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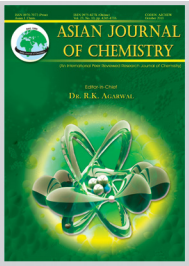

Studies on the Active Components and Antioxidant Activities of Extracts from *Aeschynanthus moningeriae*

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The objectives of the present study are to characterize the total flavonoids and total polyphenols and to evaluate the antioxidant activity of extracts from *Aeschynanthus moningeriae in vitro*. Total flavonoids content was estimated according to the $NaNO_2$-$Al(NO_3)_3$ method and total polyphenols content to the Folin-Ciocalteu method and antioxidant activity was assessed using two methods: DPPH, FRAP assays. In addition, the relativity among the content of total flavonoids, total polyphenols and antioxidant activity was also investigated. The obtained results revealed that *Aeschynanthus moningeriae* is a rich source of total flavonoids and total polyphenols and capable of strong radical scavenging activity and strong reducing power on Fe^{3+}. The present results suggested that *Aeschynanthus moningeriae* could be a potential rich source of natural antioxidants.

Key Words: *Aeschynanthus moningeriae*, Total polyphenols, Total flavonoids, DPPH, FRAP.

INTRODUCTION

Oxidative damage of cells, which is induced by free radicals usually contributes to the ageing, tumors, cardiovascular, immunology disease, *etc.*[1,2]. Antioxidants play an important role in inhibiting and scavenging free radicals, thus providing protection to human against the diseases. As the further understanding of the relation among oxygen free radical, disease and antioxidants, people pay more and more attention to antioxidants[3]. Due to the possible negative effects of synthetic antioxidants, researchers are looking for natural antioxidants with the characteristics of safe, efficient and nontoxic nature.

Aeschynanthus moningeriae, the species of *Aeschynanthus* genus of the family *Gesneriaceae*, is a perennial and originated from tropical and subtropical areas. Introducing places such as Guangxi, Hainan and Taiwan in China[4-7]. It is used in treatment of inflammation, wheezing cough, furunculosis, rheumatism, bones injuries, empyrosis, snakebite and gynecological diseases[8]. Because of the medicinal values of *Aeschynanthus moningeriae*, our laboratory will study on its extraction, isolation and identification of active lead compounds. In order to guide this study, the objectives of the present study were to characterize the total flavonoids and total polyphenols and to evaluate the antioxidant activity *in vitro* of *Aeschynanthus moningeriae*. First, The crude extract of 70 % EtOH (70 % E-CE) was obtained by extracting the powder of *Aeschynanthus moningeriae* with a solution of 70 % ethanol. Then, 70 % E-CE dissolved in distilled water was fractionated with petroleum ether (PE) and ethyl acetate (EtOAc), respectively, affording three fractions of PE-EF, EtOAc-EF and W-EF. Then, the W-EF was added to Diaion HP-20 column and the resin was eluted by distilled water, 10, 30, 50, 70 % methanol, respectively. The content of total flavonoids in different extracts and enrichment fractions was determined by the $NaNO_2$-$Al(NO_3)_3$ method and the content of total polyphenols was determined by Folin-Ciocalteu method. The antioxidant activities of different extracts and enrichment fraction were investigated by DPPH and FRAP method. In addition, the relativity among the content of total flavonoids, total polyphenols and antioxidant activity was also investigated. These provide scientific basis for rational development and utilization of *Aeschynanthus moningeriae*.

EXPERIMENTAL

Infinite M 200 universal microplate spectrophotometer (Swiss Tecan Company, Swiss) was used to measure the absorbance (DPPH and FRAP assays) and UV-2102 PCS UV-VIS spectrophotometer (Shanghai Unica Company Ltd., China) was used to measure the absorbance of $NaNO_2$-$Al(NO_3)_3$ colourimetry and Folin-Ciocalteu methods. Fe^{3+}-Tripyradyl-

triazine (TPTZ), 1,1-diphenyl-2-picryl-hydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic (Trolox) are all purchased from Sigma (USA). Standard of gallic acid and rutin were purchased from China Pharmaceutical and Bioloical Products Testing Station (The batch numbers were 10080-200306 and 110831-200302, respectively). All the other chemicals used including the solvents were of analytical grade.

Plant materials: *Aeschynanthus moningeriae* was collected from Sanya City, Hainan Province, China. It was identified as the whole grass of *Aeschynanthus moningeriae* of *Gesneriaceae Aeschynanthus* genus of the family *Gesneriaceae* by Shi-man Huang professor of plant taxonomy of Hainan University and the specimen were deposited in our laboratory. The dried plants were powdered and passed through sieve No. 40 and stored the powder in airtight containers at 4 ºC until use.

Preparation of extract of *Aeschynanthus moningeriae:* The powder of *Aeschynanthus moningeriae* was macerated with 70 % ethanol at a ratio of 1:10 (w/v) for three days under room temperature. After filtration, the residue was re-extracted twice with 70 % ethanol as described above. The filtrates were pooled together, evaporated by a rotary evaporator and freeze-dried to afford 70 %- CE. Then, 70 %-CE was dissolved in distilled water and fractionated through solvent-solvent partitioning with petroleum ether (PE) and ethyl acetate (EtOAc), respectively, affording three fractions of PE-EF, EtOAc-EF and aqueous (W-EF). The 70 % E-CE and its three fractions (PE-EF, EtOAc-EF, W-EF) were concentrated and freeze-dried. Then, the W-EF was added to Diaion HP-20 column and the resin was eluted by distilled water, 10, 30, 50, 70 % methanol, respectively. The distilled water is aim to get rid of impurity, so the water was abandoned and the other eluates were concentrated and freeze-dried. 20 mg powder of 70 % E-CE, PE-EF, EtOAc-EF, W-EF, 10 % MeOH-EF, 30 % MeOH-EF, 50 % MeOH-EF, 70 % MeOH-EF were dissolved to volumetric flasks of 25 mL using 70 % ethanol.

Determination of total flavonoids: Total flavonoids content was determined by a method based on $NaNO_2$-$Al(NO_3)_3$ assay with slight modification[9]. Briefly, 0.5 mL of sample solution (with appropriate dilution to obtain absorbance in the range of the prepared calibration curve) was dissolved to 3 mL using 70 % ethanol. The dilution solution and 1 mL 10 % $NaNO_2$ solution were mixed and allowed to stand for 6 min. Afterwards, 1 mL 10 % $Al(NO_3)_3$ solution was added and allowed to stand for another 6 min before adding 3 mL 4 % NaOH solution. The volume of the mixture was metered 10 mL with 70 % ethanol, then, was allowed to stand for 15 min at room temperature. The absorbance readings were taken at 510 nm using a UV-Visible spectrophotometer. Rutin, the concentration of 0.01775, 0.05325, 0.08875, 0.124.25, 0.159.75 mg mL^{-1}, was used as standard and for calibration. The curve of calibration is that A = 0.0057C - 0.0266, R^2 = 0.9988 (Fig 1). The total flavonoids content of samples were expressed as rutin equivalents (RE).

Determination of total polyphenols: Total polyphenols content was determined by a method based on Folin-Ciocalteu assay with slight modification[10]. Briefly, 0.3 mL of sample solution (with appropriate dilution to obtain absorbance in the range of the prepared calibration curve) was dissolved to 5 mL using distilled water. The dilution solution was mixed with 0.3 mL of Folin-Ciocalteu reagents and 2 mL of saturated Na_2CO_3 solution. The volume of the mixture was brought to 10 mL with distilled water. Then, it was allowed to stand for 0.5 h at room temperature in the dark. The absorbance readings were taken at 760 nm using a UV-Visible spectrophotometer. Gallic acid, the concentration of 0.001371, 0.002742, 0.004113, 0.005484, 0.006855, 0.008226 mg mL^{-1}, was used as standard and for calibration. The curve of calibration is that A = 0.1098C + 0.0.0845, R^2 = 0.9979 (Fig. 2). The total polyphenols content of samples was expressed as gallic acid equivalents (GAE).

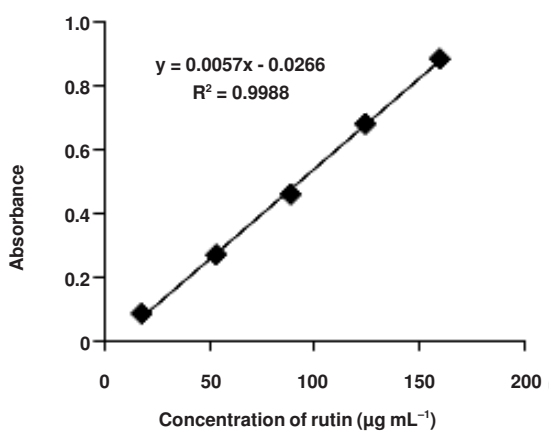

Fig. 1. Absorbance of rutin with different concentration

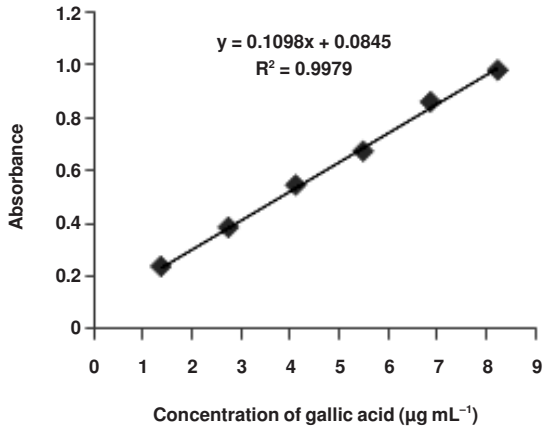

Fig. 2. Absorbance of gallic acid with different concentration

DPPH assay: The DPPH radical scavenging activity was evaluated according to the reported method[11] with a slight modification. 100 µL of Trolox solution (with the concentrations of 0.002112, 0.006336, 0.01056, 0.014784, 0.019008 mg mL^{-1}) was added to 200 µL of DPPH solution. After mixing gently and standing at 40 ºC for 1 h, the absorbance was measured at 519 nm using a microplate reader spectrophotometer *VERSA*$_{max}$. The DPPH free radical scavenging rate was calculated using the following formula: Scavenging % = 1-(A_p-A_c)/A_{max} × 100 %. Here, A_p is the stable absorbance of DPPH solution (200 µL) plus Trolox solution (100 µL), A_c is the stable absorbance of 70 % ethanol solution (200 µL) plus Trolox solution (100 µL) and A_{max} is the stable absorbance of DPPH solution (200 µL) plus 70 % ethanol solution (100 µL). The

Trolox was used as standard and for calibration. The curve of calibration is that A = 18.546C + 0.4368, R^2 = 0.9968. The scavenging activity of samples was expressed as Trolox equivalent (Fig. 3).

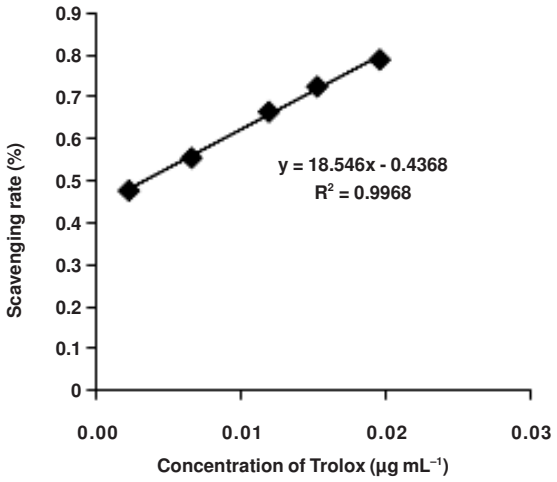

Fig. 3. Scavenging rate of Trolox on DPPH at different concentration

Total antioxidant activity (ferric reducing antioxidant power, FRAP): The total antioxidant activity of *Aeschynanthus moningeriae* were measured using FRAP assay according to the method of Ozgen *et al.*[12]. The reaction was carried out in a microplate. The antioxidant activity of the standards was estimated by using the increase in absorbance caused by the generated ferrous ion. The working FRAP reagent contained 0.01 mol mL^{-1} TPTZ 0.04 mol mL^{-1} HCl, 0.1 mol mL^{-1} acetate buffer (PH = 3.6) and 0.02 mol mL^{-1} $FeCl_3 \cdot 6H_2O$ in the ratio of 1:10:1, standing in dark for 12 h. 200 µL of this working solution was dispensed to each well of the microplate. Then, addition of 100 µL diluted sample initiated the reaction at 40 ºC and absorbance was read after exactly 1 h. The absorbance was measured at 593 nm using a microplate reader spectrophotometer *VERSA*$_{max}$. Trolox, the concentration of 0.00704, 0.01408, 0.02112, 0.02816, 0.0352 mg mL^{-1}, was used as standard and for calibration. The curve of calibration is that A = 27.715C - 0.0197, R^2 = 0.9975. The results were expressed as Trolox equivalent.

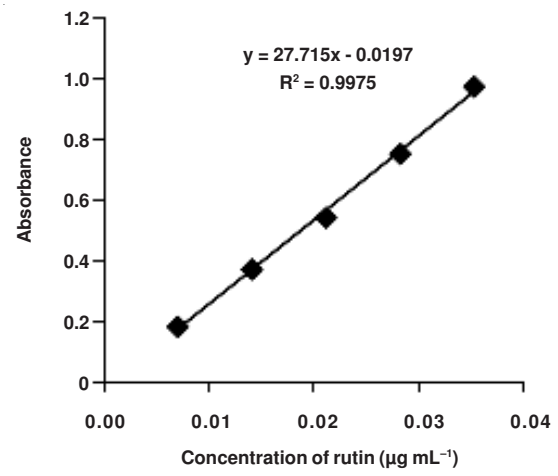

Fig. 4. Absorbance of Trolox on FRAP at different concentrations

Statistical analysis: All the data was presented as mean ± standard deviations of three determinations. Pearson's correlation test was used to assess correlations between data by using the SPSS system version 16.0 for Windows and the Figures were made by using OriginPro8.0.

RESULTS AND DISCUSSION

Total flavonoids and total polyphenols content: Phenolic compounds are ubiquitous bioactive compounds and in the form of secondary metabolites universally existed in higher plants[13]. Accordingly, bioactive phenolics have attracted special attention because they can protect the human body from the oxidative stress, which may cause many diseases, including cancer, cardiovascular dysfunction and aging[14]. In the present study, total flavonoids content of different extracts and enrichment fraction were determined according to the $NaNO_2$-$Al(NO_3)_3$ method and total polyphenols content were determined according to the Folin-Ciocalteu method. The total flavonoids and total polyphenols content of enrichment fractions were obviously higher than that of different extracts. The total flavonoids content of enrichment fractions decreased in the following order (Table-1):30 % MeOH-EF > 50 % MeOH-EF > 10 % MeOH-EF > 70 % MeOH-EF. The total polyphenols content of enrichment fractions decreased order is the same with total flavonoids. The total flavonoids content is higher than total polyphenols content in the same sample.

TABLE-1
CONTENT OF TOTAL FLAVONOIDS (TF) AND TOTAL PHENOLIES (TP) ACID OF DIFFERENT EXTRACTS AND ENRICHMENT FRACTION

Extract	Content of TF (REmg/DWg)	Content of TP (GREmg/DWg)
70 % E-CE	18.7606 ± 0.0692	4.2625 ± 0.0865
PE-EF	8.0717 ± 0.0439	0.03848 ± 0.0041
EtOAc-EF	25.7225 ± 0.0324	5.6410 ± 0.0395
W-EF	38.8651 ± 0.0885	8.6991 ± 0.0217
10 % MeOH-EF	680.7848 ± 0.0202	141.507 ± 0.0039
30 % MeOH-EF	925.9231 ± 0.0211	178.6876 ± 0.0067
50 % MeOH-EF	718.9725 ± 0.0343	168.5190 ± 0.0059
70 % MeOH-EF	467.8325 ± 0.0241	127.9025 ± 0.0089

Values in Table-1 are expressed as means ± standard deviation (n = 3)

DPPH free-radical scavenging activity: DPPH method is commonly used for detecting the free radical scavenging ability and they have been widely used in the assessment of various natural extracts[15]. The present study measured the scavenging activity of different extracts and enrichment fraction of *Aeschynanthus moningeriae* on DPPH free radicals and the results are shown in Table-2. Table-2 showed that the DPPH free-radical scavenging activities of enrichment fractions were much higher than those of different extracts. The DPPH free-radical scavenging activities of enrichment fractions decreased in the following order: 30 % MeOH-EF > 50 % MeOH-EF > 10 % MeOH-EF > 70 % MeOH-EF.

Total antioxidant activity: Ferric reducing antioxidant power assay is based on the ability of antioxidant to reduce Fe^{3+} to Fe^{2+} in the presence of TPTZ, forming an intense blue Fe^{2+}-TPTZ complex with an absorption maximum at 593 nm[16]. This reaction is pH-dependent. The absorbance increase is

proportional to the antioxidant content. The present study measured the total antioxidant activity of different extracts and enrichment fraction of *Aeschynanthus moningeriae* on FRAP and the results are shown in Table-2. Table-2 showed that the total antioxidant scavenging activities of enrichment fractions were much higher than those of different extracts. The total antioxidant activities of enrichment fractions decreased in the following order: 30 % MeOH-EF > 50 % MeOH-EF > 10 % MeOH-EF > 70 % MeOH-EF.

TABLE-2
TEAC VALUES OF DIFFERENT EXTRACTS AND ENRICHMENT FRACTION

Extract	DPPH·(TEAC) /(mgTE/DWg)	FRAP·(TEAC) /(mgTE/DWg)
70 % E-CE	22.76412 ± 0.0413	10.2674 ± 0.0444
PE-EF	5.1025 ± 0.0156	0.5658 ± 0.0077
EtOAc-EF	26.6090 ± 0.0517	14.0595 ± 0.0474
W-EF	48.1462 ± 0.0366	22.4298 ± 0.0353
10 % MeOH-EF	199.6221 ± 0.0957	511.9144 ± 0.1384
30 % MeOH-EF	263.5090 ± 0.1268	605.2754 ± 0.1333
50 % MeOH-EF	219.3589 ± 0.1307	533.3378 ± 0.1270
70 % MeOH-EF	115.5879 ± 0.0594	453.6578 ± 0.0493

Values in Table-1 are expressed as means ± standard deviation (n = 3)

Relativity analysis between the total flavonoids, total polyphenols content and the results of two methods: Here, the relativity analysis between the total flavonoids, total polyphenols content and the results of two kinds of antioxidant activity methods was made and the results were shown in Table-3. From Table-3, it is found that the total flavonoids, total polyphenols content and outcomes of two kinds of antioxidant activity methods had significant relativity. It indicates that flavonoids and phenols are the main components responsible for antioxidant behaviour of *Aeschynanthus moningeriae* and there was very high correlation between the measuring results of these two methods, so they were all available for determination of antioxidant activity of *Aeschynanthus moningeriae*.

TABLE-3
RELATIVITY ANALYSIS BETWEEN THE CONTENT OF TOTAL FLAVONOIDS (TF), TOTAL PHENOLIES (TP) AND ANTIOXIDANT ACTIVITY

	TF	TP	DPPH	FRAP
TF	1	0.990**	0.979**	0.989**
TP		1	0.964**	0.996**
DPPH			1	0.949**
FRAP				1

**Correlation is significant at the 0.01 level (2-tailed)

Conclusion

This studies report the active components and antioxidant activities of extracts from *Aeschynanthus moningeriae*. Total flavonoids content was determined according to the $NaNO_2$-$Al(NO_3)_3$ method, total polyphenols content was determined according to the Folin-Ciocalteu method and antioxidant activity was assessed using two methods: DPPH, FRAP assays. The results obtained in this work indicated that the enrichment fractions contained higher total flavonoids and total polyphenols, than the different extracts and had strong scavenging activity against DPPH and strong reducing power on Fe^{3+}. *Aeschynanthus moningeriae* could be potential rich sources of natural antioxidants. The total flavonoids, total polyphenols content and results of two kinds of antioxidant activity methods had very significant relativity. The DPPH and FRAP assays showed positive and significantly high correlation. The present findings appear useful in leading to further study in the identification and characterization of specific compounds responsible for the relatively high antioxidant activities in *Aeschynanthus moningeriae*. These studies are now in progress.

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REFERENCES

1. G.M. Williams, M.J. Iatropoulos and J. Whysner, *J. Food Chem. Toxicol.*, **37**, 1027 (1999).
2. H.H. Yu, X.G. Liu, R.E. Xing, S. Liu, Z.-Y. Guo, P.-B. Wang, C.-P. Li, P.-C. Li, *Food Chem.*, **95**, 123 (2006).
3. H.P. Xiong, *Nat. Prod. Res. Develop.*, **13**, 75 (2001).
4. Kunming Institute of Botany Chinese Academy of Sciences. Flora of Yunnan in China, p. 12 (1991).
5. Z.Y. Li and W.C. Wang, Plants of *Gesneriaceae* in China, 363 (2004).
6. Z.Y. Li, *J. Systemat. Evolut.*, **34**, 341(1996).
7. Editorial Committee for Flora of China of Chinese Academy of Sciences, Flora of China, **69**, 125 (1990).
8. M.Y. Zeng, Compendium of Chinese Medicinal Material Resources, p. 1177 (1994).
9. X.F. Yang, Y.L. Zhu and G.X. Dai, *J. Spectros. Lab.*, **23**, 812 (2006).
10. K. Dastmalchi, D.H. Damien, M. Kosar and R. Hiltunen, *LWT-Food Sci. Technol.*, **40**, 239 (2007).
11. J.K. Liu, L. Hu, Z.J. Dong and Q. Hu, *Chem. Biodiversi.*, **1**, 601 (2004).
12. M. Ozgen, J.C. Scheerens, R.N. Reese and R.A. Miller, *J. Agric. Food Chem.*, **54**, 1151 (2006).
13. A. Kumaran and R.J. Karunakaran, *Food Sci. Technol.*, **40**, 344 (2007).
14. K. Robards, P.D. Prenzler, G. Tucker, P. Swatsitang and W. Glover, *Food Chem.*, **66**, 401 (1999).
15. G.H. Naik, K.I. Priyadarsini, J.G. Satav, M.M. Banavalikar, P.P. Sohoni, M.K. Biyani and H. Mohan, *Phytochemistry*, **63**, 97 (2003).
16. F.L. Hu, R.L. Lu, B. Huang and L. Ming, *Fitoterapia*, **75**, 14 (2004).