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### An Assessment of Antioxidant Characteristics from different ratios Of Turmeric and Tamarind (*Curcuma domestica* Val.-*Tamarindus indica* L.) Leaves extracts.

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#### ABSTRACT

Turmeric and tamarind leaves have potential as an antioxidant source and combining the two different types of antioxidants can improve its effectiveness and synergism. The objective of this research is to determine the antioxidant characteristics of different ratios of turmeric and tamarind leaves extract. This research used randomized block design with the ratio of turmeric and tamarind leaves extracted as follows: (10: 0; 9 :1; 8:2; 7:3; 6: 4; 5:5; 4:6; 3:7; 2:8 ; 1:9; 0: 10). The research was repeated twice resulting in 22 experimental units. The variables measured during this research included total phenolic level, antioxidant capacity, IC50 and vitamin C. After the data was analysis based on its variables, it was then tested using the Tukey HSD test. The results showed that the ratio of turmeric and tamarind leaves extract affected the total phenolic level, IC50 and Vitamin C. The total phenolic level increased with the higher ratio of turmeric extract (2.91 to 11.15 mg GAE / 100g extract); IC50 increased with the increasing ratio of tamarind leaves extract of (0,225 - 0.830)ppm; and vitamin C increased with the increasing ratio of tamarind leaves extract of (242.6 to 752.5) mg / 100 g extract. Changing the ratio of tamarind leaves and turmeric had no effect on the antioxidant capacity the value was (2.07 -5.44) mg GAEAC /100 g. Antioxidant synergism occurred when the ratio of turmeric and tamarind leaves extract was (9:1; 8:2; 7:3)

#### INTRODUCTION

The use of traditional medication in Indonesia is increasing and it includes the use of herbal as one of cosmetic ingredients. In Bali there are several industries that use herbal extract as the main ingredient in the spa industry as it has antioxidant.

Turmeric and tamarind leaves have potential as an antioxidant source (Mulyani, *et al.* 2014). The antioxidant activity in turmeric is caused by the presence of curcumin, which had been proven by a number of research to have an antioxidant activity. (Bengmark 2006; Arranz, *et al.*, 2010; Anand *et al.*, 2008;). The three major compounds of turmeric belong to the curcuminoid group, namely curcumin, desmethoxycurcumin, and bis-desmethoxycurcumin. Turmeric contains curcumin varies between 1.8 and 5.4% depending on its variety, extraction process, solvent solution used, and the length of the extraction process (Anon, 2012).

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Essential oil plays an important role in the subsequent reaction and it has an approximate of 25 compounds in turmeric extract. There is a quantitative variation in each chemical components of essential oils depending on the location of the turmeric plantation (Jayaprakasha *et al.*, 2005). Curcumin has a low level water solubility which limits its usefulness as an oral medication. Curcumin in form of turmeric extract has antiangiogenic effect five times higher than pure curcumin as a result of the presence of its derivatives components as well as other components. Moreover, turmeric extract has been proven to have a better pharmacological potential compare with pure curcumin (Liu *et al.*, 2008).

Moreover, the main source of antioxidant in tamarind leaves is from the presence vitamin C (Mulyani, *et al.* 2014) and phenolic compounds (Bhadoriya *et al.*, 2011, De Caluwe *et al.*, 2010 and Khairunnuur *et al.* 2009). The core antioxidant substances in tamarind leaves are vitamin C (Mulyani, *et al.* 2014) and phenolic compounds (Bhadoriya *et al.*, 2011, De Caluwe *et al.*, 2010 and Khairunnuur *et al.* 2009). Several studies have found the benefits of tamarind leaves in its flavonoid content (Dahesa *et al.*, 2006). Triterpenoid lupanone and lupeol contents in tamarind leaves were discovered by Imam *et al.*, 2007 (Imam *et al.*, 2007). Tamarind leaves also contain some essential oils with benzyl benzoate and limonene as its main components (Pino *et al.*, 2002). After the same length of cooking time tamarind leaves have shown a higher antioxidant activity compared with its fruit (Mulyani *et al.*, 2014). In certain ratios, the combination of tamarind leaves and turmeric extract has shown a higher synergy in its antioxidant activity level compared with the activity level of each component independently (Mulyani *et al.*, 2012).

The GS-MS results of tamarind leaves extracted using chloroform produce 18 peaks with varieties in the form of chemical compounds consist of essential oils, fatty acids, polyphenols, etc. There are two compounds that act as antioxidants namely 6 (2,6-di-tert -Butyl-4-methylphenol) and Methyl 3,5- di-tert-butyl-4-hidroxybenzoate (Arranz, *et al.*, 2010). It has been found that tamarind leaves have antioxidant and antimicrobial activity as a result of the discovery of eight new compounds. These compounds have been confirmed to be high in fatty acids and polyphenols. In high concentrations, tamarind leaves will act as a pro-oxidant / antioxidant cation (Arranz, *et al.*, 2010).

Some research suggested that combining the two different types of antioxidants can improve its effectiveness and synergism (Smet *et al.*, 2008; Noguier, *et al.* 2014). Synergism is a phenomenon in which a net interactive antioxidant effect is higher than the sum of the individual effects (Choe and Min, 2009). There had been a study on the antioxidant synergism of turmeric and tamarind leaves in the *sinom* beverage. The result of this study shown that the combination of the two antioxidants produced a higher level of synergism compared with a partial antioxidant (Mulyani *et al.*, 2011). Combination of several antioxidants gives a better protection against free radical attack and that a combination of two antioxidants increases its activity level (Noguier, *et al.* 2014). With a combined antioxidants, free radicals receive two antioxidants, one antioxidant react against peroxy radical while the other regenerates the first one. As a result they become a very effective combination to fight free radical. Moreover, the presence of phenolic antioxidant and ascorbic acid works along with this synergism (Uri, 1961 in Brewer, 2011)

Based on the explanation above, there is substantial antioxidant synergism of turmeric and tamarind leaves. However, the right ratio of turmeric and tamarind leaves extract, in order to be synergic and able to produce the highest level of antioxidant activity, is not yet known. Therefore, it important to know the level of antioxidant activity from different ratio of turmeric and tamarind leaves extracts. The purpose of this research is first, to determine the level of antioxidant activity based on the different ratio of turmeric and tamarind leaves extract. Second, to find the extract ratio that produces the highest level of synergism in its antioxidant activity.

## MATERIALS AND METHODS

### **Material:**

#### **Preparation of Turmeric Extract and Tamarind Leaves Extract:**

First, the turmeric was washed, drained and then sliced  $\pm 1$  mm while the tamarind leaves was withered overnight. Second, both materials were dried-oven at  $55 \pm 2$  ° C until it reached the water content of a maximum 10%. 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.

### **Experimental design:**

The experiments were done by using a Random Block Design with the ratio of turmeric and tamarind leaves extract, as follows: (10: 0; 9 :1: 8:2; 7:3; 6: 4; 5:5; 4:6; 3:7; 2:8 ; 1:9; 0: 10). The experiments were twice. The results were then analyzed based on its diversity (ANOVA) before being tested by using a Tukey HSD test.

***Antioxidant Capacity, DPPH method (Yun, 2001):***

The sample, weighed 0.1 g, was dissolved in methanol extract to create a 5 ml sample. The 10  $\mu$ l sample is then added with 90  $\mu$ l of methanol, vortex. The 200  $\mu$ l sample was taken and added into the 1.4  $\mu$ l of DPPH, vortex. Left the sample in an open air for 30 minutes and then the absorbance level was measured at  $\lambda = 517$  nm. Antioxidant capacity was calculated using a standard curve of gallic acid.

***Standard curve gallic acid for DPPH:***

DPPH solution is made from 0,004 g DPPH diluted with methanol until the volume reaches 100 ml. The gallic acid standard curve is created as follows: weighed 0,01g gallic acid and then dissolved it in 100 ml of distilled water (concentration: 100 ppm). The solution then diluted to concentrations (0, 5, 10, 15, 20, 25) ppm. Next, 200  $\mu$ l gallic acid solution from each concentration was added into the 1,4 mL DPPH before being vortexed and left open for 30 minutes. The absorbance level was measured at  $\lambda = 517$  nm. The concentration and absorbance levels are used as the standard of gallic acid curve.

***Total Phenolic (Sakanaka *et al.*, 2005):***

The 0.1 g extract was diluted with methanol up to 5 ml, and then made 10  $\mu$ L. The methanol was then added into this mixture so that it reached the total volume of 500  $\mu$ L. The 200  $\mu$ L sample was taken before being added with 200  $\mu$ L of methanol, 400  $\mu$ L reagent *Folin Ciocalteu phenol* and 4.2 ml  $\text{Na}_2\text{CO}_3$ . 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 level at  $\lambda = 760$  nm. The determination process of the phenolic acids level was done by using a standard curve.

***The standard curve of total phenolic:***

The process of making gallic acid standard curve is as follows: first, dissolved 0,01g gallic acid in 100 ml of distilled water (concentration: 100 ppm). Second, dilute the solution to seven different concentrations level. They are 0, 10, 20, 40, 60, 80, and 100 ppm. Third, take 0,4 mL from each solution, mix it with folin-Ciocalteu 0,4 mL and vortex it until homogenous before being added with 4,2 mL of 5% sodium carbonate and vortex. Left the sample in an open air for 30 minutes and then the absorbance level was measured at  $\lambda = 760$  nm. Concentration and absorbance. The concentration and absorbance levels are mentioned as its standard gallic acid curve.

***Test Vitamin C (Sudarmaji *et al*, 1984):***

The 0.2 g sample was diluted with aquadest until it reached 100 ml before being filtered. The 20 ml of the filtrate was taken out and added into a 1 ml of 1% starch solution. It was then titrated with 0.01 N iodine solution until the solution turned into a light blue color.

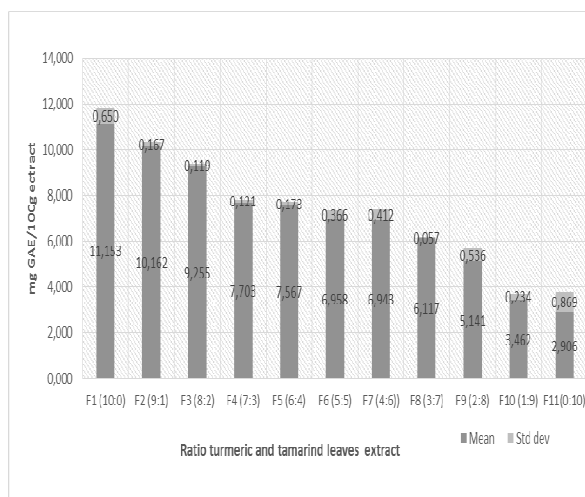
***Antioxidant capacity ( $\text{IC}_{50}$ ):***

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). The  $\text{IC}_{50}$  value showed the extract concentration or ratio that was capable of inhibiting the activity of free radical by 50% (Molyneux, 2004)

## RESULTS AND DISCUSSION

***Total Phenolic:***

Figure 1 shows the total phenolic in all treatments decreased when the ratio of tamarind leaves extract was increased. The decline in total phenolic extract was associated with the less amount of turmeric extract in the mixture. The main content of turmeric is flavonoids which is phenolic compound (Arranz, *et al.*, 2010). Another form of phenolic compounds is curcumin in turmeric, which also acts as antioxidant (Anand *et al.*, 2008). Mukhopadhyay *et al.* (2001) stated that,

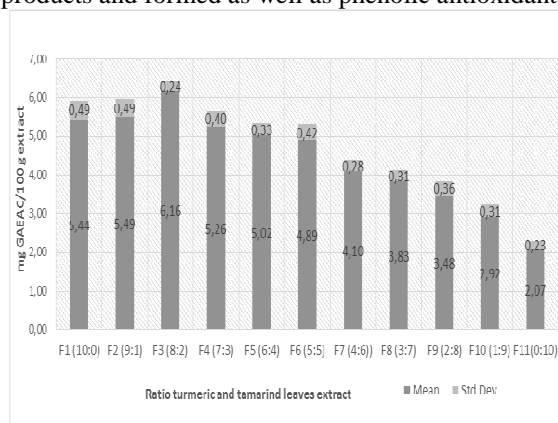


**Fig. 1:** The ratio of turmeric and tamarind leaves extract on total phenolic. Different notations indicate significant differences based on Tukey HSD test at 5% confidence level

curcumin has a potential as a highly effective free radical scavenger. Group O-methoxyphenol and hydrogenmethylenic are responsible for the antioxidant activity of curcumin. Curcumin donates an atom electron or a hydrogen reactive oxygen. Curcumin also interacts with a number of biomolecules through a non-covalent and covalent bonding (Priyadarsini, 2013).

#### **Antioxidant Capacity:**

The antioxidant capacity based on the turmeric and tamarind leaves extract can be seen in Figure 2. Antioxidant capacity showed an increasing trend in the treatment of F2, F3 subsequently decreased starting from F4. Antioxidant capacity value continues to decrease with the increasing ratio of tamarind leaves extract. This shows that in the capture of free radicals turmeric extract has a higher capacity than the tamarind leaves. The main component of turmeric extract is curcuminoid with curcumin as an antioxidant. Curcumin has a phenolic structure, which has the characteristics of a hydroxyl group (OH) attached to the benzene ring. When the reactive oxygen species (ROS) in this case DPPH reacts with extracts, free radical / DPPH will affect electron activity of curcumin to release the substituent, and present as substituents on the phenyl ring in phenolic antioxidants. As a result, O-H bond is broken and hydrogen ions are released. The hydrogen ions are available for DPPH free radicals, which then extinguish ROS reactive tendencies. The speed of reaction, depending on the relative stability of the oxidized products and formed as well as phenolic antioxidants involved



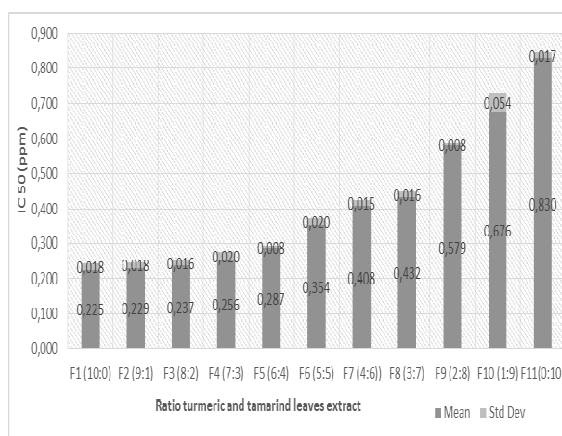
**Fig. 2:** The antioxidant capacity based on the ratio of turmeric and tamarind leaves extract. Different notations indicate significant differences at 5% confidence level

From Figure 2, the graph shows an increase in antioxidant capacity in the treatment of F2, indicating that the treatment occurred synergism. The antioxidant capacity showed an increasing trend during the F2 and F3 treatment before decreasing at F4. This condition indicated that a synergy exists due to the increased antioxidant capacity compared with other treatments. This synergy also means that the right ratio of the mixture is able to provide better protection against the free radical DPPH (Noguer, *et al.* 2014). Out of the three treatments, the F3

treatment has shown the best synergy to provide better protection against free radical attack. This was supported by information from Parasramka and Gupta (2012). They described that the synergy effect also occurs at the ratio of curcumin and garcinol (1:10, 1: 5 and 1: 2,5). In that ratio, the concentration was two to five times lower in order to produce the same effects during treatment compared with the use of curcumin alone. The combination of garcinol and curcumin has explicitly shown a strong synergism in the development of cell survival and opoptosis.

#### ***IC<sub>50</sub> Antioxidant:***

The IC<sub>50</sub> is defined as the necessary antioxidant to decrease the initial DPPH concentration by 50%. The IC<sub>50</sub> of the sample was derived from the % scavenging activity against the concentration plot and is expressed in mg / ml. Figure 3 shows that the IC<sub>50</sub> is increasing rapidly along with the increasing ratio of tamarind leaves extract. This suggests that as an antioxidant substance, curcumin has the higher ability to provide a better protection against free radical attack DPPH compared with tamarind leaves.

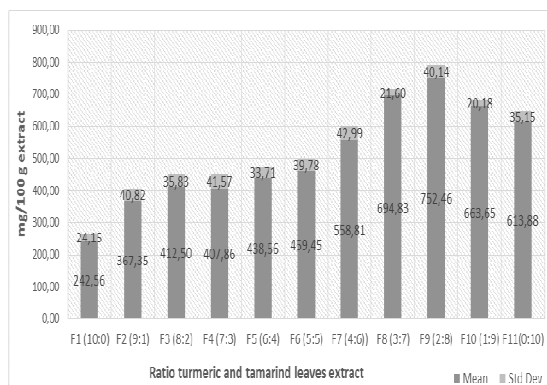


**Fig. 3:** The IC<sub>50</sub> antioxidant based on the ratio of turmeric and tamarind leaves extract. Different notations indicate significant differences based on Tukey HSD test at 5% confidence level.

The higher ability of curcumin as antioxidants is mainly due to its unique chemical and physicochemical structure. Diferuloyl methane molecules [1,7-bis (4-hydroxy-3- methoxy-phenyl) -1.6-heptadiene-3,5-dione)] contains two aromatic ring systems containing o-methoxy phenolic groups which are joined by methylene bridges. This molecule has three important functional groups. They are: aromatic o-methoxy from the phenolic groups  $\alpha$ ,  $\beta$ -unsaturated  $\beta$ -di keto and seven carbon linker. Curcumin donates an electron/hydrogen species reactive oxygen. Curcumin interacts with a number of biomolecules via non-covalent and covalent bonding (Priyadarsini, 2013). This causes curcumin has a higher ability as an antioxidant.

Curcumin exists in enolic and  $\beta$ -diketone forms. Curcumin stable at acidic pH, instability sets in at neutral and basic pH as it breaks down into ferulic acid and feruloylmethane. The presence of curcumin in a solution is for the most part in the form of an enolic (Shen, 2007) and this fact significantly influences its capacity for radical scavenging. The enolic tautomer of curcumin is deemed the major contributor to its bioactivities as well as its photo physical and photochemical features (Priyadarsini 2009 and Sharma *et al*, 2009). The stability level of curcumin is low and degradation occurs when it is exposed to adverse physiological situations.

#### ***Vitamin C:***



**Fig. 4:** Vitamin C based on the ratio of turmeric and tamarind leaves extract. Different notations indicate significant differences based on Tukey HSD test at 5% confidence level

Figure 4 shows that the vitamin C level increases along with the increasing ratio of the tamarind leaves extract. These results are consistent with the previous study which stated that the main source of antioxidant in tamarind leaves are the phenolic compounds and vitamin C (Mulyani, *et al.* 2014). The content of vitamin C in the extract affects the activity of antioxidant during treatment. When it was associated with IC<sub>50</sub>, it showed that curcumin has a more important role to capture radicals compared with vitamin C. Curcumin can normally be found in the tautomeric enol form (Roughley and Whiting, 1973). Enol tautomeric curcumin is considered as the major contributor of bioactivity as well as its photo physical and photochemical features (Priyadarsini, 2009 and Sharma *et al.*, 2009). The rapid release of H ions from curcumin is a very important antioxidant action. The release of H ions hydroxyl groups of the curcumin molecule is correlated directly with the quenching process of free radicals and ROS species. (Jovanovic, 1999).

### Conclusion:

1. The ratio of turmeric and tamarind leaves extract have a significant effect on total phenolic, IC<sub>50</sub> and vitamin C. The total phenolic level increases (2.91 to 11.15) mg GAE /100g extract, along with the increasing ratio of turmeric extract, the IC<sub>50</sub> level increases (0.225 to 0.830) ppm with the increasing ratio of tamarind leaves extract and the vitamin C increases (242.6 to 752.5) mg / 100 g extract, with increasing ratio of tamarind leaf extract.
2. The ratio of turmeric and tamarind leaves extract has no effect on the antioxidant capacity (2.07 -5.44) mg GAEAC/100 g extract. In this condition, the synergism is obtained at the ratio of turmeric and tamarind leaves extract of (9:1; 8:2; 7:3).

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