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Original Article

TOTAL PHENOLIC AND FLAVONOID CONTENTS AND ANTIOXIDANT PROPERTIES OF THAI TRADITIONAL HERBAL

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ABSTRACT

Objective: The study investigated for the several of solvent on merits Trigasornmas formula in term of phenolic, flavonoid contents and antioxidant activities.

Methods: This research studied the effects of extraction solvents in water, ethyl acetate, methanol, dichloromethane, ethanol and hexane extracts of Trigasormmas formula. Total phenolic and total flavonoid content were evaluated according to the Folin- Ciocalteu procedure and a aluminium chloride colorimetric method, respectively. 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 ferric sulfate equivalent ($FeSO_4$)/100 g sample antioxidant, respectively.

Results: The average total phenolic content of water extract was 1,955.23±60.87 mg GAE/100 g sample which was higher than the other solvents while the methanol extract showed the highest flavonoid content at about 321.15±9.12 mg $FeSO_4$/100g sample. For antioxidant properties, DPPH and FRAP assay, the highest values were found in water extract at about 158.93±12.45 mg GAE/100g sample and 2,118.87±24.38 mg $FeSO_4$/100g sample, respectively.

Conclusion: The obtained results support the use of this Thai traditional herbal formula, and suggest more investigation.

Keywords: Phenolic content, Flavonoid content, Antioxidant activities, Trigasormmas formula.

INTRODUCTION

World Health Organization (WHO) encourages, recommends and promotes traditional/herbal remedies in natural health care programmes because these drugs are easily available at low cost, safe and people have faith in them. The WHO assembly in number of resolutions has emphasized the need to ensure quality control of medicinal plant products by using modern techniques and applying suitable standards [1]. Thailand had Thai tradition medicines for treat of patient illness and disease for a long time. Thai traditional medicine is one of the most valuable heritages handed down from Thai ancestors. Which, Thai traditional medicines are still used for primary health care in Thailand because they have good therapeutic effects with fewer side effects [2].

In Thailand, the herbal medicinal products are listed in the 2013 National List of Essential Medicines contains poly herbal formula more than single herbal medicines. The knowledge of the oriental medicines usually supports the usage multi herbal formula because it takes advantage of synergy and interaction between phytochemicals in herbal recipe to achieve therapeutic efficacy with minimizing side effect [3]. In Thailand, that has more than 3000 traditional healers [4] many herbal formulas are in current use but these herbal formulas have never been investigated. Recent studies suggested that the services of traditional healers should be incorporated into contemporary health care provision of the Thai health care system. Thus, the need to justify and document ethnomedical practice is important.

Trigasornmas is a one of the Thai tradition herbal recipe. It was used and recorded in Nation list of essential medicine 2013. This formula is use for fatigue and periodic adjustments in patients recovering from an illness such as fever and diarrhea. The Trigasornmas formula that used in this research composes 3 types such as coral plant (*Jatropha multifida* L.), lotus stamen (*Nelumbo nucifera* Gaertn), and bael fruit (*Aegle marmelos* (L.) Corr). Although, these plants were reported to contain bioactive compounds imparting antioxidant, phenolic and flavonoid compounds and individual herbs were addressed as medicine but few scientific data have been reported about combination of herbs. In this article, the objectives were to investigate the relevance of bioactivity including total phenolic, flavonoid content and antioxidant activity of Thai traditional herbal formula used for patients. Moreover, these herbal formulas were additionally tested as supporting information for further in vivo studies.

MATERIALS AND METHODS

Chemical

Gallic acid, 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), ferric sulfate ($FeSO_4$) were obtained from Merck (Darmstadt, Germany). Sodium carbonate anhydrous, aluminium chloride and sodium acetate were purchased from Carlo Erba, UK. Ethyl acetate, methanol, dichloromethane, ethanol and hexane were purchased from Mallinckrodt, USA.

Plant materials and samples preparation

The Trigasornmas formula consists of coral plant (*Jatropha multifida* L.), lotus stamen (*Nelumbo nucifera* Gaertn), and bael fruit (*Aegle marmelos* (L.) Corr). All ingredients were bought from Tradition pharmacies in Bangkok, Thailand. Each ingredient (300 g) were mixed ground and homogenized with ethanol three times at room temperature. The ethanol extract was then concentrated and partitioned between 90% methanol and hexane, removed of methanol, added of water and partitioned with dichloromethane. After that, the water layer was partitioned with ethyl acetate. Each partitioned was evaporated to dryness *in vacuo* to give residues of hexane, dichloromethane, ethyl acetate and water fractions, respectively. The sample is kept in sterile bottle and stored at room temperature for future use.

Determination of total phenolic compounds

Total phenolic content was determined by the Folin-Ciocalteau method [5]. 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, 125 µl of 7% sodium carbonate ($NaCO_3$) and 100 µl of water then added. The absorbance was measure at 760 nm after 90 minutes of incubation at room temperature. The result was expressed milligrams of gallic acid equivalents per 100 g of dry weight (mg GAE/100 g of sample). All determinations were performed in triplicates.

Determination of flavonoid compounds

The content of flavonoid was determined by Chen and Li [6]. The extracted samples (25 µl) were mixed with 125 µl of water and 10 µl of 5% sodium nitrate ($NaNO_3$). After 6 minutes, 15 µl of 10% $AlCl_3$ solution was added and stand for 5 minutes before 50 µl of 1 M sodium hydroxide was added sequentially. The absorbance of the solution at a wavelength of 510 nm was detected. The total flavonoid content in each extract was then calculated using a standard curve prepared with $FeSO_4$ and expressed as $FeSO_4$ equivalent milligrams per 100 grams sample (mg $FeSO_4$/100 g of sample). All determinations were performed in triplicates.

Determination of antioxidant activities

DPPH radical-scavenging activity

The DPPH radical-scavenging activity was determined by the method of Brand-Williams [9]. DPPH assay is a common antioxidant assay. The hydrogen atoms or electron donation ability of the corresponding extract were measured from the breaching of purple color of DPPH solution. The extracted samples (100 µl) were mixed with 0.0039 g of 2, 2-diphenyl-1-picrylhydrazyl which added 50 ml of absolute ethanol to obtain 200 µM. (The mixture of DPPH reagent was shaken vigorously and allowed to stand at ambient temperature in the dark for 3 h.) After 30 minutes incubation period at room temperature, the absorbance was measured compare to blank at the wavelength of 517 nm of. Results were expressed as milligrams of gallic acid equivalents (GAE) per 100 g sample. All determinations were performed in triplicates.

Ferric reducing power (FRAP) assay

A potential antioxidant will reduce the ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}); the latter forms a blue complex (Fe^{2+}/TPTZ), The formation of blue color Fe^{2+}-TPTZ complex (Fe^{2+}tripyridyltriazine) which increases the absorbance at 595 nm. Briefly, the FRAP reagent was prepared by mixing acetate buffer (300 µM, pH 3.6), a solution of 10 µM TPTZ in 40 µM HCl, and 20 µM $FeCl_3$ at 10:1:1 (v/v/v). The reagent (270 µl) was mixed with sample solutions (30 µl) before subjected to measure absorbance at 595 nm after the mixture was incubated for 30 minutes in the dark room. Standard curve was prepared using different concentration. Results were expressed as $FeSO_4$ equivalent milligrams per 100 grams sample (mg $FeSO_4$/100 g of sample). All determinations were performed in triplicates [8].

RESULTS AND DISCUSSION

The content of total phenolic, flavonoid and antioxidant activity varied in different solvents were showed in fig. 1, 2, 3 and 4 respectively. In the fig. 1, the water extract demonstrated the highest of total phenolic content at about 1,955.23±60.87 mg GAE/100 g sample. Moreover, the total phenolic content of water extract was significantly ($P<0.05$) different from the ethyl acetate, methanol, dichloromethane, ethanol and hexane. The research conducted by Yilmaz and Toledo, [9] confirmed that water is better solvents when compared with ethanol or acetone. It appears from our work that the vast majority of polyphenol are water soluble. Moreover, Turkman [10] reported that solvents with different polarity (ethanol and water) have significant effect on polyphenol content.

The flavonoid content shown in fig. 2, among the solvents test found that methanol extract (321.15±9.12 mg $FeSO_4$/100 g sample) was highest than other solvents. This result is same with Abugri and McElhenney [11] who found that methanol was considered to be the best solvent for extraction of total flavonoid in *Ganodermaap planatum* and *Fomes fomentarius*. The effectiveness of solvent in extractions of total phenolic and total flavonoid may also depend on the moisture content and particle size of the plant species studied. It is known that some solvent is not effective in isolating certain compounds when large particle size is used within a short reaction time and temperature [12].

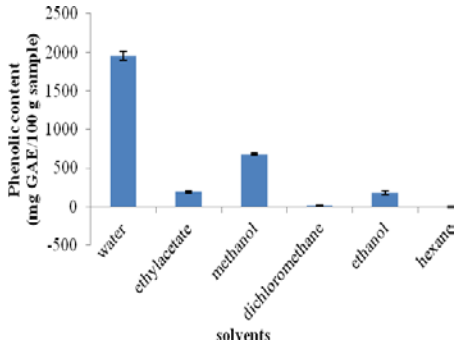

Fig. 1: Effect of solvent types on the average phenolic content of Trigasornmas formula

Because of the solvent is inability to permeate the tissue. The moisture content could also effective the extraction ability because of high water content in sample, which could dilute the concentration of the total phenolic and total flavonoid content in such tissue resulting in low absorbance reading. Since the coloumetric analysis depends solely on the intensity of the complex form. The lesser the color the lower the absorbance might be and could affect the total yield of the phenolic profile.

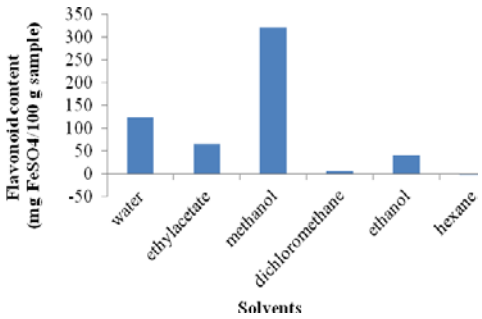

Fig. 2: Effect of solvent types on the average flavonoid content of Trigasornmas formula

Fig. 3 and 4 showed the antioxidant activity in DPPH radical scavenging and reducing ability (FRAP). It has been proved that for each solvent, with the proton gation of the extraction time, obtained solution deactivate radical to higher extent. The antioxidant in DPPH and FRAP assay of the water extract showed the highest content and following methanol extract, These data show a relation between the content of DPPH method and FRAP ability. The extraction of antioxidant activity from sample is directly related to the compatibility of the compounds with the solvent [13]. Based on these results it is assumed that formula contains diverse antioxidant compounds with different polarity. Previous studied by Thoo [14] and Durling [15] showed that the extraction of antioxidant compounds maximized at low concentration of ethanol contains higher proportion of hydrophilic compounds. In this study, scavenging activity of formula of different extracts increased considerably when the polarity of the solvents increased. Kim [16] also found similar phenomenon in mulberry leaves. Thus it is revealed that solvents with different polarity significantly can alter the DPPH radical scavenging activity.

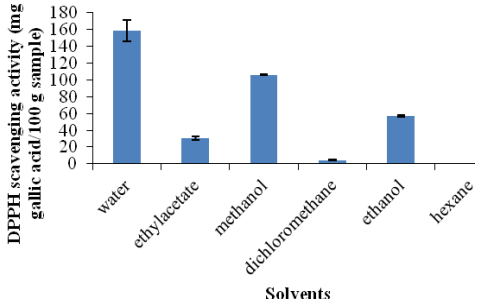

Fig. 3: Effect of solvent types on the average antioxidant activity by DPPH method of Trigasornmas formula

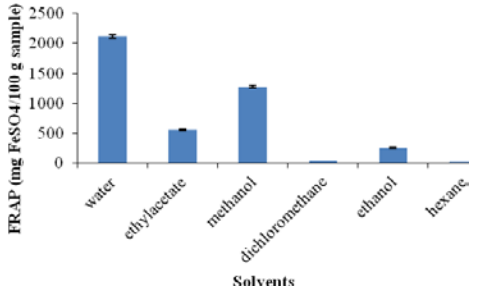

Fig. 3: Effect of solvent types on the average antioxidant activity by FRAP method of Trigasornmas formula

CONCLUSION

This study has found that water extracted is the most efficient solvent for extraction of phenolic content and antioxidant activities. While for flavonoid content, flavonoid content methanol extract is the most efficient solvent. Trigasornmas formula has high potential for patients recovering from an illness. The close investigations into the *in vitro* and *in vivo* activities by the formula are therefore warranted and current being pursed in the current author's laboratory. It may be worthwhile to investigate another activity together with studying chemical compounds responsible for the activities further.

CONFLICT OF INTERESTS

Declared None

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