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GRADUATE RESEARCH ARTICLE

Preparation and Evaluation the Efficacy of Combined Mixture of Finger Root and Robusta Coffee Extracts on Antioxidative Activity

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ABSTRACT

Finger Root (FR) and Robusta Coffee (RC) are natural substances known to be beneficial for human health and used as a traditional medicine in various parts of the world. The efficacy of two commonly used extraction techniques [Ultrasound-Assisted Extraction (UAE) and maceration with stirring (MR)] and four different extraction solvents (50% ethanol, 80% ethanol, 100% ethanol and acetone) on antioxidant activity and the Total Phenolic Content (TPC) in both herbs were investigated. The antioxidant potentials of the samples were determined by reducing capacity with Folin-Ciocalteu reagent, while the radical scavenging activity using 1,1-Diphenyl2-Picryl Hydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzothia zoline-6-sulfonic acid) (ABTS) assays. FR and RC extracts from UAE and using 80% and 50% ethanol as an extraction solvent, respectively, showed the highest antioxidant capacity compared to the other extraction processes. The combined mixtures of FR and RC (CFR) in the weight ratios of 1:1 was prepared and evaluated the antioxidant activity. The CFR extract showed a significant increase of TPC and resulted in its strong radical scavenging activity, quite the same level in comparison to the model antioxidant substance, ascorbic acid, compared to when they were assayed independently. Stability results indicated that the TPC of CFR extract was slightly decreased, by around 8.7% and the IC_{50} values of CFR extract showed slightly increased from 7.13 to 9.06 µg/mL in DPPH assay and from 725.72 to 858.65 µg/mL ABTS assay after storage at room temperature over 1 month. Findings suggested that the extraction methods and different solvent polarity significantly affect polyphenol recovery. The combined mixture of FR and RC extracts, which is quite stable and enhances radical scavenging activity, is interesting to increase economic value and utilization in cosmetic industry.

Keywords: Fingerroot, Robusta coffee, antioxidant activity, total phenolic content

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1. Introduction

Natural antioxidants play a potential role in preventing oxidative stress and also certain degenerative diseases [1]. Two medically acclaimed plants were included in this study: Fingerroot (FR) and robusta coffee (RC). The rhizome of FR (*Boesenbergia pandurata*, Family Zingiberaceae) has been widely used as food and traditionally used for several diseases [2]. Its phytochemicals, flavonoids and polyphenols, showed very promising activities, such as anti-inflammatory, anti-bacterial, anti-fungal and strong antioxidant [2, 3]. Another attractive plant, the seed of RC (*Coffea robusta*, Family Rubiaceae), is reported to contain high amounts of proanthocyanidins, phenols (chlorogenic acid, caffeic acid), caffeine, tocopherol and other constituents, that showed promising biological activity properties including remarkable antioxidant activity [4, 5]. The antioxidant capacity not only depends on the extraction method but also on the solvent used for extraction. However, little is known about how to efficiently extract these phenolic compounds from FR and RC. Such knowledge could be useful for facilitating future applications of these bioactive compounds. Plants are composed of several potential sources of potent biological compounds. Using plants in combination with other plants is a quite new approach to increase the efficacy and could lead to the optimization of bioactivities [6]. The present study was aimed to investigate the impact of different processes on TPC and antioxidant activity by in vitro methods, DPPH and ABTS, from FR and AC. Moreover, the effect of combined mixture of FE and RC extracts (1:1 by weight) on the TPC and antioxidant capacity and stability of the combined mixture were also evaluated. The results of this research will be beneficial for alternative medicine and cosmetic products.

2. Experimental Procedure

2.1 Comparison of Extraction Methods and Solvents

Two different extraction methods [ultrasound-assisted extraction (UAE) and maceration with stirring (MS)] with four different extraction solvents (50% ethanol, 80% ethanol, 100% ethanol and acetone) on the TPC and antioxidative activity of two samples (FR and RC) were investigated. Briefly, the extraction process was provided to prepare the extracts using solvent to dried sample powder ratio 100:1. The extraction time of maceration with stirring and ultrasonication techniques were carried out for 24 hours and 30 min, respectively. Three replicate extractions of each sample were carried out.

2.2 Determination of Total Phenolic Content

The total phenolic content (TPC) of the obtained extracts was determined using the Folin-Ciocalteu colorimetric assay, described by N. Siddiqui et al. [7] with minor modifications. Briefly, 1 mL of the diluted sample or gallic acid standard solution was mixed with 5 mL of 10% v/v Folin-Ciocalteu reagent, and afterwards 4 mL of 7.5% w/v Na_2CO_3 was added, then incubated in the dark at room temperature for 30 min before the absorbance was spectrophotometrically measured at 765 nm. The calibration curve was established using 5-50 µg/mL gallic acid solution (Figure 1). The amount of total phenolics in each extract was calculated from the calibration curve and expressed as mg of gallic acid equivalent (GAE) per gram of dry plant extract.

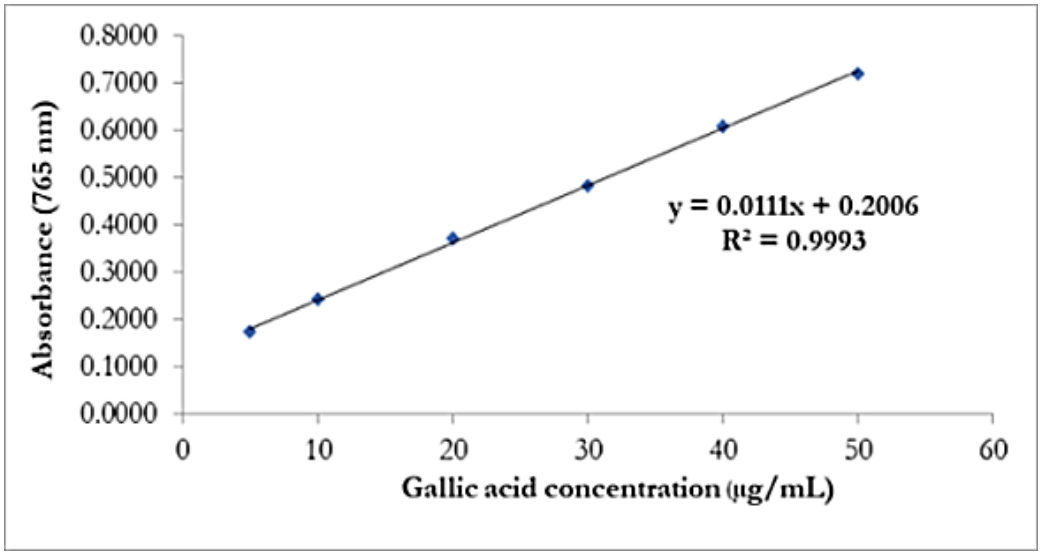

Figure 1: *Calibration curve for gallic acid (5-50 µg/mL)*

2.3 Determination of Antioxidant Capacity

2.3.1 DPPH Radical Scavenging Activity

The free radical scavenging ability of the extracts was tested by DPPH radical scavenging assay as described by R. Ahmad et al. [8] with few modifications. 100 µL of extract solutions at various concentrations were added to 1900 µL of 0.3 mM DPPH radical solution in methanol. The mixture was vortexed thoroughly and incubated in the dark at room temperature for 20 minutes, then the absorbance of the mixture was measured at 517 nm. Ascorbic acid was used as the standard for a calibration curve. The inhibition of DPPH radical by the sample was calculated according to the following equation:

$$\% \text{ DPPH Radical scavenging activity} = (A_0 - A_1)/A_0 \times 100$$

where A_0 and A_1 are the absorbance of the control and the test extract/standard, respectively. The extract concentration providing 50% of free radical inhibition (IC_{50}) was calculated from the graph of inhibition percentage plotted against test extract concentration.

2.3.2 ABTS Radical Scavenging Activity

The ABTS radical scavenging activity assay was carried out following the previously reported method by Re et al. [9] with some modifications. The radical cation ($ABTS^+$) was generated by oxidation of 7 mM ABTS solution with 2.45 mM potassium persulfate and left the mixture to stand in the dark at room temperature for 15 hours before use. This solution was diluted with methanol to an absorbance of 0.700 ± 0.02 at 734 nm. For assay, 100 µL of extract solutions at various concentrations were then added to 2 mL of $ABTS^+$ solution. The mixture was incubated at room temperature for 30 min and the absorbance at 734 nm was recorded. The standard curve was performed, as described previously, with ascorbic acid and the IC_{50} values were calculated from the curve of ABTS radical scavenging activity against extract concentration.

2.4 Effect of the Combined Mixture of FR and RC on TPC and Antioxidant Capacity

The appropriate extraction process which gave the highest extraction yields of phenolics and the lowest IC_{50} was selected for preparing FR and RC extracts. The combined mixture of FR and RC (CFR) was provided by mixing FR extract and RC extract in the weight ratio of 0.5:0.5 and evaluating the TPC and antioxidant capacity as described in triplicate.

2.5 Preliminary Stability Studies of CFR

The studies were carried out by keeping CFR which stored in airtight sealed vials for a period of one month and evaluated in terms of changes in TPC and antioxidant capacity retained in CFR.

2.6 Data Analysis and Statistics

Data expressed are means of three replicate determinations ± SD. Statistical analysis was performed using SPSS 16.0 and the level of statistical difference was considered at $p<0.05$.

3. Results and Discussion

3.1 Effect of Extraction Process on TPC

The impact of extraction process [two different methods (ultrasound-assisted extraction and maceration with stirring) and four different extraction solvents (50% ethanol, 80% ethanol, 100% ethanol and acetone] on the TPC from FR and RC extracts is shown in Figure 2. The values were compared to determine suitable method and solvent with the highest extraction of TPC efficiency. The results of analysis revealed that both extraction method and four solvents are significant factors affecting the amounts of total phenolics ($P<0.05$). Figure 2 depicted the values of TPC ranged from 38.47 ± 0.92 to 81.62 ± 2.61 mg GAE/g and extracts obtained by UAE resulted in high TPC compared to MS. The cavitation phenomenon of UAE results in the disruption effect within the matrix and increase extraction yields might be a possible explanation. The results clearly showed that, for both methods, the polarity of extraction solvents had a significant effect on the recovery of TPC from extracts. The highest value of TPC was observed in FR extract by UAE using 80% ethanol as a solvent (81.62 ± 2.61 mg GAE/g), whereas the highest value of TPC was observed in RC by UAE using 50% ethanol as a solvent (75.24 ± 1.53 mg GAE/g). The results might be due to the different dielectric constants/polarities of the solvents used, which selectively extracted targetable phenolic compounds, such as flavonoids from FR extracts and chlorogenic acid from RC extracts. These findings are in good agreement with the previous studies [10], which reported that 80% ethanol is an efficient solvent for recovery of total phenolics from FR. In addition, N. Saewan et al. [11] also found that 50% ethanol had better extraction yields of phenolics from RC in comparison with absolute ethanol, ethyl acetate and water. The results of investigation showed that the extraction process has been found to be the important role towards the TPC of extracts [12].

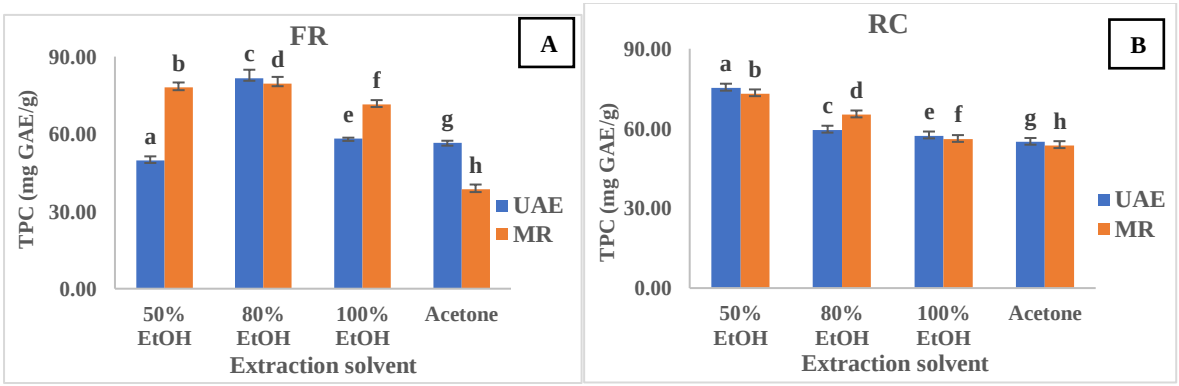

Figure 2: *Effect of different solvents on total phenolic contents in FR extract (A) and RC extract (B) obtained by UAE and MS methods (values mean ± SD for at least in triplicates. Columns with different superscript letters are significantly different at $p<0.05$).*

3.2 Effect of Extraction Process on Antioxidant Capacity

In order to obtain information about the ability of the test extracts on the radical scavenging activity two antioxidant assays, DPPH and ABTS, have been used in this study to evaluate the influence of different

extraction processes on the antioxidant properties of FR and RC. The results (Figures 3 and 4) stated that the extracts prepared by different solvents and different methods showed varying level of antioxidant properties. The IC_{50} of FR and RC extracts ranged from 50.18 - 206.21µg/mL by DPPH assay, whereas the IC_{50} of both extracts ranged from 848.17 - 3,505.50 µg/mL by ABTS assay. The results showed that the FR extracts prepared by UAE using 80% ethanol as solvent, showed the lowest IC_{50}, which is 50.18 ± 3.74 µg/mL by DPPH assay and 848.17 ± 66.79 µg/mL by ABTS assay, respectively. Similar trend was evident for RC extracts, which prepared by UAE and using 50% ethanol as solvent, showing the lowest IC_{50}, which is 56.18 ± 1.28 µg/mL by DPPH assay and 920.81 ± 0.41 µg/mL by ABTS assay, respectively. These results indicate a high capacity of both extracts to scavenge the free radicals of DPPH and ABTS. As shown in Figure 3 (D), there was not statistically significant different ($p<0.05$) in IC_{50} of RC among 50% ethanol used in UAE and MR. Therefore, UAE using 80% and 50% ethanol were selected as the extraction process of FR and RC, respectively, for the next step in the study. The results showed that phenolic compounds are important contributors to the radical scavenging activity of these plant extracts. Our findings are in agreement with several studies regarding high correlation between TPC in the extracts with their antioxidant activity [13, 14].

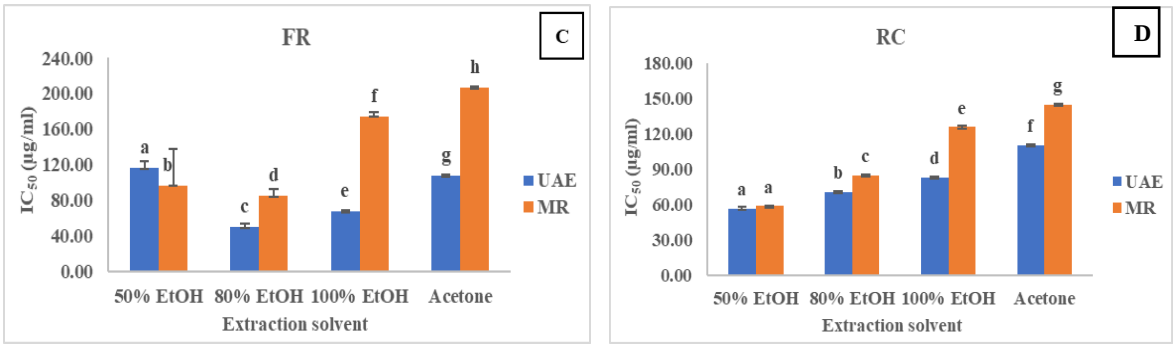

Figure 3: *The effect of different solvents on the recovery of antioxidant properties from FR extract (C) and RC extract (D) using DPPH assay (The values are mean ± SD for at least in triplicates. Columns with different superscript letters are significantly different at p<0.05).*

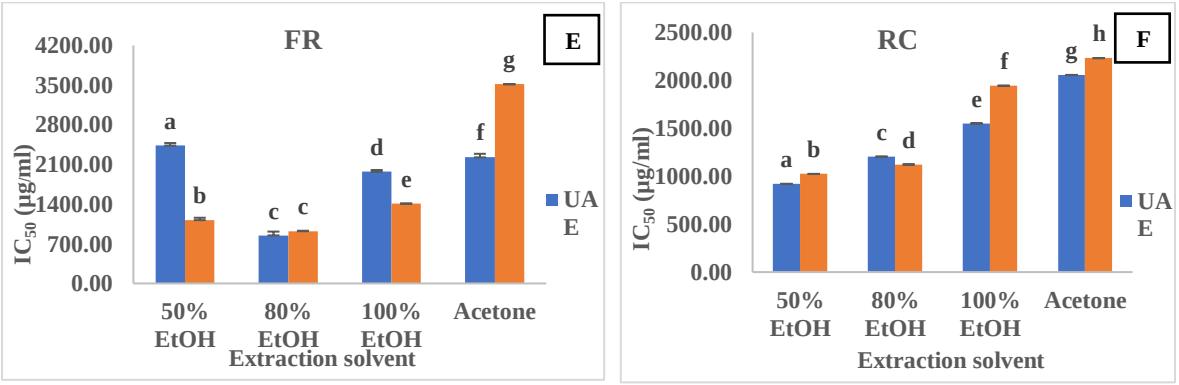

Figure 4: *The effect of different solvents on the recovery of antioxidant properties from FR extract (E) and RC extract (F) using ABTS assay (The values are mean ± SD for at least in triplicates. Columns with different superscript letters are significantly different at p<0.05).*

3.3 Effect of CFR on TPC and antioxidant capacity

The effect of each FR, RC and the combined CFR extracts on TPC and recovery of antioxidant properties were reported in Table 1 and compared to that of ascorbic acid. The IC_{50} values of ascorbic acid was 5.60 ± 0.50 µg/mL by DPPH method and 96.46 ± 0.69 µg/mL by ABTS method.

The results revealed that CFR showed a significant increase of TPC and resulted in its strong radical scavenging activity compared to when they are assayed independently. Its TPC value was 333.26 ± 0.88 mg GAE.g^{-1}, approximately 4 – 5-fold higher than the values of individual FR and RC extracts. As can be seen in Table 1, CFR exhibited the lowest value of IC_{50}, indicating the highest antioxidant property, with 7.13 ± 0.01 and 725.72 ± 0.52 µg/mL in DPPH and ABTS assays, respectively. Especially, the IC_{50} value of CFR in DPPH assay was much lower, approximately 8-fold, than the value of FR and RC extracts. Additionally, the scavenging ability of CFR using DPPH assay was quite the same level in comparison to the model antioxidant substance, ascorbic acid.

Table 1: *Total phenolic content (TPC) and IC_{50} of FR, RC, and CFR extracts*

Compound	TPC (Mg GAE/g)	IC_{50}(µg/mL)	
		DPPH	ABTS
FR extract	81.62 ± 2.61	50.18 ± 3.74	848.17 ± 66.79
RC extract	75.24 ± 1.53	56.18 ± 1.28	920.81 ± 0.41
CFR	333.26 ± 0.88	7.13 ± 0.01	725.72 ± 0.52

Each Value represents the mean (n=3) ± SD. Values within the columns are statistically significant ($p<0.05$).

3.4 Preliminary Stability Studies of CFR

The preliminary stability studies of CFR in terms of the TPC and antioxidant property during storage were also investigated. The visual assessment of CFR was also observed, prior to be analyzed, in order to evaluated changes in appearance. The results showed that color of CFR at room storage remained stable after 30 days. Table 2 indicated that the TPC of CFR was slightly decreased, by around 8.7% after storage at RT over 1 month. The strong antioxidant activity of the combined mixtures is most often correlated with high content of total phenols. Moreover, after stability period, the IC_{50} values of CFR in DPPH and ABTS assays showed slightly increased, indicating the lower scavenging ability, from 7.13 to 9.06 µg/mL and from 725.72 to 858.65 µg/mL, respectively. Results suggested that CFR was quite stable within 1 month at room storage.

Table 2: *Stability studies of CFR at RT*

Day	TPC (Mg GAE/g)	IC_{50}(µg/mL)	
		DPPH	ABTS
0	333.26 ± 0.88	7.13 ± 0.01	725.72 ± 0.52
30	304.31 ± 0.79	9.06 ± 0.01	858.65 ± 0.66

Each Value represents the mean (n=3) ± SD. Values within the columns are statistically significant ($p<0.05$).

4. Conclusions

The present study indicated that the different types of extraction method and solvents had a significant impact on the recovery yields of total phenolics and antioxidant potential of FR and RC. Comparing the extraction methods, UAE was more efficient than MR to extract the TPC exhibiting a higher antioxidant property. Regarding the polarities of solvent, 50% and 80% ethanol are the most suitable solvents and suggested for the extraction of antioxidant constituents from FR and RC using UAE. CFR containing FR and RC extracts in the weight ratio of 0.5:0.5 exerted more potent effects on free radicals than each one separately. The stability studies of CFR for a period of 1 month showed that the combined mixture is quite stable. Nevertheless, further studies are needed to identify bioactive substances in CFR that possess high amount of phenolic compounds and strong antioxidant activity. Some novel methods such as

nanoencapsulation of bioactive compounds have attracted much attentions and represent an effective approach to protect them against degradation, as well as to improve their bioavailability and stability.

5. Declarations

5.1 Competing Interests

The authors declare no conflict of interests.

5.2 Publisher's Note

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