2013; Karthika 2016) especially antihelminthic activities (Kundu *et al.*, 2014).

Though this plant has immense pharmacological activities but it was checked for its acute toxicity. During the 15 day acute toxicity studies it clearly revealed that no behavioural distress or change in skin colour, eyelids, sleep, food and water intake and no observable toxicty symptoms were observed (Klaassen, 2013).

Histopathalogical studies shows no lesions in the organs which suggest the safety level of alcoholic extract on the animals. Oral acute toxicity of the *Brucea javanica* Merril plant extract was analysed on male and female DDY-Mice. The leaves extract was administered for first 24hrs at various dose levels (562.5 mg/kg bw (body weight), 1125 mg/kg bw, 2250 mg/kg bw, and 4500 mg/kg bw) to reveal its toxic effect by testing on its various vital organs (liver, heart, lymph, lungs etc) LD50 was determined using Reed ad Munich formula. The result showed that this leaves extract is slightly toxic (Angelina *et al.*, 2012).

Abrus precatorius leaves has been in long term use for the treatment of many diseases such as cough, malaria and infertility in women. It is known for its pharmacological effects. Aqueous, 70% methanol, petroleum ether and acetone extracts were investigated for oral acute toxicity study.

In this study, the graphical method of miller and Tainter was used to analyse their LD50 using graded doses ≤ 5000 mg/kg (oral limit dose). It is concluded that acetone extract showed lowest L50 value (187 mg/Kg) indicating high toxicity and methanol extract showed LD50 value (3942mg/kg) revealing less toxicity. Histopathological study showed changes in the examined organs such as the hepatotoxicity, cardiotoxicity (Ogbuehi, 2015).

Pericampylus glaucus (Lam.) Merr was tested for acute and sub-acute oral toxicity on animal models. The oral dose (300, 2000 and 4000 mg/kg) of plant extract was administered to three groups in single dose and mortality, adverse effects were observed up to 72 hr. In sub acute toxicity the plant extracts were administered at a dose of 600 and 1000 mg/kg for 28 days and evaluated its toxicity effect. The result indicated that the oral administration of *Pericampylus glaucus* (Lam.) Merr extract doesnot show any toxic effects (Kifayatullah *et al.*, 2015).

2.13.2 Analgesic Activity

Pain plays a vital role in protective mechanism in our body but an unpleasant sensation. Analgesic are substances which reduce the pain by stimulating pain threshold to external stimuli with consciousness. Therefore drugs with analgesic activity against anti-inflammatory drugs (NSAIDS) and opiates are a great demand to the society (Zulfiker *et al.*, 2010). Normally available analgesic drugs exhibits more

adverse effects such as gastrointestinal disorders, kidney problems and other unwanted effects.

The alcoholic extract of *Leonurus cardiac* as subjected to analysis at a dosage of 500 and 250mg/Kg by intraperitoneal administration. From this analysis, it is concluded that the intraperitoneal administration produced a significant antinociceptive in first and second phase of formalin test, the increase in antinociceptive effect at a dosage of 500mg/Kg were observed in tail flick and Hot plate test. The result indicated that this plant has significant central and peripheral antinociceptive activity (Rezaee-Asl *et al.,* 2014).

Papaver somniferum with narcotic alkaloid constituent called morphine has apioid receptor. The molecular signal activates the receptors in brain stem as well as in thalamus thus it relieves pain and sedation. The brain and spinal cord has delta receptor which activates analgesic effects (Hallett *et al.,* 2013).

According to the recent report (Das *et al*., 2014) the phytochemical screening and analgesic activity of *Oroxylum indicum* was determined by hot plate, acetic acid-induced writhing and formalin test. The standard drug Ketorolac at 10mg/kg was used in analgesic activity. The ethanolic extract of the plant at 250 and 500mg/kg doses showed potential analgesic effect.

Lavandula officinalis root is significant anticonvulsant and leaves exhibits analgesic effects. It has wide ethano pharmaceutical properties as anti-depressants,anti-rheumatic and cures various skin ailments. This plant stimulates parasympathetic nervous sytem in relieving pain with 1, 8 cineol as active ingredient. This active ingredient prevent araclhidonic metabolism which is pain mediators. In clinical investigations it is proved that this plant has significant analgesic effect on humans by inhibiting degranulation of host cells and release of histamines (Hajhashemi *et al.,* 2003).

Sida tiagii Bhandari (*S. tiagii B*) prevalent in India and Pakistan traditionally used as analgesic, anti-inflammatory, sedative, anti-seizure and anti-paltelet. The study was investigated to explore the analgesic activity in this plants. The fruits were ground with 90% ethanol, n-hexane, ethyl acetate. The extract of fruit *Sida tiagii* Bhandari were evaluated by acetic acid induced writhing method, tail immersion and

tail flick test in swiss albino mice. From the above analysis it is confirmed that this Ethyl acetate extract of this plant has potential analgesic activity (Kumawat *et al., 2012).*

Kalanchoe pinnata (Lam.) Pers is a prevalent herb commonly used in Bangladesh, India etc. A study was under taken to investigate the analgesic activity in the ethanolic fractions of the leaf of *K.pinnata by* acetic acid-induced writhing and Formalin induced persistent pain test. From the study it was confirmed that the dosage at 400mg/kg body weight,displays significant analgesic activity (Ahmed 2012).

2.13.3 Anti-pyretic activity

Pyrexia or fever is the body's natural defence mechanism due to the infected or damaged tissue. The initiation of proinflammatory mediator's like cytokines, interlukin 1β, α, β and TNF-α during tissue damage increase the synthesis of prostaglandin near hypothalamus area and there by triggers the hypothalamus to elevate body temperature. (Saper & Breder, 1994).

The Temperature regulatory system is a nervous feedback mechanism that occurs when body temperature rises dilation of blood vessels occurs and increase sweat to reduce the temperature. But when body temperature is very less hypothalamus protect the body by vasoconstriction of blood vessels.

Progression of disease often increases with high fever by tissue catabolism, dehydration and existing complaints as found in AIDS (Veugelers *et al.*,1997). Anti-inflammatory drugs generally has anti-pyretic activity (e.g) Non-steriodal anti-inflammatory drugs (NSAIDS). NSAIDS inhibits prostaglandin synthesis which in turn enhances anti-pyretic activity.

Synthetic drugs such as paracetamol, aspirin, nimusulide etc with anti-pyretic activity has more side effects So, search for safe herbal remedies with anti-pyretic potential is essential.

The results of sub acute toxicity reveal that the ethanol extract of *Azima tetracantha* possess a significant antipyretic effect at a concentration of 200 mg/kg in rats compared with the control. The single bioactive substance or compounds present

in the plant contribute to the antipyretic effect. It is also assumed that the presence of flavonoids may also enhance the antipyretic effects .

Flavonoids, triterpenoids and steroids plays a vital role in anti-pyretic effect. The presence of these compounds in *Leptadenia reticulate* enhances the anti-pyretic effect. Anti-pyretic effect were observed in two models i.e baker's yeast induced prexia in albino rats and cow milk induced pyrexia albino rabbits. Aqueous extract of *Leptadenia reticulate* at 200 mg/Kg body wt and 400 mg/Kg body wt potentially reduced the elevated body temperature in both the models (Mahalakshmi *et al*., 2013).

Ethanomedicine analysis of plants employed as pain relievers is one of the productive strategies in search of new drugs (Devi *et al*., 2003 and Elisabetsky *et al*., 1995) *Aloe volkensii* (family Asphodeteceae) is of great utility worldwide (Vongtau *et al*., 2004). Phytochemical screening of this plant reveals the presence of saponins, tannins, flavonoids, alkaloids and phenols.

The analysis of *A. volkensii* scientifically confirms that dose of 150 mg/kg, 100 mg/ kg and 50 mg/kg body weight of *Aloe volkensii* aqueous extract is more effective in reducing fever. A dose of 150 mg/kg, 100 mg/ kg and 50 mg/kg body weight of *Aloe volkensii* aqueous extract is more effective in the management of fever, inflammation and pain respectively. The study, therefore, scientifically confirms and supports the aqueous leaf extract of *Aloe volkensii* efficiency towards antipyretic effect.

Mollugo pentaphylla Linn (family-Aizoaceae) commonly called as carpet weed has broad ethanopharmacological properties such as anticancer (Lin *et al*., 2004) spermicidal (Jha *et al*., 1984) antibacterial (Smita & Sharma 2010) and anti fungal activity (Nene *et al.*, 1968).

Brewer's Yeast induced pyrexia method in albino rats were used to evaluate the anti-pyretic effect of this plant. The results revealed that ethanolic extract at 400mg/Kg showed maximum antipyretic activity than the extract of petroleum ether. It indicates that *Mollugo pentaphylla* has significant antipyretic effect.

After intraperitoneal administration of methanolic extracts of *Bidens pilosa* (500mg/kg body weight) to fever induced rabbits a significant reduction in body

temperature were observed. Control were setup for experimental groups. The results confirmed that *Bidens pilosa* shows remarkable anti-pyretic effect .

2.13.4 Anti-inflammatory activity

Inflammation is a normal protective response in human body against tissue damage caused by physical trauma, toxic chemicals or pathogens (Calixto *et al*., 2004). It is a complex biological phenomenon against harmful pathogens, characterized by pain, warmth, swelling and redness (Palladino *et al*., 2003).

Acute inflammation is a initial response to harmful stimuli and chronic inflammation a major inflammatory response to the body. Cyclo oxygenase is the key enzyme involved in the secretion of prostaglandins, prostacyclins and thrombokinase as result of inflammation, pain and platelet aggregation (Pilotto *et al*., 2010).

Chronic inflammatory diseases forms the base for many diseases such as heart attack, Alzheimer's diseases and cancer (Coussens & Werb, 2002) NSAIDS are the steroidal anti-inflammatory drugs that block the production of enzyme CO_{X-1} and CO_{X-2} .Long term uses of these drugs cause hazardous effects such as gastric lesions, cardiovascular, renal failure (Huerta *et al*., 2005) and damage human biological systems such as liver, gastrointestinal tract etc.

Plants has a major role in treating chronic and even infectious diseases (Thatoi & Dutta 2009). Many medicinal plants exhibit more pharmacological activities against inflammation and it is proved using many models such as histamine, dextran,serotonin induced hind paw oedema, Freund's Adjuvant, cotton pellet induced granuloma, experimental models of acute and chronic inflammation.

Acute inflammation is characterized by dialation of blood vessels, extravasation of fluid and proteins and aggregation of white blood cells (Posadas *et al*., 2004). Failure of acute inflammation leads to chronic inflammatory diseases (Recio e*t al*., 2012). Chronic inflammatory mediators include PGE2, lipoxygenase and nitric oxide. The inflammation ailments includes chronic peptic ulcers, rheumatoid arthritis, systemic lupus, asthma etc (Nordqvist, 2015).

Anti-inflammatory activity of methanolic leaf extracts of *Kigelia africana* and stem bark extract of *Acacia hockii* showed a significant anti-inflammatory response

on carrageenan induced paw edema in swiss albino mice. These studies were consistent with other studies using animal models. The anti-inflammatory studies were carried out by comparing with control Dichloro methane. Similar studies were demonstrated in methanolic stem bark extract of *Tinospora cordifolia* Wild, rhizomes of *Cyperus rotundus* Linn and fruits of *Emblica officinalis* the response were studied in rodents (Mradu *et al*., 2013).

The methanolic leaf extract of *Kigelia africana* and stem bark extract of *Acacia hockii* showed potent response on carrageenan induced paw edema in mice. It is a dose dependent response and compared to reference drug (diclofenac). The extracts were highly active at the dose level of 150 mg/kg body weight in the fourth hour of treatment. Both the plants proved as a bioresource for generating anti-inflammatoy response.

Bamboo leaves are widely used in Ayurvedic medicine in paralytic complaints. This is due to the presence of manna a crystalline substance. Phytochemical analysis of this plant revealed that it contains flavonoids, glycosides, proteins and alkaloids. Flavanoids exhibit anti-inflammatory activities due to their inhibitory effect on chemical mediators of inflammation (Owoyele *et al*., 2005).

Many evidences shows that plants has been used in Ayurvedic medicine for treatment of inflammatory disorders and wound healing activities (Gacche *et al*., 2011).

Aqueous and ethanolic extract of *Kalanchoe pinnata* (Lam) Pers was tested for ani-inflammatory activity against carrageenan induced paw-oedema in rats. Indomethacins were taken as reference control. The extracts of dried stem of the plant showed high potential anti-inflammatory activity. The root of Bilwa was tested for anti-inflammatory response in albino rats using carrageen induced paw edema and cotton pellet induced granuloma. For reference purpose indomethacin was taken a control. The result clearly showed that the plant has inhibitory effect on inflammatory response (Benni *et al*., 2011).

Bryophyllum pinnatum, it's anti-inflammatory response was investigated by Ojewole *et al*., 2005, Fresh egg albumin-induced pedal (paw) oedema model were used along with Diclofenac (100mg/Kg) as control. The results revealed that different

flavonoids, polyphenols of this herb are speculated to have for anti-inflammatory of the plant.

The anti-inflammatory potential of whole plant of *Cassia occidentalis* in ethanolic extract was investigated for anti-inflammatory activity. The dose taken was 250 mg/kg using carrageenan induced paw edema model. The result revealed that reduction in malondialdehyde levels of hepatic microsomes significantly reduced inflammation in carrageenan induced mice at a dose of 250mg/kg (Sreejith *et al*., 2010).

The aqueous extract of *Emblica officinalis* has shown anti-inflammatory effect. This effect was tested on the synthesis of inflammatory mediators such as leukotriene B4, platelet activating factor (PAF) and thromboxane. It is a tree prevalent in subtropical and tropical parts of China, India, Indonesia and Malaypeninsula. The result revealed that the aqueous extract has potential anti-inflammatory activity (Asmawi *et al*., 1993).

Zingiber officinale has wide ethanopharmacological properties. According to recent investigations by Shimoda *et al*., 2010 40% ethanolic extract from dried red ginger valuated for its anti-inflammatory activity against acute and chronic inflammation models.

2.14 Anticancer Studies

2.14.1 MTT assay

3- [4, 5-dimethylthiazole-2-yl] -2, 5-diphenyltetrazolium bromide (MTT) The drug either from natural source or synthesized by chemical means are analysed for its safety of the host cell or for the cancer cytotoxicity effect. This cell viability test varies from simple to complex one. For cell membrane permeability exposure of cell to trypan blue can be useful to identify viable cell. Trypan blue doesnot stain dead cell (Strober, 1997).

Many methods such as enzyme activity, cell membrane permeability, celladherence, ATP production, co-Enzyme production and nucleotide uptake activity were used. In that, MTT is one of the frequently used methods.

To determine the viability of the cell, this method use colorimeter (Mosmann, 1983). In MTT assay, the linear relationship between metabolically active cells and the colour produced is established based on viability and cytotoxicity of the cells. This assay based on cellular activities due to NAD(P)H-dependent cellular Oxidoreductase enzymes (Berridge *et al*., 2005). The viable cell exhibits high rates of MTT reduction to formazan while the dead or inactive cell fails to do so. The final purple colour formazan is formed on MTT reduction which can be easily dissolved in DMSO (dimethyl sulfoxide). Viability is related with quantification of formazan at 540nm.

The determination of cytotoxic effect of methanolic extract of *Origanum vulgare* on HCT-116 and MDA-MB-231 cell line were analysed by MTT assay. The extract of this plant exhibits significantly lower cell growth activity when compare to controlled cells. The effect of inhibition is more in HCT-116 cell line than in MDA-MB-231. From the results it is concluded that methanolic extract of Origanum vulgare act as potential drug for anti-proliferative and cytotoxicity effect (Grbović *et al* ., 2013).

The cytotoxicity of aqueous and ethanolic extracts of *Ficus deltoidea* on human carcinoma cell lines were determined by MTT assay (Akhir *et al*., 2011). From the results it is observed that both aqueous and ethanolic extracts of the plant sample gave IC50 values of 224.39 + 6.24µg/ml and 143.03 ± 20.21 µg/ml respectively. The results shows that both extracts cause apoptosis at 1000 µg /ml where as aqueous extract prompted for cell detachment and the ethanolic extract tried to inhibit cell proliferation by DNA fragmentation.

The medicinal plants such as *Emblica officinalis, Terminalia bellerica, Terminalia chebulla, Withania somnifera, Cyperus rotundus, Alpinia galangal, Oscimum sanctum, Adhatoda vasica, Piper longum* were selected for potential cytotoxic activity. Alcoholic and aqueous extracts of this plants were analysed by using MTT assay against Vero cell line. The study suggested that plant extracts shows less cytotoxicity on normal Vero cell line (Bhatt *et al*., 2016).

The *Invitro* cytotoxicity effect of methanolic and aqueous extract of aerial parts of *Cocculus hirsutus* on MCF-7 breast cell lines were determined by MTT assay. The result exhibited that the standard Tamoxifen IC50 value, methanol extract were

9.3, 39.6 μg/ml respectively. Thus methanol extracts of *Cocculus hirsutushas* showed anti-oxidant anti-proliferative activities.

Numerous medicinal plants were used for the treatment of malaria in Tanzania, clinical evidences requires for their safety and efficacy. The recent report states that ethanol extracts were checked for cytotoxic activity by using MTT assay on LLC-MK2 cells and by brine shrimp lethality assay and 93.75 % of crude extracts were non-toxic where as 6.25 % were toxic on LLC-MK2 cells.

2.14.2 DNA fragmentation assay

According to the recent report (Machana *et al.*, 2011) the extracts of *Pinus kesiya* shows higher potent cytotoxicity in HepG2 cell line with IC value of 52.0 ± 5.8 μg/ml where as *Catimbium speciosum* and *Cladogynos orientalis* shows less potent cytotoxicity which includes nuclei and morphological changes.

(Prasannaa *et al*., 2009) Leaf Extract of *Cassia auriculata* was evaluated against human breast Adenocarcinoma MCF-7 and Human larynx carcinoma Hep-2 cell lines. The results showed that the extract exhibits apoptosis activity in both the cell lines targeting the leaf extract as a potential anticancer drug.

Plant based drugs are best potential for anticancer treatment, they are administered orally and non-toxic to humans (Shah *et al*., 2013). Plant based drugs includes methytransferase inhibitors, DNA damage preventive drugs or anti-oxidants, Histone deacetylases (HDAC) inhibitors and mitotic disruptors (Amin *et al*., 2009).

Recent study suggest that *Sphaeranthus indicus* has appreciable anticancer activity against HL-60 cells in vitro studies. The anticancer compound such as β-Sitosterol and 7- hydroxyfrullanolide considered as a potent anticancer compounds isolated from *Sphaeranthus indicus* (Nahata *et al*., 2013).

Trachyspermum ammi known as Ajowan is commonly used in various ailments such as digestive disorders etc. The ethanolic extract of *Trachyspermum ammi* exhibits cell shrinkage, Nuclear DNA fragmentation as a sign of apoptosis. On analysis of gene expression, mRNA levels the expression of p53 was significantly increased when compared with MCF-7 cell line. This study concludes that *Trachyspermum ammi* has significant anticancer activity (Ramya *et al*., 2017).

The above review of literature gives a vivid account of study area, medicinal uses of the plants, selected plant for the present investigation and the need for affirmation of these plants for their identity based on morphological, anatomical and organoleptic characters .The review also enumerates the recent *Invivo* and *Invitro* studies on the pharmacological properties of various medicinal plants and the techniques used for identifying the bioactive principles. With this background the three medicinal plants such as *Annona muricata* L., *Spermacoce articularis* L.f. and *Rauvolfia tetraphylla* L. were investigated for their therapeutic value.