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RESEARCH PAPER

A Non-conventional Wine from Stem of *Syzygium cumini* and Statistical Optimization of its Fermentation Conditions for Maximum Bioactive Extraction

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Abstract

Keeping in view the increased demand of functional foods and the therapeutic potential associated with the stem of *Syzygium cumini*, a novel wine was produced from it. The wine was found to possess an ethanol content of 8.0±0.03% v/v. The wine had high total phenolic content (331.5µg/mL) and consequently, high total anti-oxidant capacity (27.09±0.1 µM/mL.). Statistical modeling was employed to the wine production for increasing ethanol, total phenolic and total anti-oxidant capacity of the wine. Primarily, the three most significant factors affecting the process were identified by Plackett - Burman design. In the second phase, the concentration and interaction of the factors identified (KH_2PO_4, yeast extract and $ZnSO_4$) was studied and optimized to achieve maximum response. The model was found to be significant ($p<0.005$) and ethanol concentration increased significantly to 11.59% (v/v), total anti-oxidant capacity to 186.0 µM/mL and total phenolic content to 1049.87 µg/mL in the optimized media. Further, for the identification of major bioactive compounds of the wine, LC-MS analysis of the wine was carried out which revealed the presence of various phyto-chemicals including malvidin, dephnidin, petunidin, gallic acid etc.

Keywords: *Syzygium cumini*, stem, wine, RSM, phenolics, anti-oxidant, ethanol, anthocyanin, *Jamun*

Food, beverage and nutrition are crucial companions of human race. Therefore, the bioactive ingredients of beverages and food take care of nutritional shortfalls, stabilize physical and mental health and support complete body system. Consumers have initiated preferring a healthy lifestyle and functional foods that accompany it. In the beverage market, functional botanical ingredients are more focused now than ever. In this context, *Syzygium cumini* (syn. *Eugenia Jambolana*) also known as *Jamun*, is an evergreen tropical tree native to India, Pakistan, Bangladesh, Indonesia, Nepal, Sri Lanka and Malaysia. Fruits, seed, leaves, stem and bark of the plant have been found to possess medicinal values. The fruit has been found to be diuretic, stomachic, carminative, astringent and anti-microbial. Seed extract, both in powdered and liquid form, when given orally, have been found to control blood glucose levels and glycosuria in patients. Other reports have suggested the use of leaves for diabetes, dysentery and as an anti-bacterial agent. Further, the stem of *S. cumini* comprises tannins and carbohydrates which have been accounted to help in dysentery and diabetes. Aqueous extracts of stem have displayed significant reductions in blood glucose and cholesterol levels in diabetic rats. The same also led to increase in total red blood cells and T-lymphocytes (Ayyanar *et al.* 2013). Intervention of bark extracts have also shown

to increased levels of insulin and C-peptide and thereby demonstrated anti-hyperglycemic effect in streptozotocin induced diabetic rats (Sarvanan and Leelavinothan 2006, 2008).

With the view of functional properties of *S. cumini* stem, we prepared a novel non-conventional wine from it and optimized its production with statistical modeling by Response Surface Methodology (RSM) for enhanced polyphenolic extraction. To the best of our knowledge, this is the first report of production of wine with functional properties from *S. cumini* stem and optimization of its fermentation conditions, though methods to produce wine, have been reported earlier (Joshi *et al.* 2012).

The traditional method of optimization, one factor at a time is arduous and time consuming. The major drawback of the same is that it does not define the interaction between different factors (Lu *et al.* 2011; Prasad *et al.* 2011). In this context, Placket -Burman (PB) method is a reliable technique that provides information on the effect of each factor and not their interaction. RSM is a useful technique for obtaining bet er understanding of the factors and their interactions for achieving improved productivities (Karacabey and Mazza, 2010). Therefore, RSM was employed for bet er ethanol yield, and maximum extraction of polyphenolics and antioxidants into the wine. Further, LC-MS profiling of wine was done for identifying the major bio-active compounds in the wine as discussed in this paper.

Materials and Methods

Reagents and chemicals

All the reagents and chemicals used in the study were of analytical grade.

Microorganism

Yeast strain used in the present study, *Saccharomyces cerevisiae* MTCC 786 was acquired from Microbial Type Culture Collection, Institute of Microbial Technology (IMTECH), Chandigarh, India.

Wine production

Twenty five ml of sterilized GYE broth (yeast extract, 0.3%, malt extract, 0.3%, peptone, 0.5% and glucose, 1%, pH 4.5) was inoculated with *Saccharomyces cerevisiae* and incubated over-night at 22±2^{o}C on a rotary shaker (150 rpm). Pre-inoculum was prepared by centrifuging the cells at 10,000 rpm (4^{o}C, 15 min). Cells were washed twice, and re-suspended in normal saline to raise a final concentration of 10^{8} cells/ml. The inoculum was prepared by transferring 10 ml of the pre-inoculum to 100 ml of aqueous extract of S. cumini stems, which was prepared by adding 15% (w/v) stems to distilled water and boiling it for 30 min. Stem pieces were not removed from the mixture and the TSS of the mixture was adjusted at 5° B with cane sugar.

For the production of wine, the stem extract was exogenously supplemented with cane sugar to adjust the TSS at 20° B and pH was adjusted at 4.5. diammonium hydrogen phosphate and Magnesium sulfate at the rate of 0.1% (w/v) and 100 ppm potassium metabisulphite were added. Fermentation was carried out at 22±2°C for 20 days by the activated yeast *Saccharomyces cerevisiae* MTCC 786 (10% v/v). Af er which the wine was filtered and used for further studies.

Analysis of wine

Physio-chemical analysis of wine was carried by using standard protocols. The parameters studied includes ethanol (Caputi *et al.* 1968), titratable acidity AOAC, 1980), total phenolics (Rathee *et al.* 2006) and total antioxidant capacity by FRAP method (Benzie and Strain, 1996).

Statistical Optimization of ethanol, total phenolic content and total antioxidant capacity of wine by Placket -Burman (PB) Design

Ethanol and phenolic content of the wine is highly influenced by many factors including media components and environmental parameters. Screening of the most significant parameters affecting the fermentation of Jamun stem was achieved by PB design (Placket and Burmann 1946). A total of 11 (n)

Table 1: Coded and actual values of the independent variables used in optimization

Independent variables	Units	Symbol	Coded levels				
			-2	-1	0	1	+2
KH_2PO_4	% w/v	X1	0.05	0.075	0.1	0.125	0.15
Yeast Extract	% w/v	X2	0.05	0.075	0.1	0.125	0.15
Zinc Sulfate	% w/v	X3	0.05	0.075	0.1	0.125	0.15

variables including TSS (15, 20° Brix), inoculum size (5, 10% v/v), incubation days (5, 10 days) along with 16 nutritional factors including ammonium sulfate, urea, diammonium hydrogen ortho-phosphate, peptone, malt extract, yeast extract, soybean meal, tryptone, di-potassium hydrogen phosphate, calcium chloride, sodium chloride, potassium chloride, zinc sulfate, di-hydrogen potassium phosphate, ammonium di-hydrogen phosphate and magnesium sulfate (0, 0.1% w/v) were studied to evaluate their effect on ethanol, total phenolic content and total antioxidant capacity of the wine. The figures in the parenthesis indicate the high and low values of the respective variables.

Statistical Optimization of screened parameters by Response Surface Methodology (RSM)

Following the selection of critical variables by PB design, RSM was employed to optimize their concentration for wine production. Regression analysis was performed on the data obtained from the design experiments. Experimental designs were generated and analyzed by using the statistical software package Design-Expert. 9.0.5, Stat-Ease Inc., Minneapolis, MN, USA. Response surface methodology (RSM) with central composite design (CCD) was employed to optimize the extraction parameters, viz. concentration of ethanol (X1), total phenolic content (X2) and total antioxidant capacity (X3). The range and central point value of all the three process variables are shown in Table 1. The process variables were coded according to the following equation:

$$x = Xi - X0/\Delta X$$

where, x is the dimensionless coded value, Xi is the actual value of variables, $X0$ is the actual value of variables at the centre point and ΔX is the step change value. After the conduct of the experiment, the data were fitted with a second-order polynomial equation as follow:

$$Y = \beta 0 + \sum \beta iXi + \sum \beta iiX2i + \sum \sum \beta ij\, Xi\, Xj + \varepsilon$$

where, Y is the predicted responses (ethanol, total phenolic content and total antioxidant capacity) β0 is the model constant, βi, βii and βij are model coefficients (linear, squared and interactive effects), ε and is the error.

Data analysis

The design of experiments, analysis of the results and prediction of the responses were carried out using Design-Expert software (Version 8.0). Comparisons of means were performed by one-way ANOVA (analysis of variance), followed by Tukey's test (p-value < 0.05).

Verification of the model

The wine production conditions were numerically optimized for the maximum yield of ethanol, TPC and high antioxidant activities based on the regression analysis and the contour plots of the independent variables. The responses were determined under the recommended conditions of the fermentation. Finally, the predicted values were compared with the experimental value in order to determine the validity of the model.

Liquid Chromatography-Mass Spectrometry (LC-MS)

The MS spectrometry was carried out by LC-

MS instrument (Waters Micromass Q-Tof Micro) having quadrapole time of flight, mass equipped with electrospray ionization. Wine samples were separated on a C18 column (250 mm × 5 mm) using a mobile phase composed of methanol (A) – 0.1% formic acid (B) under gradient elution. The linear gradient increased from 10 to 17% A in 7 min, and increased to 30% A in another 1 min. Then, the solution A was continuously increased from 30 to 90% in 12 min. Finally, the solution A was linearly decreased back to the initial condition of 10%. Analysis was performed in total ion chromatography mode (TIC) with positive ESI interface. For MS, the parameters used were desolvation gas: 550Lts/Hr, Cone gas: 35 Lts/Hr, desolvation temperature: 300°C, source temperature: 110°C, Capillary Voltage: 3000V, Cone Voltage: 30V. Identifications were achieved on the basis of the molecular ion mass, time of retention and comparison with literature data.

Results and Discussion

The fermentation of Jamun stems produced a clear brownish colored wine with ethanol content of 8.0±0.03% (v/v) and pH 3.4±0.02. The total soluble solids in wine were found to be 2°Brix, titratable acidity was 0.50±0.01 g Tartaric acid/100mL, reducing sugars of 0.44±0.02 g/100mL, total phenolic content of 331.5±0.15 µg/mL. Total anti-oxidative capacity of wine in terms of its ferric reducing ability, were measured by FRAP method and was found to be 27.09±0.1 µM/mL.

Table 2: CCD design with predicated and actual values for ethanol, total antioxidant capacity and TPC

Standard Order	Run Order	Coded Variables			Response I (Y_1) Ethanol % v/v		Response II (Y_2) Total antioxidant capacity (moles/mL)		Response III (Y_2) TPC (µg/mL)	
					Experimental	*Predicted*	*Experimental*	*Predicted*	*Experimental*	*Predicted*
		X1 KH_2PO_4 (% w/v)	*X2 Yeast Extract (% w/v)*	*X3 Zinc Sulfate (% w/v)*						
12	1	0	+2	0	9.8	9.87	79.76	79.40	999.00	990.05
3	2	-1	+1	-1	8.85	8.90	96	96.39	1056.15	1052.61
20	3	0	0	0	10.05	10.15	107.32	107.20	1013.79	1013.00
18	4	0	0	0	10.1	10.15	107	107.20	1012.20	1013.00
1	5	-1	-1	-1	10.8	10.80	89	89.03	1002.00	997.76
11	6	0	-2	0	10.85	10.83	150.5	150.67	1114.50	1122.53
6	7	+1	-1	+1	9.92	9.82	153	152.80	1077.34	1081.80
14	8	0	0	+2	9.32	9.42	121	120.45	819.30	817.41
15	9	0	0	0	10.2	10.15	107.33	107.20	1012.76	1013.00
4	10	+1	+1	-1	9.4	9.34	83	82.61	910.89	922.42
7	11	-1	+1	+1	10.2	10.08	104.4	105.14	713.33	719.01
8	12	+1	+1	+1	10.8	10.75	74	74.16	889.31	894.47
13	13	0	0	-2	9.85	9.80	94	94.35	912.77	913.74
10	14	+2	0	0	10.5	10.55	107.08	107.66	804.98	797.24
2	15	+1	-1	-1	11.3	11.37	136	135.45	849.28	844.52
17	16	0	0	0	10.1	10.15	107.48	107.20	1013.20	1013.00
19	17	0	0	0	10.2	10.15	106.92	107.20	1013.63	1013.00
5	18	-1	-1	+1	9	9.01	123	123.58	940.00	929.39
16	19	0	0	0	10.2	10.15	107.33	107.20	1013.33	1013.00
9	20	-2	0	0	9.3	9.30	93	92.22	768.20	775.02

Screening of parameters by Placket -Burman design

PB experiments highlighted the significance of optimizing fermentation conditions for Jamun stem wine. Out of 19 variables which were likely to play an important role in enhancing ethanol, total phenolics and total anti-oxidant capacity of the wine, 3 factors namely, potassium di-hydrogen orthophosphate, yeast extract and zinc sulfate were found to be the most effective.

Optimization of screened variables by RSM

The three significant factors were further optimized by RSM for most aptimum concentration. Table 2 shows the results of the predicted and experimentally obtained responses for the 20 runs. Ethanol concentrations obtained varied from 8.85 to 11.3% (v/v) and the maximum yield was obtained at run 15 with experimental conditions X1=0.125, X2=0.75 and X3=0.75% w/v. Total antioxidant capacity in terms of FRAP and total phenolic content ranged from 74 to 150.5 moles/ mL and 713.3 to 1114.5 μg/ mL, respectively. The maximum yields of both were obtained at a run 6 with the concentrations, X1=0.1, X2=0.5 and X3=0.1% w/v (Table 2). Based on this data, the fermentation parameters were optimized for maximum ethanol production and improved phenolic and anti-oxidant extraction into wine.

Fit ing the model

The data were fit ed into second-order polynomial model and the results are summarized in Table 2. The model was shown to be significant by the result of analysis of variance (ANOVA). The predicted and observed values were found to be moderately close to each other and indicated the satisfactory development of the model. The coded values for the coefficients of the predicted model for the three responses are given in Table 3. The significant contribution of each coefficient was determined by p-value ($p<0.05$).

The lack-of-fit test with $p>0.05$ for ethanol, TPC and total anti-oxidant capacity depicted the suitability of the model to accurately calculate the variations. With the coefficient of determination (R2) values of 0.9895, 0.996, 0.9972 for the three responses respectively, the mathematical model was found to be quite reliable. The coded values for the coefficients of the predicted model for the three responses are given in Table 3. The significant contribution of each coefficient was determined by p-value ($p<0.05$) and no evidence for lack-of-fit model was indicated for all the three responses.

Effect of variables on ethanol production

Factors such as sugar concentration, inoculum size, temperature and pH significantly contribute to the ethanol concentration of wines. With an optimum sugar and inoculum size, the factors which play a lead role into the fermentation medium is nutritional sources including nitrogen and other essential elements. These sources are vital for optimum growth of yeast. Furthermore, a few inorganic elements including potassium, magnesium etc.

Table 3: The fitted quadratic model in terms of coded variables for Y1, Y2 and Y3 responses

Responses	2nd order polynomial equation	Regression (p- value)	R^2	R^2 Adjusted	Lack of fit	Sum of squares	F-value
Ethanol (Y1)	10.15+0.31X1-0.24X2-0.093X3-0.034X1X2+0.059X1X3+0.74X2X3-0.056$X1^2$+0.050$X2^2$-0.13$X3^2$	0.0001	0.9895	0.9801	5	7.72	104.881943
Total Antioxidant capacity (Y2)	107.20+3.86X1-17.82X2+6.52X3-15.05X1X2-4.30X1X3-6.45X2X3-1.81$X1^2$+1.96$X2^2$+0.052$X3^2$	0.0001	0.9996	0.9993	5	8519.72	2839.56419
TPC (Y3)	149.45+5223.78X1+4898.92X2+9213.92X3+9221.02X1X2+1.22X1X3-1.06X2X3-9.747.83$X1^2$+17317$X2^2$-58969$X3^2$	0.0001	0.9972	0.9947	5	226759.80	396.955611

have been found to improve ethanol productivity of yeast. In our study, the influencing factors on ethanol concentration were defined by significant coefficient of second-order polynomial regression equation given in Table 3. The F value of 104.881943 and a R2 value of 0.9801 imply that the model is highly significant. The linear terms X1, X2, X3, interactive term X2X3 and the quadratic term X12, X22 and X32 were found to be significant for ethanol production. X1X2 and X1X3 with p>0.05 was found to be insignificant.

The most substantial interactive effect on ethanol was of yeast extract and $ZnSO_4$. Ethanol concentration was predicted to be maximum (13.41% v/v) at yeast extract (0.05% w/v) and $ZnSO_4$ (0.05% w/v), keeping KH_2PO_4 at 0 coded value. With an increase in the levels of the respective variables, ethanol declined. However, at 0.12% yeast extract and 0.14%w/v $ZnSO_4$, ethanol start to increase again and the predicted value raised up to 12.0% v/v ethanol at 0.149% w/w yeast extract and $ZnSO_4$, respectively as indicated in Fig. 1.

The interactive and quadratic terms X1X3, X2X3 and X22 were found to affect the ethanol yields positively in terms of coefficient of estimate. The linear term X1 i.e. KH_2PO_4 as a single factor, with a coefficient of estimate 0.31 and F-value 188.77 was found to be only and the most effective variable for the improved ethanol yields. All the other terms were negatively affecting ethanol production.

Effect of variables on total antioxidant capacity of wine

The antioxidant capacity is the major factor of increasing popularity of wines among health conscious consumers. Factors such as substrate origin, yeast nutrients, fermentation temperature, and ethanol content majorly contribute to the extraction of antioxidants into wine (Radovanovic *et al.* 2012). RSM design employment led to an overall increase in total antioxidant capacity of Jamun stem wine. This was defined in a second-order polynomial equation described in Table 3. For total anti-oxidant capacity, KH_2PO_4, yeast extract and $ZnSO_4$ were significant in the first order linear effect (X1, X2, X3), second order quadratic effect (X12, X22) and interactive effect (X1X2, X2X3, X1X3). X32 with a p>0.005 was insignificant.

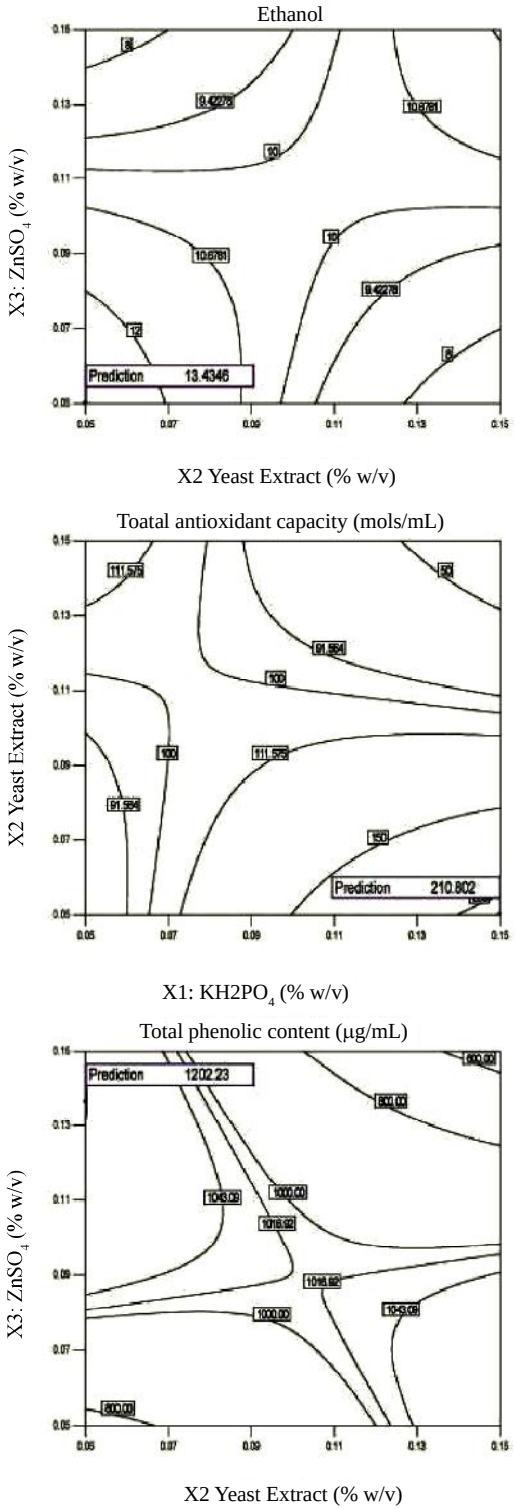

Fig. 1: Response surface plots showing the most significant interactive effect of KH_2PO_4, Yeast extract and $ZnSO_4$ on ethanol, total anti-oxidant capacity and total phenolic content

The most significant interactive effect on total anti-oxidant capacity (210.72 μg/mL) was of KH_2PO_4 with maximum concentration of 0.15% w/v and yeast extract (0.05% w/v), keeping $ZnSO_4$ at 0 coded value as shown in Fig. 1. With further increase in the concentration of yeast extract, the total anti-oxidant capacity decreased. However, at maximum concentrations of both KH_2PO_4 and yeast extract (0.15% w/v), the response was observed to be minimum (19.94 μg/mL).

The model presented a coefficient of estimate of 6.52 and F value of 2043.38 of $ZnSO_4$, as most significant factor for total antioxidant capacity, followed by KH_2PO_4 with a coefficient and F value of 3.86 and 715.09, respectively. The quadratic terms of yeast extract (coefficient 1.96, F value 96.50) and $ZnSO_4$ (coefficient 0.052, F value 0.067) were positively affecting total anti-oxidant capacity. All other terms were found to be negative.

Effect of variables on TPC of wine

Total phenols ranged statistically significant ($p<0.001$) from 713.33 (run 11) to 1114.5 μg/mL (run 6). Response methodology led to an increase in the extraction of polyphenols into the wine and the same is defined by the second-order polynomial equation described in Table 3. In the case of TPC, the linear (X1, X2, X3), interactive (X1X2, X1X3) and quadratic (X12, X22 and X32) were found to be significant statistically. Keeping, KH_2PO_4 at 0 code value, TPC increased up to 1202 μg/mL with an increase in $ZnSO_4$ (0.135% w/v) and with 0.05% w/v yeast extract (Fig. 1). With any further increase, TPC declined and at maximum concentration of both the variables, predicted TPC was found to be least (530 μg/mL). But it was observed that holding $ZnSO_4$ on 0.05% and at 0.15 % w/v of yeast extract the predicted value raised up to 1155 μg/mL and decreased with any further increase in $ZnSO_4$.

The total phenolic content of the Jamun stem wine was found to be most significantly affected by X1, KH_2PO_4 with a coefficient of estimate 5.55 and F value 7.78. The interactive terms X1X2 (coefficient 5.76, F value 4.19), X1X3 (coefficient 76.41, F value 735.92) were significant. Only the quadratic term of X2, yeast extract (coefficient 10.82, F value 46.40) was positively affecting TPC. All the other terms were insignificant in terms of coefficient of estimate.

The antioxidant activity of the wines or herbal extracts is the synergistic effect of the polyphenols present in the substrate. The increase or decrease in the polyphenolic content has been linked with the varying levels of antioxidants earlier also and it has been observed that both have a positive correlation with each other (Darsini *et al.* 2013). Our results are in good agreements with these reports as both total antioxidant capacity and total phenolic content has been found to be maximum at the same run order 6 (Table 2).

Validation of model

The optimization of the parameters was carried out on the basis of initial experiments. In order to obtain maximum ethanol, total anti-oxidant capacity and TPC in wine, the predicted optimal concentrations of KH_2PO_4 (0.127% w/v), Yeast extract (0.05% w/v) and $ZnSO_4$ (0.1% w/v) were used. The adequacy of the model was verified by comparing the predicted and observed values of the responses. No significant differences between predicted and observed values were observed as in case of ethanol (predicted 11.61%, observed 11.59% v/v), total anti-oxidant capacity (predicted 185.99, observed 186.0 μM/mL) and TPC (predicted 1050.0, observed 1049.87 μg/mL) were obtained. The responses being in quite good agreement of the predictions, produced an insignificant deviation ($p<0.05$).

Identification of bio-active compounds by LC-MS profiling

The *Jamun* stem wine was subjected to LC-MS profiling for identification of the major phytochemicals present in it. Fig. 2 shows the Diode array and ToF-MS-ES-TIC chromatograms of the wine.

Compound identification was the done on the basis of retention time and mass spectra. The bio-actives identified included major anthocyanins like cyanidin,

malvidin, petunidin and other polyphenolics listed in Table 4.

Table 4: Major bio-active compounds in Jamun stem wine identified by LC-Q-ToF-MS profiling

Compound	m/z	Retention time (min)
Bergenin	327	9.25
Gallic Acid	169	2.53
Cyanidin-3-glucoside	451	13.79
Petunidin	317	1.02
Quercetin/Ellagic Acid	301	11.47
Luteolin	285	11.47
Cyanidin/Kaempferol	287	11.47
Malvidin	331	12.17
Cyanidin-3-O-arabinoside	419	10.72
Petunidin-3-O-glucoside	479	10.72
Myricetin rhamnoside	349	0.99
Lupeol	427	0.99
Stigmasterol	411.38	10.72
Myristic acid	225.5	10.72
Kaempferol-7-O-methylester	299.58	1.41
Malic acid	133	1.41
Tartaric acid	149	1.41
Ferulic acid	193	1.41
Prtotocatechuic acid	153	0.9
Catechin	451	13.79
Methylgallate hexoside	345	11.47
Apigenin	269	1.41

Fermentation of exogenously supplemented Jamun stems yielded a wine with high anti-oxidative capacity. In order to further improve the medicinal value of the beverage, its production conditions were optimized statistically. Potassium di-hydrogen orthophosphate, yeast extract and zinc sulfate were found to be the most effective factors for improving the ethanol content, total phenolic content and the total antioxidant capacity of the wine.

KH_2PO_4 with a coefficient of estimate of 0.31 was found to be the most effective factor for increasing the ethanol levels of the wine. However, the interactive effect of $ZnSO_4$ and yeast extract was observed to be the best for ethanol. It has been related to increase in ethanol production in wine earlier also (Mary *et al.* 2010; Santiago-Urbina *et al.* 2011). A number of reports have shown that potassium is necessary for maximum utilization of glucose by living yeasts, enhances its tolerance to toxic ions and is involved in stabilizing both intercellular pH of yeast and pH of fermentation media (Jackson 2014). Our model

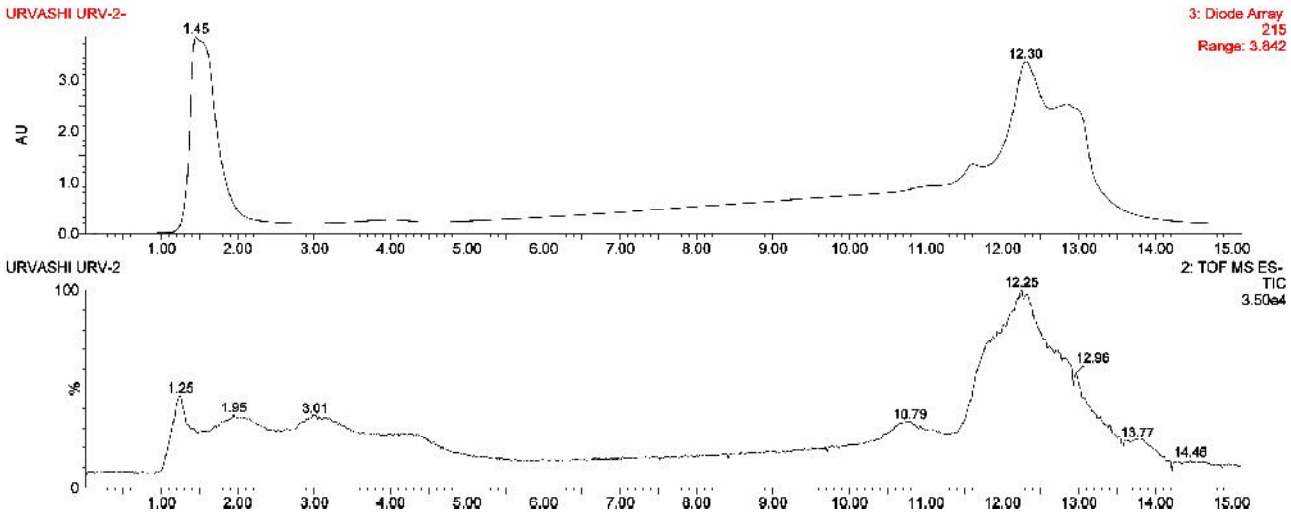

Fig. 2: LC-DAD and TIC chromatogram of Jamun stem wine

thus, is in compliance of the previous studies which show substantial positive effect of potassium on ethanol production by yeast. Ethanol has been found to be directly linked to the anti-oxidant capacities of various herbal substrates and is also a primarily used solvent for antioxidant and polyphenolic extraction systems (Prasad *et al.* 2011; Ilaiyaraja *et al.* 2015). Our results are in agreement to the earlier reports which cumulatively conclude that zinc aids in glycolysis, leading to increased ethanol production and ultimately gives an improved anti-oxidant capacity into the product (Liyana-Parthirana and Shahidi 2005; Kondrashov *et al.* 2012; Jackson 2014; Ilaiyaraja *et al.* 2015).

For TPC also, KH_2PO_4 with a coefficient of estimate of 5.55 was most suitable. However, for total anti-oxidant capacity, $ZnSO_4$ was found to be the most effective with a coefficient of estimate of 6.52. The interactive effect of various factors was also studied, as discussed earlier. It was observed that KH_2PO_4 (0.127% w/v), Yeast extract (0.05% w/v) and $ZnSO_4$ (0.1% w/v) were most suitable for achieving increased levels of all the three parameters studied.

To date, many investigations have revealed the possible mechanisms by which wines exert their beneficial effects, in particular towards hypertension and cardiovascular problems. The most significant pathways include vasorelaxation and enhancement in nitric oxide production and nitric oxide synthase activity. Polyphenolics and minerals are the major contributors of this antioxidant efficacy of red wines. Elements such as magnesium, potassium and zinc are essential for the proper functioning of endogenous antioxidant system (Kondrashov *et al.* 2012). Further, in the fermentation media, the presence of these elements leads to production of bet er ethanol levels which ultimately aids in bet er extraction of phenolic compounds into the media. Our results are in agreement with the earlier reports of the role of these elements in fermentation media (Mary *et al.* 2010; Santiago-Urbina *et al.* 2011).

When analyzed for the identification of bioactive compounds, many polyphenolics were found to be present in the Jamun stem wine. Compounds such as malvidin, luteolin, cyanidin etc., which have already proven biological activities associated with them were identified in the wine. This again emphasize on the medicinal properties of this beverage and studies must be undertaken in the future to evaluate the curative abilities of Jamun stem wine in animal models.

Conclusion

In the view of medicinal effects of *Syzygium cumini*, the stems of the plant were fermented for producing wine by exogenously supplementation with cane sugar. The beverage produced was found to be good in terms of the physico-chemical characteristics. For maximizing the ethanol, total anti-oxidant capacity and total phenolic content of the wine, mathematical modeling (PB and RSM) was employed. The most efficient variable concentrations, KH_2PO_4 (0.127% w/v), Yeast extract (0.05% w/v) and $ZnSO_4$ (0.1% w/v) gave an ethanol (11.59% v/v), total anti-oxidant capacity (186.0 moles/mL) and total phenolic content (1049.87 μg/mL) in the wine. Major polyphenolics and other bio-active compounds were identified by LC-MS analysis. The analysis depicted the presence of numerous health beneficial phyto-chemicals. *S. cumini* wine thus, can be an effective anti-oxidant beverage against the disease mediated by oxidative stress. The stem wine holds a great functional potential and our lab is also evaluating the in-vivo prospective of this beverage. These studies will further unravel the actual efficacy of the prepared beverage.

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