

## Nutritional Evaluation of *Adinandra Nitida* Leaves

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### Abstract

In this study, the nutritional evaluation of *Adinandra nitida* leaves was investigated. It was found that *Adinandra nitida* leaves were rich in necessary amino acids and unsaturated fatty acids. The content of the main flavonoid (camellianin A) was as high as  $27.57 \pm 0.92$  %. These results proved the high nutritional value of *Adinandra nitida* leaves. It has great commercial interest in the food and phyto-pharmaceutical market.

**Index Terms:** *Adinandra nitida*; nutrition; flavonoid

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### 1. Introduction

*Adinandra nitida* is a kind of particular wild plant in South China. Its leaves have been consumed as health tea (Shiyacha) and herbal medicine for hundreds years, which is reported to have many curative effects, such as reducing blood pressure, antibacterial, antitumor, analgesic *etc* [1-3]. Today, *Adinandra nitida* has been successfully cultivated in Guagnxi province, China. The objective of this study work was to evaluate the nutritional value of *Adinandra nitida* leaves.

### 2. Materials and Methods

#### 2.1. Materials and Chemicals

Leaves of *Adinandra nitida* (2006 spring production, moisture content 9.3 %) for this study were purchased in Laibin, China. Free Amino acids and Fatty acid methyl esters used as standards were from Sigma Chemicals Co. Other chemical were of analytical grade.

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## 2.2. Crude Fat and Protein Analysis

The crude fat and crude protein contents of dried samples were analyzed using the standard AOAC methods (Firestone, 1998), and the compositions were denoted by percentage on dry basis.

## 2.3. Analysis of Fatty Acid Composition

Free amino acid composition was determined with an Amino Acid Analyzer (Waters M510, USA). The different amino acids recovered were presented as g / 100 g dry basis. The 0.5 g of the sample was soaked in 10 mL of de-ionized water, and then sonicated for 15 min. The resulting mixture was then centrifuged at 10000 rpm/min for 15 min. Determination was obtained by pre-column derivatization of the sample with phenyl isothiocyanate and separating the corresponding derivatives with RP-HPLC on a PICO.TAG NH<sub>2</sub> analytical column at 38 °C and UV detection (254 nm).

A gas chromatograph (GC) (HP5890) equipped with a flame ionization detector (FID) and a HP-Innowax capillary column (30 m×0.25µm). The column, injector, and detector temperatures were set at 190, 230, and 230 °C, respectively. The flow rate of carrier gas N<sub>2</sub> with a split ratio of 1:50 was set at 70 mL/min. The fatty acids were identified with reference to the retention times of standard fatty acid methyl ester performed at the same conditions.

## 2.4. Analysis of Fatty Acid Composition

The analysis of fatty acid methyl esters was performed on a gas chromatograph (GC) (HP5890) equipped with a flame ionization detector (FID) and a HP-Innowax capillary column (30 m×0.25µm). The column, injector, and detector temperatures were set at 190, 230, and 230 °C, respectively. The flow rate of carrier gas N<sub>2</sub> with a split ratio of 1:50 was set at 70 mL/min. The fatty acids were identified with reference to the retention times of standard fatty acid methyl ester performed at the same conditions.

## 2.5. Quantitative Analysis of Camellianin A

30.8 mg camellianin A was dissolved in methanol to produce stock solution of 0.308 mg/ml. For the calibration curve, the stock solution was diluted with methanol in the concentration range from 0.0154 to 0.308 mg/ml.

1.079 g of dried powdered leaves was placed in a Soxhlet extractor and refluxed at 80 °C for 10 h with 150 ml methanol, and then the extract was collected and diluted to 250 ml with methanol for HPLC analysis.

The samples were separated on a reversed phase column, Diamonsil<sup>®</sup> C18 column (4.6 × 250 mm; 5 µm particle size) manufactured by DIKMA. The mobile phase consisted of methanol and water (1:1) with a flow rate of 0.8 mL/min. The HPLC system consisted of a Waters 1525 Binary HPLC Pump and a Waters 2487 Dual λ Absorbance Detector. The injection volume was 10 µL and the wavelength for detection was set at 265 nm. Before HPLC analysis, all samples must be passed through a 0.45 µm millipore filter. The quantitative analysis of Camellianin A in the samples was based on an external standard. The chromatographic data were recorded and processed by Breeze System 3.30 software.

## 2.6. Statistical analysis

The data obtained in this study were expressed as the mean of three replicate determinations and standard deviation (SD). Statistical comparisons were carried out using student t test. P values of < 0.05 were considered to be significant.

### 3. Results and Discussion

#### 3.1. Crude Fat Content and Fatty Acid Composition

In this study, the crude fat content was determined as 3.48 %. The GC analyses of samples were shown in Table 1. The *Adinandra nitida* leaves were abundant in unsaturated fatty acids, with only lower amounts present in saturated fatty acids.

The composition rates of unsaturated fatty acids, such as oleic acid (18:1), linoleic acid (18:2), and linolenic acid (18:3) are 7.49 %, 17.65%, and 31.85%, respectively. Meanwhile, major saturated fatty acid of *Adinandra nitida* leaves was palmitic acid (16:0) which comprised approximately 19.87 % of total fatty acids. Accordingly, only small quantity of stearic acid (18:0) was observed.

Polyunsaturated fatty acids such as linoleic acid (18:2), linolenic acid (18:3), and arachidonic acid (20:4) are called essential fatty acids (EFA) because of their necessity in the human body. Linolenic acid is a known enhancer for transporting bioactive compounds into the skin, and it is converted to arachidonic acid which serves as a precursor for powerful hormone-like compounds [4].

#### 3.2. Crude Protein Content and Amino Acid Composition

In this study, the crude protein content was determined as 10.51 %. It was considered that the high contents of necessary amino acids such as lysine, threonine, valine, methionine, isoleucine, leucine, tryptophane and phenylalanine, will provide a high nutritional value. Because lysine is the first limiting amino acid in the various protein sources, and sulfur containing amino acids are evaluated as the functional are evaluated as the functional amino acids [5].

#### 3.3. Camellianin A

Flavonoid, abundant in fruits, teas, vegetables, and medicinal plants, have received the greatest attention and have been investigated extensively, since they are highly effective free radical scavengers and are assumed to be less toxic than synthetic antioxidants such as BHA and BHT, which are suspected of being carcinogenic and causing liver damage [6]. According to our previous study [7], the main flavonoid in leaves of *Adinandra nitida* was identified as camellianin A (Fig. 1). In this study, the content of camellianin A in *Adinandra nitida* leaves was determined as  $27.57 \pm 0.92$  % (Fig. 2). To the best of our knowledge, there are few plant sources containing so much flavonoid like leaves of *Adinandra nitida*. To this day, more than 4000 kinds of flavonoids have been identified or synthesized, but few of them can be widely used in fields of food, medicine. Why? Though flavonoids ubiquitously exist in plants, few kinds of plants can contain enough flavonoids to achieve large-scale production. In this study, *Adinandra nitida* leaves were found to be a kind of flavonoid-rich plant source.

Table 1. Composition of fatty acids in *Adinandra nitida* leaves

	Retention time (min)	Fatty acids	Peak Area (pA*s)	Area ratio (%)
1	21.58	C13:0	1184	1.27
2	25.049	C15:0	2241	2.41
3	25.884	C15:1	7499	8.1
4	26.770	C16:0	18450	19.87
5	31.496	C18:0	3622	3.9
6	33.052	C18:1	6953	7.49
7	34.696	C18:2(t)	611	0.66
8	35.507	C18:2(c)	16391	17.65
9	36.498	C20:0	1088	1.17
10	38.413	C18:3	290582	31.85

Table 2. Composition of amino acids in *Adinandra nitida* leaves

Free amino acid	Content (mg/100g)	Free amino acid	Content (mg/100g)
Aspartic acid	910.96	Tyrosine	427.94
Glutamic acid	915.01	Valine	507.36
Serine	121.52	Methionine	151.58
Glycine	539.92	Cysteine	18.02
Histidine	388.48	Isoleucine	408.59
Arginine	541.73	Leucine	129.75
Threonine	372.96	Phenylalanine	470.38
Alanine	537.11	Lysine	482.63
Proline	523.32	Tryptophan	236.37
Total		Total	7683.62

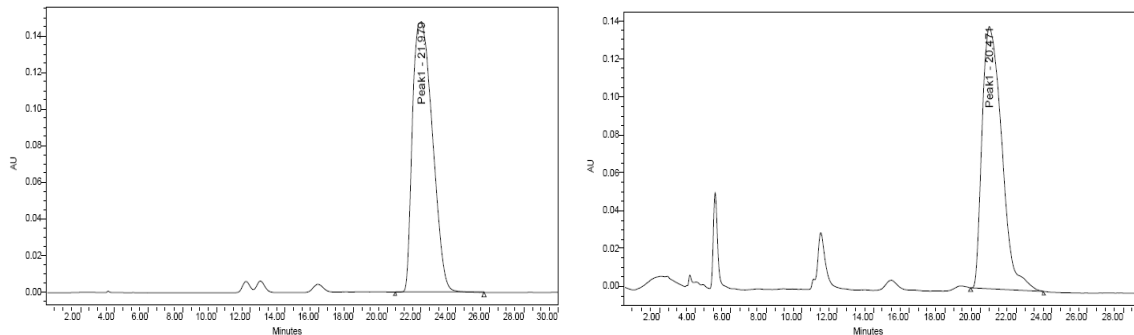


Fig. 1. HPLC profiles of camellianin A and the extract

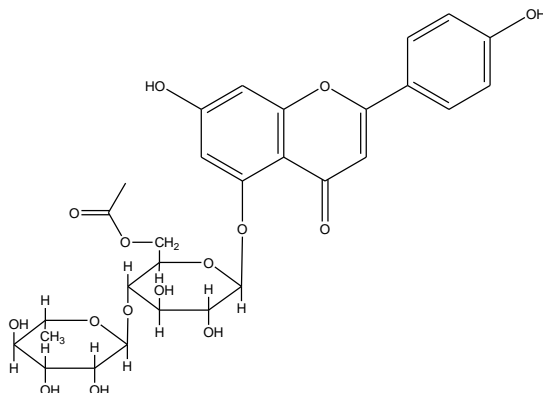


Fig. 1. Chemical structure of camellianin A

#### 4. Conclusion

In this study, it was found that *Adinandra nitida* leaves were rich in necessary amino acids and unsaturated fatty acids. The content of the main flavonoid (camellianin A) was as high as  $27.57 \pm 0.92$  %. These results prove the high nutritional value of *Adinandra nitida* leaves. It has great commercial interest in the food and phyto-pharmaceutical market.

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