

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 preservatives and storage temperature on the total phenolic content, total flavonoid content, and total saponin content were investigated over the period of 16 weeks.

Methodology: *Polyscias fruticosa* extract-based herbal drinks were formulated. Potassium sorbate and sodium benzoate were used as preservatives while storage temperature was set at 4 and 25°C. 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 content can clearly be seen after 6 weeks, while significant 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°C 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°C, 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°C) has been the optimal condition to maintain the quantity of biological phytochemicals 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.

30 1. INTRODUCTION

31 *Polyscias fruticosa* (L.) Harms. (*P. fruticosa*) belongs to the family Araliaceae, which is a
32 member of the ginseng family. In Asian countries, *P. fruticosa* leaves are used as a tonic,
33 anti-inflammatory, antitoxin, antibacterial, and digestive support [1,2]. The root extract of *P.*
34 *fruticosa* has been traditionally used for the treatment of ischemia, anti-dysentery, anti-
35 inflammatory, neuralgia, and rheumatic pains [3,4,5,6]. Phytochemical studies have shown
36 that *P. fruticosa* contains a variety of bioactive components such as alkaloids, glucosamine,
37 saponins, flavonoids, tannins, and B vitamins [7]; particularly the presence of some essential
38 amino acids including lysine, cysteine and methionine. However, the pharmacological
39 activities of *P. fruticosa* could be attributed to the presence of saponins, [8], phenolics and
40 flavonoids. Being potent antioxidants, phenolics and flavonoids in *P. fruticosa* possess
41 certain health benefits such as preventing the growth of prostate and lung cancer, improving
42 vascular health, exhibiting anti-mutagenic, anti-asthma and anti-cancer activities [9,10,11].

43 In recent years, many nutraceutical products have been developed which take advantages of
44 the availability and potential nutrition of traditional medicinal plants [12]. One such plant is *P.*
45 *fruticosa*. Herbal beverages are considered to be an excellent medium for the
46 supplementation of nutraceutical components for enrichment, mainly bioactive herbal
47 extracts [13]. It has been scientifically proven to be more convenient to consume a beverage
48 providing health benefits rather than swallow vitamins or pills for the same influences. *P.*
49 *fruticosa* herbal health drink not only brings a new yield to the beverage industry market but
50 also delivers to the customers a product that can be consumed daily and contains
51 nutraceutical ingredients for the body. In view of these considerations, the stability and
52 quality of the products under storage conditions need to be analyzed.

53 2. MATERIAL AND METHODS

54 2.1. Chemicals and materials

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

59 18. Folin-Ciocalteu reagent was obtained from Merk (Darmsradt, Germany). Catechin,
60 gallic acid, and oleanolic acid were purchased from Sigma-Alrich (USA). All other reagents
61 used in the research were of analytical grade.

62 2.2. Preparation of *P. fruticosa* (L.) Harms herbal health drinks

63 2.2.1. Preparation of plant extract

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

68 Ten grams (10 g) of each *P. fruticosa* powder was soaked in 100 mL of distilled water.
69 Subsequently, the mixture was incubated at 60.9°C for 5 hours using shaking incubator (IKA,
70 Germany) at 200 rpm, followed by vacuum filtration to obtain the transparent solution. The
71 extract process yielded 37.42±26 mg of oleanolic acid which was almost the same quantity
72 (37.18 mg) extracted by Vo (2007) [14].

73 2.2.2. Herbal Formulations

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

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

Formula	<i>P. fruticosa</i> 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

84

85 **2.3. Analytical method**

86 **2.3.1. Determination of total phenolic content**

87 The total phenolic content (TPC) of *P. fruticosa* extract was measured by Folin-Ciocalteu
88 method [15]. 2 mL of diluted sample was mixed with 2 mL of Folin-Ciocalteu (10% v/v). The
89 mixture was incubated at room temperature for 4 minutes, followed by the addition of 1.6 mL
90 of sodium carbonate (7.5% w/v) to the mixture. Then the mixture was vortexed for 10
91 seconds and incubated in dark condition for 2 hours. The absorbance was measured at 765
92 nm. Gallic acid was used to calibrate the standard curve.

93 **2.3.2. Determination of total flavonoid content**

94 The total flavonoid content (TFC) of *P. fruticosa* extract was measured by colorimetric
95 method [16]. 0.5 mL of diluted sample was mixed with 0.15 mL of sodium nitrite (5% w/v)
96 and incubated at room temperature for 6 minutes, followed by the addition of 0.3 mL of
97 aluminum chloride (10% w/v) to the reaction mixture. The mixture was kept for 5 minutes
98 before adding 1 mL of sodium hydroxide (1 M) and then mixed well by vortex mixer. The
99 absorbance was measured at 510 nm. The total flavonoid content was expressed as
100 milligram of catechin per gram of *P. fruticosa* powder.

101 **2.3.3. Determination total saponin content**

102 The method for determination of total saponin content (TSC) of *P. fruticosa* extract was
103 based on the colorimetric methods [17]. 0.5 mL of sample was mixed with 0.5 mL of vanillin
104 reagent (8% w/v in absolute ethanol). The test tubes were placed in cold water, and 5 mL of
105 sulfuric acid (72% v/v) was added slowly on the inner side of the test tubes, and allowed to
106 stand for 3 minutes. The mixture was heated to 60°C for 10 minutes in water bath. Then the
107 test tubes were removed and cooled in cold water. The absorbance was measured at 544
108 nm. Oleanolic acid was used to calibrate standard curve.

109 **2.3.4. Statistical analysis**

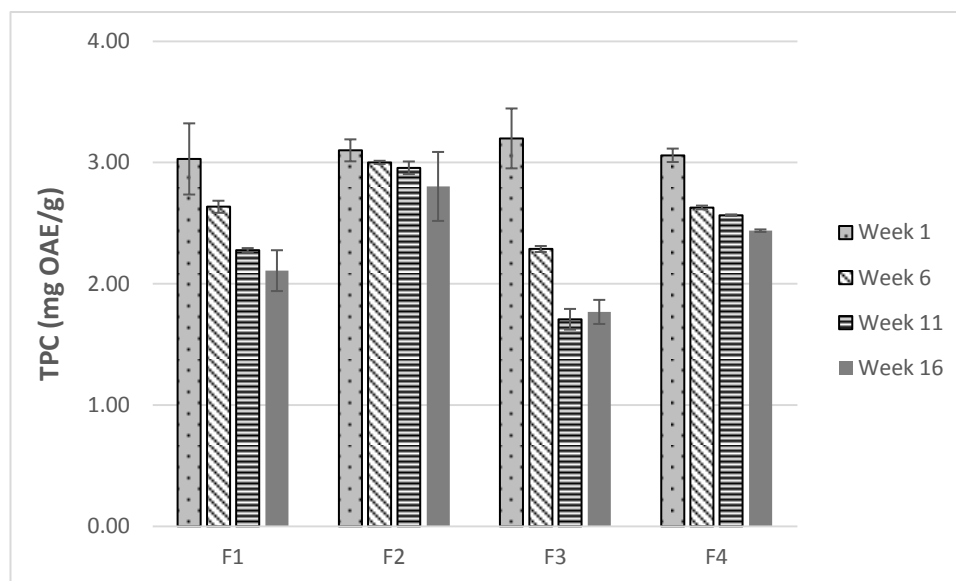
110 All experiments were conducted in triplicate, and the results were expressed as mean \pm SD.

111 Statistical analysis was performed by SPSS and analysis of variance (ANOVA) with the level

112 of significance $p = .05$.

113 **3. RESULTS AND DISCUSSION**

114 **3.1. Phenolic stability during long term storage**



115

116 **Figure 1: Total phenolic content change with the presence/absence of preservatives**
117 **under different storage temperatures**

118 The effect of preservatives and storage temperature on the change of TPC in the *P. fruticosa*

119 herbal drink had been assayed for the period of 16 weeks (Fig 1). The results were

120 expressed in a unit of milligram of gallic acid per gram of extracted powder. Gallic acid is a

121 type of phenolic acid which is known as 3, 4, 5-trihydroxybenzoic acid [18]. In general, the

122 concentration of TPC in all formulas showed the downward trends over the tested time.

123 [19,20]. However, the impact can clearly be seen for the formula with addition of

124 preservatives and kept at low temperature while the formula without the addition of

125 preservatives or maintained at ambient temperature showed significant decrease in TPC.

126 The quantity of TPC in the formula F2 was stable over this time period. The concentration of

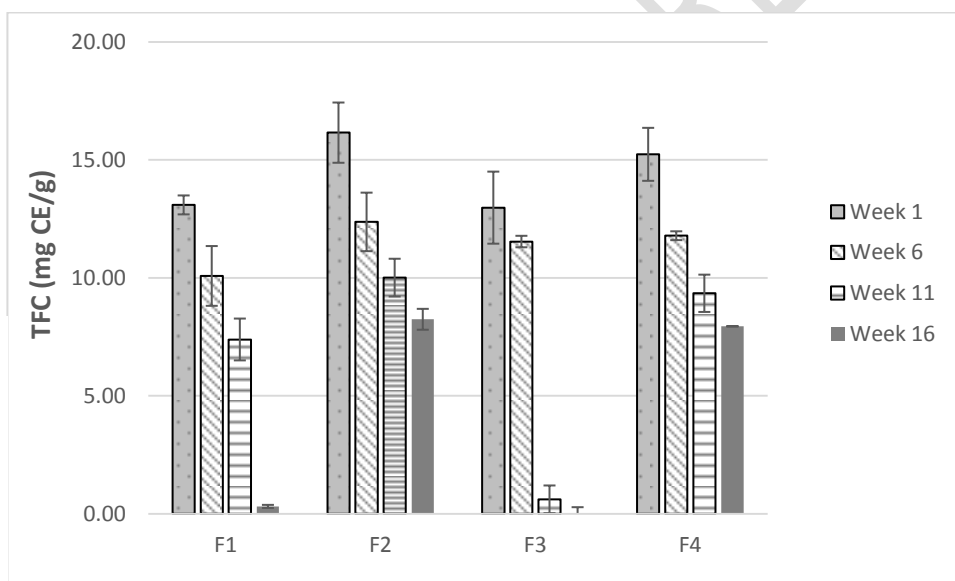
127 TPC was measured at 3.10 ± 0.59 mg GAE/g in week 1, stood at a value of 3.00 ± 0.01 mg

128 GAE/g in week 6, and then maintained almost constant at a value of 2.96 ± 0.05 mg GAE/g

129 and 2.80 ± 0.26 mg GAE/g after 11 and 16 weeks, respectively. Meanwhile, the concentration
 130 of TPC in F4 (stored at fridge temperature without the preservatives) was almost stable in
 131 the first week (3.36 ± 0.09 mg GAE/g), but slightly reduced to 2.63 ± 0.06 mg GAE/g in week 6
 132 and then to 2.57 ± 0.01 mg GAE/g and 2.44 ± 0.01 mg GAE/g in week 11 and 16, respectively.
 133 On the contrary, the impact of storage temperature on the TPC in F1 and F3 had been noted
 134 as the concentration of TPC was 3.03 ± 0.29 mg GAE/g and 3.2 ± 0.25 mg GAE/g in week 1,
 135 but fell to 2.11 ± 0.17 mg GAE/g and 1.77 ± 0.1 mg GAE/g after 16 weeks, respectively.

136 3.2. Flavonoid stability during long term storage

137 Figure 2 illustrates how the concentration of TFC in *P. fruticosa* herbal drink was altered with
 138 the interference of storage temperature and preservatives. Catechin was used as a
 139 reference since it is the primary flavonoid found in tea belonging to this group of polyphenols
 140 [18].



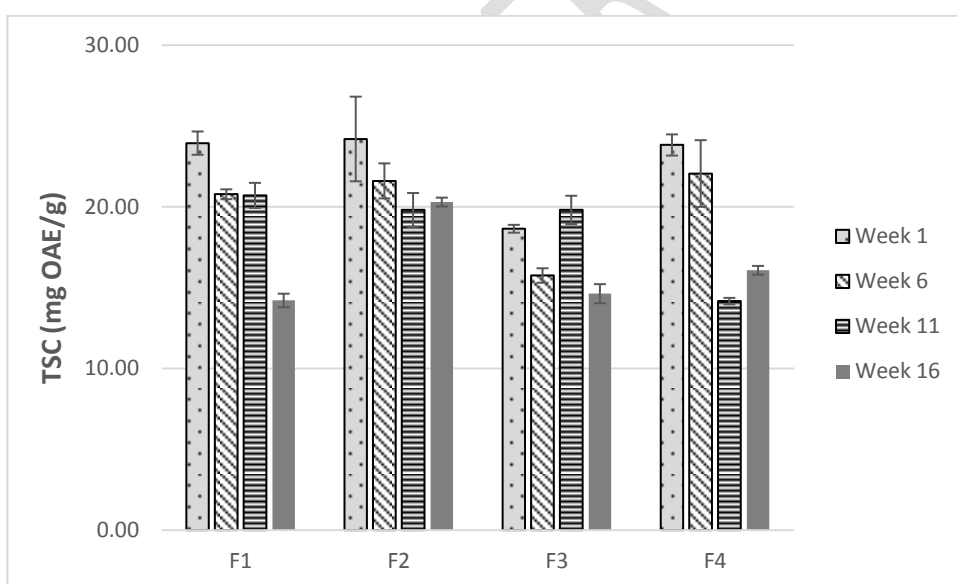
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142 **Figure 2: Total flavonoid content change with the presence/absence of preservatives**
 143 **and under different storage temperatures**

144 It can clearly be seen that the presence of preservatives at fridge temperature could not
 145 secure the quantity of TFC in herbal drink over the assay time as the concentration of TFC in
 146 F2 significantly reduced from 16.15 ± 1.28 mg CE/g in week 1 to 12.37 ± 1.24 mg CE/g in week
 147 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
 148 after 16 weeks. It should be noted, however, the fridge temperature might partially play a key

149 role in maintaining the concentration of TFC in herbal drink without adding the preservatives.
 150 Accordingly, the concentration of TFC in F4 was measured at a value of 15.23 ± 1.12 mg
 151 CE/g in the first week, went down to 11.78 ± 0.19 mg CE/g in week 6 and reduced to
 152 9.34 ± 0.79 mg CE/g in week 11. Noticeably, there was no significant difference in
 153 concentration of TFC between F2 and F4 after 16-week experiment as it was found at a
 154 value of 7.94 ± 0.01 mg CE/g for the F4 after 16 weeks. Meanwhile, the extremely significant
 155 difference can be observed for the concentration of TFC in F1 and F3 over the same period.
 156 The concentration of TFC in F1 was found to be 13.09 ± 0.4 mg CE/g in the first week,
 157 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
 158 steeply plunged to 0.31 ± 0.06 mg CE/g after 16 weeks. On the same manner, the
 159 concentration of TFC in F3 (stored at ambient temperature without preservatives) just
 160 stabilized at a value of 11.54 ± 0.24 mg CE/g for a period of 6 weeks, and then sharply fell to
 161 a value of 0.61 ± 0.59 mg CE/g in week 11 and found empty after 16 weeks.

162 **3.3. Saponin stability during long term storage**



163

164

165 ***Figure 3: Total saponin content change with the presence/absence of preservatives***
 166 ***and under different storage temperatures***

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

169 been evidenced as can be seen in F2. Accordingly, the concentration of TSC in F2 was
170 measured at a value of 24.2 ± 2.62 mg OAE/g in the first week, slightly reduced to 21.61 ± 1.08
171 mg OAE/g in week 6 and steadily declined to 19.81 ± 1.04 mg OAE/g in week 11, but still
172 maintained almost same value after 16 weeks (20.29 ± 0.27 mg OAE/g). Meanwhile, the
173 same trends had been observed in F4, but only for the first 11 weeks. The concentration of
174 TSC in F4 stayed at a value of 23.83 ± 0.65 mg OAE/g in the first week, slightly decreased to
175 22.06 ± 2.06 mg OAE/g in week 6 and slowly went down to 20.27 ± 0.2 mg OAE/g after 11
176 weeks; however, it significantly dropped to 16.08 ± 0.25 mg OAE/g in week 16. In the same
177 way, the concentration of TSC in F1 (with the presence of preservatives at ambient
178 temperature) was found at a value of 20.71 ± 0.78 mg OAE/g in week 11, but sharply fell to
179 14.21 ± 0.42 mg OAE/g after 16 weeks. In contrast, the concentration of TSC in F3 was found
180 to be low throughout the tested time. It stood at low concentration even in the first week
181 (18.64 ± 0.24 mg OAE/g), slowly reduced to 15.75 ± 0.45 mg OAE/g, but remained stable
182 between week 11 and 16 at the value of 14.17 ± 0.88 and 14.63 ± 0.59 mg OAE/g,
183 respectively.

184 In Vietnam, *P. fruticosa* has been formulated as a supplement for relieving ischemia and
185 enhancing circulation of brain blood [21]. However, the idea of formulating *P. fruticosa* in
186 form of herbal health drink came from the traditional use of this herb as tonic drink. On the
187 other hand, phenolics, flavonoids and particularly saponins are believed to play a key role in
188 determining the quality of this herb [22]. Since the *P. fruticosa* extract-based formulation was
189 prepared in liquid form, the addition of preservatives and storage temperature had been
190 taken into consideration. Indeed, significant difference in concentration of TPC and TFC can
191 clearly be seen after 11 weeks of assay. The concentration of TSC in F3 (stored at ambient
192 temperature without adding preservatives) was found almost empty even in week 11.
193 Saponins, on the other hand, seem to be less sensitive to the temperature compared to that
194 of TPC and TFC. However, for the long term storage (after 16 weeks), significant difference
195 in concentration of TSC has been clearly noted. These findings suggest that addition of
196 preservatives and storage temperature should be taken into account when formulating the *P.*
197 *fruticosa* extract-based herbal health drink, depending on the storage time. Since this
198 research mainly focus on the quantity of TPC, TFC and TSC in liquid form, these herbal

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

202 4. CONCLUSION

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

211

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213 Not applicable.

214

215 COMPETING INTERESTS

216 Authors have declared that no competing interests exist.

217

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