ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

Various Technical Aspects in Herbal Tea Production from Alisma Plantago-Aquatica Leaf

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Abstract

Alisma plantago-aquatica is abundant weed on thinned crops of rice. *A. plantago-aquatica* is resistant to flood, but being very susceptible to shade; therefore the thickened rice crops shade the weeds and detain their growth. Leaves green; lower ones only sessile, widely linear, and swimming; the other ones aerial, long-petiolate; their plates big, ovoid or widely ovoid, with cordate base usually, with pointed apex. The use of medicinal herbs as a source of antioxidants is significant because they are often utilized to heal chronic diseases. Objective of this study focused on the effectiveness of blanching temperature and time; oven drying temperature and storage condition to total phenolics (mg/g), total flavonoids (mg/g), ferric reducing/antioxidant power (FRAP, mg/g), radical-scavenging activity (DPPH, mg/mL) of the dried *Alisma plantago-aquatica* tea. Results showed that *Alisma plantago-aquatica* should be treated in hot water at 95^oC within 8 seconds in the present of citric acid 1.0% and then being dried by drying oven at 45^oC until 7.5% moisture. The final herbal tea could be preserved under vacuum in aluminum bag at 28^oC to maintain total phenolics, total flavonoids, antioxidant power and radical-scavenging activity for 12 months.

Keywords: *Alisma plantago-aquatica, Phenolic, Flavonoid, Herbal, FRAP, DPPH, Blanching, Drying.*

Introduction

Alisma plantago-aquatica is a shoreline plant, characterized by lance-shaped leaves, with many tiny pale lilac flowers [1]. Alisma plantago-aquatica was used as an adsorbent to remove heavy metal from aqueous solution. Alisma *plantago-aquatica* revealed the presence of hydroxyl, carboxyl and carbonyl groups which are participating in the adsorption of metal ions [2, 3].

The development of a biotechnological system for generating bioelectricity on closed balconies of buildings from living plants *Alisma plantago-aquatica* and soil microorganisms grown in containers with natural wetland substrate, provided with a graphite and Zn-galvanized steel electrode system was described [4]. Terpenes and phenolic acid were regard as major secondary metabolites from this medicine plant [5]. Three terpenes with Protostane type were isolated from the rhizomes of *Alisma plantago-aquatica* [6].

It was used as folk medicine for immune-modulation, anti-tumor, anti-inflammatory and antibacterial. *Alisma plantago-aquatica* var. Orientale and alisol B 23-acetate show promise as therapeutic agents for various damages involving free radical reactions [7]. There was little report on processing of dried *Alisma plantago-aquatica* leaf as herbal tea.

The utilization and awareness of the numerous benefits of *Alisma plantago-aquatica* is springing up in Vietnam and there arise the need to produce herbal tea. Therefore, objective of this study focused on the effectiveness of blanching temperature and time; oven drying temperature and storage condition to total phenolics (mg/g), total flavonoids (mg/g), ferric reducing/

antioxidant power (FRAP, mg/g), radical-scavenging activity (DPPH, mg/mL) of the dried *Alisma plantago-aquatica* leaf tea.

Materials and Method

Material

Alisma plantago-aquatica leaves were collected from Vinh Long province, Vietnam. After collecting, they must be conveyed to laboratory within 8 hours for experiments. They were washed under tap water to remove foreign matters. Besides Alisma plantago-aquatica we also used another material during the research such as citric acid. Lab utensils and equipments included digital weight balance, cooker, and oven dryer.

Figure 1: ***Alisma plantago-aquatica*** **leaves**

Effectiveness of Blanching Temperature and Time to Total Phenolics (mg/g), Total Flavonoids (mg/g), ferric Reducing/antioxidant Power FRAP (mg/g), and Radical-Scavenging Activity DPPH (mg/mL) in the Dried Alisma Plantago-aquatica Tea

Raw *Alisma plantago-aquatica* was blanched in hot water with 1.0% citric acid at different temperature and time (100°C, 4 second; 95°C, 8 seconds; 90°C, 12 seconds; 85°C, 16 seconds). Then they were dried by drying oven at 55°C until 7.5% moisture. All samples were analyzed total phenolics (mg/g), total flavonoids (mg/g), FRAP (mg/g), and DPPH (mg/mL) to demonstrate the optimal blanching condition.

Effectiveness of Drying Temperature to Total Phenolics (mg/g), Total Flavonoids (mg/g), Ferric Reducing/Antioxidant Power FRAP (mg/g), and Radical-scavenging Activity DPPH (mg/mL) in the Dried Alisma plantago-aquatica tea

Raw *Alisma plantago-aquatica* was blanched in water solution with 1.0% citric acid at 95°C in 8 seconds. Then these samples would be dried under drying oven at different temperature (45°C, 50°C, 55°C, 60°C) until 7.5% moisture. All samples were analyzed total phenolics (mg/g), total flavonoids (mg/g), FRAP (mg/g), and DPPH (mg/mL) to demonstrate the appropriate drying temperature.

Effectiveness of Preservation to Total Phenolics (mg/g), Total Flavonoids (mg/g), Ferric Reducing/Antioxidant Power FRAP (mg/g), and Radical-scavenging Activity DPPH (mg/mL) in the Dried Tea

After drying treatment, the dried *Alisma plantago-aquatica* was gone to preservation. They were preserved in aluminum bag at ambient temperature (28°C). The total phenolics (mg/g), total flavonoids (mg/g), FRAP (mg/g), and DPPH (mg/mL) will be analyzed in interval of 3 months for 12 months.

Physico-chemical and Sensory Analysis

Total phenolic (mg/g) was determined according to the Folin-Ciocalteu method [8]. Total flavonoids (mg/g) was determined according to the $NaNO_2$–Al $(NO_3)_3$ method [9]. Ferric reducing/antioxidant power (FRAP, mg/g) was measured using FRAP assay [10]. DPPH radical-scavenging activity (mg/mL) was evaluated according to the Kalaivani T method [11].

Statistical Analysis

The experiments were performed in triplicate with three different lots of samples. Data were subjected to analysis of variance (ANOVA) and mean comparison was carried out using Duncan's multiple range test (DMRT). Statistical analysis was performed by the Stat graphics Centurion XVI.

Result & Discussion

Effectiveness of Blanching Temperature and Time to Total Phenolics (mg/g), Total Flavonoids (mg/g), Ferric Reducing/Antioxidant Power FRAP (mg/g), and Radical-Scavenging activity DPPH (mg/mL) in the dried *Alisma plantago-aquatica* tea

Blanching is a thermal treatment that is usually performed prior to drying. It is essential to preserve the product quality during the long-term storage because it inactivates the enzymes and destroys microorganisms. Blanching can expel air entrapped inside plant tissues, especially intercellular gas [12, 14]. Thermal blanching can cause structural changes in plant tissues such as disruption of cell membranes, loosening of the hemi-cellulose, cellulose and pectin networks, and alternating cell wall porosity. These can improve the extraction of bioactive compounds [15]. Raw *Alisma plantago-aquatica* was blanched in hot water with 1.0% citric acid at different temperature and time (100°C, 4 second; 95°C, 8 seconds; 90°C, 12 seconds; 85°C, 16 seconds).

Then they were dried by drying oven at 55°C until 7.5% moisture. All samples were analyzed total phenolics (mg/g), total flavonoids (mg/g), FRAP (mg/g), and DPPH (mg/mL) to demonstrate the optimal blanching condition. Results were mentioned in table 1. From table 1, the *Alisma plantago-aquatica* should be blanched at 95°C in 8 seconds to maintain the most total phenolics (mg/g), total flavonoids (mg/g), FRAP (mg/g) and DPPH (mg/mL) in the dried *Alisma plantago-aquatica* tea.

Table 1: Effectiveness of blanching temperature and time to total phenolics (mg/g), total flavonoids (mg/g), ferric reducing/antioxidant power FRAP (mg/g), and radical-scavenging activity DPPH (mg/mL) in the dried *Alisma plantago-aquatica* tea

Blanching	Total phenolics (mg/g)	Total flavonoids (mg/g)	FRAP (mg/g)	DPPH (mg/mL)
Control	4.14±0.06^{a}	1.35±0.03^{a}	39.24±0.12^{a}	1.43±0.013^{a}
100°C, 4 seconds	2.85±0.03^{bc}	0.58±0.01^{bc}	22.57±0.07^{c}	0.83±0.01^{c}
95°C, 8 seconds	3.04±0.07^{b}	0.75±0.05^{b}	26.45±0.08^{b}	0.51±0.05^{d}
90°C, 12 seconds	2.61±0.02^{c}	0.42±0.01^{c}	19.92±0.04^{d}	0.97±0.02^{bc}
85°C, 16 seconds	2.05±0.08^{d}	0.17±0.06^{d}	16.07±0.03^{e}	1.15±0.03^{b}

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%)

The loss of nutrients during hot water blanching is caused mainly by leaching or diffusion [15]. Different water-soluble nutrients, such as vitamins, flavors, minerals, carbohydrates, sugars, and proteins, can leach out from plant tissues to the blanching water. In addition, hot water blanching can also lead to degradation of some thermal sensitive substances such as ascorbic acid, aroma and flavor compounds [14].

Effectiveness of Drying Temperature to Total Phenolics (mg/g), Total Flavonoids (mg/g), Ferric Reducing/Antioxidant Power FRAP (mg/g), and Radical-scavenging Activity DPPH (mg/mL) in the Dried *Alisma plantago-aquatica* tea

Fresh herbs have high moisture content to degrade its quality over a short period of time; thus limiting it unsuitable for consumption. This decomposition can be controlled by drying the fresh herb .Drying basically discards the moisture from the herb which reduces and finally eliminates the microbial activity which initially causes the degradation of the herb quality. Drying is one of the oldest and a very important unit operation, it involves the application of heat to a material which results in the transfer of moisture within the material to its surface and then water removal from the material to the atmosphere [16].

This is the most common storage technique [17]. Drying brings about substantial reduction in weight and volume, minimizing packaging, storage and transportation costs [18]. The drying features for pressure, air velocity, relative humidity, and product retention time vary according to the material and method of drying. During the drying operation physical, structural, chemical, nutritional changes in the vegetables may occur, and that can affect the quality attributes like texture, color, flavor and nutritional value [19]. The radical scavenging assay is the most widely used analysis in evaluating this activity in phytochemistry. It is a simple and rapid test that is based on the reaction rate between a stable free radical, 1, 1-diphenyl-2-pycrylhydrazyl (DPPH) and antioxidants [20].

Raw *Alisma plantago-aquatica* were blanched in water solution with 1.0% citric acid at 95^{o}C in 8 seconds. Then these samples would be dried under drying oven at different temperature (45^{o}C, 50^{o}C, 55^{o}C, 60^{o}C) until 7.5 % moisture. All samples were analyzed total phenolics (mg/g), total flavonoids (mg/g), FRAP (mg/g), and DPPH (mg/mL) to demonstrate the appropriate drying temperature. Results were mentioned in Table 2. Some of the active components in the product are temperature sensitive, and are not preserved by this drying method, reducing the product quality [17]. From table 2, the *Alisma plantago-aquatica* should be dried at 45^{o}C to maintain the most total phenolics (mg/g), total flavonoids (mg/g), FRAP (mg/g) and DPPH (mg/mL) in the dried *Alisma plantago-aquatica* tea.

Table 2: Effectiveness of drying temperature to total phenolics (mg/g), total flavonoids (mg/g), ferric reducing/antioxidant power FRAP (mg/g), and radical-scavenging activity DPPH (mg/mL) in the dried *Alisma plantago-aquatica* tea

Drying temperature	Total phenolics (mg/g)	Total flavonoids (mg/g)	FRAP (mg/g)	DPPH (mg/mL)
45^{o}C	3.25±0.04^a	0.90±0.06^a	27.07±0.07^a	0.45±0.09^c
50^{o}C	3.18±0.05^{ab}	0.83±0.03^{ab}	26.95±0.05^{ab}	0.48±0.02^{bc}
55^{o}C	3.04±0.07^b	0.75±0.05^b	26.45±0.08^b	0.51±0.05^b
60^{o}C	2.75±0.03^c	0.61±0.01^c	24.33±0.06^c	0.74±0.04^a

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%)

Various changes in physical, chemical and/or biological characteristics of sample occur. These changes alter the physical aspect such as colour and structure. Herbs were commonly dried at high temperatures (40-60°C) [17]. Our results were similar to finding by Jing Zhang et al [10]. Low drying temperatures between 30 and 50°C are recommended to protect sensitive active ingredients.

The loss of volatiles in herbs and spices during drying depends mainly on drying conditions and the biological characteristics of the herb. Some volatile compounds evaporate during drying, while others are partially retained [17]. The relative instability of most phenolic compounds from plants may indicate a sensitivity of these compounds to drying treatments [21].

Effectiveness of Preservation to Total Phenolics (mg/g), Total Flavonoids (mg/g), Ferric Reducing/Antioxidant Power FRAP (mg/g), and Radical-scavenging Activity DPPH (mg/mL) in the Dried Tea

Medicinal herbs are usually subjected to drying and longtime storage during production, and drying is considered a beneficial way to protect their phytochemical efficiency [20]. Storage is applied in herb manufacturing before processing for several purposes. This operation requires some conditions which could be offered by drying the raw materials.

Alisma plantago-aquatica needs to be dried to extend product shelf-life so it can be easily preserved. Different drying methods have been developed recently. After drying treatment, the dried *Alisma plantago-aquatica* was gone to preservation. They were preserved in aluminum bag at ambient temperature (28^{o}C). The total phenolics (mg/g), total flavonoids (mg/g), FRAP (mg/g), and DPPH (mg/mL) will be analyzed in interval of 3 months for 12 months. Dried *Alisma plantago-aquatica* leaves should be stored under vacuum in aluminum bag at 28^{o}C to maintain flavonoid content for 12 months.

Table 3: Total phenolics (mg/g), total flavonoids (mg/g), FRAP (mg/g), and DPPH (mg/mL) in dried *Alisma plantago-aquatica* during preservation

Storage time (month)	Total phenolics (mg/g)	Total flavonoids (mg/g)	FRAP (mg/g)	DPPH (mg/mL)
0	3.25±0.04^{a}	0.90±0.06^{a}	27.07±0.07^{a}	0.45±0.09^{c}
3	3.19±0.03^{ab}	0.86±0.02^{ab}	27.01±0.06^{ab}	0.47±0.06^{bc}
6	3.14±0.07^{b}	0.83±0.05^{b}	26.95±0.08^{b}	0.49±0.03^{b}
9	3.05±0.04^{bc}	0.79±0.06^{bc}	26.92±0.03^{bc}	0.53±0.07^{ab}
12	3.01±0.05^{c}	0.77±0.04^{c}	26.87±0.05^{c}	0.56±0.02^{a}

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%)

The low temperature was not an appropriate condition for enzymatic reactions that cause a decrease in the antioxidant capacity of herbs.

However, the severe chilling injury might be the cause of oxidation stress and the decline of the TPC among fresh herbs because of the high moisture content [20].

Conclusion

Herbs and spices are beneficial due to their phytochemical nutrient and essential oil component. The demand of traditional herb shows a large increase. Blanching is a crucial step before drying to inactivate enzymes. Application of a suitable blanching technology with a selection of appropriate conditions are of great importance, since blanching directly affects the quality of the dried product in terms of its physical and nutritional property. The blanching had more of a positive effect in retaining the original green color of the fresh herbs than with direct drying. Drying is important for preserving product quality, and until recently herbs were commonly dried at high temperatures. Drying of herbs and spices is essential to extend their shelf life.

This is because low moisture contents prevent the growth and reproduction of microorganisms that cause decay. The association of drying with proper storage could augment the shelf life of the product. We have successfully investigated several factors affecting to production of *Alisma plantago-aquatica* herbal tea. By this investigation, the added value of *Alisma plantago-aquatica* could be improved.

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