Postprint: Extraction Process and Antioxidant Activity of Total Tannins from Abutilon Leaves

Authors: Yang Jie(1); Chen Xiaoyun(1); Guo Yuru(1); Gao Xiang(1); Tian Chunlian(1); Liu Mingchun(1)

Date: 2018-12-24T00:00:00+00:00

Abstract

To investigate the extraction process and antioxidant activity of total tannins from Abutilon leaves, this study optimized the extraction process through single-factor experiments and response surface methodology, while simultaneously evaluating the in vitro antioxidant activity of total polyphenols, non-adsorbed polyphenols, and total tannins from Abutilon leaves using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) free radical scavenging assay, and ferric reducing antioxidant power (FRAP) assay. The results showed that the optimal extraction process for total tannins was: acetone concentration of 70%, liquid-to-material ratio (V/m) of 15.25:1.00, and extraction time of 20 min. Under these process conditions, the contents of total tannins in Abutilon leaves harvested in August, September, and October were (1.41±0.05)‰, (1.77±0.03)‰, and (1.49±0.03)‰, respectively. The total tannins from Abutilon leaves exhibited good in vitro antioxidant activity. This study established an efficient extraction process for total tannins from Abutilon leaves, and the extracted total tannins possessed good antioxidant activity.

Full Text

Preamble

A Research on Extraction Process and Antioxidant Activity of Total Tannins from Abutilon theophrasti Medic. Leaves

YANG Jie, CHEN Xiaoyun, GUO Yuru, GAO Xiang, TIAN Chunlian*, LIU Mingchun
College of Animal Husbandry and Veterinary Medicine, Shenyang Agricultural University, Shenyang 110866, China

Abstract: This study investigated the extraction process and antioxidant activity of total tannins from Abutilon theophrasti Medic. leaves. Single-factor experiments and response surface methodology were employed to optimize the extraction process, while the antioxidant activities of total polyphenols, non-adsorbed polyphenols, and total tannins were evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay, 2,2' -azinobis-(3-ethylbenzthiazoline-6-sulphonate) (ABTS) free radical scavenging assay, and ferric ion reducing antioxidant power (FRAP) analysis. The results demonstrated that the optimal extraction conditions were: acetone concentration 70%, liquid-to-material ratio (V/m) 15.25:1.00, and extraction time 20 min. Under these conditions, the total tannin contents in leaves harvested in August, September, and October were (1.41±0.05)‰, (1.77±0.03)‰, and (1.49±0.03)‰, respectively. The total tannins exhibited excellent in vitro antioxidant activity. This study establishes an efficient extraction process for total tannins from Abutilon theophrasti leaves and confirms their strong antioxidant potential.

Keywords: Abutilon theophrasti Medic. leaves; total tannins; extraction technology; response surface; antioxidant activity

Corresponding author: Lecturer, Master' s supervisor, E-mail: tianchunlian823@163.com

Introduction

Abutilon theophrasti Medic., belonging to the Malvaceae family, is a plant whose whole herb or leaves have been used in traditional medicine for clearing heat, promoting diuresis, detoxification, and restoring consciousness. It is commonly employed to treat dysentery, otitis media, tinnitus, deafness, suppurative tonsillitis, and carbuncles. The plant contains flavonoids, polyphenols, tannins, organic acids, and other chemical constituents. While numerous studies have focused on flavonoids and polyphenols from Abutilon theophrasti, research on tannins remains scarce. Tannin compounds exhibit excellent astringent properties and can be used to treat ulcers, diarrhea, wounds, and burns. Additionally, tannins possess hemostatic, antibacterial, anti-inflammatory, antiviral, antioxidant, antiallergic, antiparasitic, and blood pressure-lowering effects.

In recent years, with the intensification and scaling of animal husbandry, oxidative stress has become an increasingly serious threat to livestock and poultry health. Moreover, as reports of side effects from synthetic antioxidants continue to grow, natural antioxidants have attracted considerable attention from researchers. This study utilized ultrasonic-assisted extraction combined with single-factor and response surface experiments to investigate the extraction process of total tannins from Abutilon theophrasti leaves and evaluate their antioxidant activity, aiming to provide a foundation for the research and development of natural antioxidants.

Materials and Methods

1.1 Materials

Abutilon theophrasti leaves were collected from Tongyu County, Jilin Province. After collection, the leaves were cleaned, naturally dried in a shaded area, and stored for later use. Gallic acid standard was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (batch number: 110831-200302). Sodium tungstate was obtained from Tianjin Ruijinte Chemical Co., Ltd.; sodium molybdate and lithium sulfate were purchased from Tianjin Bodi Chemical Co., Ltd.; casein was obtained from Beijing Aoboxing Biotechnology Co., Ltd.; and other analytical reagents were purchased from Sinopharm Chemical Reagent Co., Ltd.

1.2 Methods

1.2.1 Sample Solution Preparation Precisely 0.5 g of powdered plant material was placed in a 100 mL Erlenmeyer flask, mixed with solvent, and extracted under various conditions using ultrasound. After cooling and standing, each sample was extracted twice. The filtrates from both extractions were combined, centrifuged, and concentrated to near dryness. The residue was dissolved in water and diluted to 10 mL.

1.2.2 Single-Factor Experiments Single-factor experiments were conducted to investigate the effects of extraction time, liquid-to-material ratio, and acetone concentration on the extraction yield of total tannins from Abutilon theophrasti leaves. Using the extraction yield as the evaluation index, three factors were examined at different levels: extraction time (10, 15, 20, 25, 30 min), liquid-to-material ratio (V/m) (10.00:1.00, 15.00:1.00, 20.00:1.00, 25.00:1.00, 30.00:1.00), and acetone concentration (20%, 40%, 60%, 80%, 100%). When examining the effect of any single factor, the other two factors were maintained at intermediate levels (extraction time 20 min, liquid-to-material ratio 20.00:1.00, acetone concentration 60%). Each experiment was performed in triplicate.

1.2.3 Response Surface Experiment Based on the single-factor experiments, Design-Expert 8.0 statistical software was used for response surface experimental design and analysis. According to response surface methodology principles, acetone concentration (A), liquid-to-material ratio (B), and extraction time (C) were selected as independent variables, with the extraction yield of total tannins from Abutilon theophrasti leaves as the response value.

1.2.4 Determination of Total Tannin Content 1.2.4.1 Preparation of Gallic Acid Standard Curve
Following the "Tannin Content Determination Method" in Appendix 135 of the *Veterinary Pharmacopoeia of the People' s Republic of China*, a 0.05 mg/mL

gallic acid standard solution was prepared. The standard curve of absorbance (A) versus concentration (c) was constructed, yielding the following regression equation:

$$A = 0.1709c + 0.0011 \quad (r = 0.9999)$$

The results indicated a good linear relationship within the range of 1–10 g/mL.

1.2.4.2 Tannin Content Determination

Following the "Tannin Content Determination Method" in Appendix 135 of the *Veterinary Pharmacopoeia of the People's Republic of China*, the total tannin content in Abutilon theophrasti leaves was calculated using the following formula:

$$\text{Total tannin content} = \text{Total phenol content} - \text{Non-adsorbed phenol content}$$

1.2.5 In Vitro Antioxidant Activity Tests 1.2.5.1 DPPH Free Radical Scavenging Assay

Based on the method of Tian et al. with appropriate modifications, 10, 20, 40, 80, and 100 L of sample solution were mixed with 900 L of 0.1 mmol/L DPPH working solution, then diluted with distilled water to a final volume of 1 mL. The mixture was incubated at 37 °C for 30 min in the dark, and the absorbance was measured at 517 nm (AS). For the control, 10, 20, 40, 80, and 100 L of sample solution were diluted with distilled water to 100 L, and absorbance was measured at 517 nm (A0). Additionally, 100 L of distilled water mixed with 900 L of DPPH working solution was incubated under the same conditions, and absorbance was measured at 517 nm (AC). Butylated hydroxytoluene (BHT) was used as a positive control. The scavenging rate was calculated using the following formula:

$$\text{Scavenging rate } (\%) = \left[1 - \frac{(AS - A0)}{AC}\right] \times 100$$

The DPPH concentration required to achieve 50% scavenging was defined as the IC50DPPH.

1.2.5.2 ABTS Free Radical Scavenging Assay

Based on the method of Tian et al. with appropriate modifications, 7 mmol/L ABTS solution was mixed with an equal volume of 4.9 mmol/L potassium persulfate solution and stored in the dark at room temperature for 12–16 h to generate ABTS stock solution. The following day, the stock solution was diluted with phosphate buffer to prepare ABTS working solution with an absorbance of 0.70 ± 0.02 at 734 nm. Precisely 10, 20, 40, 80, and 100 L of sample solution were mixed with 900 L of ABTS working solution and diluted with distilled water to

1 mL. After reacting in the dark at room temperature for 30 min, the absorbance was measured at 734 nm (AS). For the control, 10, 20, 40, 80, and 100 L of sample solution were diluted with distilled water to 100 L, and absorbance was measured at 734 nm (A0). Additionally, 100 L of distilled water mixed with 900 L of ABTS working solution was reacted under the same conditions, and absorbance was measured at 734 nm (AC). BHT was used as a positive control. The scavenging rate calculation method and formula were the same as in section 1.2.5.1. The ABTS concentration required to achieve 50% scavenging was defined as the IC50ABTS.

1.2.5.3 Ferric Ion Reducing Antioxidant Power (FRAP) Assay
Based on the method of Olszowy et al. with appropriate modifications, 150 L of FeSO4 solutions at concentrations of 0, 0.2, 0.4, 0.6, 0.8, and 1.0 mmol/L were mixed with 4.5 mL of FRAP reagent [prepared by mixing 300 mmol/L acetate buffer (pH 3.6), 10 mmol/L 2,4,6-tripyridyl-s-triazine (TPTZ) solution, and 20 mmol/L ferric chloride solution at a volume ratio of 10:1:1, freshly prepared before use]. The mixture was incubated in a 37 °C water bath for 10 min, and absorbance was measured at 593 nm. A standard curve was constructed with absorbance as the x-axis and FeSO4 concentration as the y-axis. Sample solutions were tested following the same procedure, replacing FeSO4 solution. The FRAP value was expressed as the amount of FeSO4 (in mmol) required to achieve the same absorbance per (g • mL) of sample. A higher FRAP value indicates stronger antioxidant activity.

1.3 Data Processing and Statistical Analysis

Response surface experimental data were analyzed using Design-Expert 8.0 software. Statistical analysis was performed using Analysis of Variance (ANOVA) in SPSS 17.0 software.

Results

2.1 Single-Factor Experiment Results

2.1.1 Effect of Acetone Concentration on Extraction Yield As shown in [Figure 1: see original paper]-A, the extraction yield of total tannins increased with increasing acetone concentration, reaching a maximum at 80% acetone concentration. Further increases in acetone concentration led to a decrease in extraction yield. Therefore, 80% acetone concentration was selected as the optimal level.

2.1.2 Effect of Liquid-to-Material Ratio on Extraction Yield As shown in [Figure 1: see original paper]-B, the extraction yield increased with increasing liquid-to-material ratio, reaching a maximum at a ratio of 15.00:1.00. Further increases in the ratio resulted in decreased extraction yield. Therefore, a liquid-to-material ratio of 15.00:1.00 was selected as appropriate.

2.1.3 Effect of Extraction Time on Extraction Yield As shown in [Figure 1: see original paper]-C, the extraction yield gradually increased with ultrasonic extraction time, reaching a maximum at 15 min. Beyond this point, further increases in extraction time led to decreased yield. Therefore, an extraction time of 15 min was selected as suitable.

Figure 1 Effects of acetone concentration (A), liquid-to-material ratio (B), and extraction time (C) on the yield of total tannins from Abutilon theophrasti Medic. leaves.

2.2 Response Surface Experiment Results

2.2.1 Response Surface Design and Experimental Results Based on single-factor experiments, Design-Expert 8.0 software was used to design and analyze the response surface experiment. According to response surface methodology principles, acetone concentration, liquid-to-material ratio, and extraction time were selected as independent variables, with the extraction yield of total tannins as the response value. The factors and levels are shown in , and the experimental design and response values generated by Design-Expert 8.0 are presented in .

Regression analysis of the experimental data in yielded the following quadratic polynomial regression equation for the extraction yield (Y):

$$Y(\%) = 1.07 - 0.15A + 0.022B + 0.040C - 0.005AB + 0.015AC + 0.01BC + 0.29A^2 - 0.38B^2 + 0.18C^2$$

where A is acetone concentration (%), B is liquid-to-material ratio (mL/g), and C is extraction time (min).

To evaluate model validity and factor effects, ANOVA was performed, with results shown in . The model was highly significant ($P < 0.0001$), indicating that the quadratic polynomial regression model effectively described the relationship between independent variables and the response function. The lack-of-fit term was not significant ($P = 0.0655$), demonstrating good agreement between the model and actual conditions, confirming the reliability and feasibility of this approach. Extraction time had an extremely significant effect on tannin extraction yield ($P < 0.01$), acetone concentration had a significant effect ($P < 0.01$), and liquid-to-material ratio had an extremely significant effect ($P < 0.01$). The interaction between acetone concentration and extraction time significantly affected extraction yield ($P < 0.05$). The coefficient of determination (R^2) was 0.9995, indicating high correlation between predicted and measured values, with only 0.11% of variation unexplained by the model [adjusted R^2 (R^2Adj) = 0.9989]. Other interaction terms showed poor significance, indicating that the relationship between experimental factors and response values was not simply linear but nonlinear.

Table 1 Design factors and levels of response surface experiment.
Table 2 Design and response values of response surface experiment.
Table 3 Analysis of variance for results of response surface experiments.

Response surface and contour plots were generated using Design-Expert 8.0 software. As shown in [Figure 2: see original paper], at a fixed extraction time, when acetone concentration remained constant, the extraction yield first increased then decreased with increasing liquid-to-material ratio, reaching a maximum at a ratio of 15.50:1.00. When the liquid-to-material ratio was fixed, extraction yield first decreased then increased with acetone concentration, reaching a maximum at 70% acetone. However, the interaction between acetone concentration and liquid-to-material ratio was not significant ($P > 0.05$).

As shown in [Figure 3: see original paper], at a fixed liquid-to-material ratio, when acetone concentration remained constant, extraction yield first decreased then increased with extraction time, reaching a maximum at 20 min. When extraction time was fixed, extraction yield first decreased then increased with acetone concentration, reaching a maximum at 72% acetone. However, due to relatively flat contour lines, the interaction between acetone concentration and extraction time showed low significance ($0.01 < P < 0.05$).

As shown in [Figure 4: see original paper], when acetone concentration was fixed, extraction yield first decreased then increased with extraction time at a constant liquid-to-material ratio, reaching a maximum at 20 min extraction time. When extraction time was fixed, extraction yield first increased then decreased with liquid-to-material ratio, reaching a maximum at a ratio of 15.00:1.00. However, the interaction between liquid-to-material ratio and extraction time was not significant ($P > 0.05$).

Figure 2 Interaction of acetone concentration and liquid-to-material ratio on the yield of total tannins from Abutilon theophrasti Medic. leaves.
Figure 3 Interaction of acetone concentration and extraction time on the yield of total tannins from Abutilon theophrasti Medic. leaves.
Figure 4 Interaction of liquid-to-material ratio and extraction time on the yield of total tannins from Abutilon theophrasti Medic. leaves.

2.3 Determination and Validation of Optimal Conditions

The optimal extraction conditions determined by Design-Expert 8.0 software were: acetone concentration 70%, liquid-to-material ratio 15.25:1.00, and extraction time 20 min, with a predicted extraction yield of 1.76‰. To verify the accuracy of this optimized protocol and improve experimental stability, validation experiments were performed in triplicate using these optimal conditions. The average extraction yield was 1.77‰, with a deviation of 0.568% between actual and predicted values, indicating good correlation and demonstrating that response surface methodology optimization was reasonable and feasible. Under these optimal conditions, the total tannin contents in leaves harvested in August, September, and October of the same year were (1.41±0.05)‰, (1.77±0.03)‰,

and (1.49±0.03)‰, respectively.

2.4 Antioxidant Activity Tests

The contents of total polyphenols, non-adsorbed polyphenols, and total tannins from Abutilon theophrasti leaves and their antioxidant activity results are shown in , , and , respectively. As shown in , the IC50ABTS values of total tannins from leaves harvested in August, September, and October were 0.84, 0.51, and 0.35 g/mL, respectively, all lower than that of the BHT positive control (2.25 g/mL). Additionally, DPPH scavenging and FRAP assays showed that IC50DPPH values for all three harvest months were lower than the BHT control, while FRAP values were higher than the BHT control. Furthermore, both total polyphenols and non-adsorbed polyphenols exhibited satisfactory antioxidant activity (Tables 4 and 5). These results suggest that total polyphenols, non-adsorbed polyphenols, and total tannins from Abutilon theophrasti leaves possess good antioxidant activity, with variations depending on harvest time, likely related to differences in chemical composition types and contents that require further investigation.

Table 4 Yield and results of antioxidant test of total polyphenol extract (TPE) from Abutilon theophrasti Medic. leaves.
Table 5 Yield and results of antioxidant test of non-adsorbed polyphenols (NAP) from Abutilon theophrasti Medic. leaves.
Table 6 Yield and results of antioxidant test of total tannin extract (TTE) from Abutilon theophrasti Medic. leaves.

2.5.1 Correlation Analysis

As shown in , the content of total polyphenols in Abutilon theophrasti leaves showed significant correlation with antioxidant activity measured by the DPPH method ($P < 0.05$). As shown in , the content of non-adsorbed polyphenols showed extremely significant correlation with antioxidant activity measured by the FRAP method ($P < 0.01$). As shown in , the correlation between total tannin extract content and antioxidant activity measured by different methods ranked as FRAP > ABTS > DPPH, while the correlation between total tannin content and antioxidant activity ranked as DPPH > ABTS > FRAP. These results suggest that different compound types exhibit different antioxidant activities and varying capacities for scavenging different free radicals or reducing power. Therefore, to objectively and accurately evaluate antioxidant activity, multiple antioxidant assays should be employed simultaneously.

Table 7 Correlation between total polyphenol content in Abutilon theophrasti Medic. leaves and antioxidant activity determined by different methods.
Table 8 Correlation between non-adsorbed polyphenols content in Abutilon theophrasti Medic. leaves and antioxidant activity determined by different methods.
Table 9 Correlation between total tannins content in Abutilon theophrasti

Medic. leaves and antioxidant activity determined by different methods.

Discussion

3.1 Optimization of Total Tannin Extraction Process

Tannin compounds contain numerous hydroxyl groups in their structure, resulting in poor stability and large molecular weights, which present certain difficulties for extraction and separation. Therefore, optimizing the extraction process is crucial for practical production applications. This study employed ultrasonic extraction combined with single-factor and response surface experiments to optimize the extraction process for total tannins from Abutilon theophrasti leaves, successfully establishing optimal extraction conditions.

3.2 In Vitro Antioxidant Activity of Total Tannins

Due to their abundant phenolic hydroxyl groups, tannins exhibit excellent free radical scavenging capabilities. This study evaluated the in vitro antioxidant activity of total polyphenols, non-adsorbed polyphenols, and total tannins from Abutilon theophrasti leaves using DPPH, ABTS, and FRAP assays, with all three fractions demonstrating strong antioxidant activity.

3.3 Relationship Between Harvest Time and Tannin Content/Antioxidant Activity

Research has shown that harvest time directly affects the yield, quality, and extraction rate of active compounds in medicinal plants. This study extracted total tannins from Abutilon theophrasti leaves harvested in different months of the same year and evaluated their in vitro antioxidant activity. The results revealed that total tannin content in leaves harvested in different months followed the order: September > October > August. ABTS and FRAP assays indicated that antioxidant activity gradually increased from August to October, while DPPH assays showed the order: September > October > August. Therefore, the optimal harvest time should be selected based on specific applications.

Conclusion

1. The optimal extraction conditions for total tannins from Abutilon theophrasti leaves were established as: acetone concentration 70%, liquid-to-material ratio (V/m) 15.25:1.00, and extraction time 20 min. Under these conditions, the total tannin contents in leaves harvested in August, September, and October were (1.41±0.05)‰, (1.77±0.03)‰, and (1.49±0.03)‰, respectively.
2. In vitro antioxidant assays including DPPH free radical scavenging, ABTS free radical scavenging, and FRAP analysis demonstrated that total tannins from Abutilon theophrasti leaves possess excellent antioxidant activity.

References

[1] Shi KM, Li CY, Li C, et al. Research progress on chemical constituents of Abutilon theophrasti [J]. Heilongjiang Medicine, 2015, 28(2): 223-227.

[2] Mao SJ. Overview of pharmacological research on tannin components in traditional Chinese medicine [J]. Chinese Traditional Patent Medicine Research, 1985(5): 22-24.

[3] Olchowik-Grabarek E, Makarova K, Mavlyanov S, et al. Comparative analysis of BPA and HQ toxic impacts on human erythrocytes, protective effect mechanism of tannins (Rhus typhina) [J]. Environmental Science and Pollution Research, 2017, 25(2): 1200-1209.

[4] Liu HW, Li K, Zhao JS, et al. Effects of chestnut tannins on intestinal morphology, barrier function, pro-inflammatory cytokine expression, microflora and antioxidant capacity [J]. Journal of Animal Physiology and Animal Nutrition, 2017, doi:10.1111/jpn.12839.

[5] Guo YJ, Zhao L, Li XF, et al. Effect of corilagin on anti-inflammation in HSV-1 encephalitis and HSV-1 infected microglias [J]. European Journal of Pharmacology, 2010, 635(1/2/3): 79-86.

[6] Hau DK, Zhu GY, Leung AK, et al. In vivo anti-tumour activity of corilagin on Hep3B hepatocellular carcinoma [J]. Phytomedicine, 2010, 18(1): 11-15.

[7] Kinoshita S, Inoue Y, Nakama S, et al. Antioxidant and hepatoprotective actions of medicinal Terminalia catappa from Okinawa island tannin corilagin [J]. Phytomedicine, 2007, 14(11): 755-762.

[8] Chen YY, Chen CH. Research progress on pharmacological activities of corilagin [J]. Chinese Journal of Modern Applied Pharmacy, 2010, 27(5): 390-394.

[9] Yu ZY, Jin ZX. Research progress on antioxidant effects of tannins [J]. Heilongjiang Medicine, 2014, 27(1): 43-46.

[10] Wu NY, Guo XY, Chen JP, et al. Extraction and content determination of tannins from Potentilla glabra branches and leaves [J]. Journal of Inner Mongolia Medical University, 2014, 36(1): 35-38, 43.

[11] Chinese Veterinary Pharmacopoeia Commission. Veterinary Pharmacopoeia of the People' s Republic of China: Part 2 [M]. Beijing: China Agriculture Press, 2015.

[12] Tian CL, Zhang DX, Yang CX, et al. Research on extraction technology, antibacterial and antioxidant activity of ethanol extract from leaves of Abutilon theophrasti medic [J]. Acta Poloniae Pharmaceutica: Drug Research, 2017, 74(3): 881-890.

[13] Olszowy M, Dawidowicz AL. Essential oils as antioxidants: their evaluation by DPPH, ABTS, FRAP, CUPRAC, and -carotene bleaching methods [J].

Monatshefte für Chemie: Chemical Monthly, 2016, 147(12): 2083-2091.

[14] Xing JJ, Cao TT, Yang F, et al. Research progress on tannin compounds [J]. Heilongjiang Medicine, 2011, 24(5): 776-780.

[15] Chang CH, Chiu HF, Han YC, et al. Photoprotective effects of cranberry juice and its various fractions against blue light-induced impairment in human retinal pigment epithelial cells [J]. Pharmaceutical Biology, 2017, 55(1): 571-580.

[16] Chen YF, Li HQ, Zhang S, et al. Anti-myocardial ischemia effect and components of litchi pericarp extracts [J]. Phytotherapy Research, 2017, 31(9): 1384-1391.

[17] Adamczyk B, Simon J, Kitunen V, et al. Tannins and their complex interaction with different organic nitrogen compounds enzymes: old paradigms versus recent advances [J]. ChemistryOpen, 2017, 6(5): 610-614.

[18] Wei Z. Study on dynamic changes of main active components in Citrus peel at different growth stages [D]. Master' s thesis. Chengdu: Chengdu University of Traditional Chinese Medicine, 2013.

Note: Figure translations are in progress. See original paper for figures.