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Research Article

EFFECT OF SOLVENT TYPES ON PHENOLIC, FLAVONOID CONTENTS AND ANTIOXIDANT ACTIVITIES OF *SYZYGIUM GRATUM* (WIGHT) S.N.

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ABSTRACT

Objective: The present study was carried out in various of solvent on *Syringium gratum (Wight) S.N.* leaves in term of phenolic, flavonoid contents and antioxidant activities.

Methods: This research was studied the effects of extraction solvents in ethanol, methanol and water extracts of leaves from *S. gratum.* Two methods of antioxidant activities were used DPPH radical scavenging assay (DPPH assay) and Ferric Reducing Antioxidant Power (FRAP) assay. According to our study, the outcomes of free radical scavenging properties were demonstrated in terms of mg gallic acid equivalent (GAE)/100 g sample and mg Trolox equivalent (TE)/100 g sample antioxidant, respectively.

Results: For DPPH assay 10,237.63 mg GAE/100g sample in ethanol and FRAP assay values was 5,713.46 mg TE/100g sample in water. IC_{50} values were 6.77, 23.25, and 50.33 µg/ml for ethanol, methanol and water, respectively. The average total phenolic content of water extract was 4,042.21 mg GAE/100 g sample, which was higher than the other solvents. The flavonoid content in water extract showed the highest amount, about 700.32 mg CE/100g sample.

Conclusion: The obtained results support the use of this plant in traditional medicine, and suggest more investigation.

Keywords: Antioxidant activities, Phenolic content, Flavonoid content, *Syzygium gratum* (Wight) S.N.

INTRODUTION

Free radicals contribute several disorders in humans including atherosclerosis, arthritis, ischemia and repercussion injury of many tissues, a central nervous system injury, gastritis and cancer [1]. An imbalance between reactive oxygen species and the inherent antioxidant capacity of the body, directed the use of dietary and/or medicinal supplements particularly during the disease attack. Studies on herbal plants, vegetables, and fruits have indicated the presence of antioxidants such as phenolics, flavonoids, tannins, and proanthocyanidins.

The antioxidant contents of medicinal plants may contribute to the protection they offer from disease. The ingestion of natural antioxidants has been inversely associated with morbidity and mortality from degenerative disorders [2]. Due to environmental pollutants, radiation, chemicals, toxins deep fries and spicy food as well as physical stress, free radicals cause depletion of the immune system antioxidants, the change in gene expression and induce abnormal protein. The oxidation process is one of the most important routes for producing free radicals in food, drugs and even living systems [3]. The phenolic compounds and their antioxidant activities present in no fewer than 3,000 plant species including some Thai plants have been studied [4].

Thai plants are widely distributed throughout the tropics particularly in Southeast Asia. Several researchers have shown that various tissues from plants grown in tropical and subtropical climate contain high concentrations of natural phenolic phytochemicals, including flavonoids [5]. The antioxidant activity of fruits and vegetables is generally positively correlated with their content of polyphenols [6].

Polyphenols a class of compound that is very broad and complex, which the flavonoids are the most widely studied. The search for novel natural antioxidants of plant origin has ever since increased. It is not known which constituents of plant are associated in reducing the risk of chronic diseases, but antioxidants appear to play a major role in the protective effect of plant medicine. The present study was designed to investigate the total phenolic and total flavonoid content and to evaluate the antioxidant activities. *Syzygium gratum* (Wight) S.N. locally known in Thai as Sa-med-Chun, belongs to the Myrtaceae family. It is also called as Sa-med or Sa-med-Dang. This plant is an evergreen shrub, hazel bark. The leaf is simple with lanceolate shape. The flower is small, pale yellow covering at the end. There are no reports of its pharmacological activities and bioactive compounds.

MATERIALS AND METHODS

Chemicals

Gallic acid, catechin, trolox, 2,4,6-tris(2-pyridyl)-s-triazin (TPTZ) and 1,1-Diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (USA). Folin-Ciocalteu phenol reagent, ferric (III) chloride hexahydrate ($FeCl_3.6H_2O$), and sodium hydroxide (NaOH) were obtained from Merck (Darmstadt, Germany). Sodium carbonate anhydrous, aluminium chloride and sodium acetate were purchased from Carlo Erba, UK. Hydrochloric acid, hexane, ethanol, methanol were purchased from Mallinckrodt, USA.

Plant material

The fresh leaves of *S. gratum* were collected from Phattalung province, Southern of Thailand.

Leaves were dried in hot air oven at 45 °C for 48 h.

Extraction

The dried leaves of *S. gratum* were ground into powered, sieved through sieve no.60 and stored in airtight containers for experiment. The powder (10g) was taken and extracted with solvents (100 mL) including ethanol, methanol and water. Each extract was concentrated in vacuole using a rotary evaporator.

Determination of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging system

The determination of antioxidant activity through DPPH scavenging system was carried out according to the method of Brand and Williams [7]. DPPH assay is a common antioxidant assay. The hydrogen atoms, or electron donation ability, of the corresponding extract were measured from the bleaching of purple color of DPPH solution. Each 100 µL of various concentrations of the extracts/gallic acid was added to 100 µL of a 200 µM ethanol solution of DPPH. After a 30 minutes incubation period at room temperature, the absorbance was read compared to a blank at the wavelength of 517 nm. Gallic acid was taken as the standard reference. The antioxidant activity was expressed as mg gallic acid equivalent (GAE) milligrams per 100 grams of dried weight (DW) sample (mg GAE/100 g of sample).

Determination of ferric reducing antioxidant power (FRAP) assay

The determination of antioxidant activity through FRAP was carried out according to the method of Benzie and Strain [8]. The FRAP assay measures the reducing ability of plant extracts. The FRAP reagent was freshly prepared by mixing of 10 mM of TPTZ in 40 mM hydrochloric acid, 20 mM FeCl3•6H2O in distilled water and 300 mM acetate buffer pH 3.6. 30 µL of sample extracted/trolox was added to a 96 well plate followed by 270 µL of FRAP reagent. The absorbance was measured at 593 nm after 30 minutes incubation at room temperature. The standard reference of trolox was constructed. Antioxidant activity was determined from a standard curve as milligrams trolox equivalent per 100 grams of sample (mg TE/100 g of sample). Antioxidant activity of each sample was compared with the standard and the presented data is an average of three replications. The antioxidant activity was expressed as mg trolox equivalent (TE) milligrams per 100 grams of dried weight (DW) sample (mg TE/100 g of sample).

Determination of total phenolic content

The total phenolic content was carried out according to the method of Folin-Ciocalteau method [9]. Each 12.5 µL of sample extracted/gallic acid and 50 µL distilled water was added to a 96 well plate. 12.5 µL of Folin-Cioculteau phenol reagent was added to the mixture and shaken vigorously. After 6 minutes, 125 µL of 7% Na2CO3 solution was added and mixed. The solution was immediately diluted to volume with 100 µL distilled water, mixed thoroughly and then allowed to stand for 90 minutes. The absorbance was measured at 760 nm using a microplate reader versus the prepared blank. Calibration curve of gallic acid was set up to estimate the activity capacity of samples. The result was expressed milligrams of gallic acid equivalents per 100 g of dry weight (mg GAE/100 g of sample).

Determination of total flavonoid content

The total flavonoid content was determined according to a modified protocol developed by Chen and Li [10]. The mixture of sample/catechin (25 µL), distilled water (125 µL) and 5% $NaNO_2$ (10 µL) were added to 96 well plate. After 6 minutes, 15 µL of 10% $AlCl_3$ solution was added. The mixture was allowed to stand for 5 minutes at room temperature.

The reagent solution was 1 M NaOH (50 µL) and shaken for 1 minute. Reading the absorbance at a wavelength of 595 nm was measured using a microplate reader. A calibration curve was made by absorbance and the concentration of catechin. Results were also expressed as catechin equivalent milligrams per 100 grams sample (mg CE/100 g of sample).

Statistics

To verify the statistical significance of all parameters, the values of mean ± standard deviation (SD) were calculated. Analysis of variance (ANOVA) was performed to determine the efficiency of the solvents for extraction as well as establish the differences in the content of polyphenols, and flavonoid and antioxidant activity. A p-value of less than 0.05 was considered to be statistically significant.

RESULTS

The results of DPPH and FRAP assay are shown in Fig. 1 which varies in different solvents. The DPPH assay in ethanol extract was significantly (p-value < 0.05) different from methanol and water extract (10,237.63, 3,025.10 and 155 mg GAE/100 g sample, respectively). The FRAP assay the water extract showed the highest value (5,713.47 mg TE/100 g sample), secondly the methanol (1983.30 mg TE/100 g sample) and ethanol extract (590 mg TE/100 g sample). In consistent to the ferric reducing antioxidant power assay, potential of the samples was expressed as the 50 percent inhibitory concentration (IC_{50}) as shown in Figure 2. The water extract, showed the highest inhibition (6.77 µg/mL), secondly the methanol extract (23.25 µg/mL) and the ethanol extract (50.33 µg/mL) showed in Fig. 2.

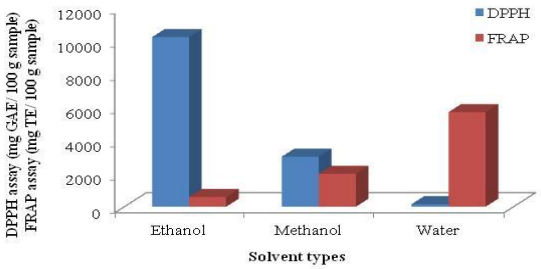

Fig. 1: Effect of solvent types on DPPH assay and FRAP assay of *S. gratum*

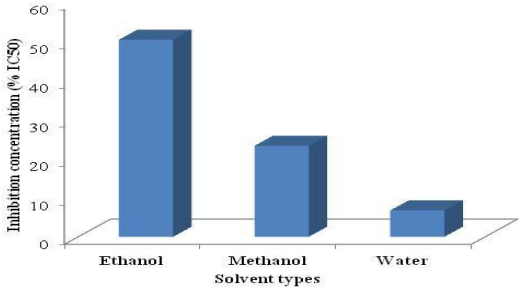

Fig. 2: IC_{50} for the test extracts

The phenolic and flavonoid contents of *S. gratum* extracts are showed in Fig. 3 and 4. The highest in total phenolic and flavonoid contents were present in the water extract as 4,042.21 mg GAE/ 100 g sample and 700.32 mg CE/ 100 g sample, respectively.

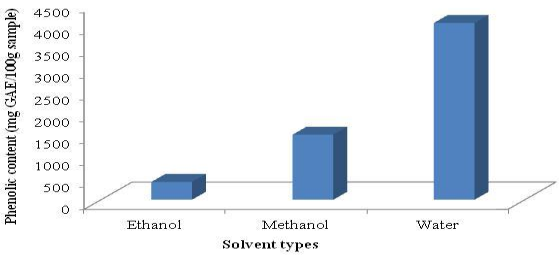

Fig. 3: Effect of solvent types on the average phenolic content of *S. gratum*

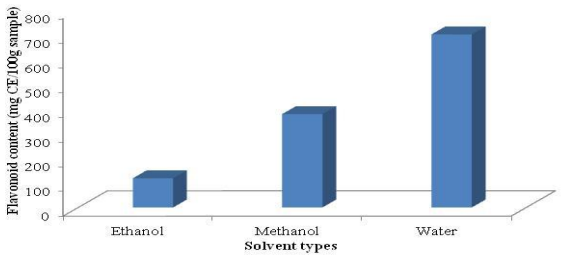

Fig. 4: Effect of solvent types on average flavonoid content of *S. gratum*

DISCUSSION

Antioxidant activities, phenolic and flavonoid contents of each extracted are shown. This may be due to suitable of solvent. In general, extractability of component depends on degree of polarity and ratio of solute and solvent. The important difference between DPPH and FRAP is that FRAP can be dissolved in buffer, in which the antioxidant activity can be measured, due to the hydrophilic nature of the compounds in samples. The FRAP assay measures the ability to reduce ferric tripyridyltriazine (Fe^{3+}-TPTZ) in samples to the ferrous form (Fe^{2+}-TPTZ) [11]. In contrast, DPPH• can only be dissolved in organic media, especially in ethanol, this being an important limitation when interpreting the role of hydrophilic antioxidants.

The content of phenolic acids and flavonoid varied in different solvents. Turkmen *et al.* (2006) [12] reported that solvents with different polarity (ethanol and water) have significant effect on polyphenol content. In addition, antioxidant activity is higher content in more polar extraction solvents. However, Ghasemzadeh *et al.* (2010) [13] showed that the ineffectiveness of water for the extraction of total phenols of grapes seeds (*Vitis vinifera*). Similar to our finding, the FRAP components is highly associated with their total phenolic and total flavonoid content. Our results showed that water extract was the best solvent for extraction of phenol and flavonoid extraction. Many researchers observed that other phytochemicals, such as ascorbic acid, tocopherol and pigment, also contributed to total antioxidant activity. While phenolic compounds undergo a complex redox reaction with the phosphotungstic and phosphomolybdic acids present in the reagent, the assay has been shown to be specific not to just polyphenols, but to any other substance that could be oxidized by the Folin reagent, Many researchers have been reported the poor specificity of the assay [14].

CONCLUSION

This study indicated that the water extract is the most efficient solvent for the extract with relatively high antioxidant activities in FRAP assay, polyphenolic and flavonoid contents from *S. gratum*. However, for total antioxidant activity, the ethanol extract is the most efficient solvent in DPPH assay.

S. gratum can therefore be further investigated for novel antioxidant or bioactive compounds which will be shown in the present work.

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REFERENCES

1. Su L, Yin J, Charles D, Zhou K, Moore J, Yu, L. Total phenolic contents, chelating capacities of black peppercorn, nutmeg, rosehip, cinnamon and oregano leaf. Food Chem. 2007; 100: 990-997.
2. Gulcin I. Antioxidant activity of food constituents: an overview. Arch. Toxicol. 2012; 86: 345–391.
3. Turkoglu A, Duru M, Mercan N, Kivrak I, Gezer K. Antioxidant and antimicrobial activities of *Laetiporus sulphureus* (Bull) Murrill. Food Chem. 2007; 101: 267-273.
4. Maisuthisakul P, Pongsawatmanit R, Gordont H. Assessment of phenolic content and free- radical scavenging capacity of some Thai indigenous plants. Food Chem. 2007; 100: 1409-1418.
5. Leong L, Shui G. An investigation of antioxidant capacity of fruits in Singapore markets. Food Chem. 2002; 76: 69-75.
6. Benzie IFF. Evolution of dietary antioxidants. Compare. biochem. physiol. Molec. Integra. Physiol. 2003; 136: 113-126.
7. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. Lebens. Wissen. Technol. 1995; 28: 25-30.
8. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal. Biochem. 1996; 239: 70-76.
9. Slinkard K. Singleton VL. Total phenol analysis: Automation and comparison with manual methods. Amer. J. Enol. Viticult. 1977; 28: 49-55.
10. Chen JJ, Li XG. Hypolipidemic effect of flavonoids from mulberry leaves in triton WR-1339 induce hyperlipidemic mice. Asia Pac. J. Clinic. Nutri. 2007; 16: 290-294
11. Wojdylo A, Oszmiariski R, Czemerys R. Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chem. 2007; 105: 940-949.
12. Turkmen N, Sari F, Velioglu, YS. Effect of extraction solvents on concentration and antioxidant activity of black and black mate polyphenols determined by ferrous tartrate and Folin-Ciocalteu methods. Food Chem. 2006; 99: 838-841
13. Ghasemzadeh A, Jaafar HZE, Rahmat A. Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia young ginger (*Zingiber officinale* Roscoe). Molec. 2010; 15: 4324-4333.
14. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. Met. Enzym. 1999; 29: 152-178.