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KEYWORDS: *Punica granatum* L., Leaf extract, Anti-oxidant activity

INTRODUCTION

Pomegranate (*Punica granatum* L.) belongs to the family Punicaceae. Pomegranate tree has been cultivated all over Thailand, mostly as a holy plants and its fresh fruits are popularly consumed or made into beverage. Since ancient times, pomegranate has been used as a traditional medicine for treating diarrhoea (1), metrorrhagia, as an antiviral (2) and its extract used as dietary supplement. The target of this study is to investigate the anti-oxidant activity of the leaves from "Pet Chom Poo", "Dang Ma Ruay" and "Siam" varieties of pomegranate for their free radical scavenging ability through the DPPH method.

MATERIALS AND METHODS

Plant Materials:

Leaves of pomegranate varieties Dang Ma Ruay, Siam and Pet Chom Poo were harvested in May 2012 from Pak Chong, Nakhonratchasima Province, They were dried at 40°C for 24 hrs, then ground to coarse powder for extraction purpose.

Figure 1 Pomegranate, variety Dang Ma Ruay

Figure 2 Pomegranate, variety Siam (3)

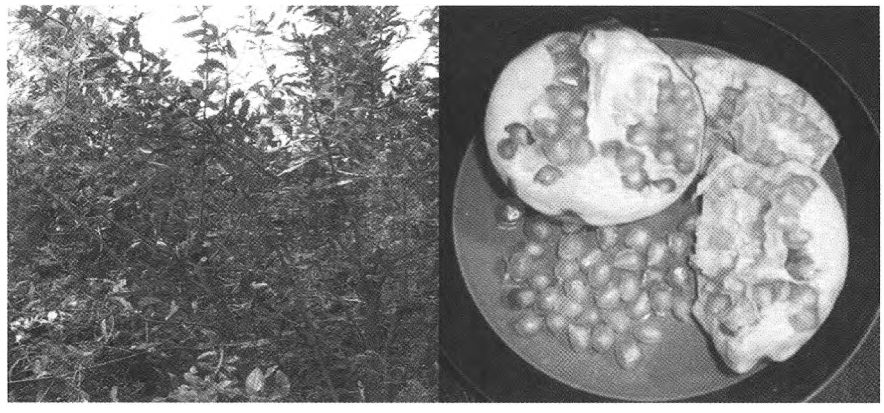

Figure 3 Pomegranate, variety Pet Chom Poo (4)

Extraction of Plant Materials: Ten gram each of powdered pomegranate leaves from varieties, Pet Chom Poo, Dang Ma Ruay and Siam were extracted with 25 ml of methanol at room temperature for 3 times with constant stirring. the filtrates were evaporated to dryness under vacuum at 40°C to yield crude extracts of Pet Chom Poo, Dang Ma Ruay and Siam varieties of pomegranate leaves for anti-oxidant activity test.

Determination of Anti-oxidant Activity by DPPH radical scavenging Assay: The scavenging activity on DPPH radical (2,2-diphenyl-1-picrylhydrazyl) of 3 different crude extracts was determined according to the method of Brand-Williams (5) method. Trolox was used as a standard anti-oxidant. An appropriate amount of sample was prepared to obtain the final concentrations of 0.2, 0.4, 0.8, 1.2 and 1.6 mg/ml. The 2,2-diphenyl-1-picrylhydrazyl in methanol was used as the control. The mixture was shaken vigorously and left to stand for 30 min in the dark at room temperature. Then, the absorbance at 517 nm of each sample was measured and the % inhibition was calculated by the following formula:

2,2-diphenyl-1-picryhydrazyl (DPPH) DPPH: H

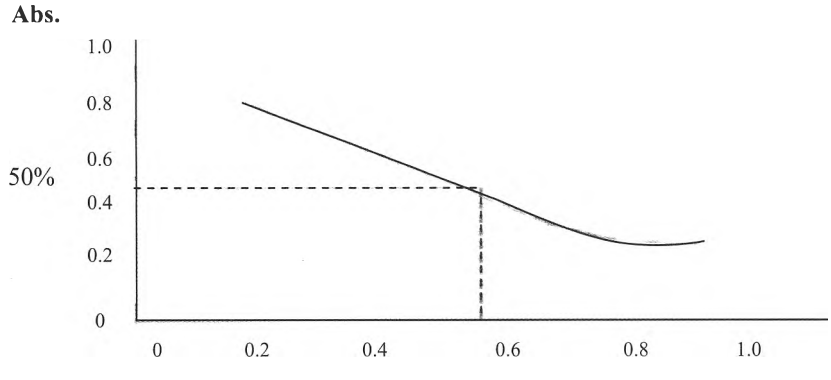

Figure 4 Curve of DPPH Scavenging

$$\text{DPPH radical-scavenging \%} = [1 - \frac{\text{Abs.sample}}{\text{Abs.control}}] \times 100$$

The calculated value of effective concentration at 50% (EC_{50}) means the effective concentration of sample or crude extract required to reduce the 50% of DPPH radical are scavenged. The anti-oxidant capacity was expressed in μmole equivalent in activity of trolox. The experiments were done in triplicate.

RESULTS AND DISCUSSION

The free radical scavenging activity determined by DPPH was expressed as the EC_{50} value (the effective concentration of extract required to inhibit 50% of the initial DPPH free radical). Results shown in Table 1 indicate that the EC_{50} value of Dang Ma Ruay extract was higher than those of Siam and Pet Chom Poo at 0.107 mg/ml, 0.100 mg/ml and 0.0846 mg/ml, respectively. Anti-oxidant activity values were 46,926TE for Pet Chom Poo, 39,700TE for Siam and 37,102.80TE for Dang Ma Ruay, respectively. The result of anti-oxidant activity is useful in showing pomegranate leaves as a good source of natural ingredients for cosmetic or dietary supplement products. The quantitative analysis of polyphenols in these extracts will be further studied.

Table 1 The EC_{50} values of 3 varieties of pomegranate leaf extract

Crude leaf extract	EC_{50} Value (mg/ml)
Dang Ma Ruay	0.107
Siam	0.100
Pet Chom Poo	0.0846

CONCLUSION

The 3 different crude methanolic extract of pomegranate leaves displayed a good anti-oxidant activity. It will be a good source of medicinal plant extract for which the waste leaves can be utilized as a dietary supplement or cosmeceutical products.

ACKNOWLEDGMENTS

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