PHENOLIC ANTIOXIDANTS FROM *Bougainvillea* SPP.

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Abstract

In this study, bract and flower parts of four different color paper flowers (*Bougainvillea* spp.), i.e., white, yellow, pink and reddish pink, were investigated for phenolic content and antioxidant capacity. Extraction process was performed by conventional shaking method with different solvents; ethyl acetate (EtOAc), ethanol (EtOH), propylene glycol (PG) and deionized (DI) water. Extractable phenolic content (EPC) of the extracts was determined by Folin-Ciocalteu method, while antioxidant capacity was investigated by DPPH radical scavenging activity and ferric reducing antioxidant power (FRAP). The EPC value of the paper flower extracts were in the range of 1.73 and 21.78 mg gallic acid equivalent per gram dry weight (mg GAE/g dw). The propylene glycol gave the highest extraction efficacy and the highest value of EPC (21.78 mg GAE/g dw) was found in the yellow paper flower extract. The efficient values of DPPH radical scavenging and FRAP were also found in the presence of propylene glycol (6.98-29.21 mg trolox equivalent antioxidant capacity per gram dry weight (mg TEAC/g dw) and 6.80-10.88 mg TEAC/g dw), respectively). The correlation plots between the EPC and DPPH radical scavenging activity and FRAP showed very high correlation between the EPC and antioxidant capacity of the all extracts.

Keywords: antioxidant; extraction; paper flower; phenolic compound

Introduction

Cosmetic market value has been increasing annually, especially in skin care product which account for 60%. This makes research and development to find new sources of active ingredients in cosmetic rapidly increase. Flowers have been attracting more attention owing to their great potential values of natural antioxidants (Elzaawely et al., 2007; Shi et al. 2009). Flower resources are abundant around the world including Thailand, of which many kinds are edible and can be used as herbs. Therefore, inclusion of bioactive compounds from flowers is attractive design.

Paper flowers (*Bougainvillea* spp.) are popular plants and they have varies colorful flowers such as white, yellow, orange, red, pink or purple which are commonly found in Thailand. Many reports indicate that flowers contain phenolic compounds and also have antioxidant activity as well (Wybranieca et al., 2010; Yin et al., 2011). Therefore, this research intended to extract phenolic antioxidant from paper flower (*Bougainvillea* spp.) which would lead to a better understanding of phenolic antioxidant in this flower and provide new promising resource for cosmetic application.

Methodology

Sample preparation
Bougainvillea spp. flowers (paper flowers) with different colors, i.e., white, yellow, pink and redish pink, were collected from Muang district, Chang Rai in 2011. The samples were weighed and dried in a hot air oven at 55 C° until the weight was constant. After drying, the paper flowers were pulverized into 250 µm size. The ground paper flowers were stored at -20 C° until used.

Preparation of phenolic antioxidants from paper flower
Five grams of each powdered sample were extracted with 4 different solvens, ethanol (EtOH), ethyl acetate (EtOAc), propylene glycol (PG) and deionized water (DI water), by suspending each sample in 50 mL of each solvent at room temperature. The mixtures were shaken on orbital shaker at 100 rpm for 24 hr. The samples were then filtered through a Whatman filter paper No.1. The filtrates were concentrated by rotary evaporator at 45 ºC. The obtained crude extracts were stored at -20 C° for further analysis of phenolic contents and antioxidant capacities.

Determination of extractable phenolic content and antioxidant capacities
The extractable phenolic content (EPC) was determined as described in Kumar et al. (Kumar et al., 2008) with some modifications. The result was expressed as milligram gallic acid equivalent per gram extract (mg GAE/g). The antioxidant capacity was determined by DPPH radical scavenging assay (Thaipong et al., 2006) and ferric reducing antioxidant power (Benzie et al., 1996) which was exhibited as mg trolox equivalent antioxidant capacity per gram extract (mg TEAC/g).

Results

Preparation of sample and crude extract.

The four paper flowers exhibiting different color were collected, dried and pulverized into 250 µm particle size. The fresh and powdered paper flowers are represented in Figure 1. The dried powdered samples were then extracted by different solvents (EtOAc,EtOH, PP and DI water) by skaing method. Of all the color of paper flowers extracted by EtOAc and EtOH solvents had dark green color because it may contained chrolophyll compound in the extract. When PG and DI water solvents were used for extraction, the yellow color extracts were obtained when white and yellow flower papers were used, while the pink to red color extracts were achieved when using ping and reddish pink flower papers as raw material. The presence of pink or red color might be due to betacyanins that are an extremely complex coloring agent in this flower.

Extractable phenolic contents.

Figure 2 shows the EPC from four paper flowers extracted with 4 different solvents. According to the obtained results, it was evident that EtOAc was the least effective solvent for phenolic extraction. It provided only 1.73 – 2.58 mg GAE/g dw range when comparing to those other solvents. Propylene glycol showed the highest capacity (12.59 – 21.78 mg GAE/g dw) for extract the phenolic antioxidant from the paper flowers.

Figure 1 Appearance of fresh (left) and dry powdered paper flowers (*Bougainvillea* spp.) (right) with different colors.

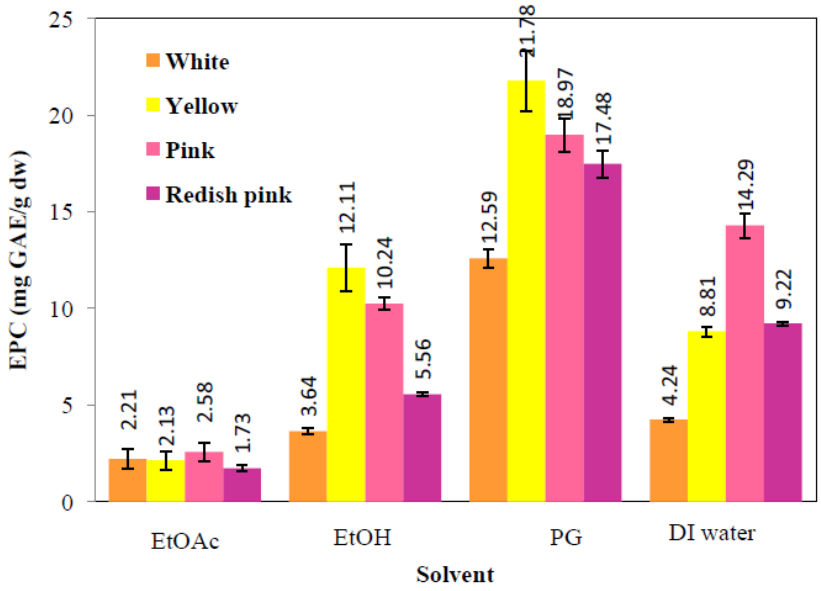

Figure 2 Extractable phenolic contents (EPC) of four color paper flowers extracted by different solvents

DPPH radical scavenging activity of flower papers.

The PG extract provided the highest antioxidant capacity exception for white color **(**Figure 3). While the EtOAc extract showed the least effective antioxidant capacity range from 0.77 – 1.34 mg TEAC/g dw. The different results suggest that the extractability of different solvents provided a variety of antioxidant capacity based upon the antioxidant property content.

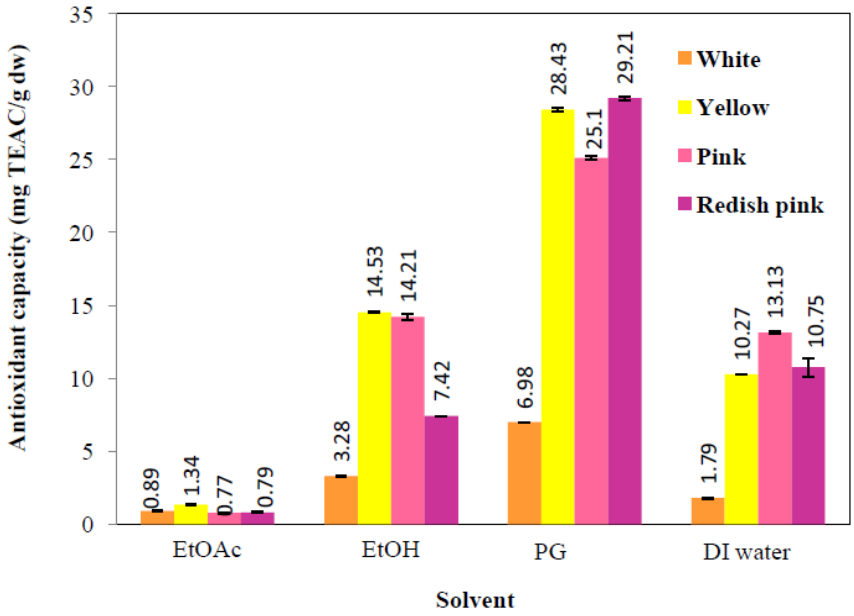

Figure 3 DPPH radical scavenging capacity of four color paper flowers extracted by different solvents

Ferric reducing antioxidant power (FRAP) of flower papers.

The effect of solvents and color of paper flowers on FRAP in the extracts are shown in Figure 4. The PG exhibited as the most efficient solvent for bioactive compound extraction. The FRAP values in PG extracts were in the range from 6.80 – 10.88 mg TEAC/g dw. When comparing the color of paper flowers, it can be seen that there is no drastic difference in the FRAP among the 4 paper flower extracts. It can be roughly summarized that the color of paper flower had no major factor on the value of FRAP, while the type and polarity of solvent played a major role in the extraction of antioxidant from the paper flower.

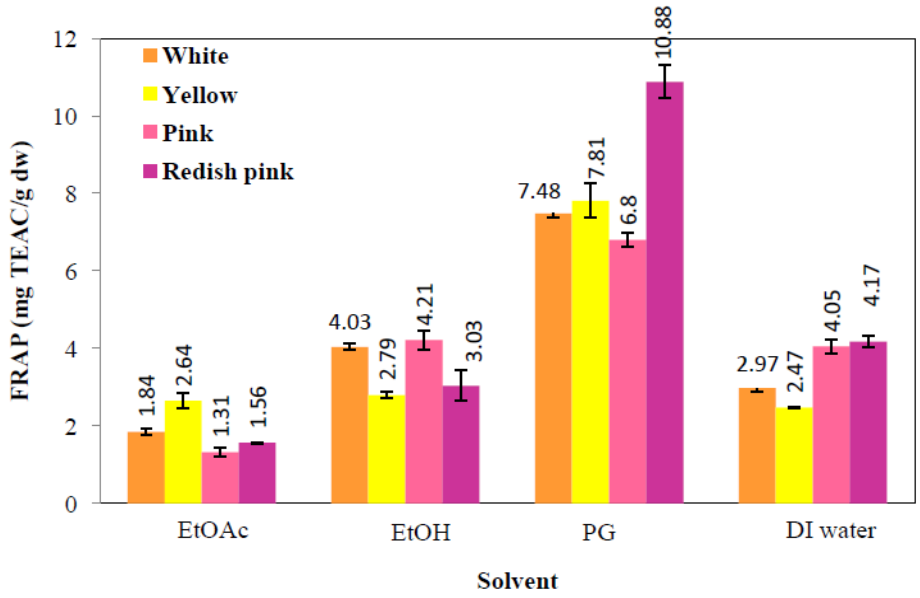

Figure 4 Antioxidant capacity based on ferric reducing antioxidant power (FRAP) of four color paper flowers extracted by different solvents

Correlation between phenolic compounds and antioxidant capacities.

Positive correlation between the value of EPC and DPPH scavenging activity, EPC and FRAP and DPPH scavenging activity and FRAP were demonstrated (Figure 5). These results indicated that phenolic compounds represented in paper flowers had a strong contribution towards their antioxidant capacity. These results are in agreement with Benzie and Strain (7), who found a strong positive correlation between total phenolic content and FRAP assay values.

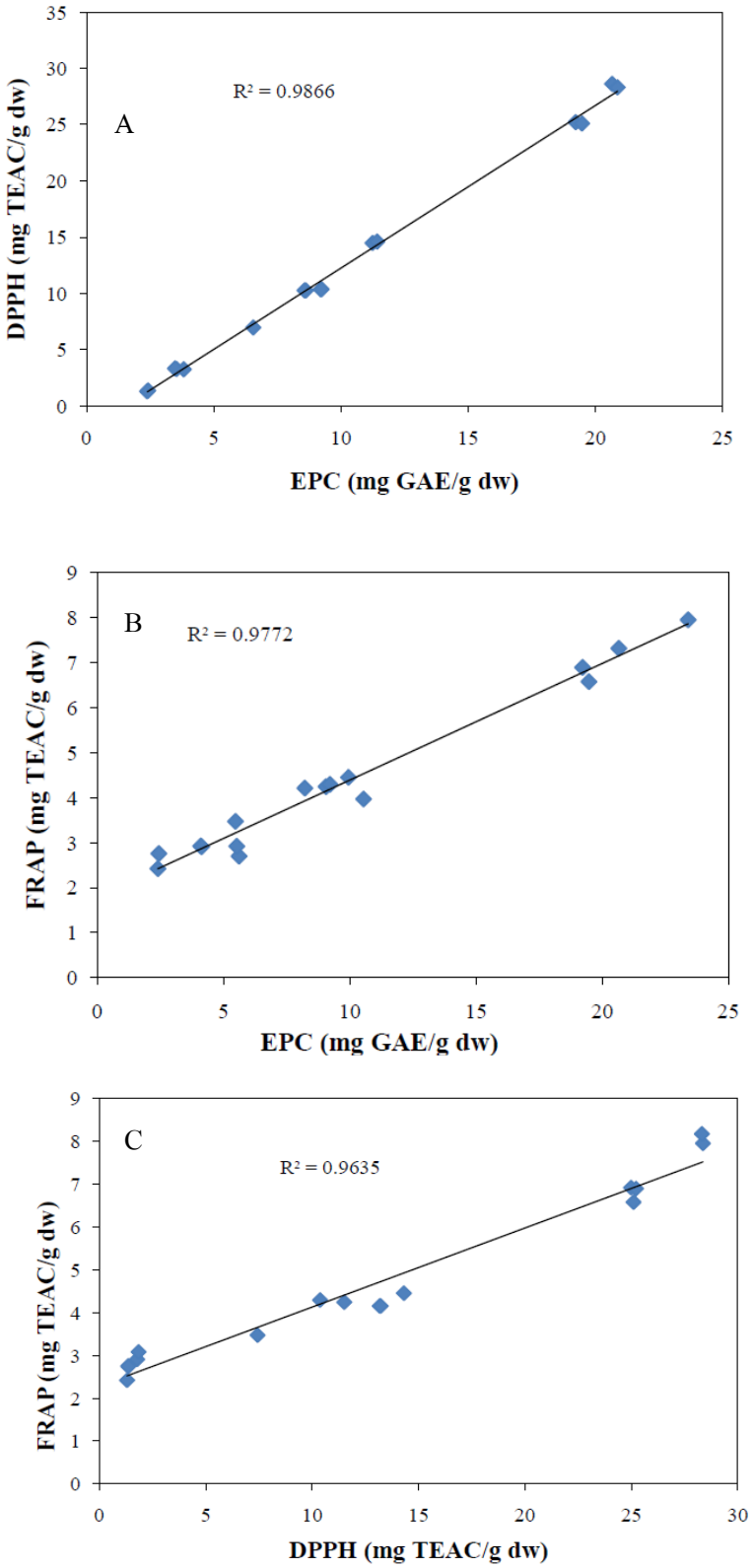

Figure 5 Correlation between the EPC and DPPH radical scavenging activity (A), EPC and FRAP (B) and DPPH radical scavenging activity and the FRAP (C) of paper flower extracts.

Discussion and Conclusion

Flower extracts, which exhibited strong antioxidant activities, were found to contain high amount of total and individual phenolics that may contribute to this activity. Phenolics are commonly found in plant and they have been reported to have at strong antioxidant activity (Elzaawely et al., 2007). For flavonoids, the major flavonoid in flowers is quercetin. The content of flavonoids and antioxidant activity are associated with the amount of quercetin present in the flowers. This may indicate that quercetin could be a potential active compound in the flowers (Vanisree et al., 2008). The principal pigments of purple *B. glabra* bract exist mostly in the 6-o-glycosilated forms of betanidin. In the case of orange and yellow pigmented varieties, biosynthesis of betaxanthin-type pigments was dominant (Yin et al., 2001, Wybranieca et al., 2010). Because of relatively high hydrophilicity of betalains it was difficult to find appropriate solvent systems capable to separate the pigments, especially the most polar ones (Wybranieca et al., 2010). Water extract of fresh Bougainvillea spectabilis showed DPPH radical scavenging activity of 21.94% positioning at the eleventh among sixty-nine flowers in Southern China. This plant also exhibited phenolic content of 4.165 mg catechin equivalent per 0.2 mg fresh weight (Yin et al., 2001).
In this study, the pink and reddish pink bracts exhibited the highest DPPH radical scavenging capacity when using DI water and propylene glycol as solvents. The solvents and bract color of paper flower have been shown that they had an effect on phenolic antioxidant extraction. In addition, The study also showed that the paper flower extract substantially contained antioxidant activity. It is, therefore, might be an attractive source for cosmetic use due to its high natural antioxidant capacity and high colorant efficiency.

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