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Effect of extraction temperatures on tannin content and antioxidant activity of *Quercus infectoria* (Manjakani)

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ABSTRACT

This study was specifically aimed to investigate the effects of extraction temperatures on tannin and antioxidant activity; the interaction between these two responses towards the processing parameter chosen. The main bioactive compound; tannin was extracted from *Quercus infectoria* (Manjakani) galls using aqueous decoction method. Few different extraction temperatures were selected prior for the extraction of tannin from *Q. infectoria* galls optimally. The selected temperatures were at 50, 75 and 100 °C and the effects of these temperatures on tannin content and antioxidant activity were examined thoroughly. High Performance Liquid Chromatography (HPLC) was used to identify and quantify the active compounds mainly tannic acid found in *Q. infectoria* galls. Alternatively, DPPH radical scavenging assay was performed to analyse the antioxidant activity and trends affected from the extraction temperature. The results demonstrated that *Q. infectoria* aqueous extract gives the highest tannin concentration of 2233.82 ± 1.311 and highest antioxidant activity approximately at 93.422 ± 0.256% at the extraction temperature of 75 °C. The outcomes of this study illustrate that extraction temperatures gave significant effects on the response variables (tannin content and antioxidant activity) respectively and the interaction between these responses were considered.

1. Introduction

Concurrently, there are numerous studies being conducted on the potential of traditional plants on their biological activity and effects towards medicinal purposes. The biological activities correlated with these plants are basically related to the bioactive compounds present within these plants which notably able to exhibit physiological actions on human body (Mohemmadi, 2011). There are wide variety of bioactive compounds that have captured numerous researcher interest nowadays since a decade ago and they are tannins, saponins, alkaloids, flavonoids and phenolic compounds (Hill, 1952). However, the extraction of active compounds is highly reliant on various factors including type of extraction, extraction temperatures, solvent of extraction and concentration, particle size of medicinal plants and duration of extraction (Liyana-Pathirana and Shahidi, 2004; Nobre et al., 2005). These crucial factors will contribute to the efficiency of the bioactive compound extraction process based on its rate of extraction and quality of the extracted bioactive compounds. In this research, the extraction temperature is mainly the principle factor to be examined. Generally, extraction temperatures can potentially affect the chemical compositions and bioactivities of the plant extracts (Chen et al., 2016).

Quercus infectoria galls on the other hand have been widely used as folk medicine to treat wide range of illness including swelling, inflammation, teeth infection and oral cavity, acute diarrhea and bleeding (Galla, 1911; Kaur et al., 2008). Since ancient time, Asians have used this medicinal plant traditionally to treat inflammation diseases. While in Malaysia, this plant is widely known as *Manjakani* which has been extensively used as herbal drink to restore uterine wall elasticity among women after childbirth (Chopra et al., 1956). It was claimed that the high content of tannins present in this herbal plant is the main contributor to these biological actions. As being reported by researchers, high content of bioactive constituents such as tannins, gallic acid and syringic acid found in *Q. infectoria* galls have greatly induced the biological reactions exhibited by these compounds (Dar et al., 1956: Ikram and Nowshad, 1977; Hwang et al., 2000).

Tannins are widely distributed among all plants (Serrano et al., 2009; Manach et al., 2004). The galls of *Q. infectoria* contained specifically about 50–70% of tannins in composition besides about 2–4% of gallic acid and traces of ellagic acids (Evans, 2002). Since galls extract was found to be high content in polyphenols, it can pose a potent reducing power. Hence, when being tested chemically, the gall extracts able to exhibit a potent antioxidant activity (Kaur et al., 2008). A

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previous study also claimed that tannins from *Q. infectoria* galls whether aqueous, methanolic or ethanolic extract have high antioxidant activities consecutively (Ayub et al., 2015). Tannic acid is also a naturally occurring plant polyphenol, which are composed of a central glucose molecule which will derivatize at its hydroxyl groups with one or more galloyl residues (Gulcin et al., 2010).

Oxidative stress on the other hand is an important risk factor that would contribute to various chronic diseases. Free radicals and other reactive oxygen species are recognized as the possible agents related in the pathogenesis of sickness such as inflammatory arthropathies, asthma, Parkinson's and Alzheimer's disease, diabetes, cancers and also artheroclerosis. Besides that, these free radicals and reactive oxygen species are also responsible in human aging (Kanwar et al., 2009; Chiavaroli et al., 2011). As such, antioxidants can be defined as a substance potentially in delaying or inhibiting the oxidative damage to a target molecule (Yamagishi and Matsui, 2011). Antioxidants are defined as compound which able to inhibit or delay the oxidation of other molecules by its inhibition or propagation of oxidizing chain reactions (Velioglu et al., 1998). An antioxidant can actually trap free radicals which the compounds could be either phenolic acids, polyphenols or even flavonoids that has the ability to scavenge free radicals including peroxide, hyperoxide or lipid peroxyl. These antioxidant compounds will eventually inhibit the oxidative mechanism that leads to degenerative diseases (Wu et al., 2011). Hence, antioxidants are potentially able to protect human body from free radicals and the ROS (Reactive Oxygen Species) effects. Since ancient time, plants antioxidant has been greatly considered as a good alternative compared to modern medicines.

Since the extraction of bioactive compounds mainly tannins from *Q. infectoria* is highly crucial for numerous purposes. Considering factors that could affect the whole extraction process is beneficial for various purposes. Consecutively, the total extraction process of plants extracts is highly impacted from the crucial factors affecting the extraction process and that includes the extraction temperatures. In extracting the desired active compounds from the plant extract, it is crucial to screen for the best extraction temperatures beforehand in order to obtain a high quality of extracted compounds for diverse usage whether in pharmaceuticals, medicinal or other industries.

However, none of the evidence on scientific studies reported previously on performing different extraction temperatures effects on antioxidant activity and tannin content of *Q. infectoria* galls using decoction as its extraction method. Generally, active compounds from different plant extract requires extraction temperatures differently in which in the whole process, extraction temperature could affect the entire extraction process of desired active compounds. Hence, this present study primarily aimed to investigate the effects of extraction temperatures on the active compounds content (Tannin) extracted from *Q. infectoria* galls as well as to evaluate its antioxidant activity trends of the extracted active compound using DPPH free radicals scavenging assay.

2. Materials and methods

2.1. Collection of plant material

The *Quercus infectoria* galls were purchased from a local herbal shop at Pasar Larkin, Johor Bahru and were sent to Food Laboratory at Institute of Bioproduct Development, Universiti Teknologi Malaysia, Skudai, Johor for further treatment.

2.2. Chemicals and reagents

2, 2-diphenyl-1-picrylhydrazyl (DPPH), Tannic Acid were purchased from Sigma-Aldrich (M) Sdn Bhd Chemicals.

2.3. Extraction of plant material

The raw material went for a pre-treatment process which includes cleaning and drying at 40 °C using a drying oven to remove excess water. The grinded galls were weighed at 5 g and placed in a beaker filled with distilled water at a solid to solvent ratio of 1:20. Decoction process were carried out at selected temperatures which were at 50 °C, 75 °C and 100 °C. All these extractions were carried at a specific extraction time which at 120 min according to the temperatures selected. Then, the extraction yield was subjected in the rotary evaporator at 40 °C to remove the excess solvent. After that, the yield of the extracted samples was calculated using the following equation:

$$\text{Percent of yield extraction (\%)} = \frac{\text{Final weight (g)}}{\text{Initial weight}} \times 100$$

2.4. Quantification of tannin using high performance liquid chromatography (HPLC)

The concentration of active constituents from the extracted compounds were analyzed using high performance liquid chromatography (HPLC) which was performed by Asghari et al. (2011) with slight modifications. 1 mg of *Q. infectoria* extract was dissolved in 1 ml of distilled water. Then, the mixture was filtered using 0.45 µm nylon filter membrane and injected into the HPLC system. Tannic acid was used a chemical marker in this experiment. High performance liquid chromatography was performed by reversed-phase HPLC on a C18 column by using a binary gradient elution. The gradient system consisted of solvent A (1% of orthophosphoric acid) and solvent B (100% acetonitrile) pumped at flow rate of 1 ml/min. The gradient started with 95% solution A and ended with 5% solution B. The column temperature was maintained at 30 °C. The sample peaks were identified by comparing with standard solution of tannic acid at 280 nm obtained from the assay. The concentration of the tannin acid was calculated using the appropriate calibration curves.

2.5. Antioxidant activity determination using DPPH radical scavenging assay

This assay was carried out according to the method of Miliauskas et al. (2004) with a slight modification. DPPH or 2,2-diphenyl-1-picrylhydrazyl is stable free radicals, which forms a purple-coloured solution when dissolved in methanol. Antioxidant components can scavenge free radicals and therefore the purple colour will be bleached out (colourless). Extract solution was prepared to a final concentration of methanolic extract at 1.0 mg/ml. Then, 1 mL of prepared methanolic extract at different concentration was mixed with 2 mL of 0.05 mM methanolic solution of DPPH. The mixture was placed in a dark place for 30 min at room temperature and the reduction of absorption reading was measured at 517 nm by using a spectrophotometer. Hence, free radical scavenging activity of the crude extracts was calculated based on the following formula:

$$\text{DPPH quenched(\%)} = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100$$

2.6. Statistical analysis

The data presented were analyzed by using SPSS 16.00. The significance difference between the data were analyzed by using one-way analysis of variance (ANOVA) and Tukey's test at 95% confidence level.

3. Results

Table 1 above shows the effects of extraction temperatures on concentration of tannic acid and its antioxidant activity. At minimum

Table 1
Concentration of tannic acid and antioxidant activity of tannin from *Q. infectoria* galls at selected temperatures of extraction.

Extraction temperature (°C)	Concentration of tannic acid (mg/g)	Antioxidant Activity (%)
50	1765.03 ± 2.373[a]	91.944 ± 0.256[a]
75	2233.82 ± 1.311[b]	93.422 ± 0.256[b]
100	2150.74 ± 0.810 [c]	90.761 ± 0.148[c]

Values are expressed as mean ± SE of triplicate measurement in which different letters for each column (a-c) are significantly different at $p < 0.05$.

Table 2
Antioxidant activity of tannin extracted from *Q. infectoria* galls at 75 °C with different range of concentrations.

Sample concentration (mg/ml)	DPPH Free Radicals Scavenging Activity (%)
0.02	6.504 ± 0.074[a]
0.04	14.708 ± 0.195[b]
0.06	44.420 ± 0.074[c]
0.08	83.888 ± 0.195[d]
0.10	92.683 ± 0.128[e]
0.12	93.422 ± 0.148[f]

Values are expressed as mean ± SE of triplicate measurement in which different letters for each column (a-f) are significantly different at $p < 0.05$.

temperature of 50 °C, tannic acid is at its minimal amount which was around 1765 mg/g with approximately 91.9% of antioxidant activity. However, as the temperature increases to 75 °C, it can be illustrated that high amount of tannic acid has been acquired with as potentially high antioxidant activity at approximately 93.4%. However, both tannin content and antioxidant activity declines as the extraction temperature reached 100 °C which is the boiling point of water (aqueous).

From Table 2 above it can be illustrated that the active compound extracted at 75 °C (optimum extraction temperature) was tested its efficacy in scavenging free radicals using at different concentration levels. Hence, the result above showed that as the active compound concentration increases, the trend of its scavenging ability also increases reaching maximum percentage of free radicals scavenge at approximately 93.4% at 0.12 mg/ml of tannin from *Q. infectoria* galls. From the trends above, it can be seen that higher concentration of tannin leads to higher ability of the extract to scavenge free radicals based on its percentage activity shown in Table 2.

Table 3 and Fig. 1 above shows the effects of tannin concentration on the extract antioxidant activity. As the concentration of tannin from extract galls (*Q. infectoria*) increases from 0.02 mg/ml to 0.12 mg/ml, the ability of the active compounds to scavenge free radicals also increases. The graph also showed the comparison between different tannin samples extracted at different temperature of extraction and how these factors affect the trend of antioxidant activity as shown in Fig. 1. IC_{50} values illustrated in Table 3 on the other hand clarifies on how effective the active compound (tannin) extracted from *Q. infectoria* galls in comparison with ascorbic acid as standard to combat free radicals at 50% inhibition.

The active compound (tannin) from *Q. infectoria* galls extract has been identified according to its retention time at wavelength of 280 nm as shown in Fig. 2 above.

Table 3
IC_{50} values of DPPH scavenging activity from *Q. infectoria* samples of various extraction temperatures.

Samples	IC_{50} values (mg/ml)
Ascorbic acid	0.048
50 °C	0.065
75 °C	0.064
100 °C	0.068

4. Discussion

Identification and quantification of tannic acid are performed by using High Performance Liquid Chromatography with UV detector was employed to identify and quantify tannic acid in *Q. infectoria* galls extract from different selection of extraction temperatures. The concentrations of tannic acid are determined by using the peak area from the calibration curves obtained as shown in Fig. 2 above.

The effects of extraction temperatures on phenolic compound mainly tannin found in *Q. infectoria* galls and its antioxidant capacities are showed in Table 2 and Fig. 1 above. The yield of active compound; tannin increased proportionally reaching its maximum peak which was at 75^{O}C with increasing extraction temperatures. However, the extraction of tannin started to subside once it reaches to its aqueous boiling point which was at 100^{O}C. On the other hand, the antioxidant activity for the corresponding tannin extracted showed similar trends whereby the highest scavenging ability achieved at 75 °C of extraction temperature whereby reaching its boiling point, the scavenging ability subside gradually.

Even though there was a study proven that heat possibly has the ability to enhance the recovery of phenolic compounds whereby higher temperatures of extraction is able to exhibit higher amount of phenolic compounds significantly in extracting both Indian medicinal plant and *mashua* respectively (Lim and Murtijaya, 2007; Silva et al., 2007). This was further clarified by scientific literature conducted by Al Farsi and Lee (2008) in which increment of extraction temperatures promote high extraction of active compounds especially those from phenolic groups whereby the increase of diffusion coefficient and its solid to solvent solubility are also increased that lead to greater extraction of active compounds.

Another related theory that could also explained the terms of extraction temperatures affecting the extraction of active compounds efficiency is that intense heat subjected to extraction solvent would also able to break the cell walls hence releasing the phenolics which result to a higher phenolic yield in an extract (Wang et al., 2007). In contrary, prolonged exposure or treatment with high temperatures could also lead to active compounds degradation. Some compounds could not withstand high temperatures which will lead to deterioration of their active compounds. Hence, a reduction in total amount of active constituents extracted from an extraction process can be comprehended. This matter could also explained the reduction of tannic acid content extracted from *Q. infectoria* galls extract above whereby at 100 °C (boiling point of water), the total amount of tannin reduced gradually. Thermal degradation of polyphenols (tannin itself) can be observed based on the trend portrayed in Table 1 and Fig. 1.

Additionally, the trend of antioxidant activity depicted in Tables 1 and 2 showed that tannic acid is proportionally related towards the scavenging ability of the extracted active compounds. High amount polyphenolic compound results in higher antioxidant activity since there are more active compounds to be able scavenging the available free radicals. Phenolic compounds commonly acknowledge as the natural antioxidant agents which act as free radical terminators (Shahidi et al., 1992). Several authors have observed a direct correlation between polyphenolic content and antioxidant activity (Rice-Evans et al., 1997). Thus, huge amount of research has focused on exploring plants with antioxidant capabilities. While on the other hand, free radicals exhibit mainly in many disorders such as neurodegenerative diseases, cancers and AIDS. There are many options in examining the antioxidant ability of plants extracts. However, DPPH instead is a stable free radical method is an uncomplicated, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts (Koleva et al., 2002). Table 3 shows the amount of each extract needed for 50% inhibition (IC_{50}).

Illustrated in Fig. 1 shows the response-dose curve of DPPH radical

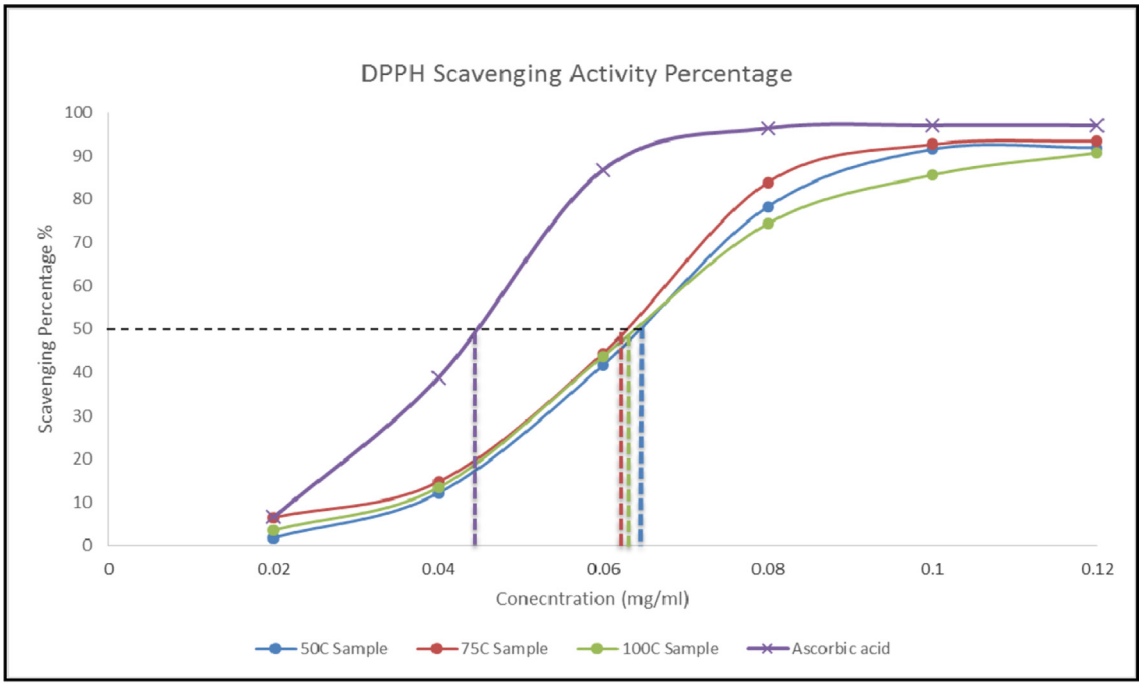

Fig. 1. DPPH Scavenging Activity of *Q. infectoria* samples from different extraction temperatures with its corresponding IC_{50} values and ascorbic acid as its standard comparison.

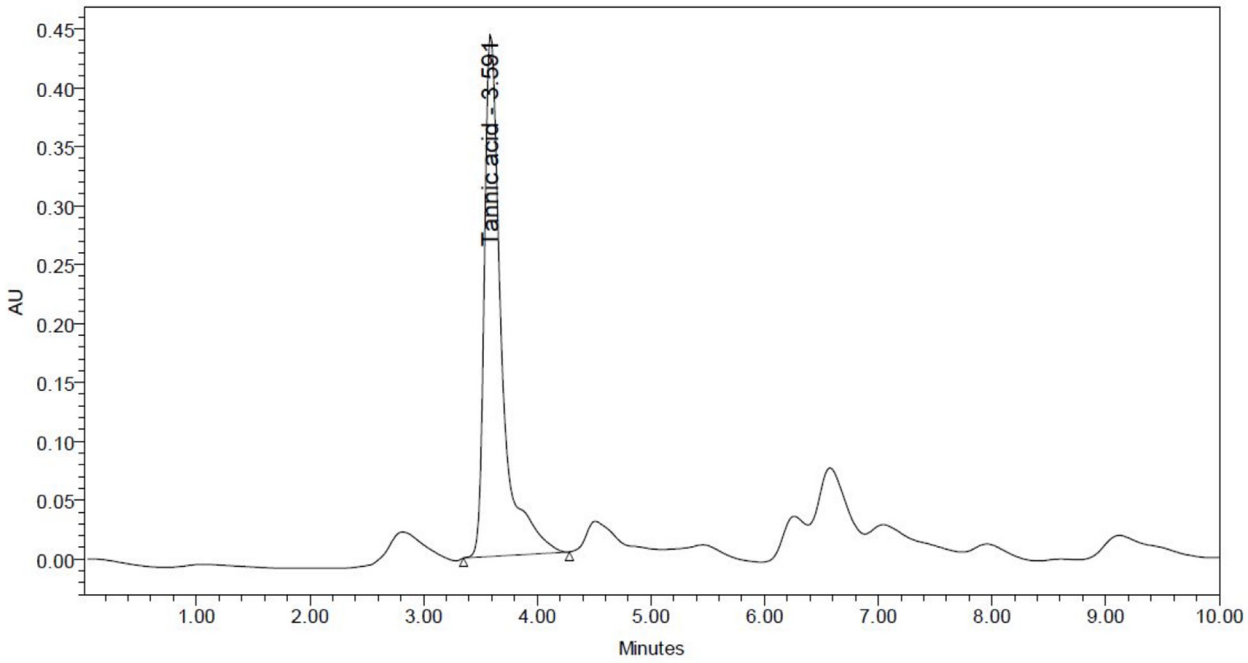

Fig. 2. Chromatogram of tannic acid from *Q. infectoria* galls extract at 75 °C extraction temperature.

scavenging activity of aqueous *Q. infectoria* galls extract from different extraction temperatures compared with ascorbic acid as its standard. It was observed that the aqueous extract of *Q. infectoria* at 75 °C extraction had higher antioxidant activity with the amount of 93.422 ± 0.256% than the other extracts. At 50 °C, with the concentration of extract only at 0.1 mg/ml, the scavenging activity of the extract maximally reached 91.574 ± 0.128% while with the same concentration at 100 °C the scavenging activity of the extract reached only 89.591 ± 0.510%. Hence, the highest antioxidant activity of the aqueous extract of *Q. infectoria* galls was at 75 °C of extraction temperature. While 0.12 mg/ml of the extract is needed to scavenge free radicals optimally about 93.422 ± 0.148%. These scavenging effects exhibited profoundly based on the proton-donor ability as it could serve as free radical scavengers, possibly acting as a primary antioxidants (Adedapo et al., 2009). Generally, plant polyphenols could act as reducing agents and antioxidants based on the hydrogen-donating property of their hydroxyl group (Aberoumand and Deokule, 2008). Hence, the antioxidant activities portrayed in this study are explained by these plant polyphenols mainly tannin which are abundantly found in the *Q. infectoria* galls extract (Mahdi-Pour et al., 2012).

On the other hand, only 0.064 mg/ml is needed to reach at least 50% inhibition of free radicals as shown in Table 3. Low IC_{50} values

depicted that the active compound available in the extract is highly effective and is efficiently able to scavenge free radicals. Hence, the highest antioxidant activity observed at 75 °C extract conveyed low IC_{50} value which contributed at best scavenging ability compared to other sample extracts. In comparison with ascorbic acid, the optimal antioxidant activity achieved was at 97.081 ± 0.145% whereby it takes only 0.048 mg/ml of its content to achieve 50% of free radicals inhibition. This can be concluded that the efficiency of the *Q. infectoria* extract in emitting scavenging ability is comparable with the ascorbic acid standard and there are not much contrast in terms of its antioxidant activity and its half maximal inhibitory concentration (IC_{50}). Therefore, high antioxidant activity will exhibit low IC_{50} value and vice versa. In general, the radical scavenging activity of *Q. infectoria* extract increases as the concentration of extract increases.

5. Conclusion

Based on this present study, it can be deduced that extraction parameter plays a significant role in achieving optimal yet highly quality of the extracted bioactive compounds. Different plants comprise different active compounds which are responsible in exhibiting various human health benefits. Extraction temperatures are greatly important to properly distil out the active compounds from plant extracts. High temperatures may either boost up the amount of active compound extracted or degrades them. HPLC analysis has identified and quantified tannic acid in *Q. infectoria* galls extract. Tannic acid extracted possess as the main polyphenol responsible for the relatively high antioxidant ability and scavenging effects. On the other hand, it can also be concluded from this study that tannic acid from *Q. infectoria* galls extract has high potential as an effective naturally occurring antioxidants based on its high antioxidant activity and scavenge effects. Besides that, different extraction temperatures did not disrupt overall yield of tannin as its antioxidant activity are greater than 90% which is greatly beneficial for consumers. However, this is only a preliminary study on how extraction temperatures could give significant effects on the content of tannic acid from an extraction as well as how it affects the antioxidant activity. In future, this study could be further enhanced by proposing a proper statistical design to study various effects of extraction (temperature, solvent concentrations and time of extractions) at different levels upon its active compound concentration, antioxidant activity, its total protein content (TPC) and total flavonoid content (TFC). This proper statistical design can further design specific experiments that may include comparative tests, optimization and many others.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101104.

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