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The Stability of Antioxidants Turmeric and Tamarind (*Curcuma domestica* Val. -*Tamarindus indica* L.) Leaves Extract During Storage.

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ABSTRACT

A mixture of turmeric and tamarind leaves extract potential as a source of antioxidants.The purpose of this study was to evaluate the stability of the antioxidant mixture of turmeric and tamarind leaves extract during storage.The experiments using complete randomized design(CRD), with the treatment: room temperature (25±2)°C; cold temperatures (5±2)°C; and frozen temperatures (-10±2)°C, observed every week for 8 weeks, treatment was repeated three times. Variablesmeasured were total phenolic, vitamin C, the antioxidant capacity and IC_{50}. Results showed: storage temperatures had no effect on all observed variables: the total phenolic content, vitamin C, antioxidants capacity, and $IC_{50}$of the extract remained stable at alltemperature storage during 8-week storage. Extract stored at (25±2)^oC, (0 ±2)^oC and(-10 ±2)^oC total phenolic (15,01315,571, 15,655) g GAE/100g;vitamin C(340,52;448,54 and 454,21) mg/100g; the antioxidants capacity (6,149, 6,516, and 6,836)g GAEAC/100g; and IC_{50}(93,333, 84,667and 79,647) µg /mL.

Keyword: turmeric, tamarind leaves, antioxidant, temperature storage

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INTRODUCTION

Traditional medicine has been increasingly used in Indonesia nowadays, not to mention herbal for cosmetics. Unfortunately, more than 60% of the herbal materials are still imported. Indonesia is the mega-center of world biodiversity, taking second place to the list of the richest. [1]. To minimize the number of traditional and herbal medicine that needs to be imported, Directorate of Binfar has been developing methods to process raw materials for traditional medicine and herbal for cosmetics. Some extract industries can be found in Bali. Extracts are the main material of spa. The active ingredients of extracts that are essential for spa are antioxidants.

Turmeric and tamarind leaves have potential as a source of antioxidants [2]. The antioxidant activity in turmeric is sourced from curcumin [3, 4]. It has been proven that curcumin performs antioxidants activity [5,6]. Meanwhile, the antioxidant characteristics of tamarind leaves come from vitamin C [2] and phenolic compounds [7,8]. Some research has suggested that the combination of these two antioxidants may result in the improved effectiveness and synergism of both [9,10]. The results of research[11] the synergism of antioxidants contained in a drink (*sinom)* that is made from turmeric and tamarind leaves. The synergism was also observed in the extract of the mixed plants. The highest synergism of antioxidants (6,25± 0,459 g GEAEC/100 g extract) in turmeric and tamarind leaves extract was found at a ratio of 8:2[12].

Antioxidants in some plants can be used in cream a active ingredients because they perform a photo-protective effect that can protect skin from ultraviolet (UV) [13]. The synergism of the antioxidants will improve the photo-protective effect so that the ability of the cream to protect skin from UV light will also increase which as a result will be beneficial for spa industry. As a traded main material, extracts become important. Extract stability is characterized by the stability of the antioxidant contained in it during storage. Therefore, it is necessary to conduct research on the stability of antioxidants found in turmeric and tamarind leaves extract during storage.

MATERIALS AND METHODS

Material:

The Turina-1 turmeric variety plants from BPPT Bogor-Indonesia which were cultivated in Antap village, Tabanan Bali were harvested at the age of nine months. The tamarind leaves used for this research were taken from the buds of local planted in Jimbaran, Badung, Bali, DPPH, gallic acid(Sigma), *Folin ciocalteu phenol*, Tiobarbituric acid and Na_2CO_3(Merck), iodine, ethanol andmethanol(Brathaco Chemical)

Experimental design:

The experiment used samples of turmeric and tamarind leavesextract ratio of 8: 2. The samples store at room temperature (25±2)°C; cold temperature (0±2)°C; and freeze temperature (-10 ± 2)°C. The the sample is packed with a closed vial, keep for 8 weeks and change of variable was observed every week. The data were analyzed descriptively by looking at the trend of change of variable during storage. Week 8 data was analyzed for its diversity and continued Least Significance Different (LSD) test if treatment had an effect.

Preparation of Turmeric Extract and Tamarind Leaves Extract:

First, the turmeric was washed, drained and then sliced ± 1 mm while the tamarind leaves was withered overnight. Second, both materials were dried-oven at 55 ± 2°C until it reached the water content of a maximum10%. Third, both ingredients were turned into powder and sieved with 80 mesh. Fourth, the turmeric and tamarind leaves powder were macerated/ soaked in ethanol 96% with a ratio of 1:6 for the powder and its solvent. The maceration process was conducted in 2 phases with each phase lasting for 24 hours. During each phase, the mixture was stirred twice. The filtrate was then separated using a rotary evaporator at 40°C and a pressure of 100 m Bar.

The sample unit is a mixture of turmeric extract and tamarind leaves extract ratio (8: 2), the sample is packed with a closed vial. Samples were stored at room temperature (25±2)°C; cold temperature (0±2)°C; and freeze temperature (-10 ± 2)°C. Samples were observed every week until week 8.

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Antioxidant Capacity, DPPH method [14]

The sample, weighed 0.1 g, was dissolved in methanol extract to create a 5 ml sample. The 10 µl sample is then added with 90 µl of methanol, vortex. The 200 µl sample was taken and added into the 1.4 µl of DPPH, vortex. Left the sample in an open air for 30 minutes and then the absorbance level was measured at λ = 517 nm. Antioxidant capacity was calculated using a standard curve of gallic acid with concentrations (0, 5, 10, 15, 20, 25) ppm

Total Phenolic [15]

The 0.1 g extract was diluted with methanol up to 5 ml, and then made 10 µL. The methanol was then added into this mixture so that it reached the total volume of 500 µL. The 200 µL sample was taken before being added with 200 µL of methanol, 400 µL reagent Folin Ciocalteu phenol and 4.2 ml Na_2CO_3. In a test tube. Next, the tube was vortexed and left in the open air for 30 minutes. Such materials were measured for its absorbance at λ = 760 nm. The determination process of the phenolic acids level was done by using a standard curve.with concentrations (0, 10, 20, 40, 60, 80,100) ppm.

Antioxidant capacity [16]

Add 100 ml of sample in test tube filled with 3 mL of methanol and homogenized with vortex. Added 1 mL DPPH and left in the dark at room temperature 15 in minutes. The decrease of DPPH absorbance was measured using a spectrophotometer at λ517 nm. Standard used gallic acid with a concentration equal to the concentration of sample solution. Theinhibition free radical of the sample was measured at concentrations (0.00, 0.02, 0.04, 0.08 and 0.12) mg / mL. Penghambatan radikal bebas DPPH pada sampel konsentrasi (0,00; 0,02; 0,04; 0,08 dan 0,12) mg/mL.The result of the antioxidant activity calculation was put into line equation y = ax + b with the concentration(mg /L) as the abscissa (x- axis) and the percentage of the antioxidant activity as the ordinate (y-axis).

RESULTS AND DISCUSSION

Figure 1 suggests a stable trend in turmeric and tamarind leaves extract until week 8. Changes in the phenolic compounds during storage were affected by water transmission, oxygen, and permeability of the package. Research conducted by [17] proved that mango extract which had been stored for 182 days in a polyethylene container had lower phenolic compared to the one wrapped with aluminum foil. Due to the condition of the tightly sealed vial, water transmission, oxygen and permeability of the package were lower than of aluminium foil. As a result, the total phenolic content of the extract remained stable.

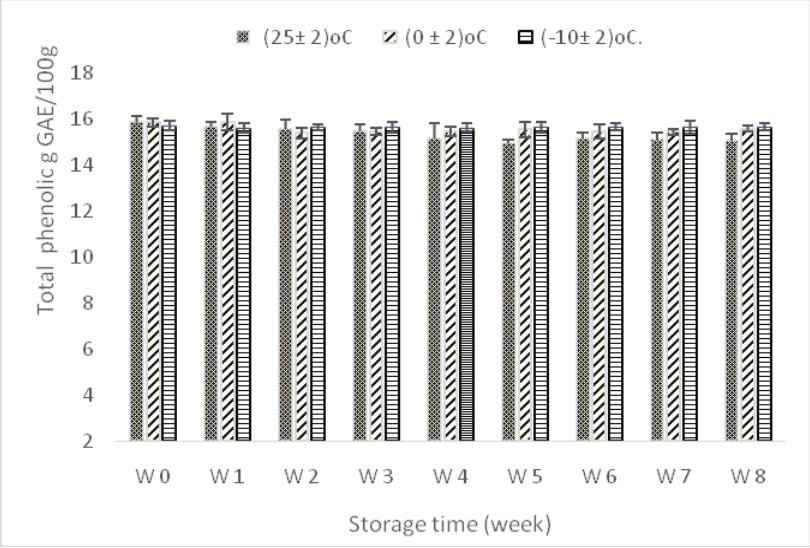

Figure 1: Trend total phenolic of turmeric and tamarind leaves extract for 8 week storage

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The results of the LSD test (Table1) indicated that the total phenolic content of the extract until week 8 were not changing despite varied storage temperatures. The extract was stored in a tightly sealed vial so that water transmission, oxygen, and permeability of the package remained the same. A study conducted by [17]indicated that mango extract which was wrapped with aluminium foil and stored at a room temperature (28-32)^{O}C contained stable phenolic until day 60. At (-20)^{O}C, the package and the storage temperature did not have any effect on the total phenolic content of the mango extract which was wrapped in aluminum foil until day 182. Similarly, [18] reported that a freezing temperature and 4-months storage did not affect the activity of bioactive components and antioxidants activity as well as total phenolic content of an extract, in particular the ones which are made from berry.

Table 1: Total phenolic of turmeric and tamarind leaves extract ratio 8:2 on 8th week of storage

Storage temperature	Total phenolic (g GAE /100g)		
(25± 2)°C	15,013	±	0,148[a]
(0 ± 2)°C	15,571	±	0,136[a]
(-10± 2) ° C	15,655	±	0,148[a]

Mean values ± SD (n = 3), the same superscript letter are not significantly different at 5%

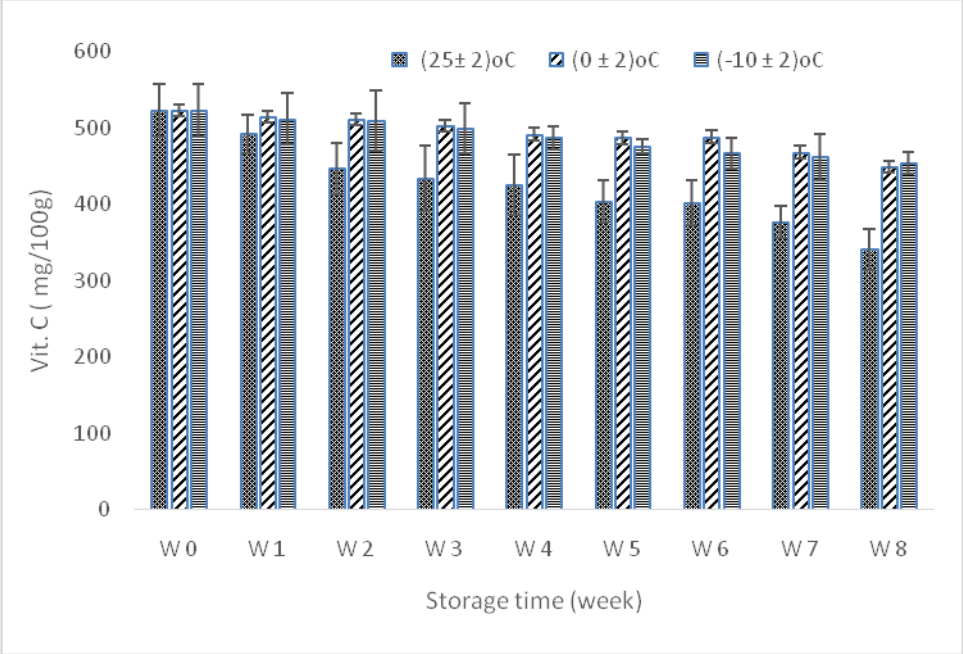

Figure 2: Trend vitamin C of turmeric and tamarind leaves extract for 8 week storage

The vitamin C content in turmeric and tamarind leaves extract during 8-week storage is presented by Figure 2. At (25±2)°C, there was a decline in vitamin C content while at(0 ± 2)°Cand (-10 ± 2)°C, vitamin C content was likely stable. Vitamin C is an unstable compound because it is susceptible to oxygen, light, and heat. Therefore, at(25±2)°C, vitamin C content decreased. Vitamin C is also easily oxidized, either in an enzymatic or non-enzymatic condition [19].

Table 2: Vitamin Cof turmeric and tamarind leaves extract ratio 8:2 on 8th week of storage

Storage temperature	Vitamin C (mg /100 g)		
(25 ± 2)°C	340,52	±	25,362[a]
(0 ± 2)°C	448, 54	±	15, 470[a]
(-10 ± 2)° C	454,21	±	13, 424[a]

Mean values ± SD (n = 3), the same superscript letter are not significantly different at 5%

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The results of the LSD test (Table 2) shows that storage temperatures did not have any significant effect on vitamin C content in extract in the eighth week. There was no difference observed in Vitamin C content on varied temperatures during storage. Asstated [20] that ascorbic acid loss in vegetables is most probably caused by oxidation which is induced by enzymes. However, at a freezing temperature, there is no enzyme activity so that ascorbic acid loss in vegetables which are stored for 12 months will not occur. High phenolic content in an extract could maintain vitamin C during storage because the enzymes are not active [21]. Extract which is stored in a place covered from the sunlight at a room temperature will also be able to keep the stability of the vitamin C content.

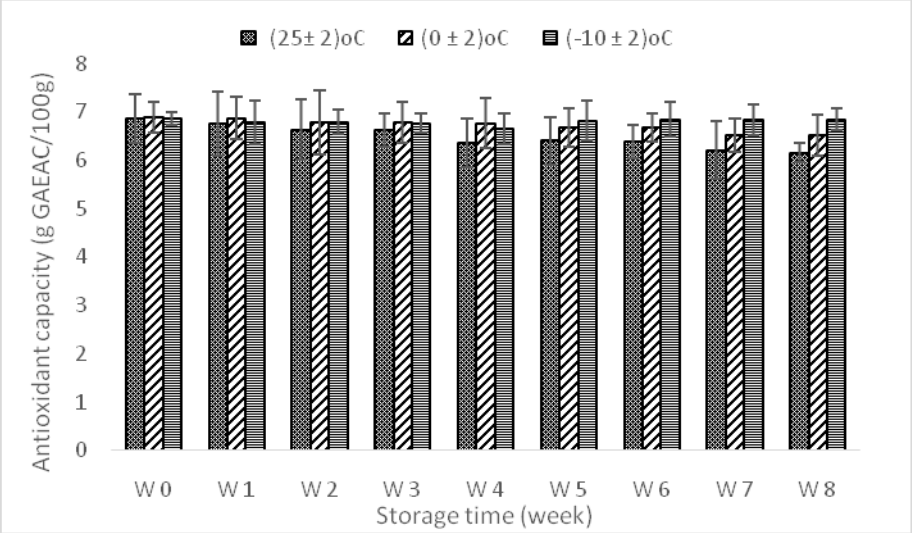

Figure 3: Trend antioxidant capacity of turmeric and tamarind leaves extract for 8 week storage

The trend shows a decrease in antioxidants capacity of turmeric and tamarind leaves extract after being stored for 8 weeks (Figure 3). No significant reduction of antioxidants was reported during the first three weeks of storage. Frozen storage did not change the capacity of the antioxidants [18,22] Also, storage in(0±2)^{o}C and (25±2)^{o}C did not change the antioxidants capacity [23, 24]. It has also been reported that there was no change in antiradical activity performed by orange juice stored in a refrigerator at 4 °C for 40 days [23]. Antioxidants activity is the result of some phytochemicals contained in an extract which generate synergism effects [24]. Synergism between turmeric extract and tamarind leaves extract maintained the capacity of the antioxidants during storage in a room temperature.

Table 3: Antioxidant capacity of turmeric and tamarind leaves extract ratio 8:2 on 8th week of storage

Storage temperature	Antioxidant capacity (gGAEAC /100g)		
(25± 2)^{o}C	6,149	±	0,201^{a}
(0 ± 2)^{o}C	6,516	±	0,428^{a}
(-10 ± 2) ^{o} C	6,836	±	0,225^{a}

Mean values ± SD (n = 3), the same superscript letter are not significantly different at 5%

The results of the LSD test (Table3) indicated that storage temperatures had no effect on the capacity of the antioxidants within 8 weeks. The ability of the extract to maintain the capacity of the antioxidants at a room temperature during storage may probably be caused by the synergism [21].In fruit, the contribution of ascorbic acid to synergism of antioxidants is 15% lower [25].The contribution of ascorbic acid to generate synergism of antioxidants capacity of a few kinds of fruit is only 0,4% [26,27, 28,29]. The stable antioxidants capacity at (25±2)^{o}Cmight be resulted from the contribution of ascorbic acid contained in the tamarind leaves extract which was more than 0,4%. The ascorbic acid stimulated synergism effects so that the antioxidants could last and remained stable during storage.

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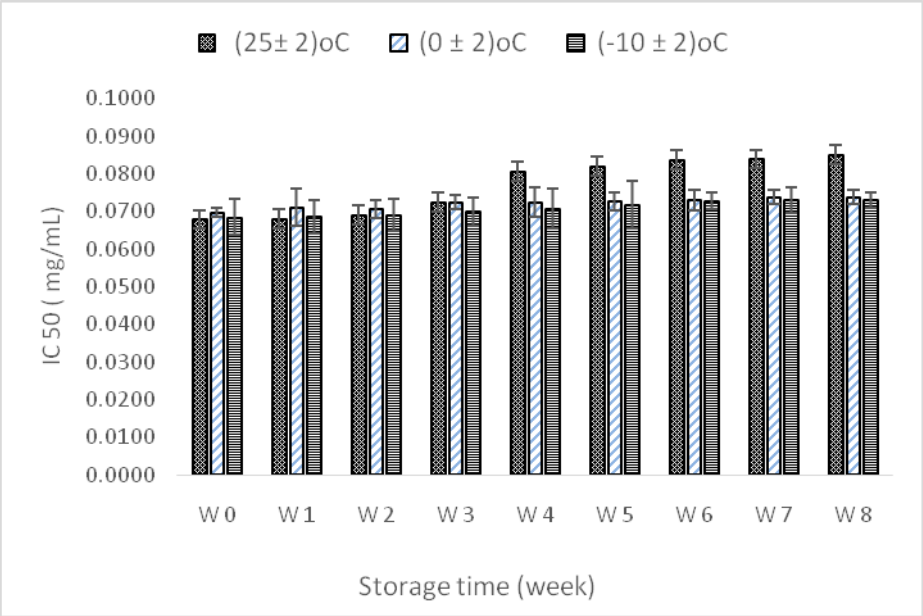

Figure 4: Trend IC_{50} of turmeric and tamarind leaves extract for 8 week storage

Figure 4 shows an increasing value of the extract IC_{50}during 8-week storage. At (25±2)°C, the IC_{50}value of the extract was higher than of other treatments. This finding indicated that at a cold (0 ±2) °C and freezing (-10 ±2) ° Ctemperature, the capacity of the antioxidants of the extract could last longer. The upward trend of IC_{50}value was observed on extract stored at (25±2)°C due to a decreased in vitamin C content. Antioxidants are compounds that are able to inhibit the activity of a free radical. The source of antioxidants is vitamin C which is easily oxidized by the surrounding condition.

Table 4: IC_{50} of turmeric and tamarind leaves extract ratio 8:2 on 8th week of storage

Storage temperature	IC_{50} (µg /mL)		
(25± 2)°C	93,333	±	4,78[a]
(0 ± 2)°C	84,667	±	2,05 [a]
(-10 ± 2) ° C	79,647	±	5,84 [a]

Mean values ± SD (n = 3), the same superscript letter are not significantly different at 5%

The results of the LSD test suggested that storage temperatures had no significant effect on the extract IC_{50} (Table 4).It showed that extract which was stored for 8 weeks with three different temperature degrees did not experience changes in inhibiting the activity of a free radical. The stability of the antioxidants capacity at (25±2)°Cmight be resulted from ascorbic acid contained in the tamarind leaves extract which produced a synergism effect [21]. Based on the range of the extract IC_{50} (79,647-93,333) µg/mL, the antioxidants of turmeric and tamarind leaves extract can be categorized as strong [30].

CONCLUSION

1. Storage temperatures had no effect on all observed variables: the total phenolic content, vitamin C, antioxidants capacity, and $IC_{50}$of the extract remained stable at (25±2)°C, (0 ±2)°C and (-10 ±2)°C during 8-week storage
2. Extract stored at (25±2)°C, (0 ±2)°C and (-10 ±2)°C had total phenolic content (15,01315,571, 15,655) g GAE/100g;vitamin C content (340,52;448,54 and 454,21) mg/100g; the antioxidants capacity(6,149, 6,516, and 6,836) g GAEAC/100g; and IC_{50}(93,333, 84,667and 79,647) µg /mL.

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