Original Research Article

Effect of storage temperature and preservatives on the stability and quality of *Polyscias fruticosa*(L.) Harms herbal health drinks.

ABSTRACT

Aims:*Polyscias fruticosa*has been well-known as a traditional medicinal herb which shares the same function as ginseng, favorable for their antioxidant capacity. In this study, the herbal health drink had been developed based on the *Polyscias fruticosa* extract. The effect of preservativesand storage temperature on the total phenolic content, total flavonoid content, and total saponin content were investigated over the period of 16 weeks.

Methodology:*Polysciasfruticosa*extract_based herbal drinks were formulated. Potassium sorbate and sodium benzoate were used as preservatives while storage temperature was set at 4 and 25^0C. Determination of total phenolic content was performed by Folin-Ciocalteu method. Meanwhile, analysis of total flavonoid and saponin content was conducted by colorimetric methods.

Results: In general, the effect of preservatives and storage temperature on the concentration of total phenolic content and total flavonoid contentcan clearly be seen after 6 weeks, whilesignificant difference in concentration of total saponin content had been evidenced from week 11. Typically, The concentration of total phenolic content, total flavonoid content and total saponin content in formulas added preservatives and kept at 4^0C were measured at 2.80±0.26 mg GAE/g, 8.24±0.44 mg CE/g and 20.29±0.27 mg OAE/g after 16 weeks, respectively; however, without adding preservatives and stored at 25^0C, these components were found at a value of 1.77±0.1 mg GAE/g, 0.0±0.28 mg CE/g, 14.63±0.59 mg OAE/g, respectively.

Conclusion: Overall, the presence of preservatives and fridge temperature (4^oC) has been the optimal condition to maintain the quantity of biological phytocomponents in herbal health drink; however, the addition of preservatives and storage temperature should be taken into consideration depending on the storage time of herbal drink.

Keywords: Flavonoid,herbal drink,phenolic,*Polyscias fruticosa*(L.) Harms, saponin.

1. INTRODUCTION

Polyscias fruticosa(L.) Harms. (*P. fruticosa*) belongs to the family Araliaceae, which is a member of the ginseng family. In Asian countries, *P. fruticosa* leaves are used as a tonic, anti-inflammatory, antitoxin, antibacterial, and digestive support [1]. The root extract of *P. fruticosa* has been traditionally used for the treatment of ischemia, anti-dysentery, anti-inflammatory, neuralgia and rheumatic pains [2].Phytochemical studies have shown that*P. fruticosa*contains a variety of bioactive components such as alkaloids, glucosamine, saponins, flavonoids, tannins, and B vitamins [3]; particularly the presence of some essential amino acids including lysine, cysteine and methionine. However, the pharmacological activities of *P. fruticosa* could be attributed to the presence of saponins, phenolics and flavonoids.Being potent antioxidants, phenolics and flavonoids in *P. fruticosa* possess certain health benefits such as preventing the growth of prostate and lung cancer, improving vascular health, exhibiting anti-mutagenic and anti-cancer activities. [4].

In recent years, many nutraceutical products havebeen developed which take advantages of the availability and potential nutrition of traditional medicinal plants [5]. One such plant is *P.fruticosa*. Herbal beverages are considered to be an excellent medium for the supplementation of nutraceutical components for enrichment, mainly bioactive herbal extracts. It has been scientifically proven to be more convenient to consume a beverage providing health benefits rather than swallow vitamins or pills for the sameinfluences.*P. fruticosa* herbal health drink not only brings a new yield to the beverage industry market but also deliver to the customers a product that can be consumed daily and contains nutraceutical ingredients for the body. In view of these considerations, the stability and quality of the products under storage conditions need to be analyzed.

2. MATERIAL AND METHODS

2.1. Chemicals and materials

The roots and leaves of 5-year-old *P. fruticosa*were collected in Dong Nai Province, Vietnam. The plant was deposited in the herbarium of Applied Biochemistry Laboratory, Department of Applied Biochemistry, School of Biotechnology, International University, Vietnam National University – Ho Chi Minh City, Vietnam with voucher No. HB-BIO-06-08-18. Folin-Ciocalteau

reagent was obtained from Merk (Darmsradt, Germany). Catechin, gallic acid, andoleanolic acid were purchased from Sigma-Alrich (USA). All other reagents used in the research were of analytical grade.

2.2. Preparation of *P. fruticosa*(L.) Harms herbal health drinks

2.2.1. Preparation of plant extract

The roots and leaves of *P. fruticosa*were first harvested, drenched, and washed with water to remove dust and soil and other contaminants. They weresubsequently chopped down into smaller pieces and left for sun drying for 2-3 weeks. The material was thenground to a fine powder and then stored in desiccators for further use.

Ten grams (10 g) of each *P. fruticosa* powder was soaked in 100 mL of distilled water. Subsequently, the mixture was incubated at 60.9^{o}Cfor 5 hours using shaking incubator (IKA, Germany) at 200 rpm, followed by vacuum filtration to obtain the transparent solution. The extract process yielded 37.42±26 mg of oleanolic acid which was almost the same quantity (37.18 mg) extracted by Vo (2007) [6].

2.2.2. Herbal Formulations

The samples were assigned into four different formulas namely F1, F2, F3 and F4 (Table 1). Each formula was initially prepared by adding the same amount of *P. fruticosa* extract (100 mL) and Stevia sugar powder (0.7 g). Both formulas F1 and F2 were given the same amount of preservatives of combined potassium sorbate (0.005 g) and sodium benzoate (0.005 g), while no preservatives were added to the formula F3 and F4. Both formulas F1 and F3 were kept at ambient temperature (25^{o}C) whereas the formula F2 and F4 were stored in fridge (4^{o}C) over the period of 16 weeks. All samples were kept in sterilized glass bottle, sealed with aluminum foil. The data were then collected after 1 week of experiment, followed by week 6, 11 and 16.

Table 1.Formulation of herbal drinks based on *P. fruticosa* extract

Formula	*P. fruticosa* extract (mL)	Stevia sugar (gram)	Potassium sorbate (gram)	Sodium benzoate (gram)	Storage temperature
F1	100	0.7	0.005	0.005	25

F2	100	0.7	0.005	0.005	4
F3	100	0.7	0	0	25
F4	100	0.7	0	0	4

2.3. Analytical method

2.3.1. Determination of total phenolic content

The total phenolic content (TPC) of *P. fruticosa* extractwas measured by Folin-Ciocalteu method [7]. 2mL of diluted sample was mixed with 2mL of Folin-Ciocalteu (10% v/v). The mixture was incubated at room temperature for 4 minutes,followed by the addition of 1.6mL of sodium carbonate (7.5% w/v) to the mixture. Then the mixture was vortexed for 10 seconds and incubated in dark condition for 2hours. The absorbance was measured at 765nm. Gallic acid was used to calibrate the standard curve.

2.3.2. Determination of total flavonoid content

The total flavonoid content (TFC) of *P. fruticosa*extract was measured by colorimetric method [8]. 0.5 mL of diluted sample was mixed with 0.15mL of sodium nitrite (5% w/v) and incubatedat room temperature for 6 minutes, followed by the addition of 0.3mL of aluminum chloride (10% w/v) to the reaction mixture. The mixture was kept for 5 minutes before adding1mL of sodium hydroxide(1M) and thenmixed well by vortex mixer. The absorbance was measured at 510 nm. The total flavonoid content was expressed as milligram of catechin per gram of *P. fruticosa*powder.

2.3.3. Determination total saponin content

The method for determination of total saponin content (TSC) of *P. fruticosa*extract was based on the colorimetric methods [9]. 0.5mL of samplewas mixed with 0.5mL of vanillin reagent (8% w/v in absolute ethanol). The test tubes were placed in cold water, and 5mL of sulfuric acid (72% v/v) was added slowly on the inner side of the test tubes, and allowed to stand for 3 minutes. The mixture was heated to 60^{o}C for 10minutes in water bath. Then the test tubeswere removed andcooled in cold water. The absorbance was measuredat 544 nm. Oleanolic acid was used to calibrate standard curve.

2.3.4. Statistical analysis

All experiments were conducted in triplicate,and the results were expressed as mean ± SD. Statistical analysis was performed by SPSS and analysis of variance (ANOVA) with thelevel of significance$p$<.05.

3. RESULTS AND DISCUSSION

3.1. Phenolic stability during long term storage

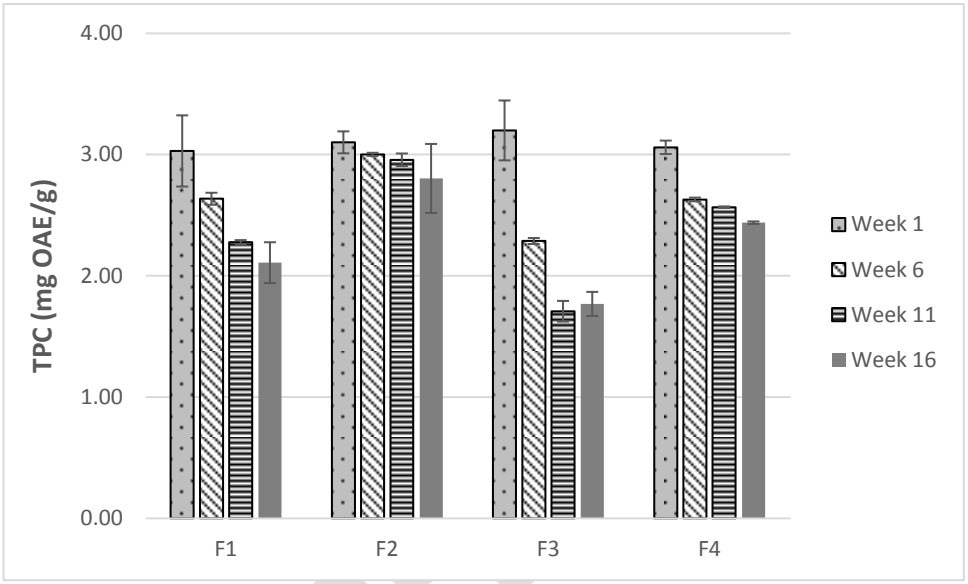

Figure 1: Total phenolic content changewith the presence/ absence of preservativesunder different storage temperatures

The effect of preservatives and storage temperature on the change of TPC in the *P. fruticosa* herbal drink had been assayedfor the period of 16 weeks (Fig 1). The results were expressed in a unit of milligram of gallic acid per gram of extracted powder. Gallic acid is a type of phenolic acid which is known as 3, 4, 5-trihydroxybenzoic acid. In general, the concentration of TPC in all formulas showed the downward trends over the tested time. However, the impact can clearly be seen for the formula with addition of preservatives and kept at low temperature while the formula without the addition of preservatives or maintained at ambient temperature showed significant decreasein TPC. The quantity of TPC in the formula F2was stable over this time period. The concentration of TPC was measured at 3.10±0.59 mg GAE/g in week 1, stood at a value of 3.00±0.01 mg GAE/g in week 6, and then maintained almost constant at a value of 2.96±0.05 mg GAE/g and 2.80±0.26 mg

GAE/g after 11 and 16 weeks, respectively. Meanwhile, the concentration of TPC in F4 (stored at fridge temperature without the preservatives) was almost stable in the first week (3.36±0.09 mg GAE/g), but slightly reduced to 2.63±0.06 mg GAE/g in week 6 and then to 2.57±0.01 mg GAE/g and 2.44±0.01 mg GAE/g in week 11 and 16, respectively.On the contrary, the impact of storage temperature on the TPCin F1 and F3 had been noted as the concentration of TPC was 3.03±0.29 mg GAE/g and 3.2±0.25 mg GAE/g in week 1,but fell to 2.11±0.17 mg GAE/g and 1.77±0.1 mg GAE/g after 16 weeks, respectively.

3.2. Flavonoid stability during long term storage

Figure 2 illustrates how the concentration of TFC in*P.fruticosa* herbal drink was altered with the interference of storage temperatureand preservatives. Catechin was used as a referencesince it is the primaryflavonoid found in tea belonging to this group of polyphenols [10].

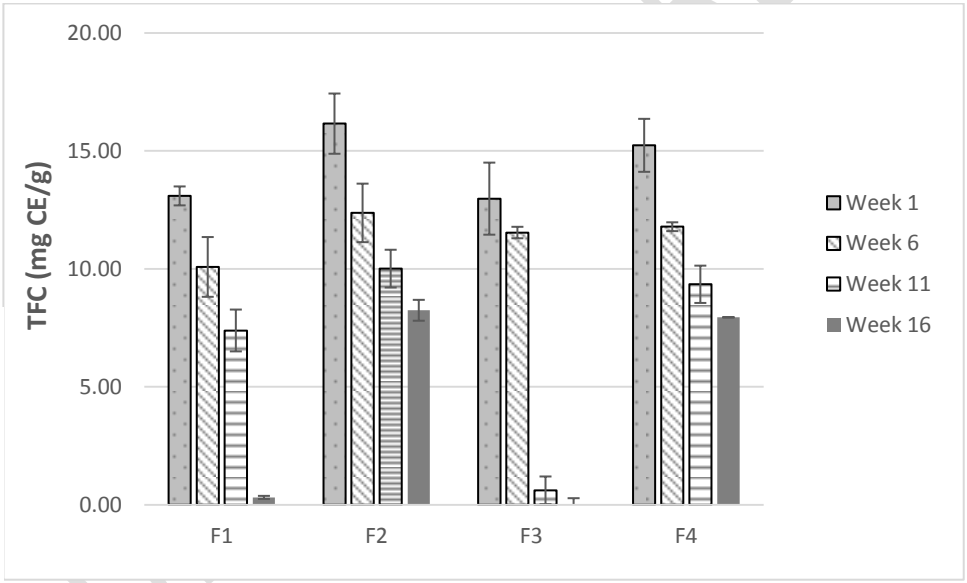

Figure 2: Total flavonoid content change with the presence/ absence of preservative and under different storage temperatures

It can clearly be seen that the presence of preservatives at fridge temperature could not secure the quantity of TFC in herbal drink over the assay time as the concentration of TFC in F2significantly reduced from 16.15±1.28 mg CE/g in week 1 to 12.37±1.24 mg CE/g in week 6 and then to 10.01±0.8 mg CE/g in week 11, and continuously fell to 8.24±0.44 mg CE/g after 16 weeks. It should be noted, however, the fridge temperature might partially play a key

role in maintaining the concentration of TFC in herbal drink without adding the preservatives. Accordingly, the concentration of TFC in F4 was measured at a value of 15.23±1.12 mg CE/g in the first week, went down to 11.78±0.19 mg CE/g in week 6 and reduced to 9.34±0.79 mg CE/g in week 11. Noticeably, there was no significant difference in concentration of TFC between F2and F4 after 16-week experiment as it was found at a value of 7.94±0.01 mg CE/g for the F4 after 16 weeks. Meanwhile, the extremely significant difference can be observed for the concentration of TFC in F1 and F3 over the same period. The concentration of TFC in F1 was found to be 13.09±0.4 mg CE/g in the first week, decreased to 10.08±1.27 mg CE/g in week 6 and then to 7.39±0.89 mg CE/g in week 11, but steeply plunged to 0.31±0.06 mg CE/g after 16 weeks. On the same manner, the concentration of TFC in F3 (stored at ambient temperature without preservatives) just stabilized at a value of 11.54±0.24 mg CE/g for a period of 6 weeks, and then sharply fell to a value of 0.61±0.59mg CE/g in week 11 and found empty after 16 weeks.

3.3. Saponinstability during long term storage

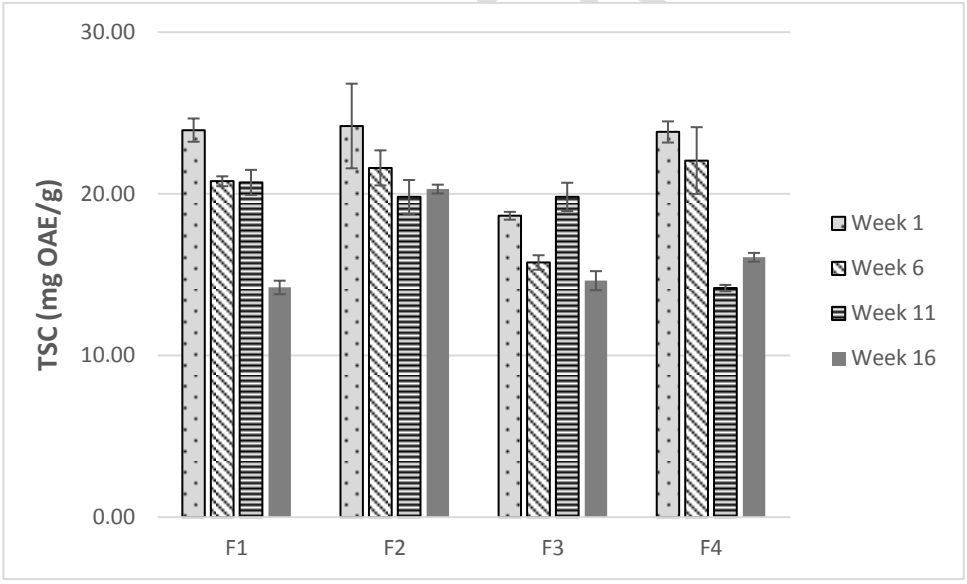

Figure 3:Total saponin content change with the presence/ absence of preservative and under different storage temperatures

Figure 3 presents the concentration of TSC in different formulas of herbal drink over the period of 16 weeks. The impact of storage temperature on the concentration of TSC had

been evidenced as can be seen in F2. Accordingly, the concentration of TSC in F2 was measured at a value of 24.2±2.62 mg OAE/g in the first week, slightly reduced to 21.61±1.08 mg OAE/g in week 6 and steadily declined to 19.81±1.04 mg OAE/g in week 11, but still maintained almost same value after 16 weeks (20.29±0.27 mg OAE/g). Meanwhile, the same trends had been observed in F4, but only for the first 11 weeks.The concentration of TSC in F4 stayed at avalue of 23.83±0.65mg OAE/g in the first week, slightly decreased to 22.06±2.06 mg OAE/g in week 6 and slowly went down to 20.27±02 mg OAE/g after 11 weeks; however, it significantly dropped to 16.08±0.25 mg OAE/g in week 16. In the same way, the concentration of TSC in F1 (with the presence of preservatives at ambient temperature) was found at a value of 20.71±0.78 mg OAE/g in week 11, but sharply fell to 14.21±0.42 mg OAE/g after 16 weeks. In contrast, the concentration of TSC in F3 was found to be low throughout the tested time. It stood at low concentration even in the first week (18.64±0.24 mg OAE/g), slowly reduced to 15.75±0.45 mg OAE/g, but remained stable between week 11 and 16 at the value of 14.17±0.88 and 14.63±0.59 mg OAE/g, respectively.

In Vietnam*, P. fruticosa* has been formulated as a supplement forrelieving ischema and enhancing circulation of brain blood. However, the idea of formulating *P. fruticosa* in form of herbal health drink came from the traditional use of this herb as tonic drink. On the other hand, phenolics, flavonoids and particularly saponins are belived to play a key role in determing the quality of this herb. Since the *P. fruticosa*_based formuation was prepared in liquid form, the addition of preservatives and storage temperature had been taken into consideration. Indeed, significant difference in concentration of TPC and TFC can clearly be seen after 11 weeks of assay. The concentration of TSC in F3 (stored at ambient temperature without adding preservatives) was found almost empty even in week 11. Saponins, on the other hand, seem to be less sensitive to the temperature compared to that of TPC and TFC. However, for the long term storage (after 16 weeks), significant difference in concentration of TSC has been clearly noted. These findings suggest that addition of preservatives and storage temperature should be taken into account when formulating the *P. fruticosa*_based herbal health drink, depending on the storage time. Since this research mainly focus on the quantity of TPC, TFC and TSCin liquid form, these herbal drinks can be

spoiled by certain microorganisms such bacteria, viruses or yeasts and molds. Thus, to secure the quality of *P. fruticosa*_based herbal health drink, preservatives and storage temperature should be regarded as factors affecting the stability of product.

4. CONCLUSION

The addition of preservatives (combined potassium sorbate and sodium benzoate) and storage temperature had evidently an impact on the quantity of total phenolic content, total flavonoid content and total saponins content in *Polyscias fruticosa*herbal health drink over the period of 16 weeks. The significant difference in concentration of total phenolic content and total flavonoid content can clearly be seen after 6 weeks of tested time, while the impact on the total saponins content can only be noted after 11 weeks of experiment. These findings could be considered to be one of the key factors when formulating the herbal health drink based on the *Polysciasfruticosa* extract.

ACKNOWLEDGEMENTS

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Huan, V. D., Yamamura, S., Ohtani, K., Kasai, R., Yamasaki, K., Nham, N. T., & Chau, H. M. (1998). Oleananesaponins from *Polysciasfruiticosa*. Phytochemistry, 47(3), 451-457.
2. Quisumbing, E. (1951). Medicinal plants of the Philippines. Department of Agriculture and Commerce, Philippine Islands Technical Bulletin., (16).
3. Đỗ, T. L. (2007). NhữngcâythuốcvàvịthuốcViệt Nam.

4. Hebbar, R. D., &Monnanda, S. N. (2013). Phytochemical screening, total phenolic content and in vitro antioxidant studies of leaf, bark and flower extracts of Schefflera spp.(Araliaceae).

5. Garg, M., & Ahuja, V. (2015). Development and Evaluation of a Nutraceutical Herbal Summer Drink. Development, 1, 24284.

6. Vo, N. L. P. 2017. Optimization of conditions for saponins extraction from *Polyscias fruticosa* (L.) Harms using response surface methodology, Ho Chi Minh City, Vietnam: International University, BSc Thesis

7. Li. B., Wong. C., Cheng. K., & Chen. F. (2008). Antioxidant properties in vitro and total phenolic contents in methanol extracts from medicinal plants. LWT-Food Science and Technology. 41, 385-390.

8. Zhishen J., Mengcheng T., Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem, 1999; 64: 555-559

9. Hiai, S., Oura, H., & Nakajima, T. (1976). Color reaction of some sapogenins and saponins with vanillin and sulfur1c acid. PlantaMedica, 29(02), 116-122

10. Groot, H. D., &Rauen, U. (1998). Tissue injury by reactive oxygen species and the protective effects of flavonoids. Fundamental & clinical pharmacology, 12(3), 249-