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Antioxidant Activity and Total Phenolic Content of Hot and Cold Water Extracts of Bitter Melon Fruit (*Momordica charantia*)

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ABSTRACT

The method of beverage preparation, whether using hot or room temperature water, significantly affects the extraction of bioactive compounds and the resulting antioxidant activity. However, limited information is available on the antioxidant properties of Momordica charantia (bitter melon) fruit when extracted using hot and cold water. In this study, bitter melon fruit was subjected to both hot and cold water extraction methods. Antioxidant activity and total phenolic content were evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay and the Folin–Ciocalteu reagent (FCR) method, respectively. Fourier-transform infrared (FTIR) spectroscopy was employed to identify functional groups present in the samples. Results indicated no significant difference in total phenolic content between the two extraction methods. A weak negative correlation (R^2 = 0.1261) was observed between antioxidant activity and phenolic content, suggesting that higher phenolic content did not correspond to increased antioxidant capacity. The highest FTIR absorption peak was observed at 2905.87 cm^{-1}, corresponding to the C–H stretching vibration. Overall, hot water extraction appeared to yield higher antioxidant activity and phenolic content, making it the preferred method for extracting bioactive compounds from bitter melon.

Keywords: Bioactive compound, FTIR analysis, Water extracts.

1. INTRODUCTION

Bitter melon (*Momordica charantia*) is usually used in food and medicine, and it tastes bitter [1]. It usually grows in tropical and subtropical countries. Bitter melon is categorized as a member of the family Cucurbitaceae. The growth of plants is about six meters or longer. The alternate leaves are about 4-12 cm across, with 3-7 deeply separated lobes and usually rounded with small points. Bitter melon gives many benefits for our health and treatment, such as killing bacteria, reducing inflammation, killing cancer cells, balancing hormones, reducing obesity, and preventing heart disease. Besides, bitter melon is also used in traditional medicine as an anti-diabetic [2]. Bitter melon fruit exhibited better radical scavenging effect and metal chelating ability according to studies by Hazra et al. (2022)[3]. Antioxidants function to protect human, animal and plant cells from harmful radicals. By taking antioxidants in the diet, will act as a cancer inhibitor because they are safe for human health [4]. Free radicals play a role in diseases such as arthritis, cancer, and atherosclerosis [5]. Bitter melon has been shown to be a good source of phenolic compounds [6], which can help protect the body against oxidative stress and reduce the risk of chronic diseases.

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Bitter melon is typically prepared as a vegetable dish or brewed into tea from dried material for both food and health benefits [7]. The yield of phenolic compounds and their antioxidant potential from plant sources is influenced by various extraction conditions, such as solvent selection, temperature, and extraction duration [8]. Hot water under atmospheric conditions has traditionally been utilized to extract polar constituents from solid substrates. As phenolic compounds are generally polar in nature, it is reasonable to suggest that moderate thermal conditions are adequate to achieve efficient recoveries using pressurized hot water extraction [9]. Previous studies have highlighted the use of water as a green solvent under moderate temperature (100–220 °C) and pressure (5–80 bar) conditions as a sustainable and scalable approach for extracting bioactive compounds from food industry by-products [10].

Several reports have indicated that water extracts exhibit higher antioxidant activity compared to methanol extracts [11],[12]. Folashade et al. [12] reported that cocoa–HFE–ginger blends showed high phenolic content in both hot and cold water extracts. Bitter melon is prepared as a functional drink in various hot and cold beverages; however, limited information exists on its antioxidant activity and phenolic content when extracted with hot and cold water. This study aims to evaluate the antioxidant activity and total phenolic content of hot and cold water extracts of bitter melon fruit. It is hypothesized that hot water extraction will yield higher antioxidant activity and phenolic content than cold water extraction, as elevated temperatures enhance compound solubility and diffusion from plant tissues into the solvent.

2. MATERIAL AND METHODS

2.1 Samples Collection and Preparation

Approximately 1 kg of bitter melon fruit was purchased from a fresh market in Perlis, Malaysia. The fruits were washed with distilled water and cut into small pieces, then oven-dried at 60 °C until a constant weight was achieved. The dried pieces were ground using a blender to obtain a fine, homogeneous powder. The powder was stored in an airtight container until further extraction and chemical analysis.

2.2 Hot and Cold-Water Extraction

Approximately 0.2 g of bitter melon fruit powder was placed in a conical flask with 20 mL of either hot water (90 °C) or distilled water (4 °C). For both methods, extraction was carried out in a water bath shaker at 180 rpm for 15 minutes. For the hot water method, the mixture was left to stand for 3 hours at room temperature, whereas for the cold water method, the flask was placed in an ice-filled container to maintain 4 °C during the 3-hour standing period. Four replicates were prepared for each extraction method prior to further analysis.

2.3 Total Phenolic Content Analysis

The total phenolic content was determined using the Folin–Ciocalteu method. An aliquot of 0.5 mL of the sample was transferred into a 10 mL volumetric flask containing 0.5 mL of Folin–Ciocalteu's reagent. Subsequently, 5 mL of distilled water and 1.5 mL of sodium bicarbonate solution (20% w/v) were added, and the volume was adjusted to 10 mL with distilled water. The mixture was left to stand for 2 hours at room temperature, after which the absorbance was measured at 765 nm against a blank.

2.4 Evaluation of Antioxidant Activity

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, a standard method for evaluating antioxidant activity in plant extracts, was performed following Katalinic et al. [13] with

minor modifications. Briefly, 2 mL of methanolic DPPH solution was mixed with 200 µL of hot or cold water extract of bitter melon, and methanol was added to adjust the final volume to 3 mL. The mixture was incubated in the dark for 60 minutes at room temperature, after which the absorbance was measured at 517 nm using a spectrophotometer, with methanol serving as the blank. A control was prepared by mixing 2 mL of methanolic DPPH solution with 1 mL of methanol. The percentage of inhibition was calculated as follows:

$$\text{Percent of inhibition (\%)} = \frac{\text{Ac of control} - \text{As of sample}}{\text{Ac of control}} \times 100 \quad (1)$$

2.5 FTIR Analysis

A Perkin Elmer Universal Attenuated Total Reflectance (ATR) sampling accessory was used to obtain the FTIR spectra of bitter melon fruit powder. Data collection and processing were tested by the Spectrum version 6.2.0.0055 software. The FTIR spectrum was used to distinguish the functional groups of the active components found in the plant sample based on the peak values in the region of IR radiation.

2.6 Statistical Analysis

All the experiments were measured in triplicate, and results were expressed as means ± S.E. Student's Analysis of variance ANOVA (Microsoft Excel 2010 Workbook) was performed to analyze for statistically significant results.

3. RESULTS AND DISCUSSION

3.1 Antioxidant Activity and Total Phenolic Content Analysis

The hot water extract exhibited a higher percentage inhibition of antioxidant activity (40.05%) compared to the cold-water extract (34.48%). This is likely due to the hot extraction process enhancing the release of phenolic compounds, resulting in greater antioxidant activity. Similar results were reported by Antony and Farid [14] and Abdelkader et al. [15]. However, there was no significant difference in the total phenolic content between the two extracts. The total phenolic content of the hot water extract was 76.045 ± 1.216 mg/g, while that of the cold-water extract was 73.284 ± 1.837 mg/g. This finding is consistent with Venditti et al. (2010) [16], who also reported no significant difference in phenolic content between hot and cold water extracts. Values reported within column are the means ± standard error, and the means are compared with ANOVA at $p < 0.05$ (n=3). The different letters represent a significant difference between the types of extraction.

Table 1: Percentage inhibition of antioxidant activity and total phenolic content in hot and cold water extracts of bitter melon fruit.

Type of extraction	% Inhibition of antioxidant	Total phenolic content (mg/g)
Hot water extracts	40.05a ±0.71	76.05a ± 1.22
Cold water extracts	34.48b ±1.92	73.28a ± 1.84

3.2 FTIR Analysis

The FTIR spectrum of bitter melon fruit powder extract (Figure 1) revealed characteristic absorption peaks corresponding to various functional groups: alkane C-H (2905.87 cm^{-1}), alkene C=C (1622.66 cm^{-1}), aromatic C=C (1404.59 cm^{-1}), alcohol C-O stretching (1050.14 cm^{-1}), and

alkyl halide (576.89 cm^{-1}). Comparable chemical constituents in bitter melon extracts have also been documented by Andayani et al. [17]. As noted by Bautista-Hernández et al. [18], the presence of C=C, C–H, and C–O vibrations is indicative of polyphenolic compounds in plant materials. Polyphenols in tea are linked to numerous health benefits, including antimutagenic, antidiabetic, anti-inflammatory, antioxidant, antimicrobial, and cancer-preventive effects, as well as cardiovascular protection [16], [19]. These effects are achieved through mechanisms such as radical scavenging, modulation of signal transduction, regulation of cell cycles and apoptosis, and induction of beneficial enzymes [20].

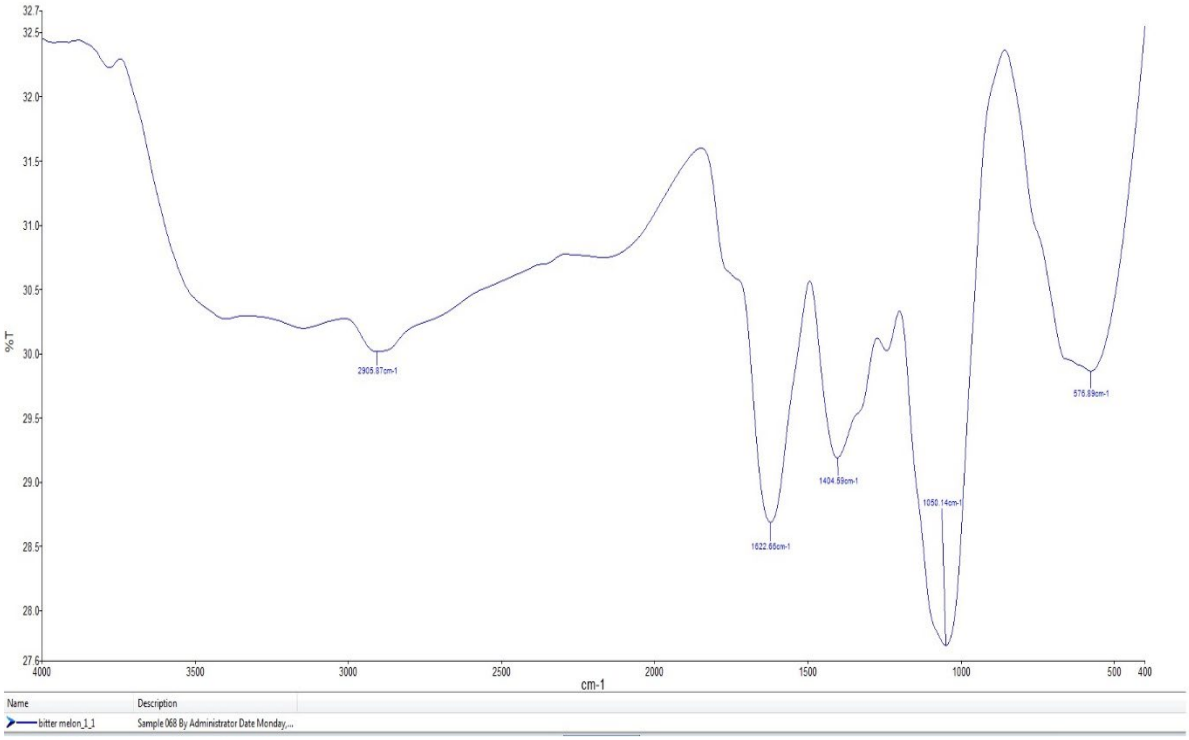

Figure 1: FTIR spectra of bitter melon fruit powder extract.

4. CONCLUSION

Hot water extraction of bitter melon fruit yielded higher antioxidant activity than cold water extraction, likely due to enhanced release of bioactive compounds, although the total phenolic content was not significantly different between the two methods. FTIR analysis confirmed the presence of functional groups such as alkanes, alkenes, aromatics, alcohols, and alkyl halides, consistent with polyphenolic constituents reported in previous studies. These findings suggest that extraction temperature influences antioxidant potential without markedly altering overall phenolic content.

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