Regular article

Comprehensive analysis of phenolic composition and antioxidant mechanisms in *Gymnema sylvestre* extracts using LC-MS and column chromatography

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Abstract

Recent studies have highlighted the potential of plant extracts as therapeutic agents for managing oxidative stress and related disorders. This study aims to elucidate the phenolic composition and antioxidant properties of *Gymnema sylvestre* extracts. Ethanolic reflux extraction followed by column chromatography was employed to isolate phenolic compounds. The total phenolic and flavonoid contents were quantified using the Folin–Ciocalteu and aluminum chloride colorimetric methods, respectively. Antioxidant activities were assessed by DPPH, ABTS scavenging assays and the ferric reducing antioxidant power (FRAP) assay. High-Performance Liquid Chromatography (HPLC) with a C_{18} column and Thermo TSQ Quantum Access Max (LC-MS) were used to determine the levels of gymnemic acid and identify other potential phenolic compounds. The analysis revealed significant antioxidant activities in the fractions. Fraction A showed the highest DPPH and ABTS scavenging activities, and Fraction C demonstrated the highest ferric reducing power. LC-MS analysis identified several phenolic compounds, indicating that these are major contributors to the antioxidant efficacy of the extract. This study provides a detailed phenolic profile and confirms the strong antioxidant potential of *Gymnema sylvestre* leaf extract, supporting its therapeutic use and further investigation.

Keywords: *Gymnema sylvestre*; antioxidants; column chromatography; phenolic compounds; LC-MS

1 Introduction

Gymnema sylvestre (Retz.) Shcult, a perennial woody vine native to tropical Asia, is not only the cornerstone of traditional Indian Ayurvedic medicine, but also a valued herb in traditional Chinese medicine, especially widely planted in southern provinces of China [1]. Known for its ability to mask sweet tastes and control glucose levels, this plant has been the subject of extensive research, especially on the hypoglycemic mechanisms of gymnemic acid [2]. However, there is still a significant gap in the comprehensive analysis of other components, especially the phenolic structures and their

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functional roles in mitigating oxidative stress and other metabolic disorders [3].

Phenolic compounds are widely present as secondary metabolites in the plant kingdom, characterized by structures where one or more hydroxyl groups are attached to an aromatic ring. Phenolic compounds can be categorized into flavonoid and non-flavonoid compounds [4]. Among them, flavonoid phenolic compounds are distinguished by two benzene rings (A ring, B ring) connected by a three-carbon chain, forming various natural phenolic compounds with different degrees of oxidation and substitutions, including hydroxyl groups, methoxy groups and glycosides. These modifications result in a diverse array of flavonoid phenolic compounds that can be primarily divided into six categories, namely flavanones, flavonols, flavan-3-ols, anthocyanidins, isoflavones and others [5].

The latest development of analytical techniques, such as *Thermo* TSQ Quantum Access Max (LC-MS), is helpful to further explore the phenolic structures in *Gymnema sylvestre* extracts. This study aims to elucidate these structures and their implications in neutralizing free radicals and reducing oxidative damage. By combining LC-MS with column chromatography, we tried to establish a comprehensive phenolic profile of *Gymnema sylvestre* and relate these phenolic constituents with their antioxidant efficacy [6].

The exploration of phenolic compounds extends beyond *Gymnema sylvestre*, touching various facets of traditional Chinese medicine [7]. Research topics usually focus on identifying bioactive compounds, understanding their mechanisms and applying these findings to develop therapeutic strategies for chronic diseases, such as diabetes, obesity and cardiovascular disorders. Given the variety and effective bioactive compounds in this plant, especially phenolic acids and flavonoids, it is very important to understand their specific contribution to its overall antioxidant capacity [8].

The focus of this study is to identify these phenolic compounds, quantify their concentrations, and evaluate their synergistic effects in scavenging free radicals [9]. The integration of these findings will contribute significantly to the existing knowledge system and may lead to the development of novel antioxidant therapies derived from this traditional herb and other related medicinal plants [10].

2 Materials and methods

2.1 Plant material

Gymnema sylvestre were purchased from the Kangmei Medicinal Herbs Market in Bozhou, Anhui, China, and are native to Guangxi Province, China. The plant material was authenticated by Professor Hu Guangwan of the Wuhan Botanical Garden, Chinese Academy of Sciences as belonging to the dried above-ground part of the *Gymnema sylvestre* (Retz.) Shcult in the family Asclepiadaceae [11].

2.2 Chemicals and reagents

Methanol, acetonitrile, acetic acid, formic acid and ammonium acetate of HPLC grade were purchased from China National Pharmaceutical Group (Sinopharm), Shanghai, China. Ultra-pure water was prepared using a Millipore Sigma water purification system. Folin-Ciocalteu reagent, DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) were of analytical grade and obtained from Vorradex Chemicals, Fuzhou, China. TPTZ (2,4,6-tripyridyl-s-triazine) and other chemicals were obtained from Aladdin Reagent Company, Shanghai, China [12].

2.3 Extraction of Gymnema sylvestre phenolic compounds

Approximately 100 g of dried *Gymnema*

sylvestre were finely ground. The powdered material underwent solvent extraction using 70% methanol (*v*/*v*) in water. This process was conducted at the controlled temperature of 70 °C for 4 h to ensure optimal extraction efficiency. After extraction, the mixture was filtered through Whatman No.1 filter paper, and the filtrate was concentrated under reduced pressure at 40 °C using a rotary evaporator [13].

2.4 Determination of total phenolic content

The total phenolic content in the *Gymnema sylvestre* extract was determined using the Folin-Ciocalteu method adapted from Singleton et al [14]. The measurement was performed at 765 nm with a UV-Visible spectrophotometer (Shimadzu, Japan), using gallic acid as the standard. The results were expressed in milligrams of gallic acid equivalents (GAE) per gram of dry extract. The standard curve for the determination of total phenolic content was constructed using gallic acid standards in the range of 0.01-0.25 mg/mL. The equation of the standard curve was $Y = 49.9939X + 01051$ ($R^2 = 0.9991$), indicating a strong linear relationship.

2.5 Antioxidant activity assays

2.5.1 DPPH radical scavenging activity

The antioxidant capacity of the extracts was evaluated by their ability to scavenge DPPH radicals. Different concentrations of the extract were mixed with a methanol solution of DPPH. After 30 min of incubation at room temperature in the dark, the decrease in absorbance was measured at 517 nm. The percentage of DPPH radical scavenging was calculated to assess the antioxidant effectiveness [15].

2.5.2 ABTS radical scavenging assay

The ABTS radical cation decolorization assay was performed following the method developed by Re et al, with minor modifications. $ABTS^{\bullet+}$ was produced by reacting the ABTS stock solution with potassium persulfate. After 12-16 h of standing in the dark at room temperature, the extract was added to $ABTS^{\bullet+}$ solution, and the absorbance was recorded at 734 nm after 6 min of reaction. The results were expressed by Trolox equivalent antioxidant capacity (TEAC) [16].

2.5.3 Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was conducted according to the procedure outlined by Benzie and Strain, with slight modifications. The FRAP reagent, prepared fresh and warmed to 37 °C before use, included acetate buffer, TPTZ solution and ferric chloride hexahydrate. The absorbance of the reaction mixture, which combined the extract with FRAP reagent, was recorded at 593 nm. The results were quantified as micromoles of ferrous equivalent (Fe(II)) per gram of extract, indicating the reducing ability of the antioxidant components [17].

2.6 LC-MS analysis of phenolic compounds

Phenolic profiling was conducted using an Agilent 1220 Infinity II LC system coupled with a Thermo TSQ Quantum Access Max triple quadrupole mass spectrometer. Chromatographic separation was achieved on a Zorbax Eclipse Plus C_{18} column (2.1 mm × 100 mm, 1.8 μm) maintained at 40 °C. The mobile phase consisted of 0.1% formic acid in water (A) and acetonitrile (B). The flow rate was set at 0.3 mL/min. The elution profile started at 5% B, increased to 95% B over 30 min, and then returned to the initial condition. The mass spectrometer was operated in MRM mode for targeted analysis, with both positive and negative ionization modes used to enhance the detection of phenolic compounds [18].

2.7 Experimental site

All experiments were conducted at the Wuhan Botanical Garden, Chinese Academy of Sciences, providing a controlled environment to ensure the consistency and reliability of the experimental results.

2.8 Statistical analysis

To robustly analyze the data, we first assessed the normality of distributions using the Shapiro-Wilk test and checked the homogeneity of variances with Levene's test. Subsequent analysis of variance (ANOVA) identified significant differences, with post-hoc comparisons using the Tukey Honest Significant Difference test for normally distributed data, while the Kruskal-Wallis and Mann-Whitney U tests were applied for non-parametric data, both adjusted for multiple comparisons using the Benjamini-Hochberg False Discovery Rate procedure. Pearson's correlation and simple linear regression analyses were used to examine the relationship between phenolic content and antioxidant activities. All analyses were conducted using SPSS version 25 and R software, with statistical significance set at $P < 0.05$.

3 Results

3.1 Compound content and extraction yield

Preliminary screening of the ethanol reflux extract using Thin Layer Chromatography (TLC) revealed the presence of active compounds in the *Gymnema sylvestre* extract. The active components were separated on a TLC plate and compared against the R_f values of known standards, including Quercetin, Gallic Acid, Caffeine and Trolox. This step was essential to identify and characterize the phytochemical profile of the extract [19].

Using silica gel as the stationary phase and the optimal TLC solvent system as the mobile phase, the crude extract was analyzed by column chromatography and 12 different fractions were obtained. These fractions were further analyzed using a UV spectrophotometer, and three pooled fractions were obtained based on their UV absorbance values:

Fraction A: Composed of fractions 1 to 4 from column chromatography.

Fraction B: Composed of fractions 5 to 10.

Fraction C: Composed of fractions 11 and 12.

Subsequent analysis of these pooled fractions revealed different levels of phenolic and flavonoid contents:

Fraction A demonstrated the highest total phenolic content at 0.294 ± 0.056 mg QE/mL. The flavonoid content was 0.274 ± 0.050 mg QE/mL, and the extraction yield was 12.60%.

Fraction B demonstrated the lowest total phenolic content at 0.085 ± 0.039 mg QE/mL. The flavonoid content was 0.309 ± 0.110 mg QE/mL. Remarkably, this fraction achieved the highest extraction yield at 39.93%.

Fraction C had a phenolic content of 0.227 ± 0.041 mg QE/mL and the highest flavonoid content among the fractions at 0.384 ± 0.007 mg QE/mL. Its yield was 11.41%.

Table 1 Total phenolic and flavonoid content and yield of the *G. inodorum* leaf extract fraction

Samples	Total Phenolic Content (mg QE/mL)	Total Flavonoid Content (mg QE/mL)	% Yield (w/w)	Positive Drug (Trolox) (mg TE/mL)
Fraction A	0.294 ± 0.056	0.274 ± 0.050	12.60	95.45 ± 0.89
Fraction B	0.085 ± 0.039	0.309 ± 0.110	39.93	93.46 ± 1.43
Fraction C	0.227 ± 0.041	0.384 ± 0.007	11.41	87.29 ± 0.45
Trolox (Positive Control)	N/A	N/A	N/A	98.15 ± 0.85

Note: Each Value represents the mean ± SEM (n = 3).

The antioxidant activity of three fractions derived from the leaf extract of *G. inodorum* was assessed using DPPH, ABTS and FRAP assays. In the DPPH assay, fraction A demonstrated the highest antioxidant activity (71.25 ± 3.15 mg TE/mL), followed by fraction B (31.57 ± 6.42 mg TE/mL), and fraction C exhibited the lowest activity (27.92 ± 3.64 mg TE/mL). Similarly, in the ABTS assay, fraction A showed the highest radical scavenging activity (95.45 ± 0.89 mg TE/mL), closely followed by fraction B (93.46 ± 1.43 mg TE/mL), with fraction C displaying comparable activity (87.29 ± 0.45 mg TE/mL). In contrast, the FRAP assay results indicated that fraction C exhibited the highest reducing activity (2.97 ± 0.25 mg TE/mL), while fractions B and A showed significantly lower ferric ion reducing power (2.65 ± 0.26 mg TE/mL and 0.85 ± 0.14 mg TE/mL, respectively) (Fig. 1).

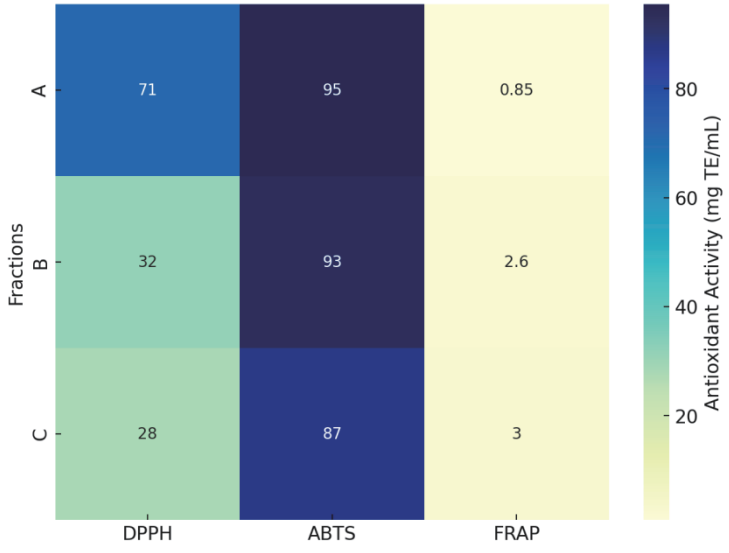


Fig. 1 The heatmap represents the antioxidant activity of the three fractions derived from the leaf extract of *G. inodorum* across the DPPH, ABTS and FRAP assays. The heatmap provides a visual comparison of the antioxidant activities, with the color intensity indicating the magnitude of activity in mg TE/mL

The three fractions were analyzed by HPLC with C_{18} column to quantify the Gymnemic acid level. Fraction C showed the highest gymnemic acid concentration at 0.393 ± 0.003 mg/g, followed by fractions B and A at 0.159 ± 0.003 mg/g and 0.142 ± 0.000 mg/g, respectively (Table 2). HPLC-C_{18} chromatogram in Fig. 2 shows the extract's standard and gymnemic acid peaks.

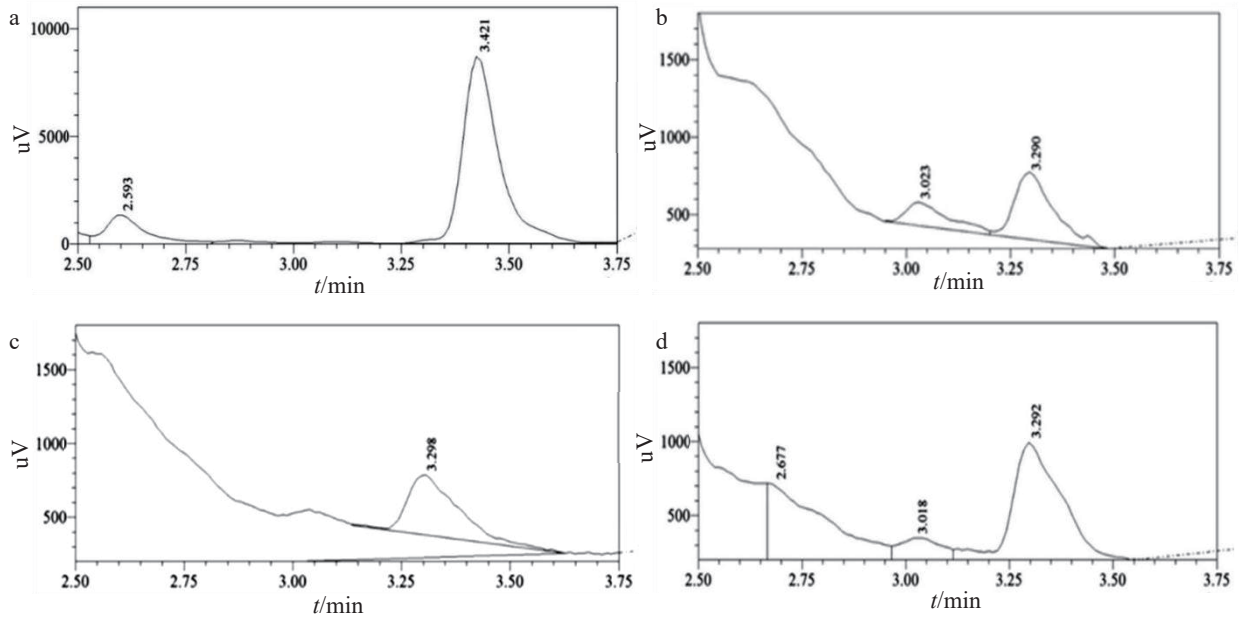


Fig. 2 HPLC-UV chromatogram of gymnemic acid. (a) gymnemic acid standard (100 μg/mL); (b) gymnemic acid in fraction A; (c) gymnemic acid in fraction B; (d) gymnemic acid in fraction C

Table 2 Gymnemic acid concentration in fraction of *G. inodorum* leaf extract

Fractions	Gymnemic Acid (mg/g)
Fraction A	0.142 ± 0.000
Fraction B	0.159 ± 0.003
Fraction C	0.393 ± 0.003

Note: Each value represents the mean ± SD (n = 3).

Phenolic compounds were identified in fraction of *G. inodorum* leaf reflux extract using the specified LC-MS system. The identification involved targeted analysis and database search through EMBL-EBI and ChemSpider by the Royal Society of Chemistry to authenticate the compounds.

Fraction A revealed several phenolic compounds, including Bufotalinin, Erysothiopine, Schisanhenol B, Zhebeiresinol, Kanzonol P, 8-[(2*Z*)-3,6-Dimethyl-2-hepten-1-yl]-5,7-dihydroxy-2-(4-hydroxyphenyl)2,3-dihydro-4H-chromen-4-one and Toyocamycin. Additional antioxidants such as fatty acids and drugs were also detected.

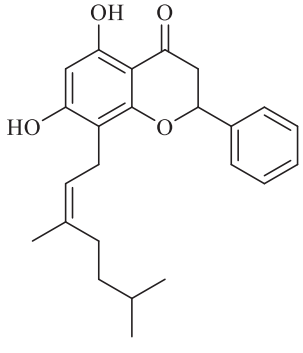
Fraction B of *G. inodorum* leaf reflux extract contained Erysothiopine, Schisanhenol B, Kanzonol P, Fluocinolone, Bufotalinin, Chamuvaritin and Tomentolide A as active phenolic compounds.

Fraction C, from the 6-h reflux extract of *G. inodorum* leaf, included 2,2-Dimethyl-3,4-bis(4-methoxyphenyl)-2H-1-benzopyran-7-ol acetate, Clausarinol, Psilostachyin and Eudesmin.

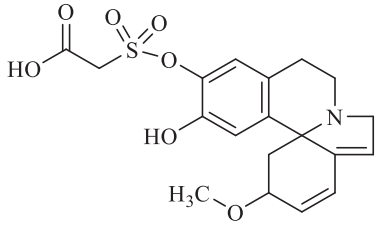
The catalog of these phenolic compounds is shown in Table 3. Fig. 3 shows the chromatograms from the LC-MS analysis of these compounds.

Table 3 Identification of phenolic compounds in *Gymnema sylvestre* extract fractions using LC-MS

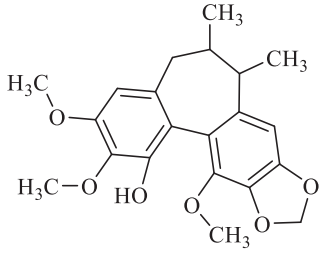
Sample	Peak NO	RT	Mass	$[M+H]^+$	Mass Diff (db/ppm)	Matching Score/%	Formula	Tentative Phenolic Compound
Fraction A	1	15.48	414.20	415.2123	1.01	97.57%	$C_{24}H_{30}O_6$	Bufotalinin
	2	13.65	407.10	425.1368	18.03	96.67%	$C_{19}H_{21}NO_7S$	Erysothiopine
	3	14.11	386.17	387.1805	1.01	95.54%	$C_{22}H_{26}O_6$	Schisanhenol B
	4	13.65	280.09	281.1021	1.01	85.66%	$C_{14}H_{16}O_6$	Zhebeiresinol
	5	13.66	368.16	369.1698	1.00	83.60%	$C_{22}H_{24}O_5$	Kanzonol P
	6	15.48	396.19	397.2010	1.01	82.43%	$C_{24}H_{28}O_5$	8-[(2*Z*)-3,6-Dimethyl-2-hepten-1-yl]-5,7-dihydroxy-2-(4-hydroxyphenyl)-2,3-dihydro-4H-chromen-4-one
	7	13.47	291.09	309.1315	18.04	82.22%	$C_{12}H_{13}N_5O_4$	Toyocamycin
Fraction B	1	13.65	407.10	425.1368	18.03	96.67%	$C_{19}H_{21}NO_7S$	Erysothiopine
	2	14.03	386.17	387.1812	1.01	94.48%	$C_{22}H_{26}O_6$	Schisanhenol B
	3	13.66	368.16	369.1698	1.00	83.60%	$C_{22}H_{24}O_5$	Kanzonol P
	4	14.03	412.17	413.1777	1.00	82.57%	$C_{21}H_{26}F_2O_6$	Fluocinolone
	5	15.48	414.20	415.2123	1.01	81.92%	$C_{24}H_{30}O_6$	Bufotalinin
	6	15.85	452.16	453.1682	1.00	80.18%	$C_{29}H_{24}O_5$	Chamuvaritin
	7	14.49	402.14	425.1369	22.99	79.90%	$C_{25}H_{22}O_5$	Tomentolide A
Fraction C	1	15.87	414.20	437.1931	22.99	98.00%	$C_{24}H_{30}O_6$	Clausarinol
	2	14.06	386.17	409.1627	22.99	91.51%	$C_2H_{26}O_6$	Eudesmin
	3	15.87	430.17	453.1669	22.99	87.85%	$C_{27}H_{26}O_5$	2,2-Dimethyl-3,4-bis(4-methoxyphenyl)-2H-1-benzopyran-7-ol acetate
	4	15.48	280.13	281.1388	1.00	85.47%	$C_{15}H_{20}O_5$	dihydroxygrosheimin



Kanzonol P



Erysothiopine



Schisanhenol B

Fig. 3 Phenolic compounds LC-MS analysis in fraction of *G. inodorum* extract, operated under the following parameters: ionization mode [positive], ionization source [Dual AJS ESI] and collision energy. Mass measurements were conducted with high resolution and accuracy 1.3 Da, 3ppm respectively, ensuring precise determination of molecular masses

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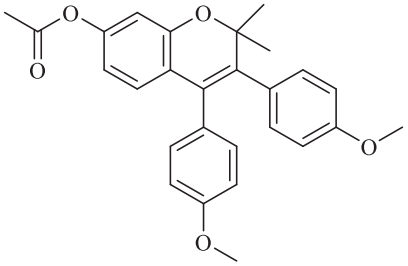
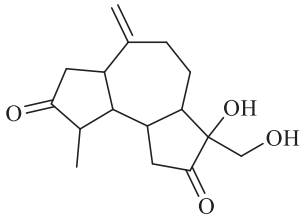
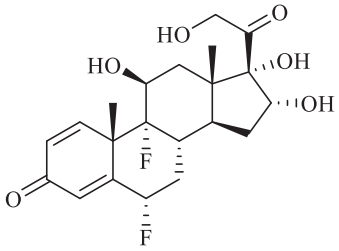


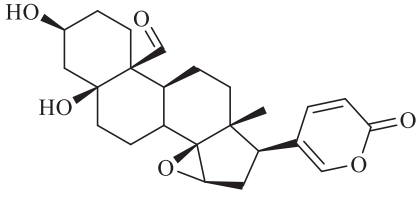
Clausarinol



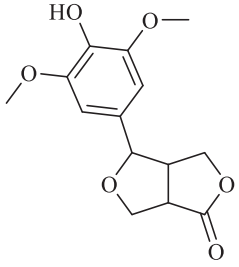
benzopyran-7-ol acetate



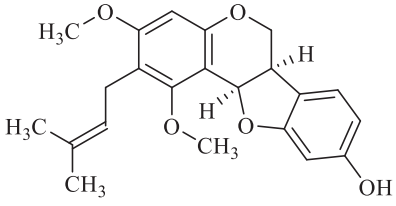
Fluocinolone



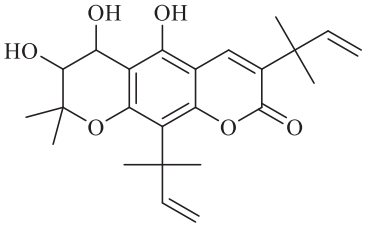
(2Z)-3,6-Dimethyl-2-hepten-1-yl



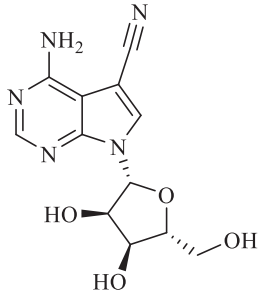
Zhebeiresinol



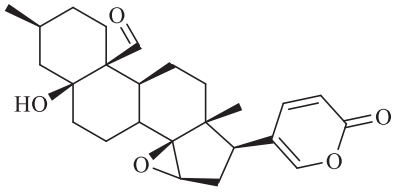
Toyocamycin



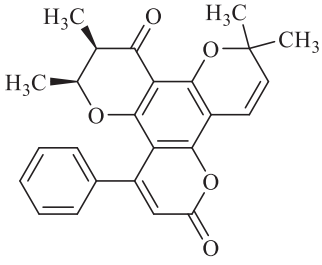
Eudesmin



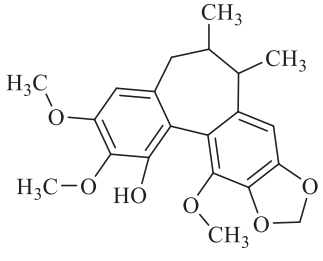
Bufotalinin



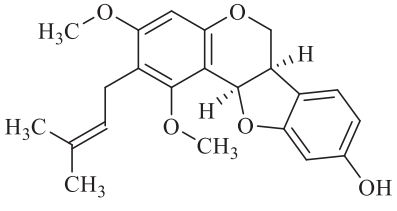
Tomentolide A



Schisanhenol B



Kanzonol P



Bufotalinin

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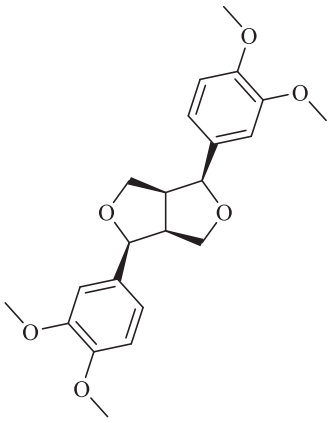
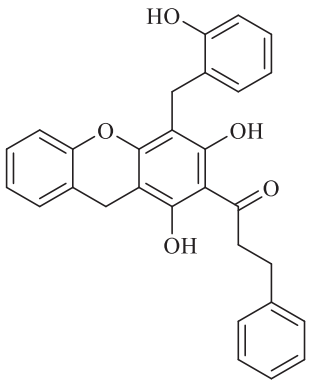


dihydroxygroshemin



Chamuvaritin

Continued fig. 3

4 Discussion

This study explored various methods to determine antioxidant potential, screen phenolic compounds, and analyze gymnemic acid levels by compound extraction techniques. Both column chromatography and thin-layer chromatography (TLC) were used to detect and separate active compounds in the extract. The results indicate that column chromatography is highly effective for isolating active compounds from the extract. Interestingly, the preliminary TLC results were consistent with column separation, which is helpful to select the best solvent for fraction separation [20].

Apocynaceae family has attracted great interest because of its phenolic compounds, which exhibit physiological functions such as scavenging free radicals, preventing mutations, inhibiting carcinogenesis and reducing inflammation [21]. The antioxidant efficacy of phenolic compounds mainly comes from their redox properties, enabling them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators. Our study detected a significantly higher concentration of phenolic compounds in the column-separated fractions of *G. inodorum* leaf extract than in the crude extract. This finding provides a scientific basis for the extensive application of this plant in traditional medicine and demonstrates its high antioxidant activity [22]. DPPH assay revealed enhanced radical scavenging activity in the column-separated fractions, suggesting an increased ability of the purified compounds to donate hydrogen ions. Fraction A exhibited the highest DPPH and ABTS radical scavenging activity, probably because it is rich in purified phytochemicals with hydrogen-reducing ability. Conversely, fractions B and C showed lower radical scavenging capacity, which may be due to factors such as light affinity, temperature and solubility of the extracts in the solvent system [23].

ABTS assay indicated that fraction A had high $ABTS^{+}$ radical scavenging activity, comparable to the Trolox standard at a 1 mg/mL concentration, underscoring the potential presence of specific phenolic compounds with therapeutic potential. FRAP assay, which measures antioxidant capacity by reducing ferric to ferrous ion, showed that fraction C had the highest antioxidant activity, which is different from the DPPH and ABTS results. This shows that the antioxidant compounds in fraction C are more favorable for FRAP assay. Quantification of gymnemic acid levels in the fractions revealed concentrations of 0.1417 ± 0.000 mg/g, 0.1591 ±

0.003 mg/g and 0.3933 ± 0.003 mg/g for fractions A, B, and C, respectively, surpassing previously recorded values in crude *G. inodorum* extract. This supports the efficacy of the column chromatography method in separating active compounds [24].

Overall, the study demonstrates that *G. inodorum* leaf extract fractions contain high concentrations of phenols and flavonoids, which are responsible for the observed antioxidant activity. The correlation between antioxidant activity and total phenolic content is consistent with previous reports. Both DPPH and FRAP assays indicated a high antioxidant capacity in fractions with high total phenolic content and gymnemic acid levels. LC-MS analysis supported the view that fractions with higher phenolic content exhibited greater antioxidant activity. This shows the relationship between phenolic compounds and bioactivity, particularly in scavenging free radicals. The presence of active phenolic compounds, such as Schisanhenol B and Zhebeiresinol known for their antioxidant and anti-inflammatory properties, further confirmed these findings. Additionally, compounds like Fluocinolone, Eudesmin, Clausarinol, Chamuvaritin and Tomentolide A, identified in the extract, showed potential health benefits [25].

The high concentration of phenolic compounds in fraction A, particularly Schisanhenol B, may explain its superior DPPH and ABTS radical scavenging activities observed in this study. These results suggest that phenolic compounds with high hydrogen-donating abilities significantly contribute to the antioxidant capacity of the extracts. Fraction B's antioxidant activity, attributed to compounds such as Bufotalinin and Chamuvaritin, demonstrates moderate radical scavenging capabilities. Bufotalinin is known for its cytoprotective effects against oxidative stress, while Chamuvaritin exhibits strong free radical scavenging and anti-inflammatory properties. Fraction C, with compounds like Eudesmin and Clausarinol, showed the highest antioxidant activity in FRAP assay, indicating their potential in reducing ferric ions to ferrous ions, which is the key aspect of their antioxidant mechanism. Eudesmin, in particular, has been studied for its neuroprotective effects against oxidative stress-induced neuronal damage, suggesting its potential in the management of neurodegenerative disease.

In conclusion, this study underscores the significant antioxidant potential of phenolic compounds in *G. inodorum* leaf extract. The findings provide scientific basis for the traditional use of the plant in the treatment of various diseases such as diabetes, hyperglycemia and premature aging. Further *in vivo* studies and exploration of different antioxidant mechanisms are needed to fully understand the therapeutic potential of these extracts[26].

5 Conclusion

In this study, the phenolic composition and antioxidant mechanisms of *Gymnema sylvestre* extracts were elucidated using LC-MS and column chromatography. The findings demonstrate that column chromatography is an effective method to separate active compounds, as supported by preliminary TLC results for solvent selection. The content of phenolic compounds in the column-separated fractions was significantly higher than in crude extracts, and the phenolic compounds showed high antioxidant activity which is consistent with traditional medicinal use [27].

DPPH and ABTS assays revealed fraction A's superior radical scavenging activity attributed to its rich purified phytochemicals, while the FRAP assay showed fraction C's highest antioxidant activity, indicating different antioxidative mechanisms. Quantification of gymnemic acid level revealed its higher concentration in the fractions than in crude extracts, validating the effectiveness of the column chromatography. LC-MS analysis identified phenolic compounds such as Schisanhenol B and

Zhebeiresinol, known for their antioxidant and anti-inflammatory properties, along with other beneficial compounds like Fluocinolone, Eudesmin, Clausarinol, Chamuvaritin and Tomentolide A [28].

Significance and future directions

In this study, the direct correlation between total phenolic content and antioxidant activity was established, and the therapeutic potential of *Gymnema sylvestre* extracts in the treatment of diseases like diabetes, hyperglycemia and premature aging is emphasized. These findings support the traditional medicinal use of the plant, and call for further *in vivo* studies and exploration of different antioxidant mechanisms to fully harness its therapeutic benefits.

Future research should focus on:

Mechanistic studies: Conducting *in vivo* studies to explore the detailed mechanisms of these phenolic compounds to exert their antioxidant effects and their potential interactions with other bioactive compounds.

Therapeutic applications: Investigating the therapeutic efficacy of these phenolic compounds in clinical settings, particularly in controlling chronic diseases such as diabetes, cardiovascular diseases and neurodegenerative disorders.

Synergistic effects: Exploring the synergistic effects of different phenolic compounds in *Gymnema sylvestre* to develop more effective antioxidant therapies.

Bioavailability and metabolism: Studying the bioavailability and metabolic pathways of these phenolic compounds in the human body to optimize their therapeutic use.

By providing a comprehensive phenolic profile and confirming the strong antioxidant potential of *Gymnema sylvestre* extract, this study lays a solid foundation for developing novel antioxidant therapies from this traditional herb. Further research in these areas will significantly contribute to the understanding and utilization of phenolic compounds in medicinal plants [29].

References

[1] Dunkhunthod B, Talabnin C, Murphy M, et al. Gymnema inodorum (Lour.) Decne. Extract Alleviates Oxidative Stress and Inflammatory Mediators Produced by RAW264.7 Macrophages. Oxid Med Cell Longev, 2021, 4: 1-20.

[2] Kasote D, Katyare S, Hegde M, et al. Significance of Antioxidant Potential of Plants and its Relevance to Therapeutic Applications. Int J Biol Sci, 2015, 11: 982-991.

[3] Dai J, Mumper R. Plant Phenolics: Extraction, Analysis and Their Antioxidant and Anticancer Properties. Molecules, 2010, 15: 7313-7352.

[4] Ounjaijean S, Romyasamit C, Somsak V. Evaluation of Antimalarial Potential of Aqueous Crude Gymnema inodorum Leaf Extract against Plasmodium berghei Infection in Mice. Evid Based Complement Alternat Med, 2021, 27: 9932891.

[5] Suwan N, Baison W, Chuajedton A. Purification of Gymnema inodorum Leaf Extract and Its Antifungal Potential Against Colletotrichum gloeosporioides. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences, 2022, 92: 667-677.

[6] Jeytawan N, Yadoung S, Jeeno P, et al. Antioxidant and Phytochemical Potential of and Phytochemicals in Gymnema inodorum(Lour.) Decne in Northern Thailand. Plants, 2022, 11: 3498.

[7] Chiabchalard A, Tencomnao T, Santiyanont R. Effect of Gymnema inodorum on postprandial peak plasma glucose levels in healthy human. Afr J Biotechnol, 2010, 9: 1079-1085.

[8] Boonyapranai K, Sarakul O, Somsak V, et al. Effects of Gymnema inodorum Leaf Extract on the Alteration of Blood Coagulation Parameters and Platelet Count in Plasmodium berghei-Infected Mice. J Parasitol Res,

2022, 11: 4225682.

[9] Cianciosi D, Forbes-Hernández T, Afrin S, et al. Phenolic compounds in honey and their associated health benefits: A review. Molecules, 2018, 23: 2322.

[10] Baba S, Malik S. Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of Arisaema jacquemontii Blume. JTUSCI, 2015, 9: 449-454.

[11] Kasote D, Katyare S, Hegde M, et al. Significance of antioxidant potential of plants and its relevance to therapeutic applications. Int J Biol Sci, 2015, 11: 982.

[12] Tiwari P, Mishra B, Sangwan N. Phytochemical and pharmacological properties of Gymnema sylvestre: An important medicinal plant, Biomed Res Int, 2014, 2014: 830285.

[13] Bajpai V, Majumder R, Park J. Isolation and purification of plant secondary metabolites using column-chromatographic technique. Bangladesh J Pharmacol, 2016, 11: 844-848.

[14] Zhang Y, Gan R, Li S, et al. Antioxidant Phytochemicals for the Prevention and Treatment of Chronic Diseases. Molecules, 2015, 20: 21138-21156.

[15] Abeyrathne E, Nam K, Huang X, et al. Plant- and Animal-Based Antioxidants' Structure, Efficacy, Mechanisms, and Applications: A Review. Antioxidants, 2022, 11: 1025.

[16] Onanong Nuchuchua, Wanwisa Srinuanchai, Chaisak Chansriniyom, et al. Relationship of phytochemicals and antioxidant activities in Gymnema inodorum leaf extracts, Heliyon. 2023, 10: 23175.

[17] Nwozo O, Effiong E, Aja P, et al. Antioxidant, phytochemical, and therapeutic properties of medicinal plants: a review. Int J Food Prop, 2023, 26: 359-388.

[18] Srinuanchai W, Nooin R, Jarussophon S, et al. Determination of gymnemic acid level in Gymnema inodorum leaves using multiple reaction monitoring mass spectrometry. J Chem Metrol, 2019, 13: 75-79.

[19] Srinuanchai W, Nooin R, Pitchakarn P, et al. Inhibitory effects of Gymnema inodorum(Lour.) Decne leaf extracts and its triterpene saponin on carbohydrate digestion and intestinal glucose absorption. J Ethnopharmacol, 2021, 266: 113398.

[20] Nuchuchua O, Srinuanchai W, Chansriniyom C, et al. Relationship of phytochemicals and antioxidant activities in Gymnema inodorum leaf extracts. Heliyon, 2023, 10: e23175.

[21] Wang X, Ma S, Lai F, et al. Traditional Applications, Phytochemistry, and Pharmacological Activities of Eupatorium lindleyanum DC.: A Comprehensive Review. Front Pharmacol, 2020, 8: 577124.

[22] Montoro V, Asensio C, Martínez Á, et al. Efficacy and safety of fluocinolone acetonide 0.025% otic solution in patients with otic eczema: a randomized, double-blind, placebo-controlled clinical trial. J Int Med Res, 2018, 46: 4050-4060.

[23] Abu F, Mat Taib C, Mohd Moklas M, et al. Antioxidant Properties of Crude Extract, Partition Extract, and Fermented Medium of Dendrobium sabin Flower. Evid Based Complement Alternat Med, 2017, 2017: 2907219.

[24] Gini T, Jeya Jothi G. Column chromatography and HPLC analysis of phenolic compounds in the fractions of Salvinia molesta mitchell. Egypt J Appl Sci, 2018, 5: 197-203.

[25] Islam M, Prottay A, Sultana I, et al. Phytochemical screening and evaluation of antioxidant, anti-inflammatory, antimicrobial, and membrane-stabilizing activities of different fractional extracts of Grewia nervosa (Lour.) Panigrahi. Food Biosci, 2023, 1: 54.

[26] Jeeno P, Tongban S, Yana P, et al. Tentative Identification of Phytochemicals from Smilax glabra and Smilax corbularia Extracts by LC-MS and Their Bioactive Potential. Plants, 2022, 11: 2089.

[27] Chaves N, Santiago A, Alías J. Quantification of the Antioxidant Activity of Plant Extracts: Analysis of Sensitivity and Hierarchization Based on the Method Used. Antioxidants, 2020, 9: 76.

[28] Devi K, Jain N. A validated HPLC method for estimation of Gymnemic acids as Deacyl gymnemic acid in various extracts and formulations of Gymnema sylvestre. Int J Phytomedicine, 2014, 6: 78-88.

[29] Arjin C, Hongsibsong S, Pringproa K, et al. Effect of Ethanolic Caesalpinia sappan Fraction on In Vitro Antiviral Activity against Porcine Reproductive and Respiratory Syndrome Virus. Vet Sci, 2021, 8: 106.