8.0. GENERAL DISCUSSION

The world health organization has defined as traditional medicine comprising therapeutic practices that have been in existence for hundreds of years (Kamboj, 2000). The traditional preparations comprise medicinal plants, minerals and organic matter. Herbal drugs constitute only those traditional medicines which primarily use medicinal plant preparations for therapy (Hota and Pathi, 2003). It is estimated that, 70-80% of people all over the world largely depends on traditional herbal medicine to meet their health care needs (Hamayun *et al*., 2006). The global demand for herbal medicine is growing (Omino and Kokwaro, 1991; Muregi *et al*., 2003; Zowari *et al*., 2003) while, plant species in traditional medicines continue to be a reliable source for discovery of useful compounds. Screening of plants growing under various environmental conditions could provide another source for pharmacological activities (Mureugi *et al*., 2003; Rajkaruna *et al*., 2002). Considerable researches on pharmacognosy, chemistry, pharmacology and clinical therapeutics have been carried out on ayurvedic medicinal plants (Dhanukar *et al*., 2000). Plants and plant extracts have been used for thousands of years for the treatment of various diseases. More recently, (Cohen *et al*., 2000) the renewed interest on the use of natural products is due to reduced side effects on the human body compared to synthetic medicinal

drugs (Baillion, 1884). However, few of the herbal medicines available to the public have undergo testing for safety, efficacy and potential drug interaction with other drugs taken for an ailment (Cohen *et al*., 2000; O'Hara *et al*., 1998). Mangrove plants have been used in folklore medicine and recently extracts from mangroves have proven activity against human, animals and plant pathogens but few studies were done with liver diseases (Bandaranayake, 2002). In view of this, the present study was carried out to find out the effectiveness of the mangrove plants against the carbon tetrachloride induced hepatotoxicity in rats.

The present study was made an attempt to test the different mangrove plant extracts against the carbon tetrachloride induced hepatotoxicity in rats. Of the 18 different mangrove plant part extracts, *Bruguiera cylindrica* leaf extract showed maximum percentage (72.20%) of hepatoprotective activity.

The protective effect of *Bruguiera cylindrica* leaf extract could be due to the presence of unique biochemical constituents such as reducing sugar, proteins, phenolic groups and tannins. Generally, the polyphenols are considered to have potent antioxidant effects (Lee *et al.,* 2007). Masella *et al.* (2005) reported that, hepatoprotective activity of the *Camella oleifera* is due to the presence of polyphenols and flavonoids. Dose dependant activity of the *Bruguiera cylindrica* leaf extract are also carried out by the present study. It

reveals that, all the three (75, 150 and 300mg.kg^{-1}.bw) doses showed hepatoprotective activity but the high dose of 300mg.kg^{-1}.bw showed maximum effect (73.54%) of hepatoprotection.

Hence, the present study was undertaken to find out the *in vitro* antioxidant activity of the *Bruguiera cylindrica* leaf extract by DPPH antioxidant activity (Guleria *et al.,* 2010). It reveals that, the *Bruguiera cylindrica* leaf extract having maximum antioxidant potential and hence it might prevent the reactive radical species from damaging biomolecules such as lipoproteins, poly unsaturated fatty acids (PUFA), DNA, amino acids, proteins and sugars in biological system (Halliwell *et al.,* 1995; Ara and Nur, 2009).

Formation of hydroxyl radicals damage every molecule found in living cells (Hochestein and Atallah, 1988) by join with nucleotides and cause strand breakage, which leads to cytotoxicity (Kappus, 1991). The present study observed that, the leaf extract of *Bruguiera cylindrica* is proved to have the ability in reducing the formation of hydroxyl free radicals which might reduce the hepatotoxicity. Vidyalakshmi *et al.* (2006) observed that, the maximum percentage (80%) reduction of hydroxyl radical scavenging activity in the leaf extract of *Mussaenda glabra* showed hepatoprotection in rats.

The present study also made an attempt to find out the lipid peroxidation activity of *B. cylindrica* as because the formation of free radicals can enhance the lipid peroxidation activity there by the liver toxicity may be increased. It is interesting to notice that, the addition of *B. cylindrica* leaf extract not only reduce the hydroxyl free radicals and also it suppress the lipid peroxidation activity. Khan and Shahidi, (2001) reported that, the TBARS values of borage and evening primrose triglycerides showed maximum inhibition of lipid peroxidation activity.

In a biological system, nitric oxide (NO*) and superoxide (O_2*-) anion free radical causes ischemic renal injury (Okada and Okada, 1998). Suppression of NO* is attributed to reduce the amount of nitrite generated from the decomposition of sodium nitro pruside under *in vitro* condition. Reduction in the level of NO* could reduce the tissue damage. To check this, an experiment was conducted with the addition of *B. cylindrica* leaf extract on the level of NO*. It reveals that, the extract from *B. cylindrica* reduced the level of NO* and hence the extract have the capacity to cure the liver damage. Earlier investigation reveals that, seaweed extract of *E. cava* showed nitric oxide radical scavenging activity (Senevirathne *et al.,* 2006). Heo *et al.* (2005) reported that, *Sargassum* sp. was showed the maximum (60%) hydroxyl radical scavenging property.

The reducing power ability is exhibited antioxidative potential by breaking free radical chain and there by donating hydrogen atom and further the liver damage is reduced (Gordon, 1990; Pen- Der- Duh, 1998). To find out this an experiment was conducted to find out the reducing power of the leaf extract of *B. cylindrica.* It reveals that, the antioxidant reductants in the *Bruguiera cylindrica* leaf extract at a concentration of 250 $\mu g.ml^{-1}$ causes the reduction of the Fe^{3}/ferricynanide complex to the ferrous ion. Guo *et al.* (2001) reported that, the aqueous and methanol extracts of stem and leaf of broccoli showed higher reducing power at the concentration of 4 $mg.ml^{-1}$. Interestingly, the *Bruguiera cylindrica* leaf extract showed maximum percentage of inhibition by 16 fold reduction in concentration and found to be a promising plant than broccoli.

The total antioxidant activity of the *Bruguiera cylindrica* leaf extract was increased with increasing concentration of the extracts. The present result indicates that, the *Bruguiera cylindrica* leaf extract are having the capacity to donate the electrons which can react with free radicals to convert them as more stable products and strongly inhibiting radical chain reaction (Rao *et al.,* 2010). Shajiselvin and Muthu (2010) reported that, the methanolic extract of *B. hispidia* showed maximum (80.01%) percentage inhibition of total antioxidant activity at 1000 $\mu g.ml^{-1}$ but, the *Bruguiera cylindrica* leaf extract showed maximum antioxidant activity at the concentration of 62.5

μg.ml^{-1} which is found to be 10 fold lower concentration and hence the promising hepatoprotectivity of *B. cylindrica* is authenticated and confirmed.

FRAP method describes the reducing ability of antioxidant compounds from Fe^{III}-TPTZ to Fe^{II} complex (Iris *et al.,* 1996). In the present study, the formation of Fe^{II} complex and colour reduction has been reduced by the *Bruguiera cylindrica* leaf extract at the IC_{50} value of 54.20±0.93μg.ml^{-1}. Shajiselvin and Muthu, (2010), reported that, the methanolic extract of *B. hispidia* showed the FRAP reduction at the IC_{50} value of 65.00μg.ml^{-1}.

The present study was also made an attempt to identify the relationship between the polyphenol content and *in vitro* antioxidant assays. It reveals that, all the antioxidant assays such as DPPH (0.982), FRAP (0.985), hydroxyl radical scavenging (0.936), lipid peroxide (0.965) and superoxide radical scavenging (0.979) activity were showed statistically significant ($p<0.05$) and positive correlation with polyphenols content. The antioxidant property of *Bruguiera cylindrica* leaf extract could be due to the presence of phenolic content (0.983). These results suggest that, the polyphenolic constituents of the extract might play in free radical neutralization and lipid peroxidation inhibition. Rajkumar *et al.* (2010) reported that, *Bergenia ciliata* extract showed significant ($p<0.05$) positive correlation with the polyphenolic content and antioxidant properties.

In histopathological assay, the centrilobular hepatic necrosis, fatty changes and congestion in central vein were displayed in carbon tetrachloride intoxicated rats. But, pretreatment of *Bruguiera cylindrica* leaf extract treated rats have significantly reduced the destruction of paranchymal cells and architecture. These results further corroborate the inhibition of liver function markers elevation.

The present study was made an attempt to find out the organoleptic, physical, chemical and microbiological standardization of most potential activity of *Bruguiera cylindrica* leaf extract. It reveals that, the organoleptic properties of taste, colour, odour and consistency and physical properties of such as total ash, acid soluble ash, insoluble ash, were found within the acceptable limits as per the WHO guidelines (WHO, 2000). It was already reported that, marine environment is known to accumulate a variety of heavy metal contaminants derived from drainages. For the future development of herbal drugs, the presence of such contaminates could produce side effects rather than the curative effects. The herbal drugs contain the large quantum of heavy metals when they are in combination with other metals which can utilise the nascent effect of particular heavy metal will be harmful in the traditional drug. Keeping this in mind, the present study was also analysed with toxic heavy metals of iron (Fe), copper (Cu), zinc (Zn), chromium (Cr), manganese (Mn), nickel (Ni), lead (Pb), cadmium (Cd) and

arsenic (As) and all the metals are found within the limits of WHO guidelines (WHO, 2000).

In general, the herbal drugs normally carry a large number of bacteria and moulds, often originating in soil or derived from manure. While a large range of bacteria and fungi form the naturally occurring micro flora of medicinal plants, aerobic spore-forming bacteria frequently predominate. Proliferation of microorganisms may result from failure to control the moisture levels of herbal medicines during transportation and storage, as well as from failure to control the temperatures of liquid forms and finished herbal products. The presence of *Escherichia coli*, *Salmonella* sp. and moulds may indicate poor quality of the drug. In this connection, the present study was made attempt to identify the microbial load of *E. coli, Salmonella* sp. *Enterobacter sp.* and fungal species in *Bruguiera cylindrica* leaf extract. It reveals that, all the selected microbes were found up to the limits of WHO guidelines (WHO, 2002).

The establishment of herbal standardization also depends upon the adulteration and chemical fingerprinting of the secondary metabolites. The present study was made an attempt to identify the important constituents of *Bruguiera cylindrica* leaf extract by HPTLC analysis. Which could be used as a blue print for checking the adulteration if any in the finished product of the herbal medicine used for the treatment of alcoholic liver damage.