Comparative Study of Total Flavonoid Contents from the Different Tissues and Varieties of *Abelmoschus Esculentus*

Shuai Hu[a#], Congying Yuan[a#], Cheng Zhang[a], Ping Wang[a], Qiang Li [c], Jie Wan[a], Hongping Chang[a], Jiazhuo Ye[b*], Xinhong Guo[a*]

[a]School of Biology, Hunan University, Changsha 410082 P.R.China

[b]College of Electrical and Information Engineering, Hunan University, Changsha 410082 P.R.China

[c]Jiangxi Wugongshan Agriculture Development Limited Company, Luxi 337200 P.R.China

[*]**Corresponding author:**

[#]Shuai Hu and Congying Yuan contributed equally to this work.

Abstract

Flavonoid intake from vegetables, fruit, berries and beverages has been favorably linked with reduced risks of a number of diseases. One possible mechanism behind the health effects of flavonoids is their antioxidant effects. An abundant intake of antioxidants from the diet may help the body to protect against harmful effects of pollution, UV light, cigarette smoke and radiation caused by free radicals. Okra (*Abelmoschus Esculentus)* is a vegetable widely grown in the tropics, sub-tropics and warmer areas of the temperate zones. It is known to have significant amounts of flavonoids and phenols. The 13 accessions of Okra were collected from different geographic regions of China. The objective of the study was to assess the difference of total flavonoid contents from the different tissues and varieties of Okra. Results indicated that the flavonoid content of the flowers was quite high compared to that of the root, stem, leaves, leaf stalk, and pod. Among the 13 different Okra varieties, the content of total flower flavoniods of Xiangkui No.3 was the highest. The content of total flavoniods of Xiangkui No.3 in pod was the highest among Xiangkui No.1, 3, 4, 5, 8. The above results provide a new way for the quality assessment and industrialization study of Okra.

KEYWORDS： *Abelmoschus Esculentus*, flavonoid extraction, spectrophotometry

1. Introduction

Okra (Abelmoschus esculentus L.) is one of the most important vegetables widely grown in Nigeria for its tender fruits and young leaves. It is widely planted from Africa to Asia, South European to America since it's easy to be cultivated and grows well in both tropical and temperate zones. In recent years, it is widely cultivated in Northern China and Southern China with the discovery of its nutritional value (Camciuc *et al*., 1998). Okra is the preferred vegetable for athletes at the Beijing 2008 Olympics Games (Kolawole and Bukola, 2010). It is named as 'plant viagra' in the USA and 'green pana' in Japan and South Korea because it is rich in vitamins and minerals (Liao *et al*., 2012; Oyelade *et al*., 2003). It is becoming a popular vegetable among both the consumers and farmers all over the world. The previous studies

showed this plant had been used to treat a variety of disorders, such as cell proliferation, microbial infection, antioxidant activity, apoptosis, hypoglycemia, constipation, urine retention and inflammation (Kumar, *et al*., 2009; Vayssade *et al*., 2010; Gul *et al*. 2011). In the present study, we analysed and compared the total flavonoid contents from the different tissues and varieties of Okra to provide a new way for the quality assessment and industrialization study of this palnt.

2. Materials and Methods

2.1 Apparatus

The JY92-IID ultrasonic cell disruptor (Ningbo Scientz Biotechnology Zhejiang, People's Republic of China) was used here and its power was 900 W with a frequency range from 20 kHz to 25 kHz. The absorbances of the samples were determinated by SP-756PC ultraviolet visible spectrophotometer (Spectrum Instruments Co., Ltd., Shanghai, People's Republic of China).

2.2 Materials and reagents

The Okra (Xiangkui No.1-13) plants harvested from The Okra Base, Hunan University. The standard rutin was purchased from J&K scientific Co., Ltd. The $NaNO_2$, $Al(NO_3)_3$, NaOH and ethanol employed were of the analytical reagent grade. Double-distilled water was used in all experiments.

2.3 Standard preparation

A total of 50 mg/L of the rutin standard solution was prepared by dissolving rutin reference material in 70% ethanol.

2.4 Procedures for the determination of total flavonoids

Each dried plant material was crushed and passed through a 20-mesh sieve. Each sample was accurately taken, mixed with 40 ml of 70% ethanol, and then pipetted into a 100 ml volumetric flask. In order to establish a reliable method for determining total flavoniods from Okra (*Abelmoschus Esculentus*), the four methods including sonication, water bath, water bath and sonication, or water-bath microwave for extracting total flavonoids were applied. (1) Sonication. The sample was extracted ultrasonically for 60 min. (2) Water bath. The sample was warmed in a water bath at 70℃ for 150 min. (3) Water-bath sonication. The sample was extracted ultrasonically for 30 min after warming in a water bath at 70℃ for 120 min. (4) Water-bath microwave. The sample was extracted using microwave (400 W) with shaking it well each 15 s for 30 min after warming in a water bath at 70℃ for 150 min. The sample solution was filtered into a flask to make a total volume of 50 ml with 70% ethanol.

A total of 1 ml of each sample extraction was pipetted into a 10-ml volumetric flask. The solution was treated with 0.30 ml of the 5% $NaNO_2$ solution for 5 min and evenly mixed, into which 0.3 ml of the 10% $Al(NO_3)_3$ solution was added and shaked up, then 6 min later, 2 ml of the 1mol/L NaOH solution was added to it. The mixture was added to the volume with 30% ethanol, and allowed to stand for 10 min before analyzing against the blank solution. Values are averages for three independent experiments.

3. Results and discussion

3.1 Selection of the detection wavelength

The $NaNO_2$-$Al(NO_3)_3$-NaOH spectrophotometry was used to determine the total flavone content in detected samples according to the fact that the maximum absorbency can be obtained when the chelate formed by the combination of flavonoids and metal ion is at 493 nm (Wu *et al.*, 1998). Comprehensively, 493 nm was chosen as the detection wavelength.

3.2 Standard curve drawing

The linearity of the method was tested by analyzing different amounts of the standard solutions of rutin (0, 1, 2, 3, 4, 5 ml, respectively) coupled with the $NaNO_2$-$Al(NO_3)_3$-NaOH spectrophotometry under the detection wavelength of 493 nm. A good linear relationship was observed in the range of 0.00～0.067 mg/ml with the regression equation of y=12.046x-0.0015 and a correlation coefficient of 0.9993 (Fig.1).

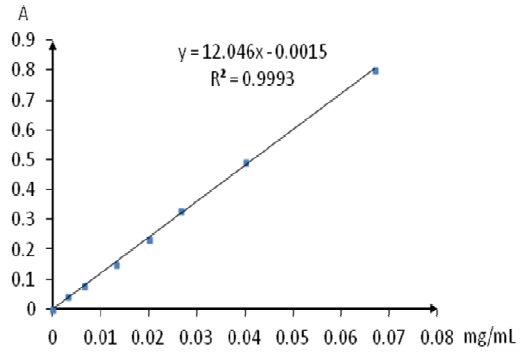

Fig.1 Calibration plot for flavonoid determination using $NaNO_2$-$Al(NO_3)_3$ method

3.3 Comparison of extraction methods for total flavonoids

In order to establish a reliable method for determining total flavoniods from Okra, the four methods including sonication, water bath, water-bath sonication, or water-bath microwave for extracting total Flavonoids in Flower of Xiangkui No.5 were compared. The contents of total Flavonoids in flower of Xiangkui No.5 extracted using sonication, water bath, water-bath sonication, or water-bath microwave were respectively 2.50%, 2.85%, 3.12%, and 0.815% (Table 1). Therefore, water-bath sonication method had an excellent effect on the extraction of total flavonoids from Okra. It can save more time and enhance efficiency (Hu *et al.*, 2013).

Table 1 Comparison of extraction methods for total flavonoids in flower of Xiangkui No.5

Extraction methods	Dry sample (g)	Absorbance (A)	Total flavonoids quality (mg)	Contents of total flavonoids (%)
Sonication	0.4004	0.24	10.02407	2.50
Water bath	0.3913	0.267	11.14478	2.85
water-bath sonication	0.3843	0.287	11.97493	3.12
water-bath microwave	0.3831	0.073	3.092313	0.81

3.4 Determination of total flavonoids in different tissues of Okra

Flavonoids are probably the most important natural phenolics because they are one of the most diverse and widespread group of natural compounds. These compounds possess a broad spectrum of chemical and biological activity including antioxidant, radical scavenger, antileukemic and vasodilator. Using the standard plot of quercetin (y=12.046x-0.0015, R^2= 0.9993), the flavonoid contents of dry samples from the different tissues of Okra were determined. The percentage contents of total flavonoids of root, stem, leaves, leaf stalk, pod and flower ranged from 0.39% to 5.29% (Table 2). Results showed the

content of total flavoniods of the different tissues of Okra was highest in the flowerer.

Table 2 Determination of total flavonoids in different tissues of Okra

Tissue	Dry sample (g)	Absorbance (A)	Total flavonoids quality (mg)	Total flavonoids (%)
Main root	0.503	0.049	2.0961315	0.42
Lateral root	0.5065	0.098	4.1300017	0.82
Stem	0.5102	0.047	2.0131164	0.39
Leaf stalk	0.5051	0.059	2.511207	0.50
Leaf	0.5131	0.287	11.974929	2.33
Old pod	0.5137	0.053	2.2621617	0.44
Green pod	0.5045	0.121	5.0846754	1.01
Flower	0.5110	0.650	27.0	5.29

3.5 Determination of total flavonoids in flower among different Okra varieties

The 13 varieties of Okras were selected in the test. The dried flower samples of Xiangkui No.1-13 Okras was crushed and passed through a 20-mesh sieve. Then the dried powder samples were accurately taken, mixed with 70% ethanol. The total flavonoids in flowers were determined by spectrophotometry with rutin standard and $NaNO_2$-$Al(NO_3)_3$-NaOH system.

Table 3 Determination of total flavonoids in flower among different okra varieties

Samples	Dry sample (g)	Absorbance (A)	Total flavonoids quality (mg)	Contents of total flavonoids (%)
No.1	0.5100	0.328	13.7	2.68%
No.2	0.4996	0.296	12.3	2.47%
No.3	0.5110	0.650	27.0	5.29%
No.4	0.4960	0.392	16.3	3.29%
No.5	0.5059	0.387	16.1	3.19%
No.6	0.4919	0.276	11.5	2.34%
No7	0.4965	0.217	9.1	1.83%
No.8	0.5074	0.206	8.6	1.70%
No.9	0.5106	0.259	10.8	2.12%
No.10	0.5131	0.255	10.6	2.07%
No.11	0.5118	0.282	11.8	2.30%
No. 12	0.5114	0.326	13.65	2.67%
No.13	0.5295	0.271	12.34	2.33%

There were differences in their total flavonoids in flower among different Okra varieties. Results indicated the content of total flavoniods of Xiangkui No.3 in flower was the highest (5.29%), but lowest (1.70%) in Xiangkui No.7 among the 13 Okra flower samples (Table 3). The total flavonoids in flower from Xiangkui No.1-11 Okras were determined in previous study (Hu et al., 2013). The above results provide a new way for the quality assessment and industrialization study of Okra.

3.6 Determination of total flavonoids in pod among different Okra varieties

To determine total flavonoid contents in pod of different Okra varieties, the spectrophotometry with rutin standard and $NaNO_2$-$Al(NO_3)_3$-NaOH system was applied. In the tested samples including Xiangkui No.1, 3, 4, 5, 8, the content of total flavoniods of Xiangkui No.3 in pod was the highest (2.71%), but lowest (0.75%) in Xiangkui No.8 (Table 4). The above results will establish the academic base for the exploitation and utilizing of the Okra resource.

Table 4 Determination of total flavonoids in pod among different Okra varieties

Samples	Dry sample (g)	Absorbance (A)	Total flavonoids quality (mg)	Contents of total flavonoids (%)
No.1	0.2875	0.1010	4.254524	1.48%
No.3	0.5608	0.364	15.17101	2.71%
No.4	0.5076	0.1560	6.53744	1.29%
No.5	0.1378	0.0540	2.303669	1.67%
No.8	0.3715	0.0660	2.80176	0.75%

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