ISSN No:-2456-2165

The Antioxidant Effect of Mongolian Honey Products

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Abstract:- Honey is one of the leading functional food praised for its health benefits in the world market. In our study, a total of 10 honey products derived from native plants grown on Central Asian steppe are studied for its antioxidant properties. For determination of antioxidant effects total phenolics content (TPC), total flavonoids content (TFC) were tested by colorimetric assays; vitamin A, E, C was analyzed by HPLC; antioxidant capacity was measured by DPPH radical scavenging activity assay and IC50% was calculated by GraphPad Prism software. Honey products collected from plants like buckwheat, scabiosa species, thymus species, and *allium senescens* presented outstanding results. The highest results in TPC, TFC and vitamin E were detected in honey collected from buckwheat with 2.79 GAE mg/g, 0.18 RE mg/g, and 1.27 µg/g. The honey product derived from thymus species showed significant high data in vitamin C with 6.41 µg/g and the second-highest result in vitamin A with 0.92 µg/g. In TPC, TFC the thymus honey also displayed moderate-high data with 1.98 GAE mg/g, 0.16 RE mg/g and DPPH IC50% was 32.18 mg/ml. The highest DPPH IC50% record with 31.53 mg/ml and vitamin E content with 0.94 µg/g showed the honey produced using scabiosa species and *allium senescens*. The honey products collected from different floral origin and locations of Mongolia have been tested and buckwheat, thymus, scabiosa, and *allium senescens* honey products showed the best antioxidant effects and contained antioxidative compounds.

I. INTRODUCTION

Honey is a natural carbohydrate product produced by honeybees and has been used in traditional medicines of China, Greece, and the Roman Empire [1,2]. This precious product is known for its wide specter of activities including antioxidant, antibacterial, anti-inflammatory, wound healing and antidiabetic effects [3]. The compound concentrations and unique contents of honey can be different based on its plant groups, geographical locations, entomology source, weather climate and product processing [4]. On the international level bee products such as honey and propolis from different regions and companies are studied well and can be purchased around the world. One salient example is New Zeland's Manuka honey, the products have been researched well and have a high value on the global market [5].

The country Mongolia is located in central Asia and known for its extreme climate conditions and plants are resistant to environmental stress factors. The honey completed through these plants may have properties different from tropical or commercial honey products. The examination of bee products in Mongolia is not researched well, thus a study of Mongolian honey is principal work to complete.

Honey is mainly consisted of fructose and glucose and is rich with secondary metabolites like phenolics, flavonoids, carotenoids; vitamins ascorbic acid, alpha-tocopherol and enzymes like glucose oxidase, catalase [6]. These minor compounds are normally the main effective factors of the antioxidant activity of honey bee. Numerous researches prove that there are correlations and causations between phenolics, flavonoids and antioxidant activities [3,4,6,7].

Oxidative stress term used when any alteration in homeostasis followed by an increased release of free radicals, beyond the detoxifying capability of the local tissues [8]. The excessive free radicals interact with different molecules in tissue and cells causing oxidative damage to proteins, membranes, and nucleic acids. During this procedure, more free radicals are produced and the chain of damage and destruction continues. The free reactive oxidative species (ROS) and reactive nitrogen species (RNS) radicals lead to harmful effects and potential biological damage. Thus, these effects are termed as oxidative stress and nitrosative stress [9, 10, 11].

When there is excessive production of ROS/RNS and a deficiency of enzymatic or non-enzymatic antioxidant defense, the harmful effects can be seen clearly. The detoxification of ROS/RNS is a complex and complicated process and it depends on different factors, but still, it's important and one of the main processes of detoxification of the organism body [12]. Oxidative damage has been the cause of many diseases, predominantly cardiovascular diseases, neuronal degeneration, and cancer. Oxidative stress also has a negative effect on the human body's aging process and is one of the causes of many diseases [13]. Therefore, it is crucial to use antioxidant functional foods in daily life to protect our bodies against harmful oxidative stresses.

ISSN No:-2456-2165

For the last decades, the Mongolian bee industry is growing fast. The rise of honey bee products made in Mongolia is significantly increased over the years. Many companies are stepping into the bee production field and promoting their goods in both domestic and international markets. The main goal of the research is to examine the antioxidant activities of Mongolian honey products from several companies, derived from different regions. The research results would identify the best products sold in the Mongolian market and will help increase their commercial value as bio-active food.

II. METHODS

❖ *Honey Samples*

Totally 10 samples were acquired from 3 commercial companies and one research center located in 4 different locations with different plant origins. Permaculture LLC provided 4 different products; Mihachi LLC, which exports its products to Japan, supplied 3 honey samples; and Ikh Aurag Ord LLC provided 2 types of honey and the Research center for honeybee health and pharmaceuticals at Institute of veterinary medicine (IVM), Mongolia gave 1 sample for the experiments. Mongolian weather is harsh, therefore all samples were gathered during summertime in August and were stored at -4^0C in lightless conditions packaged in their original product bottles.

❖ *Antioxidant Activity*

➢ *Total Phenolics Content (TPC)*

The TPC was determined by the spectrophotometric Folin-Ciocalteu method described in Socha *et. al.* 2009 with slight modification [14]. Honey samples extracts were mixed with 7.5% Na_2CO_3 and incubated for 2 minutes before adding 10% Folin-Ciocalteau reagent. All mixtures were incubated in dark condition for 5 minutes at 50^0C in a water bath and light absorbance was measured at 765 nm. Gallic acid was used as a standard. The total phenolic content described the reduction of the phosphor-wolframatephosphomolybdate complex by phenolics to a blue reduction product. The final data were calculated by comparison between a standard curve from gallic acid. The phenolic content was reported as milligrams of gallic acid equivalents per gram (GAE mg/g) with mean±SD.

➢ *Total Flavonoids Content (TFC)*

The TFC in honey samples was measured by the $AlCl_3$ method described in Zhishen *et. al.* 1999 with slight modification [15]. Rutin was used as a standard, so flavonoid content was calculated, using the calibration curves of rutin and results were expressed as mg of rutin equivalent per gram with mean±SD. Honey samples were mixed with $AlCl_3*6H_2O$ and 120µmol CH_3COONa. The honey extracts were mixed with CH_3COONa and incubated for 30 minutes at room temperature in lightless conditions at the shaker. The absorbance was detected at 415nm. Rutin was used as a standard and TFC was calculated using the calibration curve of rutin and results were expressed as mg/g of rutin equivalent (RE mg/g).

➢ *DPPH Radical Scavenging Activity*

The effect of honey samples on DPPH radical scavenging was determined as described by Huang *et al.,* 2005 with slight modification and rutin was used as a positive standard. Honey samples were prepared in different concentrations and mixed with DPPH reagent and shook for 30 min at room temperature in dark condition. The absorbance was measured at 517 nm. The results were calculated as a percentage of DPPH discoloration using the equation described in the assay. The experiments were done in triplicate and results were expressed as mean values ± SD. The IC50 value was analyzed by statistics software GraphPad prism 7.

➢ *Determination of Vitamin Content by HPLC*

Vitamins A, E, C were selected to be measured due to their high antioxidant effects. The total content of these vitamins was determined by high-performance liquid chromatography (HPLC). The HPLC test was performed at the State professional inspection agency, Mongolia. The content analysis was tested by methods described in Mongolian standards.

III. RESULTS AND DISCUSSION

The content of honey depends on multiple factors and one of the main factors is the plant origins. All of the studied honey samples were collected from different parts of Mongolian with diverse plant distribution. Products from all organizations contained high results in phenolics compounds. The highest data in TPC were detected in S1 2.79 GAE mg/g and S5 1.98 GAE mg/g. In TFC the same samples showed the best data of 0.18 RE mg/g and 0.16 RE mg/g in S1 and S5, respectively. The high amount of TPC and RE flavonoids in S1 is possibly due to the high content of rutin in buckwheat. Flowers of buckwheat contains the highest amount of rutin compared with leaves and stem [17]. The honey product S1 has been collected at buckwheat cropland. The most commercially popular honey product is Manuka honey from New Zealand, which has a TPC of 0.89 GAE mg/g [18]. In TFC determination assay the concentrations calculated using different types of flavonoids such as catechin, quercetin or rutin calibration curves, therefore results may have a high scale.

The antioxidant effect measured by DPPH radical scavenging activity assay. DPPH is an oxidative compound that is commonly used for the study of natural subjects' antioxidant effect. DPPH radical scavenging activity of honey products was tested at 50, 100, 150, 200, and 250 mg/ml concentrations. All samples displayed an increasing antioxidant effect with concentration increase. Most samples showed the highest results at the 25 mg/ml concentration. Honey product S5 honey made of nectars and pollen's from herbaceous plants *Thymus spp*. showed the highest result with

ISSN No:-2456-2165

66.68% and S1 honey collected from buckwheat showed the same high result with 66.28% of scavenging activity. The IC50% indicates the concentration that reduces 50% of oxidative free radicals, which in our case is DPPH. The lower the IC_{50} value, the higher the antioxidant activity. The data showed the best samples were S4 and S5 with 31.53 mg/ml and 32.18 mg/ml, respectively. In the research lead by Greece scientists Dimitris Mossialos at the University of Thessaly, Greece the data of Greece honey products collected from Mount Olympus showed an IC_{50} range of 10 mg/ml up to 110 mg/ml. The well studied New Zeland Manuka honey showed 68 mg/ml of IC50 value [19].

In vitamin content test vitamins A, E, C was selected due to high antioxidant effects. Honey products contain a moderate amount of vitamin C, however, vitamin A and E research is not common research object on honey products. The main antioxidative effect of fat-soluble vitamin A and it's derivatives are connected by a hydrophobic chain of polyene parts that neutralize singlet oxygen and stabilize peroxyl radicals. The structures of vitamin A and carotenoids can autoxidize when O_2 tension is increased [20]. The highest content of vitamin A was detected at multi-floral honey sample S10 from the capital city of Mongolia 2.07 µg/g and the second-highest was detected at S2 0.92 µg/g.

The fat-soluble vitamin E has a specific function to protect long-chain polyunsaturated fatty acids and preserve them for signaling events in various cells [21]. The best result of 1.27µg/g was presented by S1 honey, which produced by using buckwheat. Vitamin C is a water-soluble antioxidant compound due to its electron donor and reducing agent. Ascorbic acid donates two electrons from a double bond between the second and third carbons of the 6- carbon molecule [22]. The vitamin C content was highest in S5 with 6.41 µg/g. The study of vitamin C content is widely researched and data are possibly related to floral variance and geographical positions. The examination of vitamin C with RP-HPLC presented that eucalyptus honey showed the highest amount from 2.0-5.8 µg/g. Some honey products collected from citrus, asphodel, and strawberry-tree also showed a moderate-high concentration of vitamin C with 3, 4, 4.4 µg/g, respectively [23].

Comparing with other researches the amount of vitamin C in S5 was high and the reason may be in plant medicinal plant thymus, which is known for its rich natural constituents and multiple benefits. The thymus spp. has digestive, antispasmodic, antimicrobial, antioxidant, antiviral, anti-inflammatory, antiseptic, expectorant activities and used for the treatment of colds. In different traditional medicines, it is used in herbal preparations of gastroenteric and bronchopulmonary disorders. Thyme oil is one of the best essential oils used as a food preservative and used for immune-stimulating activity [24].

Sample no.	Organization	Location	Plant origin
1	Permaculture LLC	The northern part of Mongolia, Selenge province, Eruu sum	Buckwheat
2			Multiple florae
3			*Chamaenerion angustifolium*
4			*Scabiosa spp., Allium senescens L*
5	Mihachi LLC	The northern part of Mongolia, Selenge province, Altanbulag sum	*Thymus spp*
6			*Dracocephalum foetidum* Bunge
7			*Dracocephalum foetidum* Bunge
8	Ikh Aurag Ord LLC	Central part of Mongolia, Tuv province, Erdene sum	*Brassica napus* (canola)
9			Multiple flora including *Scabiosa spp., Astragalus spp., Geranium spp., Trigolium spp., Chamaenerion angustifolium, Sanguisorba officinalis, Allium senescens, Delphinium grandiflorum*
10	Research center for honeybee health and pharmaceuticals, IVM	The central part of Mongolia, Capital city Ulaanbaatar	Multiple florae

Table 1:- Mongolian honey samples

ISSN No:-2456-2165

Sample no.	TPC, GAE mg/g mean±SD	TFC RE mg/g mean±SD	DPPH, mg/ml mean±SD sults	DPPH, IC50% mg/ml	Vit A µg/g	Vit E µg/g	Vit C µg/g
1	**2.79±0.11**	**0.18±0.07**	**66.28±3.6**	44.95	0.65	**1.27**	0.28
2	0.70±0.13	0.12±0.32	55.74±7.1	107.8	0.65	0.33	**4.74**
3	0.82±0.09	0.03±0.14	39.41±1.8	81.98	0	0.27	0
4	2.37±0.17	0.09±0.03	53.54±1.9	**31.53**	0.78	**0.94**	0.37
5	**1.98±0.03**	**0.16±0.08**	**66.68**±2.4	**32.18**	**0.92**	0.21	**6.41**
6	1.43±0.08	0.04±0	49.21±5.9	72.55	0.78	0.48	0.56
7	1.0±0.25	0	51.62±5.2	41.96	0	0.67	3.74
8	0.60±0.05	0	47.3±7.1	152.5	0.72	0.46	1.81
9	0.74±0.03	0	38.5±2.7	64.5	0.72	0.42	2.09
10 IVM	1.11±0.11	0.01±0	62.3±2.9	46.94	**2.07**	0.46	1.13

Table 2:- Antioxidant effect of mongolian honey samples

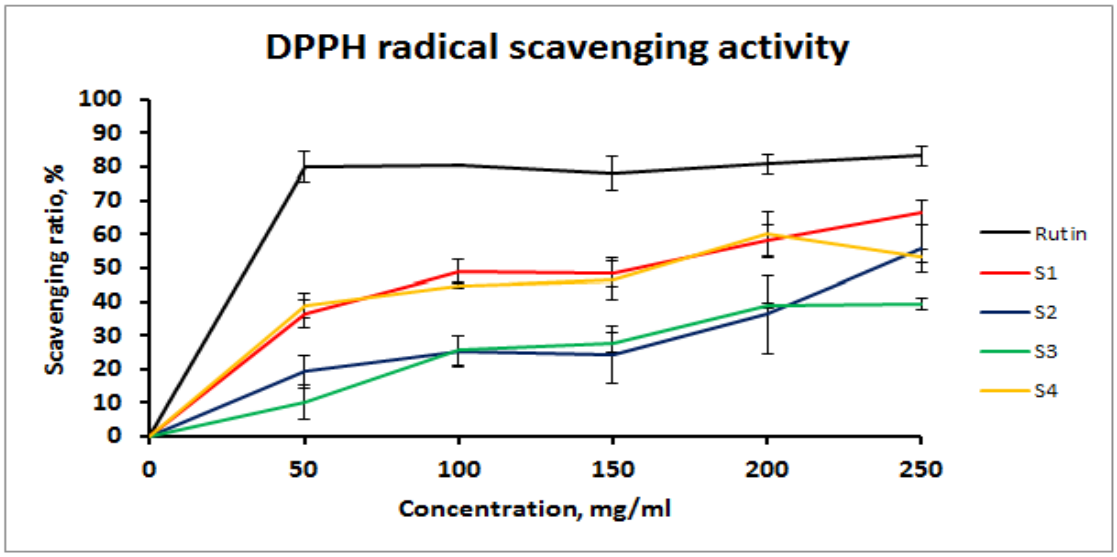

Fig 1:- DPPH radical scavenging activity results in Permaculture LLC honey products. All values are expressed as the means±SD (n=3).

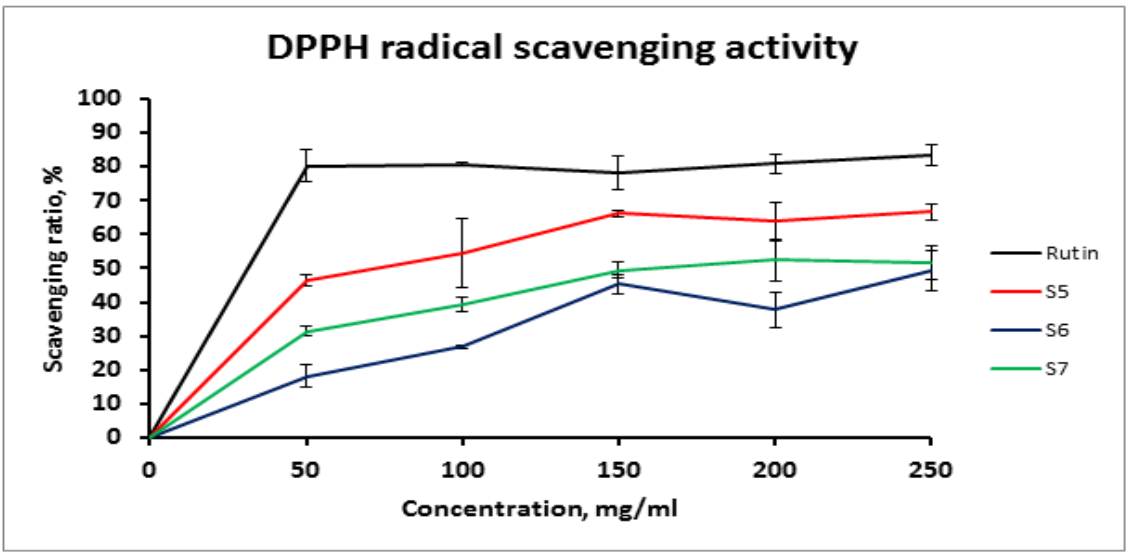

Fig 2:- DPPH radical scavenging activity results in Mihachi LLC honey products. All values are expressed as the means±SD (n=3).

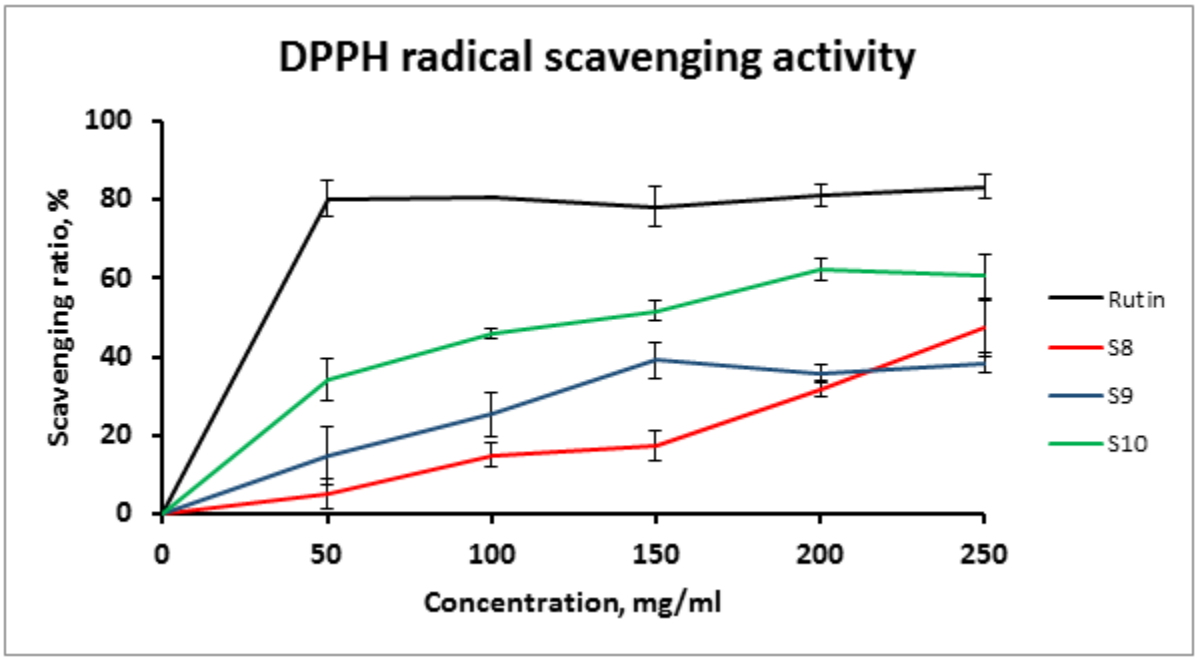

Fig 3:- DPPH radical scavenging activity results in Ikh-Aurag Ord LLC and IVM honey products. All values are expressed as the means±SD (n=3).

IV. CONCLUSION

This is the first specialized research on the antioxidant properties of Mongolian honey products derived from the different geographical and botanical origins of Mongolia. Our study results will benefit the companies which cooperated with us and science-based data will be used for the improvement of honey products. The results indicate that some products have moderate-high antioxidant potentials. Among ten honey products, the S1 and S5 presented the highest antioxidant activities and has the highest amount of antioxidant compounds. Moreover, the highest data of TPC, TFC and high content of vitamin A, E, C were displayed by these honey products. These high results are presumably due to their plant species of buckwheat and thymus species.

ACKNOWLEDGMENTS

We would like to express sincere thanks to the Asian Development Bank for financing our project. Authors also want to express gratitude to Permaculture LLC, Mihachi LLC, Ikh-Aurug Ord LLC and Research center for honeybee health and pharmaceuticals, IVM for supplied honey products.

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