http://www.scirp.org/journal/ns Natural Science, 2019, Vol. 11, (No. 4), pp: 99-105

Optimizing Extraction of Phenolics and Flavonoids from *Solanum ferox* Fruit

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Keywords: Phenolics, Flavonoid, *Solanum ferox*

Received: March 24, 2019 **Accepted:** April 16, 2019 **Published:** April 19, 2019

ABSTRACT

Various phenolic and flavonoid compounds that are secondary plant metabolites are known to contribute to physiological wellbeing. Extraction efficiency of such compounds from plant sources is dependent on the extraction solvent type and composition, and its pH. In this study, different extraction variables were examined: heating time (20 to 180 min), temperature (60˚C, 75˚C and 90˚C) and pH (2.5, 3.0, 4.0, 5.0, 6.0 and 7.0). Hot water was used in the extraction of dry samples. For phenolics, the most efficient extraction was by using water at 60˚C for 180 min, whereby 5.95 mg GA equivalent/dry extract was achieved. The most efficient extraction of flavonoids was achieved with water at 60˚C for 150 min, whereby 43 µg Quercetin equivalent/dry extract was obtained. Adjusting the solvent to pH 2.5 increased the yield to 45.3 µg Quercetin equivalent/dry extract.

1. INTRODUCTION

Terung Asam or *Solanum ferox* is traditionally planted in Malaysia for its fruit, commonly alongside hill paddy, and is popularly cultivated throughout the state of Sarawak. The average yield of this crop is between 16 to 20 tonnes per hectare. With current good market prices fetching RM6.00 - 10.00 per kg, depending on the size and quality, this plant has good prospects as a cash crop that should be promoted. In October 2010, the Department of Agriculture Sarawak filed Terung Asam for Geographical Indication (GI) certification. This plant has been listed in the ethnobotanical inventory of medicinal plants (Barbosa Filho *et al.* 1991) [1] for its medicinal properties, reportedly effective for the treatment of fever, vomiting, sore throat, and gonorrhoea. In India it is used to treat female sexual disorders. Its roots and berries are said to be anti-bechic, anti-asthmatic, anti-rheumatic, anti-viral, anti-cancer, and spermicidal (Joy *et al.*, 2001) [2].

The *Solanum* ferox fruit is rich in phenolic compounds, equipping it with powerful free radical scavenging activity (Oszmiański, 2014) [3]. Phenolics, together with flavonoids, are reported to have multiple

biological effects, including anti-oxidant and anti-inflammatory properties. Recent evidence suggests that diets rich in polyphenolic compounds play a significant role in combating oxidative stress-related disorders (Laouini *et al*., 2016) [4]. The extraction of polyphenols and flavonoids from plant sources is demanding owing to their chemical structure and their interaction with other food components. Many factors, such as solvent composition, extraction duration, temperature, pH, solid-to-liquid ratio and particle size may significantly influence the solid-liquid extraction process (Wang and Provan, 2004; Kos *et al*., 2005) [5, 6]. In this regard, there is a growing interest in the development of efficient and environmentally acceptable extraction methods. The desirable features characterizing "green" extraction methods are low solvent consumption, short extraction time, and high extraction yield. The extraction process and further stages in preparing the product and specifying its bioactive agents require a great deal of caution. In spite of the development of new extraction techniques, the efficiency of the process can be broadly determined by the selection of suitable solvents, optimal temperature, and extraction duration. The aim of this study is to examine the extraction parameters: temperature, extraction time and pH on phenolic and flavonoid yield extraction from *Solanum ferox* fruits.

2. MATERIALS AND METHODS

2.1. Sample Preparation and Extraction

Fruits of "terungasam" (**Figure 1**) were obtained from a farm in Kuching, Sarawak, Malaysia in November 2017. The fruits were cut and dried immediately in an oven at 55˚C for 3 days. They were then ground using a blender and packed in plastic bags for storage in a dark, dry and cool environment. Distilled water was used for the extraction that was performed at three different temperatures (60˚C, 75˚C or 90˚C) for varying durations: 20, 30, 60, 90, 120, 150, and 180 mins. Approximately 50 g of fruit sample was extracted with 1000 mL distilled water contained in a 2 L beaker that was heated using hot plate and stirrer with speed of 90 rpm was used. The extraction mixture was then centrifuged for 15 mins, 5000 rpm at 4˚C and the recovered supernatant was stored at −18˚C until analysis (within 7 days).

2.2. Determination of Phenolic Content

Total phenolic content (TPC) in each extract was determined using the Folin-Ciocalteu (FC) method described by McDonald *et al.* (2001) [7]. The extract (100 µl) was added to 0.2 mL FC reagent (5-fold diluted with distilled water) and mixed thoroughly for 3 minutes. Sodium carbonate (0.2 mL, 10% w/v) was added to the mixture; the mixture was then allowed to stand for 30 minutes at room temperature. The absorbance of the mixture was measured at 760 nm using a Jasco V-550 UV-VIS spectrophotometer (Jasco, Tokyo, Japan). TPC was expressed as milligram gallic acid equivalent per gram dry extract (mg GAE/mg dry extract).

Figure 1. Whole fruit of *Solanum ferox* (terungasam).

2.3. Determination of Total Flavonoid Content

The total flavonoid content (TFC) in each extract was investigated using the aluminium chloride colorimetry method described by Chang *et al.* (2002) [8]. The sample extract (0.1 ml) was mixed with 0.1 mL of 10% (w/v) aluminium chloride solution and 0.1 mL 0.1 mM potassium acetate solution. The mixture was kept at room temperature for 30 minutes, following which its absorbance was measured at 415 nm using a UV-VIS spectrophotometer. TFC was expressed as microgram quercetin equivalent per gram dry extract (µg QCE/mg dry extract).

2.4. Statistical Analyses

A completely randomised design was used for the experiment. Statistical analysis was carried out using the SPSS Package. Measurements were expressed as the mean ± standard deviation.

3. RESULTS AND DISCUSSION

The natural phenolics, including polyphenols, are secondary metabolites of plants which exhibit antioxidant activity that confers various health benefits (Bravo, 1998) [9]. Polyphenols are among the large numbers of natural phenolic compounds found abundantly in plants. Research on phenolic compounds has been growing lately because of their rising demand worldwide and increasing application in the food industry. Their beneficial effects are attributed to anti-oxidant, anti-cancer and superoxide radical scavenging activity (Djeridane *et al.*, 2006) [10]. Polyphenols in plant extracts react with the Folin-Ciocalteu reagent to form a blue complex that can be quantified by visible-light spectrophotometry.

In the present study, the total phenolic compounds of *Solanum ferox* obtained by water extraction ranged from 2.29 to 6.01 mg GAE/g dry extract (**Figure 2**). Extraction was most successful at 90˚C for 120 mins (yielding 6.01 mg GAE/mg dry extract), followed by extraction at 60˚C for 180 mins and 150 mins to give 5.95 and 5.90 mg GAE/mg dry extract, respectively. Rowena *et al.* (2009) [11] reported in their study that *Solanum tuberosum* var Bengueta had the highest extracted phenolic content of 50.0 ± 1.5 mg GAE)/100 g (dry basis).

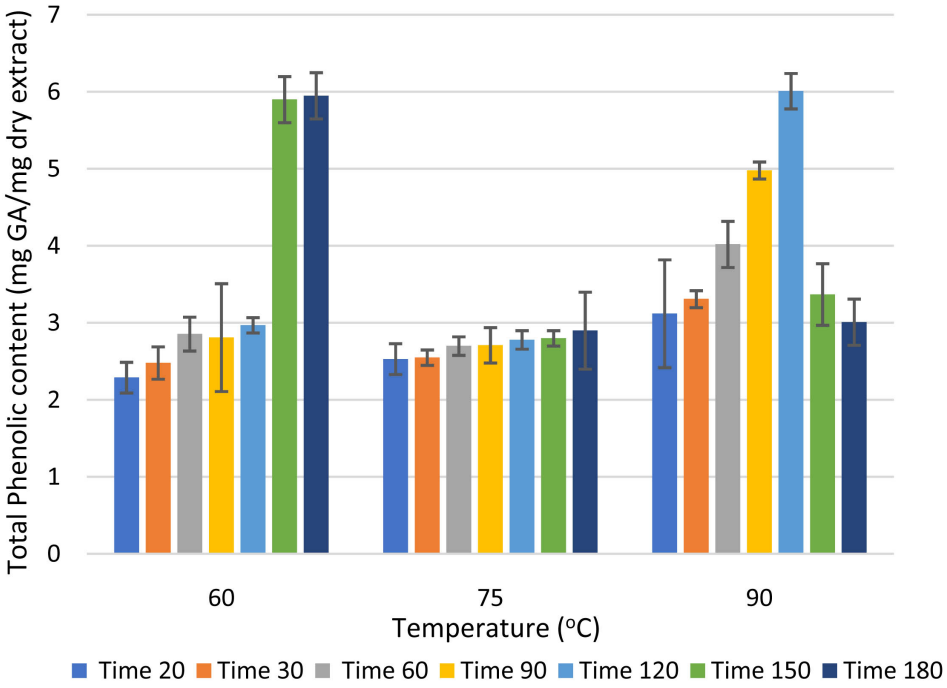

Figure 2. Total *Solanum ferox* fruit phenolic content extracted at different temperatures and durations.

TPC was markedly higher in fresh matured brinjals with dark purple lines (skin colour) (*Solanum melongethan*) than in the other samples (Somawathi *et al.* 2014) [12]. Nisha *et al.* (2009) [13] found the TPC of brinjal to be in the range of 49.02 ± 1.3 - 106.98 ± 2.2 mg GAE/100 g.

Aqueous extracts of pH 4 had the highest TPC at 3.68 ± 0.1 mg GAE/mg DW (**Figure 3**). This was followed by extractions at pH 3 (3.26 ± 0.3 mg GAE/mg DW), pH 2.5 (3.21 ± 0.2 mg GAE/mg DW), pH 6 (3.18 ± 0.1 mg GAE/mg DW) and pH 7 (2.45 ± 0.5 mg GAE/g DW).

The phenolic constituent plays an important role in plants as they scavenge injurious free radicals such as superoxide and hydroxyl radicals (Dewick, 2001) [14]. Phenolic acids have reportedly been implicated as natural antioxidants in fruits, contributing to their nutritional value in providing health beneficial effects (Sulaiman and Indira, 2012) [15]. In general, the results of plant extraction analysis for phenolic content changed according to the pH treatment. Aqueous extractants with low pH enhanced the phenolic content, with an optimum at pH 4. TPC from aqueous extracts of Algerian *Matricaria pubescens* was highest at pH 5, reaching 9.76 ± 0.32 mg GAE/mg DW (Laouini *et al.*, 2016) [4].

The total flavonoid content of water extracts from *Solanum ferox* fruit obtained at different temperatures and over different time durations is given in **Figure 4**. The extract had the highest flavonoid content when performed at 60˚C for 150 mins (43 µg QCE/mg dry extract). This was followed by the treatment at 60˚C for 180 mins (40 µg QCE/mg dry extract) and 90˚C for 120 mins (37 µg QCE/mg dry extract). Hsu *et al.* (2011) [16] found higher total flavonoid contents in aqueous extracts as compared with ethanolic extracts of *Solanum muricatum* fruits. Sudha *et al.*, (2011) [17] also made a similar observation; aqueous extracts were found to have higher flavonoid content than ethyl acetate extracts of *Solanum muricatum*. Flavonoids of fruits and vegetables, including those of *Solanum* spp., are known for their antioxidant capacity. In the present study, the water extract fruits of *Solanum ferox* were similarly found to have considerable amounts of flavonoids, indicating that this fruit might be a good source of antioxidant compounds.

The total flavonoid content of *Solanum ferox* fruits when extracted in water at different pH varied from 29.7 to 45.3 µg FA/mg dry extract (**Figure 5**). Extraction at pH 2.5 gave 45.3 µm FA/mg dry extract, but extraction efficiency declined with increasing pH. Thus, extractions at pH 3, 4, 5, 6 and 7 yielded 41.3, 36.9, 36.8, 31.3 and 29.7 µm FA/mg dry extract, respectively. This observation was contrary to that of Laouini *et al.* (2016) [4] who found total flavonoids extracted from Algerian *Matricaria pubescens* to increase with pH, being highest at pH 7. Accordingly, the effect of pH on flavonoid recovery was pH 7 > pH 6 > pH 4 > pH 3 in their results.

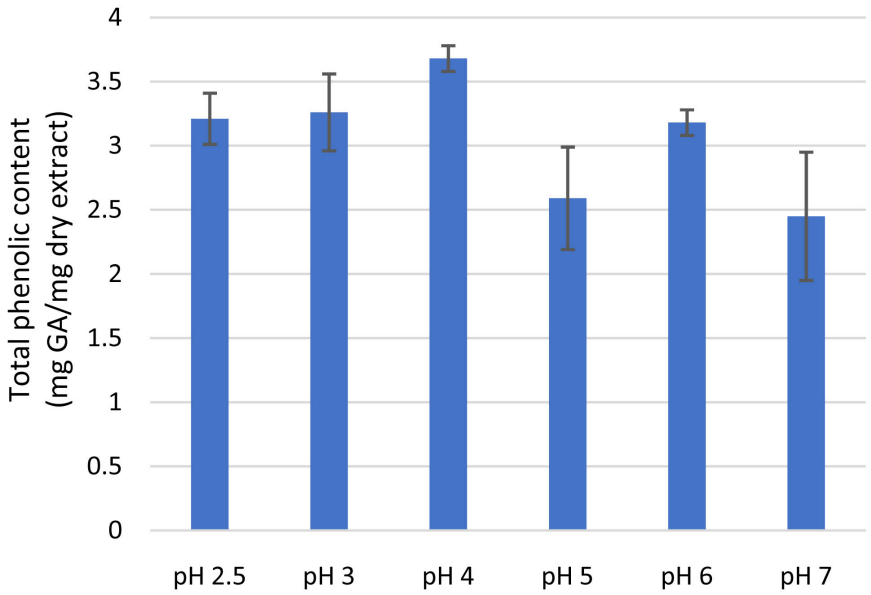

Figure 3. Effect of pH on extraction yield of phenolics from *Solanum ferox* fruits.

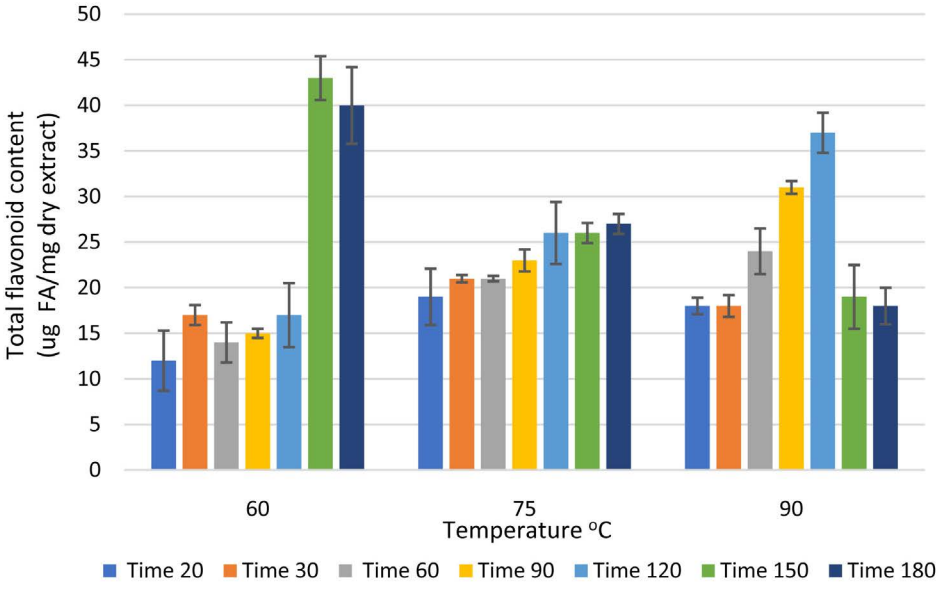

Figure 4. Total *Solanum ferox* fruit flavonoid content extracted at different temperatures and durations.

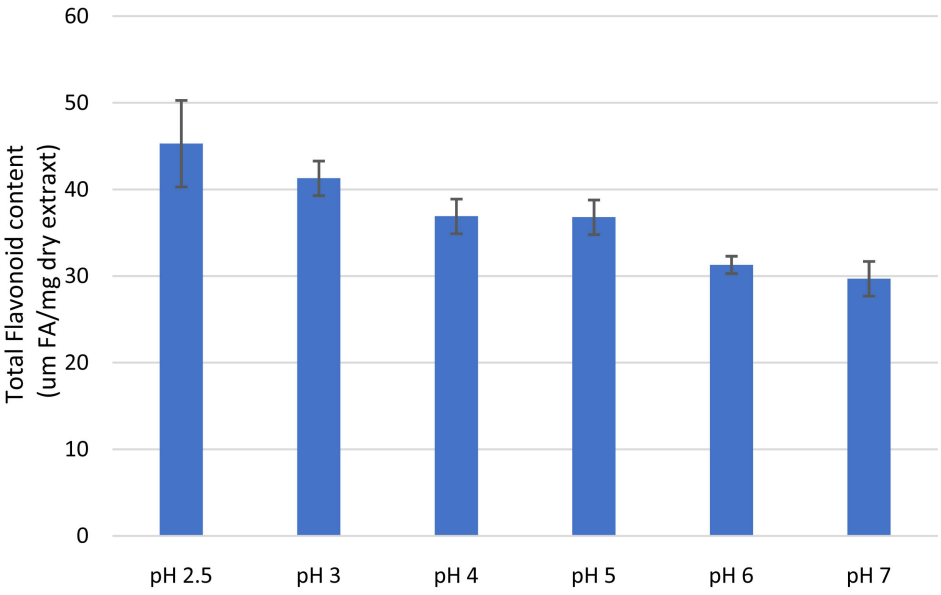

Figure 5. Effect of pH on extraction yield of flavonoids from *Solanum ferox* fruits.

It can be seen from the above that efficiency on the extraction of flavonoids is influenced by parameters such as temperature, solvent, pH, extraction pressure, among others, and their consequences may be either independent or interactive (Lu *et al.*, 2011) [18].

4. CONCLUSION

This study focused on maximizing the extraction of total phenolics and flavonoids contents in the *Solanum ferox* fruit. The effects of temperature of the extraction water, heating duration, and pH on yield were examined. The optimal extraction process for phenolics required a water temperature of 60˚C for 180 min. Maintaining the pH value at 4 was advantageous, as compared to pH values that were higher or lower. For flavonoids, the highest yield was obtained at a water temperature of 60˚C for a duration of 150

min. The extraction yield of flavonoids decreased as the pH rose from 2.5 to 7, indicating that the pH was crucial to the extraction yield of flavonoids. These findings further illustrate that water temperature, heating time, and pH affect the extracting process of these phytochemicals. Further studies should be conducted to explore and identify the specific components in the bioactive extracts of *Solanum ferox.*

CONFLICTS OF INTEREST

The authors declare no conflicts of interest regarding the publication of this paper.

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