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Development of Health Functional Wine produced from *Emblica officinalis* and *Phyllanthus niruri* and a Comparative Study of them over Commercial Wine

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Abstract

Wine is one of the functional fermented foods that have many health benefits. Using fruits and herbs having medicinal and nutritional value as a substrate for wine production, the health benefits of them can be improved widely. Bhumi Amla and Indian gooseberry, which are known for its high medicinal and nutritional value, are used as the substrate here. Fermentation is carried out with *Saccharomyces cerevisiae*. Daily monitoring was done to study the composition and characteristics of the wine. The wine produced resembled the commercial wine in terms of its composition, taste and aroma. After the fermentation period the wine was analysed for pH, total soluble solids, alcohol and antioxidant profile, post aging. The wines with *Phyllanthus niruri* and *Emblica officinalis* displayed high quantum of tannins, phenols and free radical scavenging activity. After the aging period parameters such as Antioxidant profile, Alcohol content, Total Suspended Solids (°Brix), pH, Titratable Acidity were analysed. These parameters were compared with that of commercial wine. Thus the studies showed that the pH, TSS, Tannin and Alcohol content were higher for commercial wine. But the phenol, reducing sugar and % inhibition of free radicals is higher for homemade wine.

Keywords: Antioxidant, *Emblica officinalis* Gaertn, *Phyllanthus niruri*, Wine

Home winemaking is an enjoyable, educational and satisfying hobby. Winemaking recipes make the process easy and simple instructions ensure success. The basic steps are easy to learn and practice. The traditional homemade wine base ingredient is the grape because it naturally contains the correct mix of sugar, moisture, tannin, and nutrients required for fermentation and preservation, and it even carries its own yeast. But in reality, wine can be made from almost any non-toxic plant or plant part if additional ingredients are supplied in the correct amount. So the process of making wines from various types of fruits, vegetables, herbs and spices.

One of the cherished fruits of Ayurveda is amla and it is known to provide systematic support, natural body benefits and rejuvenation. Amla is being regarded as a significant rejuvenating food and is revered as a natural plant medicine. Its nutritive profile has been chronicled in medical studies and schools of pharmacy from scientific laboratories (Basak, 2005). Fruit is a rich source of vitamin-C. Phyllemblin from the fruit pulp is identified as ethyl gallate. Besides these the fruits also contain 1, 3, 6- trigalloylglucose, terchebin, corilagin, Ellagic, phyllenblic acids, alkaloids like phyllantidine and phyllantine (Suresh, 2015). Amla fruits contains very high amount of ascorbic acid. On an average 600 mg/100g of fruit ascorbic acid is analgesic, anti-inflammatory and antipyretic (Basak, 2005). Besides, this compound is also antihepatotoxic, antinephrotoxic, antioxidant and impart chromosomal stabilization The fruit

contains around 28% phenolic compounds of which ellagic acid, gallic acid, corilagin etc. are important ones.

Bioactive constituents commonly found in herbs, and other plants have been shown to have possible health benefits with anti-oxidative, anti-carcinogenic, anti-hypertensive, antimutagenic properties. Wine being one of the rich sources of antioxidants can be considered as an effective nutraceutical. Many wines are also made from herbs with perceived medicinal value and such wines have additional health benefits (Nambiar, 2016).

In addition, antioxidants of herbal origins have been proved to be beneficial in reversing the hepatotoxicity and oxidative stress. According to World Health Organization, the medicinal plants are the best source to obtain a variety of medicines. The use of herb extracts, having recognized antimicrobial properties, can be of great importance in the treatment of various diseases.

In order to fulfil the human needs, there is the need for creating a synergistic formulation that has universal acceptance and taste sensation. To have a healthy lifestyle, efforts are being made to develop a product by blending constituents of "*Bhumi Amla"* and Indian Gooseberry (*"Amla"*) which are highly useful, and cost effective medicinal plants.

MATERIALS AND METHODS

Collection of materials

Fully matured, disease free, amla fruits were procured from local market in Kolkata for the experiment. The Fermentation jars and bottles (1000 ml capacity) used for the experiment were also procured from local markets of Kolkata and were sterilized, and then used for preparing amla wine. A freeze dried pure culture of *Saccharomyces cerevisiae* (K1-1116) was procured from the Lallemand Inc., Canada. This yeast strain is an industrial strain of yeast, used for wine making, hence complete genetic profile is not known.

Yeast preparation

The Lalvin active dried wine yeast was rehydrated by opening the 5 gram sachet and pouring contents into 50 mL (1/4th cup) clean 40°C (104°F) water. The yeast suspension was stirred lightly and allowed to stand for 20 minutes, then stirred again.

Preparation of Amla wine

300 gm. of matured, disease free amla fruits were taken. They were washed with water and put in a basket to drain excess water. The fruits were cut into small pieces and slightly crushed. One litre of sugar syrup with different sugar concentrations was prepared. It was boiled for five minutes and the boiling syrup was poured over amla fruits in such a way that it covered the amla fruits completely. It was closed and allowed to cool. After it came to room temperature, ten ml of inoculum was added. Lid was closed and fermentation was allowed for the next 21 days, it was allowed to ferment under airtight condition in the absence of light.

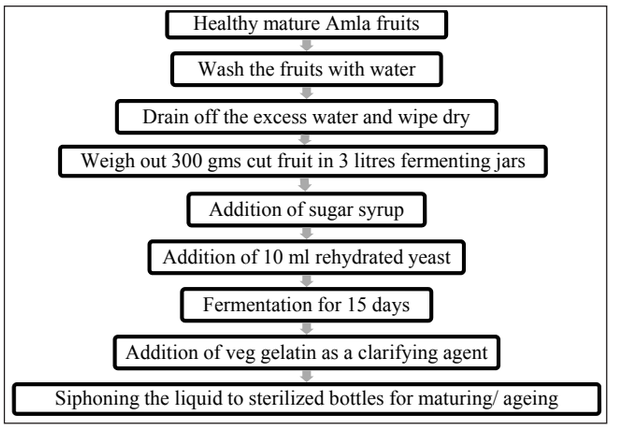

Fig. 1: Flowchart of Amla Wine Making

Blending the herb extracts into wine

Fresh green leaves of the herb were collected and washed to remove impurities. About 5 gm leaves were weighed and taken for crushing. The leaves were crushed evenly by adding water. Later, it was added to 100 ml distilled water and boiled. Then it was filtered with the help of filter paper. The extract was prepared just before adding to wine to ensure it is added when fresh. Before blending, the extract

was examined in well-lit surroundings for clarity. Presence of particulate components in them would make the wine hazy in appearance. 5, 10, 15 and 20 ml extract of "*Bhumi Amla"* was added to Amla wine sample. Addition of extracts was followed by thorough mixing to ensure uniformity.

Organoleptic Evaluation

The prepared amla wine with different sugar concentrations were evaluated after five months of storage for the quality attributes like appearance, colour, sweetness, astringency and overall acceptability by a panel of 40 panel members by following the Hedonic rating scale. The following chart was used for evaluating the products where the samples were ranked for sensory parameters from higher to lower in the descending order of acceptability.

Biochemical profile analysis

(a) ***pH:*** The pH of the amla wine was measured by using digital pH meter. The temperature of all samples was kept constant while taking observations for all the samples.

(b) ***Total Soluble Solids (TSS ºB):*** The content of total soluble solids (TSS) in the wine was determined with the help of digital hand refractometer and expressed as degree Brix (ºB). Care was taken that the prism of the refractometer was washed with distilled water and wiped dry before every reading.

(c) ***Reducing sugars (%):*** Estimation was conducted using the Fehling's Titrimetric method. The sample was titrated against 10 ml of Fehling mixture. The titer value was used for calculations of reducing sugars (%). (Ranganna,1986)

(d) ***Tannins:*** Tannin estimation was conducted using the Folin-Ciocalteau Spectrophotometric assay. Using 1 ml sample, absorbance was determined and mg tannic acid/l00 ml was obtained from standard curve (Ranganna, 1986).

(e) Total phenolic content: Total phenolic content of wines was determined using the Folin-Ciocalteau micro method. The absorbance of each sample solution is determined at 765 nm against the blank (the "0 ml" solution) and absorbance vs. concentration is plotted.(FSSAI, 2017)

(f) DPPH free radical scavenging activity: 0.06 mM/ L solution of DPPH in ethanol was prepared. An aliquot (100 µl: 10 µl wine and 90 µl ethanol) of sample was added to (2.9 ml) of DPPH solution. For control, (50 µl) of ethanol was added to DPPH solution, the decrease in absorbance was measured at 515 nm after 30 mins of incubation at 37 ^{o}C. All tests were performed in duplicates. Antioxidant activity was defined as the % inhibition of the initial concentration of DPPH radical caused by each ml of diluted wine sample.

RESULTS AND DISCUSSION

Effect of Fortification on Biochemical Parameters of Amla Wine

The pH and TSS of wine did not vary significantly among the treatments. Amla wine with 20mlherb extract (F4) was found to have higher pH (3.06). Amla wine with 20 ml herb extract (F4) was found to have highest TSS (11 ºB).

There was no significant difference between the treatments with respect to acidity content of the Amla wine. The citric acid content of *"Bhumi amla"* leaves can be the possible reasons for slight increase in acidity. As the wine ages, the acidity acts as a buffer and helps to preserve the wine longer.

There was not much significant increase in the treatments with respect to ascorbic acid content of the Amla wine. The highest ascorbic acid content was observed in Amla wine with 20 ml extract (F4) i.e., 193.71 mg/100ml, whereas, the lowest ascorbic acid contentwas recorded in control sample i.e., 193.54 mg/100ml.Ascorbic acid content of the wine increased with the increase in herb extract. *"Bhumi amla"* (*Phyllanthus niruri*) is a rich source of ascorbic acid (18.8 ± 0.32 mg/100 gms). Human trial studies indicate that administration of aqueous extract of *Phyllanthus niruri* led to an increase in the Vitamin C

levels in liver tissues by 28% and comparatively lesser depletion of ascorbic acid stores when administered along with alcohol, as compared to alcohol intake alone (Chatterjee, 2006).

Amla wine (control) was found to be significant with lowest tannin content of 21.9 mg/100ml. The highest tannin content was observed in Amla wine with 20 ml herb extract (F4) i.e., 49.32 mg/100ml. The increase in tannin content was observed with the increase in herb extract. *Phyllanthus niruri* contains around 381.67±7.64 mg/100 gm of tannin compounds, which includes ellagic acid, gallic acid, corilagin etc. Tannins being the highest phytochemical component of the leaves are well known for its antioxidant, antimicrobial, hepatoprotective, nephroprotective properties as well as for anti-inflammatory and dieresis effects.

The phenolic content was found highest in the Amla wine prepared by using 20 ml herb extract (F4) with 347.6 mg GAE/100ml. There was significant difference between the treatments with respect to phenols. The lowest level of phenols was observed in the control sample. The increasing trend in the phenolic content was observed in sequential treatments.

As the concentration of *Phyllanthus niruri* in the wine increased, the phenolic content increases in all the treatments. *Phyllanthus niruri* contains high amounts of phenolic compounds (44.17 ± 0.21) like Phyllanthin, Rutin, and Quercetin etc. Presence of phenols in the extract may help among others, in preventing oxidative stress by scavenging free radicals and bioactivation of carcinogens for excretion in the liver. This shows that the plant could bevery useful in fighting diseases that can lead to cell injury like neuroinflammation due to presence of free radicals and could also be useful to fight the aging process.

The data on % inhibition of free radicals by Amla wine as influenced by different concentrations of *Phyllanthus niruri* extract, after four months of storage. There were significant differences between the treatments with respect free radical inhibition of the Amla wine. A higher % inhibition was observed when herb extract concentration is increased. It was found to be significantly highest in Amla wine with 20 ml herb extract (F4) with 89.76%. These phenols exhibit higher radical scavenging activity. Higher amounts of phenols are found in *Phyllanthus niruri* extract. It contains natural antioxidants like ascorbic acid, flavonoids, tannins and other polyphenols, all of which exhibit an anti-radical activity and serve as potent free radical scavenger.

Effect of fortification with herb on the organoleptic properties of wine sample

The quality of fortified Amla wine samples was

Table 1: Effect of Fortification on Biochemical Parameters of Amla Wine

Parameter	Control	F1	F2	F3	F4
pH	3.03	3.03	3.03	3.03	3.03
TSS (o Brix)	10	10	10	10	10
Titratable Acidity (mg citric acid/100ml)	0.67	0.67	0.67	0.67	0.67
Ascorbic Acid (mg/100ml)	193.54	193.54	193.54	193.54	193.54
Tannins ((mg/100ml)	34.9	34.9	34.9	34.9	34.9
Total Phenols (mg GAE/100ml)	304.91	304.91	304.91	304.91	304.91
Free Radical Scavenging Action(%inhibition)	85.33	85.33	85.33	85.33	85.33

Control: Amla Wine (26° Brix), without herb extract, F1: Amla Wine (26 °Brix), blended with 5 ml herb extract, F2: Amla Wine (26 °Brix), blended with 10 ml herb extract, F3: Amla Wine (26 °Brix), blended with 15 ml herb extract, F4: Amla Wine (26 °Brix), blended with 20 ml herb extract.

Table 2: Effect of Fortification on Organoleptic Properties of Amla Wine

Attribute	Mean Sensory Score				
	Control	F1	F2	F3	F4
Appearance	4.34 ± 0.12	4.35 ± 0.26	4.25 ± 0.11	4.55 ± 0.41	4 ± 0.17
Colour	4.03 ± 0.36	4.10 ± 0.37	4.50 ± 0.34	4.53 ± 0.22	4.23 ± 0.13
Acidity	3.73 ± 0.13	3.75 ± 0.16	3.76 ± 0.22	3.80 ± 0.2	3.75 ± 0.29
Sweetness	3.51 ± 0.48	3.50 ± 0.44	3.45 ± 0.63	3.42 ± 0.45	3.35 ± 0.5
Body	4.10 ± 0.2	4.09 ± 0.19	4.07 ± 0.13	4.02 ± 0.07	4.01 ± 0.04
Astringency	3.89 ± 0.21	3.85 ± 0.11	3.84 ± 0.27	3.84 ± 0.29	3.80 ± 0.31
Overall Acceptability	4.89 ± 0.88	4.88 ± 0.54	4.9 ± 0.44	4.94 ± 0.62	4.94 ± 0.62

Control: Amla Wine (26 °Brix), without herb extract, F1: Amla Wine (26 °Brix), blended with 5 ml herb extract, F2: Amla Wine (26 °Brix), blended with 10 ml herb extract, F3: Amla Wine (26 °Brix), blended with 15 ml herb extract, F4: Amla Wine (26 °Brix), blended with 20 ml herb extract.

Table 3: Comparison between fortified amla wine and commercial wine

Parameter	Fortified Amla Wine	Commercial Wine	
		Commercial Red Wine	Commercial Red Wine
pH	3.03	3.39	3.04
Alcohol %	9.79	13.2	11.48
Reducing Sugars %	4.32	3.47	3.58
Tannins (mg/100ml)	43.68	64.43	47.13
Total Phenols (mg GAE/100ml)	386.12	310.02	140.04
Free Radical Scavenging Action (%inhibition)	88.61	84.6	62.8

Control: Amla Wine (26 °Brix), without herb extract, F1: Amla Wine (26 °Brix), blended with 5 ml herb extract, F2: Amla Wine (26 °Brix), blended with 10 ml herb extract, F3: Amla Wine (26 °Brix), blended with 15 ml herb extract, F4: Amla Wine (26 °Brix), blended with 20 ml herb extract.

assessed by organoleptic or sensory evaluation by a panel of judges, using a 5-point hedonic scale. The wine was evaluated based on the various parameters, for its overall quality. The score obtained by sensory evaluation are presented and discussed here under.

Organoleptic evaluation suggests that, the best wine could be made with 15 ml *Phyllanthus niruri* extract, since it exhibited the perfect combination of aroma, taste, astringency and appearance. Sensory scores by panel members demonstrated that Amla wine fortified with 15 ml *Phyllanthus niruri* extract recorded highest overall acceptability. This may be due to the presence of sufficient sweetness, pleasant flavour, moderate astringency and acidity, colour and appearance. All the treatments were found acceptable with respect to quality.

- pH is an important factor that strongly influences the properties of wine, such as colour, odour, taste, biological and chemical stability. Lower pH values are known to improve the stability and inhibit microbial and bacterial growth, so wine makers usually prefer a pH range of 3.0 to 3.5 as a crucial guideline in wine making.
- Alcohol in wine is produced naturally from the fermentation process when yeast is added. The presence of alcohol in wine aids polyphenol absorption and contributes to their bioavailability because before absorption,

polyphenols are hydrolysed by intestinal enzymes or by colonic micro flora, then undergo intestinal and liver metabolism; hence, the presence of alcohol protects phenols from their loss.

- In the wine-making process, during fermentation yeast converts sugar through enzymaticaction into CO_2 and alcohol. Once the fermentation is complete, sugar enhances sweetness in wine.
- Wines are rich in simple and complex phenolic compounds mainly represented by phenolic acids, flavonols, monomeric catechins, and tannins. The composition of phenolics in wine also depends on the type of fruits used for vinification, their extraction procedures employed for wine making,and the chemical reactions that occur during aging of the wine.
- Wine contains a variety of low molecular mass of polyphenol molecules and many of these have a primary antioxidant role. In order to survive, these anti-oxidative compounds counteract with reactive oxygen species (ROS). As wine is the complex mixture of phenolics, antioxidant activity of wines is not a property of single phenolic compound. During maceration, the phenolic compounds from the fruits are extracted into wine.

CONCLUSION

From the above comparison it is evident that the fortified Amla wine samples are at par or rather a better alternative when compared to commercial wines, due the fact that it showed a better antioxidant profile and lower alcohol %, making it a good replacement to commercial red and white wines, which are produced from grapes. Upon comparison of fortified Amla wine samples to the commercial wine varieties, it was revealed that Amla wine had a better antioxidant profile and lower alcohol% when compared to red and white wines produced from grapes.

Hence, it is concluded that Amla and *Bhumi Amla* can be used as a valuable ingredient for the production of an herbal wine with all the important properties of wine having medicinal characteristics of amla fruits. Wine acquires its properties due to the various components either arising from its source or during its preparation or processing. Ideally, wine must benefit the consumer in some or the other manner and must be quite acceptable. In this regards, development of wine with components having potential healthful properties renders the wine more beneficial to the consumer. The herbal extracts used in the fortification of Amla wine were found to complement the basic attributes of the wine when used in the specified amounts. Also regular, but limited administration of these fortified wines would help in receiving benefits of the herbal extracts, thus, minimizing the need for synthetic medicines for treating various disorders. The combinations gave a novel product with better qualities, increased acceptability and wider applications. Such fortifications need to be explored for developing products that could be included in the realm of Health-oriented products. Also the application of such fortified wines in the field of therapeutics is a conceivable approach.

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