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Development of Alcoholic Beverages of Punicagranatum Blend with Kalanchoe Pinnata and Emblica Officinalis using Saccharomyces Cerevisiae

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Abstract—*The present study has attempted to develop a technology to produce alcoholic and self-fermented fruit and herb-based beverage using a yeast strain Saccharomyces cerevisiae NRRL-Y-12632 which is isolated from sugar. Marketing flooding of unhealthy, high calorie synthetic drink has led to obesity especially in children. The kalanchoe pinnata herb is an innovative for beverages which can used in a fortified product which is essential for vitamin and mineral which is produced using or innovative process technology.Juice Patharchatta (kalanchoe pinnata), Pomegranate juice (Punica granatum L) and Gooseberries (Emblica) were optimized to a blended beverage which was stored for 1 week in a pet bottles (100ml capacity) at a refrigerated temperature. Physic-chemical, antioxidant property and statistical analysis were evaluated. The properties were check in 21 days. The Physic-chemical properties of 1st days blend juice was pH (5.4), TSS(11 ^{0}B), titratable acidity (0.18g/100ml), moisture content (92.10%), ash content (0.23%) and fat content (0.97%). The changes in the physicochemical properties after 7 days fermented beverages blend recorded were pH (5.3), TSS 6 ^{0}B, titratable acidity (0.11g/100ml), moisture content (92.05%), ash content (0.36%), fat content (0.72%), carbohydrate (6%), protein content (0.134%) and alcohol content (4.07%). The changes in the physicochemical properties after 14th days recorded were pH (5.3), TSS 6 ^{0}B, protein content (0.151%). After 21st days pH (5.0), TSS 6 ^{0} B, ascorbic acid (5mg/100ml). Among the three beverages,* Pomegranate: *Patharchatta: Amla (6:3:1) was highly acceptable when compared on 9 hedonic scale.*

Keywords*: Blended juice, alcoholic, Beverages, Physic-chemical properties, Antioxdant activity, self-fermentation, yeast and statistical ANOVA.*

1. INTRODUCTION

Self-fermented and non-self-fermented beverages which produced from herbs and fruits. The herbs are very nutritional and pharmaceutical. Such products are isolated from nutrients, dietary and supplements. There is a three class of alcoholic beverages such as malted beverages (beer and ale), fermented fruit juices (wine and cider) and distilled liquors (brandy, whiskey, rum and vodka) are common. The mostly we used yeast is of genus *Saccharomyces* and the species is *Cerevisiae*. So, they manufactured of many foods, with special strains used for the leavening of bread and to produce ale, wine, alcohol, glycerol and invertase. Top yeast are active fermenters and grow rapidly at 20^0 C. The clumping of the cells and the rapid evolution of carbon dioxide sweep the cells to the surface and hence the name top yeasts. Bottom yeast do not clump and settle to the bottom hence the name bottom yeast. They grow more slowly and ferment at lower temperatures of 10-15^0 C.

In the current study, a technology was developed to produce self- fermented, alcoholic beverages fromPatharchatta *(kalanchoe pinnata*), pomegranate and gooseberries. The patharchatta herbis a perennial herb which generally grown in throughout the world. They are very nutritional for human health. Patharchatta are folkloric for curing the diseases. It contains n-alkane, n-alkanol, alpha- and beta-amyrin and sitosterol. Nowadays, the research interest on pomegranate fruit is increased because of reports establishing its benefits on human health (**Faria, Calhau*et al.*, 2011**).immune deficiency system. Pomegranate are good for ingestion which contains 85% water, 10% total sugars, 1.5% pectin, ascorbic acid, and polyphenols (**Aviram *et al.*, 2000**). Amla is known as Indian gooseberry (*Embilica officinalis*Family- *Euphorbiaceae*). It is reportedthat Amla possesses greater antioxidant, antibacterial, antimutagenic, anti-ulcer and hepatoprotective activities and also useful in giving relief from cough, bronchitis haemoptysis, tuberculosis and scurvy (**Argade*et al., 2016***). Gooseberries is good in Ayuvedic product like Chyawanprashand Triphala one of the best rejuvenating herb. They are healthful for properties of wine using some different microbial species including *Saccharomyces cerevisiae*, lactic acid and acetic acid bacteria. It contains maximum percent of vitamin C – 69%. The fermentation of blend juice for the

beverages they enhance the flavour and other desirable qualities associates with digestibility and edibility of food product (**Kolawole *et al.*, 2007**). Yeasts play a central role in the fermentation of foods and beverages, with high carbohydrate content (**Jimoh*etal.*, 2012**). According to the BCAP code of the United Kingdom, beverage containing less than 1.2% (v/v) alcohol is considered as low-alcoholic a beverage that contains less than 0.5% Alcohol by Volume (ABV) is non-alcoholic (**HeenaChilana*et al.*, 2015**).

Table 1: Prepare blend juice in a different blending ratio (Awsi Jan, Er.Dorcus Masih 2012).

S.no.	Juice	Blending Ratio	Treatment symbol
1	Patharchatta	0:100:0	T0
2	Pomegranate:Patharchatta:amla	70:20:10	T1
3	Pomegranate:Patharchatta:amla	60:30:10	T2
4	Pomegranate:Patharchatta:amla	50:40:10	T3

2. MATERIAL AND METHODS

Collection of herbs and fruit

The main materials selected for the preparation of Alcoholic beverages, in the present study were as patharchatta plant, pomegranate fruited and gooseberries fruits. These are commonly available fruits and herb were collected from local market of Dughalpur in Greater Noida.

Preparation of Juice extract

Firstly, the fresh fruit of pomegranate and gooseberries were taken for juiced extraction because fresh fruits are more nutritious than dried fruits and are good for ingestion. The pomegranate fruit was peeled, and gooseberries was taken as whole and both were separately grinded for juice extraction the juices was strained and waste material was discarded. The leaves of patharchatta were collected, washed and grinded in a mixer with added distilled watered to make juice of patharchatta, this juice was also strained, and waste material was discarded. All three juices were refrigerated at 4^0C for 1 week.

Preparation of blended juice

After storage of 1st week, the juice was prepared for 4 different ratios of 0:100:0, 70:20:10, 60:30:10, 50:40:10 respectively. Then, citric acid was added to all the four blends of juiced properly to maintain the pH for fermentation. The product was filled in 200ml petted bottles. After filling of juice, 1.5ml of *Saccharomyces cerevisiae* culture was added to 200 ml bottled. Juice was fermented at 37^0C for 2 days.

Preparation of alcoholic beverages

After fermentation, the pH, TSS of product was determined. There was a decrease in the pH and TSS valued of juices. So, it confirmed the development of alcohol content in juices and then the alcoholic concentration of juice was checked by a spectrophotometer. the test it confirmed that the juice had converted into an alcoholic beverage.

Physicochemical and Phytochemical Evaluation

Various physicochemical and phytochemical parameters like pH which is measured by pH meter (AOAC 1985), TSS were determined directly with a refractometer ATAGO (0 - 50^O Brix), moisture content, ash was performed. Moreover, the prepared drink was analyzed for the presence of carbohydrates, proteins, fat, and vitamin C using standard procedures. The carbohydrate content was determined by Anthrone reagent/ toluene acid using spectrophotometer method. The Fat content was determined by Soxhlet method. Protein content was determined by Kjeldhal method. Vitamin C content determined by Spectrophotometer. (Ranganna 1986).

Determination of Antioxidant activity

Antioxidant helps to scavenge the free radicals and prevent the damage from them. The antioxidant property of the alcohol beverage was measured by using 1, 1- diphenyl-2-picryl-hydrazyl (DPPH) assay.

Statistical analysis

The statistical analyses using the analysis of variance (ANOVA) and the Duncan Multiple range test with significance level at $p<0.05$ (Ihekoronye and Ngoddy, 1985).

3. RESULT AND DISCUSSION

Preparation of juice extract

The juice extracts prepared as mentioned result varying yield as detailed below.

Herb and fruits	Extracting yield/ juice yield
Patharchatta	60ml/100g leaves
Pomegranate	80ml/100g seeds
Gooseberries	80ml/100g fruit

Preparation of alcoholic beverages

Final Composition of Alcoholic Beverages

Patharchatta	30%v/v
Pomegranate	60%v/v
Gooseberries	10%v/v
Soda	3.6 %w/v

4. DISCUSSION

Physico-chemical Analysis

Physico-chemical properties of pure pomegranate, Patharchatta, gooseberries beverages were executed after 2 days static fermentation at 37^O C. After fermentation without using preservative, the beverages are refrigerated in 7 days. The fermentation process isprohibiting the growth of contaminating microorganism. It was observed that, the pH, TSS (^{O}B), Titratable acidity and carbohydrates was increase

before fermentation. But after fermentation, the yeast convert sugar which mainly to alcohol and carbon dioxide that's why the pH, TSS, TA and carbohydrates are decrease. Similar results were obtained by (**Sahota *et al.*, 2010**) who reported gradual increase in carbon dioxide content, totaled acidity (% citric acid), alcohol % (v/v) and gradual decrease in pH, TSS(°B) and brix acid ratio of low alcoholic naturally carbonated blended guava and lemon beverage.

Table 2: Approximate content of fresh juice and fermented juices

S. no.	Sample	Fresh juice %	Fermented juice%
1	Moisture content	92.10	92.05
2	Ash content	0.23	0.36
3	Fat content	0.97	0.72
4	Carbohydrate	-	6%

Table 3: Variation of pH during storage

	T0	T1	T2	T3
1stDAY	5.5	5.4	5.4	5.8
7th DAY	4.5	4.7	5.3	4.9
14th DAY	4.2	4.4	5.3	4.7
21st DAY	3.	4	5	4.6

On the first day, I have taken patharchatta juice of volume 100 ml and measured its pH 5.5 and keep it in the refrigerator for 7 days. On, the seventh day I have measured pH and it was 4.5 then, kept it in the refrigerator for next 7 days. On, the 21st day I have checked sample's' pH and it was 3.2. The next sample was the mixture of patharchatta, pomegranate and Amla in the different ratios of 70:20:10 ml respectively and; calculated the pH which was 5.4 and kept it in the refrigerator for 1 week. After, 1 week taken out this sample and calculated this pH which was 4.7 and keep it in the refrigerator for 1 week and calculated the pH 4.4 and Similarly after 1 week it was 4.0. Then, I have taken 3rd sample with same three mixture in a ratio of 60:30:10 ml respectively and calculated the pH and it was 5.4 after maintained the pH and keep it in the refrigerator for next 7 days and calculated pH it was 5.3 and repeat this procedure on 14th day and 21st day and their pH were 5.3 and 5.0 respectively. Now, I had prepared3rd solution with all those 3 juices in the ratio of 50,40,10 ml respectively, and calculated the pH 5.8 and keep that solution in the refrigerator for 7 days after that calculated pH on the 7th day it was 4.9 and repeated this procedure for next 2 week. After, 14th and 21st day I had measured their pH which was 4.7 and 4.6.

Table 4Variation of pH during storage

	T0	**T1**	**T2**	**T3**
1st DAY	2	10	11	8
7th DAY	1	4	6	4
14th DAY	1	4	6	4
21st DAY	1	4	6	4

With above 4 solutions I had checked their TSS value on the respective days. The TSS value of first solutionOn, the first day it was 2 and after 1 week it was 1. With, solution 2 the TSS value was 10 on the first day and4 on the 7th day. With, sample 3 the TSS value was 11 on the first day and 6 on the 7th day.With, 4th solutionthe TSS value on first and 7th day it was 8 and 4.

Table 5

Sample /Days	**Fresh juice**	**Fermented juice**
T0	2.0 (0.092g/100ml)	5.6(0.25g/100ml)
T1	2.6(0.11g/100ml)	5.0(0.23g/100ml)
T2	2.5(0.11g/100ml)	4.0(0.18g/100ml
T3	2.4(0.11g/100ml)	3.5(0.16g/100ml)

The effect of Titratable acidityof fresh juices are 0.18g/ml and the fermented juices are decrease 0.11g/ml which are sample of T2. Calculated by using formulae.

Carbohydrate

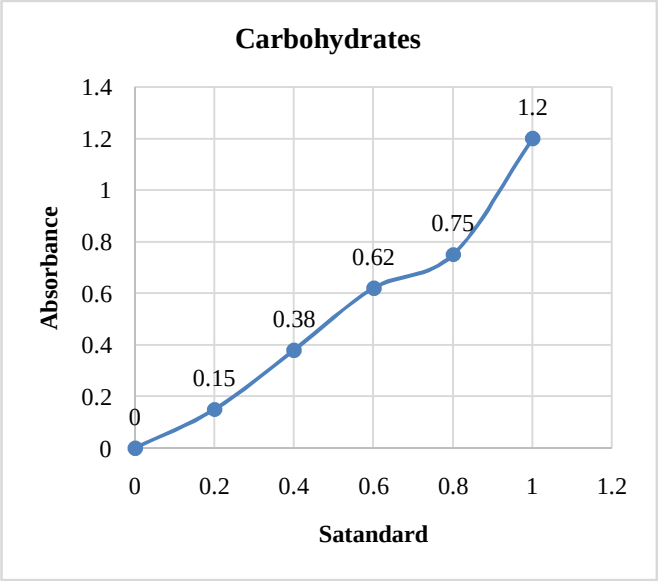

Fig. 1: Absorbance of carbohydrate

With sample 2, I have calculated certain finding. The moisture content of fresh juicewas 92.10 and after fermented it was 92.05. The ash content of fresh juice was 0.23 and after fermented it was 0.36. Fat content of fresh juice was 0.97 and after fermented it was 0.72. Fermented juice of carbohydrate content 6%.

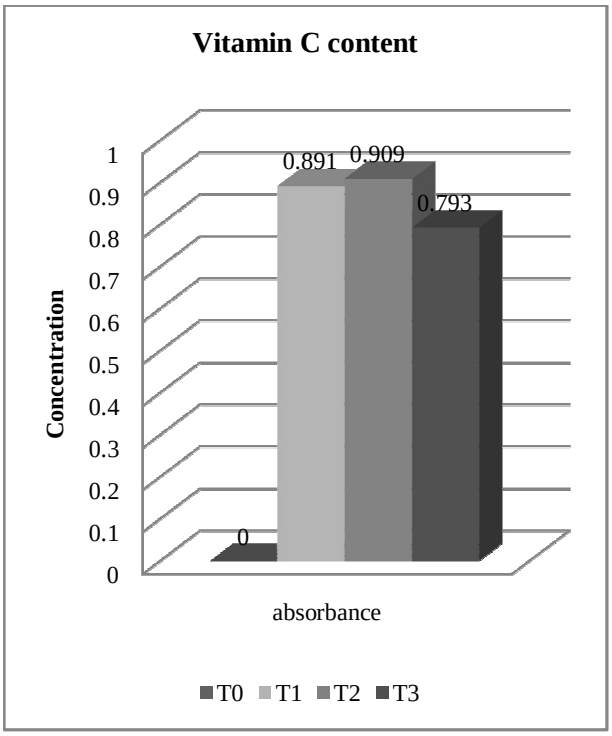

Fig. 2: Ascorbic acid content

The ascorbic acid activity of alcoholic beverages was determined after 14 days of fermentation when stored at 4^0C. It was seen that the absorbance of all three blends were slightly different from each other. The maximum absorbance was seen in T2 Sample and least was seen in T3 as it can be due to the different blending ratio of juice at the start before fermentation. The maximum absorbance seen in T2 was taken and ascorbic acid was calculated as 0.5ug/ml.

Protein content

The average nitrogen content of the different fruit and herbs categories measured with the Kjeldhal method. The results show that the highest variation is found in different samples. In 64% of the samples assayed, the Kjeldhal method gave slightly higher nitrogen values(**Rossi *et al.*, 2004**). These findings suggest that a more complete digestion is achieved with the Kjeldhal method, which takes a digestion time 1hrs. So, the fresh juice of beverages without fermentation we observed the nitrogen% was found to be 0.044% (Sample 1), and 0.134%(Sample 2) for patharchatta beverages respectively. Sample 3 has nitrogen% 0.223. Protein content of fresh juice which are calculated by using formulae is 0.134%. Fermentation we observed that nitrogen% was found to be 0.062% (sample 1), and 0.151% (sample 2) for patharchatta beverages respectively. Sample 3 has nitrogen%0.187.The protein content of fermented beverages are increased 0.151% (**Shafiya Rafiq *et al.*, 2016**)

Calculation:

Nitrogen % = 1.4007x (sample titre - blank titre) x N of HCl)/ g sample

Protein % = nitrogen % x 6.38

Antioxidant Activity

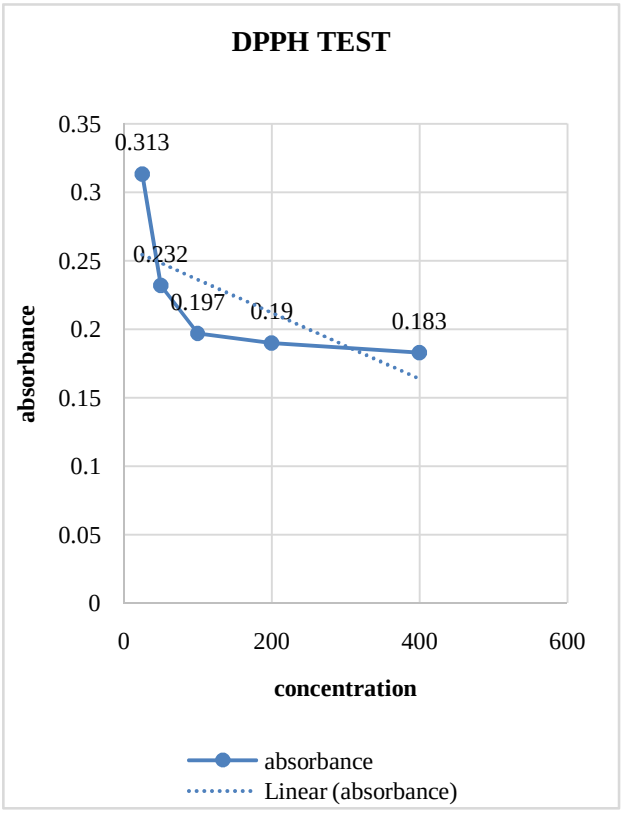

Fig. 3: Absorbance of DPPH

DPPH was a free radical that was used to measure the free radical scavenging activity of Beverages. Sample T2 was found to be a rich source of phenolics and flavonoids which imparted more antioxidant potential to drink. The free radical scavenging activity of fresh nutraceutical summer soft drink by DPPH assay was found to be 75.52%. There was no significant decrease founded in the antioxidant activity during the storage period of three months.

Alcohol content

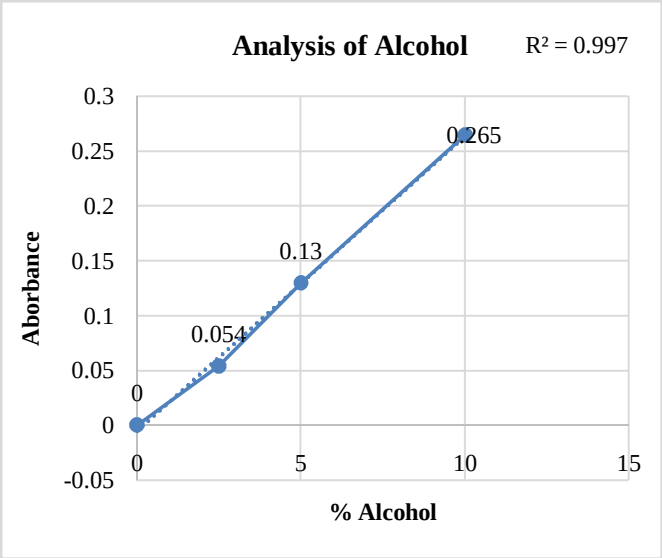

Fig. 4: Absorbance of Alcohol content

To determine the alcohol content of alcoholic drink of sample T2 in different concentration 0%,2.5%, 5.0%, 10%, firstly the solvent which is Bis(O, O'-dipropyl dithiophosphato) nickel(II)are soluble in ethanol and the absorbance at 524nm.So, the solvent mixed in a water – ethanol which gives the complex dissociated into nickel(II) and 0, O'-dipropyl dithiophosphate ions which depend on the water content. This complex property is exploit for the spectrophotometric determination of the alcohol content. After that, the mixture is warmed at 25^{O}C at 524nm, alcohol content calculated by using calibration curve. The alcohol content is 4.07% (**Sasaki *et al.*,2006**)

Statistical analysis (ANOVA)

Table 6: ANOVA for carbohydrate

Carbohydrate	Sum of square	Degree of freedom	Mean of square	F-test	Significance
Between group	2.926	6	488	3.845	.018
Within group	1.775	14	127		
Total	4.701	20			

Samples	% Carbohydrate
Samples 1	0.0100 ± 0.576^{a}
Samples 2	0.1500 ± 0.111^{ab}
Samples 1	0.3210 ± 0.121^{ab}
Samples 2	0.3800 ± 0.539^{ab}
Samples 3	0.6200 ± 0.519^{abc}
Samples 4	0.7500 ± 0.547 ^{bc}
Samples 5	1.2000 ± 0^{c}

Sig a= .077, Sig b= .081, Sig c= .078

Table 7: ANOVA for Vitamin C at different sample

Vitamin C	Sum of square	Degree of freedom	Mean of square	F-test	Significance
Between group	.024	2	.012	.047	.954
Within group	1.519	6	.253		
Total	1.543	8			

Samples	Vitamin C
Sample1	0.8900 ± 0.486 ^{ab}
Sample2	0.9067 ± 0.441 ^{ab}
Sample3	0.7900 ± 0.570 ^{ab}

Table 8: ANOVA for Antioxidant activity using DPPH reagent

DPPH	Sum of square	Degree of freedom	Mean of square	F-test	Significance
Between group	100	4	.025	4.022	.034
Within group	.062	10	.006		
Total	.162	14			

Samples	DPPH
Sample1	0.3130 ± 0.105^{a}
Sample2	0.2320 ± 0.100$^{a\ b}$
Sample3	0.1970 ± 0.058$^{a\ b}$
Sample4	0.1900 ± 0.060 ^{b}
Sample5	0.610 ± 0.051 ^{b}

Sig a= .070, Sig b= .105

5. CONCLUSION

It was concluded that the pomegranate juiced blend with *K. pinnata* and gooseberries juice (60:30:10) was most effective fermented beverages blend for minimum changes in pH, TSS, Titratable acidity, Ash Content, Vitamin C, Fat, Carbohydrate, Protein. On, the basis of above results revealed in the present study it may be concluded that the formulation of mixed fermented blend beverages is possible to satisfy consumer taste and preference. So, this, beverages could be stored for 1 month. Till date there is no functional fermented beverage available in the market, such functional beverages are those which are alcoholic that has been formulated by herbs, vitamins, minerals and amino acids. These drinks have health promoting benefits such as heart health and boon for immune system. So, in future we can look forward towards energy drink, syrup, powder, fortified baker's product and Ayurvedic proprietary medicine from Pinnata leaf extract. This drink will not only be good in taste but will also contribute to high nutritional benefits (**Ekta Batra *et al.*, 2015**).

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