ANTIOXIDANT ACTIVITY OF THE FLOWER FRUIT AND LEAF, ANITDESMA VELUTINUM (TULAS) – A COMPARISON

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ABSTRACT
The point of this research is to compare some parts (flowers fruits and leaves) of Antidesma velutinum extracted, antioxidant activity, total phenolic content and total flavonoid content of ethanol by soxhlet method. Correlation was considered by statistical analysis. The result showed, The highest of percent yield found in leaf 29.13 ± 0.01 and total phenolic and flavonoid contents relates with this have 87.65 ± 0.18 mg GAE/100 mg and 10.45 ± 0.01 RE/100 mg respectively. The comparison of phenolic and flavonoid contents of Antidesma velutinum, in each part was significantly different p < 0.01. For antioxidant activities, DPPH scavenging activity assay have IC\textsubscript{50} of leaf was 13.96 ± 0.29μg/ml, the higest. white positive control, BHT presented higher activity with IC\textsubscript{50} 9.08 ± 0.74μg/ml. The ABTS radical cation showed higher at BHT with IC\textsubscript{50} 76.4 ± 2.44μg/ml, the IC\textsubscript{50} of ABTS radical cation of leaf the higest all of the organs, 69.4 ± 5.98μg/ml. The methods (DPPH and ABTS) for detected has a high correlation with the total phenolic analyzed by DPPH and ABTS, r = 0.831 and 0.793 respectively. However, the development product of this seems to be an interesting for further studies.

KEYWORD: antidesma velutinum, antioxidant activity, thai traditional medicine, total phenolic, total flavonoid.

INTRODUCTION
For hundred of years, herbs have played an important role throughout the word in treating and preventing human diseases. Antidesma velutinum (AV) or Sommao (name in Thai) is a plant distributed in Southern of Thailand. [1] It is classified in the Euphorbiaceae family, have
shrub of 70-130 cm in height. It has oval shaped leathery evergreen leaves up to about 17-23 cm long and 5-7 wide. The flowers have a mild, Thai traditional medicine (TTM) beliefs about aroma therapy by inhaling dizziness while traveling. The staminate flowers are arranged in small bunches and the pistillate flowers grow on long racemes which will become the long strands of fruit. The fruits are ovary and just under a centimeter wide. When they are still white they have sour and astringent taste, sour taste when they are red and have sweet and sour taste when they are black. The variation of properties about parts of AV, leaf fruit and flower (LFW) are used in TTM, antitussive, antiseptic and antitoxic for decoction. Preservation of this information can be a valuable policy for good usage of natural sources and investigation in this field.

Major compound of LFW of AV were polyphenol and flavonoid showed against antioxidation, free radicals are atoms with unparired electrons. Many diseases are caused by free radicals, cancer, coronary heart disease, allergies etc. However, there are no reports about variation of properties form AV to antioxidant but have been reported about fruits of AV in 2012 for the highest to antioxidant that the hypothesize.

The quantitative method for antioxidant determination include DPPH radical scavenging assay, ABTS radical radical cation decolorization assay etc. Finally, I will adjusted for determination method for suitable the aims to finded.

MATERIALS AND METHODS
Reagents
Folin-Ciocalteu’s reagent (Fluka, Germany), Gallic acid and Rutin (Sigma, USA), 2,2-Diphenyl-1-picrylhydrazyl, Butylatedhydroxytoluene (BHT) and Potassium acetate (Fluka, Germany), Sodium carbonate (Merck, Germany). Potassium persulphate (Hitiba, India)

Plant material
The plant used in the present study were LFW of AV collected on January 2019 form Dongbung Farm, Prachinburi, Thailand. The plant material were washed and dried at 60°C for 24 hrs in a hot air oven and were reduced to powder.

Plant extraction
Soxhlet extraction method was used. Ten grams of plant powder, LFW of AV were packed in Whatmann No. 1 filter paper and placed in extraction chamber which was suspended below
in the round bottom flask containing the solvent (100 ml of ethanol). The samples were extracted at 73.5 °C for 24 hrs and then concentrated under reduced pressure at 60 °C with the rotaryevapalationer. The extract were calculated in % yield and stored at 4 °C for further studies.

% yield = [ weight of extract (g) / weight of fresh plant (g) ] x 100

**Total phenolic content procedure**

The total phenolic content were determined using the Folin-Ciocalteu assay which is modified from Kamtekar S et al.[7] An aliquot 50 µl of the extracts (2 mg/ml) were mixed with 250 µl of 10 % Folin-Ciocalteu’s reagent. Incubation at room temperature for 10 min. Add 100 µl of 7% sodium carbonate in 96-well microplate. The mixture was shaken and kept in dark room for 2 hrs. The absorbance were measured against blank (without extract) at 765 nm using UV-Visible spectrophotometer. The calibration curve was prepared using the standard gallic acid solution, various concentrations (50, 100, 150, 200, 250, 300 and 350µg/ml). Total phenolic content was expressed as milligram of gallic acid equivalent GAE/100 mg. The experiment were carried out in triplicate.

**Total flavonoid content procedure**

The total flavonoid content were determined using the aluminium chloride colorimetric assay which is modified from Tungmungmee S et al.[8] An aliquot 120µl of the extracts (2 mg/ml) were mixed with 320µl of 95 % ethanol, 40µl of (4g/ml) aluminium chloride, 600µl distilled water and 40µl of potassium acetate. Incubation at room temperature for 1 hr. The absorbance were measured against blank (without extract) at 510 nm using UV-Visible spectrophotometer. The calibration curve was prepared using the standard rutin solution, various concentrations (100, 150, 200, 250, 300, 350 and 400µg/ml). Total flavonoid content was expressed as milligram of rutin equivalent RE/100 mg. The experiment were carried out in triplicate.

**Radical scavenging activity using DPPH method**

The radical scavenging activity (RSA) which is modified from Pengkumsri N et al.[9] Different concentrations of the extracts were taken in test tubes. 500µl of extracts were transferred into a 96-well microplate. Then 100µl of 9µM DPPH (in absolute ethanol) were added into each well. Incubation at the dark room temperature for 25 min. The blank was prepared as above without the extract and ethanol was used for the baseline correction. Adjust in the absorbance of the extract samples were measured at 516 nm using the UV–visible spectrophotometer. The RSA were calculated in the equation here.
% of inhibition RSA = \[ \frac{(A_{516 \text{ blank}} - A_{516 \text{ sample}})}{A_{516 \text{ blank}}} \] \times 100.

**Free radical scavenging activity by the use of a stable ABTS radical cation method**

The ABTS radical cation method which is modified from Dhanani T et al.\(^{[10]}\) ABTS was dissolved in water to a 8 μM concentration and radical cation (ABTS) was produced by reacting ABTS solution with 3.50 μM potassium persulphate. Incubation at the dark room temperature for 18 hrs. For assay, Mixture was diluted with ethanol bring to an absorbence value of 0.75 ± 0.02 at 734 nm. Used 15 μl of the diluted sample and bring ABTS 10 mg. The ABTS were calculated compared BHT standard.

**Statistical analysis**

Statistical analysis were performed ANOVA method, confidence level 99 % of the comparison were compared by individual pair, Tukey’s test. The correlations of method was detected by the coefficient of correlation of Pearson.

**RESULTS AND DISCUSSION**

**Percent yield topic**

Extraction percent yield (PY) of flowers fruits and leaves of AV in table 1 showed that the highest form leaves.

The experiments showed variation in PY of organs, LFW. Similar results have been reported by Crepin I et al.\(^{[11]}\) Some part of plant were variated of PY. But this reports were conflict reported by Bhanuz D et al.\(^{[6]}\) them show that the PY of fruits, *Antidesma velutinum* extracted by maceration have the highest. My opinion, the factors can influence the PV content: The particle size of sample, solvent and the extraction method etc. In addition, wisdom of Thai traditional medicine was recommend time place and day, harvest have been influence for PV.

**Total phenolic and flavonoid content**

The total phenolic content of each extracted was using the folin-ciocalteu reagent. The result showed variations of phenolic content parts of the plant (table 2), the highest presented in the leaves that association flavonoid content. The comparison of phenolic and flavonoid content of AV. In each part was significantly different p < 0.01 of phenolic content, post hoc multiple comparison by Tukey’s method. The phenolic content was highest of the leaves, flowers and fruits in descending order. While the amount of flavonoid that abundant in leaves but not significant different p > 0.01. That was showed, secondary metabolites are present
different parts that has specific at compartment. Compared with the principles of air, clear of air there may be a dust.

**Free radical scavenging activity by DPPH and ABTS methods**

For antioxidant activities, DPPH scavenging activity assay have IC$_{50}$ of leaves was 13.96 ± 0.29μg/ml, the higest all of LFW (table 3). white positive control, BHT presented higher activity with IC$_{50}$ 9.08 ± 0.74 μg/ml. The power of ABTS radical cation showed higher at BHT with IC$_{50}$ 76.4 ± 2.44μg/ml. The IC$_{50}$ of ABTS radical cation of leaves the higest all of LFW, 69.4 ± 5.98μg/ml. Therefore, a statistical test of the antioxidant efficiency by all methods in the leaves of AV compared to positive control showed unsignificant differences p> 0.01. Which shows that the TTM landscape with a history of AV uses in the treatment of diseases in the past may be caused by the antioxidant properties above. However, applying these knowledge to develop into a drug in the present pattern still requires further study and clinical trials.

**Relationships between polyphenol and antioxidant methods**

Previous studies, it was found the antioxidant activity of herbal extracts was associated with the total phenolic content, depends on the properties of the extract and the method used to analyze, the method used. Moreover, it was found that the study of the antioxidant activity by different methods may give results in different directions.$^{[12]}$ Which the relationship in this study It was found that the total phenolic content has a high correlation with the antioxidant activity analyzed by ABTS and DPPH (r = 0.731 and 0.693) respectively (table 4). This research, it was found that the results from the FRAP test lack the ability to replicate. And does not correspond to the results obtained from the DPPH and ABTS methods. Therefore, the researcher chose the two methods above as stated just because, The FRAP test showed lower than underestimated, especially in complex examples or contains many types of antioxidants. This is because each group of antioxidants also has kinetics and the mechanism of reaction to each test method varies.

**Table 1: Percent yield of the various parts of Antidesma velutinum were extracted by Soxhlet method.**

<table>
<thead>
<tr>
<th>Parts of <em>Antidesma velutinum</em></th>
<th>Percent yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>flowers</td>
<td>17.16 ± 0.12</td>
</tr>
<tr>
<td>fruits</td>
<td>19.33 ± 0.19</td>
</tr>
<tr>
<td>leaves</td>
<td>29.13 ± 0.01</td>
</tr>
</tbody>
</table>
Table 2: Phenolic and flavonoid content of the various at organs of plant.

<table>
<thead>
<tr>
<th>Organs of plant</th>
<th>Total phenolic content</th>
<th>Total flavonoid content</th>
</tr>
</thead>
<tbody>
<tr>
<td>flowers</td>
<td>35.16 ± 0.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.06 ± 0.09&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>fruits</td>
<td>55.33 ± 0.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.98 ± 0.03&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>leaves</td>
<td>87.65 ± 0.18&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.45 ± 0.01&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*The different letters in the same column showed the significant difference p < 0.01

Table 3: Antioxidant activities of AV.

<table>
<thead>
<tr>
<th>Organs of plant</th>
<th>DPPH IC&lt;sub&gt;50&lt;/sub&gt; (μg/ml)</th>
<th>ABTS IC&lt;sub&gt;50&lt;/sub&gt;(μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>flowers</td>
<td>95.87 ± 0.85&lt;sup&gt;d&lt;/sup&gt;</td>
<td>19.06 ± 1.19&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>fruits</td>
<td>35.33 ± 1.54&lt;sup&gt;c&lt;/sup&gt;</td>
<td>28.98 ± 0.73&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>leaves</td>
<td>13.96 ± 0.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>69.4 ± 5.98&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>BHT (positive control)</td>
<td>9.08 ± 0.74&lt;sup&gt;a&lt;/sup&gt;</td>
<td>76.4 ± 2.44&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*The different letters in the same column showed the significant difference p < 0.01

Table 4: Quantity relationship Phenolic and antioxidant methods.

<table>
<thead>
<tr>
<th>Methods</th>
<th>Total phenolic</th>
<th>Total flavonoid</th>
<th>DPPH</th>
<th>ABTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phenolic</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total flavonoid</td>
<td>0.812</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPPH</td>
<td>0.831</td>
<td>0.121</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>ABTS</td>
<td>0.793</td>
<td>0.311</td>
<td>0.601</td>
<td>1</td>
</tr>
</tbody>
</table>

CONCLUSION

Extraction form ethanol of AV found have higher as leaf antioxidant as well. Activity in antioxidant of this plant related to the total phenolic and total flavonoid content.

REFERENCES


