2. REVIEW OF LITERATURE

The herbal preparation symbolizes more beneficial than the synthetics that are regarded as unsafe for human health. A medicinal herb contains variety of natural chemical constituents, therapeutic agents and active chemical constituent with different medicinal property (Velμrugaran, 2011).

Medicinal plants being an important natural resource and potentially safe drugs can play an important role in assuaging human health by contributing herbal medicines. The high cost of allopathic medicine and their potential side effects, encouraged the people to use traditional medicine (Zaidi, 1998). The increasing demand of plant extracts the use in the cosmetic, food and pharmaceutical industries suggests that systematic studies of medicinal plants are very important in order to find active compounds and their use as a medicine for curing various diseases (Nostro et al., 2001).

2.1. Stachytarpheta jamaicensis

Stachytarpheta jamaicensis have been discovered to be rich in secondary metabolites, such as tannins, terpenoids, alkaloids, flavonoids, phenols, steroids and volatile oil. These compounds are responsible for their therapeutic activities (Cowan, 1999; Rabe and Vanstoden, 2000). Also, since times past, some plant parts have been used as antimicrobial agents, especially their extracts either as decoctions, infusions, or oral
administration (Okemo et al., 2001). Importantly, plants have been known to exhibit medicinal properties on internal organs in animals and human. If the toxic effect after administration is low, there is a possible chance of introduction of such drugs for therapeutic purposes (Ibeh, 1998).

Sasidharan et al. (2007) reported that the methanol extract of the leaves of *Stachytarpheta jamaicensis* showed the diarrheal activity at the doses of 250 and 500 mg/kg. Jude et al. (2008) carried out *in vivo* antimalarial activity of ethanolic leaf extract of *S. jamaicensis* was screened for blood schizonticidal activity against chloroquine sensitive *Plasmodium berghei* in mice. Okwu and Ohenhen (2009) showed that chemical investigation of the bioactive compound, antioxidant, anti-inflammatory, analgesic, antacid, anti-anaphylactic activity from the leaves of *S. jamaicensis*. Idu et al., (2009) and Ogie-Odia et al. (2009) reported that *S. jamaicensis* morphological and anatomical features of the leaves and stems. The potential dose-related effect of powdered *S. jamaicensis* leaves known for treating different ailment was investigated for changes in the body weights and its effects on the liver of albino rats.

Okwu and Ohenhen (2010) reported that steroids represents essential group of natural products which exhibits a broad spectrum of pharmacological profile. Meena and Pitchai (2011) carried out the antibacterial activity and preliminary phytochemical analysis of whole plant of *S. jamaicensis*. Rejender et al. (2011) carried out microproagation
of *S. jamaicensis* highest of 84% rooting was observed at 0.5 mg/L level of IBA and BA. The *in vitro* derived plantlets were hardened and acclimatized in soil. About 75% of plantlets survived in the field condition.

### 2.2. PHARMACOGNOSTICAL STUDIES

Since the beginning of human civilization, medicinal plants have been used by mankind for its therapeutic value. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Many of these isolations were based on the use of agents in traditional medicine. The plant-based, traditional medicine systems continues to play an essential role in health care, with about 80% of the world’s inhabitants relying mainly on traditional medicines for their primary health care (Owolabi *et al*., 2007). The ancient texts like Rig Veda (4500-1600 BC) and Atharva Veda mentioned the use of several plants as medicine. The books on ayurvedic medicine such as Charaka Samhita and Susruta Samhita refers the use of more than 700 herbs (Jain, 1968).

According to the World Health Organization (WHO, 1977) “a medicinal plant” could be any plant, which in one or more of its organ contains substances that can be used for the therapeutic purposes or which, are precursors for the synthesis of useful drugs. This definition distinguishes those plants whose therapeutic properties and constituents have been established scientifically and plants that are regarded as medicinal but which have not yet been subjected thorough investigation.
The use of traditional medicines and medicinal plants in most developing countries as therapeutic agents for the maintenance of good health has been widely observed (UNESCO, 1996). Modern pharmacopoeia still contains 25% drugs derived from plants and many others, which are synthetic analogues, built on prototype compounds isolated from plants. Interest in medicinal plants as re-emerging health aid has been fuelled by the rising costs of prescription drugs in the maintenance of personal health and well being and the bioprospecting of new plant-derived drugs (Lucy and Edgar, 1999). The ongoing growing recognition of medicinal plants is due to several reasons, including escalating faith in herbal medicine (Kala, 2005). Furthermore, an increasing reliance from the use of medicinal plants in the industrialized societies has been traced to the extraction and development of drugs and chemotherapeutics from these plants as well as from traditionally used herbal remedies (UNESCO, 1998).

2.2.1. Pharmacognostical studies on medicinal plants

The leaf morphology of *Alternanthera brasiliiana* was studied by Tekeda and Farango, 2001; Delaporte *et al.*, 2002 and Lorenzi and Matos, 2002. Pectic polysaccharides have been isolated from the fruits of *Naringi crenulata* by extraction with water. The water extract contains large amount of protein. The polymers present in the water extract are fractionated by graded precipitation with ethanol, anion exchange chromatography and size exclusion chromatography.
Characterization of the sub fractions obtained from various chemical and physico-chemical methods of analysis reveals the water extract contain pectic polymers substituted to various degrees with side chains comprising mainly of terminal, 1,4-, 1,6-, 1,3,6-linked galactose, together with lesser amounts of 1,2,4- and 1,3-linked galactose residues. Arabinose residues are terminal, 1,5-, 1,3,5-linked. These polymers contain acetyl groups and give viscous solution in water (Saroj et al., 2003).

Phytochemical analysis of *Enicostemma littorale* showed that presence of terpenoids, saponins, tannins, phenols, anthraquinine, coumarin, flavonoids, proteins and sugars in different extracts. Previous studies have showed presence of alkaloids, steroids, catechins and flavonoids exhibit antioxidant effect in *E. littorale* (Prince and Srinivasan, 2005). *Plectranthus amboinicus* revealed pharmacognostical studies like macroscopical characters, histochemical characters (Nirmala Devi and Periyanayagam, 2008). Physico-chemical analysis of *Naringi crenulata* showed the moisture content and loss of drying were respectively (Mayuree et al., 2009).

Mammen *et al.* (2010) reported that the analysed the various parameters such as, ash analysis, extractive values and moisture content for three plants *Aerva lanata*, *Hedyotis corymbosa* and *Leptadenia reticulata*. Mathur *et al.* (2010) carried out microscopic, macroscopic and phytochemical analysis of leaves of *Amaranthus spinosus* which includes leaf constants, physiochemical parameters such as ash
values, extractive values and moisture content and total ash, acid insoluble ash, water soluble ash values and sulfated ash were observed to be 6.33, 3.60, 2.44 and 0.80% w/w respectively. Alcohol soluble and water soluble extractive values of the leaves were observed to be 6.40, 3.30%, respectively.

Preliminary phytochemical screening of the leaf extracts showed the presence of alkaloids, flavonoids, saponins glycosides, terpenoids, tannins, proteins and carbohydrates. The roots of *Capparis zeylanica* contain alkaloid, phytosterol, acids and mucilage (Sunil *et al*., 2011). Pulak (2011) reported that *Peperomia pellucida* the physical parameters was carried out for the determination of methanol-soluble extractive value, water-soluble extractive values; ash value which includes total ash, acid insoluble ash and water-soluble ash, foaming index, swelling index, fibers measurement and moisture content. Sutar (2011) reported that *Achyranthes aspera* pharmacognostical as well as phytochemical studies. These observations will help the Pharmacognostical identification and standardization of the drugs in the crude form and also to distinguish the drug from its adulteration.

*Enicostemma littorate, Euphorbia hirta, Tephrosia purpurea* and *Desmodium laxum*, were found physicochemical, fluorescence analysis and phytochemical analysis showed the presence of steroids, terpenoids, alkaloids, tannins, phenols, coumarins and saponins
(Kala et al., 2011). Pharmacognostical studies on the root tuber of *Pimpinella tirupatiensis* like physicochemical, fluorescence and phytochemical analysis (Sudhakar et al., 2011). Vijay (2011) investigated the histological and physicochemical studies of *Macrosolen parasiticus* stem. Physicochemical studies of powdered plant material like determination of foreign matter, total ash value, acid insoluble ash value, water soluble extractive value, alcoholic soluble extractive values and hydroalcoholic soluble extractive value, microscopical study in *Cynodon dactylon* (Renu and Badri, 2012).

Prakash and Rao (2012) reported that has been studied the morpho-anatomical characters and physicochemical analysis of *Fumaria indica* such as foreign matter, loss on drying, total ash, alcohol and water soluble extractives, sugar, starch and tannins. They reported the chloroform, methanol extracts from *Fumaria indica* (hausskn) for the use of TLC and phytochemical studies. Lakshman, (2012) have reported the methanol, acetone, chloroform and hexane extraction of *Cyamppsis tetragonoloba* positive tests for few phytochemicals. Some chemical components cannot extract some solvents. Compared to other solvent methonolic extract is good for phytochemical studies. Many chemical compounds cannot show positive results for other solvents like chloroform, acetone and hexane.
2.3. ANTIOXIDANT ACTIVITY

Generally antioxidant means “against oxidation”. An antioxidant is any substance that delays or prevents weakening, loss or destruction by oxidation (Hatano et al., 1988). A free radical is a compound by means of one or more unpaired electrons in its outer orbit (Jesberger, 1991). Such unpaired electrons make these species are unstable, therefore relatively reactive with other molecules due to the existence of unpaired electrons (Karlsson, 1997) and they try to pair their electrons and generate a more stable compound. Free radicals are formed uninterruptedly in regular by-products of oxygen metabolism during mitochondrial oxidative phosphorylation. Thus the mitochondrion is the main basis of free radicals (Przedborski et al., 1998; Fahn et al., 1992).

The most hazardous free radicals are the atomic and molecular weights of oxygen, which is known as Reactive Oxygen Species (ROS). While ROS are not strictly free radicals, they are highly reactive with the molecules around them (Sharma, 1998). ROS is a combined term, which includes not only the oxygen radicals (O and (Hydroxide) OH) but also some non-radical derivatives of oxygen, but also hydrogen peroxide (H₂O₂), hypochlorous acid (HOCl) and ozone (O₃) (Sjodin, 1990). However, all organisms are protected from free radical attack by resistance mechanisms such as a defending antioxidant system that declines the rate of free radical formation and another system to produce chain-breaking antioxidants that scavenge
and stabilize free radicals. When free radical manufacturing rate exceeds the capability of the antioxidant defense mechanisms considerable tissue injury occurs (Rahman, 2007). Therefore, antioxidants having free radical scavenging activities may have some great significance in the prevention and therapeutics of free radical aided diseases.

Before doing any purification and isolation work, natural products have to be extracted from the biomass. The main aim could to isolate a known metabolite or to isolate and illustrate as many compounds as conceivable for an organized phytochemical analysis. An initial extraction is executed characteristically on a small quantity of material to attain primary extract. Once definite metabolites have been recognized in the initial extract, then it may be desirable to isolate them in larger magnitudes. As natural products are diverse and show distinct physicochemical properties, the question arise is how can these metabolites being extracted proficiently from the material under exploration. Solvent-extraction methods can be used for small initial scale laboratory research on in bulk for industrial purpose.

### 2.3.1. Plants with antioxidant activity

In recent years much attention has been devoted to natural antioxidant and their association with health benefits. Plants are potential sources of natural antioxidants and produce various antioxidative compounds that have therapeutic potentials. Antioxidant-based drug
formulations are used for the prevention and treatment of many complex diseases. Naik et al. (2003) examined *Momordica charantia*, *Glycyrrhiza glabra*, *Acacia catechu* and *Terminalia chebula* as antioxidants. Piao et al. (2004) reported the DPPH radical-scavenging activity in *Furano coumarins* and its correlation with the number of phenolic hydroxyl groups present in their structures.

The methanol extract of *Helichrysum plicatum* has been reported to have antioxidant activity using two *in vitro* methods, namely DPPH and β-carotene linoleic acid assays (Tepe et al., 2005). Lie et al. (2005) showed the examined antioxidant activities of twenty-six medicinal herbal extracts that have been popularly used as folk medicines in Taiwan. The results of scavenging DPPH radical activity show that, among the 26 tested medicinal plants, *Ludwigia octovalvis*, *Vitis thunbergii*, *Rubus parvifolius*, *Lindernia anagallis* and *Zanthoxylum nitidum* exhibited strong activities and their IC$_{50}$ values for DPPH radicals were range between 4, 6, 24, 27, 36 and 50 µg/mL, respectively. As for the superoxide anion scavenging activity (IC$_{50}$ µg/mL), the five most significant activities were observed in plant extracts of *Ludwigia octovalvis* (26 µg/ml), *Vitis thunbergii* (58 g/mL), *Prunella vulgaris* (113 µg/mL), *Saurauia oldhamii* (124 µg/mL) and *Rubus parvifolius* (151 µg/mL). The IC$_{50}$ values for DPPH and superoxide anion of catechin (positive control) were 2.5 and 7.2 µg/mL, respectively.
Pourmorad et al. (2006) carried out a systematic record of the relative antioxidant activity in selected Iranian medicinal plant species methanolic extracts, 50, 100, 150, 200, 250 mg L$^{-1}$. The total phenol ranged from 24.1 ± 1 to 289.5 ± 5 mg g$^{-1}$ in the extracts. Flavonoid contents were ranged between 25.15 ± 0.8 and 78.3 ± 4.5 mg g$^{-1}$. 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging effect of the extracts was determined spectrophotometrically. The highest radical scavenging effect was observed in *Melilotus officinalis* with IC$_{50}$ values of 0.018 mg/ml$^{-1}$. The potency of radical scavenging effect of *M. officinalis* extract was about 4 times greater than synthetic antioxidant butylatedhydroxytoluene (BHT). The greater amount of phenolic compound leads to potent radical scavenging effect as shown by *M. officinalis* extracts.

Antioxidant activity of *Cyperus rotundus* rhizomes extract was evaluated in a series of *in vitro* assay involving free radicals and reactive oxygen species and IC$_{50}$ values were determined. CRRE exhibited its scavenging effect in concentration dependent manner on superoxide anion radicals, hydroxyl radicals, nitric oxide radical, hydrogen peroxide and property of metal chelating and reducing power. The alcohol–water extracts of *Ichnocarpus frutescens* leaves possessed 1,1-diphenyl-2-picrylhydrazyl radical and superoxide anion radical scavenging activity (Kumarappan et al., 2007). The scavenging effect of *Andrographis paniculata* was demonstrated against DPPH and ABTS which showing its ability to convert unpaired electrons to paired ones (Rakshamani and Kamat, 2007).
Sidduraju and Becker (2007) reported that antioxidant and free radical scavenging activities of processed cowpea seed extracts, where in DPPH radical and ABTS cation radical scavenging activities were correlated with the ferric reducing antioxidant capacity of the extracts. A crude aqueous extract of *Chlorophytum borivilianum* has been shown to scavenging DPPH free radicals and decrease TBARS, revealing a promising anti-stress agent as well as a potential antioxidant (Kenjale *et al.*, 2007). The antioxidant activity of the aqueous extracts of the leaves of *Bauhinia forficata* and *Cissus sicyoides* were determined using several different assay systems, namely (ABTS) decolorization, superoxide anion radical (O$_2^-$) scavenging and myeloperoxidase activity (Najeh *et al.*, 2008).

The methanol extracts of *Annona squamosa* and *Sapium macrocarpum* showed two times more DPPH scavenging activity than the commercial antioxidant butylated hydroxyl anisole (Ruiz *et al.*, 2008). An aqueous extract from *Choerospondias axillaries* showed a potent scavenging effect on DPPH (Wang *et al.*, 2008). Methanol extract of bark, fruits and leaves of *Ficus microcarpa* exhibited excellent ABTS scavenging activity (Changwei *et al.*, 2008).

Desai *et al.* (2008) reported the free radical scavenging potential of the aqueous extract of roots of *Baliospermum montanum* by DPPH and nitric oxide (NO) scavenging assay, which showed a high concentration-dependent free radical scavenging activity. The antioxidant
and antibacterial properties of the acetone and methanol extracts of leaves and roots of *Sansevieria hyacinthoides* were investigated. The leaves extracts at 1 mg/mL exhibited over 80% DPPH activity, while acetone and methanol extracts from the roots at 0.75 mg/mL showed 91.4 and 92.8% DPPH scavenging activity (Aliero et al., 2008).

The ethanolic extracts of the leaves of *Carica papaya*, *Psidium guajava* and *Vernonia amygdalina*, stem bark of *Mangifera indica* were screened for the presence of phytochemicals and their effect on 2,2-diphenyl-1-picryl-hydrazyl radical (DPPH) was used to determine their free radical scavenging activity and concentrations of the plant extracts required for 50% inhibition of DPPH radical scavenging effect (IC$_{50}$) were recorded as 0.04 mg/mL, 0.313 mg/mL, 0.58 mg/ml, 2.30 mg/mL and 0.054 mg/mL for *P. guajava*, *M. indica*, *C. papaya*, *V. amygdalina* and Vitamin C, respectively. All plants showed potent inhibition of DPPH radical scavenging activity, *P. guajava* being the most potent (Ayoola et al., 2008).

A study was carried out to determine the antioxidant activity *Albizia amara*, *Achyranthes aspera*, *Cassia fistula*, *Cassia auriculata* and *Datura stramonium* by inhibition of lipid peroxidation technique. The highest inhibition of lipid peroxidation activity was observed in *A. amara* (96%) followed by *C. fistula* (89%) and *C. auriculata* (89%). The potency of protective effect of *Albizia amara* was about 4 times greater than the synthetic antioxidant butylated hydroxyl toluene (BHT). The total alkaloid contents were ranged from 24.6 ± 0.18 to 72.6 ± 2 mg g$^{-1}$
in the extracts. Flavanoid contents were between 23.15 ± 0.2 and 63.3 ± 0.6 mg g⁻¹ in the methanolic extracts of these plants (Suresh Kumar et al., 2008).

The methanolic crude extracts of some commonly used medicinal plants were screened for their free radical scavenging properties using ascorbic acid as standard antioxidant. Free radical scavenging activity was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. The overall antioxidant activity of green tea (Camellia sinensis) was the strongest, followed in descending order by black tea (Camellia sinensis), Eugenia caryophyllus, Piper cubeba, Zingiber officinale and Piper nigrum. Trigonella foenum-graecum and Elettaria cardamomum showed weak free radical scavenging activity with the DPPH method. All the methanolic extract exhibited antioxidant activity significantly. The IC₅₀ of the methanolic extracts values ranged between 6.7 ± 0.1 and 681.5 ± 8.4 µg/mL and also standard of ascorbic acid was 8.9 ± 0.1 µg/mL. The study reveals the consumption of these spices would exert several beneficial effects by virtue of their antioxidant activity (Nooman et al., 2008).

Shahin et al. (2008) reported that Amaranthus paniculatus, Aerva lanata, Coccinia indica and Coriandrum sativum indicating the plants could be the source of dietary antioxidant supplies. The antioxidant activity and radical scavenging activity of methanolic extracts of selected plant materials, traditionally used by Iranian
population as folk remedies was evaluated against linoleic acid peroxidation and 2,2-diphenyl-1-picrylhydrazyl radical. The antioxidant activity expressed as $IC_{50}$ values of 1.28 $\mu$g/mL in *Biebersteinia multifida* to 63.48 $\mu$g/mL in *Polypodium vulgare*. Radical scavenging activities expressed as $IC_{50}$ 1.83 $\mu$g/mL in *Salix* sp. to 187.88 $\mu$g/mL in *Allium hirtifolium* (Effat *et al*., 2008).

Abu Bakar *et al.* (2009) showed that antioxidant activity of different parts of *Mangifera pajang* and *Artocarpus odoratissimus*. The results showed that kernel and peel from *M. pajang* contains a broad range of polyphenol phytochemicals, which might be responsible for the cytotoxicity activity against selected cancer cell lines. Chan *et al.* (2009) reported that antioxidant properties of tealeaves and ginger species. All methods of thermal drying (microwave, oven and sun-drying) resulted in drastic declines in total phenolic content, ascorbic acid equivalent antioxidant capacity and ferric-reducing power, with minimal effects on ferrous ion-chelating ability and lipid peroxidation inhibition activity.

Methanolic extracts of *Plumbago zeylanica* (Root), *Acorus calamus* (Rhizome), *Hemidesmus indicus* (Stem) and *Holarrhena antidysenterica* (Bark) were evaluated for their antioxidant activity. The order of antioxidant potential according to ferric thiocyanate assay (FTC) assay was found to be the highest in *P. zeylanica* followed by *H. antidysenterica*, *A. calamus* and *H. indicus*. While there is slightly difference in activities as measured by TBA method.
The antioxidant activity of medicinal plants have commercial antioxidant butylated hydroxy toluene (BHT), L-Ascorbic acid and \( \alpha \)-tocopherol. Further, the radical-scavenging activity of the extracts was measured as decolourizing activity followed by trapping of the unpaired electron of DPPH. The percentage decrease of 1,1-diphenyl-2-picrylhydrazylradical (DPPH) standard solution was recorded maximum for \( H. \text{indicus} \) (77.0\%) followed by \( P. \text{zeylanica} \) (73.41\%), \( A. \text{calamus} \) (20.88\%) and \( H. \text{antidysenterica} \) (20.06\%) extracts at a concentration of 100 \( \mu \text{g/mL} \) (Maryam \textit{et al.}, 2009).

Zhou \textit{et al.} (2009) studied the effect of water quality on the nutritional components and antioxidant activity of green tea extracts. Results suggested that the synergistic effect of catechins, caffeine and other components are more important than any single component in free radical scavenging. Giovanelli and Buratti (2009) showed polyphenolic composition and antioxidant activity of wild Italian blueberries and some cultivated varieties. Results showed that total phenolics and total anthocyanin concentrations were, respectively two fold and three fold higher in the wild fruits. Lin \textit{et al.} (2009) assessed antioxidant property of buckwheat enhanced wheat bread results showed that it has good antioxidant activity, reducing power and 1,1-diphenyl-2-picrylhydrazyl radical scavenging ability. Mohsen \textit{et al.} (2009) reported total phenolic contents and antioxidant activity of \textit{Corn tassel} in water, ethanol, methanol, acetone, hexane, chloroform, butanol,
petroleum ether and methylene chloride extracts and results revealed that ethanol exhibited the highest extraction ability for phenolic compounds, followed by methanol and water.

The aqueous, methanol and ethanol extracts of *Melissa officinalis*, *Matricaria recuttia* and *Cymbopogan citrates* were found to posses DPPH scavenging activity (Pereira *et al.*, 2009). The methanol extracts of leaves and flowers of *Lippia alba* exhibited significant DPPH radical scavenging activity compared to the standard antioxidant ascorbic acid (Ara and Nur, 2009). The methanol extract of *Manilkara zapota* showed strong activity on scavenging DPPH radical, which implicated an essential defense against the free radicals (Kaneria *et al.*, 2009). Sharififar *et al.* (2009) showed that crude extracts of *Teucrium polium* isolated compounds for their antioxidant and free radical scavenging activities. Slusarczyk *et al.* (2009) showed the antioxidant properties of the different extracts from *Lycopus lucidus* and to correlate their antioxidant potential to the composition of polyphenols.

Robert *et al.* (2009) studied ten herb honeys of various origin reveal the differences in their antioxidant activity and profiles of phenolic acids and flavonoids. Thyme herb honey had high quercetin content. Zhang *et al.* (2009) studied antioxidant phenolic compounds from walnut kernels (*Juglans regia*). The results of this study, suggested the antioxidant activities of these phenolic compounds may be influenced by the number of hydroxyls in their aromatic rings.
Oke et al. (2009) evaluated antioxidant activities of the essential oil and the methanolic extract from *Satureja cuneifolia* by using DPPH radical scavenging, β-carotene linoleic acid bleaching and metal chelating activity assays. They concluded the minimum inhibitory concentration of plant extracts in different models. The hot water extract of *Perilla frutescens* stalk showed moderate DPPH radical scavenging abilities than the leaf and seed extracts (Chou et al., 2009).

Antioxidant activity of chloroform extracts of flower of *Saccharum spontaneum* was investigated in the crude chloroform extract and standard ascorbic acid showed antioxidant activity with the IC$_{50}$ value of 51.04 µg/mL and 43.04 µg/mL respectively (Farhana, 2009). Crude extracts from *Inula aucherana*, *Fumaria officinalis*, *Crocus sativus*, *Viscum album*, *Tribulus terrestris*, *Polygonatum multiflorum*, *Alkanna tinctoria* and *Taraxacum officinale* were screened for their in vitro antioxidant and antimicrobial properties by Memnune et al. (2009). Prakash et al. (2009) reported the methanolic crude extracts of *Desmodium gangeticum*, *Eclipta alba*, *Ocimum sanctum*, *Piper longum*, *Solanum nigrum* and *Amaranthus caudatus* were screened for their free radical scavenging properties using ascorbic acid as standard antioxidant. Free radical scavenging activity was evaluated using 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical. The overall antioxidant activity of *D. gangeticum* was found to be the strongest, followed in descending order by *A. caudatus*, *S. nigrum*, *P. longum*, *E. alba* and *O. sanctum*. The IC$_{50}$ values of the
extracts were ranged between 0.05 ± 0 and 0.19 ± 0 mg/g. The ascorbic acid levels ranged from 3.86 ± 0.20 to 21.33 ± 1.49 mg/g and the carotenoids content were observed between 9.0 ± 0.24 to 24 ± 1.16 mg/g in plant extracts. The highest total phenols content were found to be in *Ocimum sanctum* with the value of 48.93 ± 0.24 mg/g.

The antioxidant activity of rhizome extracts from *Zinger montanum* was investigated. The antioxidant activities of the ethanolic extracts were screened by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. Samples from different sources showed significantly different DPPH scavenging activities (p < 0.05). The antioxidant activities of the rhizomes extracts obtained from the north showed the highest activity (80.88%), followed by those from the east (76.47%), the south (72.51%), the northeast (67.38%), the west (66.66%) and the central region (57.63%) (Saowaluck and Yingyong, 2009). The antioxidant activity in the extracts of three medicinal plants (*Tilia argentea*, *Crataegus folium* leaves and *Polygonum bistorta* roots). The results indicated that acetone extracts of *Tilia argentea* had the highest antioxidant capacity as free ABTS radical scavengers (Demiray et al., 2009). The *n*-hexane, carbon tetrachloride, chloroform soluble fractions of methanol extract from *Centella asiatica* was subjected to the antioxidant. All the fractions showed moderate, to potent antioxidant activity, of which the chloroform and aqueous soluble fraction demonstrated the strongest antioxidant activity with the IC₅₀ value of 4.0 µg/mL and 7.0 µg/mL, respectively (Obayed et al., 2009).
In-vitro antioxidant activity of methanolic leaves and flowers extract of *Lippia alba* was determined by DPPH free radical scavenging assay. The reducing power of extracts was also determined. Ascorbic acid was used as standard and positive, which controls both the analysis. The methanolic leaves and flowers extracts of *L. alba* had shown very significant DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging activity compared to standard antioxidant. The DPPH radical scavenging activity of the extract was increased with the increasing concentration. In DPPH free radical scavenging assay IC$_{50}$ value of leaves and flowers extracts of *L. alba* was found to be 34.4 µg/mL. The results concluded that the extracts have a potential source of antioxidants of natural origin (Naznin and Hasan, 2009) that have reported a high radical scavenging activity in the stem of *Kigelia* followed by leaf of *Hibiscus, Gemelia* and *Kigelia*.

The essential oils of *Myrtus communis* Contained compounds such as 1,8-cineole and methyl eugenol that showed considerable DPPH scavenging activities (Mimica-Dukic *et al.*, 2010). In vitro antioxidant activity of methanolic seed extracts of *Canavalia ensiformis* and *C. gladiata* were determined by DPPH free radical scavenging assay (Doss *et al.*, 2010). The DPPH radical scavenging activity of the extract was increased with the increasing concentration. The highest radical scavenging effect was observed in *C. gladiata* with IC$_{50}$ value of 59.23 ± 1.6 µg/mL.
The greater amount of phenolic compounds leads to more potent radical scavenging effect as shown by *Canavalia gladiata* extract. The results concluded that the extracts have a potential source of antioxidants of natural origin. Rudrappa and Mohmoud (2010) studied free radical scavenging activity of *Echinops echinatus* Roxb., root. Extracts of *Echinops echinatus* Roxb., roots were evaluated for radical scavenging activities using different *in vitro* models like scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, nitric oxide radical and superoxide anion.

**2.4. PHYTOCHEMICAL STUDIES**

Phytochemicals are chemical compounds formed during the plants normal metabolic processes. These chemicals are often referred to secondary metabolites of which there are several classes including alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides, phenols, tannins, terpenes and terpenoids (Harborne, 1973; Okwu, 2004). Phytochemicals are present in a variety of plants, which is utilized as important components of both human and animal diets. These include fruits, seeds, herbs and vegetables (Okwu, 2005). Diets containing an abundance of fruits and vegetables are protective against a variety of diseases, particularly cardiovascular diseases (Cadenas and Packer, 1996). Herbs and spices are accessible sources for obtaining the natural antioxidants (Okwu, 2004). In addition to these substances, plants
contain other chemical compounds. These can act as agents to prevent undesirable side effects of the main active substances or to assist in the assimilation of the main substances (Anonymous, 2007).

There are several standard methods used for the phytochemical screening of medicinal plants. They are described as alkaloids (Harborne, 1973), steroids (Trease and Evans, 1989), phenolics and flavonoids (Awe and Sodipo, 2001) saponins and cardiac glycosides (Sofowora, 1993) and tannins (Odebiyi and Sofowora, 1978). In contrast to synthetic pharmaceuticals based upon single chemicals, many medicinal and aromatic plants exert their beneficial effects through the additive or synergistic action of several chemical compounds. It acts as single or multiple target sites associated with a physiological process. As pointed out by Tyler (1999) these synergistic pharmacological effects can be beneficial by eliminating the problematic side effects associated with the predominance of a single xenobiotic compound in the body. Kaufman et al. (1999) extensively documented how synergistic interactions underlie the effectiveness of a number of phytomedicines. Most of these phytochemical constituents are potent bioactive compounds found in medicinal plant parts, which are precursors for the synthesis of useful drugs (Sofowora, 1993).

Ghosh et al. (1982) found that Limonia acidissima fruits contain flavonoids, glycosides, saponins and tannins. Berberine is an isoquinoline alkaloids found in the roots of Coptis japonica
(Sato and Yamada, 1984). Phytochemical analysis in fifty-one medicinal plants, which are used as indigenous systems in medicine as well as by local inhabitants either as single drugs or in combinations, to cure the various ailments. The study carried out so far, revealed that the presence of alkaloids in thirty-one plants of flavonoids in twenty eight glycosides in thirty four, saponins in thirty four, sterols in thirty seven and terpenoids in thirty three plants (Agarwal et al., 1989).

Sener et al. (1998) studied the biological activities of some Turkish medicinal plants as a resource of new chemistry for public health and plant protection. A systematical approach to the discovery of drugs from these plants had resulted in the identification of active compounds representing a wide range of structures, including alkaloids, terpenoids and phenolic compounds.

Iqbal and Beg (2001) studied qualitative phytochemical tests, TLC and TLC bioautography of certain active extracts demonstrated the presence of common phytocompounds in the plants that extracts including phenols, tannins and flavonoids as active constituents in 12 medicinal plants. Catalan and Lampasona (2002) studied alkaloids, terpenoids, steroids, flavonoids and tannins found in Aloysia triphylla and that these chemical constituents were found in significant amount, namely in terpenoids and flavonoids.
Mojab et al. (2003) studied a phytochemical analysis of fifty five Iranian plants belonging to 21 families were carried out. A qualitative phytochemical analysis was performed for the presence of alkaloids, tannins, saponins and flavonoids. The medicinal uses of these plants are also reported. Krasteva et al. (2004) reported the phytochemical analysis of ethyl acetate extract from Astragalus corniculatus and brain antihypoxic activity. Gonzala et al. (2004) reported that phytochemical screening showed the alkaloids, tannins and flavonoids presence in the aqueous extracts of Melissa officinalis.

Krasteva et al. (2004) ethyl acetate extract containing flavonoids was obtained from above ground parts of Astragalus corniculatus. Seven flavonoids were isolated and identified as rutin, hyperoside, iso quercitrin, narcissin, quercetin, kaempferol and isorhamnetin for the first time. The ethyl acetate extract was chromatographed on a cellulose column, using a 0.95% ethanol linear gradient. Seventy fractions were collected and analysed by TLC on silica gel. Identical fractions were put together and rechromatographed, further purified by column chromatography. Five flavonol glycosides and three flavonol glycones were isolated.

Venkata et al. (2010) reported that different medicinal plant methanolic extracts qualitative preliminary phytochemical analysis was performed on aforesaid extracts for the presence of alkaloids, flavonoids, steroids and terpenoids and each analysis was carried out in triplicate,
which resulted a total of 22, 19, 37 and 30 plant species were found to give positive results for alkaloids (41%), flavonoids (35%), steroids (69%) and terpenoids (56%), respectively. Plants which are rich in a wide variety of secondary metabolites belonging to chemical classes (tannins, terpenoids, alkaloids and polyphenols) are generally superior in their biological activities suggesting that this strength is dependent on the diversity and quantity of such constituents (Geyid et al., 2005).

The present study regarding the qualitative analysis of the selected medicinal plants is in agreement with the previous studies.

Steroids, terpenoids, flavonoids and tannins, were present in Melissa officinalis and Matricaria chamomilla but alkaloids were found absent. In Olea europaea leaves, all the chemical constituents were present except alkaloids, and terpenoids. Aloysia triphylla showed the presence of all the constituents. Alkaloids have been associated with cytotoxic properties and steroids have been reported with antibacterial properties (Raquel, 2007).

The bioactive chemical constituents to evaluate the antimicrobial activity of the ethanolic extract of traditionally used right medicinal plants of Nepal. A qualitative phytochemical analysis was performed for the detection of alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins and reducing sugar. The highest yield of ethanolic extract was found in Azadiracta indica (29.08%). Ocimum sanctum
contained all the chemicals except flavonoids and reducing sugar hormone. The *Colquhounia coccina* locked alkaloids and reducing sugar. The antimicrobial activities of these plants extract were also observed. The extract of *Rhododendron setosum* and the essential oil of *Eucalyptus globules* were most effective against *Escherichia coli* and *Staphylococcus aureus* respectively. But the extracts of *Azadiracta indica* and *Elshotlzia feucticosa* found to be most effective against *Klebsiella* species (Himal Paudel *et al.*, 2008).

Monalisha *et al.* (2010) reported that methanolic extraction of the modified stem of *Achras sapota* by standard method and chemical constituents of *A. sapota* including alkaloids, steroids, flavonoids, saponins, reducing sugars, tannins, amino acid, protein, anthraquinone glycosides, deoxy sugar and phenolic compounds. The main biological activity was found as an anti-oxidant.

### 2.4.1. Terpenoids

The terpenoids have also been shown to be of great ecological significance (Degenhardt *et al.*, 2003; Pichersky and Gershenson, 2002). The steroids and sterols in animals are biologically produced from precursors of terpenoids. Sometimes terpenoids are added to proteins to increase their attachment to cell membrane the process is known as isoprenylation (Sacchettini and Poulter, 1997). These compounds
and their derivatives are belong to other drugs such as validol, menovasin, turpentine, bromkamfora etc., Turpentine is extensively used as external drugs, and it is the main raw material for other products on the base of terpenoids.

2.4.2. Flavonoids

Flavonoids are polyphenolic compounds that are ubiquitous in nature and are categorized, according to their chemical structure into flavones, anthocyanidins, isoflavones, catechins, flavonols, chalcones and flavanones. More than 4,000 flavonoids have been recognised, many of which occur in vegetables, fruits and beverages like tea, coffee and fruit drinks.

The flavonoids have provoked considerable interest recently, because of their potential valuable effects on human health, they have been testified to have been shown to have several biological properties including anti-inflammatory, hepatoprotective anti-thrombotic and antiviral activities many of which may be associated, partially at least, to their antioxidant and free-radical-scavenging ability (Robak et al., 1988). The antiradical property of flavonoids is directed mostly towards HO and O2 as well as peroxyl and alkoxy radicals (Husain et al., 1987). Furthermore, as these compounds present a strong affinity for iron ions their antiperoxidative activity could also be ascribed to a concomitant capability of chelating iron. (Morel et al., 1993; Afanasav et al., 1989).
One of the undeniable functions of flavonoids and related polyphenols is defending plants against microbial attack. This not only comprises their presence in plants as constitutive mediators but also their accumulation as phytoalexins in response to microbial attack (Grayer et al., 1994; Harborne, 1999). Because of their extensive ability to prevent spore germination of plant pathogens, they also have been suggested for using against fungal pathogens. There is an ever growing interest in plant flavonoids for treating human diseases and particularly for monitoring the immune deficiency virus which is the contributing agent of AIDS.

The majority of flavonoids documented as constitutive antifungal agents in plants are flavanones, isoflavonoids or flavans. It is recognized that a flavone glycoside, namely luteolin 7-(200-sulphatoglucoside), is an antifungal component of the marine angiosperm. Thalassia testudinum is remarkable claim that acylated flavone glycosides existing in the leaf hairs of Quercus ilex affords the plant useful protection against the damage of UV radiation (Jensen et al., 1998; Skaltsa et al., 1994).

Haraguchi et al. (1998) reported that consistent presence of antibacterial activity among flavonoids. Thus, the retrochalcone licochalcone C (4,40-dihydroxy-20-methoxy-30-prenyl) is active against Staphylococcus aureus with a MIC of 6.25 µg/mL. The compound 5,7-dihydroxy-3,8-dimethoxy favone has an MIC of 50 µg/mL towards Staphylococcus epidermis (Iniesta et al., 1990) and the substance
5,7,20,60-lavandulyl-40-methoxyfavanone completely inhibits the progress of *S. aureus* at concentrations among 1.56 and 6.25 µg/mL (Inuma *et al.*, 1994). The above favanone is chiefly active against antibiotic resistant strains of *S. aureus* and could have some activity towards treating patients, who unintentionally pick up this infection while in hospital.

Das and Pereira (1990), have shown that a carbonyl group at C-4 and a double bond between C-2 and C-3 are also important features for high antioxidant activity in flavonoids. Butein and other 3,4-dihydroxychalcones are more active than analogous flavones because of their ability to achieve greater electron delocalisation (Dziedzic and Hudson, 1983). Likewise, isoflavones are frequently more active than flavones because of the stabilizing effects of the 4-carbonyl and 5-hydroxyl in the former (Dziedzic and Hudson, 1983). In the antioxidant action of *o*-dihydroxyfavonoids metal chelation is an important factor (Shahidi *et al.*, 1991).

Flavonoids have been stated to possess many useful properties, containing anti-inflammatory activity, enzyme inhibition, antimicrobial activity, oestrogenic activity (Havsteen *et al.*, 1983; Harborne *et al.*, 1999) anti-allergic activity, antioxidant activity (Middleton *et al.*, 1986) vascular activity and cytotoxic antitumor activity (Harborne and Williams, 1992). For a group of compounds of relatively homogeneous structure, the flavonoids inhibit a mystifying
number and variety of eukaryotic enzymes and have an extremely wide range of activities. In the event of enzyme inhibition, this has been assumed to be due to the interaction of enzymes with different parts of the flavonoid molecule such as carbohydrate, phenyl ring, phenol and benzopyrone ring (Havsteen et al., 1983).

A recent area of research that is of particular interest is the deceptive inhibitory activity of some flavonoids against human immunodeficiency virus (HIV). *In vitro* studies have shown that baicalin inhibits HIV-1 infection and replication. Inhibition of HIV-1 entry into the cells expressing CD4 and chemokine co-receptors (Li et al., 2000), and antagonism of HIV-1 reverse transcriptase (Li et al., 1993). Baicalein (Ono et al., 1989), robustaflavone and hinokiflavone (Lin et al., 1997) have also been shown to inhibit HIV-1 reverse transcriptase, as have several catechins, but catechins inhibit other DNA polymerases and their interaction with the HIV-1 enzyme is therefore thought to be of non-specific nature (Moore et al., 1992). In addition, it has been demonstrated that several flavonoids, including demethylated gardenin A and 3,2′-dihydroxyflavone, inhibit HIV-1 proteinase (Brinkworth et al., 1992).

Robinetin, myricetin, baicalein and quercetagetin (Fesen et al., 1994) and quercetin 3-O-(2′-galloyl)-1-arabinopyranoside (Kim et al., 1998) inhibit HIV-1 integrase, although there are concerns that HIV enzyme inhibition by quercetagetin and myricetin is non-specific (Ono et. al., 1990). It has also been reported that the flavonoids chrysin,
apigenin and acacetin prevent HIV-1 activation via an unusual mechanism that possibly involves inhibition of viral transcription (Critchfield et al., 1996). Interestingly, chrysin was reported to have the highest therapeutic index of 21 natural and 13 synthetic flavonoids against HIV-1 (Hu et al., 1994). Furthermore, at least two groups have proposed mechanisms of action for HIV-1 enzyme inhibition (Brinkworth et al., 1992; Fesen et al., 1994). Flavonoids show interactions with cytochrome P450 (Ng et al., 1996), anti-leukemic properties and mild vasodilators properties useful for the treatment of heart disease (Hodek et al., 2002).

2.4.3. Saponins

Saponins are a group of secondary metabolites found widely distributed in the plant kingdom as plant glycosides, characterized by a skeleton resulting of the 30-carbon precursor oxidosqualene to which glycosyl residues are attached along with it they have study foaming property. Conventionally, they are subdivided into triterpenoids and steroids glycosides, or into triterpenoids, which are found primarily in dicotyledonous plants also in some monocots, spirostanol, and furostanol saponins (Hostettmann et al., 1995). Steroids saponins occur chiefly in monocotyledons family such as the Liliaceae, Agavaceae, Drosoraceae and in certain dicotyledons, such as foxglove (Hostettmann et al., 1995). Oats are unusual because it contains both
triterpenoids and steroids saponins (Price et al., 1987). Steroidal glycoalkaloids are found principally in members of the family belonging to solanaceae, which includes potato and tomato.

The saponins formed by oats and tomato have been studied in detail in relation to their potential role in the defences of plants against phyto-pathogenic fungi (Osborn, 1996). They are stored in plant cells as inactive precursors but are readily converted into biologically active antibiotics by plant enzymes in reply to pathogenic attack. These compounds can also be regarded as preformed O, since the plant enzymes that activate them are already present in healthy plant tissues (Osborn, 1996). The natural role of saponins in plants is thought to be protection against attack by pathogens and pets (Price et al., 1987; Morrissey et al., 1999). These molecules also have substantial marketable value and are processed as drugs and medicines, foaming agents, sweeteners, taste converters and cosmetics (Hostettmann et al., 1995).

Saponin based adjuvants have the ability to modulate the cell mediated immune system as well as to enhance antibody production and have the advantage that only a low dose is needed for adjuvant activity (Oda et al., 2000). Saponins isolated from plants such as fenugreek (Petit et al., 1993), Phellodendron and Aralia cortex (Kim et al., 1998), Pueraria thunbergiana (Lee et al., 2000) and
Calendula officinalis (Yoshikawa et al., 2001) revealed to have hypoglycaemic effects in saponins. Petit et al., (1993) established chronically higher plasma insulin levels, possibly initiated by stimulation of the β-cells in male wister rats were given 10 and 100 mg fenugreek extract/300 g body weight mixed with food while (Matsuda et al., 1999) did not find insulin-like or insulin-releasing activity in rats were given oleanolic acid glycosides orally. The hypoglycaemic act here was due to suppression of transportation of glucose from the stomach to the small intestine and the inhibition of glucose transport across the brush border of the small intestine.

2.4.4. Tannins

The biological activity of tannins such as antioxidant or radical scavenging activity as well as inhibition of lipid peroxidation and lipoxygenases In vitro (Amarowicz et al., 2000; Gyamfi et al., 2002), antimicrobial and antiviral activity (Dolara et al., 2005; De Bruyne et al., 1999), antimitmutagenic activity (Dolara et al., 2005; Carlsen et al., 2010) and antidiabetic properties (Matsui et al., 2001; Anderson et al., 2002). The antioxidant activity of tannins results from their free radical and reactive oxygen species-scavenging properties, as well as the chelation of transition metal ions that modify the oxidation process (Serrano et al., 2009). Antioxidants have also been reported to provide synergistic benefits for the treatment of diabetes because of their insulin enhancing potential (Madhujith et al., 2004).
2.4.5. Phenolic compounds

Phenolic compounds are some of the most widespread molecules among plant secondary metabolites, acting as natural antioxidants and antinitrosating agents which are of great significance in plant development. They are involved in various processes comprising including rhizogenesis (Curir et al., 1990), vitrification (Kevers et al., 1984), resistance to biotic and abiotic stress (Deladonde et al., 1996) and redox reactions in soils (Takahama et al., 1992). This coumarins was identified in some species, among which Euodia borbonica (Valenciennes et al., 1999), Citrus limon (Salvatore et al., 2004) and Citrus medica sarcodactylis (Gao et al., 2002). It is a phototoxic coumarins (Salvatore et al., 2004); lately, some preliminary works about its anticancer activity have been carried out (Kawaii et al., 1999).

Kaempferol has antiviral (Mitrocotsa et al., 2000), antioxidant and antiprotozoic (Calzada et al., 1999) activities; quercetin is the major flavonoid in the human diet and has been reported to exhibit antioxidative (Chopra et al., 2000), anti-inflammatory (Ferry et al., 1996), antiaggregatory (Pignatelli et al., 2000) and vasodilating (Perez-Vizcaíno et al., 2002) effects. Apigenin showed to possess antitumor and a strong antioxidant activities (Cardenas et al., 2006), as well as genistein (Jeune et al., 2005; Rufer and Kulling, 2006) and myricetin (Valdez et al., 2004; Maggiolini et al., 2005).
2.4.6. Friedelin

Friedelin is one of the most abundant terpenes presents cork and cork-derived materials. The recent studies shows that Friedelin particularly it have anti-cancer activity (Moiteiro et al., 2001; Moiteiro et al., 2004; Zhang et al., 2004), analgesic, antiulcerogenic, anticancer, antibacterial, antitumor, antiviral and cytotoxic (Linuma et al., 1994; Satori et al., 1999; Yimdjo et al., 2004), anti-inflammatory capability (Nakamura et al., 1997), vascularizing agent (Harsh Mohan, 2002), have potential can be used in pharmaceuticals or functional foods for the treatment or prevention of cardiovascular and cerebrovascular diseases and tumours (Zhang et al., 2006) and as agrochemical (Moiteiro et al., 2006).

2.4.7. Ursolic acid

Recent reports indicates that the anticarcinogenic, anti-inflammatory, and proapoptotic effects of ursolic acid were due to its ability to inhibit immunoregulatory transcription factor NFκB in response to a wide variety of carcinogens and inflammatory agents (Shishodia et al., 2003). However, there are no detailed reports on the immunosuppressive effects of UA in T cells which are the primary cell type involved during an adaptive immune response.
2.4.8. Hispidulin

Flavonoids are chemical constituents widely present in plants and possess many pharmacological activities (Wollenweber and Volker, 1981; Beretz et al., 1982). Reported biological activities attributed to *Scoparia dulcis* (Osei et al., 2009) hispidulin include antimutagenicity (Chulasiri et al., 1992) hepato-toxicity (Ferrandiz et al., 1994), antioxidant activity (Gu et al., 2001), positive allosteric properties and anticonvulsant activity (Kavvadias et al., 2004) and antifungal activity (Tan et al., 1999).

2.4.9. Alfa-spinasterol

The biological activities of Alpha- spinasterol was identified as the Antimicrobial activity of *Luffa cylindrica* (Velmurugan et al., 2011), *Phytolacca americana* (Jeong et al., 2004), Spinasterol, which is biologically active compound isolated from the aerial parts of *Aster scaber* (Asteraceae), exhibits various pharmacological activities, including anti-tumor, antiulcerogenic, and anti-carcinogenic activities (Villasenor and Domingo, 2000; Jeon et al., 2005).