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ORIGINAL ARTICLE

Constituents and Antioxidant Activity of Bleeding Sap from Various Xinjiang Grapes

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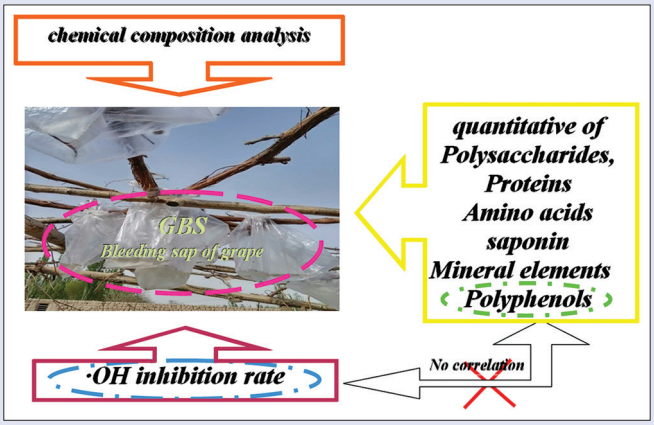
ABSTRACT

Objective: Wine grape sap or bleeding sap of grapes (GBS) is commonly used in Xinjiang (China) for therapeutic aims. Do variations in composition related to region and variety affect its properties? **Methods:** GBS samples originating in various parts of Xinjiang (Turpan, Hotan, Kashgar, and Atush) were tested for phenols and polyphenols, polysaccharides, saponin, proteins, individual amino acids, and minerals. Their antioxidant activity was measured using ascorbic acid as reference. **Results:** Polyphenol content varied from 2.6 to 6.6 mg/L, polysaccharides 18.3–816 mg/L, saponin 6.25–106 mg/L, and protein 3.0–22.4 mg/L. Mineral elements and amino acids ranged from 6.20 to 201.2 mg/L and 0.06–118.7 mg/L, respectively. ·OH scavenging ability varied from 70% to over 90%, higher than Vitamin C. Grapes from Turpan had lower antioxidant activity than other grapes even though the polyphenol content was generally higher. **Conclusion:** Bleeding sap of Xinjiang grape is rich in amino acids, polysaccharides, polyphenols, and protein. The contents are different according to the origin, related possibly to species, climate, and environment. Antioxidant effects were not correlated with polyphenol content.

Key words: Antioxidant activities, bleeding sap of Xinjiang grape, polyphenols

SUMMARY

- Antioxidant activity of plants or plant extracts is often associated with polyphenols
- Bleeding sap of grapes has strong antioxidant properties
- Bleeding sap from different grape varieties from different parts of Xinjiang (China) had different polyphenol concentrations
- There was no correlation of polyphenol concentrations with antioxidant activity.



Abbreviations used: GBS: Bleeding sap of grapes; PITC: phenyl isothiocyanate.

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INTRODUCTION

In Xinjiang (China), over fifty different varieties of *Vitis vinifera* grapes are grown in various areas (Turpan, Hotan, Kashgar, Atush, etc.).[1] Grapes are eaten fresh or dried (raisins) or used for wine, mostly in Turpan. Grapes used for winemaking are mostly grapes of French origin, such as cabernet or merlot, whereas table grapes and raisins come from traditional local varieties such as Munage. Grapes and raisins from Turpan, Atush, Kashgar, or Hotan are renowned for their taste.[1] Dried Hotan grapes are also used in traditional medicine, whereas others are more often used for daily consumption or in traditional cooking recipes.

When the vines are pruned in the springtime, the cut branches exude sap, called bleeding sap of grape (GBS).[2-4] GBS contains many nutrients, especially calcium, potassium and glutamic acid,[4-7] other components such as kinines,[8-10] and polyphenols. GBS is commonly used in traditional Uyghur medicine as a tonic and more generally to promote health and prevent aging,[11,12] which may be related to its radical scavenging properties.[13,14]

Since the organoleptic properties of grapes and wines from these grapes are modified by the nature, location, and weather of the land on which they grow, among other factors, we attempted to identify the components in the bleeding sap of various grapes from Xinjiang and test for differences in composition and antioxidant properties.[11]

Instruments and materials

Instruments

Shimadzu 2550 ultraviolet (UV)-visible spectrophotometer (Shimadzu, Japan); Vista-PRO inductively coupled plasma emission spectrometer

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(VARIAN, America); e2695 high performance liquid chromatography (Waters, America); Shim-pack VP-ODS column (YMC, Japan, 4.6 mm × 250 mm, 5 μm); HWS26 electric-heated thermostatic water bath (Shanghai Yiheng technical Co. Ltd.); and N-1001 rotary evaporator (Shanghai Ailang Technical Co. Ltd.).

Reagents

Gallic acid standard (National Institutes for Food and Drug Control, 110831-200803); oleanolic acid (National Institutes for Food and Drug Control, 110709-201206); Ca, K, Mg, Na, Fe, Mn, Cu, Zn, P, S standard solutions were purchased from National Research Centre; amino acid reference substances, sodium acetate (Tianjin Guangfu Fine Factory, pure grade); acetonitrile, methanol (HPLC grade); hydroxyl radical kit (Nanjing Jiancheng Bioengineering Institute, 20120306); glucose, bovine serum, albumin, phenyl isothiocyanate (PITC), hexane, triethylamine (TEA), sodium tungstate, sodium molybdate, lithium sulfate, anhydrous sodium carbonate, ninhydrin, sodium hydroxide, α-naphthol, copper, magnesium, silicon acid, mercuric chloride, potassium iodide, phosphoric acid, hydrochloric acid, sulfuric acid, acetic acid, acetic anhydride, bromine water, ethanol (analytical grade); and twice-distilled water.

Sample materials

GBS was collected from vineyards and grape fields in Hotan, Kashgar, Atush, and Turpan, Xinjiang, in April 2012 and April 2013, and preserved at 4°C.[2] The original plants providing the sap were identified as *Vitis vinifera* L. (Vitaceae).

METHODS

Pretest experiments of chemical composition

The different pretests for chemical composition of GBS and the positive reactions are described in Table 1.

Ninhydrin reaction: 1 mL concentrated solutions from different varieties and areas is mixed with 2-3 drops ninhydrin reagent. After heated in boiling water for 10–15 min, we observe the color change.

Biuret reaction: 10d concentrated solutions from different varieties and areas is mixed with 10d 10% NaOH and 2d 1% $CuSO_4$ and heated.

α-naphthol/concentrated sulfuric acid reaction: 1 mL GBS from different varieties and areas is mixed with 4d Molish reagent and 1 mL concentrated sulfuric acid. The mixed solutions separate into two distinct layers, and if positive at the junction, a purple ring appears.

Foam reaction: 2 mL GBS from different varieties and areas is vigorously shaken in 1 min after that we observe the bubble duration.

Acetic anhydride/concentrated sulfuric acid reaction: 2 mL concentrated solutions from different varieties and areas is dried under reduced pressure and dissolved with 1 mL acetic anhydride. After heating in boiling water for 10–15 min, we observe the color change. After adding 1d concentrated sulfuric acid slowly, we observe the color change.

HCl-Mg reaction: 2 mL concentrated solutions from different varieties and areas are mixed with Mg and 3–4d concentrated HCl. After heating, we observe the color change.

Silicon acid, potassium mercuric iodide, iodine-potassium iodide reaction: 2 mL concentrated solutions from different varieties and areas are mixed with some silicon acid reagent, potassium mercuric iodide reagent, or iodine-potassium iodide reagent.

Folin–Ciocalteau reaction 0.4 mL concentrated solutions from different varieties and areas are mixed with 0.5 mL Folin–Ciocalteau reagent and 3 mL H_2O. The mixed solutions are placed for 4 min. After adding 1.5 mL 10% Na_2CO_3, we observe the phenomenon.

Quantitative measurement of contents

When the pretest showed the presence of the chemicals as described above, they were quantified with the following tests:

Polyphenols

Using gallic acid as a standard, the phenol content in GBS was determined by Folin–Ciocalteau colorimetry:[15] 0.4 mL concentrated solutions were mixed with 0.5 mL Folin–Ciocalteau reagent and 3 mL H_2O. The mixed solutions were left to rest for 4 min, then added with 1.5 mL 10% Na_2CO_3 and distilled water to 10 mL; after 120 min, the absorbance at 760 nm was determined. The equation of the standard curve for gallic acid was $A = 0.1228C + 0.0295$, $R^2 = 0.9997$. It has a good linearity between 0 and 6 mg/mL.

Polysaccharides

Using glucose as a standard, the polysaccharide content of GBS was determined by the anthrone-sulfuric acid method: bleeding sap is added with distilled water to 2.0 mL, then mixed with 0.5 mL 0.02 mg/mL anthrone-ethyl acetate and 5 mL concentrated sulfuric acid. After cooling at room temperature, the absorbance was measured at 621 nm.

Proteins

Using bovine serum albumin as a standard, the protein content was determined by the Coomassie brilliant blue method. Concentrated solutions are added with distilled water to 1.0 mL then mixed with 5.0 mL 10% Coomassie brilliant blue reagent. After 5 min, the absorbance at 595 nm was measured.

Amino acids

Seventeen amino acids were quantified by HPLC, using PITC as a precolumn derivatization reagent.[16] Chromatographic conditions: Shim-pack VP-ODS column (4.6 mm × 250 cm, 5 μm), column temperature: 35°C, mobile phase A: 0.1 mol/L sodium acetate-acetonitrile (93:7), mobile phase B: methanol:acetonitrile:water = 20:60:20, elution gradient: 0–5 min, 0%–2% B; 5–6 min, 2%–6% B; 6–15 min, 6%–9% B; 15–19 min, 9%–21% B; 19–32 min, 21%–45% B; 32–34 min, 45%–55% B; 34–38 min, 55%–100% B; 38–47 min, 0% B; flow rate: 1.0 mL/min, detection wavelength: 254 nm, injection volume: 10 μL, and derivative reaction: 200 μL concentrated solution from Xinjiang GBS is mixed with 100 μL 0.1 mol/L PITC and 100 μL 1 mol/L TEA. After 20 min at room temperature, the mixed solution was centrifuged for 5 min at 10000 r/min and added with 500 μL n-hexane. After removing the supernatant, the solution was filtered and measured.

Table 1: Results of pretest experiments on grape bleeding sap

Pretest objective	Experimental reaction	Endpoint	Result
Amino acid, peptides	Ninhydrin test	Blue-purple	Positive
protein	Biuret test	Blue	Positive
Sugars, polysaccharides, and glycosides	α-naphthol/concentrated sulfuric acid reaction	Purple ring	Positive
Saponin	Foam test	Lasting over 10 min	Positive
Flavonoid and glycoside	Hydrochloric-magnesium reaction	Faint tea-red	Positive
Alkaloid	Silicon acid, potassium mercuric iodide, iodine-potassium iodide reaction	No precipitation	Negative
Polyphenol or tannin	Folin-Ciocalteau reaction	Blue-gray	Positive

Determination of saponin

Saponin concentrations were measured using UV-visible spectrophotometry at 548 Nm with oleanolic acid as standard: oleanolic acid solutions from 0 to 0.8 mL were evaporated at 90°C completely, then added with 0.2 mL 0.5 mL/L vanillin ice acetic acid solution and 0.8 mL perchlorate, set in a 70°C water bath 15 min, cooled, added with 4 mL ethyl acetate and shaked well. The least square method is used to build a standard curve. The equation obtained by the least square method is $A = 0.0085C - 0.0099$, $r = 0.9999$. It has a good linearity between 0 and 80.8 μg. The same process was followed for samples.

Mineral elements

The mineral elements were determined by ICP-OES:[17] 5 mL GBS was mixed with 8 mL nitrification liquid ($V[HNO_3]:V[HClO_4]$ = 4:1) in a Kjeldahl flask and rested overnight. After heating, refluxing and digesting to colorlessness, and completing volume to 50 ml with distilled water, the mixed solution was determined.

OH inhibition rate

This test follows ·OH reagent kit instruction: 0.2 mL GBS was added with 0.2 mL substrate solution and 0.4 mL NO3 solution. After 1 min at 37°C, reagent was added immediately to stop the reaction. The amount of remaining· OH was detected at 550 nm as absorbance. Results are indicated by inhibition rate, where inhibition rate (%) = (the absorbance of control tube − the absorbance of measuring tube)/the absorbance of control tube × 100%. Vitamin C (ascorbic acid, VC) was tested as reference (0.2 mg/mL).

Table 2: Polyphenols, polysaccharides, and protein contents of Xinjiang grapevine bleeding sap (mg/L)

Grape type	Polyphenols	Polysaccharides	Protein
Turpan seedless white	5.7	18.3	5.0
Turpan Kashyyyk	4.9	596	4.3
Hotan County red grape	4.3	556	4.2
Hotan red grape	2.6	101	9.6
Hotan Munage	6.6	63.6	22.4
Kashgar Munage	3.4	193	3.0
Atush Munage	4.5	816	21.1

Table 3:·Saponin content in Xinjiang grapevine bleeding sap

	Saponin μg/mL
Hotan County red grape	17.3
Hotan green grape	65.4
Hotan red grape	47.8
Hotan Munage	75.5
Kashgar Munage	39.0
Atush Munage	6.50
Turpan seedless white	15.7
Turpan Kashyyyk	106
Turpan white gac	25.5

RESULTS

The results of pretest experiments of the chemical composition of GBS are described in Table 1. From this general pretest, GBS contains amino acids, protein, polysaccharides, saponin, flavonoids, and polyphenols but no alkaloids.

In the final testing, the polyphenol content varied from 2.6 to 6.6 mg/L [Table 2]. The polyphenol content of Turpan GBS was generally higher than that of Hotan. Polysaccharide content was very variable with no clear difference between the origins of the GBS. GBS from Hotan and Atush Munage but not Kashgar Munage varieties has higher protein content than other varieties. There was no obvious association between these elements.

GBS from different origins has very different saponin content [Table 3]. The order is Turpan Kashyyyk > Kashgar Munage > Hotan County red grape > Artux Munage > Turpan white gac > Hotan green grape > Turpan seedless white > Hotan Munage > Hotan red grape GBS contained most amino acids to various degrees [Table 4] though four GBSs from Hotan had low concentrations of essential amino acids. There were no clear differences in mineral contents between GBS [Table 5].

When the ·OH scavenging abilities [Table 6] are sorted by increasing scavenging activity, GBS seems to segregate in two groups, one of the Turpan grapes, around 70%–80%, and the other of the grapes from the Western end of Taklamakan, all above 90%. They were all superior to the reference antioxidant, VC (0.2 mg/mL). There was no correlation

Table 4: Amino acid contents of grape bleeding sap in seventeen amino acids (mg/L)

Amino acid	Turpan white gac	Turpan kashyyk	Turpan seedless white	Hotan munage	Hotan red grape	Hotan segezi township red grape	Hotan green grape	Kashgar munage	Atush munage
Asp	2.38	1.99	0.53	100.2	7.51	6.94	6.54	0.87	4.72
Glu	1.49	1.46	75.33	77.23	6.91	6.76	2.00	0.67	10.49
Ser	13.38	-	10.06	-	0.18	0.14	0.10	-	-
Gly	0.56	0.55	0.32	0.26	-	0.19	-	0.19	1.48
His	3.28	1.03	1.95	1.24	0.15	0.13	-	0.54	2.40
Arg	-	-	0.89	-	0.13	0.54	-	0.13	3.79
Thr*	1.19	0.47	0.42	0.47	-	0.19	0.20	0.23	1.03
Ala	0.20	0.20	0.27	2.66	0.28	0.50	-	0.12	0.78
Pro	0.28	0.15	0.22	0.97	-	0.27	0.15	0.19	1.16
Tyr	1.43	0.65	0.81	0.62	0.13	0.13	0.15	0.25	1.37
Val*	4.18	2.23	4.09	4.58	0.16	0.24	0.13	1.44	2.72
Met*	1.70	0.41	2.49	0.49	0.11	0.15	0.13	0.14	0.17
Cys	-	-	-	-	0.14	0.15	-	0.12	0.13
Leu*	2.47	1.23	3.18	2.38	-	_	-	0.84	0.15
Ile*	0.86	0.46	1.45	1.03	0.38	0.32	0.14	0.46	0.85
Phe*	4.63	1.98	6.28	4.13	0.78	0.73	3.96	1.65	5.12
Lys*	4.68	3.65	3.95	4.91	3.65	3.11	0.72	1.83	5.85
E	19.71	10.43	21.86	17.99	5.08	4.74	5.28	6.59	15.89
T	42.71	16.46	112.24	201.22	20.51	20.49	14.22	9.67	42.21

*Essential amino acids. E: Essential amino acids; T: Total amino acids; -: Not detected

Table 5: Mineral elements contents of Xinjiang grapevine bleeding sap (*n*=3, mg/L)

	Turpan seedless white	Hotan County red grape	Hotan red grape	Hotan Munage	Kashgar Munage	Atush Munage	Average
Ca	111.71	88.39	74.94	104.22	90.16	120.38	98.3
Fe	0.34	0.25	0.42	0.58	0.32	0.44	0.39
K	127.91	90.72	54.22	156.72	151.08	131.58	118.71
Mg	17.25	12.40	11.13	23.35	16.56	31.41	18.68
Mn	0.13	0.18	0.09	0.02	0.31	0.93	0.28
Na	19.93	31.46	22.33	19.90	18.80	21.13	22.26
Zn	0.03	0.05	0.32	0.35	0.08	0.40	0.21
Cu	0.07	0.07	0.07	0.06	0.07	0.01	0.06
P	5.07	0.60	0.60	10.30	4.98	12.48	5.67
S	8.24	1.43	1.24	8.41	5.43	9.10	5.64

Table 6: Hydroxyl radical scavenging ability of Xinjiang grapevine bleeding sap

Specie	Inhibition rate (%)
Vitamin C (0.2 mg/mL)	64.55
Turpan seedless white	70.49
Turpan white gac	75.98
Turpan Kashyyyk	80.04
Hotan green grape	90.45
Hotan County red grape	91.40
Kashgar Munage	91.62
Hotan Munage	92.30
Atush Munage	92.64
Hotan red grape	92.80

between the GBS contents in polyphenols or saponins and their ability to scavenge ·OH radicals.

DISCUSSION

Grapes in Xinjiang are mostly found in two distinct areas: the region of Turpan in the Northeast of the Taklamakan desert and the region at the Western end of the Taklamakan which includes Kashgar, Atush, and Hotan, 1400 km away from Turpan. GBS from the various regions is used similarly.

GBS contains minerals, protein, and essential amino acids with up to 10-fold variation from one GBS to another with no clear distinction between the types of grapes or their provenance. GBS has an antioxidant activity, generally superior to that of VC, which appeared lower in GBS from plants in the Turpan area. Differences in the content and effect of GBS may be related to a number of factors, such as the grape type, temperature,[10] water availability or irrigation or more generally the weather, or again the soil, and perhaps the amount and intensity of pruning and other stresses.[18,19] These are essential to the differences between wines from the same varieties of grapes in different regions or different grapes in the same region as winemakers know well. What affects the grapes may also affect the sap from the vines, which was the object of this study.

From a nutritional and health point of view, drinking GBS does not seem detrimental, and the health allegations in traditional Uyghur medicine may not be without merit. However, there are clearly differences in the content and the antioxidant effect of GBS from different origins. Because of this, the potential benefits of GBS may vary according to its origin.

In the present case, the role of the extreme heat, dryness, and high atmospheric pressure in Turpan, which is under sea level (150 m below the sea level, the second lowest place on earth), and the irrigation with karez (underground aqueducts bringing water from distant mountains) remain to be explored, compared to higher altitude (Kashgar is around 1200 m) and milder weather of the Kashgar area.

The benefits of grapes and wine (and GPS) are often ascribed to their polyphenol content and their antioxidant activity. However, this study differentiates between the polyphenol content of GBS and its antioxidant activity. Clearly, the antioxidant activity is not limited to polyphenols as was also found with other grape-derived products.[20] We confirm the need to specify the origin and composition of plant extract when describing effects. In addition, we did not study change in product composition over the years: if the variable taste of wine according to vintage indicates change in its composition, then surely this could affect GBS too. What is true for grape should be true for all plants.

Further studies will be needed to identify the source of the variations we found and further explore the possible benefits of GBS on human health.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Liu JJ. Xinjiang grape cultivation, storage and processing. Urumqi: Xinjiang People's Publishing House; 1987.
2. Alexou M, Peuke AD. Methods for xylem sap collection. Methods Mol Biol 2013;953:195-207.
3. Biondi E, Galeone A, Kuzmanovic N, Ardizzi S, Lucchese C, Bertaccini A. *Pseudomonas syringae* pv. Actinidiae detection in kiwifruit plant tissue and bleeding sap. Ann Appl Biol 2013;162:60-70.
4. Wang Z. Plant Physiology. Beijing, China: Agriculture Press; 2000.
5. Friedman R, Levin N, Altman A. Presence and identification of polyamines in xylem and Phloem exudates of plants. Plant Physiol 1986;82:1154-7.
6. Wormall A. The constituents of the sap of the vine (*Vitis vinifera* L.). Biochem J 1924;18:1187-202.
7. Yu T, Wang YX, Liu HK. Analysis on nitrogen form in bleeding sap of three fruit trees. J Northwest Forestry Univ 2006;21:34-6.
8. Skene KG. Gibberellin-like substances in root exudate of *Vitis vinifera*. Planta 1967;74:250-62.
9. Skene KG. Cytokinins in the xylem sap of grape vine canes: Changes in activity during cold-storage. Planta 1972;104:89-92.
10. Skene KG, Kerridge GH. Effect of root temperature on cytokinin activity in root exudate of *Vitis vinifera* L. Plant Physiol 1967;42:1131-9.
11. Liu YM, Ikem S. Uygur Medicine. Urumqi: Xinjiang People's Publishing House; 2009.
12. Tohit I, Mtre T, Ababkli E. Research on biological activity of gum of oleaster for recovering antioxidant capacity of deteriorating grape sap. Mod Agric Sci 2009;16:32-4.
13. Ilbraim A, Abdurxit A, Li IJ, Mahmut M. Study on the antioxidant activities and heat

stabilization for bleeding sap from Xinjiang grape tree. Food and Fermentation Industries 2009,35:14-16

14. Wang YY, Zhao JF, Tang ZZ, Chen MM, Tohit I, Lu CM. Preliminary study of antioxidants in bleeding sap from *Vitis vinifera* L. Food Sci Technol 2009;34:166-70.
15. Fu-Rong ZH, Tohit I, Ibraim A, Huang Q. Study on the antioxidant activities and polyphenol content determination for bleeding sap of Xinjiang grape tree. Food Res Dev 2013;34:12-5.
16. Abdureyim M, Aikemu A, Hajinissa, Wan Y. Content measurement of quercetin and analysis of amino acids in *Capparis spinosa* L. J Xinjiang Med Univ 2009;32:1422-3.
17. Ibraim A, Zhu FR, Mamat R, Huang Q. Principal component analysis and clustering analysis of the mineral elements in bleeding sap of Xinjiang grape tree. Food Ferment Ind 2013;39:208-11.
18. Du TS, Kang SZ, Zhang BZ, Li SE, Yang XY. Stem sap flow of grape under different drip irrigation patterns and its relationships with environmental factors in arid oasis region of Shiyang River basin. Ying Yong Sheng Tai Xue Bao 2008;19:299-305.
19. Hochberg U, Degu A, Toubiana D, Gendler T, Nikoloski Z, Rachmilevitch S, *et al*. Metabolite profiling and network analysis reveal coordinated changes in grapevine water stress response. BMC Plant Biol 2013;13:184.
20. Al Awwadi NA, Borrot-Bouttefroy A, Umar A, Saucier C, Segur MC, Garreau C, *et al*. Effect of Armagnac fractions on human platelet aggregation *in vitro* and on rat arteriovenous shunt thrombosis *in vivo* probably not related only to polyphenols. Thromb Res 2007;119:407-13.