Antioxidant Activity and Irritation Test of Acanthus ilicifolius Linn. Extract

Theeraya Krisdaphong1, Ampa Jimtaisong1,* Surapol Natakankitkul2, Narunan Wuttisin1
1 School of Cosmetic Science, Mae Fah Luang University
2Faculty of Pharmacy, Chiang Mai University
*Corresponding Author Email: ampa@mfu.ac.th Phone +66 53916843 Fax +66 5391 6831

ABSTRACT

Acanthus ilicifolius Linn. was a tropical plant commonly found in Thailand yet it has been known as a beneficial resource for antioxidant activity. This study aimed to evaluate antioxidant activity and irritation of ethanolic A. ilicifolius leave extract. The major antioxidant compounds in the extract were screened by thin layer chromatography (TLC) and measured by UV-spectrophotometer, which revealed 14.07% polyphenol, % 8.07flavonoid and % 20.96triterpenoid. The obtained crude extract indicated satisfying free radical scavenging activity with IC50 of 68.67 μg/ml in comparison to gallic acid (2.60 μg/ml), BHT (26.00 μg/ml) and quarcetin (6.12 μg/ml). A chromatogram obtained from High Performance Liquid Chromatography (HPLC) illustrated the content of trigonelline at Rt of 6.321 minutes. The A. ilicifolius extract also presented no irritating effect upon twenty subjects after a 24 hours single patch test measured at 30 minutes and 48 hours, the extract is therefore considered as safe natural ingredient. Further study on the anti-inflammatory effect of the extract shall be considered.

Keywords: Acanthus ilicifolius/Polyphenol/Flavonoid/Triterpenoid/Antioxidant/Irritation test

INTRODUCTION

The development of traditional knowledge to suit new lifestyle would result huge benefit upon livings. The global consumers were aware on the benefits of the natural products. The global market value of Herbal/Traditional Products reached US$ 30 billion in the year 2010. Thus, the development of Thai natural product industry to reach international standard would widen the current market share and increase national income in the form of value-added products.

A. ilicifolius has been well known in a Thai traditional medicine as a treatment to eczema, which is a form of dermatitis or inflammation of the epidermis layer of the skin. Eczema includes dryness and recurring skin rashes that is observed as redness, swelling, itching and dryness. Thus, utilizing A.
*ilicifolius* as a cosmetic ingredient would be very beneficial in cosmetic industry refer to its antioxidant or anti-inflammatory properties in the traditional data and its concept of natural origin in marketing term. In order to safely utilize the *A. ilicifolius* in cosmetic application, this independent research focus on the extraction of the *A. ilicifolius* by using ethanol as a solvent. The ethanolic fraction of the extract will be tested on the antioxidant activities and further on to the anti-inflammatory activities.

**MATERIALS**

1. Chemical Reagents and Standards
   1.1 Aluminium Chloride
   1.2 Ethanol
   1.3 Folin – Ciocalteu’s phenol reagent
   1.4 Gallic acid
   1.5 Methanol
   1.6 2,2-diphenyl-1-picrylhydrazyl (DPPH)
   1.7 Potassium acetate
   1.8 Quercetin
   1.9 Sodium Carbonate

**EXPERIMENTAL**

This study aimed to evaluate antioxidant activity and irritation of ethanolic *A. ilicifolius* leave extract. The major antioxidant compounds in the extract were screened by thin layer chromatography (TLC) and measure by UV-spectrophotometer, and then determination of total phenolic content, total flavonoids, triterpenoid, free radical scavenging activity. A chromatogram obtained from High Performance Liquid Chromatography (HPLC) and irritation test by covered patch test.

**RESULTS**

1. The Thin Layer Chromatography (TLC) Screening Test
   1.1 The Screening test of Total Phenolic Content

   The TLC plate from Toluene: Ethyl acetate system was observed. The unclear blue spot, which indicate the present of phenolic compound, was observed under UV visible light at 365 nm.

   1.2 The Screening test of Total Flavonoids
The TLC plate from Ethyl acetate: Methanol: Water system was observed. The yellowish orange spot, which indicate the present of flavonoid, was observed under UV visible light at 365 nm.

1.3 The Screening test of Saponin

The TLC plate from Chloroform: Methanol: DI water system was observed. A light blue spot, which indicate the present of saponin, was visibly observed.

2. Determination of Total Phenolic Content

The absorbance of A. ilicifolius extract are 0.3268 and 0.3268, the content of total phenolic compound present of gallic acid equivalent in the A. ilicifolius extract was 14.07% phenolic content of dry crude. (Waterman & Mole, 1994)

3. Determination of Flavonoids

The absorbance of A. ilicifolius extract are 0.4312 and 0.4300, the content of total flavonoids present of Quercetin equivalent in the A. ilicifolius extract was % 8.07 flavonoids of dry crude.

4. Determination of Triterpenoids

The absorbance of A. ilicifolius extract were 0.3770 and 0.3503, when referred to the standard curve (Figure 4.7), the content of total triterpenoid present of saponin equivalent in the A. ilicifolius extract was 20.96% triterpenoid of dry crude.

5. Antioxidant Activity of Acanthus ilicifolius Leaves Extract

Antioxidant activity of crude A. ilicifolius extract was considered as relatively satisfying with the IC50 value of 68.67 µg/ml whereas the IC50 values of standards were 26.00 µg/ml for BHT, 2.60 µg/ml for gallic acid and 6.12 µg/ml for quercetin.

6. High Performance Liquid Chromatography (HPLC)

The chromatogram of A. ilicifolius extract illustrated the content of trigonelline content peak presented at Rt of 6 min.

7. Irritation Test by Covered Patch Test

The readings at 30 minutes and 48 hours after removal of the patch produced the following M.I.I. values:

<table>
<thead>
<tr>
<th>M.I.I</th>
<th>30 minutes</th>
<th>48 hours</th>
</tr>
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<tbody>
<tr>
<td>Results</td>
<td>non irritating</td>
<td>non irritating</td>
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</table>
CONCLUSION

*A. ilicifolius* leaves were proven in containing various beneficial compounds for antioxidant and anti-inflammatory effects. The analysis of thin layer chromatography (TLC) and UV spectrophotometer illustrated the content of major antioxidant compounds in the extract including % 14.07 of polyphenol, of % 8.07 flavonoid. The obtained crude extract showed satisfying free radical scavenging activity with IC50 equivalent of 68.67 µg/ml in comparison to gallic acid (2.60 µg/ml), quercetin (6.12 µg/ml) and BHT (26.00 µg/ml) standards. Anti-inflammatory effect of the extract shall be further studied due to the high content of % 20.96 triterpenoids. Moreover, HPLC chromatogram of the extract also suggests the present of trigonelline, which contains several therapeutic properties. The *A. ilicifolius* extract also shows no irritating effect by 24 hours single patch test upon subjects after 30 minutes and 48 hours therefore is considered as safe as a cosmetic ingredient. Nevertheless, repeated patch test shall be considered to comply with international requirement. Due to the high content of triterpenoid and trigonelline of the plant, further study on anti-inflammatory such as in-vitro analysis of COX-1/COX-2 should be considered in order to obtain a new natural and sustainable source of cosmetic ingredient.

REFERENCES


