5. DISCUSSION

The well known traditional systems of medicine, namely Unani, Ayurveda, Siddha and Chinese medicine etc. regardless of having wider distinction in their principles of treatments and agreed upon the point that diseases occur due to imbalance within the constituents of the body and aim of treatment is to restore the balance with the help of herbs (Wadud et al., 2007).

The medicinal plants are not only used throughout the world as home remedies, but also provide raw materials for the pharmaceutical industries and represent a substantial proportion of the global drug markets. Despite the great advances observed in modern medicine in recent years, plants still make an important contribution to health care. It is estimated that about 25% of all modern medicines are directly or indirectly derived from higher plants and sources for new lead structures and also for the development of standardized phytotherapeutic agents with proved efficacy, safety and quality.

According to WHO, because of poverty and lack of access to modern medicine, about 75-80% of world population, which lives in developing countries, depends essentially on plants for primary health care because of better cultural acceptability, compatibility of plants with the human body and lesser side effects (Yadav and Dixit, 2008).
The sources of raw material and good practices of manufacturing processes are certainly the essential steps for the quality control of herbal medicines.

The study of plant drugs from the pharmacognostical standpoint would include the study of the habitat, general characters of the plant from which the drug is derived, its place in the botanical system, the organ or the organs of the plant used, their gross, minute structures in the whole and in the powdered conditions and the chemistry of the constituents especially of those which may be used in therapeutics.

The macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and the degree of purity of such materials. This should be carried out before any tests are undertaken. Lack of proper standards of medicinal plants may result in the usage of improper drugs which in turn will cause damage not only to the individual using it, but also to respect gained by the well known ancient system of medicine and the entire work on the plant becomes invalid. Thus, in recent years there has been an emphasis in pharmacognostical standardization of medicinal plants of therapeutic potential. So, the present study is undertaken to standardize *Stachytarpheta jamaicensis* L. (Vahl) pharmacognostically which will help in the correct identification of the drug.
The physico-chemical parameters are mainly used in judging the purity and quality of the drug. An ash value of a drug gives an idea of the earthy matter or inorganic composition or other impurities present along with the drug. The pH values of various parts in medicinal plants may also be considered as the parameters for the purity of a drug.

The extractive value is also important parameters for detecting adulteration in drugs. The amount of extracts that drug yields to a certain constituents or a group of related constituents that a drug contains. The amount of extracts in a particular substance plays an important role in establishing the index of the purity of drug because it is adulterated or exhausted drugs, which will give different extractive values than original drug. The selection of solvents also plays an important role and is taken into consideration, which depends on the type of components to be extracted. The specific solvents extracts the specific phytochemical compounds are dissolved. Fats, lipids, phospholipids, sterols etc. have more solubility in petroleum ether, benzene, ethanol and methanol. Amino acids and glycosides, which are soluble in alcoholic solvents and some alkaloids and glycosides are soluble in water.

The result of fluorescence analysis of *S. jamaicensis* powdered of various parts like leaves, stems and roots and their extracts in different solvents like petroleum ether, chloroform, acetone, ethanol, methanol, aqueous and different chemicals. The results of fluorescent studies of the powdered plants material using by the different chemical
reagents and solvents. It is stated an important phenomenon exhibited by various chemical constituents present in the plant material (Kamil and Paramjyothi, 2010). Visual inspection is the simplest and quickest way to establish identity, purity and quality; colour, consistency, odour and taste that are also significant and important parameters.

5.1. ANTIOXIDANT STUDIES

5.1.1. DPPH free radical scavenging assay

The DPPH free radical scavenging assay in different extracts of leaf, stems and roots. The highest antioxidant capacity was observed in leaves (83.14 ± 0.04) in methanolic extract, the IC$_{50}$ value of 25 µg/mL. Similar investigation was reported in Romulea ramiflora and Gagea fibrosa leaves in the methanolic extract (61.16 ± 1.46) has higher DPPH radical scavenging activity (Mammadov et al., 2011). Charalampos et al. (2013) reported the IC$_{50}$ values of Nepeta melissifolia, Mentha pulegium and Phlomis lanata (5.1 ± 0.4, 13.5 ± 0.5, 23.9 ± 0.4 µg/mL) were found to be similar in BHT and ascorbic acid (18.5 ± 0.4, 3.9 ± 0.3 µg/mL). The IC$_{50}$ values of the examined plant extracts and essential oils are presented respectively.

Amit et al. (2012) reported that IC$_{50}$ values of extracts were found to be 3.37 µg/mL and that of ascorbic acid was 2.27 µg/mL. The highest DPPH scavenging activity was observed in methanolic extracts of Olax psittacorum (91.14%) followed by Ipomoea cairica (83.49%),
Leucas aspera (83.25%), Commelina benghalensis (81.4%), Bauhinia purpurea (77.2%) and Cassia tora (63.84%) (Rajani et al., 2013). Anjali and Sheetal (2013) reported that the highest antioxidant activity was given by Mentha spicata extract at the concentration of 170 µg/mL among the methanolic leaves which was found to be more than the ascorbic acid while activity of Oscimum sanctum was found to close to the standard.

The IC₅₀ values of whole plants methanolic extract of Ocimum sanctum (16.8 µg/mL), Achyranthes aspera (36.2 µg/mL), Cassia auriculata (68.4 µg/mL), Coccinia indica (54.6 µg/mL), Mentha spicata (47.3 µg/mL), Hygrophilla auriculata (59.5 µg/mL), Datura stramonium (48.3 µg/mL), Delonix regia (38.4 µg/mL), Coriandrum sativum (89.3 µg/mL) and Pterospermum acerifolium (26.3 µg/mL), and the low IC₅₀ values of methanolic extract of Ocimum sanctum might be due to the presence of high polyphenolics and flavonoids (Subhendu et al., 2011). Kognou et al. (2011) observed that the percentage inhibition between the concentrations of 500 and 1000 µg/mL, in the methanolic extract of stem bark of Pteleopsis hylochryson.

The whole plant of Launaea procumbens methanol extract of (IC₅₀ values of 2.6 ± 0.004 µg/mL) possessed the highest antioxidant activity (least IC₅₀ value of 2.6 ± 0.004 µg/mL) where as, Launaea procumbens n-hexane extract showed the lowest scavenging effect (with higher IC₅₀ value of 19 ± 0.04 µg/mL) (Rahamat et al., 2012). Arul Raj et al. (2012) reported the highest scavenging activity (DPPH radical)
of *Alpinia purpurata* was 68.42% for ethyl acetate extract at the concentration of 500 µg/mL and this extracts was close to that of BHT standard (88.37%).

5.1.2. Superoxide radical scavenging activity

The highest SOD capacity was observed in leaves (53.63 ± 0.02) IC$_{50}$ values of 456 µg/mL. Similar observations was found in *Alstonia scholaris*, the ethyl acetate fraction and dicholromethane fraction had a strong superoxide radical scavenging activity comparable to that of BHA and ascorbic acid (Arulmozhi *et al.*, 2010). The superoxide scavenging activity of ethyl acetate and dicholromethane were statistically significant (p<0.01) from the control. *Casalpinia bonduc* has the scavenging of hydrogen peroxide radicals for the ethanol extract was 51.17 ± 0.14 (Sivasankari *et al.*, 2011). Ramani *et al.* (2012) discussed the values of superoxide radical scavenging activity of two species *Leucas lavandulifolia* with IC$_{50}$ values of 6.637 ± 0.342 µg/mL was found to be higher than *Leucas nagalapuramiana* with IC$_{50}$ values of 7.100 ± 0.469 µg/mL. *Withania somnifera* and *Aloe vera* effects on superoxide scavenging activity was observed that *Withania somnifera* possesses better dose dependent superoxide scavenging potential than *Aloe vera* Patel *et al.* (2012).

5.1.3. Iron chelating activity

The maximum chelating of metal ions at 600 µg/mL for leaf extracts and EDTA were found to be 51.96 ± 0.02 and 61.64 ± 0.01 respectively. The IC$_{50}$ values of leaf extracts and EDTA were recorded as methanol extracts of IC$_{50}$ value of 373 µg/mL. The leaf extracts are
having lower activity compared with standard but, higher than stems and roots. *Ocimum basilicum, Petroselinum crispum, Laurum nobilis, Juniperus communis, Elettaria cardamomum, Zingiber officinalis, Pimpinella anisum, Foeniculum vulgare, Carum carvi* were identified significantly (P < 0.01) the best iron chelation (178 ± 1.68 mg Na$_2$EDTA/g extract), followed by the fennel (Iris *et al.*, 2006).

In *Mentha arvensis*, the most active extracts interfered with the formation of ferrous and ferrozine complex, suggesting that it has chelating activity and captures ferrous ion before ferrozine. IC$_{50}$ values of the extract for chelating activity was 80 ± 0.01 µg/mL which is lower than the positive standard EDTA (IC$_{50}$ values of = 17 µg/mL). The IC$_{50}$ of chelating effects of other extracts on Fe$^{2+}$ and ferrozine complex (Ebrahimzadeh *et al.*, 2008). Patralekh and Mukherjee (2010) recorded *in vitro* studies on the Antioxidant and iron chelating activity of *Enhydra fluctuans* has been conducted, iron – chelating activity IC$_{50}$ values of the plant extracts 6.38 mg/ml.

Chandra mohan *et al.* (2012) reported that *Kalanchoe pinnata* metal ion chelating capacity was significant. Since, it reduces the concentration of the transition metal that catalyzes lipid peroxidation. According to the results, the plant extract is not good as the standard of EDTA; but decrease in concentration dependent colour formation in the presence of the extract indicates that it has iron chelating activity the IC$_{50}$ values of EDTA (50.70 µg/mL) and the IC$_{50}$ values of *Kalanchoe pinnata* leaf extract (909.91 µg/mL) respectively.
5.1.4. Nitric oxide radical scavenging activity

The present study, nitric oxide radical scavenging activity was observed and highest IC\textsubscript{50} values of leaves extracts was recorded as (IC\textsubscript{50} values of 349 µg/mL). The effect was comparable to the standard ascorbic acid with IC\textsubscript{50} values of 474 µg/mL. Sanja et al. (2009) was reported the methanolic extract of \textit{Portulaca oleracea} has increased in nitric oxide the IC\textsubscript{50} value of standard curcumin is 41.37 ± 5.05 µg/mL. The results are in agreement with the present investigation. In \textit{Bridelia scandens} the reduction of nitric oxide radical by the methanolic extract and ascorbate, the maximum scavenging activity of methanolic extract and ascorbate at 1000 µg/mL were found to be 79.31% and 75.23% respectively. The IC\textsubscript{50} values of methanolic extract and ascorbate were recorded as 130 µg/mL and 410 µg/mL (Senthil Kumar et al., 2010).

\textit{Pongammia pinnata} were recorded the percentage inhibition of nitric oxide radical generation by three extracts compared to standard curcumin the 50% inhibition (IC\textsubscript{50}) by leaves, seeds and flowers were found as 140.63 ± 9.46 µg/mL, 958.51 ± 47.61 µg/mL and 595.26 ± 12.28 µg/mL respectively, whereas curcumins showed 90.82 ± 4.75 µg/mL as IC\textsubscript{50}. The leaf extracts has best activity among the three extracts (Bibhabasu et al., 2011). Rozina et al. (2012) reported that \textit{Phyllanthus freternus} a greater inhibitions comparatively to other plants extracts but less than ascorbic acid which has shown 96.27% inhibition of nitric oxide. The maximum nitric oxide scavenging of leaves of \textit{Triumfetta rhomboidae}, barks of \textit{T. rhomboidae}, roots of \textit{T. rhomboidae}
and *Casuarina littorea* were 53.94%, 50.43%, 33.23% and 54.02% with IC$_{50}$ values of 97.81 µg/mL, 196.89 µg/mL > 200 µg/mL and 168.17 µg/mL respectively.

### 5.1.5. Hydroxyl radical scavenging activity

The present study of *Stachytarpheta jamaicensis*, the highest hydroxyl radical scavenging activity was observed in leaves (40.30 ± 0.03) IC$_{50}$ values of 708 µg/mL. Li *et al.* (2005) reported that the ethanol extract of the roots of *Phygonum multiforum* which showed a better hydroxyl radical scavenging activity than that by resveratol, which is structurally similar to the stilbene glycosides. The *Acanthopanase senticosus* work by providing hydrogen atoms from the phenolic hydroxyl groups to scavenge hydroxyl radicals generated from hydrogen peroxide (Park *et al.*, 2006).

Patil (2009) reported that the *Gmelina arborea* showed the less scavenging activity (H$_2$O$_2$) than that of ascorbic acid. The IC$_{50}$ values of the extracts and standard in this assay were 34 ± 0.82 µg/ mL and 27 ± 0.92 µg/ mL. The IC$_{50}$ value of the extract was less than that of the standard. At 100 µg/mL, the percentage inhibition values were 63.36% and 66.00% for *G. arborea* and ascorbic acid. *Inula graveolens* recorded the scavenging of hydroxyl radicals by the methanolic extract was increased in a dose dependent manner the scavenging percentage achieved 91.38% at a concentration of 20 mg/mL (Adnan, 2010). *Curcuma amada* showed the administration of plants extracts reversed
the damage caused by H$_2$O$_2$ with methanolic extract showing higher potential followed by chloroform and aqueous extracts in both the parts that studied as indicated (Sivaprabha et al., 2011).

5.1.6. Total antioxidant activity

The present study recorded the highest total antioxidant capacity was observed in leaves (85.04 ± 0.02) IC$_{50}$ values of 12 µg/mL compared with stems and roots, as opposed to that of ascorbic acid (63.23 ± 0.02) IC$_{50}$ values of 793 µg/mL in leaves. The results obtained in the present study indicate that C. rotundus rhizomes extract can be a potential source of natural antioxidant (Nagulenran et al., 2007). The highest antioxidant capacity was observed in Viscum album (82.23%), followed by Inula aucherana (80.10%), Alkanna tinctoria (79.80%), Fumaria officinalis (78.93%), Polygonatum multiflorum (75.85%), Tribulus terrestris (69.25%), Crocus sativus (63.15%) and Taraxacum officinale (43.05%) respectively (Memnune et al., 2009). The antioxidant capacity of standard BHA and BHT was 93.21 and 90.71%. Methanolic extracts of Ionidium suffruticosum was found to be extremely effective in free radical scavenging activity than that other extracts in total antioxidant activity (Satheeshkumar et al., 2011).

Mentha arvensis, Moringa oleifera, Spinacia oleracea, Trogonella foenum-graecum, Tamarindus indica and Amaranthus viridis were recorded. The extracts of M. arvensis showed the highest total antioxidant capacity and it was 215 µg/mL calculated as ascorbic acid
equivalents was detected. The total antioxidant activity of methanolic extract exhibited the following order: *M. arvensis* (215 µg/mL) > *M. oleifera* (93 µg/mL) > *T. foenumgraecum* (78 µg/mL) > *T. indica* (72 µg/mL) > *A. viridis* (70 µg/mL) > *S. oleracea* (66 µg/mL) (Raghavendra et al., 2013).

5.1.7. Ferric reducing antioxidant power assay

The gallic acid had higher FRAP activity (73.56 ± 0.07) the IC₅₀ values of 326 µg/mL compared with plant extracts *S. jamaicensis*. The highest FRAP capacity was observed in leaves of (55.82 ± 0.04) IC₅₀ values of 456 µg/mL. Chang et al. (2007) reported that the antioxidant and free radical scavenging activities of *Phellinus merrillii* extracts in ferric reducing antioxidant power of crude extract and hexane, EtOAc, *n*-BuOH, water soluble and water insoluble fractions were expressed as mM of trolox equivalent. The EtOAc fraction had the highest reducing antioxidant power of 19.09 ± 0.03 mM, crude extract and *n*-BuOH fraction had the reducing antioxidant power of 12.00 ± 0.01 mM and 13.98 ± 0.02 mM, water insoluble fraction and water fraction had the reducing antioxidant power of 3.37 ± 0.01 mM and 1.01 ± 0.01 mM.

The FRAP values for the methanol extract of the leaves and stems of *Celtis africana* were significantly lower than that of ascorbic acid, quercetin and catechin, but higher than that of BHT (Adeolu et al., 2009). *Terminalia arjuna* the hexane fraction had the lowest reducing antioxidant power of 0.68 ± 0.01 mM. Acetone and methanol extracts of *Terminalia arjuna* bark was compared for its total antioxidant status by FRAP assay. Total antioxidant status was found
to be significantly (P<0.01) higher in acetone extract (212.5 ± 11.55 µM) as compared to methanol extract (35.50 ± 4.70 µM). This indicates higher antioxidative property of *Terminalia arjuna* bark acetone extract in comparison to methanol extract (Chandan *et al.*, 2013).

### 5.1.8. Total phenols

The highest total phenols observed in leaves and the phenolic contents (6.98 ± 0.04) was low in all the parts compared with flavonoids. The similar results were revealed in the total phenolic content of *Inula aucherana, Fumaria officinalis, Crocus sativus, Vicum album, Tribulus terrestris, Polygonatum multiflorum, Alkanna tinctoria* and *Taraxacum officinale* ranged from 4.04 mg GAE/g (*Polygonatum multiflorum* L.) to 42.29 mg GAE/g (*Crocus sativus*) per g dry weight basis. Earlier, a wide variation was observed on total phenolic content in different aromatic and medicinal plants was 6.80-32.10 mg gallic acid equivalents per g dry weight basis (Bajpai *et al.*, 2005). Moreover, total phenolic content of *Polygonatum* species were found 1.27-8.69 mg/GAE.

Senthil Kumar *et al.* (2010) recorded the *Bridelia scandens* total phenolic content of various extract of whole plant which was based on the results of methanolic extract *Bridelia scandens* was found higher content of phenolic components than that of petroleum ether (1.50 mg) and ethyl acetate extracts (2.60 mg) and total phenolic content of standard gallic acid. The total phenolic content of methanolic extracts exhibited the following order: *Mentha arvensis* (75 µg/mL) > *Moringa oleifera* (60 µg/mL) >
Spinacia oleracea (20 µg/mL) > Trigonella foenum-graecum (12 µg/mL) > Tamarindus indica (10 µg/mL) > Amaranthus viridis (5 µg/mL) (Raghavendra et al., 2013).

5.1.9. Total flavonoids

The highest total flavonoids (6.98 ± 0.04) was observed in leaves of Stachytarpheta jamaicensis. Raghavendra et al. (2013) reported that the extract of Mentha arvensis showed highest total phenolic content and it was 675 µg/mL calculated as quercetin equivalent flavonoids was detected. The results are positively correlated with the present study. The total flavonoids content of methanolic extracts exhibited the following order: Mentha arvensis (675 µg/mL) > Moringa oleifera (415 µg/mL) > Tamarindus indica (205 µg/mL) > Trigonella foenum-graecum (190 µg/mL) > Amaranthus viridis (155 µg/mL) > Spinacia oleracea (125 µg/mL).

5.2. PHYTOCHEMICAL STUDIES

5.2.1. Preliminary phytochemical studies

The present investigation preliminary phytochemical studies on various parts such as leaves, stem and root of Stachytarpheta jamaicensis extracts showed the presence of phytochemicals such as tannins, flavonoids, saponins, phytosterols, alkaloids and glycosides. The present study coincide with previous report by Murugan et al. (2012). That the preliminary phytochemical analysis of the methanol extracts of Gymnema sylvestre and Morinda pubescens leaves and stems of
*Gymnema sylvestre* revealed the presence of alkaloids, anthraquinones, catechins, coumarins, flavonoids, phenols, steroids, tannins, terpenoids and xanthoprotein.

### 5.2.2. Analysis of bioactive compounds

Successive isolation of botanical compounds from plant material is largely dependent on the type of solvents used in the extraction procedure. The traditional healers use primarily water as the solvents, but in the present investigations the plant extracts by ethanol provided more phytocompounds followed by acetone in comparison to the aqueous extraction which are also agreement with previous researchers (Romero *et al.*, 2005; Pandith *et al.*, 2009). The qualitative changes in the phytochemical analysis of tested plant species are correlated to methods of preparation. The preliminary phytochemical tests are therefore significant and helpful in finding chemical constituents in the plant material that may lead to their quantitative estimation and also in locating the source of pharmacologically active chemical compounds (Sharanabasappa *et al.*, 2007).

**Thin layer chromatography**

The present investigation TLC of different parts of various extracts was discussed. It gives better results of leaves extracts in methanol. The major components were presented clearly compared with stems and roots extracts. TLC analysis provide an idea about the
polarity of various chemical constituents, in a such way that compounds showing high \( R_f \) value in less polar solvents system have low polarity and with less \( R_f \) values have high polarity. Ravishankar et al. (2012) reported that *Asparagus racemosus* has phytoconstituents, confirmed by thin layer chromatography and their \( R_f \) values have been presented as 0.7 and yellow colour indicates presence of tannins and flavonoids and pink colour indicates the presence of steroids.

5.2.3. Isolation of biological Compounds

**Friedelin**

The present investigation the separation of compound Friedelin from leaves extract of *Stachytarpheta jamaicensis*. It was identified by using \(^1H\) NMR spectrum, \(^{13}C\) NMR spectrum, GC and GC-MS and IR spectrum steps of isolation compounds. Friedelin has shown antimicrobial, anticandidal, anti-inflammatory and anticancer activities (Niero et al., 2006; Moiteiro et al., 2006). Sharaf et al. (1967) reported that Friedelin, had strong biological activities in *Punica granatum* such as hypotensive, antispasmodic and anthelmintic effects. Antimicrobial activity of following microorganisms were used such as *Bacillus cereus*, *Enterobacter cloacae*, *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus*, *S. saprophyticus* and *Streptococcus agalactiae*, *C. albicans* and *Candida tropicalis* (Pretto et al., 2004).
**Ursolic acid**

The present investigation the separation of compound ursolic acid from leaves extract of *Stachytarpheta jamaicensis*. It was identified by using $^1$H NMR spectrum, $^{13}$C NMR spectrum, GC and GC-MS and IR spectrum steps of isolation compounds. Potent anti-inflammatory activity of ursolic acid, a triterpenoid antioxidant (Checker *et al.*, 2012). Ursolic acid is the part of traditional medicine and it has been shown to possess many biological activities, such as antioxidative, anti-inflammatory, anticancer and hepato-protective activities (Suh *et al.*, 1998).

**Hispidulin**

The present investigation the separation of compound hispidulin from leaves extract of *Stachytarpheta jamaicensis*. It was identified by using $^1$H NMR spectrum, $^{13}$C NMR spectrum, GC and GC-MS and IR spectrum steps of isolation compounds. Recently hispidulin isolated from number of species such as *Arnica montana* (Bourdillar *et al.*, 1988), *Millingtonia hortensis* (Chulasiri *et al.*, 1992; Hase *et al.*, 1995), *Artemisia* species (Tan *et al.*, 1999), *Salvia plebeia* (Gu *et al.*, 2001) and *Salvia officinalis* (Kavvadias *et al.*, 2003) has been reported to inhibit platelet aggregation. A number of flavonoids are also known to have free radical scavenging activity (Kandaswami and Middleton, 1994). Hispidulin having free radical scavengers and inhibit
lipid peroxidation (Sanz et al., 1994; Yoshino et al., 1997; Gao et al., 1999) and baicalein hispidulin have anti-inflammatory activities.

**Alpha-spinasterol**

The present investigation the separation of compound Alpha-spinasterol from roots extract of *Stachytarpheta jamaicensis*. It was identified by using $^1$H NMR spectrum, $^{13}$C NMR spectrum, GC and GC-MS and IR spectrum steps of isolation compounds. Several published studies have shown that spinasterol also has anti-inflammatory effects (Zhou et al., 1985). However, the mechanisms underlying the anti-inflammatory effect of spinasterol remain to be elucidated.

*Stachytarpheta jamaicensis* contains flavonoids, terpenes, phenols and steroids. Several of these plant chemicals have been documented (Raintree Nutrition, 1996). Iridoid glycosides known as verbascoside or acetoside have been isolated from the plant (Liu, 2003). In clinical research, this powerful antioxidant phytochemical has been documented with neuroprotective, antiviral, antibacterial, liver protective, cardioactive and antitumor effects (Sanz, 1994; Sheng, 2002; Daels, 2000). Hispidulin has been reported to have anti-asthmatic, bronchodilator and antispasmodic properties; liver detoxifying actions and helps to normalize sticky blood (Xiong, 1998; Ferrandiz, 1994).