ANTIOXIDANT POTENTIAL OF CENTELLA ASIATICA

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Abstract - This research aims to study the effectiveness of the active antioxidants of centella (Centella asiatica). The treatments were divided to 3 groups according to the methods used to prepare the plant including fresh and dry (either by hot-air oven or natural light) samples of centella leaves. Plants were extracted by 95% ethanol to test antioxidant activities by the DPPH method, using measurement of light absorbance at 517 nm wavelength. In comparison with the standard curve of trolox, the results showed the highesttrolox equivalent antioxidant capacity (TEAC) of 10.80 ± 4.06 (mmol/mg) which was obtained in centella prepared by hot air oven drying.

Keywords - Centella Asiatica, Antioxidant, Drying

I. INTRODUCTION

Free radicals refers to an atom or molecule that has at least one unpaired electron in the outer valence orbitals. Free radicals occur when the bonds between atoms split, causing radical instability and susceptible to quick reaction with molecules around them by pulling electrons from adjacent molecules to become stable and causes new unstable free radical that reacts with other molecules as a chain reaction [1]. Cells are made of molecules. Normally, a molecule or atom in our body has an even number of electron but it can lost electrons to free radicals, resulting in an imbalance that causes cell damage as cells are made of molecules. Radicals are generated from external sources including air pollution, ozone, nitrous oxide, nitrogen dioxide, dust, cigarette smoke, foods containing unsaturated fatty acids, sunlight, gamma ray and the internal source within the body including free radicals produced during metabolic reactions in our body. Examples of free radicals areO2•- superoxide anion, hydroxyl (˙OH) radical, ROO peroxy radicle, H_2O_2 hydrogen peroxide, LOO• lipid peroxyl [1,2].

Although free radicals are beneficial as it may help to destroy foreign substances which enter to the body, the body still needs a defense mechanism itself against accumulation of free radicals by producing antioxidant enzymes to balance the amount of free radicals, especially when the body receives external toxins via smoking, exposure to sunlight, dining with oily, grilled or scorched food, etc, leading to a less effective system to control the toxin level and more accumulation to the point that it becomes toxic to the body. Even breathing produces free radicals which steadily attack cells resulting in the cause of illnesses and diseases, i.e cancer, heart disease, lung disease and diabetes [2].

Control mechanism of antioxidants from the body alone is not enough. It is necessary to also rely on a group of antioxidants derived from food such as vitamin a, vitamin c, vitamin E, beta-carotene, polyphenols and flavonoids that are found in fruits and vegetables to help strengthen the anti-oxidative system to retain effective.Variety of natural native plant species have potential as medicinal herbs that can be used in healthcare [3]. Antioxidants, found in these plants, are substances that neutralize free radicals formed by the oxidation reaction within the body. Therefore, the body needs external sources that have antioxidant potential in order to reduce these free radicals which occur continuously by itself.

Centella asiatica is in the family Umbelliferae. It is a herbaceous, frost-tender perennial plant, found in European countries, South Africa, India, Pakistan and Sri Lanka. In Thailand, it is used for preparing a drink or can be eaten in raw. There are reports of the medicinal benefits in lowering high blood pressure, cancer cell inhibition, inflammation treatmentas it contains many active ingredients including terpenoid compounds, flavonoid glycosides, free amino acids and polyacetylenic compounds, etc, making it become the plant with numerous health benefits[4],[5]. This research aims to study another property aspect to determine antioxidant potential of the extracts of both fresh and dry Centella asiatica.

II. EXPERIMENTAL

Fresh leaves of centella were washed with water and cut into small pieces. A portion was dry heated with hot-air oven at a temperature of 45 degrees Celsius. Another portion was air-dried. Leaves were grinded and extracted (10% w/v) with 95% ethanol for 24 hours. The extract solution samples of different concentrations were filtrated for further concentration adjustment with methanol in order to determine antioxidant capacity by DPPH radical scavenging assay [6]. The 500 µL extract solution of different concentrations of fresh, air-dried and oven-dried samples was added to 1,500 µL 0.1 mM DPPH methanol solution (2,2-diphenyl-1-picrylhydrazyl, Aldrich) and left in the dark at room temperature for 30 minutes. Absorbance at 517 nm wavelength was measured. The result of antioxidant activities were

expressed asthe trolox equivalent antioxidant capacity using a calibration curvey=572x +1.138.

III. RESULTS

The antioxidant capacities of cantella extracts were analyzedby the DPPH assay.The percents of DPPH inhibition of different extract concentrations were presented in Table 1.

To calculate % inhibition, the following equation was used:

% inhibition = 100 x [(A_{517}control-A_{517} test sample)/ A_{517}control].

They were also mutually plotted as shown in Fig1-3 to obtain IC_{50} as indicated inTable 2. The inhibition ranged from 2.67 to 10.8µmol TE g^{-1}. The highest antioxidant capacity was observed in oven-dried leaves (10.80µmol TE g^{-1}), followed by air-dried (6.66 µmol TE g^{-1}) whilethe lowest was observed in fresh leaves (2.67 µmol TE g^{-1}) as presented in Table 3.

Table 1. DPPH inhibition of different concentrations of centella extract

conc (mg/ml)	Fresh centella				
	A_{517}(1)	A_{517}(2)	A_{517}(3)	A_{517}(x̄)	% inhibition
30.0	0.346	0.523	0.461	0.443	50.94
25.0	0.465	0.426	0.592	0.494	45.29
20.0	0.597	0.594	0.589	0.593	34.33
15.0	0.553	0.61	0.522	0.562	37.76
10.0	0.648	0.632	0.64	0.640	29.13
5.0	0.579	0.688	0.673	0.647	28.34
2.5	0.670	0.667	0.659	0.665	26.36
1.0	0.678	0.674	0.683	0.678	24.92
conc (mg/ml)	Air-dried centella				
	A_{517}(1)	A_{517}(2)	A_{517}(3)	A_{517}(x̄)	% inhibition
30.0	0.226	0.228	0.233	0.229	74.64
25.0	0.238	0.242	0.246	0.242	73.20
20.0	0.291	0.300	0.298	0.296	67.22
15.0	0.352	0.371	0.404	0.376	58.36
10.0	0.431	0.456	0.484	0.457	49.39
5.0	0.638	0.611	0.626	0.625	30.79
2.5	0.647	0.637	0.679	0.654	27.57
1.0	0.633	0.628	0.627	0.629	30.34
conc (mg/ml)	Oven-dried centella				
	A_{517}(1)	A_{517}(2)	A_{517}(3)	A_{517}(x̄)	% inhibition
30.0	0.222	0.213	0.211	0.215	76.19
25.0	0.204	0.215	0.212	0.210	76.74
20.0	0.199	0.193	0.200	0.197	78.18
15.0	0.214	0.220	0.265	0.233	74.2
10.0	0.366	0.366	0.363	0.365	59.58
5.0	0.520	0.531	0.498	0.516	42.86
2.5	0.606	0.59	0.581	0.592	34.44
1.0	0.634	0.632	0.627	0.631	30.12

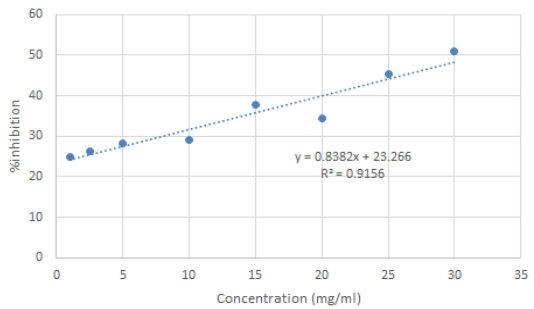

Fig 1. DPPH inhibition effect of fresh centella leaves

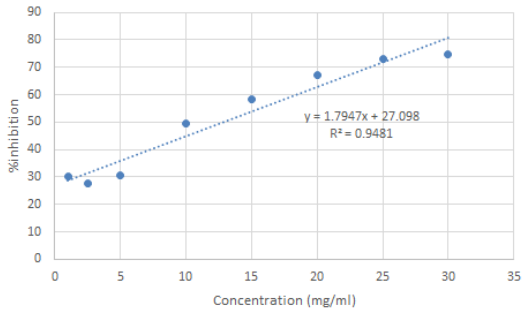

Fig 2. DPPH inhibition effect of air-dried centella leaves

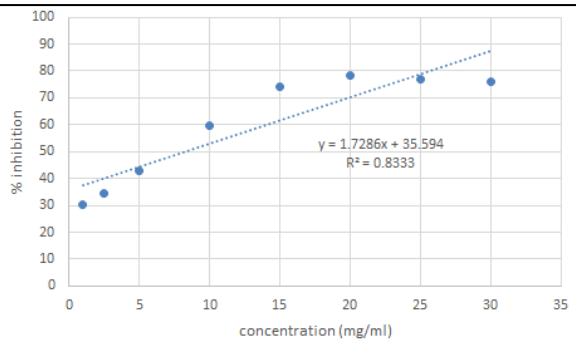

Fig 3. DPPH inhibition effect of oven-dried centella leaves

Table 2. Equations for calculation of DPPH inhibition effects

Samples	equation	Ic_{50}
Fresh	y = 0.3732x + 25.437 R^2 = 0.9156	65.82
Air-dried	y = 1.7947x + 27.098 R^2 = 0.9481	17.79
Oven-dried	y = 0.5548x + 25.139 R^2 = 0.8333	44.81

Table 3. Trolox Equivalent Antioxidant Capacity (TAEC) in extracts of centella leaves

Samples	Antioxidant capacities (µmol TE g^{-1})
Fresh	2.67
Air-dried	6.66
Oven-dried	10.80

CONCLUSIONS & DISCUSSIONS

From the study on capacities of the active ingredient as antioxidants in Centella asiatica, it showed that all fresh, air-dried and oven-dried plant leaves had antioxidant potential to reduce the level of free radicals when tested with DPPH assay which was considered a valid accurate, easy and economic method to evaluate radical scavenging activity of antioxidants [7]. The highest antioxidant activity was observed in the sample extracted from oven-dried leaves (10.88 µmol TE g^{-1}), followed by the air-dried (6.66 µmol TE g^{-1}) and fresh ones (2.67 µmol TE g^{-1}). Centella asiatica is a famous herb, confirmed to have medical properties and many benefits for health. It comprises several important substances such as glycosides which acts as antioxidants. It is also rich in vitamins and minerals. Study of nutrition revealed that phenolic compounds and vitamin C in Centella asiatica had the ability to defend against free radicals,detected by Folin-Ciocalteu method and 2,4 dinitrophenylhydrazine assay respectively. It was reported to contain an average of 15.3. GAEmM/gFW phenolic compounds and 1.3 AEACmM/gFW vitamin C [8,9, 10]. Both phenolic compound and vitamin c had relatively high efficiency for antioxidation.

The extract of centella with the most antioxidant activity was found to be from dry leaves heated by hot-air oven. The plant prepared by other methods seemed to give lower potential of antioxidants. The result corresponded to the research from Assoc. Prof. Dr. Manop Cherinchaitrakun (2018), the Lecturer of department of chemical engineering, faculty of engineering, Kasetsart University, who reported a study in the appropriate preparation method for extraction of the active ingredient from centella. A specific part of a leaf was washed thoroughly, aired and drained. Preparation was performed by using a hot air oven to dry heat samples at a temperature of around 50 degrees Celsius for 24 hours. Dry centella was then blended to be used as raw material for the extraction of the active ingredients. After soaking with solvent, it was found that a single round of ethanol extraction of the centella powder was adequate to obtain effective active ingredients in large quantities. The average quantity of all substances was approximately 65 percent, including high level of antioxidants [11].

It might be said that centella should undergo drying to generate more or valuable anti-free radical agents. The heat caused water in plant cells to evaporate, resulting in higher extraction efficiency or the heat might influence anti oxidation activities in plants, which corresponded to the work of Hajimehdipoor (2012) that the heat through drying helped raising activity of antioxidants and even increased the phenolic compounds of some plants, such as Mentha piperita, Mentha spicata and Thymus vulgaris [12]. Activity expansion could be caused by other types of compounds which were produced as by-products, induced by heat, and also had capabilities of antioxidant activity [13].

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