



ANTIOXIDANT ACTIVITY OF TAMARIND PULP (*Tamarindus indica* L.) EXTRACT IN METHANOL COMPARED TO EXTRACT IN WATER USING DPPH (1,1-Diphenyl-2-Picryl Hydrazyl)

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ABSTRACT

Now a day, there is a strong demand in public to replace syntetic antioxidant by natural ones. Usually, Tamarind pulp (*Tamarindus indica* L.) is added as a flavor in culinary, fresh drink and snack, also available from traditional to modern market. In order to find a natural antioxidant, a simple methode using DPPH (1,1-Diphenyl-2-Picryl Hydrazyl) was carried out qualitatively and quantitatively on testing antioxidant activity of Tamarind pulp extract in methanol compare to extract in water. The violet colour of DPPH (1,1-Diphenyl-2-Picryl Hydrazyl) fades after the addition of extract indicated the antioxidant activity. Quantitative analysis was done by visible spectrophotometry at maximum wavelength 516 nm. The result showed antioxidant activity of Tamarind pulp extract in methanol by $EC_{50} = 5650,31$ ppm and extract in water by $EC_{50} = 15043,22$ ppm

Keywords : antioxidant activity, extract in methanol, extract in water, tamarind pulp (*Tamarindus indica* L.), DPPH

INTRODUCTION

Consuming antioxidant is the simple way to prevent body cells from damages caused by free radicals. Vitamin E, C, β Carotene are potential antioxidant. Synthetic antioxidant which are used in many food and drink industry are *butylatedhydroxytoluene* (BHT) and *butylatedhydroxyanisole* (BHA). Some research proved these substances are carcinogenic agent. Recently, there is a strong tendency in searching a natural antioxidant because of their safety. According to a research of one cosmetic industry in Swiss, Unitamuron H-22 which contains tamarind seed's extract with polysaccharide inside, showed immunostimulant potency (**Hernani dan Mono Rahardjo, 2005; Gormley & Lieberman, 2005**). Strickland et al showed galactoxiloglican from tamarind seed is a molcul with a signal function to avoid cell damages from environment influences like UV radiation or other destroyer factors. A natural antioxidant was isolated and identified has a similar function with α -tocopherol (**Induchem, 2007**). In line with this report, research on antioxidant activity of tamarind pulp (*Tamarindus indica* L.) extract which is usually added as a flavor in culinary, fresh drink, and snacks will be done by using DPPH (1,1-Diphenyl-2-Picryl Hydrazyl).

MATERIAL AND METHODS

Before extracting with methanol or water by kinetic maseration, tamarind pulp (*Tamarindus indica* L.) from Desa Tulungagung Kecamatan Baureno, Bojonegoro, Jawa Timur, was preliminary dried.

The next step is preparing a methanol solution of extract to detect antioxidant activity by adding DPPH solution. The violet colour of DPPH (1,1-Diphenyl-2-Picryl Hydrazyl) fades after the addition of extract indicated the antioxidant activity. Quantitative analysis

was done by visible spectrophotometry on various concentration of sample solution at maximum λ 516 nm and EC_{50} was calculated. Samples solution is extract which was solved in methanol and 0,004% DPPH solution in methanol was added before absorbance observation. A blank solution is 0,004% DPPH solution in methanol.

Data analysis :

$$\% \text{Scavenging} = 1 - \frac{\text{Absorbance}_{\text{Sample}}}{\text{Absorbance}_{\text{Blank}}} \times 100\%$$

(Joyeux *et al.*, 1995)

0% means there is no free radical scavenging activity. On the other hand 100% means fully scavenging activity and needed to dilute sample to observe limit activity concentration. All data % scavenging at various concentration was calculated into a regression equation and EC_{50} is stated (Joyeux *et al.*, 1995)

RESULT AND DISCUSSION

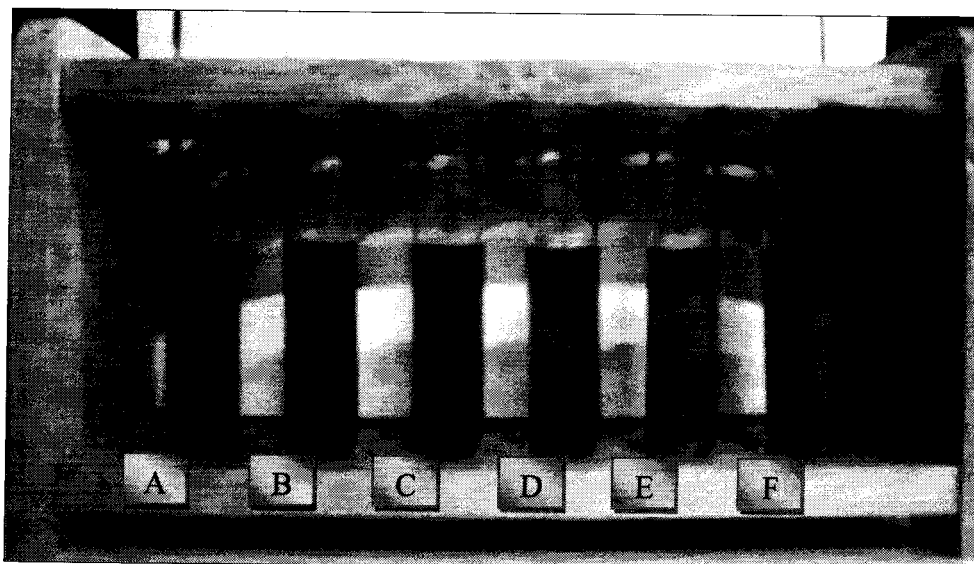


Figure 1. The result of Free Radical DPPH Scavenging Test Qualitatively on Tamarind Pulp Extract in Methanol by Color Reaction

Figure notes:

- A : 4,0 ml DPPH solution 0,004% + 2,0 ml Metanol p.a
- B : 4,0 ml DPPH solution 0,004% + 2,0 ml tamarind pulp methanol extract 400 ppm
- C : 4,0 ml DPPH solution 0,004% + 2,0 ml tamarind pulp methanol extract 800 ppm
- D : 4,0 ml DPPH solution 0,004% + 2,0 ml tamarind pulp methanol extract 2000 ppm
- E : 4,0 ml DPPH solution 0,004% + 2,0 ml tamarind pulp methanol extract 4000 ppm
- F : 4,0 ml DPPH solution 0,004% + 2,0 ml tamarind pulp methanol extract 6000 ppm

DPPH was used to indicate free radical scavenging of tamarind pulp methanol extract. DPPH is a relative stable free radical compared to the other (Larson, 1997). Other advantage is the violet color of DPPH solution that fades after reaction with free radical scavenging substances. The color changes can be observed visually and instrumentally using spectrophotometer. (Joyeux *et al.*, 1995). The figure showed that the violet color of solution B,C,D,E,F tubes faded if compared to A tube as a blank solution. These proved qualitatively capability of tamarin pulp methanol extract as free radical DPPH scavenger.

Table 1. Absorbance and Calculation % DPPH Scavenging of Tamarind Pulp Extract in Methanol.

Replication	Extract weight (g)	Concentration (ppm)	Absorbance (A)	%Scavenging
I	2,0352	Control	0,7613	
		407,04	0,7126	6,40
		814,08	0,6734	11,55
		2035,2	0,5953	21,80
		4070,4	0,4580	39,84
		6105,6	0,3246	57,36
II	2,0369	Control	0,7888	
		407,38	0,7463	5,39
		814,76	0,7047	10,66
		2036,9	0,6159	21,92
		4073,8	0,5030	36,23
		6110,7	0,4010	49,16
III	2,0365	Control	0,7698	
		407,3	0,7212	6,31
		814,6	0,6811	11,52
		2036,5	0,5880	23,62
		4073	0,4599	40,26
		6109,5	0,3358	56,38
IV	2,0242	Control	0,7432	
		404,84	0,7099	4,48
		809,68	0,6757	9,08
		2024,2	0,6030	18,86
		4048,4	0,4778	35,71
		6072,6	0,3674	50,56
V	2,0516	Control	0,7443	
		410,32	0,6981	6,20
		820,64	0,6695	10,05
		2051,6	0,5823	21,76
		4103,2	0,4583	38,43
		6154,8	0,3603	51,60

Results showed free radical scavenging effect of tamarind pulps methanol extract by fading the violet color of DPPH in line with increasing in free radical scavenging capability by higher concentration. These were happened because DPPHydrazil radical has one atomic N react with unpairing electron. If the atomic N react with free radical scavenging substance, DPPHydrazil is transformed to a stable DPPHydrazin (Senba, *et al*, 1999; Molyneux, 2004).

Table 2. Result on Calculation Linear Regretion Equation

Replication	Linear Regretion Equation	r calculation	EC ₅₀ (ppm)	(extract weight equal)	(dried material equal)
I	$y = 0,0088x + 3,6535$	0,9996	5245,44	262,27	551,36
II	$y = 0,0076x + 4,3769$	0,9954	6044,64	302,23	635,37
III	$y = 0,0087x + 4,3180$	0,9982	5270,46	263,52	553,98
IV	$y = 0,0080x + 2,1347$	0,9992	5920,09	296,00	622,27
V	$y = 0,0079x + 4,0408$	0,9971	5770,93	288,55	606,60
Average			5650,31	282,51	593,92

Table 3. Result on correlation coefficient (r calculation)

Replication	r calculation	r table
I	0,9996	0,959
II	0,9954	
III	0,9982	
IV	0,9992	

V	0,9971
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From the data above, r calculation $>$ r table that means there was a significant correlation between methanol extract of tamarind pulps with free radical DPPH scavenging. Using methanol as solvent in detecting free radical scavenging because almost all substances will be solved and methanol has no absorbance at wavelength 516 nm. (Depkes RI, 1986)

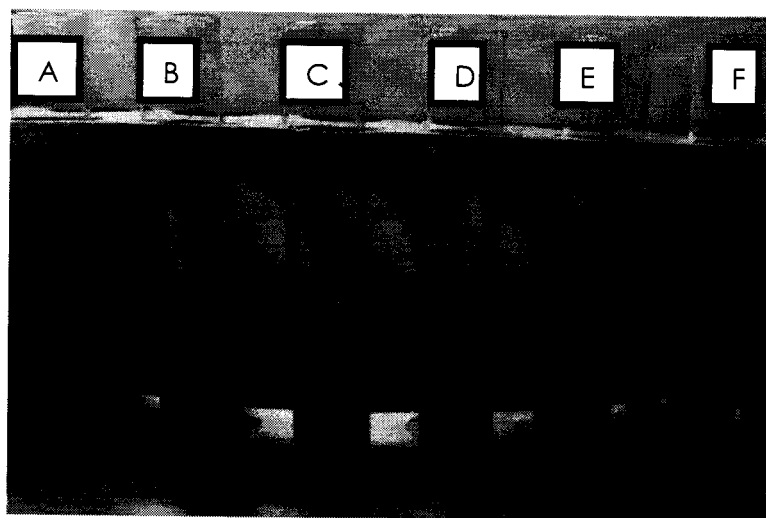


Figure 2. The result of Free Radical DPPH Scavenging Test Qualitatively on Tamarind (*Tamarindus indica* L.) Pulp Extract in Water by Color Reaction

Figure notes:

- A = 4,0 ml DPPH solution 0,004 % + 2,0 ml metanol p.a
- B = 4,0 ml DPPH solution 0,004% + 2,0 ml tamarind pulp water extract 3000 ppm.
- C = 4,0 ml DPPH solution 0,004% + 2,0 ml tamarind pulp water extract 6000 ppm.
- D = 4,0 ml DPPH solution 0,004% + 2,0 ml tamarind pulp water extract 9000 ppm.
- E = 4,0 ml DPPH solution 0,004% + 2,0 ml tamarind pulp water extract 15000 ppm.
- F = 4,0 ml DPPH solution 0,004% + 2,0 ml tamarind pulp water extract 18000 ppm

The figure showed that the violet color of solution B,C,D,E,F tubes faded if compared to A tube as a blank solution. These proved qualitatively capability of tamarin pulp water extract as free radical DPPH scavenger.

Table 4. Absorbance and Calculation % DPPH Scavenging of Tamarind Pulp Extract in Water

Replication	Weight (g)	Concentration (bpj)	A _{sample}	A _{blank}	% Scavenging
1	2,9368	2936,8	0,6008	0,7002	14,19
		5873,6	0,5167		26,21
		8810,4	0,4351		37,86
		14684	0,3437		50,91
		17620,8	0,2694		61,53
2	2,9219	2921,9	0,7219	0,7623	5,29
		5843,8	0,6738		11,61
		8765,7	0,6025		20,96
		14609,5	0,4991		34,53
		17531,4	0,3704		51,41
3	2,9242	2942,2	0,6036	0,7153	15,52
		5848,4	0,5197		27,35
		8772,6	0,4492		37,20
		14621	0,3329		53,59
		17545,2	0,2851		60,14
4	2,9470	2947	0,5915	0,7097	16,65
		5894	0,5177		27,05
		8841	0,4539		36,04
		14735	0,3349		52,81
		17682	0,2889		59,29
5	2,9437	2943,7	0,6554	0,7563	13,34
		5887,4	0,5748		23,99
		8831,1	0,5072		32,94
		1471 EC ₅₀ 8,5	0,3960		47,64
		17662,2	0,3304		56,31

Table 5. Result on Calculation Linear Regretion Equation and EC₅₀ of Tamarind Pulp Extract in Water

Replication	Regretion Equation	r calc	EC ₅₀ (ppm)	(Extract weight equal)	(Dried material equal)
1	y = 0,0038x + 7,3937	0,9931	13.836,76	0,691	1,686

2	$y = 0,0030x + 5,2034$	0,9867	18.320,84	0,916	2,232
3	$y = 0,0030x + 8,8422$	0,9957	13.668,42	0,683	1,665
4	$y = 0,0029x + 9,4277$	0,9979	14.047,06	0,702	1,712
5	$y = 0,0028x + 6,4081$	0,9978	15.343,04	0,767	1,869
Average			15.043,22	0,752	1,833
r calculated range			0,9867-0,9979		

Table 6. Result on correlation coefficient (r calculation)

Rentang r hitung	r tabel	N	α
0,9867-0,9979	0,959	5	0,01

r calculation larger than r table means there is a correlation between concentration and % scavenging.

Just like methanol extract, tamarind pulp water extract also showed increasing in free radical scavenging effect by increasing sample solution concentration. Water was chosen as a solvent because in culinary, drink and snacks, tamarind is only stirred with water and there are enough solubility of some constituent. Chemical constituents in tamarind pulp that are predicted has antioxidant activity are flavonoid, tannin, sterol/terpene, citric acid, tartaric acid, malic acid, vitamin A, vitamin C, (Hernani & Mono Raharjo 2005; Agromedia, 2008). Lower EC₅₀ means stronger antioxidant effect and also the contrary (Rohman, 2005). Based on EC₅₀ methanol extract of tamarind pulp lower than water extract tamarind pulp, that is predicted in methanol more constituent that have antioxidant activity will be solved.

CONCLUSION

1. Tamarind Pulp Extract in methanol and water (*Tamarindus indica* L.) from Desa Tulungagung Kecamatan Baureno, Bojonegoro, Jawa Timur have antioxidant activity.
2. EC₅₀ average as Free Radical DPPH Scavenger for Tamarind Pulp Extract in methanol is 5650,31 ppm ~ 0,59 g dried material, and for Tamarind Pulp Extract in water 15.043,22 ppm ~ 1,833 g dried material
3. Antioxidant activity of Tamarind Pulp Extract in methanol stronger than Tamarind Pulp Extract in water

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