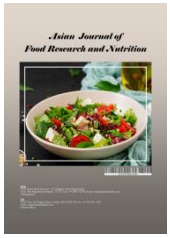

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Effects of Different Solvents and their Purity on the Extraction of Total Phenolic Content, Total Flavonoid Content and Antioxidant Activity from the Peels of Lotkon (*Baccaurea Motleyana Müll. Arg*.) and Longan (*Dimocarpus Longan Lour.)*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The objectives of this study was to investigate how three types of solvent (ethanol, methanol, and acetone) affected the yield of total phenolic content (TPC), total flavonoids content (TFC) and DPPH radical scavenging activity of extracts from lotkon and longan peels. The results showed that the 50% methanol extracts for longan peel displayed the highest levels of TPC, TFC, and DPPH free radical scavenging activities. The TPC varied for both peel extracts (longan and lotkon) from 343.39 ± 0.22^{j} to 425.55 ± 0.19^{a}, 121.63 ± 0.29^{j} to 321.54 ± 0.12^{a} mg GAE/100g dry weight; TFC from 48.24 ± 0.09^{j} to 86.24 ± 0.11^{a} , 6.47 ± 0.37^{g} to 15.37 ± 0.11^{a} mg QE/100g dry weight and DPPH radical scavenging activity from 30.79 ± 0.03^{j} to 60.01 ± 0.02^{a} %,25.84 ± 0.01^{j} to 54.76 ± 0.03^{a} % respectively. These findings showed that total phenolic, total flavonoid and antioxidant activity of lotkon and longan peel extracts are significantly influenced by the extraction solvents and their purity. The findings of this study serve as a valuable guide for the food sector in determining the ideal conditions for extracting antioxidants and desirable phenolic compounds from lotkon and longan peels, as well as a solid foundation for more research in the future.

Keywords: Antioxidant; extraction solvent; phenolic substances; lotkon and longan peels.

1. INTRODUCTION

Lotkon (*Baccaurea Motleyana Müll. Arg*.) is one of the most widely consumed fruits in Bangladesh and is a family member of the Phyllanthaceae. Lotkon is a significant evergreen, sluggish, dioecious fruit tree native to tropical and subtropical regions. Longan (*Dimocarpus Longan Lour*.), sometimes referred to as Kath Lichu regionally is a popular fruit that originates from China and Southeast Asia that that is a subtropical evergreen tree belongs to the Sapindaceae family . Due to the juicy and rich acidic flesh of longan, it is widely prized for its distinctive taste and flavor and is denoted as a snack eaten frequently [1]. According to Sruamsiri and Silman [2], longan contains approximately 12.4-19.6% peel. While lotkon contains approximately 36.11% peel of the whole weight [3]. According to Rakariyatham et al. [4], the peels of longans were found to contain more than 50 phenolic chemicals. Phenolics, flavonoids, tannins, related hydrolysis products, and variations are among the various types of phenolic chemicals. As stated by Nurmayani et al. [5], the fruit and its peel both contain antioxidants and phenolics, two naturally occurring protectors, in addition to the lotkon's nutritional and dietary worth. The major bioactive compounds present in lotkon peel are polyphenols, flavonoids, and tannins [6].

These bio-wastes can be used to make these antioxidants for practical use because longan and lotkon peels are both abundant sources of bioactive substances, and phenolic compounds are well known for their health benefits related to antioxidant properties as well as their potential application in food processing industries as bio preservatives that enable the production of food without synthetic chemicals for consumers [7]. Numerous investigations showed that the kind of solvent used has a significant impact on whether bioactive chemicals may be successfully extracted from plant material. Various phenolic compounds, including simple phenolics, phenolic acids, coumarins, lavonoids, stilbenes, hydrolyzable and condensed tannins, lignans, and lignins, are present in plants [8]. Extraction of bioactive compounds from plant material is typically performed using solvent extraction. Since the solvent system is simple to use and effective for the extraction of various chemicals, different solvents are employed to prepare extracts from plant material. The extraction yield of the compounds is affected by the physical and chemical properties of the sample, the type of solvent used, the length of time and temperature of the extraction process, and the ratio of the sample to the solvent [9]. Depending on the solvent's polarity and the solubility of phenolics in the solvent, polyphenols can be recovered from plant material [10]. It is common practice to extract antioxidants from plants using water and

aqueous solutions of ethanol, methanol, and acetone [11]. Solvent diffuses into the solid plant material during extraction, solubilizing substances of a comparable polarity [12]. The majority of polyphenols are extracted from plant material using polar solvents. Solvents like acetone, ethanol, methanol, and ethyl acetate are optimal for extracting polyphenolic chemicals [9]. The type of solvent used is crucial to the efficient extraction of bioactive chemicals from organic material [13].

Methanol was used to extract tea polyphenols [14] and acetone was used to extract wheat total phenolics [15], both of which were more successful than water. The polarity of the solvents was important to the extraction process, according to Alothman et al. [16] since it boosted the total solubility of antioxidant compounds from three tropical fruits pulps (honey pineapple, banana and thai seedless guava). According to Muhamad et al. [10], the extraction parameters and solvent polarity had an impact on the antioxidant, total flavonoid, and total phenolic content yields from *Averrhoa bilimbi* extract. There is a lack of research comparing how various solvents affect the extraction and antioxidant activity of longan and lotkon peel extract. This study aims to assess the effects of solvent type and their purity on the yields of total phenolic, total flavonoids and antioxidant activity in extracts of longan and lotkon peels.

2. MATERIALS AND METHODS

2.1 Chemical Reagent

Aluminum chloride, sodium hydroxide, ethanol, acetone, Follin's reagent were bought from Merck (Darmstadt, Germany). All additional chemicals and reagents utilized in the experiment were of analytical grade.

2.2 Sample Preparation

From the Jashore area of Khulna, Bangladesh, fruit samples of the lotkon (*Baccaurea Motleyana Müll*. *Arg*.) and longan (*Dimocarpus Longan Lour.)* were collected. Fruits were selected to ensure ripe as well as uniformity in size and color. The peels were hand detached after the fruits had been carefully cleansed with distilled water. Lotkon and longan peels were dried in an oven (Hanyangs Scientific Equipment Co. Ltd., Korea) at 60 ºC for 48 hrs and 60 ºC for 24 hrs respectively. Then peels were ground (maximum particle size 0.4 mm) into powder in a lab mill (FW100, Taisite Instrument Co., Ltd, Tianjin, China). The supplies were stored in a desiccator at room temperature before to use.

2.3 Extraction Procedures

With a small adjustment, extracts from samples of lotkon and longan fruits were made using the technique outlined by Addai et al. [17].

Using the maceration process, antioxidant and bioactive chemicals were extracted from fruit samples. For this procedure, 10 ml of solvent (1:10 w/v) was applied to universal bottles containing 1 gm of peel powder. Pure acetone, ethanol, and methanol are among the solvents, along with their corresponding aqueous solutions at 50% and 70% concentrations. After that, an ultra-turrax homogenizer was used to thoroughly blend the samples (peel powder with solvents) for 5 min. A tabletop centrifuge (DSC-200A-2, Digisystem Laboratory Instrument Inc., Taiwan) was used to centrifuge all of the extracted samples for 10 min at 3000 rpm. The supernatants were collected and stored at -20°C for further analysis.

2.4 Analysis

2.4.1 Total Phenolic Content (TPC)

The total phenolic content was calculated using a modified Folin-Ciocalteu method [18]. Briefly, 5 ml of Folin-Ciocalteu reagent (1:10 v/v in distilled water) and 4 ml of 7.5% (w/v) sodium carbonate solution were mixed with 1 ml of each extract (1 g/ml). The mixture was vortexed for 15 sec to enhance the color before being allowed to stand at 40°C for 30 min. In order to measure the absorbance at 765nm, a double beam UV-Vis spectrophotometer from Thermo Scientific was used (T60, UVV-is Spectrophotometer, USA). In place of the sample, water was used to prepare the blank. A collection of gallic acid reference solutions was used to compare a blank too. A calibration curve using gallic acid was used to determine the result as mg GAE/100g dry sample.

2.4.2 Total Flavonoid Content (TFC)

The total amount of flavonoid was measured by spectrophotometric method as described by Csepregi et al. [19]. After being combined with 0.3 mL of 5% $NaNO_2$, 1 mL of the sample was diluted with 3.5 mL of demineralized water. 0.4 mL of $AlCl_3$ (10% w/v) was added after 6 min.

Following the addition of 2.1 mL of 1 M NaOH, 6 min later, 2.7 mL of demineralized water was added right away, bringing the total to 10 mL. After thorough mixing, the solution's absorbance was determined using a standardized ultraviolet-visible spectrophotometer at 510nm. By comparing the result to the quercetin calibration curves, which were created under the same circumstances, the result was represented in mg quercetin equivalent/100gdried sample.

2.4.3 Antioxidant Activity

The modified approach reported by [20] was used to measure the stable DPPH radical-scavenging activity. In order to conduct this test, 2 ml of extract solutions in a range of concentrations were added to 2 ml of 0.1 mM DPPH solutions. The mixture was then vigorously stirred for 15 sec. The solutions were then allowed to stand for 30 min at room temperature in a dark environment in order for a reaction to happen. Utilizing a twin-beam Scientific UV-Vis Spectrophotometer at 517nm (T60, UV-Vis Spectrophotometer, USA), absorbance was measured after 30 min against a blank. Equation (1) was used to direct the calculation:

$$\% \text{ DPPH free radical scavenging activity} = \frac{\text{Ab (blank)-Ab (sample)}}{\text{Ab (blank)}} \times 100 \ldots\ldots\ldots \quad (1)$$

2.5 Statistical Analysis

Statistical Tool for Agricultural Research (STAR) software system was used to do an ANOVA on the triplicate data at a 5% level of significance.

3. RESULTS AND DISCUSSION

3.1 Total Phenolic Content (TPC)

Natural phenolics' major health benefits are mostly brought about by their antioxidant activity [21]. There is a correlation between the extraction yields of phenolic compounds and the kind of solvent used for the extraction, which might range from polar to non-polar [22]. Total phenolic content extraction yields from lotkon and longan peels were significantly (p<0.05) affected by solvent type and their purity.The findings demonstrated that longan peel extract had significantly more total phenolic content than lotkon peel extract (Fig. 1). Comparing the different solvent concentrations, 50% methanol had the highest amounts of TPC (425.55±0.19^{a} and 321.54±0.12^{a} mg GAE/100g dry weight) in longon and lotkon peel extracts respectively. While compared to other solvents, water resulted in lower yields of TPC (343.39±0.22^{j} and121.63±0.29^{j} mg GAE/100g dry weight) in longon and lotkon peel extracts respectively. The TPC value was influenced by the extracting solvents in the following order, from high to low: 50% methanol >50% ethanol>50% acetone. These results agree with Park et al. [23], who discovered that methanol provided the best TPC recovery from orange peel. Similar findings were reported by Ali et al. [24], who found that methanol was the most effective solvent for extracting phenolic content from ginger fruit. According to Turkmen et al. [25], solvents with various polarities considerably affected phenolic compounds. Muhamad et al. [10] also found that *Averrhoa bilimbi* had the maximum total phenolic levels at a solvent concentration of 50% methanol. Our findings also agree with Addai et al. [17], who discovered that methanol was the most effective solvent for extracting phenolic content from papaya fruit. The highest extraction yield was observed with methanol, which may be a result of the reduced polyphenol oxidase (PPO) activity in these extracts which is an enzyme that is responsible for the oxidation of phenolic substances [13].

3.2 Total Flavonoid Content (TFC)

Flavonoids, which include flavones, flavanols, and condensed tannins, are common secondary metabolites of plants. Fig. 2 shows the total flavonoid content extraction yields from lotkon and longan peels were significantly (p<0.05) affected by solvent type and their purity. Methanol at 50% had the highest amounts of TFC (86.24±0.11^{a} and 15.37±0.11^{a} mg QE/100g dry weight) in longon and lotkon peel extracts respectively. While water resulted in lower yields of TFC (48.24±0.09^{j} and 6.47±0.37^{g} mg QE/100g dry weight) in longon and lotkon peel extracts respectively. The TFC yields by extraction solvents were as follows, from highest to lowest: 50% methanol >50% ethanol>50% acetone.

These findings agree with Ma et al. [26], who found that methanol was the most efficient solvent for extraction for the flavonoid compound from Penggan (*Citrus reticulata*) peel. Similar findings were reported by Gonzalez et al. [27], who found that extraction efficiency of TFC changes with polarity of solvents in lemon peels. Muhamad et al. [10] reported that *Averrhoa*

bilimbi had the maximum total flavonoid content at a solvent concentration of 50% methanol. Our findings also agree with Addai et al. [17], who discovered that methanol proved to be the most efficient solvent for extracting phenolic content from papaya fruit. Abad-García et al. [13] reported that methanol produced highest for extraction for the flavonoid compound due to reduced the activity of polyphenol oxidase (PPO) enzymes which responsible for the oxidation of flavonoid compounds.

3.3 DPPH Free Radical Scavenging Activity

Antioxidants are recognized to have a crucial function in preventing oxidative cell damage [28]. Dietary antioxidants, which can scavenge free radicals, can reduce the risk of the disease. As a result, determining the radical scavenging efficacy of antioxidants is essential [29]. Fig. 3 shows that extraction solvents and their purity significantly affected the antioxidant activity of the lotkon and longan peel extracts. The results showed that longan peel extract had significantly (p<0.05) higher scavenging activity than lotkon peel extract. The DPPH values of both extracted peels (lotkon and longan) decrease as the concentration of the organic solvent increases until it reaches 100%. Methanol at 50% had the highest value of DPPH (60.01±0.02^{a} and 54.76±0.03^{a} %) in longon and lotkon peel extracts respectively. Water extracts produced the lowest DPPH values for longan (30.79±0.03^{j} %) and lotkon (25.84±0.01^{j} %). The DPPH value was influenced by the extracting solvents in the following order, from high to low: 50% methanol >50% ethanol>50% acetone.

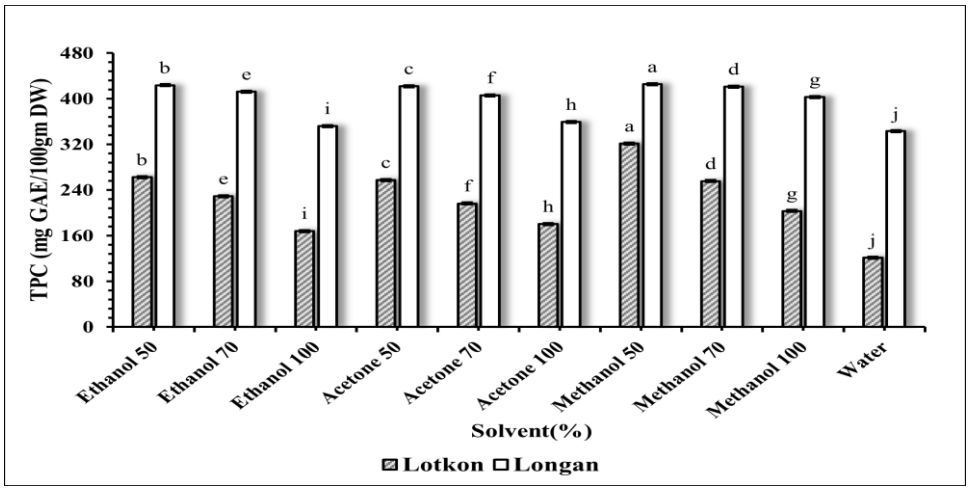

Fig. 1. Effect of different solvents and their purity on TPC of lotkon and longan peels extract

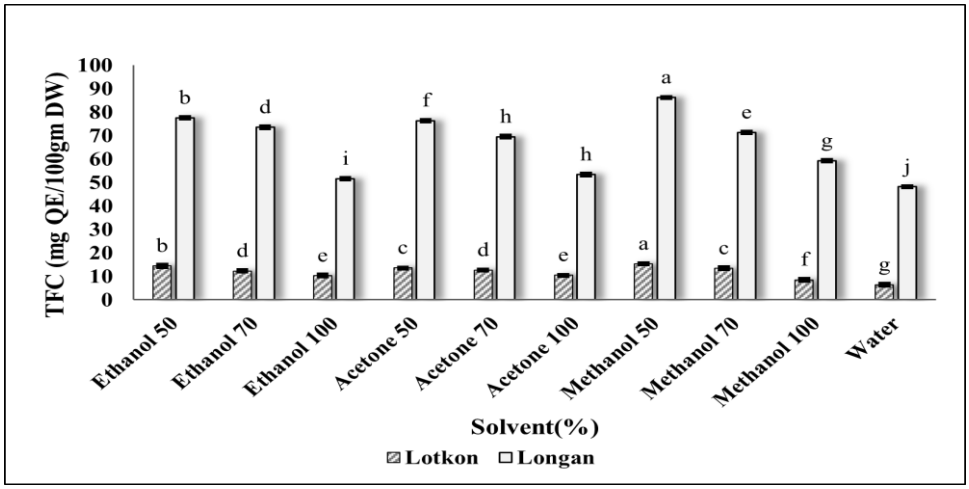

Fig. 2. Effect of different solvents and their purity on TFC of lotkon and longan peels extract

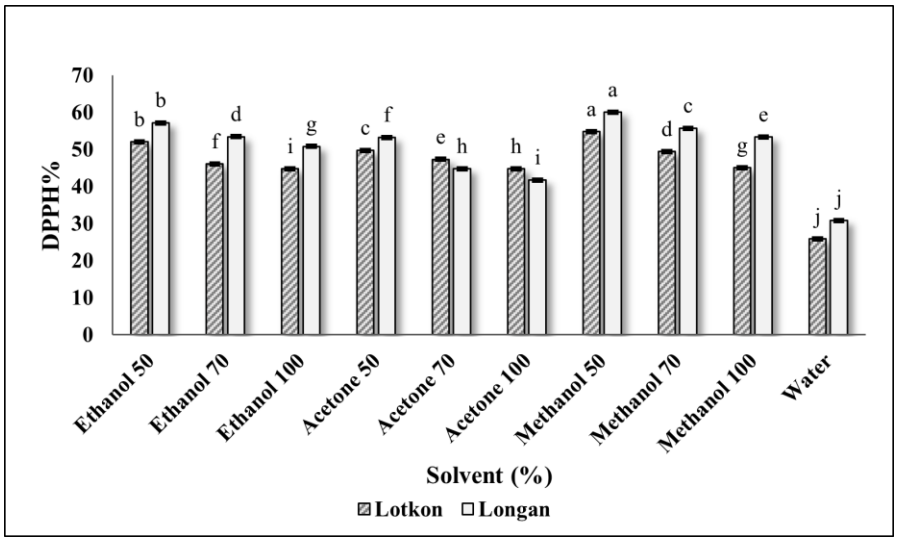

Fig. 3. Effect of different solvents and their purity on DPPH% of lotkon and longan peels extract

Our results agree with Muhamad et al. [10], who discovered that extracting antioxidant activity from *Averrhoa bilimbi* fruit needed a solvent concentration of 50% methanol. Similar findings were reported by Chavan et al. [9], who found that methanol had the highest level of antioxidant capacity for Saptarangi (*Salacia chinensis L.)* fruit pulp, whereas ethanol, acetone, and water had the lowest levels. According to Addai et al. [17] and Grant et al. [30], the lowest levels of DPPH radical scavenging activity were seen in pure solvents in the extraction process due to polarity of solvents plays an indirect role since it can increase the solubility of antioxidant chemicals. Alothman et al. [16] demonstrated that the chemical composition of the solvent has a substantial effect on the yield of DPPH value recovered from tropical fruits in Malaysia. According to Addai et al. [2], antioxidant activity results varied depending on the type of fruit, growth conditions, harvest maturity stage, storage settings, extraction time, and sample preparation method. Turkmen et al. [25] also reported that different extraction solvents probably had an impact in producing inconsistent results of antioxidant activity. The DPPH test is known as an electron transfer assay; the high value produced by methanol can be attributed to the quick transfer of electrons from the phenoxide anion to the radical as a result of partial ionization [31].

4. CONCLUSION

In summary, our findings clearly demonstrated that different solvent extracted significantly varied phenolic and flavonoid levels as well as antioxidant activity of both peel (lotkon and longan) extracts. The yield of total phenolic, total flavonoid and antioxidant activity was influenced by the characteristics of the longan and lotkon peels as well as the extracting solvents. All of the available solvents were used to evaluate the extracts of longan and lotkon peels, 50% methanol was shown to be the most effective at extracting total phenolic, total flavonoid and antioxidant activity (DPPH).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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