

Analysis and comparison of the active components and antioxidant activities of extracts from *Abelmoschus esculentus* L

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ABSTRACT

Background: *Abelmoschus esculentus* L. is a healthy vegetable belonging to the family Malvaceae. This article reports the contents of total phenolics (TP) and total flavonoids (TF) in 80% methanol extracts of the flower (FL), fruit (FR), leaf (L), and seed (S) of *A. esculentus*, and in 0, 10, 30, 50, and 70% methanol eluates (ME), through the HP-20 column chromatography of 80% of the methanol fruit extract after it is defatted with petroleum and extracted with ethyl acetate. All the names of the samples are shortened for AEE-FL, AEE-FR, AEE-L, AEE-S and 0% MEF-WE, 10% MEF-WE, 30% MEF-WE, 50% MEF-WE, 70% MEF-WE respectively. In addition, the effects of the aforementioned extracts on 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) radical-scavenging and on ferric reducing antioxidant power (FRAP) have been evaluated. **Materials and Methods:** The antioxidant activity of the extracts and the enrichment fraction of *A. esculentus* were also evaluated by two assays, the DPPH radical-scavenging and ferric reducing antioxidant power (FRAP). The content measurement of TF and TP adopts the UV-2102 PCS method, and the measurement of the antioxidant activity adopts the Infinite M 200 method. **Results:** The experiment results show that all the different parts and different enrichment fractions of the water extracts of *A. esculentus* contain phenolics and flavonoids. Through the research of antioxidant activity we know that all the parts of the methanol extracts and different enrichment fractions of water extracts in the *A. esculentus* have the effect of scavenging free radicals, among which the antioxidant activity in the 50% MEF-WE part is the strongest. Here, the main components of antioxidant activity must be the flavonoids and phenolics, and furthermore, we know that there is a direct relationship between the contents of flavonoids and phenolics and the antioxidant activity. **Conclusion:** The study suggests that *A. esculentus* may be the potential rich source of natural antioxidant. The experiment result provided a scientific basis for the further research and development of *A. esculentus*.

Key words: *Abelmoschus esculentus* L, antioxidant activity, 1,1-Diphenyl-2-picryl-hydrazyl, ferric reducing antioxidant power, the total flavonoid, the total phenolic acid

INTRODUCTION

A. esculentus, an annual herb belonging to the family of Malvaceae, is one of the most important vegetables grown in Nigeria. It is widely grown for its tender fruits and young leaves. It is easy to cultivate and grows well in both tropical and temperate zones, that is, it is widely planted from

Africa to Asia, and from Southern Europe to America.^[1] Since the discovery of its nutrition, it is widely cultivated in north and south China, in recent years. It has been the preferred vegetable for the Olympic athletes of the Beijing Olympic Games.^[2] For its functional characters, it has some interesting names, such as 'green panax' in Japan and 'plant viagra' in the USA.^[3] As a kind of health vegetable, *A. esculentus* is becoming a lot more popular all over the world.

Nutritionally, it has been reported that there are many useful substances in the seeds of *A. esculentus*, such as,

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flavones, polysaccharide, pectin, trace elements, and amino acids.^[4,5] Modern medical research provides that the extract of the fruit has the ability of resisting fatigue, and has anti-aging, and anti-oxidant properties.^[6] With the larger consumer demand for Functional Food, much more attention is paid to *A. esculentus* by our society, for its special functional and nutrition value. Therefore, it is meaningful to research the chemical compositions of *A. esculentus*, to develop its health function.

Although extensive information on cultivation, breeding, and physiology^[7,8] is available on *A. esculentus*, of late, the antioxidant properties have surprisingly not been investigated to the same extent. Therefore, the objective of the present study is to determine the TF and TP content of the different parts of the plant and different enrichment fraction of the water extracts to evaluate the antioxidant activity using two different assays, respectively.

MATERIALS AND METHODS

Experimental materials

The sample of *A. esculentus* was collected from the botanical garden of Zhejiang Agriculture and Forestry University, China. It was identified as the whole grass of *Abelmoschus esculentus* L., of the Malvaceae genus of the family *Abelmoschus Medic*, by Lu-Huan Lou, professor of plant taxonomy of the Zhejiang Agriculture and Forestry University, and the specimen were deposited in our laboratory. The dried plant organs were powdered and passed through sieve No. 4 and the powder was stored in an airtight container at 4°C till use.

Instruments and Reagent

The infinite M200 Universal Microplate Spectrophotometer (Swiss Tecan company, Swiss) was used to measure the absorbance (DPPH and FARP assays) and the UV-2102 PCS UV-Vis spectrophotometer (Shanghai Unica Co., Ltd. China) was used to measure the absorbance of the Folin-Ciocalteu assay, using the $\text{NaNO}_2\text{-Al(NO}_3)_3\text{-NaOH}$ method. DGG-924A drying machine (Shanghai Senxi Laboratory Instrument Co., Ltd. China), R201B rotary evaporator (Shanghai Shensheng Biotech Co., Ltd. China).

2, 4, 6-tri(2-pyridyl)-s-triazine (TPTZ), 1,1-Diphenyl-2-picryl-hydrazyl (DPPH), 6-Hydroxy-2,5,7,8-tetramethylchroman-2- carboxylic (Trolox), and Folin-Ciocalteu were all purchased from Sigma Company (USA). The standard samples of rutin and gallic acid were purchased from the China Pharmaceutical and Biological Products Testing Station (The batch numbers were 10080-200306 and 110831-200302). All the other chemicals, including the solvents of methanol and ethanol, used in the experiment were of analytical grade.

Preparation of the sample solution

The dried powder of the flower, fruit, leaf, and seed of *A. esculentus* (1.0000 g) was critically weighed and then extracted in an ultrasonic cleaner at 50°C with 40 times of 80% methanol, thrice (30 minutes each time). The solution was then filtered through a filter paper each time and the filtered extracts were combined. The extracts were concentrated into a dry powder by the rotary evaporator at 50°C, and then dissolved in 70% ethanol and put in 25 mL volumetric flasks. After shaking, the sample solutions of AEE-FL, AEE-FR, AEE-L, AEE-S were obtained.

The dried fruit powder of *A. esculentus* (10 kg) was extracted with three times of 80% methanol, on four occasions, at room temperature (three days each time), and the extract was concentrated into a volume of 5 L. It was then extracted by the solvent of petroleum ether (60 – 90°C boiling range) to get rid of the fat-soluble components. After extraction by EtOAc, the water solution was added to the top of the Diaion HP-20 column chromatographer, and the resin was washed with distilled water and 10, 30, 50, and 70% methanol individually. Then, the samples of 0% MEF-WE, 10% MEF-WE, 30% MEF-WE, 50% MEF-WE, and 70% MEF-WE were obtained and concentrated into a dry powder using the rotary evaporator at 50°C, respectively. The 0% MEF-WE, 10% MEF-WE, 30% MEF-WE, 50% MEF-WE, and 70% MEF-WE powder weighed 23.20, 23.32, 20.46, 23.10, 20.28 mg, respectively. They were dissolved separately, in 25 mL volumetric flasks, in 70% ethanol. Finally, the solution of the samples of 0% MEF-WE, 10% MEF-WE, 30% MEF-WE, 50% MEF-WE, and 70% MEF-WE were obtained.

Determination of TP and TF

The Folin-Ciocalteu assay,^[9] with some modification, was adopted to determine the TP of the samples. The best colored conditions were: 0.3 mL Folin-Ciocalteu solution, 2 mL 10% Na_2CO_3 solution, 30°C reaction temperature, and half an hour reaction time. Gallic acid of 29.42 mg was accurately weighed and dissolved in distilled water in 100 mL volumetric flasks. Zero, 0.05, 0.1, 0.15, 0.2, 0.25, and 0.3 mL of gallic acid solution was drawn, and put into 10 mL volumetric flasks separately, treated with the previous conditions, and fixed to 10 ml with distilled water. The absorbance was tested at 760 nm of the reaction solution and subtracted from the reagent blank. The linear regression equation was calculated, with sample concentrations as the X coordinate axis and the absorbance at 760 nm of the reaction solution as the Y coordinate axis. From Figure 1, a good liner relationship could be seen, to some extent, between the sample concentrations and the absorbance at 760 nm of the reaction solution. Meanwhile, the absorbance at 760 nm of the sample solution was tested following the treatment by the gallic acid solution. The amount of TP could be calculated as the gallic acid

equivalent from the calibration curve: $y = 0.1023x + 0.0845$, $R^2 = 0.9979$.

NaNO_2 - $\text{Al}(\text{NO}_3)_3$ - NaOH assay^[10,11] with some modification was adopted to determine the TF of the samples. Rutin of 17.75 mg was accurately weighed, and dissolved in distilled water and put into 100 mL volumetric flasks. Zero, 0.5, 1, 1.5, 2.0, 2.5, and 3.0 mL of rutin solution was drawn and put into 10 mL volumetric flasks separately, and then 70% ethanol was added up to 5 mL. The best colored conditions were as follows: adding 1 mL of 5% NaNO_2 , setting for six minutes, then adding 1 mL of 10% $\text{Al}(\text{NO}_3)_3$ and setting for six minutes, and finally adding 3 mL of 4% NaNO_2 and fixing with 70% ethanol, and allowing it to react for 15 minutes. The absorbance at 510 nm of the reaction solution was tested and then subtracted from the reagent blank. The linear regression equation was calculated with sample concentrations as the X coordinate axis and the absorbance at 510 nm of the reaction solution as the Y coordinate axis. From Figure 2, a good liner relationship could be found between the sample concentrations and the absorbance at 510 nm of the reaction solution, to some extent. Meanwhile, the absorbance at 510 nm of the sample solution was tested following the treatment by the gallic acid solution. The amount of TF could be calculated as rutin equivalents from the calibration curve: $y = 0.0057x - 0.0256$, $R^2 = 0.9989$.

Determination of the antioxidant ability

Determination of the free radical scavenging activity (FRSA) in the DPPH assay

The scavenging effect of the extracts on a DPPH radical was monitored as described.^[12] Briefly, the scavenging ratio of the sample and Trolox on DPPH at the same time was tested, and then a suitable concentration range of the Trolox and its scavenging percentage was found, a linear regression equation between the Trolox concentration

and its scavenging percentage was built, and the Trolox equivalent antioxidant capacity (TEAC) was calculated through the equation, according to the scavenging percentage of the sample solution to the DPPH radical solution.

Trolox of 21.45 mg was accurately weighed, and then dissolved in 70% ethanol and put into 100 mL volumetric flasks and fixed to the concentration of 0.2145 mg mL⁻¹. It was diluted to make its concentration up to 0.00212, 0.006336, 0.01056, 0.014784, and 0.019008 mg mL⁻¹. DPPH of 11.83 mg was accurately weighed and then dissolved in 70% ethanol and put into 100 mL volumetric flasks and fixed to the concentration of 0.1183 mg mL⁻¹. It had to be diluted before use.

Trolox solution of 100 μL was mixed with 200 μL of 70% ethanol solvent, 100 μL Trolox solution was mixed with 200 DPPH, and 200 μL DPPH solution with 100 μL of 70% ethanol solvent, to make three different reaction systems. The three different mixtures were put into the holes of 96-well microplates, respectively, and reacted under 40°C for one hour without illumination, and then their absorbance at 517 nm were read by the Infinite M 200. For convenience, they were represented as A_0 , A_1 , A_2 , so the scavenging effect of Trolox on the DPPH radical could be expressed as the following formula; $(A_1 - A_0) / A_2 \times 100\%$.^[13,14]

A linear regression equation was calculated with the Trolox solution concentrations as the independent variable (X) and the percentage of scavenging effect on the DPPH radical as the dependent variable (Y). From Figure 3, a good liner relationship could be found between the Trolox concentrations (0.002112 – 0.019008 mg mL⁻¹) and the scavenging percentage on the DPPH radical, to some extent.

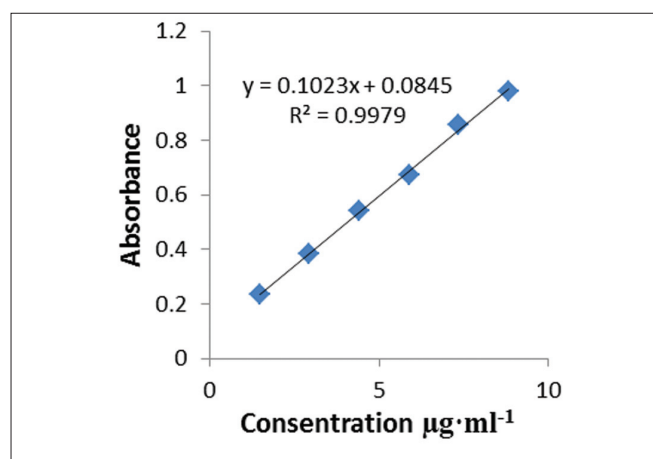


Figure 1: The absorbance of gallic acid with different concentrations

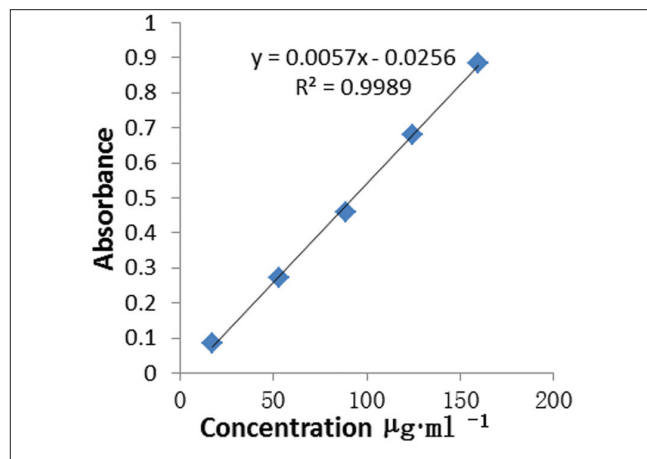


Figure 2: The absorbance of rutin with different concentrations

Meanwhile, the scavenging percentage on the DPPH radical of sample solution was tested following the treatment to Trolox solution. The scavenging effect on the DPPH radical of the samples could be calculated as the Trolox equivalent's antioxidant capacity from the calibration curve: $y = 0.1023x + 0.0845$, $R^2 = 0.9939$.

Ferric reducing antioxidant power assay

The antioxidant capacities of the sample extracts were estimated according to the procedure described by the literature.^[15]

The Ferric reducing antioxidant power (FRAP) reagent contained 2.5 mL of 20 mmol / L TPTZ solution in 40 mmol / L HCl plus 2.5 mL of 20 mmol / L $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, and 25 mL of 0.3 mol / L acetate buffer (pH 3.6), as described by the literature.^[16,17]

Trolox solution was taken and diluted to make its concentration up to 0.00704, 0.01408, 0.02112, 0.02816, and 0.0352 $\text{mg} \cdot \text{mL}^{-1}$ separately. FRAP reagent of 200 μL was mixed with 100 μL Trolox solution, and then the mixture was allowed to react under 40°C for an hour, without illumination. The absorbance at 593 nm was read via the Infinite M 200 and the absorbance of reagent blank was measured. A linear regression equation was calculated with the Trolox solution concentrations as the independent variable (X) and the absorbance at 593 nm as the dependent variable (Y). From the Figure 4, a good liner relationship could be found between the Trolox concentrations (0.00704 ~ 0.0352 $\text{mg} \cdot \text{mL}^{-1}$) and the mixture's absorbance at 593 nm, to some extent. The calibration curve is shown in Figure 4. Obviously, through a treatment like the Trolox mixture, the antioxidant capacities of the sample extracts could be calculated as the Trolox equivalent's antioxidant capacity (TEAC) from the calibration curve: $y = 27.715x - 0.0211$, $R^2 = 0.9966$.

Statistical analysis

All the final data were presented as means $\pm$ standard deviations (S.D.) of the three determinations. The Pearson's correlation test was used to assess the correlations between the content of TP, TF, and the antioxidant ability of the sample solution by using the SPSS system version 16.0 for Windows, and the figures were produced by the using Excel 2007.

RESULTS AND DISCUSSION

Total phenolic content of the samples

The total phenolics content of all the parts of the methanol extracts and different enrichment fraction of water extracts in the *A. esculentus* are presented in Table 1. Among the

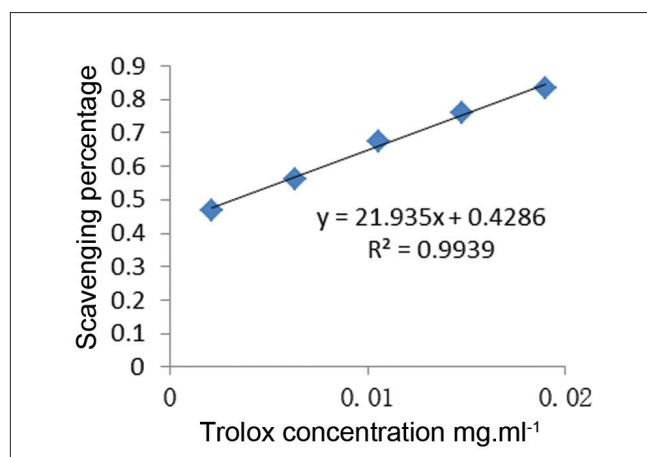


Figure 3: The reducing power of the Trolox on DPPH with different concentrations

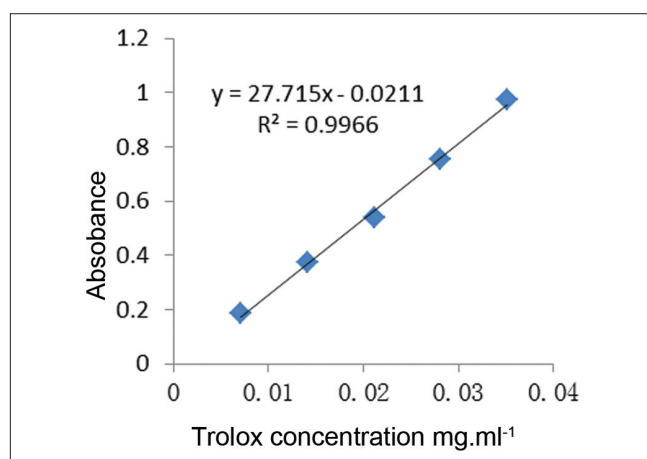


Figure 4: The reducing power of the Trolox on Ferric with different concentrations

Table 1: The TP content of *A. esculentus* extracts (mg GAE / g DW)

Samples	TP content			Average content
AEE-FL	17.5751	17.6068	17.5482	17.5767 $\pm$ 0.0294
AEE-FR	12.4753	12.4738	12.2510	12.4001 $\pm$ 0.12
AEE-L	4.7532	4.9189	4.6000	4.7574 $\pm$ 0.1595
AEE-S	2.0198	1.9687	1.8534	1.9473 $\pm$ 0.0853
0%MEF-WE	19.2880	18.4980	18.6244	18.8035 $\pm$ 0.4244
10%MEF-WE	103.7260	108.5151	107.5091	106.5834 $\pm$ 2.5252
30%MEF-WE	256.5397	264.9604	263.0493	261.5165 $\pm$ 4.4146
50%MEF-WE	326.9614	319.8010	327.7443	324.8386 $\pm$ 4.3724
70%MEF-WE	152.0875	157.1245	158.8356	156.0160 $\pm$ 3.5080

Values in Table 1 are expressed as means $\pm$ standard deviation ($n = 3$). TP expressed as mg gallic acid equivalents (GAE) / g dry plant material from the first to the fourth row, mg gallic acid equivalents (GAE) / g dry extract powder from the fifth to the ninth row

various parts of the methanol extracts, the maximum content was obtained from AEE-FL, and then from AEE-FR, AEE-L, and AEE-S. Through enrichment with Diaion

HP-20 column chromatography, the TP content in all the fractions of water extracts became more.

Total flavonoid content of the samples

The total flavonoid content of all the parts of the methanol extracts and different enrichment fractions of water extracts in the *A. esculentus* are presented in Table 2. The methanol extract of *A. esculentus* flower has a higher phenolic content than the fruit, leaf, and seed samples. The total flavonoid content of the different enrichment fractions of the water extracts are in order of 30% MEF-WE $\geq$ 50% MEF-WE > 70% MEF-WE > 10% MEF-WE > 0% MEF-WE.

Determination of the free radical scavenging activity on 1,1-Diphenyl-2-picryl-hydrazyl

The free radical scavenging activity (FRSA) of all the parts of the methanol extracts and different enrichment fraction of water extracts in the *A. esculentus* are presented in Table 3. In the free radical scavenging power, AEE-FL exhibits a higher free radical scavenging activity than AEE-

FR, AEE-L or AEE-S. There is a significant difference in scavenging activity among the enrichment fraction of water extracts in the *A. esculentus*. Fifty percent of MEF-WE and thirty percent of MEF-WE perform a stronger free radical scavenging activity than the other fractions.

Determination of the reducing power on ferric

The reducing power of all parts of the methanol extracts on ferric and different enrichment fraction of water extracts in the *A. esculentus* are presented in Table 4. Obviously, the reducing power of the enrichment fraction of water extracts in the *A. esculentus* becomes stronger after enrichment with the help of Diaion HP-20 column chromatography. Different parts have different antioxidant abilities. The orders are AEE-FL > AEE-FR > AEE-L > AEE-S and 50% MEF-WE > 30% MEF-WE > 70% MEF-WE > 10% MEF-WE > 0% MEF-WE.

The relativity analysis between the total phenolic content, total flavonoid content, and the antioxidant results of two assays

Here, the relativity analysis between the TP content, TF content, and the results of the two assays was made by SPSS.16.0, and the results are shown in Table 5. From table 5, we can see that the TP content, TF content, and the outcome of two antioxidant activity assays, all have

Table 2: The TF content of *A. esculentus* (mg RUT / g DW)

Samples	TF content			Average content
AEE-FL	28.0120	28.1234	27.7304	27.9553 $\pm$ 0.2025
AEE-FR	28.4782	28.4809	28.0560	28.3384 $\pm$ 0.2446
AEE-L	10.4071	10.4727	10.4450	10.4416 $\pm$ 0.0329
AEE-S	5.8282	6.1006	6.1690	6.0326 $\pm$ 0.1802
0%MEF-WE	27.8472	28.5061	26.5820	27.6451 $\pm$ 0.9778
10%MEF-WE	167.5676	162.9784	166.8716	165.8059 $\pm$ 2.4732
30%MEF-WE	977.5064	983.2086	975.8986	978.8712 $\pm$ 3.8413
50%MEF-WE	974.7760	974.3392	979.0670	976.0607 $\pm$ 2.6126
70%MEF-WE	322.8135	307.1804	322.5312	317.5084 $\pm$ 8.9454

Values in table 2 are expressed as means $\pm$ standard deviation (n = 3). TF expressed as mg rutin equivalents (RUT) / g dry plant material from the first to the fourth row, mg rutin equivalents (RUT) / g dry extract powder from the fifth to the ninth row

Table 4: The reducing power on the ferric of *A. esculentus* extracts (mg Trolox / g DW)

Samples	FRAP (TEAC)			Average FRAP (TEAC)
AEE-FL	25.5828	26.2772	26.4398	26.1000 $\pm$ 0.4552
AEE-FR	21.4970	21.6208	21.6544	21.5908 $\pm$ 0.0828
AEE-L	11.9547	12.3748	12.4074	12.2456 $\pm$ 0.2524
AEE-S	1.9328	1.8887	1.8630	1.8948 $\pm$ 0.0352
0%MEF-WE	20.6590	20.6626	20.6193	20.6470 $\pm$ 0.0240
10%MEF-WE	86.2132	88.1666	82.8094	85.7298 $\pm$ 2.7112
30%MEF-WE	326.3014	317.156	324.8646	322.774 $\pm$ 4.9176
50%MEF-WE	379.1556	384.8112	388.7162	384.2276 $\pm$ 4.8070
70%MEF-WE	198.5108	195.1081	201.5872	198.4020 $\pm$ 3.2409

Values in table 4 are expressed as means $\pm$ standard deviation (n = 3), TEAC is expressed as mg Trolox equivalents / g dry plant material from the first to the fourth row, mg Trolox equivalents / g dry extract powder from the fifth to the ninth row

Table 3: The scavenging activity of *A. esculentus* extracts (mg Trolox / g DW) on DPPH

Samples	DPPH (TEAC)			Average DPPH (TEAC)
AEE-FL	9.9214	10.0575	9.9758	9.9849 $\pm$ 0.0685
AEE-FR	6.8437	6.8468	7.1283	6.9396 $\pm$ 0.1634
AEE-L	4.8827	4.8441	4.8634	4.8634 $\pm$ 0.0193
AEE-S	3.7456	3.6944	3.7830	3.7410 $\pm$ 0.0444
0% MEF-WE	2.3874	2.8560	2.3787	2.5407 $\pm$ 0.2731
10% MEF-WE	26.0236	26.3444	25.3260	25.8980 $\pm$ 0.5206
30% MEF-WE	207.7650	204.7563	210.0741	207.5318 $\pm$ 2.6666
50% MEF-WE	230.2140	233.9490	234.8644	233.0091 $\pm$ 2.4634
70% MEF-WE	41.8730	41.3460	40.5572	41.2587 $\pm$ 0.6622

Values in table 3 are expressed as means $\pm$ standard deviation (n = 3) TEAC expressed as mg Trolox equivalents / g dry plant material from the first to the fourth row, mg Trolox equivalents / g dry extract powder from the fifth to the ninth row

Table 5: The relativity analysis among the TP, TF content, and results of the two methods of antioxidant activity

	Total flavonoids	Total phenolics	DPPH	FRAP
Total flavonoids	1	0.972**	0.990**	0.979**
Total phenolics		1	0.950**	0.994**
DPPH			1	0.957**
FRAP				1

**Correlation is significant at the 0.01 level (two-tailed)

very significant relativity to each other ($P < 0.01$). It can be easily found that the TP content and TF content of the extract of *A. esculentus* play an important role in the antioxidant activities.

CONCLUSION

To the best of our knowledge, this article is the first report on the TP and TF contents, as also on the antioxidant ability of different organs and different enrichment fractions of water extracts of the *Abelmoschus esculentus* L. plant. It has confirmed that there are fruitful TP and TF contents in all the extracts of the plant organ, although the content amount varies to some extent. The results also show that there is more TP and TF content in the extract of the *Abelmoschus esculentus* L. flower than in the other parts. Meanwhile, the contents of TP and TF in the 50% enrichment fraction are higher than in the other fractions.

Furthermore, a significant correlation exists between the contents of TP, TF, and the DPPH radical scavenging ability, reducing power. Thus, it is easy to conclude that both the TP and TF content attribute to the antioxidant ability of the extract. This study indicates that *Abelmoschus esculentus* L has a high utilization value based on its high content of TP and TF, as well as a strong antioxidant activity.

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