Bio-utilization of wild berries for preparation of high valued herbal wines

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The fruits of wild berries, viz. Berberis lycium Royle, Pyrus pashia Buch.-Ham ex D. Don, Actinidia deliciosa (A.Chev.) C. F. Liang & A. R. Ferguson, Syzygium jambos (L.) Alston, Emblica officinalis Gaertn., Prunus cerasoides D. Don, Rubus ellipticus Sm, Crataegus sp. and Citrus karma Raf. were collected from the forests of various regions of Kangra, Himachal Pradesh (India) for preparation of low alcoholic and economical herbal wines along with their total qualitative evaluation, using standard methods. Various herbal wines were prepared from wild berry fruits and their total antioxidant activity, polyphenol content and flavonoid content were estimated by spectroscopic methods. Though, these herbal wines were prepared from those berry fruits which have poor market value but the wines prepared from them showed promising antioxidant activity of 75 mg-187 mg/L, total polyphenol content of 162 mg-2845 mg/L and total flavonoid content of 15 mg-183 mg/L. These herbal wines have strict range of alcohol (10-15%), °brix (8-10), pH (3.5-3.8) with acceptable sensory parameters like; colour, taste, sweetness, astringency and flora. So these highly valuable wild berry fruits from high altitudes of Himalayas could be processed for obtaining herbal wine with health and wealth exploring potential.

Keywords: Wild berries, Herbal wines, Polyphenols, Flavonoids, Antioxidants

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Introduction

Fruit wine is an undistilled, low alcoholic beverage containing all the natural ingredients of the fruits like, vitamins, amino acids, polyphenols, flavonoids, tannins, anthocyanins and minerals in it, which together makes it a nutritive health drink of high commercial value. The red wines are abundant source of natural antioxidants and polyphenols1 and represent an important dietary component for some populations. In recent years, various studies have been carried out to study the potential cancer chemopreventive activities and other health beneficial properties of red wine polyphenols2,3.

The polyphenols in the red wines are the major components which are responsible for the taste, colour, astringency, bitterness and mouth feel4. These polyphenolic compounds are the secondary metabolites which are naturally present in the wines while some are produced during vinification process. The formation of polyphenolic compounds during the ageing of wines occurs by oxidation, hydrolysis and various transformation reactions5. Studies have reported that moderate consumption of red wines have beneficial health effects in the prevention of a number of diseases like atherosclerosis and coronary heart disease6. Though the consumption of red wines has been hypothesized to be the most likely cause for the phenomenon known as the “French Paradox”7.

The berry fruits have a lot of potential to form various value added products8 and are also considered to be as rich source of highly valuable chemical components9. In Himalayas various types of these berry fruits are found in abundance and are consumed from decades by some local peoples with few of them have their medicinal values9. So, the present study aims towards the bioutilization of these wild Himalayan berry fruits which have poor market value as such, for wine making. The study also involves comparative quality evaluation of total antioxidant activity, polyphenol content and total flavonoid contents of the wines made from these fruits along with the sensory studies.

Materials and Methods

Chemicals and solvents

Chemicals 2, 2′-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu’s phenol reagent, gallic acid,
trolox, and quercetin, were purchased from Sigma–
Aldrich Chemie. All other solvents and chemicals
were of analytical grade and obtained from S. D. Fine
Chem. Ltd. (India).

Production of herbal fruit wines

Collection of wild berries fruit

Fruits of different wild species, viz. Berberis lycium
Royle (Berberidaceae), Pyrus pashia Buch.-Ham ex D. Don (Rosaceae), Actinidia deliciosa (A.Chev.)
C.F.Liang & A.R.Ferguson (Actinidiaceae), Syzygium
jambos (L.) Alston (Myrtaceae), Emblica officinalis
Gaertn. (Euphorbiaceae), Prunus cerasoides D. Don
(Rosaceae), Rubus ellipticus Sm. (Rosaceae),
Crataegus sp. (Rosaceae) and Citrus karma Raf.
(Rutaceae) were collected from the forests of various
regions of Kangra, Himachal Pradesh, India. These
berry fruits were then washed thrice with distilled
water and then soaked in 0.5% Sodium metabisulfite
solution for one hour for sterilization.

Preparation of fruit wines

Washed and sterilized berry fruits were ground
with the help of grinder machine. Took 1 kg each of
the ground fruit pulp in different food grade HDPE
containers with narrow mouth. Sucrose solution 12%
of the final volume was added followed by addition of
food for yeast and activated yeast (Saccharomyces
cerevisiae). The active dry wine yeast was purchased
from Montrachet and was activated according to
manufacturer’s procedure with some modifications.
The containers were sealed with food grade lab seal
so as to prevent the contamination. The must was then
allowed to ferment for 30-45 days at 25±3°C
temperature in a dark room away from direct light
source. When first fermentation was completed again
added food for yeast and activated yeast in it, if
required. Sucrose solution was added according to the
tested parameters like pH and brisk of the first
fermentation. Again sealed the containers and then
left it for further ageing, till it becomes appropriate
for use (at least 6 months). The prepared wines were
then decanted carefully in to other containers and then
left for natural settling (racking) for 30-50 days,
followed by filtration.

Qualitative study of the wines

Estimation of °Brix, alcohol and pH

For the estimation of °Brix of different wines the
Erma Hand Refractometer (0-32 °Bisk) was used. The alcohol percentage of the wines was measured
with a Wine Analyser Infrascan+ and the pH values of
the wines were measured using Lab India pH meter.

Determination of total polyphenols

Total polyphenol content (TPC) was determined spectrophotometrically with Hitachi
Spectrophotometer, according to a modified method using Folin-Ciocalteu’s reagent. Briefly 100 µL of
samples were taken in a 25 mL volumetric flask and
then add 500 µL of Folin reagent (1N) in it. Added
1 mL of 30% sodium carbonate solution and then
made the volume to 25 mL with distilled water
followed by vigorous shaking. Incubated it for 60 min. The appearance of blue colour was measured
spectrophotometrically at 730 nm against a sample
blank. The total polyphenol content of different wines
were calculated as gallic acid equivalent from the
calibration curve of gallic acid standard solutions (20,
40, 60, 80, 100 µL aliquots of 0.1% aqueous gallic
acid) and expressed as mg gallic acid equivalent
(GAE)/litre of the wine. All the measurements were
done in triplicate.

Determination of total flavonoids

For the determination of total flavonoid content
(TFC), the samples were measured using a modified
colorimetric method. Aliquots (100 µL) of various
wines were taken in 5 mL volumetric flasks and then
added 100 µL of 10% aluminium chloride (w/v) and
100 µL of 1 M potassium acetate solution in it and
then made the volume to 5 mL with distil water.
Afterwards incubated it at room temperature (25°C)
for 30 min, the absorbance of the reaction mixture
relative to blank was measured at 415 nm. The total
flavonoid content of samples was expressed as mg
quercetin equivalent/litre of wine. For the preparation
of calibration curve 25, 50, 75, 100 and 125 µL of
standard quercetin in ratio of (0.5 mg/mL) were
mixed with the same reagents as described above and
after 30 minutes, the absorbance at 415 nm was
measured for determination of total flavonoids. All
measurements were done in triplicate.

Determination of antioxidant activity

The total antioxidant activities of different wines
were measured against stable DPPH spectrophoto-
metrically. DPPH on reaction with an antioxidant
compound, which have tendency to donate hydrogen,
it gets reduced. The change in colour from deep-violet
to yellow was measured at 517 nm. The radical
scavenging activity was measured by slightly
modified method adopted by Brand-Williams et al. Aliquots 100 µL of the wines were added to 2.9 mL of the 100 µM DPPH solution prepared in 80% aqueous ethanol. The mixture was shaken vigorously and allowed to stand at 25°C in dark for 30 minutes and decrease in absorbance of the resulting solution was monitored at 517 nm against a sample blank. All the measurements were done in triplicate. The radical scavenging activity was calculated by the following formula:

\[
\text{% Inhibition} = \left( \frac{(A_B - A_A)}{A_B} \right) \times 100
\]

Where \(A_A\) - Absorbance of test; \(A_B\) - Absorbance of blank.

For the estimation of antioxidant activity with the DPPH radical, trolox (0.01%) in various concentrations (20, 40, 60, 80, and 100 µL) was used for preparation of the standard curve.

Sensory analysis
Sensory evaluation studies were performed by a panel of wine tasters. A blind sensory evaluation for various parameters like colour, taste, sweetness, astringency and flora was performed by serving different wines at regular intervals followed by rinsing of the palate between tastings with water. The scores for various attributes given by 5 trained panellists were in the range of 1-5 for different wines.

Statistical analysis
Chemical composition data were expressed as mean ± SD of 3 parallel measurements. The data were subjected to analysis of variance (ANOVA) as per Randomised Complete Block design having 3 replications for chemical composition data and 5 for the sensory evaluation studies. Homogenous grouping was done using Duncan’s multiple range test at \(p = 0.01\). STATISTICA version 7 software of Stat Soft Inc., Tulsa, OK, USA was used for the data analysis.

Results and Discussion
Preparation of various herbal wines using wild berry fruits from Himalayas by an economical process gives various high valued antioxidants enriched low alcoholic fruit wines. These wild berry fruits which are also rich source of antioxidant compounds, vitamins, minerals and amino acids also have lot of potential in upliftment of the local economy. The wines were prepared from these berry fruits by conventional procedure of wine making. The °Brix, total alcohol percentage, pH value, total polyphenol and total flavonoid content along with total antioxidant potential of the these herbal wines have also been estimated. The result of the study showed that wines made from selected wild Himalayan berry fruits have 6-10 °Brix, 10-15% of alcohol content and have pH value in range of 3.3-3.8. Results are given in Table 1.

The total polyphenol content (TPC) and total flavonoid content (TFC) of the wines were measured spectrophotometrically against standard curve obtained by gallic acid and quercetin and compared the content in various wines. The different wines

<table>
<thead>
<tr>
<th>Fruit plant species</th>
<th>°Brix</th>
<th>% Alcohol</th>
<th>pH</th>
<th>TPCa</th>
<th>TFCb</th>
<th>TEACc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Berberis lyceum</td>
<td>8</td>
<td>15.1</td>
<td>3.8</td>
<td>622.3 ± 2.3</td>
<td>52.1 ± 0.65</td>
<td>186.7 ± 0.35</td>
</tr>
<tr>
<td>Pyrus pashia</td>
<td>8</td>
<td>12.5</td>
<td>3.6</td>
<td>162.0 ± 2.7</td>
<td>15.9 ± 0.65</td>
<td>75.1 ±0.99</td>
</tr>
<tr>
<td>Actinidia deliciosa</td>
<td>6</td>
<td>11.2</td>
<td>3.6</td>
<td>293.0 ± 1.3</td>
<td>21.6 ± 0.75</td>
<td>168.6 ± 0.57</td>
</tr>
<tr>
<td>Syzygium jambos</td>
<td>8</td>
<td>13.8</td>
<td>3.5</td>
<td>319.1 ± 2.7</td>
<td>37.4 ± 0.35</td>
<td>184.7 ± 0.40</td>
</tr>
<tr>
<td>Emblica officinalis</td>
<td>6</td>
<td>10</td>
<td>3.3</td>
<td>2845.6 ± 2.5</td>
<td>43.3 ± 0.40</td>
<td>187.6 ± 0.21</td>
</tr>
<tr>
<td>Prunus cerasoides</td>
<td>6</td>
<td>10.8</td>
<td>3.6</td>
<td>1496.1 ± 4.7</td>
<td>180.6 ± 0.75</td>
<td>187.7 ± 0.23</td>
</tr>
<tr>
<td>Rubus ellipticus</td>
<td>8</td>
<td>14</td>
<td>3.5</td>
<td>357.2 ± 3.6</td>
<td>33.9 ± 0.40</td>
<td>168.6 ± 0.57</td>
</tr>
<tr>
<td>Crataegus sp.</td>
<td>8</td>
<td>14</td>
<td>3.7</td>
<td>560.4 ± 4.1</td>
<td>55.2 ± 0.35</td>
<td>184.0 ± 0.25</td>
</tr>
<tr>
<td>Citrus karne</td>
<td>10</td>
<td>10.4</td>
<td>3.5</td>
<td>354.9 ± 1.3</td>
<td>31.7 ± 0.65</td>
<td>91.7 ± 0.17</td>
</tr>
<tr>
<td>S. Em. ±</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1.8</td>
<td>0.32</td>
<td>0.28</td>
</tr>
<tr>
<td>CD at 1 % Level</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>4.3</td>
<td>0.77</td>
<td>0.67</td>
</tr>
</tbody>
</table>

Values are means ± S.D. for \(n = 3\)

a Data expressed as mg of gallic acid equivalent/Litre of wine

b Data expressed as mg of quercetin equivalent /Litre of wine

c Data expressed as mg of Trolox equivalent antioxidant capacity (TEAC)/litre of wine
contain 162-2845 mg of total polyphenols/litre of wine measured as gallic acid equivalent. The wine made from ripe fruits of *Emblica officinalis* shown highest amount of total polyphenol content and *Pyrus pashia* fruit wine contains least amount of total polyphenols. In case of the total flavonoid content (TFC) 15-180 mg of total flavonoids/litre of wine was present in various wine samples and measured as quercetin equivalent. The wine made from fruits of *Prunus cerasoides* contains highest flavonoids and *Pyrus pashia* wine again contains least amount of total flavonoids. Results are given in Table 1.

The result of the total antioxidant activity of the different wine samples as measured by DPPH radical scavenging assay as trolox equivalent and shown presence of 75-187 mg of total antioxidant compounds/litre of wine. These results show that *Prunus cerasoides* fruits wine contains highest amount of antioxidant compounds and *Pyrus pashia* wine contains lesser amount of antioxidant compounds. Results are given in Table 1. After total qualitative evaluation of different herbal wines we can say that the wines made from fruits of *Emblica officinalis* and *Prunus cerasoides*, better withstands within all quality parameters. Each have 6 °Brix, alcohol 10% and 10.8% and pH value 3.3 and 3.6, respectively. Total antioxidant activity and polyphenol content were also found to be highest in wines made from these fruits. The results of sensory evaluation studies were performed by a panel of 5 wine experts for various parameters like colour, taste, sweetness, astringency and flora Table 2. The results of the sensory evaluation studies show the wide acceptability of these herbal wines.

**Conclusion**

In conclusion, the present study reports an economical process for utilization of highly valuable wild berry fruits from high altitudes of Himalayas for production of low alcoholic herbal wines along with their quality evaluation studies. The study involves the usage of simpler technique for wine preparation on small industrial scale which would be very much beneficial as the scaling of the process can be adjusted according to the availability of the plant material. The present work of utilizing wild fruits in wine making and their quality assessment study gives better option for bio-utilization of highly valuable wild fruits which are otherwise of no commercial value.

**Acknowledgements**

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**References**


**Table 2—Sensory evaluation studies of various wines**

<table>
<thead>
<tr>
<th>Fruit plant species</th>
<th>Color</th>
<th>Taste</th>
<th>Sweetness</th>
<th>Astringency</th>
<th>Flora</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Berberis lyceum</em></td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.5</td>
<td>2.8</td>
</tr>
<tr>
<td><em>Pyrus pashia</em></td>
<td>3.0</td>
<td>3.0</td>
<td>3.3</td>
<td>2.4</td>
<td>2.7</td>
</tr>
<tr>
<td><em>Actinidia deliciosa</em></td>
<td>2.4</td>
<td>2.8</td>
<td>3.5</td>
<td>3.0</td>
<td>2.4</td>
</tr>
<tr>
<td><em>Syzygium jambos</em></td>
<td>3.0</td>
<td>3.6</td>
<td>3.0</td>
<td>3.4</td>
<td>3.4</td>
</tr>
<tr>
<td><em>Emblica officinalis</em></td>
<td>3.0</td>
<td>3.0</td>
<td>3.5</td>
<td>2.2</td>
<td>2.4</td>
</tr>
<tr>
<td><em>Prunus cerasoides</em></td>
<td>3.4</td>
<td>2.8</td>
<td>2.9</td>
<td>3.0</td>
<td>2.8</td>
</tr>
<tr>
<td><em>Rubus ellipticus</em></td>
<td>3.0</td>
<td>3.3</td>
<td>3.0</td>
<td>3.5</td>
<td>2.8</td>
</tr>
<tr>
<td><em>Crataegus sp.</em></td>
<td>3.0</td>
<td>2.5</td>
<td>3.3</td>
<td>3.4</td>
<td>2.7</td>
</tr>
<tr>
<td><em>Citrus karne</em></td>
<td>2.2</td>
<td>2.2</td>
<td>3.2</td>
<td>3.0</td>
<td>2.4</td>
</tr>
<tr>
<td>S. Em. ±</td>
<td>0.20</td>
<td>0.18</td>
<td>0.20</td>
<td>0.25</td>
<td>0.14</td>
</tr>
<tr>
<td>CD at 1% Level</td>
<td>0.34</td>
<td>0.31</td>
<td>NS</td>
<td>0.44</td>
<td>0.24</td>
</tr>
</tbody>
</table>

NS = Non significant. (n = 5 panelists)


8 Chakraborty I, Chaurasiya AK and Saha J, Quality of diversified value addition from some minor fruits, *J Food Sci Technol*, 2011, **48**, 750-754.


