CHAPTER II
LITERATURE REVIEW

Literature review of *A. monilifer*, Antidiabetic and Antioxidant activities.

*Manikya Kumari et al., 2012* reported the hepatoprotective effect on *A. monilifer* Linn., which is a widely used plant in the north coastal districts of Andhra Pradesh, India, to treat various liver disorders and other common ailments. The methanolic extract of the whole plant at dose levels of 200 mg/kg, 400 mg/kg and 800 mg/kg b.w., was tested in CCl₄ induced hepatotoxicity rats followed by histopathological examination of the isolated livers of the control and the treated groups. The potential effects in protecting liver function by reducing the elevated levels of various serum biochemical parameters (SGOT, SGPT, ALP & T. Bil.) in a dose dependent manner, reducing oxidative stress, and histopathological alterations in the rat model of CCl₄–induced liver damage was demonstrated. The experimental results demonstrated the potential effect of methanolic extract of *A. monilifer* and its bioactive molecules vitexin and isovitexin in protecting liver function, reducing oxidative stress, and improving histopathological structures in the rat model of CCl₄–induced liver damage.

*Shadma shahin and Naheed Ahmed 2013* reported the morphological as well as the seed storage protein band pattern of *Alysicarpus*. Found spread in four different sites of Patna. The sample collecting is done using extensive survey in the various region of Patna District, while the analysis of protein band pattern is done using SDS - PAGE. The result of the research of morphology is analyzed descriptively and presented in the form of tables. The analysis of seed protein band is done using quantitative and qualitative analysis that is based on band patterns, percentage similarity and dissimilarity index (DSI) and Rf values of bands. A total 32 bands
were observed and out of 3 bands in identical pattern. The results of protein diversity study shows that *A. monilifer* and *Alysicarpus vaginalis* formed close similarity clump, while *Alysicarpus bupleurifolius* and *Alysicarpus rugosus* var heyneanus form the other clade. The present morphological and biochemical study was thus helpful in establishing the distinct identity of the four species.

**Purvi H. Kakrani et al., 2011** reported the pharmacognostic and preliminary phytochemical evaluations of aerial parts of *A. monilifer* to develop its monograph. Pharmacognostical investigations were carried out in terms of macroscopical and microscopical characters, physio-chemical constants, extractive values in different solvents, fluorescence analysis of dry powder- its reaction after treatment with chemical reagents under visible light, and UV light at 254 nm and 366 nm. The dried root powder was subjected to successive Soxhlet extraction was carried out using petroleum ether, chloroform, ethyl acetate, methanol, ethanol and water. These solvent extracts were subjected to preliminary phytochemical screening to detect the different chemical principles present viz., carbohydrates, steroids, glycosides, alkaloids, tannins and phenolic compounds. The phytochemical evaluation revealed the presence of carbohydrates, phytosterols, triterpenoidal saponins, fixed oils, phenolics and tannins.

**Hariprasath L et al., 2015** reported that the *Senecio candicans* DC is an endemic sub-shrubby climber and the hot water extract of this plant is being used traditionally for the treatment of gastric ulcer. The present study was to design a standardized protocol for *in vitro* regeneration of *S. candicans* from leaf explants and compare the antioxidant activities of aqueous extracts of *in vitro* calluses and *in vivo* leaves by
three different methods. Callus was initiated on Murashige and Skoog's (MS) basal media supplemented with various concentrations (0.5 mg L\(^{-1}\) to 2.0 mg L\(^{-1}\)) and combinations of auxins and cytokinins under dark incubation for 2 weeks. The percentage of callus induction (PCI) and the fresh weights were recorded after 28 days of culture. The fresh weights were again recorded after 4 weeks of sub-culture to determine the relative fresh weight growth (RFWG). The highest PCI of 87% and RFWG of 3.245 were obtained on an MS medium supplemented with 6-benzyl amino purine (BA) 2mgL\(^{-1}\) and indole-3-acetic acid (IAA) 2 mg L\(^{-1}\). The callus obtained after 28 days of culture were inoculated on MS medium supplemented with different growth regulators. The maximum average shoot length (4.28 ± 0.27 cm) and maximum percentage of shoot induction (81%) was observed on MS medium supplemented with BA (2.0 mg L\(^{-1}\)) + IAA (1.0 mg L\(^{-1}\)). The maximum root induction percentage (90%) from shoots was observed on half strength MS medium fortified with 3.0 mg L\(^{-1}\) α-naphthalene acetic acid (NAA) alone. The antioxidant activity of *in vivo* leaf was significantly high compared to the *in vitro* callus in all the three antioxidant assays. A linear correlation between antioxidant activity and the total phenolic content was observed. The current protocol for *in vitro* regeneration would pave the way for further research on the isolation of bioactive compounds including antioxidants by cell suspension culture.

**Zhanyong Wang et al., 2015** revealed that the preliminary characterization and antioxidant activity *in vitro* and *in vivo* investigation of the polysaccharide fraction named as RCSP II, which was extracted from *Rana chensinensis* skin, were performed. Results indicated that RCSP II comprised glucose, galactose, and mannose in a molar ratio of 87.82:2.77:1.54 with a molecular weight of 12.8 kDa. Antioxidant activity assay *in vitro* showed that RCSP II exhibited 75.2% scavenging
activity against 2,2'-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid) radicals at the concentration of 2500 mg/L and 85.1% against chelated ferrous ion at 4000 mg/L. Antioxidant activity assay in vivo further showed that RCSP II increased the activities of antioxidant enzymes, decreased the levels of malondialdehyde, and enhanced total antioxidant capabilities in livers and sera of D-galactose induced mice. These results suggested that RCSP II could have potential antioxidant applications as medicine or functional food.

**Bun Tsoi et al., 2015** revealed that the most commonly applied strategies for the evaluation of antioxidant capacity are the chemical- or cell-based approaches. However, the results obtained from these methods might not reflect the antioxidant ability of test samples within organisms. In this study, we propose a combination of experiments, including oxygen radical absorbance capacity (ORAC), cellular antioxidant activity assay (CAA), and the chick embryo model, as an efficient trio to evaluate antioxidant capacity of food components. Taking purine alkaloids as example, results demonstrate that chemical and cellular method might misinterpret their true ability on antioxidation. In chick embryo model, caffeine and theacrine can significantly improve vessel density on chorioallantoic membrane and myocardial apoptosis. The mechanism can be involving multiple targets within the organism. We believe that the trio proposed can be widely utilized in screening massive number of antioxidant in a cost-effective way. It will also help discovering new antioxidants that are easily being omitted due to their relatively poor in vitro activities.

**Pingping Wu et al., 2015** reported that this study investigated the in vitro and in vivo antioxidant activities of the flavonoids rich extract from *Rhodomyrtus tomentosa*
Hassk (*R. tomentosa*) berries. The *in vitro* antioxidant assay demonstrated that the flavonoids rich extract (62.09% rutin equivalent) extracted by ethanol and purified by AB-8 macroporous resin was strong in reducing power, superoxide radical, hydroxyl radical and DPPH radical scavenging activity, as well as inhibiting lipid peroxidation. In the *in vivo* assays, the flavonoids rich extract significantly enhanced the activities of antioxidant enzymes in serums of mice after they were administered with the extract. The results suggested that the flavonoids rich extract from *R. tomentosa* fruits possesses potent antioxidant properties. In addition, the chemical compositions of flavonoids rich extract were identified by UPLC–TOF-MS/MS. Six flavonoids were tentatively identified as myricetin, quercetin, dihydromyricetin, kaempferol, quercetin 7,4-diglucoside and vitexin. Therefore, *R. tomentosa* berries could be used as a new source of antioxidant ingredient.

Pietro Maria Chagas *et al.*, 2015 revealed that the organoselenium compounds have been reported for many biological properties, especially as potent antioxidants. The compound bis (phenylimidazoselenazolyl) diselenide (BPIS) is a novel diaryl diselenide derivative, which shows antinociceptive and anti-inflammatory properties in mice, but whose antioxidant activity has not been studied. The present study aimed to investigate the antioxidant and toxicological potential of BPIS in brain of rats in vitro, and the effect of BPIS against the oxidative damage induced by sodium nitroprusside (SNP) in mouse brain. BPIS, at low molecular range, reduced lipid peroxidation (LP) and protein carbonyl (PC) content in rat brain homogenates (IC$_{50}$ values of 1.35 and 0.74µM, respectively). BPIS also presented dehydroascorbate reductase-like and glutathione-S-transferase-like, as well as DPPH and NO-scavenging activities. Related to toxicological assays, BPIS inhibited δ-ALA-D and Na, K$^+$-ATPase activities in rat brain homogenates and [$^3$H]glutamate uptake in
synaptosomes in vitro, but these effects were observed at higher concentrations than it had antioxidant effect (IC$_{50}$ values of 16.41, 26.44 and 3.29 µM, respectively). In vivo, brains of mice treated with SNP (0.335 µmol per site; i.c.v.) showed an increase in LP and PC and a reduction in non protein thiol content, however, it was not observed significant alterations in antioxidant enzyme activities. BPIS (10 mg/kg; p.o.) protected against these alterations caused by SNP. In conclusion, the results demonstrated the antioxidant action of BPIS in in vitro assays. Furthermore, BPIS protected against oxidative damage caused by SNP in mouse brain, strengthening the potential antioxidant effect of this compound.

Min-Cheol Kang et al., 2015 reported in this study, the antioxidative effects of a purified polysaccharide isolated from the stems of Acanthopanax koreanum Nakai (ASP) on hydrogen peroxide-induced oxidative stress was investigated both in vitro and in vivo using a zebrafish model. A. koreanum Nakai stem was hydrolyzed using five carbohydrases and five proteases for the enzyme-assistant extraction. Of the enzyme-assistant extracts, the Protamex extract exhibited in the highest yield and a potent scavenging activity against free radicals. Ethanol-added separation and anion exchange chromatography were conducted to identify the active polysaccharide. The purified polysaccharide significantly scavenged hydrogen peroxide and reduced hydrogen peroxide induced cell death in Vero cells and in zebrafish. The results reveal that ASP is a useful antioxidant polysaccharide and might be available for relevant industrial applications.

Hongyan Li et al., 2014 showed that the bioaccessibility, antioxidant activities and anti-inflammatory activities of phytochemicals in a purple tomato (Solanum
lycopersicum L.) V118 was studied using a simulated gastrointestinal digestion model, chemical and cell based antioxidant assays. The total phenolic and carotenoid contents and the antioxidant activities were significantly lowered (37–72%) and degradation seemed to have occurred during the in vitro digestion. Results indicated that these phytochemicals were bioavailable to the cells as demonstrated by the cell based antioxidant assay. Extracts from the purple tomato showed significant and dose dependent anti-inflammatory effect in the in vivo carrageenan-induced paw oedema rat study (oedematous inhibition: 7.48% and 13.8%), suggesting that anthocyanins may play a role in the anti-inflammatory effect. Direct antioxidant actions as indicated by reduced MDA and NO production and indirect actions as shown in increased GPx and SOD activities in oedematous tissue support the conclusion that tomatoes containing anthocyanins can potentially provide better protection against oxidative stress related chronic diseases of humans.

Gao Zhou et al., 2013 showed that the Meconopsis integrifolia (Maxim.) Franch is a high mountain endemic species used as a traditional Tibetan and Mongolian herb to treat hepatitis, pneumonia, and edema. This study aims to investigate the hepatoprotective and antioxidant effects of Meconopsis integrifolia ethanolic extract (MIE) in vitro and in vivo. The in vitro antioxidant property of MIE was investigated by employing various established systems. Rats with carbon tetrachloride (CCl₄)-induced liver injury were used to assess the hepatoprotective and antioxidant effect of MIE in vivo. The level or activity of alkaline phosphatase (ALP), alanine amino transferase (ALT), aspartate aminotransferase (AST), and total bilirubin (TB) in the blood serum and thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) in the liver and kidney of the rats were assayed using standard procedures. MIE exhibited strong antioxidant
ability in vitro. In the rats with CCl$_4$-induced liver injury, the groups treated with MIE and silymarin showed significantly lower levels of ALT, AST, ALP, and TB. MIE demonstrated good antioxidant activities in both the liver and kidney of the rats in vivo. MIE exhibits excellent hepatoprotective effects and antioxidant activities in vitro and in vivo, supporting the traditional use of Meconopsis integrifolia in the treatment of hepatitis.

Engin Celep et al., 2013 showed the antioxidant activity of the 80% methanolic extract of Cornus mas L. leaves (CMM) was evaluated by various methods both in vitro and in vivo. In vitro screening tests indicated that CMM had high antioxidant activity in terms of free radical scavenging and metal reducing activity. In vivo antioxidant activity studies in normal healthy rats demonstrated that the total antioxidant capacity of liver homogenates were increased, although no changes were observed in the activities of antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase or in the level of lipid peroxidation. Studies on CCl$_4$-treated rats also showed that CMM restored the activities of antioxidant enzymes, lowered the level of lipid peroxidation and elevated the total antioxidant capacities of both the total blood and liver homogenates of the animals. Further activity-guided fractionation studies led to the isolation of gallic acid, a well-known antioxidant, as one of the active components.

Jun Liu et al., 2013 revealed that the antioxidant activities of ethanolic extract from edible mushroom Agaricus bisporus (A. bisporus) were evaluated by various methods in vitro and in vivo. In antioxidant assays in vitro, ethanolic extract of A.bisporus was found to have strong reducing power, superoxide radical, hydroxyl
radical and 2, 2 diphenyl-1-picrylhydrazyl radical scavenging activity, and moderate hydrogen peroxide scavenging activity. In antioxidant assays in vivo, mice were administered with ethanolic extract of *A. bisporus* via gavage for 30 consecutive days. As a result, administration of ethanolic extract significantly enhanced the activities of antioxidant enzymes in serums, livers and hearts of mice. In addition, the total phenolic content in the extract determined by Folin–Ciocalteu method was 6.18 mg of gallic acid equivalents per gram of dry weight. The main phenolic compounds in ethanolic extract analyzed by ultra-high performance liquid chromatography tandem mass spectrometry were determined as gallic acid, protocatechuic acid, catechin, caffeic acid, ferulic acid and myricetin. These results suggested that ethanolic extract of *A. bisporus* had potent antioxidant activity and could be explored as a novel natural antioxidant.

He et al., 2012 Meconopsis quintuplinervia, a medicinal herb endemic to the Tibetan is used to treat hepatitis. The aim of this study is to evaluate the antioxidant potential of the ethanolic extract of this herb using different assays. The antioxidant capacity of *Meconopsis quintuplinervia* was investigated using various established *in vitro* systems. An *in vivo* study of carbon tetrachloride (CCl₄)-induced antioxidant activity in mice was also conducted by examining the levels of malondialdehyde (MDA) and the activities of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH). The extract showed strong *in vitro* antioxidant ability. In the *in vivo* study, CCl₄ -induced oxidative stress caused significant decreases in the SOD, CAT, and GSH levels and a significant increase in the MDA level, most of which were significantly reversed (except for SOD in the liver.) by treatment with the extract and standard Vitamin E. This study clearly indicates that the ethanolic extract of Meconopsis quintuplinervia is a valuable source
Yue Chen et al., 2011 reported that the Swertia chirayita, a medicinal herb endemic to the Tibetan region, is used as a special remedy for liver disorders. The hepatoprotective activity of its plant extracts has been associated with its antioxidant activity. This paper aims to investigate the in vitro and in vivo antioxidant effects of Swertia chirayita extracts (SCE). Antioxidant ability of Swertia chirayita was investigated by employing several established in vitro methods. In vivo antioxidant activity was tested against CCl₄-induced toxicity in mice. The levels and activities of malondialdehyde (MDA) and antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH), were then assayed using standard procedures. SCE exhibited strong antioxidant ability in vitro. The liver and kidney of CCl₄-intoxicated animals exhibited a significant (p < 0.001) decrease in SOD, CAT, and GSH levels. Additionally, these organs exhibited a significant (p < 0.001) increase in MDA level. CCl₄ did not exhibit toxicity on mice treated with SCE and Vitamin E. The effects of Swertia chirayita (three dosages) were comparable to those of Vitamin E, except in MDA level in the liver and GSH level in the kidney (p < 0.05). This study suggests that the ethanolic extract of Swertia chirayita possesses in vitro and in vivo antioxidant effects. This supports the traditional use of Swertia chirayita in Tibetan medicine to cure liver diseases.

Jun Liu et al., 2010 reported that the antioxidant activities of exopolysaccharides (EPS) from endophytic bacterium Paenibacillus polymyxa EJS-3 were evaluated by various methods in vitro and in vivo. In antioxidant assays in vitro, both crude EPS
and its purified fractions (EPS-1 and EPS-2) were found to have moderate 2, 2-diphenyl-1-picrylhydrazyl radical scavenging activity, hydrogen peroxide scavenging activity, lipid peroxidation inhibition effect, and strong ferrous ion chelating activity. And the antioxidant activities *in vitro* of EPS decreased in the order of crude EPS > EPS-2 > EPS-1. In antioxidant assays *in vivo*, mice were subcutaneously injected with d-galactose (d-Gal) for 6 weeks and administered EPS-1 via gavage simultaneously. As a result, administration of EPS-1 significantly increased the thymus and spleen indices of d-Gal induced aging mice. Moreover, EPS-1 administration significantly enhanced the activities of antioxidant enzymes and total antioxidant capacity and decreased the levels of malondialdehyde in both serums and livers of aging mice. These results suggested that EPS had potent antioxidant activities and could be explored as novel natural antioxidant.