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ORIGINAL ARTICLE **OPEN ACCESS**

DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity of raw and gamma-irradiated honeys from *Apis mellifera* and *Trigonabiroi*

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ABSTRACT

The study aims to determine the antioxidant activity of raw and gamma-irradiated honey samples produced by Apismellifera and Trigonabiroi honeybees. The DPPH scavenging activity test was conducted at concentrations of 62.5 ppm, 125 ppm, 250 ppm, 500 ppm and 1000 ppm, respectively through the DPPH assay method. In assessing the percent inhibition of the honey samples from the different species of honeybees, it was observed that it has significant antioxidant activity against the DPPH radical. The average percent inhibition of raw and gamma-irradiated honey samples utilized in this study were observed to likely increase the honeys average percent inhibition against the DPPH radical as the concentrations also increases. Raw and irradiated honey samples of Apis mellifera has higher percent inhibition than the honey samples produced by Trigona biroi. The highest percent inhibition of honey samples both from Apis mellifera and Trigonabiroi are at 1000 ppm concentrations. Further study in the conduct of other methods of identifying antioxidant properties of honeys and their significant differences are recommended to fully explore the other beneficial properties of the locally produced honey.

Keywords: *Antioxidant property, gamma-irradiated, honeys*

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INTRODUCTION

Oxidative stress is attributed to play a role in a variety of chronic diseases. [15]. Oxidative stress is the absence of balance between the free radicals produced and the antioxidant protection activity in a specific organism [8]. Some chronic diseases, such as cardiovascular disease, stroke, cancer, chronic respiratory diseases, and diabetes, are thought to be prevented by antioxidant defense against oxidation.

Honey's medicinal efficacy is linked in part to its therapeutic properties [10, 18]. Different floral sources, as well as meteorological and environmental factors, influence the content and composition of different varieties of honey [12, 13, 14, 20]. Although there have been studies on the antioxidant activity of many types of honey from various nations and botanical origins 4, 8, 10, 12, 13] the antioxidant properties and other parameters of honeys produced in the province of Isabela has not been well documented.

Various microorganisms frequently contaminate honey during harvesting and packing. A proper method of sterilizing, such as gamma irradiation [3], is strongly suggested when using honey for medical research. With this, the aim of this study to determine the antioxidant properties of raw and gamma-irradiated honey samples produced by *Apis mellifera* and *Trigonabiroi* at Isabela State University- San Mariano Campus.

MATERIAL AND METHODS

Collection of Honey Samples

Honey samples were collected using a mechanical extractor for *Apis mellifera* while dripping method was done for *Apisdorsata.* Honey samples are placed in sterilized and clear glass bottle.

Gamma-irradiation

The honey samples were irradiated at 25 kGy dose by exposing the samples to a Cobalt-60 source using the PHI-5030 gamma irradiator of Philippine Nuclear Research Institute (PNRI) Multipurpose Irradiation Facility at Diliman, Quezon City. Samples were placed at 20 cm from the source shroud, 20 cm from the centerline using 2-sided irradiation.

2,2-diphenyl-a- picrylhydrazyl (DPPH) Antioxidant Assay

The method was adapted from Clarke, G. *et al*. [7], where twenty microliters (20 µL) of the extract diluted appropriately in Dimethylsulfoxide (DMSO) with 180 µL of DPPH in methanol (40 µg/mL) in wells of a 96-well plate. The plate was kept in the dark for 15 minutes after which the absorbance of the solution was measured at 540 nm in a plate reader. DMSO served as a blank and Ascorbic Acid served as the standard.

RESULTS AND DISCUSSION

DPPH (2,2-diphenyl-a- picrylhydrazyl) scavenging activity of honeys

Honey samples in this study produced by*Apis mellifera* and *Trigonabiroi* show significant antioxidant activity against the DPPH radical (see Table 1). The average percent inhibition of raw and gamma-irradiated honey samples were observed in concentrations of 62.5 ppm, 125 ppm, 250 ppm, 500 ppm and 1000 ppm, respectively.

As shown in table 1, the percent inhibition varies as the concentration of the sample increases. Raw and irradiated honey samples of *Apis mellifera* has higher percent inhibition than the honey samples produced by *Trigona biroi*. Moreover, it is observed that the highest percent inhibition of honey samples both from *Apis mellifera* and *Trigona biroi* are at 1000 ppm concentrations. However, a decrease in percent inhibition when the honey samples were gamma-irradiated was also observed.

The variations of percent inhibition with increasing level of concentration shows that some radical scavengers could display high activity in percent inhibition at very low or high concentrations. This can be attributed to the sources of nectar that are generally a mixture of compounds with numerous interactions. In addition, Prasad, *et.al*., [16] revealed high-altitude honeys contained a higher level of antioxidants than low altitude honey, justifying that there would be the chance of synthesis of highly potent antioxidative secondary metabolites by the nectars of plants that grows at high altitude region.

In relation to other related studies, Hussein, *et.al*.,[8] studied that the antioxidant activity of raw Gelam and Nenas honeys significantly differ when gamma-irradiated. Moreover, *Apis mellifera* honeys of Polish [22], Algeria [1] and Chile [5]) has the highest percent inhibition among other honeybee species. While Moniruzzaman *et.al.*, [11] observed that in Malaysia, raw Tualang honey (*Apis dorsata*) has the highest antioxidant activity when compared to *Apis mellifera* honeys (Acacia honey, pineapple honey) and Borneo honeys (*Apis cerana*).

The antioxidants of different honeys are largely dependent on the floral source or honey variety because these are derived from both enzymatic and nonenzymatic substances [21, 17, 19, 9, 5]. Moreover, the type of containers of which the honey was stored [2] is also considered a factor in the changes of phenol, radical scavenging activity and total flavonoid values of honeys.

Table 1. Mean percent inhibition of raw and irradiated honey by species, by type and concentration level.

Concentration Level	Species	Type of Honey		Average
		Raw	Irradiated	
1000 ppm	*Apis mellifera*	28.100	20.200	24.150
	Trigonabiroi	25.400	21.200	23.300
	Average	25.250	21.650	23.450
500 ppm	*Apis mellifera*	23.200	18.900	21.050
	Trigonabiroi	22.300	17.600	19.950
	Average	21.425	19.125	20.275
250 ppm	*Apis mellifera*	21.300	18.800	20.050
	Trigonabiroi	21.500	17.800	19.650
	Average	21.875	18.425	20.150
125 ppm	*Apis mellifera*	22.800	6.600	14.700
	Trigonabiroi	19.700	18.000	18.850
	Average	21.225	15.525	18.375
62.5 ppm	*Apis mellifera*	23.800	19.400	21.600
	Trigonabiroi	19.800	19.300	19.550
	Average	21.350	19.675	20.513
Average	*Apis mellifera*	23.840	16.780	20.310
	Trigonabiroi	21.740	18.780	20.260
	Average	22.225	18.880	20.553

CONCLUSION

When honey samples from *Apis mellifera* and *Trigona biroi* honeybee species were tested for percent inhibition, it was discovered that the raw and gamma-irradiated honey samples have significant antioxidant activity against the DPPH radical. The average percent inhibition of raw and gamma-irradiated honey samples used in this investigation was shown to increase as the concentrations increased, indicating that the honey's average percent inhibition against the DPPH radical increases Therefore, it is recommended to conduct other methods of identifying antioxidant properties of honeys and their significant differences such FRAP assays, TBARS assays, and beta-carotene bleaching assay to explore the other beneficial properties of honeys produced in the locality.

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