Preliminary researches on conditions to extract alcohol soluble proteins of leaves of *Wedelia trilobata* (L.) Hitchc.

Liguo Qiu, Gan Zhao*, and Tuanna Li

College of Life Sciences, South China Agricultural University, Guangzhou 510642, China

Abstract. The conditions to extract alcohol soluble proteins of leaves of *Wedelia trilobata* (L.) Hitchc. were discussed. When the extractant was alcohol, the weight of leave(g) -to- volume of 75% alcohol solution (mL) ratio was 1:10, temperature for extraction was 50°C and the extraction period was 2hrs, the concentration of alcohol solution needed for the best extraction effect was 95% (V/V). When the extractant was 75% alcohol solution, the temperature for extraction was 50°C and the extraction period was 2hrs, the weight of leave(g) -to- volume of 75% alcohol solution (mL) ratio needed for the best extraction effect was 1:20. When the extractant was 75% alcohol solution, the weight of leave(g) -to- volume of 75% alcohol solution (mL) ratio was 1:10 and the extraction period was 2 hrs, the temperature needed for the best extraction effect was 55°C. When the extractant was 75% alcohol solution, the weight of leave(g) -to- volume of 75% alcohol solution (mL) ratio was 1:10 and the temperature for extraction was 50°C, the extraction period needed for the best extraction effect was 2hrs.

1 Introduction

Alcohol soluble proteins have been studied in common wheat [1], onion [2], corn [3], soybean [4], *Pinus tabulaeformis* [5], sorghum [6], rice [7], proso millet [8], Sudan grass [9], *Ganoderma lucidum* [10], coconut [11], *Zanthoxylum armatum* [12], Moso bamboo [13], white kidney bean [14], beer grains [15] and in *Roegneria*, *Elymus*, *Hystrix* and *Kengyilia* [16]. Xu et al [17] studied moisture-resistance and antioxidation of alcohol soluble proteins for application in food preservation. He et al [18] showed that zein could be used to preserve green peppers, tomatoes, sausages and salted meat fresh. The mice fed the proso millet prolamin (supplemented with 1.82% Lys and 0.23% Trp) showed a higher HDL-c content and a lower atherogenic index [8]. Alcohol soluble proteins have been paid attention to in pharmaceuticals [19] and as carriers to load nutrients [20]. As one of alien invasive plants, the comprehensive utilization of *Wedelia trilobata* (L.) Hitchc has been attractive [21]. Here a try was done on the conditions to extract alcohol soluble proteins from leaves of it.

2 Material, reagents and instruments

Leaves of *Wedelia trilobata* (L.) Hitchc were collected on campus in South China Agricultural University. Analytical reagents such as alcohol was made in China. 752 Type -Ultraviolet Grating Spectrophotometer was made by Shanghai Accurate Scientific Instruments Corporation. HWS24 Type Electro-thermal Constant

Temperature Water Bath was made by Shanghai Yiheng Technology Corporation. HF-200 Type Electronic Balance was made by A&D Company, limited. 5810(R) High-speed Refrigerated Centrifuge was made by Eppendorf Ltd.

3 Experimental methods

3.1 The method to extract alcohol soluble proteins from leaves of *Wedelia trilobata* (L.) Hitchc

2.0 g leaves of *Wedelia trilobata* (L.) Hitchc were mixed with 20 mL extractant and a little of quartz sand, and were ground. And after being dealt with at 50°C for 2hrs, the mixture above was centrifugated at 10000 rpm for 10 minutes. The supernatant was collected for determination.

3.2 The influences of alcohol on the effects to extract alcohol soluble proteins from leaves of *Wedelia trilobata* (L.) Hitchc at different concentrations

The experiments were done basically according to the method 3.1, but the alcohol concentration was used as 60%, 75% and 95% respectively, according to the volume of alcohol -to- volume of alcohol mixed with DDW ratio.

*Corresponding author is Gan Zhao whose E-mail is zg200010@163.com.

3.3 The influences of 75% alcohol solution on the effects to extract alcohol soluble proteins from leaves of *Wedelia trilobata* (L.) Hitchc with different leaf-to-solution ratios

The experiments were done basically according to the method 3.1, but the leaf-to-solution ratio used was changed as 1:5,1:10,1:15 and 1:20 respectively, according to the weight of leave(g) -to- volume of 75% alcohol solution (mL) ratio.

3.4 The influences of 75% alcohol solution on the effects to extract alcohol soluble proteins from leaves of *Wedelia trilobata* (L.) Hitchc at different temperatures

The experiments were done basically according to the method 3.1, but only the 75% alcohol solution was used at 35°C, 42°C, 45°C, 50 °C and 55°C respectively.

3.5 The influences of 75% alcohol solution on the effects to extract alcohol soluble proteins from leaves of *Wedelia trilobata* (L.) Hitchc for different extraction periods

The experiments were done basically according to the

Table 1: The influences of alcohol on the effects to extract alcohol soluble proteins from leaves of *Wedelia trilobata* (L.) Hitchc at different concentrations

Serial number	Weight of leaves of <i>Wedelia trilobata</i> (L.) Hitchc (g)	Concentration of alcohol (V/V)	Usage of the extractant (mL)	alcohol soluble proteins content (µg/mL) (S.E.)
1	2	60%	20	244.2442±6.364285
2	2	75%	20	333.4728±3.543484
3	2	95%	20	506.0142±4.055204

4.2 The influences of 75% alcohol solution on the effects to extract alcohol soluble proteins from leaves of *Wedelia trilobata* (L.) Hitchc with different leaf-to- solution ratios

According to Table 2, when extracting alcohol soluble proteins using 75% alcohol solution from leaves of

Table 2: The influences of 75% alcohol solution on the effects to extract alcohol soluble proteins from leaves of *Wedelia trilobata* (L.) Hitchc with different leaf-to-solution ratios

Serial number	Weight of leaves of <i>Wedelia trilobata</i> (L.) Hitchc (g)	Weight of leave(g) -to- volume of 75% alcohol solution (mL) ratio	Usage of the extractant (mL)	alcohol soluble proteins content (µg/mL) (S.E.)
1	2	1:5	10	110.1549±4.589352
2	2	1:10	20	343.3323±3.807949
3	2	1:15	30	292.0628±4.580517
4	2	1:20	40	238.8215±2.186204

4.3 The influences of 75% alcohol solution on the effects to extract alcohol soluble proteins from leaves of *Wedelia trilobata* (L.) Hitchc at different temperatures

According to Table 3, temperatures could influence the

method 3.1, but only the 75% alcohol solution was used for 30mins, 60 mins, 90 mins and 120 mins respectively.

3.6 Measurement of protein content

The experiments were done basically according to the Bradford's method [22]. When measuring the protein content of the extracts, the extractants used were used as contrasts in stead of DDW.

4 Results and Discussion

4.1 The influences of alcohol on the effects to extract alcohol soluble proteins from leaves of *Wedelia trilobata* (L.) Hitchc at different concentrations

According to Table 1, when extracting alcohol soluble proteins using different alcohol solution from leaves of *Wedelia trilobata* (L.) Hitchc ,the extraction effect by 95% alcohol solution was the best, and the one by 75% alcohol was the second. The one by 60% alcohol was the lowest.

Wedelia trilobata (L.) Hitchc , the different ratio between the weight of leave(g) and the volume of 75% alcohol solution (mL) could influence the extraction effects. When the ratio was 1(g):20 (mL), the extraction effect was the best. And when the ratio became 1(g):5 (mL) ,1(g):10 (mL) and 1(g):15 (mL) respectively, the extraction effects declined respectively.

extraction effects of alcohol soluble proteins from leaves of *Wedelia trilobata* (L.) Hitchc using 75% alcohol solution. When temperatures changed from 35°C, 42°C, 45°C, 50°C to 55°C, the extraction effects gradually rised, and the best effect could be get at 55°C.

4.4 The influences of 75% alcohol solution on the effects to extract alcohol soluble proteins from leaves of *Wedelia trilobata* (L.) Hitchc for different extraction periods

According to Table 4, extraction periods could influence

Table 3: The influences of 75% alcohol solution on the effects to extract alcohol soluble proteins from leaves of *Wedelia trilobata* (L.) Hitchc at different temperatures

Serial number	Weight of leaves of <i>Wedelia trilobata</i> (L.) Hitchc (g)	Temperature (°C)	Usage of the extractant (mL)	alcohol soluble proteins content (µg/mL) (S.E.)
1	2	35	20	235.3707±3.509025
2	2	42	20	273.3298±3.543484
3	2	45	20	341.8533±3.11785
4	2	50	20	343.3323±8.302893
5	2	55	20	457.2096±2.608581

Table 4: The influences of 75% alcohol solution on the effects to extract alcohol soluble proteins from leaves of *Wedelia trilobata* (L.) Hitchc for different extraction periods

Serial number	Weight of leaves of <i>Wedelia trilobata</i> (L.) Hitchc (g)	extraction period (min)	Usage of the extractant (mL)	alcohol soluble proteins content (µg/mL) (S.E.)
1	2	30	20	79.59042±2.415077
2	2	60	20	228.962±3.257619
3	2	90	20	334.4587±5.77013
4	2	120	20	340.8674±3.644908

5 Discussion

Liu et al [23] got new process of preparing zein with the optimum extraction conditions including ratio of liquid to solid 14:1 (mL/g), temperature 50°C, pH 9.0 and time 2hr. Ao et al [10] got the the optimal conditions to extract alcohol soluble proteins from *Gnaoderm lucidum*, with concentration of ethanol 60%, pH 2.5, material-liquid ratio 1:40 and extration temperature 60 °C. Ma et al [24] studied the the optimal conditions to extract alcohol soluble proteins from corn gluten meal, showing that liquid-solid ratio and time of microwave-treating could have a greater impact on extraction of the zein than microwave power and particle size. And the optimal conditions including that concentration of ethanol was 80%, one-time extract, liquid-solid ratio was 14: 1, particle size was 20 , microwave power was 420W, extraction times was 8 and the total time was 360s. Wang et al [25] showed that the best conditions to extract alcohol soluble proteins from gluten included 70% of ethanol concentration , solid-liquid ratio of 1:4, extraction temperature of 50 °C, pH of 5.0 and soaking time of 6 hours. Xu et al [26] showed that the best conditions to extract alcohol soluble proteins from goose millet included ethanol concentration 65%, solid/liquid (goose millet/ ethanol solution) ratio 1:6, temperature 75°C and time 1h. Feng et al [14] got the the best conditions to extract alcohol soluble proteins from (*Phaseolus vulgaris* L.), showing that the highest extraction effect was obtained when ethanol concentration, solid-liquid ratio, extraction

the extraction effects of alcohol soluble proteins from leaves of *Wedelia trilobata* (L.) Hitchc using 75% alcohol solution. When extraction periods changed from 30mins, 60mins, 90mins to 120mins, the extraction effects gradually rised, and the best effect could be get when leaves of *Wedelia trilobata* (L.) Hitchc were treated by 75% alcohol solution for 120mins.

temperature and extraction time were 70%, 1:10, 30°C and 3.0 h, respectively. Jiang et al [15] showed that the best conditions to extract alcohol soluble proteins from beer grains included 81% of ethanol concentration , solid-liquid ratio of 1:21 (g/mL), extraction temperature of 48 °C and oscillation time of 50 min. Otherwise, Li et al [5] found that the complex reagent consisting of 2-mercaptoethanol (1%) and urea (12%) was the best for extracting prolamins from *P. tabulaeformis* seeds for acidic polyacrylamide gel electrophoresis (A-PAGE). Wang et al [6] found that 60 % τ -butanol plus 4 % DTT at a sample-weight to extraction-solution ratio of 1:8 (g: mL) was optimum for kafirin extraction for SDS-PAGE. Deng et al [27] found that 70% propylalcohol supplemented with 0.2% DTT was the best extraction solution and the ratio of sample-weight to extraction-solution 1:8 (g / mL) could give optimal results to extract alcohol soluble proteins from seeds of *Sorghum sudanense* for SDS-PAGE.

It is obvious that different extraction conditions are fit for different materials for different researches. For its low prices and innocuity, maybe ethanol will be an important extractant to extract alcohol soluble proteins in the future. For the special applications of alcohol soluble proteins, the conditions to extract them will be studied further.

References

1. C.H.Dong, R.H.Xu, Q.Q. Zhang, Journal of Mountain Agriculture and Biology. 22(2), 164-168 (2003)
2. Y.P.Zhang, H.H.Liu, D.W.Ma, S.Z.Gao, Seed.(2), 16,

- 20 (2003)
3. Z.G.Liu, L.L.Hu, X.Shi, Journal of Wuhan Food Industry College. (3), 6-9 (1997)
4. Y.G.Shi, Z.H. Yang, B.Y.Sun, Journal of the Chinese Cereals and Oils Association. 20(4), 65-66, 76 (2005)
5. C. Li, B.F. Chai, A.H. Liang, M.B. Wang, Chin J Appl Environ Biol. 12 (1), 125-127 (2006)
6. W. Wang, L.P. Yin, Q. Chen, Journal of Shanghai University (Natural Science Edition). 13 (6), 746-750 (2007)
7. D.H.Jiang, B.F. Yang, M.J.Cai, D.N.Huang, X.H. Wei, Acta Agriculturae Zhejiangensis.,19 (3),174-178(2007)
8. Y. Liu, H.Y. Yao, Q. Wang, Journal of East China University of Science and Technology (Natural Science Edition). 33(2), 591-595(2007)
9. W. Wang, L.P.Yin, X.J.Liu, Q.Chen, Acta Botanica Boreali-Occidentalia Sinica.27 (1), 21-27(2007)
10. H. Ao, S.Q.Huang, J.X.Chen, Q.X. Zeng, Chin Med Biotechnol. 4 (5),359-363 (2009)
11. Y.J.Zheng, Y.Li, S.L.Zhao, H.Chen, W.J.Chen, Chinese Journal of Tropical Crops.30 (7), 1035-1038 (2009)
12. Q.P. Wen, C. Li, D.P. Xu, Q.Y. Cai, B. Pu, China Oils and Fats. 44(6), 50-55(2019)
13. J.L.Yang, H.M. Yang, L.R. Wu, J.M.Carney, C.H.Light, W. Cao, Y.H. Pan, Journal of Bamboo Research.38 (1), 1-8 (2019)
14. X.L. Feng, C.Q. Huang, Y.C. Xu, J.Y. Shen, W.T. Xie, Y.F. Yang, S.D. He, Farm Products Processing. (9), 30-34(2019)
15. F.J. Jiang, S.Y. Zhai, Y.F. Dai, J.X. Du, H. Wu, J.Q. Ma, T.T. Ma, S. Ma, J.F. Lu, Science and Technology of Food Industry.37(23), 239-242 (2016)
16. Y.Zhang, L. Zhang, H.Q. Zhang, C.B. Ding, Y.H. Zhou, Journal of Sichuan Agricultural University. 24(3), 256-262 (2006)
17. L.P. Xu, Y.N. Wang, H.L.Ma, Journal of the Chinese Cereals and Oils Association.14(5), 40-42 (1999)
18. H. He, X.G. Wang, L.Kong, Q.Z.Gu, Food Science. 25 (3), 184-187(2004)
19. H.L. Liu, J. Liu, P.T. Ding, Journal of Shenyang Pharmaceutical University.30(5), 403-408(2013)
20. H. Huang, X.Y. Li, J.W. Wang, W.H.Wu, R.H.Guo, Q.M.Yu, C.Y.Zhang, Food Science. 40(19), 318-325(2019)
21. Z.H. Chen, X.L. Gong, C. Cai, L.J. Zhang, Tianjin Pharmacy. 17(4), 1-2(2005)
22. M. M. Bradford, Anal Biochem. (72) ,248- 254 (1976)
23. X.Y.Liu, L.J. Yin, E.N. Yang, Journal of the Chinese Cereals and Oils Association. 11 (2), 23-27(1996)
24. Q. Ma, S.Y. Wang, L.L. Jiang, L.Z. Liu, Journal of Wuhan Polytechnic University. 28 (2), 5-10(2009)
25. J.H. Wang,X.M.Zhan, S. Yan, Journal of Anhui Agri. Sci.. 37 (34), 17080 - 17081, 17084(2009)
26. Z.H. Xu, H.T. Gao, Y.N. Ding, L.J. Wang, Q. Shen, Science and Technology of Food Industry.34(16), 276-279(2013)
27. Z.R. Deng, J.J. Sun, W.J. Zhang, Q. Chen, Journal of Shanghai University (Natural Science Edition).18(4), 430-434(2012)