CHAPTER-II

2. LITERATURE REVIEW

In recent years, medicinal plants & plant extracts have gained immense importance, since they are the major source of herbal drugs. Medicinal plants like *Moringa oliefera* (Ghasi *et al.* 2000; Arabshahi-Delouee *et al.* 2007; Urooj & Reddy 2010), *Allium sativum* (Yeh & Liu 2001), *Solanum melongena* (Sudheesh *et al.* 1997), *Apium graviolans, Achyranthes aspera, Terminalia arjuna, Zinziber officinalis, Phylanthus niruri, Momordica charantia, Ocimum sanctum* (Phadke 2007), *Trigonella foenum graecum* (Shashikumar *et al.* 2018), *Azardiracta indica* (Igwenyi *et al.* 2017), *Amaranthus spinosus* (Zeashan *et al.* 2009), *Launaea procumbens* (Khan *et al.* 2012) and numerous other plants are scientifically proven during the last few decades as natural antioxidants, nutraceuticals, lipid lowering as well as hepatoprotective agents.

2.1 Survey review

In a survey reported by Kpodar *et al.* 2016, traditional healers of the Togo utilize a total of 99 plant species belonging to 88 genera and 49 families that are used for hepatic diseases. Among the reported families *Caesalpiniaceae* was the highest with 8 species, followed by *Euphorbiaceae* with 7 species, *Apocynaceae* and *Asteraceae* with 6 species each. The plant parts used mainly were the leaves, followed by roots, whole plant, rhizome and the bark accounting for more than 10% each. The herbal medicines for the treatment of liver disorder were prepared mostly by decoction and were administered through orally.

A total of 40 medicinal plants were reported by Sharma *et al.* 2012, which belongs to 31 families and 38 genera were recorded to be used by the Bhoxa, nomadic Gujjars and Tharu communities in 45 formulations (single plant or in combination of plants) that uses 15, 23 and 9 plants, respectively as a remedy of jaundice. Survey shows that: *Amaranthus spinosus* L., *Cissampelos pareira* L., *Ehretia laevis* Roxb., *Holarrhena pubescens* Wall., *Ocimum americanum* L., *Physalis divaricata* D. Don, *Solanum incanum* L. and *Trichosanthes cucumerina* L. were reported for the first time in India. The literature survey by Sharma *et al*. 2012, revealed that total of 214 plants (under 181 genus & 78 families) are used as internal, 19 plants (under 18 genus & 12 families) are used as external and 14 plants (under 14 genus & 11 families) are used as magico-religious remedy for jaundice by various communities in different parts of India.

Hepatoprotective medicinal plants survey by Thockchom *et al.* 2018, in 13 different villages (under Bishnupur, Kakching and Thoubal districts) of Manipur, India, have reported a total of 34 different families and 52 plant species. They have also calculated Disease Consensus Index (DCI) on the basis of information collected to determine most significant plants. DCI value was found to be highest in *Engelhardtia spicata*, which is followed by *Saccharum officinarum*, *Averrhoa carambola*, *Andrographis paniculata*, *Justicia adhatoda* and *Cuscuta reflexa*.

2.2 Plant phytochemicals and antioxidant review

Medicinal plants have high contents of natural phyto-constituents viz., polyphenols, flavonoids, terpenoids, tannins, beta-carotene, vitamin C and E, etc., which are excellent antioxidants that can prevent incidence of several free radical induced human disorders viz; cancer, atheroschlerosis, retinopathy, liver disorder, ulcerative colitis, diabetes, hypertension, chirrosis, allergy arthritis, inflammation, etc. (Anderson *et al.* 2001; Alam *et al.* 2013; Ksouri *et al.* 2015). Several studies investigated that polyphenols are directly attached with biological activity such as hepatoprotective activity (Santillan *et al*. 2014; Wu *et al*. 2017).

Presence of phenolic, flavonoids, terpenoides, saponins, tannins and reducing sugar from the ethanolic extracts of different medicinal plants viz; leaves of *Psidium guajava*, *Carica papaya*, *Vernonia amygdalina*, stem bark of *Magnifera indica* (Ayoola *et al*. 2008), *Enicostemma littorale* (Selvaraj *et al.* 2014) and leaves, stem, bark of (Ganesan *et al*. 2016). In addition to that, *Enicostemma littorale* & *Datura stramonium* were also found to have steroids, alkaloids and glycosides. Further the DPPH radical scavenging activity showed IC_{50} values of 0.04, 0.313, 0.58, 2.3 and 0.054 mg/mL from *P. guajava*, *M. indica*, *C. papaya*, *V. amygdalina* and vitamin C respectively.

In a different research, various *in-vitro* antioxidant activities were studied taking experimental models like iron (III) reducing capacity, total antioxidant capacity, DPPH, FRAP & H_2O_2 radical scavenging activity, β-carotene bleaching assay, total phenolic, flavonoids, ascorbic acid content, nitric oxide and inhibition of ferrous sulphate-induced oxidation of lipid system by various researchers with different extracts on the plants *viz*; *Morus indica* (leaves), *Averrhoa carambola* (green & ripe fruit peel, stem, tart & honey type fruits), *Averrhoa bilimbi* (different types), *Parkia javanica* (seed) and leaves of *Phlogacanthus thyrsiflorus* (Delouee & Urooj 2007; Lim & Lee 2013; Sindhu *et al.* 2013; Asna & Noriham 2014; Chanu *et al*. 2012). Based on the outcome of above research, it was found that the antioxidant activity of *M. indica* leaves remained unchanged at 50°C and was maximum at pH-7. *A. carambola* fruit antioxidant capacities were increased significantly with ripening, except for the total ascorbic acid content. The ripe star fruit peel contained higher total polyphenol, flavanol and ascorbic acid contents than green star fruit peel. Ripe star fruit peel also demonstrated stronger FRAP and DPPH (75% inhibition) values than the green star fruit peel. *A. carambola* tart type possessed highest total phenolic, flavonoids and strong DPPH radical scavenging activity and the *A. carambola* honey type showed higher value in FRAP and β-carotene bleaching assay. *A. carambola* stem showed strong DPPH, Nitric oxide, H_2O_2 radical scavenging activity in ethanolic extract which showed IC_{50} values of 878.06 µg/mL, 391.69 µg/mL, 83.29 µg/mL respectively. Methanolic leave extract of *P. thyrsiflorus* showed highest value of 61.07 ± 0.61 and 77.29 ± 0.51 mg Trolox equivalent/g in DPPH and FRAP assay respectively whereas ethanolic extract caused maximum inhibition of lipid peroxidation at 200 µg/mL concentration (81.28 ± 0.1%) and *P. javanica* water extract showed highest phenolic content with 51.09 ± 0.78 GAE mg/g of extract.

Study by Chatterjee *et al.* 1983 and Balasubramanian *et al.* 2005, revealed that *Morus indica* (root and leaves) extracts significantly inhibited carrageenan-induced oedema in wistar rat. Root extract inhibited histamine, serotonin, bradykinin induced oedema, hyaluronidase-induced oedema and also reduces the intensity of peritoneal inflammation by decreasing exudation of plasma protein. The average mean ±SE value of total protein exudate in artificial peritoneal inflammation induced by acetic acid was 39.33 ± 3.5 mg, but with prior administration of 400 mg/kg & 800 mg/kg of *M. indica* root extract, the average mean (± SE) amounts of protein exudation were 32.75 ±1.8 mg and 20.9 ± 1.9 mg, respectively. The leave extract also reduced carrageenan induced edema by 56.76% on oral administration of 100 mg/kg, whereas indomethacin 10 mg/kg inhibited edema by 64.86% as compared to untreated control group. The extract also inhibited leukocyte migration, pleural exudates reduction and decreased granuloma weight in the cotton pellet granuloma method. The inhibition of *M. indica* extract and indomethacin drug was found to be 53.1 and 64.4%, respectively.

2.3 Hepatoprotective activity

Hepatic disease is a term that indicates damage to the cells, tissues, structure, or liver function and this damage can be induced by biological factors (bacteria, virus, and parasite infection), autoimmune diseases (immune hepatitis, primary biliary cirrhosis), as well as by the action of different chemicals/ drugs (high doses of paracetamol and antitubercular drugs), toxic compounds (carbon tetrachloride, thioacetamide, dimethylnitrosamine, *D*-galactosamine/lipopolysaccharide, and unquestionably, excessive consumption of alcohol (Upur *et al*. 2009; Kumar *et al.* 2013; Santillan *et al.* 2014). Unfortunately, conventional or synthetic drugs used to treat liver diseases are unsatisfactory because they can exert serious long-term side effects (Velioglu *et al*. 1998; Giacometti *et al*. 2016).

Oh *et al.* 2010, studied immunomodulatory and hepatoprotection effect induced by CCl_4 from *Morus indica* Linn. (MIL) glycoproteins and found that reduced activities of ALT, LDH and TBARS in serum and reduced activity of Cyclooxigenase-2 (COX-2) and expression of TNF-α and IL-1β in liver from CCl_4-treated mice were reported. Moreover MIL glycoprotein also suppressed the stress-activated protein kinase/c-jun N-terminal kinase phosphorylation and activator protein-1 transcriptional activation in liver from CCl_4-treated mice. The result indicated that MIL glycoprotein protects against liver injury effectively by down-regulation of oxidative stress and also by the inflammatory response.

In several studies, significant elevation of ALP, AST, ALT, GGT, LDH, bilirubin, glucose, urea, cholesterol level and subsequent liver injury with decreased levels of liver antioxidant enzymes (SOD, CAT, GSH-Px, GSH) content are observed in liver toxicity induced by CCl_4 (Dutta *et al.* 2018; Bahashwan *et al.* 2015; Rahmat *et al.* 2014; Ahsan *et al.* 2009). Various studies hepatoprotective activities have shown that medicinal plants/ plant extracts which are traditionally being used in many liver disorders were reported to be very much useful in maintaining the various liver enzymes activities and also in restoring the liver histopathological structure (Maheshwari *et al.* 2011; Rahmat *et al.* 2014; Dutta *et al.* 2018; Reddy & Urooj 2017; Ahsan *et al.* 2009).

Amaranthus spinosus whole plant ethanolic extract was found to contain 336 ±14.3 mg/g gallic acid equivalent total polyphenolics, while the reducing capacity was 2.26 times of BHA, whereas significant antioxidant activity in DPPH assay (IC_{50} of 29 µg/mL), superoxide scavenging assay (IC_{50} ~66–70 µg/mL), hydrogen peroxide assay (IC_{50} ~120–125 µg/mL), hydroxyl radicals assay (IC_{50} ~140–145 µg/mL) and nitric oxide assay (IC_{50} ~135–140 µg/mL) were observed in ASE. ASE (6-10 µg/mL) was able to normalise the levels of biochemical parameters in isolated rat hepatocytes intoxicated with CCl_4. The effect of the ASE at 10 µg/mL was found to be comparable to control group. The investigation carried out in human liver derived HepG2 cells against CCl_4 induced damage also confirmed the hepatoprotective nature of the ASE in a dose dependent manner (Zeashan *et al.* 2009).

Hepatoprotective activity of *Averrhoa carambola* stem ethanolic extract (ACSEE) was evaluated by CCl_4 induced hepatotoxicity in rats and estimated the serum hepatic enzyme levels (Eswaraiha *et al.* 2013). Treated animals with ACSEE (250 mg/kg) and ACSEE (500 mg/kg) showed reduction in serum enzyme levels and were comparable with standard silymarin. Histopathological studies of normal rat liver showed normal hepatocytes, sinusoids. Liver section of rat treated with CCl_4 exhibited severe necrosis, disappearance of hepatocytes and area of inflammation with increased sinusoidal spaces. Liver section of rat treated with ACSEE (250 mg) and CCl_4 exhibited mild degree of necrosis, reduced sinusoidal dilation and less inflammation. Liver section of rat treated with ACSEE (500 mg) and silymarin showed normal hepatocyte, sinusoids with no inflammation.

2.4 GC-MS review of study conducted by using GC-MS

The combination of an ideal separation technique (GC) with the best identification technique (MS) made GC–MS an ideal technique for qualitative and quantitative for volatile and semi-volatile compounds (Lordache *et al*. 2009). Because of which, in recent years, gas chromatography and mass spectrography (GC–MS) has been applied unambiguously to identify the structures of different phyto-constituents from plant extracts and biological samples with great success. GC-MS is a reliable technique to identify the phytoconstituents of volatile matter, long-chain branched hydrocarbons, alcohols, acids, esters, etc. Peak area, retention time and molecular formula were used for the confirmation of phytochemical compounds (Venkatesh *et al*. 2014; Rukmini & Devi 2014).

The *Morus alba* leave extract components were identified using gas chromatography and decomposition products were characterized by mass analyser detector GC-MS which shows three components viz; 9,12,15-octadecatrienoic acid ethyl ester, linolenic acid ethyl ester and gibberallic acid respectively (Emniyet *et al*. 2014). Saravanan *et al.* 2014, reported 13 different compounds from GC-MS analysis of *Ficus religiosa,* that were found to have antioxidant and antimicrobial activity: phenol, 4-methoxy phenol, ethyl isoallocholate and octadecanoic acid. In another study, GC-MS analysis showed the presence of 9,12,15-octadecatrienoic acid in *Vitis sitosa*, that possesses many biological activities which includes anti-inflammatory, cancer preventive, hepatoprotective, etc., (Gobalakrishnan *et al*. 2014).

2.5 Docking review

Molecular docking is a tool of molecular modelling by which we can understand the mechanism of action/interaction of medicinal plant's bioactive compounds that allows to present antagonist/agonist the best suitable orientation for most stable complex formation and the best position for molecular binding (ligand) with target proteins (Sousa *et al*. 2013; Kahraman *et al*. 2007; Lu *et al.* 2010; Ganguly & Panigrahi 2009). Docking reduce expenses and time due to carrying out the procedure that is similar to high-performance biological screening and is also possible to calculate the strength of binding (affinity) between them (Kitchen *et al.* 2004). Affinity is equal to the concentration of the ligand, in which half of the targets binds with the ligand (Meng *et al*. 2004; Morris *et al*. 2005; Zsoldos 2007). The measure of biological activity depends on ligand concentration at which the cell response is equal to half the maximum. Therefore, ligands with the highest affinity provided will block or activate the molecular target in biological experiments best of all (Cerqueira *et al*. 2009; Scigress explorer ultra, 2012; Huang *et al*. 2010). Glushchenko *et al*. 2015, stated that ligand affinity in relation to the receptor is assessed both by geometric criteria of surface complementarity of the ligand with that of receptor cavity and by physico-chemical criteria (like electrostatic interaction, van der Waals repulsion, hydrogen bonds formations etc.).

Molecular docking analysis with PDB- 3i7h complexed with H-Box motif of HBX, it was observed that the compound α-amyrin which was identified by the GC-MS analysis in the leaf extract of *Croton bonplandianus* Baill. have shown better potentiality to protect hepatocellular damages than the standard drug Silymarin (Dutta *et al*. 2018).

Gunalan *et al*. 2014, have studied docking analysis by the GC-MS compounds identified from *Bauhinia variegate* ethanolic leaf extract to inhibit COX-2 and iNOS. Among the 33 ligands of the active fractions, four ligands were found to have best glide score. The ligand: Benzofuranone was found to have better binding affinity with glide score of -3.147224 (-24.739633 glide energy Kcal/mol) in COX-2 biotarget and in case of iNOS the ligand Dioctylphthalate have shown better binding affinity with glide score of -9.359683 (-48.046527 glide energy Kcal/mol).

Glushchenko *et al.* 2015, conducted the docking studies by the method of a flexible molecular docking using the SCIGRESS software in order to determine the possible mechanisms of the hepatoprotective effect of *Bupleurum aureum* plant components for the inhibition of protein NFκB (nuclear factor kappa B) (code 2I9T). The best docking score of -35.050, -37.870 and -28.004 was obtained from the flavonoids (quercetin), alcohols (xylitol) and monoterpenoids (lolioside) respectively from *B. aureum* plant.