

Development of antioxidant rich beverage from Mahua (*Madhuca indica*) and Amla (*Emblica officinalis*)

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An antioxidant rich, sugar free (no added sugar) beverage was developed using a novel source of natural sugar i.e. mahua (*Madhuca indica*) flower juice concentrate and amla (*Emblica officinalis*) juice. Three different concentrations of mahua (20, 40 and 60 °Brix) were blended with amla in six different proportions (90%, 75%, 50%, 40%, 25% and 10%) and were analyzed for total phenol content, DPPH radical scavenging activity, sugar profile and organoleptic property. The blend prepared from 40°Brix mahua having 50% amla was found to be the blend of choice.

Keywords: Mahua, Amla, Blended juice, Antioxidant, Sugar free, Novel drink.

Introduction

Mahua (*Madhuca indica*) flowers are a rich source of natural sugars, vitamins and mineral¹⁻². They are one of the most important non timber forest product collected by the rural people in central India and used as a source of livelihood generation in the dry season (March-May). Intensive research was taken on various aspects of these flowers to increase their utility as a food³⁻⁵. Mahua juice concentrate, a natural liquid sugar was the most important product developed from the juice of fresh flowers⁶. Present research work is an attempt towards development of a high antioxidant beverage using this natural liquid sugar from mahua. Amla was selected as the source of natural antioxidant due to its extraordinary vitamin C and polyphenol content⁷⁻¹².

Materials and methods

Plant materials and chemical reagents

Mahua juice concentrate was prepared following the process developed earlier⁵ and amla juice was extracted in the laboratory. Samples were stored frozen at (-20°C) till the study. Sodium hydroxide, methanol, gallic acid, Folin-Ciocalteu reagent and acetonitrile were purchased from Merck India, New Delhi. DPPH, glucose, fructose and sucrose were from Sigma Aldrich, New Delhi.

Experimental Design

Three different concentrations of mahua juice (C1) (20°B, 40°B and 60°B) were blended with amla juice (C2) in six different proportions as T1 (90:10), T2 (75:25), T3 (50:50), T4 (40:60), T5 (25:75) and T6 (10:90). All the samples were centrifuged (10 min at 3000rpm) and benzoic acid (100 mg/L) was added in order to prevent spoilage. Pure mahua juice and amla juice were used as control. All analyses were done in triplicate, and results were reported as mean ± Standard deviation.

Preliminary analysis

Total soluble solids, pH and titratable acidity (TA) were evaluated as quality indexes. The pH values were measured using a pH-meter (HANNA INSTRUMENTS, Italy). The TA was determined by titrating 2 mL of the mixture (rising 60 mL final volume with Milli-Q water) with 0.1 N NaOH (pH 8.1). Results were expressed as g citric acid per 100 mL of sample, in accordance with AOAC. The TSS contents were recorded in a digital refractometer, PAL-3(ATAGO, Japan) and the values were expressed as degree Brix (°B).

Analysis for sugar profile

The sugar profile of different MJC and its blends were analyzed by the method of Ewaidah (1993)¹³, with slight modification. Waters HPLC with Refractive Index detector (Waters 241) system consisting of a quaternary pump, solvent degasser and column oven was used for the analysis. Appropriately

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diluted standards and samples were centrifuged and filtered through Millipore filter (0.5 μ m) before analysis. A carbohydrate column (250mm $\times$ 4.6mm with 5 μ m particle size) at 35°C was used for analysis. The solvent system used was water: acetonitrile (30:70 v/v), at a flow rate of 1 mL/min and run time was 15 minutes. Waters Empower software was used to quantify the individual sugars by comparing the retention time of the standards with that of sample.

Determination of total phenols

Total phenol content (TPC) of the above samples were determined according to the Folin–Ciocalteu method¹⁴ with slight modification. 200 μ L sample of juice was transferred into a test tube containing, 1 mL of freshly diluted (10 fold) Folin–Ciocalteu reagent. The mixture was allowed to stand at room temperature. After 8 min, 3 mL of 7.5% (w/v) sodium carbonate was added to the mixture and shaken gently. Then the mixture was incubated at room temperature for 60 min. The absorbance was recorded at 765 nm using UV-Visible spectrophotometer (PerkinElmer, LAMBDA 25, Massachusetts, USA). Acidified methanol was used as blank. The calibration curve was plotted against gallic acid and expressed in terms of mg GA equivalents per mL (mg GAE/mL).

DPPH Assay

The free radical scavenging activity of experimental samples using DPPH $\cdot$ radical scavenging method has been done according to the methods described earlier¹⁴⁻¹⁶ with some modifications. 100 μ L sample was reacted with 3.9 mL of 0.004% DPPH solution prepared. The reaction mixture was allowed to incubate in dark for 60 min. Then absorbance was measured against methanol at 515 nm using UV-Visible spectrophotometer (Perkin Elmer, LAMBDA 25, Massachusetts, USA). All the measurements were performed in triplicate.

Sensory Evaluation

Sensory quality of food is of great importance, since it satisfies the aesthetic and gustatory sense of the consumers. It is a combination of different senses of perception coming into play in choosing and eating a food. Depending upon the requirement, different methods are used to study the sensory property. Hedonic-rating test was used, to measure the

consumer acceptability of MJ and its blends, which relates to pleasurable or unpleasurable experiences. The drinks were analyzed for colour, flavour, taste and overall acceptability. A group of 10 semi-trained panelists analyzed the products at a 9- point hedonic rating scale¹⁷. The samples scoring an overall quality of 6 or above were considered acceptable.

Statistical analyses

Statistical analyses were conducted using SPSS (Statistical Program for Social Sciences, SPSS Corporation, Chicago, IL) version 16.0 for windows. One way ANOVA with Tukey's B test was performed to analyze the results. All the reported data were recorded in triplicates and results were expressed as a means $\pm$ standard deviation and means were accepted as significantly different at 95% confidence interval ($p < 0.05$).

Results and Discussion

The TSS, pH and TA of amla juice used in the present study was $8 \pm 0.18^\circ\text{B}$, 2.8 ± 0.01 and 3.5 ± 0.23 respectively. HPLC analysis of the amla juice showed no sugar peak. So we assumed that amla juice did not contain detectable amount of sugar. Total phenol content of amla juice was found to be 28.936 ± 0.28 mg GAE/mL. These values are slightly different from the earlier reports¹⁸⁻²¹. The antioxidant activity of amla juice determined in terms of DPPH radical scavenging activity was 100% (Table 1). Amla is one of the

Table 1—Total Phenol and Antioxidant activity of mahua-amla blends

Parameter	Blends	20 °Brix	40 °Brix	60 °Brix
TPC	C1	1.42 $\pm$ 0.03	2.25 $\pm$ 0.05	6.15 $\pm$ 0.23
	T1	4.16 $\pm$ 0.14 ^a	5.73 $\pm$ 0.58 ^a	8.35 $\pm$ 0.67 ^a
	T2	8.13 $\pm$ 0.55 ^b	8.80 $\pm$ 0.67 ^b	10.27 $\pm$ 0.81 ^b
	T3	15.17 $\pm$ 0.89 ^c	15.94 $\pm$ 0.68 ^c	15.92 $\pm$ 0.92 ^c
	T4	17.04 $\pm$ 0.83 ^d	17.75 $\pm$ 0.59 ^d	17.90 $\pm$ 0.24 ^d
	T5	18.58 $\pm$ 0.39 ^e	20.99 $\pm$ 0.61 ^e	22.20 $\pm$ 0.64 ^e
	T6	23.22 $\pm$ 0.39 ^f	25.18 $\pm$ 0.71 ^f	26.88 $\pm$ 2.47 ^f
% DPPH	C1	29.28 $\pm$ 0.04	33.63 $\pm$ 0.02	34.29 $\pm$ 0.03
	T1	31.11 $\pm$ 0.03 ^a	37.97 $\pm$ 0.02 ^a	36.15 $\pm$ 0.08 ^a
	T2	57.67 $\pm$ 0.06 ^b	61.14 $\pm$ 0.02 ^b	73.50 $\pm$ 0.05 ^b
	T3	87.05 $\pm$ 0.03 ^c	91.22 $\pm$ 0.06 ^c	91.98 $\pm$ 0.06 ^c
	T4	89.38 $\pm$ 0.05 ^d	92.03 $\pm$ 0.01 ^d	93.93 $\pm$ 0.05 ^d
	T5	90.84 $\pm$ 0.03 ^e	93.55 $\pm$ 0.05 ^e	94.04 $\pm$ 0.04 ^d
	T6	93.12 $\pm$ 0.03 ^f	94.25 $\pm$ 0.03 ^f	94.36 $\pm$ 0.08 ^d

Values in the same column with different superscript varies significantly ($P < 0.05$)

C2: TPC value for pure amla juice : 28.93 $\pm$ 0.28

C2: % DPPH scavenging for pure amla juice: 100

richest source of antioxidant due to presence of different group of compounds like vitamin C, polyphenols, tannins, phyllembelic acid, phyllembelin, rutin, gallic acid, emblicol etc²²⁻²⁴. For mahua, the pH of different concentration was found to be nearly 4.5 with no significant difference among the different dilutions. However, the TA, sugar content and the total phenol content of mahua concentrate decreased with increasing dilutions. TA value decreased from 0.67±0.02 to 0.18±0.01, TPC decreased from 6.15±0.23 to 1.42±0.03 and the DPPH scavenging activity decreased from 34.29±0.03% to 29.28±0.04% for diluting the mahua concentrate to 20°B from 60°B. The analytical results for the TPC and DPPH scavenging activity of different blends have been compiled in Table 1. There was significant difference ($P<0.05$) in the total phenol content of all the blends used for the study. Lowest value of TPC was found for the blend prepared with diluted mahua juice (20°B) and lowest percent of amla juice (10%). Accordingly this blend also showed the lowest DPPH radical scavenging activity. For all mahua concentrations, an increasing trend in TPC was observed corresponding to the increase in percentage of amla juice and the highest value of 26.88±2.47 was reported for the blend prepared from 60°B mahua having 90% amla juice. DPPH scavenging activity showed that addition of amla juice at a concentration of 50% or above resulted in more than 90% scavenging of the radicals except for two blends prepared from the 20°B mahua. The high antioxidant activity of mahua-amlam blend can be attributed to the high vitamin C and polyphenol (200-900 mg/100g) content of amla¹⁹. All the above blends were analyzed for sugar profile using HPLC and the results have been compiled in Table 2. According to the findings, glucose and fructose was present in all the eighteen blends and sucrose was completely absent in most of the blends except the one having high mahua concentration. Sucrose was present in all the control mahua juice (20 °B, 40°B and 60 °B) and absent in amla juice. Blending of amla juice with mahua might have resulted in the degradation of sucrose. Percentage of both glucose and fructose showed a decreasing trend with increasing concentration of amla juice. Blending experiments were designed to find out the optimum concentration and proportion of mahua juice that is to be blended with amla juice for an organoleptically acceptable high antioxidant

Table 2—Sugar profile of mahua-amlam blends

Parameter	Blends	20 °Brix	40 °Brix	60 °Brix
Glucose	C1	4.05±0.07	9.71±0.10	11.90±0.03
	T1	3.48±0.86	9.38±0.01	12.64±0.00
	T2	3.02±0.05	8.87±0.01	8.82±0.03
	T3	3.65±0.02	7.54±0.01	9.99±0.01
	T4	2.87±0.02	5.18±0.03	8.64±0.03
	T5	1.10±0.07	4.61±0.01	4.76±0.01
	T6	0.67±0.01	1.24±0.02	1.80±0.01
Fructose	C1	5.07±0.07	12.18±0.00	15.22±0.01
	T1	7.73±1.09	11.91±0.02	16.83±0.00
	T2	6.00±0.00	6.34±0.02	11.00±0.00
	T3	3.40±0.02	5.8±0.01	11.84±0.01
	T4	3.66±0.01	7.02±0.01	10.19±0.02
	T5	1.45±0.01	5.72±0.01	5.98±0.01
	T6	0.94±0.01	1.77±0.00	2.96±0.01
Sucrose	C1	2.29±0.00	5.32±0.02	6.85±0.04
	T1	0	3.31±0.00	7.8±0.03
	T2	0	0	3.22±0.01
	T3	0	0	0
	T4	0	0	0
	T5	0	0	0
	T6	0	0	0

Values are means ± standard deviations of triplicates determinations

C2: Amla juice: No peaks were detected for glucose, fructose and sucrose

beverage. Amla juice was not included in the study due to its strong astringent taste. The results of 9-point hedonic test for all the 18 blends and control mahua samples have been presented in Table 3. Among the control mahua samples, 20°B was found to be superior for the sensory parameters colour, flavor, taste and overall acceptability with readings 6.40±2.07, 4.8±1.92, 6.00±1.58 and 6.60±0.54 respectively. Blend T3 (40°B) got the highest score for colour on the other hand blend T5 (20°B) obtained the highest score for flavor. With hedonic scores of 6.96±0.61 for taste and 6.76±0.36 for overall acceptability blend T3 (40°B) was found to be the most acceptable blend. Taste is one of the most important parameter in hedonic test. Therefore depending on the result of taste, colour and overall acceptability, blend T3 (40°B) was selected as the desired nutra beverage from mahua and amla with no added sugar.

Table 3—Sensory property of pure and blended juices

	C1	T1	T2	T3	T4	T5	T6
Colour							
20 B	6.40±2.07	5±1.58	6±1.22	6.2±0.84	5.2±0.84	5.4±0.89	5.4±1.14
40 B	6.00±1.58	6±0.65	6.36±0.59	6.48±0.50	5.84±0.93	5.2±1.10	4.96±0.71
60 B	6.00±1.58	5.4±1.14	5.6±1.34	6.00±1.0	6.26±0.70	6.42±0.72	6.46±0.68
Flavour							
20 B	4.8±1.92	5.6±1.14	5.8±1.30	5.00±1.00	6.2±0.84	6.4±0.89	6.2±0.84
40 B	4.00±1.58	5±0.79	5.8±0.32	5.76±0.23	4.8±0.84	4.62±0.58	4.4±0.55
60 B	3.4±1.14	3.8±0.84	4.5±1.0	4.7±1.20	5.2±0.84	5.0±0.70	4.8±0.44
Taste							
20 B	6.00±1.58	6.4±0.55	4.2±1.30	3.4±1.14	2.00±0.71	1.66±0.55	1.2±0.45
40 B	4.2±1.30	5.5±0.53	6.08±0.47	6.96±0.61	5.02±0.63	3.92±0.55	1.58±0.49
60 B	3.8±0.83	5.0±0.70	5.6±0.89	6.1±0.74	5.32±0.41	2.22±0.23	1.44±0.61
Overall Acceptability							
20 B	6.60±0.54	6.08±0.64	5.24±0.68	4.88±0.81	2.94±1.18	1.84±0.91	1.2±0.45
40 B	5.6±2.07	5.6±0.45	6.0±0.49	6.76±0.36	4.36±0.50	2.6±0.45	1.8±0.63
60 B	5.00±1.58	5±1.58	5.6±1.67	6.4±0.89	5.44±0.46	2.3±0.90	1.5±0.5

Values are means ± standard deviations of triplicates determinations

Conclusion

Present study was focused on the development of a sugar free (no added sugar), antioxidant rich beverage using mahua and amla. Eighteen different blends were formulated using three different concentrations of mahua juice. The blends were analyzed for TSS, pH, TA, total phenol content, antioxidant activity (DPPH radical scavenging activity), sugar profile and organoleptic property. Depending on the colour, taste and overall acceptability blend T3 (40°B) was selected as the most appreciable blend. Analysis of sugar profiles for T3 (40°B) showed the presence of glucose and fructose with complete absence of sucrose. The TPC value for T3 (40°B) was 15.94±0.68 mg GAE/mL and it showed 91.22 % DPPH radical scavenging activity. Comparatively low hedonic score for all the formulated blends may be attributed to the lack of sucrose (added sugar) in it. However the aim of the paper was to find out the suitable mahua concentration that is to be blended with amla juice for the most acceptable blend with high antioxidant activity. This objective was successfully achieved. Present findings will be useful for food and beverage industry in formulation of low sugar, high antioxidant beverage.

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