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การศึกษาผลของสารต้านออกซิเดชันบางชนิดต่อปริมาณเคอซิทินในสารสกัดมะรุม

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บทคัดย่อ

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บทนำ: Moringa (*Moringa oleifera* Lam.) เป็นพืชที่มีประโยชน์มากมายทั้งเป็นผักและสมุนไพร มีชื่อเรียกเป็นภาษาไทยคือ มะรุม จากรายงานการศึกษาพบว่าประกอบด้วยสารสำคัญหลายชนิดทั้งที่อยู่ในกลุ่มของแอลคาลอยด์ ฟลาโวนอยด์ และ ซินนาเมท โดยเฉพาะอย่างยิ่งเคอซิทิน (ฟลาโวนอยด์) เป็นสารสำคัญที่พบได้มากในใบมะรุมและมีฤทธิ์ต้านการอักเสบที่ดี ดังนั้นในงานวิจัยครั้งนี้จึงมีวัตถุประสงค์เพื่อศึกษาผลของสารต้านออกซิเดชันบางชนิดที่มีผลต่อการวิเคราะห์เคอซิทินในใบมะรุม **วัสดุและวิธีการทดลอง:** ทำการศึกษาผลของสารต้านออกซิเดชันโดยเติม Butylated hydroxyanisole (BHA) ในปริมาณที่แตกต่างกันลงไปในปฏิกิริยาแล้วนำไปวิเคราะห์หาปริมาณเคอซิทินโดยวิธี HPLC **ผลการทดลอง:** พบว่าเมื่อเติม butylated hydroxyanisole ลงไปในปฏิกิริยาจะทำให้ได้ปริมาณเคอซิทินสูงกว่าเมื่อไม่ได้เติม (11.24 มิลลิกรัมต่อกรัมของสารสกัด) **สรุปผล:** ดังนั้นจากการศึกษาสามารถสรุปได้ว่า ควรจะต้องมีการเติมสารต้านออกซิเดชันลงไปในการวิเคราะห์ เพื่อให้การวิเคราะห์หาปริมาณเคอซิทินเป็นไปอย่างถูกต้องและมีความน่าเชื่อถือมากที่สุด

คำสำคัญ: มะรุม, เคอซิทิน, butylated hydroxyanisole

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Abstract

Study of Some Antioxidant to the Amout of Quercetin in Marum Extract.

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Introduction: Moringa (*Moringa oleifera* Lam.) is one of the multipurpose plants that can be used as vegetable and medicinal plants. It is known as Marum in Thai. This plant is reported to contain some compounds such as alkaloids, flavonoids and cinnamates. Quercetin is one of the flavonoid constituents shown in the leaves of this plant and showed anti-inflammation activity. Therefore, this research aimed to study the effect of some antioxidant to the amount of quercetin in *Moringa oleifera* leaves. **Materials and Method:** This study focused on the effect of some antioxidant to the analysis of quercetin. So, Butylated hydroxyanisole (BHA) in various amounts was added into the process of determination. The amount of quercetin was then analyzed by HPLC method. **Results:** The crude extract of *Moringa oleifera* leaves was prepared by ethanol extraction and used in this study. It was found that butylated hydroxyanisole added into the reaction could increase the amount of quercetin when analyzed by HPLC (11.24 mg per g extract). **Conclusion:** Thus, it could be concluded that the

analysis of quercetin in *Moringa Oleifera* leaves should add some of the antioxidant such butylated hydroxyanisole in the process of analysis. This could confirm the actual amount found in the leave of this plant and promote the accurate and reliable results to use in the other studies.

Keywords: *Moringa oleifera*, quercetin, butylated hydroxyanisole

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1. Introduction

Moringa oleifera Lam (drumstick tree or horse radish tree) is the most widely cultivated plant in family Moringaceae. It is one of the multipurpose plants that can be used as vegetable and medicinal plants. Marum is the Thai name of this plant. At a present time, this plant is already an important crop in some countries such as India, Ethiopia, Philippines and Sudan, and is being grown in West, East and South Africa, Tropical Asia, Latin America, Caribbean and Pacific Islands. All parts of this plant are edible and have long been consumed by humans (Fahey, 2005). This plant has greatest potential for benefitting people suffering from poverty, poor health and malnutrition. It has been used in the traditional medicine in many countries for skin infections, anemia, anxiety, asthma, bronchitis, conjunctivitis, cough, diarrhea, eye and ear infections, fever, headache, abnormal blood pressure, pain, psoriasis, respiratory disorder, sore throat, diabetes (Fuglie, 2001; Ramachandran *et al*., 1980). Moreover, this plant has been used for nutritional purpose, which comes from vitamins (vitamin C, vitamin A etc.), minerals (potassium, calcium, magnesium etc) and essential amino acids found in various parts (Babu, 2000). It is especially as a food source because the tree is in full leaf at the end of the dry season while it is difficult to find the other food sources.

Many studies about the pharmacological activities and phytochemical constituents of this plant were also reported (Sreelatha and Padma 2009; Bharali *et al*., 2003; Caceres *et al*., 1991). Some active constituents found in this plant have been reported to have antioxidant, hypotensive, spasmolytic, antifungal, anticancer, antibacterial activities and hypocholesterolemic effects (Caceres *et al*., 1992; Chuang *et al*., 2007; Mehta *et al*., 2003; Kumar and Pari, 2003; Pari and Kumar, 2002; Vieira *et al*, 2010).

This plant was reported to contain benzyl glucosinolates, ß-sitosterol, glycosides, sugars, alkaloids, flavonoids, proteins and saponins (Goyal *et al*., 2007). Quercetin belongs to an extensive class of polyphenolic flavonoid compounds and have some biological activities such as antibacterial, antioxidant and anticarcinogenic activities (Rahman *et al*., 2009; Lamson and Brignall, 2000; Williamson *et al*., 1996). Quercetin occurs as glycosides and is found in the leaves of this plant (Lako *et al*., 2007). However, the analytical method to determine flavonoid glycoside is quite difficult. Hydrolysis reaction need to used and the resulting aglycone, quercetin, can be identified and quantified. In order to determine the amount of quercetin in the leave of this plant without degrading the aglycone itself during the acid hydrolysis in sample preparation procedures, the effect of some antioxidant, butylated hydroxyanisole was tested in this study. Butylated hydroxyanisole is an antioxidant which commonly used as preservative in food and medicines. Thus, this present research aimed to study the effect of some antioxidant to the analysis of quercetin in *Moringa oleifera* leaves.

2. Materials and Method

2.1 Plant Material: The *Moringa oleifera* leave collected in May 2011 from Khon Kaen province was identified by Dr. Prathan Luecha, Faculty of Pharmaceutical Sciences, Khon Kaen University. A vocher specimen (SD 5501) has been deposited at the Herbarium of Faculty of Pharmaceutical Sciences, Khon Kaen University.

2.2 Chemicals: Chemicals were obtained from the following sources: butylated hydroxyanisol and formic acid from Fluka®, methanol and hydrochloric acid from BDH®, 2-propanol and ethanol from Merck®, standard quercetin from Sigma Aldrich®.

2.3 Preparation of the extracts: The leaves were dried at 50 ˚C, powdered and extracted with ethanol for 7 days. The extracts were then filtered and concentrated to dryness by a rotary evaporator.

2.4 Hydrolysis of crude extract: The hydrolysis method from the study of Hertog in 1992 (19) was used in this study. Crude extract (50 mg) was hydrolysed by refluxing at 80 degree Celsius for 2 hours in 1.2 M hydrochloric acid in 50% aqueous methanol. The effect of BHA in various amounts (0, 2, 5, and 10) was also tested in three replication.

2.5 HPLC analysis of quercetin: The hydrolysed samples were analysed by using reversed-phase HPLC system on a Hypersil ODS column (Agilent®, 4x250 mm, 5 µm) using 2-propanol, acetonitrile and 1% formic acid solution (8:22:70) as mobile phase and UV detection (370 nm) at the flow rate of 1 mL/min. Chromatograms were compared with the chromatograms from standard quercetin solution (range from 0.48-9.60 µg/mL). The amounts of quercetin were then determined by using standard curve.

Quercetin contents were subjected to statistical analysis to verify and evaluated the difference between the various amounts added of BHA. The data were expressed as mean ±SD (n=3) where 'n' represents the number of replication. Results were analyzed statistically by one-way ANOVA. The difference was considered significant if $P<0.05$.

3. Results and Discussion

Figure 1A showed the amount of quercetin content in crude ethanol extract of *M. oleifera* leaves at various amounts of BHA added. It was found that 10 mg of BHA, added in the extract prior to analysis, showed the highest amount and gave the best yield of quercetin when analyzed by HPLC (11.24 mg/g extract) using calibration curve prepared from standard quercetin solution (Figure 1B). However, there was no statistical difference when compared between the various amounts of BHA. The results from this study were conformed to the study of Nuutila in 2001 which the quercetin content in red spring onion was higher after addition with some antioxidants such as tertiary butylhydroxyquinone or ascorbic acid and conformed to the previous study of Punya in 2012 which the amount of quercetin from the extract of *M. oleifera* leaves increased due to the effect of tertiarybutylhydroxyquinone

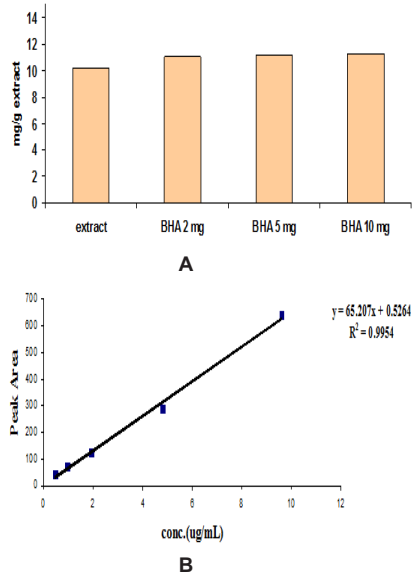

Figure 1: The amount of quercetin content in crude ethanol extract of *M. oleifera* leave at various amount of BHA (A) and Calibration curve of standard quercetin solution (B).

4. Conclusion

From the results of this study, it could be concluded that the analysis of quercetin in *M. Oleifera* leaves should add some of the antioxidant such as butylated hydroxyanisole (BHA) in the process of analysis even though there was no statistical difference between with and without BHA. This could confirm the actual amount found of quercetin in the leaves of this plant and promote the accurate and reliable results to use in the other studies such as the standardization of quercetin content in the crude extract or plant material. However, this work only studied the effect of BHA. The effect of tert-butylhydroxyquinone was previously studied and affected to the amount of quercetin the same as in this study. Moreover, There are many antioxidants that can be used such as butylated hydroxytoluene (BHT) and ascorbic acid. Thus, the further studies about the effect of such antioxidants should be performed to ensure the analysis and could be applied for the determination of the other flavonoid glycosides in plant.

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