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Physicochemical Characterization, Antioxidant Potential and Sensory Quality of Wine from Wild Edible Fruits of *Flacourtia montana* J. Graham

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HIGHLIGHTS

- Wine preparation from wild edible *F. montana* fruits.
- Physicochemical characterization of wine.
- Antioxidant evaluation of wine using multiple *in vitro* tests.
- Sensory analysis of wine.

Abstract: The objective of the present study was to produce wine from wild edible fruits of *Flacourtia montana* J. Graham. The various physicochemical attributes including total phenolic content and total flavonoid content were analyzed. Further, the prepared wine was evaluated for the antioxidant potential using four different assays, viz., 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), reducing power assay and total antioxidant activity. Finally, the wine was subjected for the sensory evaluation. Experimental results revealed

that wine had an alcohol content of 7.20%, total phenolic content of 0.776 ± 0.032 mg GAE/ml and total flavonoids of 0.121 ± 0.012 mg QE/ml. High performance liquid chromatography analysis revealed the presence of four major phenolic acids, viz., gallic acid (0.009 ± 0.0005 mg/ml), chlorogenic acid (0.623 ± 0.091 mg/ml), catechin (0.063 ± 0.011 µg/ml) and epicatechin (0.060 ± 0.009 mg/ml). *In vitro* antioxidant analysis of wine was able to successfully scavenge the free radicals in a dose dependent manner. Sensory scores indicated wine to be good in overall acceptability. Thus, present study highlighted the therapeutic nature of wine prepared from this underutilized fruit which could provide possibilities for enhancing socio-economic benefits among rural communities.

Keywords: *Flacourtia montana*; Underutilized fruit; Fruit wine; Polyphenols; Antioxidant activity; Sensory evaluation.

INTRODUCTION

Flacourtia montana J. Graham. belongs to the family of flowering and willow tree often growing up to 20m tall. The fruits of this species are pleasantly acrid, eaten raw and used also for jelly preparation. In India, this species is distributed across semi-evergreen and moist deciduous forests of Western Ghats. Fruiting begins in the month of January and fruit is often consumed raw, due to lack of suitable processing and storage technologies. Ripe fruits are delicious to taste and comprise following nutritional value: moisture (%): 77.10, total sugars (%): 64, reducing sugars (%): 9.88, non-reducing sugars (%): 54.11, calcium (%): 0.30, magnesium (%): 0.60, potassium (%): 0.89, sodium (mg/100g): 57.10, phenolics (%): 1.63, flavonoids (%): 0.66, and ascorbic acid (mg/100g): 23.30 [1].

Wine is one of the oldest fermented beverages produced from fruits and consumed across globally. Grapes being the most preferred fruit for winemaking, alternative source have been attempted for their better nutritional and therapeutic values. Several successful attempts on wine production from cultivated fruits such as Apple, Pineapple, Orange, Peach and Strawberry have been reported previously [2-4].

Physicochemical properties attribute to the great taste, nutritive and therapeutic value of wines produced from wild fruits. Polyphenols are the natural antioxidants that forms the most abundant category of phytochemicals found in wild fruits [5]. Several studies have highlighted the antioxidant potential of wine that is attributable to its polyphenolic content. Polyphenols especially anthocyanins and flavanols or catechins have proven to be effective against several chronic diseases including cardiovascular diseases [6]. Moreover, they are also proven to be potential natural antioxidants found abundantly in fruits. The present study was undertaken to analyze the suitability of *F. montana* fruits for winemaking and further to study its physicochemical, antioxidant and sensory properties.

MATERIAL AND METHODS

Chemicals

Aluminum chloride, potassium ferricyanide, ferric chloride, Folin-Ciocalteu reagent, sodium phosphate (monobasic and dibasic), sodium carbonate, trichloroacetic acid, glacial acetic acid, acetonitrile and methanol solvents (HPLC grade) were purchased from Merck (Bangalore, India). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), gallic, catechin, epicatechin, chlorogenic acid, vanillic, caffeic, syringic, p-coumaric, ferulic and trans-cinnamic acids were obtained from Himedia (Mumbai, India). All other chemicals other than listed above were of analytical grade.

Fruits collection and juice extraction

The fully ripened fruits of *F. montana* (3 kg) were procured from the local market of Uttara-Kannada District in Karnataka, India in the month of January 2015. The fruits were

cleaned with deionized water, deseeded manually, fruit pulp was pressed using stainless steel strainer and mashed carefully in a sterile container until juice was extracted. The juice was clear, orange in colour and acidic (pH 3.3) with a sweet taste and was stored in deep freezer at -20°C until further use.

Fermentation process and analysis

Fermentation substrate was prepared by thawing the *F. montana* juice (2 liters) to room temperature. Various physicochemical attributes were analyzed and the juice was ameliorated with the required quantity of pre-sterilized sucrose solution to adjust the final substrate to required brix and pH. Potassium metabisulphite as a source of sulphite was added at the concentration of 100 ppm to inhibit the growth of unfavourable microorganisms present in the juice and kept for incubation for 5 h at 20°C . In the next step, the juice was divided into two portions in to a 2 L Erlenmeyer flask for the duplicate experiments. Finally, yeast *Saccharomyces cerevisiae* (which was prepared in the same juice before 12 h) of 10% (v/v) was added to the ameliorated juice which was kept for fermentation at $22\pm 2^{\circ}\text{C}$ for the period of 21 days. Intermittent samples were drawn at the interval of every 3 days till the termination of fermentation process for analysis of process parameters and biochemical contents. After 21 days and process completion, the wine was racked at low temperature (8°C) to facilitate further sedimentation and decantation for the period of 3 days. Bentonite clay powder was added for faster clarification of wine. Further, wine was siphoned in to a sterilized glass bottles without leaving any headspace at mouth. The clarified wine was stored at 4°C in a refrigerator for further analysis.

Total phenolic content

Total phenolic content (TPC) was determined following the method of Singleton *et al.* [7]. Folin-Ciocalteu's phenol reagent (0.5 ml, 50 %) with a known aliquot of standards (20-100 $\mu\text{g/ml}$) was taken to which Na_2CO_3 solution (1.5 ml, 15 %) was mixed and further deionized water was added to make up the final volume to 10 ml. Finally, the tubes were incubated in dark at room temperature for the duration of 30 min. The absorbance was read at 760 nm using a double beam UV-visible spectrophotometer (UV-1800, Shimadzu, Japan). The values were expressed as milligrams gallic acid equivalents per ml (mg GAE/ml).

Total flavonoid content

Total flavonoid content of *Flacourtia montana* Must (FMM) and *Flacourtia montana* Wine (FMW) was determined by spectrophotometric method described by Chang *et al.* [8]. The samples (100 μl) made up to 1 ml using methanol were mixed with 4 ml of deionized water and 300 μl of NaNO_2 (5%), to which 300 μl of aluminum chloride (10% w/v) was added. After 6 min incubation, 2 ml of NaOH (1 M) and distilled water (2.4 ml) were added. The reaction mixture was thoroughly mixed and allowed to stand for 15 min at room temperature, then absorbance was recorded at 515 nm using double beam UV-visible spectrophotometer (UV-1800, Shimadzu, Japan). The total flavonoid content was calculated with the calibration curve of standard Quercetin and expressed as mg of Quercetin equivalents per ml (mg QE/ml).

In vitro antioxidant assays

Total antioxidant capacity

Total antioxidant activity was determined following the protocol of Prieto *et al.* [9]. Briefly, 0.2 ml of extract was taken in a test tube. To this, 1.8 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) was added. The tubes were incubated at 95°C for 90 min and allowed to cool. The absorbance was measured at 695 nm against a blank using a double beam UV-Vis spectrophotometer (UV-1800, Shimadzu, Japan). The values are expressed as mg ascorbic acid equivalents (AAE) per ml of sample.

Reducing power assay

Reducing power activity of FMW was determined following the protocol described by Oyaizu [10] and Rai *et al.* [11]. Briefly, 100 μ L of juice and sample (made up to 1.0 ml with methanol) was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) followed by 2.5 ml potassium ferricyanide (1%), further reaction mixture was incubated at 50°C for 20 min. After incubation, 2.5 ml of trichloroacetic acid (10%) was added and the content was centrifuged for 10 min at 650 g. Supernatant (2.5 ml) was taken, mixed with deionized water (2.5 ml), followed by 0.5 ml 1% FeCl₃ reagent and absorbance was recorded at 700 nm. Higher concentrations of samples resulted in higher absorbance values and thus indicate higher reducing power.

DPPH radical scavenging activity

DPPH radical scavenging activity of FMW was evaluated spectrophotometrically according to the protocol of Brand-Williams *et al.* [12] and as mentioned by Jagtap *et al.* [13]. DPPH stock solution was prepared by weighing 24 mg of DPPH and dissolving it in 100 ml of methanol and working solution was prepared by diluting stock solution with methanol until the absorbance was achieved to 1.1 \pm 0.01 units at 517 nm using a double beam UV-visible spectrophotometer (UV-1800, Shimadzu, Japan). 3 ml of DPPH solution was added to various concentrations of wine (100-500 μ L), vortexed and incubated in dark for 30 min at 37 \pm 2 °C. Methanol with no added samples was considered as blank control. Percentage radical scavenging activity was calculated using the formula: % RSA = (A control-A wine/A control) \times 100, where, A=absorbance at 517 nm.

ABTS radical scavenging activity

ABTS assay was done following the procedure described by Thaipong *et al.* [14]. Stock solutions were prepared by taking 7.4 mM ABTS solution and 2.6 mM potassium persulfate solution. Working solution was prepared by mixing these two stock solutions in equal volume and reaction mixture was kept for incubation in dark at room temperature. Finally, this solution was diluted by taking 1 ml reacted ABTS solution with 60 ml methanol to obtain an absorbance of 1.170 \pm 0.02 units at 734 nm using a double beam UV-visible spectrophotometer (UV-1800, Shimadzu, Japan). For each assay, ABTS solution was prepared freshly. A 300 μ L of diluted wine samples were mixed with 2700 μ L of the ABTS+ working solution that was allowed to react in dark. Finally, absorbance was recorded at 734 nm using a UV-visible spectrophotometer.

HPLC analysis of sugars, organic acids and phenolic acids

For HPLC determinations, samples were filtered using PVP membrane filter (0.45 microns) and finally 10 μ L of sample was injected. The analytical column used for sugars, organic acids and polyphenols were Kromasil-NH₂ (250mm \times 4.6) column (Dr. Maisch GmbH, Germany), RP-C18 (Intek Chromosol, India) and Luna-C18 (2) 100 Å (250 \times 4.6 mm) (Phenomenex Inc., USA), respectively. HPLC analysis of sugars was done following the protocol of Rai *et al.* [11], and organic acids by Jayprakash and Sakariah [15], whereas for polyphenols, a method of Mradu *et al.* [16] was followed.

FT Raman analysis of wine

FT-Raman spectra was obtained using a FT-Raman Module (NXR6700, Thermo Scientific, Madison, WI) with a resolution of 8 cm⁻¹ and 256 scans. Spectra were obtained in the Raman shift range between 200 and 3600 cm⁻¹. The system was operated using NXR FT-Raman OMNIC 9.1 software.

Sensory analysis

For sensory analysis, quantitative descriptive analysis (QDA) technique was followed to evaluate the sensory properties of wine. A sensory panel consisting of 10 trained panelists

were served 30 ml of *F. montana* wine (10-12°C) at room temperature (22±3 °C) to evaluate the taste, aroma, flavour and overall acceptance based on descriptive sensory analysis [11,17].

Statistical analysis

All values are represented as mean±SD of triplicate analyses. ANOVA analysis was performed using GraphPad prism (Ver. 5.0, GraphPad Software, USA) and XLSTAT (Ver. 2014.5.03, Addinsoft, USA) software.

RESULTS

The physicochemical properties of *F. montana* initial must and *F. montana* wine are tabulated in Table 1. In the present study, it was observed that wine composed of 8 °Brix at the end of fermentation. In present study it was observed that, pre-fermentation of the initial must comprised a pH of 3.5 which showed a steady decrease reaching up to 3.02 at the end of fermentation. In addition, a positive correlation was observed between the pH and the titratable acidity which can be attributed to increase in organic acid content wherein the decline in pH was evident.

HPLC analysis of sugars in FMM and FMW indicated the presence of three sugars, viz., glucose, fructose and sucrose. FMM comprised glucose (66.4±1.84 mg/ml), fructose (85.52±1.66 mg/ml) and sucrose (64.38±1.19 mg/ml). Whereas, the FMW indicated a complete utilization of reducing sugars especially fructose during the fermentation process. However, glucose and sucrose were found to be minimal with a concentration of 20.84±2.29 mg/ml and 7.4±0.31 mg/ml, respectively.

Organic acid profile of FMM and FMW showed the presence of malic acid, citric acid, succinic acid and lactic acid. In addition, considerable variation in organic acid profile was observed between the initial and final day. Malic acid (2.04±0.45 mg/ml) was found to be the most predominant organic acid in FMM followed by citric acid (1.32±0.37 mg/ml) and succinic acid (1.17±0.17 mg/ml), while, FMW composed of malic acid (1.61±0.17 mg/ml), citric acid (1.29±0.08 mg/ml), succinic acid (1.12±0.12 mg/ml), lactic acid (0.75±0.01 mg/ml) and acetic acid (0.10±0.04 mg/ml).

The TPC in the initial must and wine was found to be 1.05±0.029 and 0.776±0.015 mgGAE/ml, respectively. TFC in FMM and FMW was found to be minimal with a concentration of 0.133±0.002 and 0.121±0.005 mg/ml, respectively.

The HPLC analysis of polyphenols in FMW revealed the occurrence of four vital polyphenols, viz., gallic acid (0.009±0.0005 mg/ml), chlorogenic acid (0.623±0.091 mg/ml), catechin (0.063±0.011 µg/ml) and epicatechin (0.060±0.009 mg/ml).

Assessment of *in vitro* antioxidant activity using various free radicals indicated that FMW successfully scavenged free radicals in dose dependent manner. Figure 2 depicts the total antioxidant capacity, reducing power activity, antioxidant activity against DPPH and ABTS radicals. Total antioxidant activity by phosphomolybdenum assay highlighted the total antioxidant potential of the FMW which was found to be 4.0±0.29 mgAAE/ml (Fig. 2a). Reducing power assay showed the increase in absorbance with the increase in wine quantity which was indicative of FMW antioxidant potential (Fig. 2b). DPPH assay is one of the most widely accepted techniques to evaluate the antioxidant potential of test samples. In this study, FMW exhibited highest activity at the concentration of 500 µL whereas at 100 µL, the activity was found to be 29.05±1.84% (Fig. 2c). In addition, the TPC showed a positive correlation of 0.977 with a significance alpha value of 0.004. FMW successfully scavenged ABTS radical in a dose dependent manner wherein the highest activity of 99.11±0.23 % was observed at the 100 µL (Fig. 2d). Further Pearson's correlation between the TPC and ABTS radical scavenging activity was found to be 0.828 with a significance alpha value of 0.082 which can be correlated with previous report on fruit wines [18].

Table 1. Physicochemical characteristics of *Flacourtia montana* must and *Flacourtia montana* wine.

Attributes	<i>Flacourtia montana</i> must	<i>Flacourtia montana</i> wine
TSS (°Brix)	22.00±0.081 ^a	08.20±0.016 ^b
pH	3.50±0.009 ^a	3.08±0.010 ^b
Titrateable acidity (% CAE)	0.820±0.020 ^a	1.01±0.080 ^b
Total sugars (%)	19.76±0.328 ^a	07.30±0.030 ^b
Reducing sugars (%)	07.34±0.223 ^a	5.800±0.150 ^b
Protein (%)	0.870±0.070 ^a	0.301±0.040 ^b
Total polyphenols (mg/ml)	1.050±0.002 ^a	0.776±0.003 ^b
Total flavonoids (mg/ml)	0.133±0.030 ^a	0.121±0.005 ^a
Alcohol (% v/v)	NE	7.820±0.112 ^b
Calorific value (Kcal/100ml)	73.91±0.980 ^a	79.84±1.180 ^b

Note: Values represented as mean ± standard deviation (n=3), Means representing different superscript are significantly different from each other (Tukey's HSD, P>0.05).

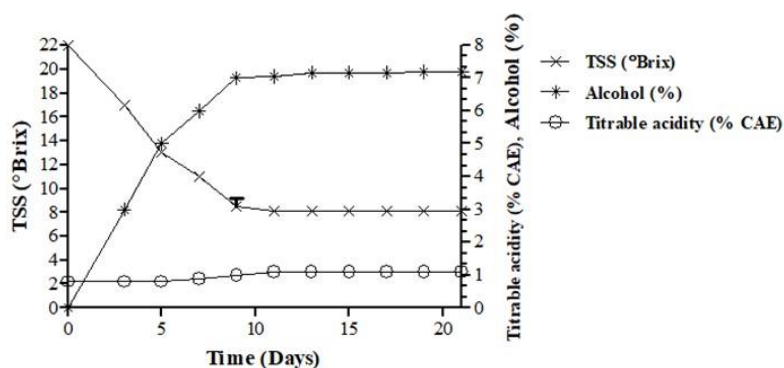


Figure 1. Fermentation kinetics of *F. montana* wine with respect to total soluble solids (°Brix), alcohol (%) and titrateable acidity (% Citric acid equivalents).

The QDA is the most followed and preferred method for evaluation of sensory characteristics of wines, wherein the panelists are trained and it is quite quick and easy process. In the present study, it was observed that wine descriptors such as color, odour, taste, flavour and mouth feel showed a potential impact. Ten trained panelists assessed the FMW and their average ratings for each descriptor were taken into consideration. The results are depicted in Figure 3, which explains the sensory characteristics of FMW. Wine received highest score for colour and clarity with respect to visual appearance, whereas the odour, taste and mouth feel was found to be fruity, sour and thin body, respectively. FMW was found to be good with respect to overall acceptance. Thus sensory profile of FMW was organoleptically accepted.

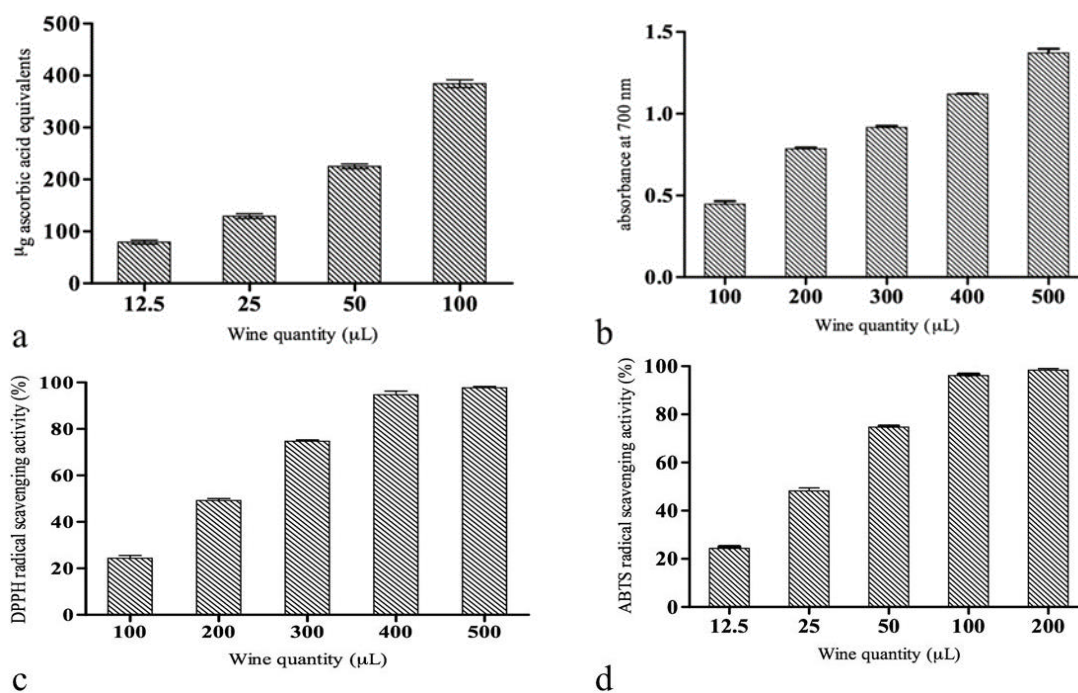


Figure 2. Antioxidant activity of *F. montana* wine against various free radicals: (a) Total antioxidant activity, (b) Reducing power assay, (c) DPPH free radical scavenging activity and (d) ABTS free radical scavenging activity.

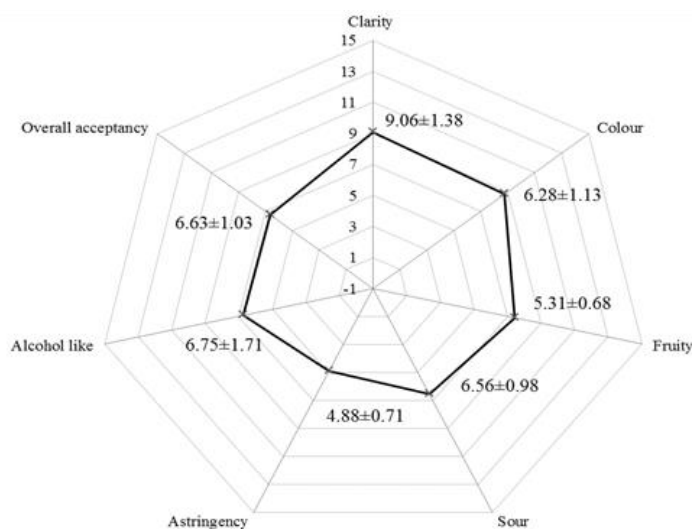


Figure 3. Spider web indicating sensory characteristics of *F. montana* wine.

The FT-Raman spectrum of *F. montana* wine is shown in Figure 4. The spectrum observed in this study was in accordance with spectra of previous report by Wu *et al.* [19] with slight variations in the wave number, wherein, their study reported 879 cm^{-1} for ethanol. According to Rodrigues-Júnior *et al.* [20] band 426 cm^{-1} was representative of skeletal modes of carbohydrate rings (C-C-O). However, in the present study, ethanol peak was observed at 878 cm^{-1} . Reference database was used for assigning bands for Raman spectrum of FMW, the peaks in between 3000-3700 show O-H stretching and the peak at 2810-3000 is due to CH_x asymmetric and symmetric stretching, the peak at 1440-1460 is due to CH_x bending, the bands observed at 1290-1200 is due to CH₂ twisting and 1000-1130 is due to CH₂ rocking including CO stretching at 1040-1100 [21,22].

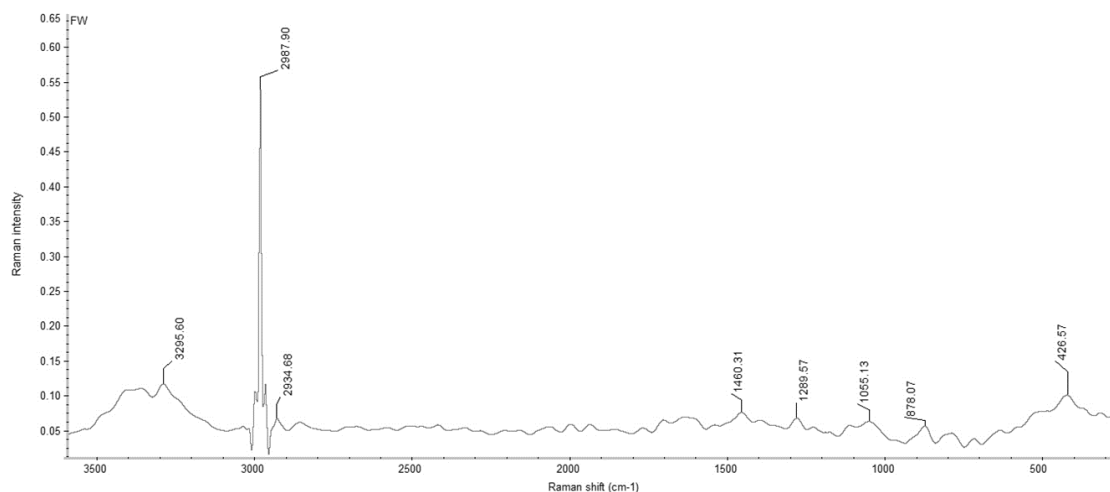


Figure 4. FT-Raman spectrum of *F. montana* wine.

DISCUSSION

The Brix value showed a rapid decline until the 7th day of fermentation process, whereas after 7th day onwards, a very slower declining trend was observed until 21st day (Fig. 1). This is due to the rapid utilization of sugars by the yeast for their initial growth thereby increasing alcohol content in the fermentation medium [23]. Increased alcohol concentration on day 7 with a concurrent decrease in Brix value was observed. The pH of the must is very essential for the yeast growth since decreased pH affect the growth rate and ceases the fermentation. Several studies have reported previously that pH is an important attribute that can stimulate the flavour of wine [23].

FMW comprised 1.1% CAE of titratable acidity which was significantly higher when compared to other fruit wines such as *Mangifera indica*, *Citrus sinensis*, *Ananas comosus*, and *Cornus officinalis* with a titratable acidity of 0.8, 0.9, 0.6 and 0.6% respectively and comparatively less with respect to *Syzygium cumini* and *Crataegus pinnatifida* var. *major* wines with titratable acidity of 1.11, and 1.8%, respectively [24-29]. The alcohol content of the wine in the present study was found to be 7.820 ± 0.122 %, which showed the moderate concentration of ethanol when compared to *Garcinia xanthochymus* fruit wine [11]. GC-FID analysis of alcohols revealed the presence of methanol and isoamyl alcohol along with ethanol as a major alcohol. The relative percentages of ethanol, methanol and isoamyl alcohol was found to be 98.10, 0.94 and 0.86%, respectively. Calorific value of FMW observed in the present study was 79.84 ± 1.180 which was comparatively less than *Garcinia xanthochymus* [11]. Table 2 highlights the correlation matrix between the different variable analyzed. It was observed that total flavonoid showed a significant correlation with the DPPH activity. Several studies indicate that the polyphenols including flavonoids exhibit strong inhibition against reactive oxygen species [30].

Acetic acid in wine gives vinegar flavor [28]. FMW contained very less quantity of acetic acid. Several other studies have reported the evolution of organic acids during the winemaking process. Generally, wines contain trace amount of lactic acid due to decarboxylation of malic acid and rarely bacterial activity. Lactic acid in wine influences the wine flavor [29,31,32].

Polyphenols plays a vital role in enhancing the quality of wine, several studies have highlighted their prominence and beneficial role. Phenolic compounds may also interact with volatiles and contribute to the specific release of aroma in a wine [33]. Natural polyphenols in wine include a large group of several hundred chemical compounds that are known to affect the taste, colour and mouthfeel of wine. Dietary phenols have shown wide array of benefit in health ailments and they are mainly attributed to antioxidant properties against various reactive oxygen species. Moreover, polyphenols in beverages including wine are responsible for the taste of bitterness and astringency [34]. The TPC in our study was comparatively higher than reported jackfruit (0.053 mgGAE/mL) and reported custard apple

wine (0.098 mgGAE/mL) [13,35]. The decrease in TPC and TFC was observed over the end of fermentation which may be due to the polymerization, precipitation and adherence of some of polyphenols to yeast cell wall [36-39].

Table 2. Correlation matrix [Pearson(n)] of physicochemical variables.

Variables	TSS	pH	TA	TS	RS	Protein	TP	TF	Ethanol	DPPH
TSS	1	-0.655	0.564	0.984	0.997	0.955	0.994	0.968	-0.849	0.982
pH		1	-0.993	-0.778	-0.596	-0.849	-0.569	-0.444	0.156	-0.500
TA			1	0.700	0.500	0.782	0.471	0.338	-0.041	0.397
TS				1	0.968	0.993	0.960	0.908	-0.742	0.933
RS					1	0.931	0.999	0.984	-0.886	0.993
Protein						1	0.918	0.850	-0.654	0.882
TP							1	0.989	-0.901	0.997
TF								1	-0.954	0.998
Ethanol									1	-0.933
DPPH										1

Values in bold are different from 0 with a significance level $\alpha=0.05$, TSS=Total soluble solids, TA=Titrateable acidity, TS=Total sugars, RS=Reducing sugars, TP=Total phenolics, TF=Total flavonoids

Recent studies have shown that chlorogenic acid is known to prevent fat accumulation and increase in body mass [40,41]. Flavonols such as catechin and epicatechin exhibit potential antioxidant activity by inhibiting LDL oxidation. Moreover, catechins are abundantly found in the beverages such as green tea and apple wine, and exhibit multiple biological activities [37,38]. The findings of the present study was in accordance with the recent report on fruit wines by Ramalho *et al.* [42] wherein their study revealed the occurrence of catechin and epicatechin among fruit wines, was comparatively higher than grape wines. The antioxidant profile of *Flacourtia montana* wine was on par with the Jackfruit (*Artocarpus heterophyllus* Lam.) wine and Custard apple (*Annona squamosa* L.) wine [13,35]. Raman spectroscopy (RS) is a simple, rapid and most reliable analytical instrument that is extensively used for the food analysis. More recently, RS has been used for the wine analysis [43].

CONCLUSION

In the present study, wine was prepared out of underutilized fruit *F. montana* in a quest to expand and analyse the extensive nutritive value in addition to the existing category of fruit wines. The resultant wine was evaluated for the physicochemical characteristics, polyphenolic content and antioxidant potential. The wine was having 7.820 % of ethanol content and catechin, chlorogenic acid and epicatechin were major polyphenols detected, that are value addition to the wine produced from *F. montana*. However, polyphenolic content decreased during the course of fermentation. According to sensory analysis, wine received good scores against overall acceptance. Thus, considering the abundant availability of *F. montana* fruits it could be subjected for a commercially viable wine production that could generate revenue among the rural communities. However, larger-scale fermentation need to be undertaken as future studies can endorse the promising beneficial results obtained in this work for its commercial viability.

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