CHAPTER-5

Summary and Conclusion

Experimental and epidemiological studies have shown that moderate intake of red wine impart numerous therapeutic benefits and provides shielding effect to the consumer, particularly against cardiovascular ailments. Therefore, the popularity of wine, one of the most acceptable classes of beverages, is on an increase due to its alleged health assistances. Not only from grape, but wines from other substrates are also being prepared and evaluated for their potential medicinal attributes. In a similar thought, the present work entitled "**Production of non-traditional wines from *Syzygium cumini* and assessment of their medicinal efficacies in animal model"** was undertaken to produce wine variants from different parts of *Syzygium cumini*, also known as Jamun in Hindi, and evaluate their efficacy against diabetes mellitus, dyslipidemia, atherogenesis and associated oxidative stress in a rat model. The chief verdicts of the study are listed below.

1. Process development for the fermentation of Jamun plant parts

Stem, leaves, seed and pulp of Jamun were taken and their respective extracts were prepared, supplemented with cane sugar to a raise TSS of 20^{o}B and were used as a medium for the growth of *S. cerevisiae* and consequent production of ethanol. After 10 days of fermentation, observed ethanol content was 6.8±1 % (v/v) with the fermentation efficiency of 72±11 %.

2. Standardization of various process conditions for the production of Jamun wines

The process parameters for the production of Jamun wines were standardized by varying various environmental factors, one at a time. The effect of environmental factors was studied by varying the sugar level, incubation temperature, inoculum level, pH of the medium and fermentation time. While the effects of nutritional factors were evaluated by employing statistical modelling which included the supplementation of various nitrogen sources and metal salts in the Jamun extract based media. Of the various environmental factors, an incubation temperature of 25^{o}C, 10% (v/v) inoculum, a pH of 4.5 and initial sugar concentration of 25°B, resulted in maximum production of ethanol and total

phenolic content (TPC) and total antioxidant capacity (TAC) in all the four variants of Jamun wine.

Amongst the nutritional factors, for stem wine, KH_2PO_4 (0.108 % w/v), yeast extract (0.05 % w/v) and $ZnSO_4$ (0.089 % w/v) supplementation yielded 11.59 % (v/v) ethanol, total anti-oxidant capacity of 42.57 µM/mL and TPC of 1047.87 µg/mL, after statistical optimization.

For leaf wine, supplementation of DAP (0.145 % w/v), malt extract (0.063 % w/v) and K_2HPO_4 (0.121 % w/v) led to the production of an ethanol level of 11.84 % (v/v), TAC of 49.2 µM/mL and TPC of 2179.1 µg/mL, after statistical optimization.

In case of seed wine, addition of DAP (0.105 % w/v), K_2HPO_4 (0.077 % w/v) and $MgSO_4$ (0.150 % w/v) yielded an ethanol concentration of 11.4 % (v/v), TAC of 66.48 µM/mL and TPC of 5141.4 µg/mL, after statistical optimization.

In the production of pulp wine, the foremost effect amongst the studied nutritional factors, was found to be of yeast extract (0.075 % w/v), KH_2PO_4 (0.149 % w/v) and $MgSO_4$ (0.050 % w/v). Supplementation of these factors led to the production of ethanol levels of 11.82 % (v/v), TAC of 45.9 µM/mL and TPC of 1613.9 µg/mL, after statistical optimization.

3. Production of Jamun wines under optimized process conditions

Production of Jamun wines under standardized environmental and nutritional conditions resulted in fermentation efficiencies of 92.4, 93.9, 90.6 and 94.8 % in stem, leaf, seed and pulp wine, respectively. Clear brown stem wine with 11.5 (% v/v) ethanol, yellowish green leaf wine with 11.8 (% v/v) ethanol, light brown seed with an ethanol of 11.4 (% v/v) and purple colored pulp wine with an ethanol content of 11.9 (% v/v), were recorded. The levels of other constituents like acidity, total soluble proteins, phenolics, antioxidant activity in the prepared wine were quite similar to the components found in various commercial wines. The aging of Jamun wines in amber colored glass bottles containing 20g/L oak wood chips was also done and it improved the characteristics of wines in terms of clarity, colour and proved to be very useful in bringing down the acidity of the wines from 0.41, 0.50, 0.45 and 0.57 gTartaric acid/100mL to 0.39, 0.43, 0.39 and 0.45 in stem, leaf, seed and pup wine, respectively. There was a significant increase in the total antioxidant capacity (TAC) of the matured wine samples to 47.3, 50.6, 71.8 and 47.7 µmol/L as compared to 45, 48.7, 66.8 and 45.7 µmol/L of TAC of unmatured stem, leaf,

seed and pulp wine samples, signifying the importance of maturation on wines' anti-oxidative potential. The wines were also found to be similar to that of commercial red wines in terms of ethanol content, pH, sugar, total phenolic content and total titratable acidity.

Phytochemical profiling of both unmatured and matured Jamun wines revealed the presence of medicinally active constituents including tannins, flavonoids, glycosides, polysaccharides, free amino acids etc. Chemical characterization of Jamun wines with LC-MS-Q-ToF showed the presence of numerous bio-active compounds like cyanidin, petunidin, luteolin, dephnidin, gallic acid, ellagic acid and acids like malic acid and tartaric acids etc. The existence of these compounds in Jamun wines, pointed towards their bio-rich composition and potential medicinal attributes.

4. Assessment of safety of prepared Jamun wines

For a new food product to be consumed by humans, it is important to evaluate their safety in terms of any harmful/toxicological reactions or damage to hepatic and renal functions. The safety of prepared Jamun wines were assessed in a rat model by measuring the important parameters of liver and kidney function as well as histological alterations after oral administration of 4mL/kg of Jamun wines to male wistar rats for a period of four weeks. No significant differences were observed in the markers of renal and liver function test after 30 days as compared to control group, which were not fed with wine. This was paralleled by the histological evidence which showed zero alterations in the morphology of liver and kidney of the wine fed animals and the overall histology was same as that of the control group. This indicated that administration of 4mL/kg Jamun wines to rats for a period of 4 weeks had no harmful effect and were found to be safe for oral consumption.

5. Organoleptic evaluation of Jamun wines

Sensorial evaluation of Jamun wines was done by a panel of 10 judges. It was found that all the 4 matured wines variants had a better sensorial acceptability as compared to their unmatured counterparts. Maturation improved aroma and organoleptic characteristics of Jamun wines. The best overall score was found to be in the order of matured leaf followed by stem, pulp and seed wines and all the wines were found to possess outstanding sensorial quality.

6. Evaluation of Jamun wines against diabetes mellitus in rat model

The anti-diabetic efficacy of Jamun wines was assessed in a diabetic rat model. Animals were diabeticized by injecting 55mg/kg streptozotocin, intraperitoneally. Diabetes was

confirmed by checking fasting blood glucose (FBG) levels and animals displaying FBG>250mg/dL were selected for further analysis. All the four wine variants were fed to the respective animal groups at a daily single of 4mL/kg. The wines were evaluated for their efficacy by both, prophylactic and therapeutic treatments and were compared with a commercial red wine, glibenclamide and insulin, taken as positive controls. The highlights of the outcomes witnessed are:

a) All the therapeutic treatments were found to be superior to the respective prophylactic treatments with the wines as well as extracts. All the wines were better than their respective extracts.

   Stem wine controlled FBG and brought it down to 171 and 134 mg/dL from 330 and 340 mg/dL after prophylactic and therapeutic treatments, respectively. Leaf wine brought down the FBG to 143 and 96 mg/dL after prophylactic and therapeutic treatments, respectively. Seed wine decreased FBG to 135 and 94 mg/dL after prophylactic and therapeutic treatments, respectively. Pulp wine arrested hyperglycemia and brought it to 180 and 125 mg/dL after prophylactic and therapeutic treatments, respectively. Therapeutic treatments with red wine, glibenclamide and insulin also controlled the elevated FBG and brought it down to 113, 98 and 112 mg/dL, respectively.

b) Insulin levels of the observed animals were also found to increase after all the treatments. The four Jamun wine variants were found to increase the insulin levels at a higher level as compared to their respective extracts and therapeutic treatments led to improved levels of insulin as compared to prophylactic treatments.

   After prophylactic and therapeutic treatments with stem wine, insulin levels were recorded to be 82 and 97 pmol/L as compared to 46 pmol/L of diabetic control. Leaf wine elevated insulin levels to 89 and 117 pmol/L, after prophylactic and therapeutic treatment, respectively. Seed wine was found to be most efficient in improving insulin levels to 91.8 pmol/L after prophylactic treatment and 124 pmol/L after therapeutic treatment, respectively. Pulp wine administration also improved insulin levels and the observed levels were 78 and 89 pmol/L after prophylactic and therapeutic treatments, respectively. Red wine, glibenclamide and insulin injections led to 91, 88 and 98 pmol/L of the insulin levels.

c. The increase in insulin levels and decrease in FBG were paralleled by the histological evidence which suggested regeneration of β-cells of pancreas after wine treatments as compared to almost nil β cells in the pancreatic histology of diabetic rats. The increase in the number of β cells must have led to increased production of insulin.

d. Diabeticization of rats led to decrease in their body weight (BW) due to insulin deficiency and inability of the body to utilize glucose for energy production, thereof. Jamun wine treatments improved BW of the animals and were found to be better than that of their respective extracts. Prophylactic treatments increased BW to 82, 89, 91 and 78 g after administering the animals with stem, leaf, seed and pulp wine, correspondingly. In contrast to this, untreated diabetic animals displayed a BW of 46g at 30^{th} day of the study. Therapeutic treatments elevated BW to 97, 113, 134 and 89 g after administration of stem, leaf, seed and pulp wine, correspondingly. Red wine, glibenclamide and insulin treatments also improved BW and brought it to 91, 88 and 98 g, respectively.

7. Effect of Jamun wines on Lipid profile

The efficacy of Jamun wines was also evaluated in terms of their ability to correct the dyslipidemia caused by induction of diabetes. Total cholesterol (TC), triglycerides (TG), high density lipoprotein-c (HDL-C), low density lipoprotein-c (LDL-C) and very low density lipoprotein-c (VLDL-C) were chosen as markers of dyslipidemia. Efficacy of the wines was found to be better than that of their respective extracts. The ameliorative effect of Jamun wines on the lipidemic markers is discussed at length.

a. Diabeticization of animals increased their TC from 69 to 105mg/dL. However, prophylactic treatment with stem, leaf, seed and pulp wine decreased TC to 78.29, 60.26, 57 and 80.3 mg/dL, respectively. Effect of therapeutic treatment was more pronounced as the observed TC levels were 58, 51, 48 and 73 mg/dL, respectively. TC was also found to decrease by administration of red wine, glibenclamide and insulin.

b. TG also increased to 228 from 104 mg/dL after induction of diabetes. Jamun wine treatments bought TG under control and their effect was superior to that of the respective extracts. Prophylactic treatment decreased TG to 171, 153.4, 150 and 160.2 mg/dL after administration of stem, leaf, seed and pulp wine, correspondingly.

After therapeutically treating the animals, the TG levels were observed to be 116, 90.7, 81 and 150 mg/dL, individually.

c. HDL-C, good cholesterol, was found to decline to 29 from 38 mg/dL, post diabeticization. Treatment with Jamun wines increased HDL-C to normal levels. It was observed that prophylactic treatments increased HDL-C to 32, 35, 36 and 31 mg/dL, with stem, leaf, seed and pulp wine, respectively. Therapeutic treatments boosted HDL-C to 36, 39, 39 and 34 mg/dL, correspondingly. Jamun wines also improved HDL-C levels in healthy rats, showing their cardio-protective effect.

d. LDL-C, also known as bad cholesterol was observed to elevate after streptozotocin injection. An LDL-C of 147 mg/dL was recorded in diabetic subjects, in contrast to 39 mg/dL of healthy subjects. Wine treatments decreased LDL-C to 88.6, 61.5, 56 and 85 mg/dL after administration of stem, leaf, seed and pulp wine, respectively. Therapeutically also, wines decreased LDL-C to 46, 27, 22 and 71 mg/dL after stem, leaf, seed and pulp wine treatments, individually.

e. VLDL-C also shot up in diabetic animals and was recorded to be 45.6 mg/dL as compared to 20 mg/dL in healthy rats. Prophylactic treatment decreased VLDL-C to 34, 30, 30 and 32 mg/dL after administration of stem, leaf, seed and pulp wine, correspondingly. After therapeutically treating the animals, VLDL-C levels were observed to be 25, 22, 21 and 30 mg/dL, individually.

f. Atherogenic index (AI), a prominent marker for the occurrence of atherogenesis, was also calculated in the present study. It was interesting to note that administration of Jamun wines decreased AI in healthy rats, thereby, protecting them against atherogenesis and cardio-vascular ailments. In diabetic animals however, AI was as high as 0.95 as compared to 0.42 of healthy rats. Prophylactic treatment decreased AI to 0.72, 0.64, 0.56 and 0.70 after administration of stem, leaf, seed and pulp wine, correspondingly. After therapeutically treating the animals, AI were observed to be 0.55, 0.45, 0.43 and 0.84, respectively.

8. Effect of Jamun wines on Hepatic and Renal Functions

Liver (hepatic) function test (LFT) and renal (kidney) function test (RFT) are the symbolic elements which ascertain the integrity, functionality and damage recovery of the two vital organs. Induction of diabetes created havoc in liver and kidney as the markers of their functionality, alkaline phosphatase, alanine phosphatase, alanine transaminase,

creatinine, uric acid and blood urea nitrogen were found to be significantly altered. Jamun wines administrations corrected these alterations and brought the indicators of hepatic and renal functions, into a desirable range. The efficacy of the wines was observed to be better than that of their extracts. The efficacy of the wine was recorded to be in the order of seed wine~leaf wine>pulp wine~stem wine. When compared to the positive controls, red wine, glibenclamide and insulin, seed and leaf wine were found to be more effective than these controls, however, stem and pulp wine were found to be at-par with their efficiency.

9. Effect of Jamun wines on oxidative stress

Reactive oxygen species are severely involved in the pathogenesis of diabetes mellitus and therefore, the biological markers of oxidative stress were assessed in liver, kidney and brain tissues of the diabetic animals. It was observed that after Jamun wines' administration, anti-oxidant status of the animals was improved in healthy rats. In order to evaluate their effect on diabetic animals, levels of malondialdehyde (MDA), superoxide dismutase (SOD), nitrite and catalase were recorded I diabetics as well as Jamun wine treated subjects. MDA and nitrite levels were noted to be increased in diabetic animals, however, the levels of free radical scavenging enzymes, catalase and super oxide dismutase were found to deteriorate. In Jamun wine treated subjects, these markers were brought to their normal ranges, thereby, protecting and curing the oxidative stress generated during diabetic state. Therapeutic treatments were superior to that of the prophylactic treatments ($p<0.01$). All the wine treated animals showed better anti-oxidant status as compared to the extract treated animals ($p<0.01$). MDA levels were diminished by up to 70% in liver, 73 % in kidney and 70% in brain, respectively. Therapeutic treatment increased SOD in liver, kidney and brain with seed wine (129, 300 and 115%) followed by leaf wine (117, 291 and 108%), stem wine (85, 158 and 64%) and pulp wine (82, 100 and 67%), correspondingly. Catalase (CAT) activity improved significantly and it was observed that in therapeutically treated animals, CAT in liver, kidney and brain were highly amended by seed wine (410, 458 and 484%) showing maximum increase followed by leaf wine (359, 395 and 400%), stem wine (170, 165 and 196%) and pulp wine (181, 190 and 216%), respectively. Therapeutic treatment exerted their enhanced efficiency and they increased nitrite in liver, kidney and brain with seed wine (91.6, 95 and 94.8%), leaf wine (87.5, 94 and 94%), stem wine (78.5, 85 and 85%) and pulp wine (78, 83.7 and 84%), respectively. The efficacy of the wines were recorded to be in the

order of seed wine>leaf wine > pulp wine~stem wine. It was also observed that even the Jamun wines' administration to healthy rats led to increase in CAT and SOD and simultaneously decreased MDA and nitrite levels. This shows that Jamun wines arrest free-radical generated havoc in diabetic animals and enhance the anti-oxidant status of healthy animals.

In conclusion, the current work was objectified at process development and optimization of production of herbal wines from various plant parts of *Syzygium cumini*, also known as Jamun. The next step constituted the pre-clinical evaluation of the prepared wines against diabetes mellitus, dyslipidemia, atherogenesis and associated oxidative stress. The work led to production of wines from stem, leaf, seed and pulp of Jamun. The wines were found to be of good bio-chemical constituency. All the 4 Jamun wines were observed to be safe for consumption and scored excellent in sensorial evaluation. Chemical profiling of the wines was done by LC-MS and it showed the presence of phytochemicals like gallic acid, myricetin, catechin, petunidin, malvidin, malic acid, naringenin etc.

Evaluation of wine in diabetic rat model showed their efficiency in combating the disease. Our data indicate that Jamun wines inherits the health benefits of Jamun plant parts and therefore, are a potential candidate of functional beverages with *in-vivo* capabilities similar to those of red wine, glibenclamide and insulin. The 4 Jamun wine variants effectively reduced blood glucose levels and improved insulin production and secretion. They also brought the markers of lipidemic configuration to standard range. In addition to this, indicators of hepatic and renal damage as well as the markers of oxidative stress were brought back to their normal ranges after treatments with Jamun wines. Seed wine administration was found to be the best treatment for all the pre-clinical parameters studied, followed by leaf, stem and pulp wine. Seed and leaf wine were also found to be better anti-diabetic agents as compared to the commercial red wine and at-par with the commercial drugs. However, stem and pulp wine were observed to be at par in their efficacy with red wine and glibenclamide for combating diabetes. Wine treatments led to regeneration of pancreatic beta cells, which was not evident in insulin and glibenclamide treated animals. Moreover, the anti-diabetic, hypolipidemic, hepato-renal protective and anti-oxidative activity of Jamun wines was found to be better than that of their respective extract, which is the main highlight of the study. The same can be accredited to the presence of ethanol and acids which might lead to improved extractions and thereby, enhanced pharmacological activities exerted by the bio-active compounds present in it.

This suggests that Jamun wines are an improved version of Jamun extracts, are safe for consumption and excellent in organoleptic terms. Thus, they can be a potent candidate for functional beverages. And it is proposed that this beverage alone as an alternative therapy or in conjunction with conventional therapies may be used as a protective or curative measure for the metabolic disorder. Further, human trials are also required for complete validation of their medicinal attributes.