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Research Article

# ANTIOXIDANT AND FREE RADICAL SCAVENGING ACTIVITY OF *HIBISCUS ACETOSELLA* LEAVES EXTRACTS

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**ABSTRACT**

**Objective:** *Hibiscus acetosella* (HA) or Chaba Maple is native plant and cultivated in tropical western in Africa and north of America. The characteristics of HA are red to purple in stem, leaf, and flower that are the pigment of antioxidant compound as anthocyanins. Anthocyanins are in the group of flavonoid and have the role as functional foods which have several health benefits such as obesity and diabetes control, cardiovascular disease prevention, and others. Hence, the aim of this study was to investigate the antioxidant and free radical scavenging activity of HA leaves extracts.

**Materials and Methods:** HA (Chaba Maple) leaves were collected in Pathum Thani province, Thailand, and were dried and extracted by maceration technique with three solvents – water, ethanol, and methanol. The antioxidant properties of extracts were carried out using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assay and ferric reducing antioxidant power (FRAP) assay. The extracts were examined for their scavenging effect on hydroxyl radical (•OH) using hydroxyl radical scavenging assay and nitric oxide (NO) radical (•NO) using NO radical scavenging assay.

**Results:** For ABTS, FRAP, and hydroxyl radical scavenging assay, ethanol extract showed the highest antioxidant property which the percentage inhibitions were 69.04%, 2381.84 µM/mg extract, and 62.88 mg/ml, respectively. For NO scavenging activity, methanol extract showed highest ability to scavenge NO which percentage inhibition was 101.28±0.73 mg/ml.

**Conclusion:** The results of this study showed that ethanolic, methanolic, and water extract of HA leaves had scavenge and reducing antioxidant properties.

**Keywords:** *Hibiscus acetosella,* Antioxidants, Radical scavenging activity, Hydroxyl radical, Nitric oxide radical.

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## INTRODUCTION

Aging is biological process in the body that is beyond the control of human. Aging occurs from the physical changes of cells and tissues and accumulative of wastes in the body together with the increasing of age which can lead to incidence rate of diseases and death [1]. In the present, the progress of medical technologies has more effect on aging process. However, aging is still undesirable. Theory of aging divides into two categories: Programmed theory and damage or error theory. Programmed theory identifies that aging is the process of biological timetable. The progress of aging is controlled by gene expression that can affect on maintenance, repair, and defense system of the body. Programmed theory divides into three subcategories [2].

1. Programmed Longevity. This theory explains that aging is depend on the functions of genes. The declination of genes is correlative with the time of age
2. Neuroendocrine Theory. The pace of aging is controlled by biological clocks through hormones
3. Immunological Theory. The immune system is overtime declination which can lead to increase incidence in infectious diseases then aging occur and continues to death.

Damage or error theory divides into 3 subcategories.

1. The Wear and Tear Theory. This theory was mentioned in the first time by Dr. August Weismann in 1882. Aging is the result of cells and tissues declination that are used by the process of body.
2. Free Radical Theory. The theory was presented by Dr. Gerschman in 1954 and was developed by Dr. Denham Harman [2]. Free radicals are unstable molecules that contain an unpaired electron. Free radicals are important role in the process of aging that is not imbalance between free radical and antioxidant. The high rate of free radical in the body leads to damage cells and tissues by oxidative reaction [3].
3. Cross-linking theory. Cross-linking theory was presented by Johan Bjorksten in 1942. This theory explained that aging occurs from the accumulative of damage cross-linked proteins which damages cells and tissues [2].

Reactive oxygen species (ROS) is a group of free radicals that have high potent free radicals. They effect on human body both harmful and beneficial [4]. ROS is produced from metabolism in human body and exogenous factors. Overproduction of ROS is major result of oxidative stress that can damage nucleic acid bases, lipids, and proteins and leading to functional of cells and viability of cells. The highest potent free radicals in ROS are hydroxyl radicals (•OH). The high amount of ROS production in cells can stimulate the inflammatory process, secretion of chemotactic factors, growth factors, proteolytic enzymes, lipoxygenases, and cyclooxygenases. ROS and their metabolites can direct effect on cell injury and may stimulate the promoting of diseases [5]. Furthermore, nitric oxide (NO) radical (•NO) is the important free radical that can affect to human body. It is classified as free radical in reactive nitrogen species (RNS) when there is high amount of NO in human body, and it can associate with various carcinomas and inflammation conditions [6].

*Hibiscus acetosella* (HA) is a spicy of plant in *Malvaceae* family. It is native plant and cultivated in tropical western in Africa and north of America [7]. The characteristics of HA are red-purple in stem, leaf, and flower that are the pigment of antioxidant compound as anthocyanin [8]. Anthocyanin

is an antioxidant in the group of phenolic compounds that can inhibit free radicals and lipid oxidation, reduce inflammation, and reduce risk in atherosclerosis [9]. Hence, the aim of this study was to investigate the antioxidant and free radical scavenging activity of HA leave extracts.

## MATERIALS AND METHODS

### Plant material

The fresh leaves of HA were collected from Pathum Thani province, Thailand. The leaves were dried at 45℃ in incubator for 24 h. The dried leaves were grinded into small pieces by grinder.

### Extraction of plant material

Dried leaves of HA were extracted by maceration technique with three solvents – water ethanol and methanol and soaked with solvents for 48 h. Then, the solutions were filtrated and evaporated by rotary evaporator at 50°C.

$$\%\,\text{Yield}: \left[\frac{\text{Weight of dry extract}}{\text{Weight of taken for extraction}}\right] \times 10$$

### 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assay

To determine ABTS radical scavenging ability, ABTS was dissolved with water to 7 mM of concentration. 2.45 mM of potassium persulfate was added into ABTS stock solution and ABTS radical cation (ABTS+) produced by the reaction of ABTS stock solution and potassium persulfate. After that, ABTS+ solution was kept in the dark at room temperature for 12–16 h. ABTS+ solution was diluted with ethanol with the ratio 1:89 v/v before assay. The wavelength was set at 734 nm for assay. The absorbance of ABTS+ solution should be 0.700±0.02 in 1 cm cuvette. Water, ethanol, and methanol extracts of HA were diluted with solvent at various concentrations (0.0625, 0.125, 0.25, 0.5, and 1 mg/ml), and 10 µl of samples were added to 1 ml of ABTS+ solution and kept in the dark at 30°C for 30 min and measured the absorbance at 734 nm with ultraviolet visible spectrometer [10,11]. The inhibition percentages were calculated by the following equation:

$$\text{ABTS radical scavenging activity}\,\% = \left[\frac{A_0 - A_1}{A_0}\right] \times 100$$

Where,

$A_0$ is the absorbance of control,

$A_1$ is the absorbance of sample.

### Ferric reducing antioxidant power (FRAP) assay

The ability of HA leave extracts to reduce ferric ion was measured using the method described by Rabeta and Faraniza[12] with some modification based on the reduction of ferric-tripyridyltriazine (TPTZ) (Fe3±TPTZ) to a blue-colored ferrous TPTZ (Fe2±TPTZ). The 20 µL of the extract was added to 150 µL of the FRAP reagent (10 mL of 0.3 M sodium acetate buffer solution, 1.0 mL of 10 mM TPTZ, and 1.0 mL of 20 mM $FeCl_3$) and incubated at 37ºC for 4 min. The absorbance of solutions was measured at 600 nm using a microplate reader. With 1 mM $FeSO_4$ solution as standard, FRAP value of the sample was expressed as µM, using linear calibration obtained with different concentration of $FeSO_4$.

### Hydroxyl radical scavenging assay

Hydroxyl radical scavenging assay was measured using the method of Pavithra and Vadivukkarasi [13]. The various concentrations of water, ethanol, and methanol extracts of HA leaves (0.0625, 0.125, 0.25, 0.5, and 1 mg/ml) were added with 1mL of iron-ethylenediaminetetraacetic acid (EDTA) solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5 mL of EDTA solution (0.018%), and 1 mL of dimethyl sulfoxide (DMSO) (0.85%, v/v in 0.1 M phosphate buffer, pH 7.4). 0.5 mL of ascorbic acid (0.22%) was added to the mixture and incubated at 80–90°C for 15 min in a water bath for initiated reaction. After incubation, 1 mL of ice-cold trichloroacetic acid (17.5%, w/v) was added followed by 3 ml of Nash reagent and left the mixture at room temperature for 15 min. The intensity of the color formed was measured spectroscopically at 412 nm. The inhibition percentages were calculated by the following equation:

$$\%\text{HRSA} = \left[1 - \frac{A_{\text{sample}}}{A_{\text{control}}}\right] \times 100$$

Where $A_{sample}$ is the absorbance of the sample and standard,

Where $A_{control}$ is absorbance of the control without the tested sample.

### NO radical scavenging assay

In this study, sodium nitroprusside (SNP) (Sigma-Aldrich, USA) was used as the NO donor. Specifically, 10 mM/L of SNP in a pH 7.4 PBS solution was incubated with 1 ml of variable concentrations of extracts (0.0625, 0.125, 0.25, 0.5, and 1 mg/ml) at 25°C for 180 min. Approximately 100 µl of the testing solution was withdrawn to react with a Griess Reagent kit (Promega, USA). The NO scavenging capacity of extracts was calculated as a percentage of NO radical inhibition using the following formula:

$$\%\text{NO radical inhibition} = \left[\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}\right] \times 100$$

### Statistical analysis

All measurements were performed in triplicates, and the experiment results were reported as mean±standard. Statistical analysis was performed using ANOVA. Differences were considered as statistically significant when $p \leq 0.05$.

## RESULTS AND DISCUSSION

HA leaves are red to violet color, and the characteristic of leaves is similar with maple leaves and the flower is similar with *Hibiscus syriacus*. In Thailand, HA is called "Chaba Maple." The dried leaves of HA were extracted with water, ethanol and methanol with maceration technique and were evaporated with rotary evaporator. The characteristic of crude extracts was purple viscous liquid. The maximum yield was obtained for water extract of HA (26.67%) followed by methanol extract (13.61%) and ethanol extract (12.75%).

### Radical scavenging activity using ABTS radical scavenging assay

The ABTS assay was preferred to use for evaluating the antioxidant activity of natural compounds in various food and herbals. The antioxidant capacity was measured from the scavenging the ABTS+ by the reaction of ABTS solution with potassium persulfate [14]. Water, ethanol, and methanol extract of HA were evaluated at various concentrations (0.0625, 0.125, 0.25, 0.5, and 1 mg/ml) and were compared with L-ascorbic acid as positive control. The results showed that the free radical scavenging ability increases with increasing concentration of extracts, and the maximum inhibition was 69.04% in 1 mg/ml of ethanol extract of HA leaves as shown in Table 1. The concentration of water, ethanol, and methanol extracts which required to inhibit free radicals by 50% was 0.86, 0.80, and 0.68 mg/ml, respectively. As the results, methanol extract exhibited lowest concentration to inhibit free radicals by 50%. The potential antioxidant activities were compared for extraction solvent factor. There was no statistically significant different between methanol extract and ethanol extract. Previous studies indicated that the use of methanol solvent extraction can be observed the highest anthocyanin and phenolic acid content from strawberry that correlated with the results of our study [15]. Therefore, ethanol and methanol solvent may suitable for HA leaves extraction more than water to scavenge the ABTS+.

### FRAP assay

The FRAP assay was used to evaluate the reducing power of antioxidant by reducing the TPTZ–Fe (III) complex to TPTZ–Fe (II) and can be observed the change of colors from colorless to blue color and measured the absorbance at 600 nm [16,17]. The 10 mg/ml of water,

**Table 1: % inhibition of ABTS radial scavenging activity of *Hibiscus acetosella* leaves extracts**

| *Hibiscus acetosella* leaves extracts | Concentrations (mg/ml) | | | | |
|---|---|---|---|---|---|
| | **1.00** | **0.50** | **0.25** | **0.125** | **0.0625** |
| Methanol extract | 57.60±0.04 | 39.72±0.01 | 20.04±0.01 | 7.38±0.01 | 1.53±0.01 |
| Ethanol extract | 69.04±0.02 | 43.69±0.01 | 23.46±0.29 | 10.51±0.01 | 2.70±0.01 |
| Water extract | 57.79±0.01 | 30.06±0.01 | 11.97±0.01 | 4.10±0.01 | 1.26±0.01 |

*Each value in the table is represented as mean±SD (n=3). ABTS: 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid). SD: Standard deviation

**Table 2: % inhibition of hydroxyl radical scavenging activity of *Hibiscus acetosella* leave extracts**

| *Hibiscus acetosella* leaves Extracts | Concentrations (mg/ml) | | | | |
|---|---|---|---|---|---|
| | **1.00** | **0.50** | **0.25** | **0.125** | **0.0625** |
| Methanol extract | 54.98±0.00 | 39.14±0.01 | 19.71±0.01 | 7.94±0.01 | 1.223±0.01 |
| Ethanol extract | 62.88±0.01 | 41.13±0.00 | 21.36±0.29 | 8.22±0.01 | 1.536±0.00 |
| Water extract | 53.67±0.00 | 37.09±0.00 | 19.00±0.01 | 3.98±0.01 | 1.166±0.00 |

*Each value in table is represented as mean±SD (n=3). SD: Standard deviation

**Table 3: % inhibition of nitric oxide radical scavenging activity of *Hibiscus acetosella* leaves extract**

| *Hibiscus acetosella* leaves Extracts | Concentrations (mg/ml) | | | | |
|---|---|---|---|---|---|
| | **1.00** | **0.50** | **0.25** | **0.125** | **0.0625** |
| Methanol extract | 101.28±0.73 | 72.69±0.93 | 57.45±1.59 | 48.85±1.37 | 34.82±1.61 |
| Ethanol extract | 59.76±0.91 | 50.99±0.66 | 47.08±1.25 | 41.93±1.02 | 30.58±0.49 |
| Water extract | 37.52±0.54 | 35.20±0.64 | 34.87±0.94 | 31.29±0.95 | 24.08±1.04 |

*Each value in table is represented as mean±SD (n=3). SD: Standard deviation

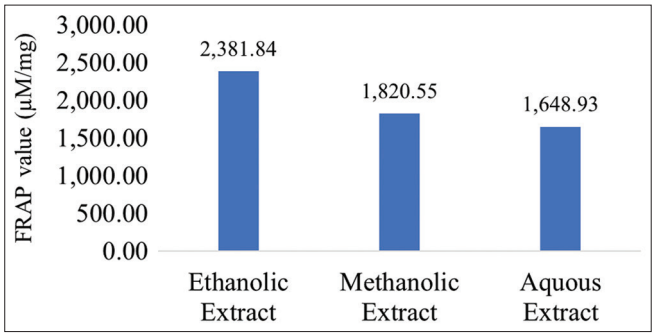


**Fig. 1: Ferric reducing antioxidant power of *Hibiscus acetosella* leave extracts**

ethanol, and methanol extracts of HA was evaluated the reducing power using FRAP assay. The results showed that the ethanol extract had highest ability to reduce TPTZ–Fe (III) complex to TPTZ–Fe (II) followed by methanol extract and water extract. The FRAP value was 2381.84±19.95, 1820.55±12.55, and 1648±12.08 µM/mg extract, respectively, as shown in Fig. 1. Therefore, HA leave extracts had potential in reducing antioxidant properties by hydrogen or electron donation for free radicals.

**Hydroxyl radical scavenging assay**

Hydroxyl radical is free radical in ROS which can generate in human body through the respiratory chain by Fenton reaction [18]. It is highest potency free radical that can be the cause of many diseases such as atherosclerosis, cancer, and neurological disorders and can lead to lipid peroxidation process [11,19]. Hydroxyl radical scavenging assay measured water, ethanol, and methanol extract of HA leaves at various concentrations and had L-ascorbic acid as positive control. Hydroxyl radical was generated by ascorbic acid–iron–EDTA and DMSO by oxidation reaction [19]. 1 mg/ml of HA leaves were extracted by ethanol, methanol, and water that were the highest hydroxyl radical scavenging activity, and the percentage of inhibition were 62.88, 54.98, and 53.67, respectively (Table 2). The inhibitory concentration of the ethanol extract had highest ability to scavenge the hydroxy radical followed by methanol extract and water extract. The $IC_{50}$ value was 0.74, 0.84, and 0.86 mg/ml, respectively.

**NO radical scavenging ability of HA leaves extracts**

NO radical is free radicals in RNS that appears in every cellular and organ in human body. NO was produced both exogenous and endogenous cells. NO production within cells are produced from enzymatic and non-enzymatic pathway, and it involves in several biological functions of human body such as vascular tone and blood flow regulations, necessary for leucocyte adhesion, and platelet aggregation [20]. Overproduction of NO in human body is associated with several diseases and inflammation conditions such as osteoarthritis, colitis, atherosclerosis, and diabetes [21]. HA leave extracts were evaluated NO radical scavenging activity Griess Reagent kit. No was produced from the interaction of SNP in aqueous solution with oxygen to produce nitrite ion [22]. The results showed that the inhibition of NO production was highest in methanol extract followed by ethanol extract and water extract of HA leave extracts at 1 mg/ml concentrations. The percentage of inhibition were 101.28, 59.76, and 37.52, respectively (Table 3). From the results, the increasing of ability in NO inhibition was increased with the increasing of the concentration of extracts, and solvents were a factor which contribute to NO inhibition capacity of extracts. We assume that HA leave extracts have anti-inflammatory effect and can reduce the risk of many diseases that related with the inflammation.

**CONCLUSION**

HA leaves extracts had potential in antioxidant and free radical scavenging properties at various solvent extractions (water, methanol, and ethanol). All of extracts had good ability to scavenge and reducing power to eliminate free radicals which may be the potential of anthocyanin content in HA leaves according to previous studies that indicated the plants in Hibiscus genus always showed the antioxidant, anti-inflammation, anticancer properties and have the potentials in free radical scavenging, due to their chemical constituents which consist of flavonoids and polyphenolic compounds [23]. The differentiation of extract solvents was a factor which effected to the ability in antioxidant of the extracts. Although the ethanol and methanol extracts had more

antioxidant potential than water extract, they also had the toxicity issue. Therefore, the extraction HA leaves with water may be more suitable and safer than ethanol and methanol for using in dietary supplements [18].

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