Summary
and
Conclusions
SUMMARY

The summary of the systematic work that has been carried out on elephant-foot yam is presented below.

CHAPTER 1

Important fruits and vegetables like ber (Ziziphus jujube), woodapple (Feronia limonia), pseudo stem of banana (Musa paradisiaca), brinjal (Solanum melangina), chow-chow (Sechium edule), cluster bean (Cyamopsis tetragonoloba), elephant foot yam (Amorphophallus paeoniifolius) and kenaf (Hibiscus cannabinus) which are less investigated but are extensively used in various Ayurvedic preparations to cure many ailments were selected and screened for their antioxidant activity. Dried powders of above plant material were extracted serially by using different solvents viz. hexane followed by chloroform, ethyl acetate, acetone and methanol. These extracts were analyzed for their potential antioxidant activity by DPPH assay. Among the different extracts tested, acetone extract of elephant foot yam exhibited the highest free radical scavenging activity (75.34%) followed by acetone extracts of brinjal (65.32%) and banana pseudo stem (63.44%). Hence the corm of elephant foot yam was selected for detail investigation in this thesis.

CHAPTER 2

Samples of elephant foot yam were collected periodically at different stages of development of corm from the field to evaluate the peak accumulation period for various bioactive constituents. Studies on changes in physico-chemical parameters like pH, TSS, acidity, sugars, phenols, carotenoids and flavonoids were carried out during development of elephant foot yam corm. Based on peak accumulation of
bioactive constituents and antioxidant activity, the optimum nutraceutical maturity stage for elephant foot yam was determined as 210 days, when compared with commercial harvest which extends to 240-270 days from planting. At optimum nutraceutical maturity the corm has pH- 5.98, TSS (%) -5.58, Total Acidity (%) -0.27, Moisture content (%) -76.82, Pulp/Peel ratio -18.86, Penetration (Newton) -11.47, Compression (Newton) -50.66, Shear (Newton) -8.01, NEB -0.247, Total Carotenoids (µg/100g) -601.69, Total Phenols (mg/100g) -117, Total Flavonoids (mg/100g) -69.37.

CHAPTER 3

Dried powder of elephant foot yam corm was extracted sequentially by using different solvents viz. hexane followed by chloroform, ethyl acetate, acetone and methanol. These extracts were analyzed for their total phenols and flavonoid content. Further, these extracts were evaluated for their functional properties like antioxidant activity, antimicrobial activity, anti-platelet aggregation activity and cytotoxicity.

Among the different extracts tested, acetone extract recorded the highest total phenol (866.27 mg GAE/gm of extract) and flavonoid (585.7 mg catechin equivalents/ gm of extract) content. It has exhibited highest antioxidant activity for DPPH radical scavenging activity, superoxide radical scavenging activity, β-carotene bleaching inhibition assay, anti-lipid peroxidation activity, hydrogen peroxide scavenging activity, nitric oxide scavenging activity and total reducing power assay.

Antimicrobial activity was carried out agar-well diffusion method using thirteen bacterial clinical isolates, including seven Gram –ve bacteria (Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi, Klebsiella pneumonia, Enterobacter aerogenes, Proteus mirabilis, Yersinia enterocolitica), six Gram +ve bacteria
(Micrococcus luteus, Staphylococcus aureus, Enterococcus fecalis, Bacillus cereus, Bacillus subtilis, Listeria monocytogenes) and also six post harvest spoilage causing fungal strains (Aspergillus niger, A. flavus, A. fumigatus, A. parasiticus, Penicillium rubrum and Fusarium moniliforme). Among the extracts tested acetone extract exhibited wide range of anti microbial activity.

The anti-platelet aggregation activity was conducted by using chronolog dual channel aggregometer. The platelet-rich plasma (PRP) isolated from venous blood from healthy human volunteers was tested with different concentrations of extracts against platelet aggregation induced by ADP, collagen and arachidonic acid. Among the extracts tested, acetone extract exhibited a maximum of 80%, 84%, and 86% inhibitory activity against ADP (50µM), collagen (0.5 mg/ml) and arachidonic acid (1mM) agonists respectively. Methanol and ethyl acetate extracts with the same concentration showed very less potency in inhibiting the human platelet aggregation. Hexane and chloroform extracts showed no anti-platelet aggregation activity against these three agonists.

Cytotoxicity was studied by using HepG-2 cell lines. High cytotoxicity was shown by chloroform extract followed by acetone, ethyl acetate, methanol and hexane.

In vitro evaluation of various functional properties of acetone extract of elephant foot yam indicated as a potential source of nutraceutical activities. Hence the extract was tested for in vivo antidiabetic activity and hepatoprotective properties in rat model.
CHAPTER 4

Anti-diabetic effect of elephant foot yam in streptozotocin induced diabetic rats

The acetone extract of yam showed highest antioxidant activity concurrently with highest bioactive constituents like total phenols and flavonoids. Hence, the effect of feeding acetone extract at 0.1 and 0.25% level in diet was studied in streptozotocin induced diabetic rats. The study involved a comparison between starch fed diabetic (SFD), acetone extract at 0.1% fed diabetic (CFD<sub>0.1</sub>), acetone extract at 0.25% fed diabetic (CFD<sub>0.25</sub>) and aminoguanidine fed diabetic (AFD) groups. The rats were examined for fasting blood sugar (FBS), glomerular filtration rate (GFR), lipid peroxidation and activities of antioxidant enzymes like catalase, superoxide dismutase, peroxidase, glutathione peroxidase and glutathione-s-transferase.

Fasting blood sugar of CFD<sub>0.1</sub> and CFD<sub>0.25</sub> groups showed 23% and 37% reduction, respectively, whereas, AFD group showed 45% reduction in comparison to SFD group. Glomerular filtration rate of experimental rats in CFD<sub>0.1</sub> and CFD<sub>0.25</sub> groups showed 28% and 41% reduction, respectively, whereas, AFD group showed 54% reduction compared to SFD group. Amelioration of intestinal maltase activities by 18% 26% and 48% was observed in CFD<sub>0.1</sub>, CFD<sub>0.25</sub> and AFD groups respectively when compared to SFD group. Intestinal sucrase activity was high in SFD group and was ameliorated in CFD<sub>0.1</sub>, CFD<sub>0.25</sub> and AFD groups to about 28, 45 and 56% respectively. Similarly, intestinal lactase activity was high in SFD group and was ameliorated in CFD<sub>0.1</sub>, CFD<sub>0.25</sub> and AFD groups to about 36, 52 and 64%, respectively. In contrast decrease in renal maltase, sucrase and lactase were observed in SFD group while ameliorated in CFD<sub>0.1</sub>, CFD<sub>0.25</sub> and AFD groups.
Streptozotocin is known to induce oxidative stress resulting in induction of diabetic condition in rats. A significant decrease in the activity of antioxidant enzymes viz., catalase, superoxide dismutase, glutathion peroxidase, glutathion S-transferase and glutathion reductase were observed in the blood serum, kidney and liver of diabetic induced rats (SFD) were observed when compared to non diabetic control rats (SFC, YFC\textsubscript{0.1}, YFC\textsubscript{0.25} and AFC). Supplementation of diet with 0.1 (YFD\textsubscript{0.1}) and 0.25% (YFD\textsubscript{0.25}) of acetone extract to diabetic rats resulted in significant amelioration in the activity of antioxidant enzymes viz., catalase, superoxide dismutase, glutathion peroxidase, glutathion S-transferase and glutathion reductase, when compared to diabetic control rats (SFD). The lipid peroxidation as measured by malondialdehyde content significantly increased in streptozotocin induced diabetic rats (SFD), when compared to non diabetic control rats (SFC, YFC\textsubscript{0.1}, YFC\textsubscript{0.25} and AFC). Streptozotocin induced diabetic rats treated with acetone extract (YFD\textsubscript{0.1} and YFD\textsubscript{0.25}) exerted a significantly improved effect by decreasing the concentration of malondialdehyde content, when compared to streptozotocin treated diabetic rats (SFD).

The reduction in glycemic conditions and amelioration of antioxidant enzymes activity and lipid peroxidation value may be attributed to antioxidant-rich phenols particularly flavonoids present in acetone extract. The results clearly indicated that acetone extract of elephant foot yam is an effective anti-diabetic agent to streptozotocin induced diabetic rats.

**Hepatoprotective effect of elephant foot yam**

Experiment was carried out to demonstrate the hepatoprotective action of acetone extract of Yam (at 100 and 200 mg/kg body weight) in rats wherein, liver
damage was induced by carbon tetrachloride (CCl₄) with or without pretreatment. Results revealed that CCl₄ induced oxidative stress by a significant increase in the activity of the serum enzymes like alkaline phosphatase (ALP), glutamate oxaloacetate transaminase (SGOT), glutamate-pyruvate transaminase (SGPT) and thiobarbituric acid reactive substances (TBARS) along with reduced activity of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) in liver. Pretreatment of rats with acetone extract (doses 100 and 200mg/kg body weight) significantly lowered serum enzymes and TBARS levels. Significant restoration of antioxidant enzymes activity was observed in liver. The results clearly indicated that elephant foot yam extract protects liver from CCl₄ induced oxidative damage.

Conclusions

- Nutraceutical maturity of the corm of elephant-foot yam was demonstrated for the first time.

- Antiplatelet aggregation activity of elephant-foot yam was demonstrated for the first time.

- *In vivo* antidiabetic activity of elephant-foot yam was demonstrated for the first time.