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Antioxidant profiling of Latkan (*Baccaurea ramiflora* Lour.) wine

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Baccaurea ramiflora Lour. (Family: Euphorbiaceae), locally know as Latkan, is a versatile plant with number of uses. Latkan wine is traditionally used in Malayasia and parts of India. In the present study, an attempt has been made to evaluate the total phenols, flavonoids, flavonols and proanthocyanidins as well as DPPH scavenging activity of the wine, prepared from the fruit pulp, with respect to aging in comparison with the juice. The total phenols, flavonoids, flavonols and proanthocyanidins ranged from 141.27-313.78 mg GAE/L, 149.2-531.2 mg QE/L, 103.2-179.2 mg QE/L and 1.46-8.45 µg catechin/L, respectively. The free radical scavenging activity by DPPH method showed the inhibition (%) of the samples in between 5.85-37%.

Keywords: *Baccaurea ramiflora*, DPPH, flavonoid, Latkan, phenol, wine

Baccaurea ramiflora Lour. syn. *B. sapida* (Roxb.) Muell.-Arg. (Family: Euphorbiaceae) is native to Southeast Asia region and is found distributed in the sub-Himalayan tract, mainly from Nepal to Sikkim, Darjeeling hills, Arunachal Pradesh, Tripura, Assam, Bhutan, Burma, Penninsular Malaysia, Tibet and Andaman islands[1]. It is a semi-evergreen tree[2] reaching a height of about 5-10 m. Fruit is yellowish and velvety, 2-3 cm in diam with leathery pericarp, three seeded arillus embedded in pinkish white pulp. *B. ramiflora* fruit finds its importance as a novel food additive because of its high content of vitamin C, protein and iron[3]. The fruit juice is mainly used for the treatment of constipation, whereas different parts of the plant are used to treat arthritis, abscesses and injuries[4]. They are also stewed or made into wine.

Fruit wines are fermented alcoholic beverages prepared from various kinds of fruits, which have an intoxicating or exhilarating effect[5]. Fruit wines are traditionally popular with home winemakers in the areas of cool climatic zones. Most fruits and berries have the potential to produce wine but few have the balanced quantities of sugar, acid, tannins and nutritive salts for yeast feeding, and water to naturally produce a stable, drinkable wine[6]. With reference to the phenomenon of "French paradox", studies have been focused on the physico-chemical characteristics and antioxidative properties of different wines[7]. Now, it has been established that phenolics and flavonoid constituents present in wine have therapeutic potential because of their antioxidant and anti-inflammatory properties[8-10].

Latkan wines are traditionally prepared in Malaysia and parts of India. However, available literature reveals that so far no work has been done to estimate the antioxidant properties of Latkan wine. Keeping this in mind, the present work deals with the suitability of fruit juice concentrate of *B. ramiflora* for the production of wine and estimation of the total phenolic, flavonoids, flavonols and proanthrocyanidin compounds along with aging of wine for 5 months at 1 month interval. The antioxidant capacity of the wine was also compared with the fruit juice by commonly used spectrophotometic method.

B. ramiflora fruits were collected from the local market of Siliguri during June-July and authenticated by the plant taxonomist. A voucher specimen has been preserved in the Botany Department of University of North Bengal, Siliguri. The fruit was peeled to remove the external hard core and squeezed mechanically to separate the seed and the juice. The juice was stored at 4°C until required. The fruit wine was prepared following conventional method of using *Saccharomyces cerevisiae*. In brief, the fruit juice was kept in fermenting vessel. The water was boiled and sugar was added at a desired concentration. Once the sugar syrup was ready it, was poured over the juice in the fermentation vessel. The mixture was allowed to cool and to it yeast was sprinkled and mixed. The vessel was sealed and allowed to ferment at 25°C for 14 d. The resultant wine was strained and bottled.

The total soluble phenolics (TPC) were determined by method of Singleton and Rossi[11] with slight modifications. The total flavonoid content (TFC) was

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determined according to Zhishen *et al*[12] with minor modifications using quercetin (QE) as a standard. The protocol developed by Kumaran and Karunakaran[13] was used to estimate the total flavonols (TFLC) using QE as a standard. The total proanthocyanidin contents (TPrC) was determined as per protocol previously reported by Sun et *al*[14]. The antioxidant activity of the wine and juice was assessed on the basis of the radical scavenging effect of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical by modifying the method of Vichitphan *et al*[15]. Absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity.

The content of phenolic compounds for the Latkan wine and fruit juice at different period of aging is depicted in Table 1. Total phenolic compound ranged from 141.27-313.78 mg GAE/L. The phenolic content decreased up to 2 months of aging in case of both wine and juice in a similar manner, and then increased suddenly at 3rd month of aging in both the cases. Further, the content decreased slightly and became almost constant in both the cases at 4th and 5th month of aging. Storage condition plays a vital role in determining the phenolic compounds present in the sample because during storage they can undergo modifications like hydrolysis, oxidations and complexations[16]. The decrease in the phenolic compound might also be due to other factors like light and temperature[17]. However, all compounds are not degraded with time. Some phenolic acids like caffeic acid, ferulic acid and *p*-coumeric acid tend to increase due to either enzymatic hydrolysis or with the increase in storage time[18], which result in the total increase in phenolic content with aging.

The flavonoid and flavonol contents for both wine and fruit juice are also depicted in Table 1. Total flavonoid content ranged from 149.2-531.2 mg QE/L, while the flavonol content ranged between 103.2-179.2 mg QE/L. Both the flavonoid and flavonol content decreased within 2 months of aging and then started to increase slightly. The decrease in the level of both flavonoid and flavonol might be due to the formation of flavonol gylcosides generated by the combination of sugar and flavonol aglycones[19]. The elevation in the level of flavonoid and flavonol with the aging might be attributed to the fact that during the process there was a huge accumulation of flavonol glycosides, which in due course of time were hydrolysed themselves into flavonol aglycones[20].

The presence of total proanthocyanidins content of Latkan wine and juice ranged from 1.46-8.45 µg catechin/L (Table 1). It was found that the total proanthocyanidins decreased gradually within 3 months of aging and then started increasing. The result clearly shows a similar trend as that in case of total flavonoids and total flavonols, probably because proanthocyanidins, a group of important secondary metabolites synthesized *via* the flavonoid pathway[21], are condensed tannins.

In vitro antioxidant activity of wine and fruit juice sample was determined by DPPH radical scavenging method for 5 months of aging process. Inhibition of the samples ranged from 5.85-37% (Table 1). In the course of aging both wine and fruit juice showed lower DPPH radical scavenging activity. Similar observation was made by Vichitphan *et al*[15]. It is, therefore, apparent from the present study that Latkan fruit is suitable for making good wine like other commercially used berries. Moreover, since the shelf life of the fruit is very short, so wine making seems to be a good idea to prevent the wastage of the fruits. Besides, the present study also clarifies that the wine is rich in natural antioxidants, including phenols, flavonoids, flavonols and proanthocyanidins, which in turn can have health benefits if consumed in limited amount.

Table 1—The total phenols, flavonoids, flavonols, proanthocyanidins and antioxidant activity measured as DPPH in both wine and fruit juice

Aging period (months)	Wine					Juice				
	TPC (mg GAE/L)	TFC (mg QE/L)	TFLC (mg QE/L)	TPrC (µg catechin/L)	DPPH (%)	TPC (mg GAE/L)	TFC (mg QE/L)	TFLC (mg QE/L)	TPrC (µg catechin/L)	DPPH (%)
0	262.06	267.2	145.2	4.95	12.74	313.78	399.2	159.2	8.45	23.75
1	247.46	229.2	131.2	3.79	33.39	268.59	337.2	167.2	7.28	37.01
2	141.27	149.2	103.2	2.33	5.85	181.48	305.2	115.2	3.79	8.61
3	219.11	345.2	111.2	1.46	28.23	256.56	415.2	121.2	3.25	34.25
4	188.18	411.2	123.2	3.58	24.10	198.45	531.2	123.2	5.83	28.74
5	185.77	305.2	159.2	4.66	14.11	196.28	515.2	179.2	6.70	17.38

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