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Assessment of Hypoglycemic Trace Elements in Bitter Gourd (*Momordica charantia*) Fruit by Neutron Activation Analysis

Penilaian Unsur Jejak Hipoglikemik pada Buah Pare (Momordica charantia) dengan Analisis Aktivasi Neutron

A.H. As'ari*, Instanto, S. Suprapti, S. Yusuf

Center for Science and Technology of Advanced Materials, National Nuclear Energy Agency
Puspiptek Area, Tangerang Selatan 15310, Indonesia
* E-mail : ahmad.hasan@batan.go.id

ABSTRACT

The fruit of bitter gourd (*Momordica charantia*) or commonly known in Indonesia by pare is believed to reduce glucose levels in the blood. It is known that several trace elements affect insulin performance, such as chromium, magnesium, zinc, and manganese. This study aims to determine the content of hypoglycemic trace elements in bitter gourd fruits using neutron activation analysis techniques. The gourd fruits were taken from four regions in Java, i.e. Bangkalan, Magelang, Cianjur, and Pandeglang. The results showed that bitter gourd fruit contained elements with average contents in mg/kg dry weight: chromium (1.647), magnesium (2881), zinc (30.1), and manganese (57). The gourd fruit from Pandeglang has three elements with the highest concentration, namely chromium, magnesium, and manganese, whereas the highest zinc concentration comes from Cianjur. Based on the recommended dietary allowance of these elements and the level of consumption, bitter gourd fruit contributed chromium, magnesium, zinc, and manganese by 31%, 6%, 2%, and 7-18% daily intake. Furthermore, in vivo studies need to be carried out based on elemental content to determine the effectiveness of bitter ground fruit against diabetes.

Keywords: bitter gourd, hypoglycemic trace elements, neutron activation analysis

ABSTRAK

Buah pare (*Momordica charantia*) dipercaya dapat menurunkan kadar glukosa dalam darah. Diketahui bahwa beberapa elemen jejak mempengaruhi kinerja insulin, seperti kromium, magnesium, seng, dan mangan. Penelitian ini bertujuan untuk mengetahui kandungan elemen jejak hipoglikemik pada buah pare menggunakan teknik analisis aktivasi neutron. Buah pare diambil dari empat daerah di Jawa, yaitu Bangkalan, Magelang, Cianjur, dan Pandeglang. Hasil penelitian menunjukkan bahwa buah pare mengandung unsur dengan kandungan rata-rata dalam mg/kg berat kering: kromium (1,647), magnesium (2881), seng (30,1), dan mangan (57). Pandeglang memiliki tiga unsur dengan konsentrasi tertinggi, yaitu kromium, magnesium, dan mangan, sedangkan konsentrasi seng tertinggi berasal dari Cianjur. Berdasarkan asupan makanan yang direkomendasikan dari unsur-unsur tersebut dan tingkat konsumtosi, buah pare menyumbang kromium, magnesium, seng, dan mangan sebesar 31%, asupan harian 6%, 2%, dan 7-18% dalam sehari. Selanjutnya, penelitian secara *in vivo* berdasarkan pada kandungan unsur perlu dilakukan untuk menentukan efektivitas buah pare dalam melawan diabetes.

Kata kunci: pare, unsur jejak hipoglikemik, analisis aktivasi neutron

INTRODUCTION

In developing countries, 80% of the population uses traditional medicine as a primary solution to medical problems. For decades, research has focused on evaluating the use of traditional medicines from origin plants. Bitter gourd (*Momordica charantia*) is one of the herbal plants and has been used many ingredients for traditional medicine. Besides being used as a medicinal ingredient, bitter gourd or pare in Indonesia, especially Java, is widely used as a vegetable and consumed in a cooked condition.

Bitter gourd is commonly found as an herbal medicine in Asia, Africa, and South America because of its function as antidiabetic, anthelmintic, abortifacient, antibacterial, antiviral, and chemopreventive [1], [2]. Bitter gourd has been shown to have hypoglycemic functions that can treat diabetes [3], [4]. Bitter gourd contains

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phytochemical compounds, such as flavonoids, saponins, terpenoids, coumarin, emodin, alkaloids, proteins, cardiac glycosides, anthraquinones, anthocyanins, steroids, and tannins [5]. Bitter gourd also contains Gurmarin as a polypeptide whose function is similar to insulin enzymes and rich in vitamins and minerals [6]. Minerals in the form of trace elements (such as chromium, magnesium, zinc, and manganese) have been shown to affect insulin performance. The hypoglycemic trace elements activate insulin receptors, function as cofactors or enzyme system components involved in glucose metabolism, increase insulin sensitivity, and act as antioxidants to prevent tissue peroxidation [7]–[10]. For example, zinc is needed for the synthesis and storage of insulin, and insulin is secreted as zinc crystals that can maintain the integrity of the structure of insulin. Zinc has a vital role in modulating the immune system and dysfunction in diabetes mellitus. Magnesium has an essential role in the reaction of glucose phosphorylation and its metabolism. Deficiencies contribute to insulin resistance, carbohydrate intolerance, and diabetes complications [11], [12]. Therefore, determining the hypoglycemic trace elements in bitter gourd fruit is considered necessary, and research on it has not been done much.

Many analytical techniques to determine the trace element content in plants, especially bitter gourd such as atomic absorption spectroscopy (AAS) [13], [14], laser-induced breakdown spectroscopy (LIBS) [15], inductively coupled plasma optical emission spectrometry (ICP-OES) [9], inductively coupled plasma mass spectrometry (ICP-MS) [16], [17], energy dispersive X-ray fluorescence (EDXRF) [10], particle-induced X-ray emission (PIXE) [18], and neutron activation analysis (NAA) [17].

The purpose of this study is to determine the hypoglycemic trace element content in cooked bitter gourd fruit using NAA. NAA is very powerful as a sensitive analytical technique for performing both qualitative and quantitative analysis of trace element contents in various samples. NAA sensitivity in elemental analysis can be seen in Table 1 [19]. Samples were taken from four different regions in Java, Indonesia, i.e. Bangkalan, Magelang, Cianjur, and Pandeglang. The results of the content analysis will be compared to each other to see its distribution.

Table 1. Estimated detection limits for INAA using decay gamma rays. Assuming irradiation in a reactor neutron flux of $1x10^{13}$ ncm-2s-1[19]

Sensitivity (picograms)	Elements
1	Dy, Eu
1–10	In, Lu, **Mn**
10–100	Au, Ho, Ir, Re, Sm, W
100–1000	Ag, Ar, As, Br, Cl, Co, Cs, Cu, Er, Ga, Hf, I, La, Sb, Sc, Se, Ta, Tb, Th, Tm, U, V, Yb
1000–10^4	Al, Ba, Cd, Ce, **Cr**, Hg, Kr, Gd, Ge, Mo, Na, Nd, Ni, Os, Pd, Rb, Rh, Ru, Sr, Te, **Zn**, Zr
10^4–10^5	Bi, Ca, K, **Mg**, P, Pt, Si, Sn, Ti, Tl, Xe, Y

EXPERIMENTAL METHOD

Sample collection

Bitter gourd fruits were randomly purchased and collected from local markets in four regions. These regions are Bangkalan (East Java), Magelang (Central Java), Cianjur (West Java), and Pandeglang (Banten). These regions are four of the cities/regencies with high cases of stunting in Indonesia [20].

Sample preparation and irradiation

Bitter gourd fruits were cleaned with ~~clean~~ water and boiled at temperatures above 80ºC until cooked (ready-to-eat conditions). The samples were cut into smaller sizes and put in a special bottle for the freeze-drying process to remove the water content. Dried samples were mashed using mortar to become a homogeneous powder (100 mesh in size). Each sample (approximately 100 mg) was put into a low-density polyethylene vial (LDPE) for the neutron irradiation process. Capsules containing samples and standard reference materials (SRM) were irradiated through the rabbit system facility located at the G.A. Siwabessy multi-purpose reactor. The irradiation process was carried out inside the reactor core with a thermal neutron flux of about 5×10^{13} neutrons.cm^{-2}.s^{-1}. Radionuclide parameters, energy γ, half-life, and other analysis details are presented in Table 1.

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Table 2. Details of analysis parameters

Isotope target	Isotope product	Half time	E (keV)	Abd (%)	T_{irr}	T_{decay}	T_{count}
Mg-26	Mg-27	9.46 m	1014.43	28.60	60 s	±10 m	120 s
Mn-55	Mn-56	2.58 h	1810.72	27.19	60 s	±10 m	120 s
Cr-50	Cr-51	27.7 d	320.08	10.08	3 h	±17 d	4 h
Zn-64	Zn-65	34.84 w	1115.55	50.70	3 h	±17 d	4 h

s=second, m=minute, h=hour, d=day, w=week

Data acquisition

After reaching the decay time, all samples and SRM were analyzed by Canberra gamma spectrometry using a high purity germanium detector (HPGe) with a 30% relative efficiency and 1.63 keV resolution for a peak of 1332.5 keV of 60Co. Energy calibration in the system was carried out using two radionuclide sources, Cs-137 and Co-60, to cover the energy range of 661 keV to 1332.5 keV. Gamma spectra were processed and analyzed using Genie2000 software.

RESULT AND DISCUSSION

The content of magnesium, manganese, chromium, and copper was determined using the standard comparison method. The precision and accuracy of the measurement results were evaluated using SRM 1547 (Peach leaves, NIST). The precision and accuracy shown by the U-test score and the Z-score are presented in Table 2. The RSD values of all elements are bellow 20%, except for chromium due to its small concentration in the SRM and its gamma energy bellow the range covered by the system. Nevertheless, all elements have the U-test score <2.58 and the |Z-score| <2 [21]. Therefore, these results indicate there is no significant difference between the value of the measurement results with the certificate value.

The results show different concentrations of the hypoglycemic trace elements from each region (see Table 3). The cooked bitter gourd fruit from Pandeglang has the highest content of magnesium (3243 ± 190 mg/kg), manganese (111 ± 5 mg/kg), and chromium (2.68 ± 0.18 mg/kg). Magnesium is a cofactor in various enzymes involved in glucose oxidation. Low magnesium levels are usually seen in people with diabetes. Magnesium supplementation in food may help insulin resistance. The recommended dietary allowance for adults is 310 mg per day [22], [23]. Manganese is an activator and also a constituent of several enzymes and is often considered a toxic element when given orally. Safe and adequate intake of manganese is 2.0-5.0 mg/day for adults in the United States [24], [25]. Chromium is a trace element that has been known as an essential nutrient for humans and animals for a long time. Its primary role is to maintain normal glucose tolerance in the body. The minimum population mean intake likely to meet normative needs for chromium might be approximately 33 μg/day [24], [26], [27]. The highest content of zinc is from Cianjur. Zinc is an essential micronutrient that has an important role in many enzymes and acts as an efficient antioxidant. Zinc plays a role in the synthesis, storage, and secretion of insulin by pancreastic islet cells. The mean daily intake of zinc is 11.2 mg [28], [29].

Table 3. Result of internal quality measurement

Element	Certified value	Measured value	RSD (%)	Ratio	Z-score	U-test value
Mg	4320 ± 80	4780 ± 170	10.6	1.11	0.53	2.41
Mn	246 ± 8	249 ± 15	1.2	1.01	0.06	0.18
Cr	1.99 ± 0.66	1.42 ± 0.04	28.6	0.71	-1.43	0.86
Zn	30.9 ± 0.7	28.1 ± 1.8	9.1	0.91	-0.45	1.45

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Table 4. The concentration of hypoglycemic trace elements in bitter gourd fruit

Elements	Concentration in mg/kg dry weight				Mean (mg/kg dry weight)
	Pandeglang	Cianjur	Magelang	Bangkalan	
Mg	3243 ± 190	3013 ± 181	2336 ± 136	2932 ± 153	2881
Mn	111 ± 5	75 ± 4	15 ± 1	26 ± 2	57
Cr	2.68 ± 0.18	1.83 ± 0.10	1.23 ± 0.07	0.84 ± 0.05	1.647
Zn	17.8 ± 0.9	52 ± 2.5	17.7 ± 0.9	32.7 ± 2.7	30.1

Table 5. Estimated intake contribution of hypoglicemic trace elements in a day

Element	Mean content (mg/kg)	Comsumption level in a day (g)	Intake estimation (mg)	Dietary allowance (mg)	Dietary intake contribution
Mg	2881	3243	18.10914	310	6%
Mn	57	111	0.356714	2-5	7-18%
Cr	1.647	2.682	0.010349	0.033	31%
Zn	30.1	52	0.188886	11.2	2%

Data on bitter gourd fruit consumption in Indonesia, especially Java, are not specifically reported, but the estimated consumption level is 44 g ~~in~~ a week [30]. Based on the recommended dietary allowance of these elements and the level of consumption, bitter gourd fruit contributed chromium, magnesium, zinc, and manganese by 31%, 6%, 2%, and 7-18% daily intake (see Table 4).

In order to determine the effectiveness of bitter gourd fruit contribution in supplying the intake of the hypoglycemic trace elements to maintain glucose levels in the blood, in vivo studies based on elemental content need to be performed.

CONCLUSIONS

The results showed that bitter gourd fruit contains the hypoglycemic trace elements with different concentrations of each region. Pandeglang has three elements with the highest concentration, namely chromium, magnesium, and manganese, whereas the highest zinc concentration comes from Cianjur. The contribution of bitter gourd fruit in the intake of the hypoglycemic trace elements is sufficient up to 30% for chromium, whereas the other elements are below 10%.

Furthermore, in vivo studies need to be carried out based on elemental content to determine the effectiveness of bitter ground fruit against diabetes.

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REFERENCES

[1] J.K. Grover, S.P. Yadav, "Pharmacological actions and potential uses of *Momordica charantia*: a review," *J. Ethnopharmacol.*, vol. 93, pp. 123–132, 2004.

[2] E. Basch, S. Gabardi, C. Ulbricht, "Bitter melon (*Momordica charantia*): A review of efficacy and safety," *Am J Heal. Pharm*, vol. 60, no. 4, pp. 356–359, 2003.

[3] D.S. Sekar, K. Sivagnanam, S. Subramanian, "Antidiabetic activity of *Momordica charantia* seed on streptozotocin induced diabetes in rats", *Pharmazie*, vol. 60, pp. 383–387, 2005.

[4] I. Ahmed *et al.*, "Beneficial effects and mechanism of action of *Momordica charantia* juice in the treatment of

p ISSN 1907-0322
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streptozotocin-induced diabetes mellitus in rat," *Mol. Cell. Biochem.*, vol. 261, pp. 63–70, 2004.

[5] P. Daniel, U. Supe, M.G. Roymon, "A review on phytochemical analysis of *Momordica charantia*," *Int. J. Adv. Pharmacy, Biol. Chem.*, vol. 3, no. 1, pp. 214–220, 2014.

[6] G.Y. Yeh *et al.*, "Systematic review of herbs and dietary supplements for glycemic control in diabetes," *Diabetes Care*, vol. 26, pp. 1277–1294, 2003.

[7] S. Praveena, S. Pasula, K. Sameera, "Trace elements in diabetes mellitus," *J. Clin. Diagnostic Res.*, vol. 7, no. 9, pp. 1863–1866, 2013.

[8] M. Basaki *et al.*, "Zinc, copper, iron, and chromium concentrations in young patients with Type 2 Diabetes Mellitus," *Biol. Trace Elem. Res.*, vol. 148, no. 2, pp. 161–164, 2012.

[9] Y. Savsatli *et al.*, "Trace elements in bitter melon (*Momordica charantia* L.) and their distribution in different plant parts," *ARPN J. Agric. Biol. Sci.*, vol. 11, no. 11, pp. 437–443, 2016.

[10] C.C. Srinivasu *et al.*, "Concentration of trace elements in selected Indian antidiabetic medicinal plants by using EDXRF technique," *Int. J. Recent Sci. Res.*, vol. 7, no. 2, pp. 9104–9108, 2016.

[11] L.M. Resnick *et al.*, "Cellular ions in hypertension, diabetes, and obesity," *Hypertension*, vol. 17, pp. 951–957, 1991.

[12] E. Mocchegiani *et al.*, "Zinc-dependent low thymic hormone level in type I diabetes," *Diabetes*, vol. 38, no. July, pp. 932–937, 1989.

[13] R. I. Bakare *et al.*, "Nutritional and chemical evaluation of *Momordica charantia*," *J. Med. Plants Res.*, vol. 4, no. 21, pp. 2189–2193, 2010.

[14] P.B. Ayoola, A. Adeyeye, O.O. Onawumi, "Trace elements and major mineral evaluation of *Spondias mombin, Vernonia amygdalina* and *Momordica charantia* leaves," *Pakistan J. Nutr.*, vol. 9, no. 8, pp. 755–758, 2010.

[15] N.K. Rai *et al.*, "Application of LIBS in detection of antihyperglycemic trace elements in *Momordica charantia*," *Food Biophys.*, vol. 4, pp. 167–171, 2009.

[16] A.M. Basha *et al.*, "Trace metals in vegetables and fruits cultivated around the surroundings of Tummalapalle uranium mining site, Andhra Pradesh, India," *Toxicol. Reports*, vol. 1, pp. 505–512, 2014.

[17] R.K. Dutta *et al.*, "Analysis of bioaccessible concentration of trace elements in plant based edible materials by INAA and ICPMS methods," *J. Radioanal. Nucl. Chem.*, vol. 300, no. 1, pp. 185–189, 2014.

[18] R. Gowrishankar *et al.*, "Trace element studies on *Tinospora cordifolia* (Menispermaceae), *Ocimum sanctum* (Lamiaceae), *Moringa oleifera* (Moringaceae), and *Phyllanthus niruri* (Euphorbiaceae) using PIXE," *Biol Trace Elem Res*, vol. 133, pp. 357–363, 2010.

[19] Z.B. Alfassi, *Activation Analysis, Volume I and II*. Boca Raton, FL: CRC Press, 1990.

[20] TNP2K, *100 Kabupaten/Kota Prioritas untuk Intervensi Anak Kerdil (Stunting) (100 Priority Districts/Cities for Stunted Child Intervention)*, 1st ed. Jakarta Pusat: Sekretariat Wakil Presiden Republik Indonesia, 2017.

[21] N. Siddique, S. Waheed, "Evaluation of laboratory performance using proficiency test exercise results," *J Radioanal Nucl Chem*, vol. 291, pp. 817–823, 2012.

[22] R. Lopez-Ridaura *et al.*, "Magnesium intake and risk of type 2 diabetes in men and women," *Diabetes Care*, vol. 27, no. 1, pp. 134–140, 2004.

[23] M.P. Guerrera, S.L. Volpe, J.J. Mao, "Therapeutic uses of magnesium," *Am Fam Physician*, vol. 80, no. 2, pp. 157–162, 2009.

p ISSN 1907-0322
e ISSN 2527-6433

[24] WHO, "Trace element in human nutrition and health," 1996.

[25] N.R. Council, *Recommended dietary allowances*, 10th ed. Washington, DC: National Academy of Sciences, 1989.

[26] R.A. Anderson, "Chromium as an essential nutrient for humans," *Regul. Toxicol. Pharmacol.*, vol. 26, no. 1, pp. 35–41, 1997.

[27] I. Geyikli, S. Bayil, "Chromium associates with insulin sensitivity," *Gaziantep Med. J.*, vol. 14, pp. 59–63, 2008.

[28] W.B. Kinlaw *et al.*, "Abnormal zinc metabolism in type II diabetes mellitus," *Am. J. Med.*, vol. 75, no. 2, pp. 273–277, 1983.

[29] R.K. Chandra, "Excessive intake of zinc impairs immune responses," *JAMA*, vol. 252, no. 11, pp. 1443–1446, 1984.

[30] W.B. Komalasari, *Statistik Konsumsi Pangan Tahun 2018 (Food Consumption Statistics in 2018)*. Jakarta Selatan: Pusat Data dan Sistem Informasi Pertanian Sekretariat Jenderal Kementerian Pertanian, 2018.