Study On DPPH* Free Radical - Scavenging Activity Of Antioxidant Compounds In Plants Composing BIN-5 Biological Active Preparation

Purevjav Urjintseren, Munkhbat Enkhtur, Badamkhand Demberel, Odbayar Tseyen-Oidov

Abstract: Recently, there has been common trend among people to refuse from food and medications produced via synthetic method, but try to consume natural products as much as possible instead. In this regard, wild berries and medicinal plants are considered to be highly essential for human health as these kinds of plants serve as rich sources of biological active substances-phenol compounds. As a result of conducting research on source and spread of herbs which are commonly used as anti-diabetic medication, we have developed a technological method to extract preparations from medicinal herbs such as Peony (Paeonia lactiflora Pall), Dandelion (Taraxacum officinalis Wigg.), Huckleberry (Vaccinium myrtillus L), Blueberry (Vaccinium uliginosum L), Cranberry (Vaccinium vitisidaea L), and Stinging nettles (Urtica dioica), accordingly studied chemical composition and antioxidant activity, and conducted pharmacological study. With the use of Folin Denis & Folin Ciocalteu reagent method,it was determined that the content of polyphenol compounds was 4.14-5.17% and 27.5 – 101.5mg/ml. The study was also aimed to investigate DPPH* free radical-scavenging activity in connection with term, temperature and concentration to identify the most rational technological procedure. As a result of study, it was identified that free radical-scavenging activity of herbs selected for the study was generally estimated at 564.25-1750.00 mcg/ml, whereas antioxidant activity of solvents with 2-10 mg/ml concentration was 417.20-1750.00 mcg /ml, respectively. This shows that such activity is dependent on concentration. However, in temperature of 30 – 100^{0}C degrees, their activity has slowly been decreased by 1750 mcg/ml – 476.7mcg/ml depending on temperature. Regarding the stinging nettles, the activity was grown directly dependent from temperature. DPHH* free radical-scavenging activity was gradually increased in 1-10 minutes, but was relatively stable and active in 11-16 minutes.

Key words: antioxidant activity, free radicals

Introduction

Antioxidants are considered as the most important substance that detoxify free radicals and protect cells. Antioxidant compounds wholly protect tissues, cells and organisms by supplementing electrons lacking in free radicals. In this way, human organism releases from excessive fat and carbohydrates. Free radicals are emerged in organism through two different ways: endogenous and exogenous ways. Radicals of endogenous origin are emerged as a result of metabolism and different kinds of inflammation whereas radicals of exogenous origin depends on external factors such as air pollution, solar radiation, X-rays, alcohols, cigarettes etc.[5], [6]

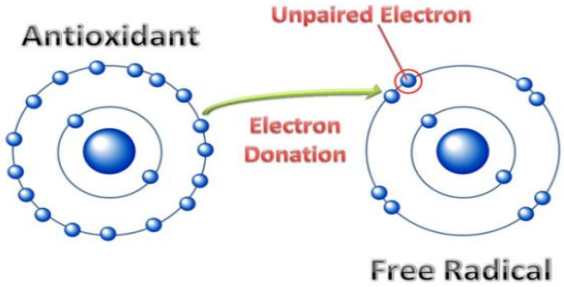


Fig 1. *Antioxidant compound and free radical connection process.*

- *Purevjav Urjintseren, Munkhbat Enkhtur, Badamkhand Demberel, Odbayar Tseyen-Oidov*
- *Mongolian State University of Education, School of Mathematics and Natural Sciences, Ulaanbaatar, Mongolia. upuujee2015@gmail.com*
- *Academy of Sciences. Institute of Chemistry and Chemical Technology. Ulaanbaatar, Mongolia. munkhbatbiotech@gmail.com*
- *Mongolian University of Science and Technology, School of Industrial Technology, Ulaanbaatar, Mongolia*

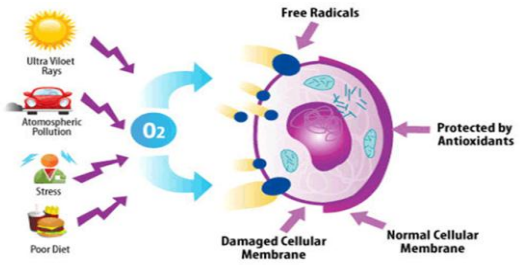


Fig 2. *External factors of emergence of free radicals*

Research materials and methodology

With the aim to extract preparation preventing from diabetes,Bog blueberry (berry)- Vaccinium uliginosum L, cranberry or cowberry (berry, leaf) - Vaccinium vitis idaea L, and stinging nettle (leaf) – Urtica dioica Peony (root) - Paeonia albiflora Pallwere selected for the study in consideration of their practices of using them in traditional medicine, current spread and resource.[2] Antioxidant activity in experimental samples were tested by the “determination method of the antioxidant activity in fruit and vegetable by spectrophotometer MNS 6292 : 2011”, [8] DPPH* radicals methods and FolinCiocalteu Method for the Determination of the Total Phenolic Content [15], respectively. DPPH* free radicals scavenging activities were experimented according to concentration, term and temperature in order to identify the opportunity to determine the rational technological mode. **DPPH - $C_{18}H_{12}N_5O_6$**

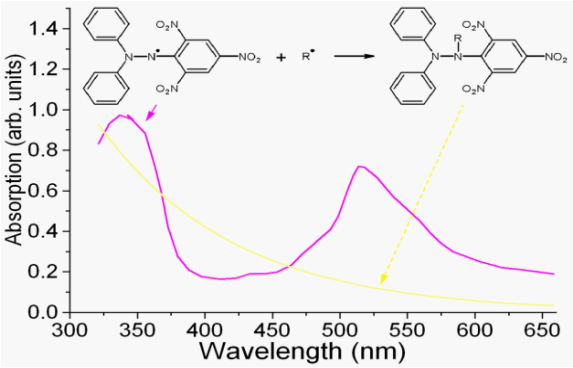


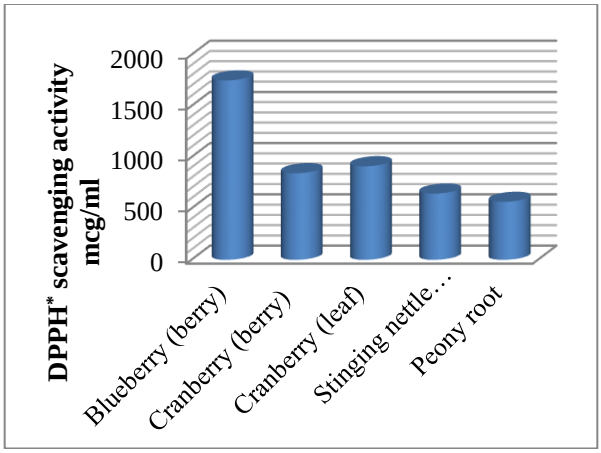
Fig 3. *DPPH* - di (phenyl) - (2,4,6-trinitrophenyl) iminoazanium*

Research outcome

With the aim to determine the content of antioxidant compounds, we constructed a calibration curve of ascorbic acid and conducted measurement in the spectrophotometric wavelength of 540 nm. Using the calibration curve, the contents of antioxidant compounds contained in 5 types of samples were calculated in formulas, which are shown in graphs and tables below.

Table 1.*Free radical scavenging activity*

Berries	DPPH*mcg/ml
Blueberry /berry/	1750.00
Cranberry /berry/	843.75
Cranberry /leaf/	911.25
Stinging nettle /leaf/	645.25
Peony /root/	564.75

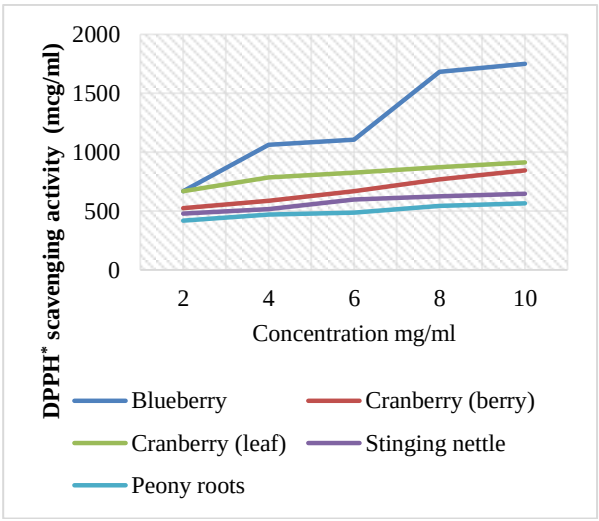


Graph 1. *Free radical scavenging activity*

DPPH*radical scavenging activities can be listed in descending orders as follows: Blueberry Cranberry leaf Cranberry berry Stinging nettle Peony. Tables 1. As seen from the Graph 2, DPPH free radicals scavenging activity of these plants equals to 843.75-1750mcg/ml, which is considered active and closer to results of other studies.

Table 2. *Dependence of free radicals scavenging activity on concentration.mcg/ml*

Concentration mg/ml	Blue berry	Cranberry (berry)	Cranberry (leaf)	Stinging nettles	Peony root
2	668.75	525.00	668.25	476.50	417.20
4	1062.50	587.50	784.50	515.25	469.75
6	1106.25	668.75	826.25	598.50	487.25
8	1681.25	768.75	873.00	624.00	543.00
10	1750.00	843.75	911.25	645.25	564.75



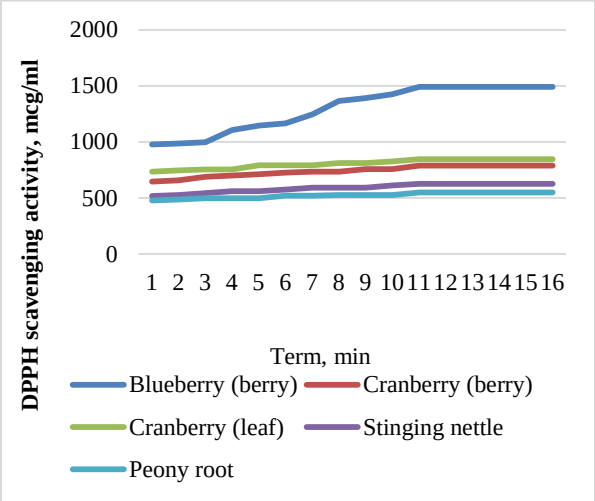
Graph 2. *Dependence of free radical scavenging activity on concentration*

As shown in the table 2 and Graph 2, free radical scavenging activity in 5 samples with different concentration (2-10 mg /ml) were determined at 417.20-1750.00 mcg/ ml, which demonstrates apparent increase in activity. This confirms the general activity results shown in Table 2 and their directly dependence on concentration. Among all 5 samples, activity for blueberries was relatively high.

Table 3. *Indicators showing the dependence of free radical scavenging activity on term.mcg/ml*

Period, min	Blue berry	Cran berry (berry)	Cranberry (leaf)	Stinging nettle	Peony root
1	976.0	645.7	734.4	515.6	476.4
2	984.23	656.4	743.4	523.6	484.2
3	995.6	687.1	754.5	541.1	497.4
4	1103.5	698.6	754.5	557.9	497.4

5	1145.7	711.3	789.5	557.9	497.4
6	1165.7	723.5	789.5	573.6	518.1
7	1245.5	734.5	789.5	589.1	520.0
8	1365.8	734.5	811.2	589.1	523.7
9	1389.6	756.6	811.2	591.4	523.7
10	1423.7	756.6	823.6	611.5	523.7
11	1489.5	786.3	845.1	623.4	546.6
12	1489.5	786.3	845.1	623.4	546.6
13	1489.5	786.3	845.1	623.4	546.6
14	1489.5	786.3	845.1	623.4	546.6
15	1489.5	786.3	845.1	623.4	546.6

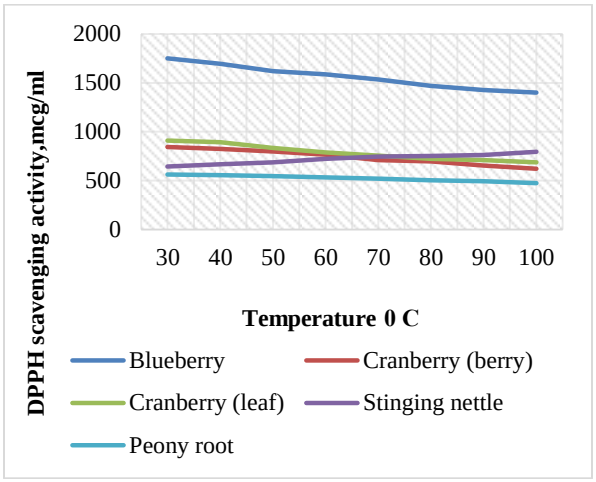


Graph 3. *Indicators showing the dependence of free radical activity on term*

As seen from Table 3 and Graph 3, antioxidant activity in 5 samples were increased within 1-11 minutes and accordingly got stabilized in 11-16 minutes, respectively. This shows that their activities to neutralize the free anions and suspend the chain reaction as well as property to change oxygen compounds are relatively high. Antioxidant activities are equal to 400-1500 mcg/ml, which confirms again the results shown in the Tables 1 and 2.

Table 4. *Indicators showing the dependence of free radical scavenging activity on temperature*

Temperature /°C/	Blueberry	Cranberry (berry)	Cranberry (leaf)	Stinging nettle	Peony root
30	1750	843,5	911,3	645,3	564,8
40	1695	824,3	894	667,1	556
50	1620	798,6	834,6	688,9	546,6
60	1587	765,7	789,2	723,5	533,8
70	1535	711,5	756,7	745,2	519,8
80	1467,7	697,5	722,3	753,8	505,7
90	1425,25	654,45	711,4	764,5	495
100	1400	621,1	688,6	795,8	476,7



Graph 4. *Indicators showing the dependence of free radical scavenging activity on temperature*

As seen from Table 4 and Graph 4, the antioxidant activity of stinging nettles has direct relationship with temperature. However, the antioxidant activities of blueberry, cranberry leaf and berry and peony were slightly decreased with the raise of temperature. The activity equals to 450-1400 mcg/ml, which shows that antioxidant activity is relatively high. In order to determine the content of phenolic compounds, we have constructed the calibration curve for Gallic acid and conducted measurement in wavelength of 540nm.

Table 5. *Total content of phenolic compound*

№	Samples	Total phenol compoundmg/gGallic acid
1	Cranberry (leaf)	101.5
2	Cranberry (berry)	33.0
3	Blueberry (berry)	27.5
4	Peony root	55.5
5	Stinging nettles	11.0

Total content of phenolic compunds can be listed in descending order as follows: Cranberry leaf Peony Cranberry (berry) Blueberry Stinging nettle Cranberry leaves contain high amount of phenolic compounds whereas stinging nettles have the lowest content of phenolic compounds. However, it is observed that the content of phenolic compounds for some samples have weak relationship with antioxidant activity.The content of phenolic compounds in peony roots were estimated to be relatively high or at 55.5 mg/g.

Table 6. Comparative indicators of the research results

Samples /leaves/	Press review	
	DPPH* mcg/ml	Total phenolic compounds mg/g
Cranberry	923.26	175.16
Gooseberries	324.05	62.46
Black currant	405.15	64.06
Rosehip	1299.81	126.04
Seabuckthorn	796.55	95.55
Strawberry	560.78	99.69
Hawthorn	250.81	58.11

Samples	Research outcome	
	DPPH* mcg/ml	Total phenolic compounds mg/g
Cranberry (leaf)	911.25	101.5
Cranberry (berry)	843.75	33.0
Blueberry (berry)	1750.00	27.5
Peony root	564.75	55.5
Stinging nettles (leaf)	645.75	11.0

In the table 6, we have determined the DPPH* free radical scavenging activity for 5 samples selected for the study and compared with the data specified in press review. According to press materials, the general activities were estimated at 250.81-1299.81mcg/ml and phenolic compounds at 58.11-175.16 mg/g, respectively. However, as seen from the study results,the general activities were estimated at 564.75-911.25 mcg/ml and total phenolic compounds at 11.0-101.5mg/g, which demonstrates that DPPH free radical scavenging activity is high and total phenolic compounds are relatively lower in some plants.

Conclusion

1. Free radical scavenging activity of plants selected for the study are equal to 564.25-1750.00 mcg/ml, which are relatively high compared to other studies and published articles, thus they can serve as natural resource of antioxidant compounds.
2. Antioxidant activity in solutions with 2-10 mg/ml concentration prepared from 5 samples was equal to 417.20-1750.00 mcg/ml, which shows their direct relationship with concentration.
3. As seen from indicators showing relationship between antioxidant activity and temperature, the activity in cranberry, blueberry, peony and cranberry leaf were slightly decreased with the increase of temperature whereas for stinging nettles this indicator is raised directly depending on the temperature increase. This is associated with the general composition of the sampled plants.
4. Regarding the 5 samples selected for the study, their DPHH free radical scavenging activities were gradually increased in 1-10 minutes and accordingly stabilized in 11-16 minutes of experiment. Generally, the activity was kept in stabilized level in 1-16 minutes of the experiment
5. As a result of determination of total amount of phenolic compounds, it was identified 11.0-101.5 mg/g Gallic acid, which apparently demonstrates that the 5 plants selected for the study can serve as important source of antioxidant compounds usable for food and cosmetic production.

References

[1] B.Odonbayar, S.Badamtsetseg Study on herbal tea with antioxidant action extracted from medicinal plant of Mongolia

[2] Volodya. Ts. “Medicinal Plants of Mongolia”, 2008. ,345-352.

[3] Dash-Ochir. “Study on food with regulatory actions”, 2011.106-110.

[4] Gogiya.V.Y.,Biochemistry of subtropical plants, 1984., 8,11.

[5] Velioglu, Y.S., Mazza, G., Gao, L. and Oomah, B.D. 1998. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. J.Agric. Food Chem. 46.

[6] Unursaikhan.S.,Govigerel.B., “Modern methods for determination of antioxidant amount in food”, UB.,2011.

[7] Ligaa.U.,Davaasuren.B., Ninjil.N., “The use of medicinal herbs of Mongolia in western and eastern medicine”, UB., 2005., 99,143.

[8] Determination method of the antioxidant activity in fruit and vegetable by spectrophotometer (MNS 6292: 2011 стандарт)

[9] Markham.K.R., Techiques of flavonoids identification, London, 1982., 113-114.

[10] Ulziikhutag.N., “Guidelines for identification of nutritional plants in pasture of Mongolia”

[11] Singleton.V.L., and Rossi.J.A., Colorimetry of total phenolics with photosphomolybdic-phosphotungstic acid reagents. American Journal of Enology Viticuture1965., 16: 144.

[12] Tserenbaljid.G., “Illustrated gallery of flora of Mongolia”UB.,2002., 62

[13] Gulyaev.V.N.,Technology for food concentrates” , Moscow 1972., 27.

[14] Tsevegsuren.N.,Monkhoobor.D., “Organic chemistry II”, UB., 2013.,401-404.

[15] Andressa Blainski, Gisely Cristiny Lopes and João Carlos Palazzo de Mello, Application and Analysis of the Folin Ciocalteu Method for the Determination of the Total Phenolic Content from Limonium Brasiliense L. 2013.