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Original Research Article

Determination of Phenolics, Antioxidant Activity and Antidiabetic Potential of *Sphagneticola calendulacea* (L.) Pruski Leaves Grown in Vietnam

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ABSTRACT

Sphagneticola calendulacea (L.) Pruski, a member of the Asteraceae family, is commonly found growing in India, Sri Lanka, China, and Southeast Asian countries. It has been utilized in traditional medicine for treating various illnesses in many Asian nations. The objective of this research was to compare the phenolic content, antioxidant activity, and inhibitory effect on α-glucosidase of *S. calendulacea* leaf extracts obtained through conventional solvent, ultrasound, and enzyme-assisted methods. Eight phenolic compounds were quantified in the extracts, and the results revealed that the methanol extract comprised the greatest amount of phenolic compounds (228.10 µg/ml) in comparison with the other extracts. The extract from the ultrasound method exhibited the highest ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) antioxidant activity. The ethanol extract presented the strongest inhibition of α-glucosidase. Generally, the applications of ultrasound and enzyme to extraction helped enhance phenolic content and bioactivities of the resulting extracts. These findings give a deeper insight into the phenolics and health endorsing activities of *S. calendulacea* leaves, which is beneficial for the development of new drugs for disease prevention and treatment.

Keywords: *Sphagneticola calendulacea*, phenolics, antioxidant activity, antidiabetic, glucosidase

Introduction

Sphagneticola calendulacea (L.) Pruski, also known as *Wedelia calendulacea* or *Wedelia chinensis*, is a perennial herb in the Asteraceae family. It is found growing in India, Sri Lanka, China and many Southeast Asian countries.[1] The plant has been traditionally used in various systems of medicine, such as Ayurveda, Siddha, and Unani, for the treatment of a variety of ailments, such as fever, jaundice, hepatitis, and other liver disorders.[2] Decoction prepared from the plant is often used to treat menorrhagia and skin diseases.[3] Additionally, leaves of *S. calendulacea* are sometimes used for promoting hair growth, preventing hair fall, and treating scalp infections.[4] Studies have shown that *S. calendulacea* possesses a wide range of potential health endorsing properties, such as antioxidant, anti-inflammatory, antibacterial, antidiabetic, wound healing, and antiproliferative activities.[3,5-9] For example, treatments with methanol extract of *S. calendulacea* leaves significantly reduced blood glucose level and exerted an ameliorative effect on triglycerides, total cholesterol, low density lipoprotein, very low density lipoprotein, and high density lipoprotein in diabetic mice.[8] Additionally, the treatments considerably reduced the activity of serum alanine aminotransferase and serum aspartate aminotransferase, as well as C reactive protein levels in the serum of diabetic mice in comparison with untreated diabetic mice. Extracts of the plant were shown to stimulate apoptosis in GBM8401 cells and autophagy in U-87MG cells.[9]

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Furthermore, the extracts significantly reduced the cell survival and invasive capability of both cell lines. Reportedly, *S. calendulacea* are composed of various chemical groups, including flavonoids, benzofuran derivatives, saponins, and triterpenoids.[5,10] Research has shown the presence of phenolics, such as apigenin, luteolin, jaceosidin and wedelolactone, in extracts of the plant species.[10, 11] Phenolics were demonstrated to have correlation with antioxidant activity of *S. calendulacea.*[12] In addition, jaceosidin was reported to inhibit the activities of α-amylase and α-glucosidase.[13] These compounds have been shown to possess multiple bioactivities of importance to human health.[14,15] For instance, a glycoside form of a triterpene derivative (19-α-hydroxyurs-12(13)-ene-28-oic acid-3-O-β-D-glucopyranoside) isolated from the plant suppresses oxidative stress and release of pro-inflammatory cytokines via inhibiting NF-κB expression.[16] In a rat model, it was shown that wedelolactone oral treatment (100 mg/kg) significantly reduced productions of IL-1α, IL-1β, IL-2, TNF-α, INFγ, STAT3 and CCL-5 in colons treated with dextran sulphate sodium.[17]

In Vietnam, *S. calendulacea*, commonly known as "sài đất", is believed to have various medicinal properties and is often used in traditional medicine. Its fresh leaves can be used as a vegetable which helps detoxify liver and maintain balance and harmony in the body's heat. The whole plant is used in many forms, such as drinking tea, boiling for bathing water, or grinding for external application on the skin.[18] The utilization of enzymes and ultrasound has proven to be a successful method in extracting phytochemicals from plants and enhancing the bioactive properties of the resulting extracts.[19] Thus, in this study, phenolics, antioxidant activity, and inhibitory activity on α-glucosidase of *S. calendulacea* leaf extracts obtained from different methods using organic solvents, ultrasound, or enzyme were researched.

Materials and Methods

Sample collection

The *S. calendulacea* leaf sample was collected from gardens located in Ho Chi Minh city, Vietnam (10°51'40.1"N 106°40'40.2"E) in December 2022. The plant (voucher specimen number: SGN-GĐ005) was identified by a botanist (Nguyen Quoc Dat) and deposited at the Southern Institute

of Ecology, Ho Chi Minh city. After collection, the sample was dried at 50 °C overnight, followed by storage in a refrigerator (4°C).

Chemicals
Flavonoid and phenolic acid analytical standards were obtained from Chengdu Biopurify Phytochemicals (China) and Sigma-Aldrich (USA). Organic solvents were purchased from Fisher Scientific (USA).

Extraction
Phenolics in the leaf sample was extracted using methanol, ethanol, and 80% acetone. Briefly, a mixture of the sample (10 g) and a solvent (100 ml) was shaken at 25 °C. After 18 h, the mixture was filtered through a Whatman filter paper. Besides, enzyme and ultrasound assisted extractions were employed to extract phenolics in the leaf sample. Briefly, 10 g of the sample were combined with 20 ml of a sodium acetate buffer (pH 5.5) and 2 ml of cellulase solution (Novozyme, Denmark). After continuous shaking on a shaker for 1 h at 35°C, 80 ml of acetone were added in the mixture, followed by a 17 h shaking. After the extraction step, the mixture was filtered through a filter paper. Ultrasound-assisted extraction was initiated on a mixture of the leaf and 80% acetone (1:10, g/mL) placed in an ultrasonic bath operated for 1 h at the frequency set at 40 kHz. Afterwards, the mixture was treated similarly to those described above.
An aliquot of each filtrate was transferred to a vial used for analysis of phenolics on a high-performance liquid chromatograph interfaced to diode array detector (HPLC-DAD).[20]

Crude extract preparation
The remaining filtrates obtained above were subjected to evaporation in a rotary evaporator (40°C). The resultant residues were used in bioassays.

Antioxidant activity
ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay was used to predict antioxidant activity of the *S. calendulacea* extracts. Briefly, a 7 mM ABTS solution was combined with 2.45 mM potassium persulfate in phosphate buffered saline (1:1, v/v). After incubation for 15 h at 25°C in darkness, the mixture was mixed with a crude extract diluted in DMSO or vitamin C (a reference standard). Changes in absorbance of the mixture was analyzed at 734 nm.[21] IC_{50} (μg/ml) was used to evaluate antioxidant activity of the extracts.

Inhibition of α-glucosidase
The assay was conducted by mixing a diluted *S. calendulacea* extract (50 μl) with 40 μl of *α*-glucosidase (0.05 U) in phosphate buffer (pH 6.8), followed by 20 min incubation at 37 °C. Subsequently, 5 mM 4-nitrophenyl-*β*-D-glucopyranoside (40 μl) was added, and another 20 min incubation step was carried out at 37°C. Sodium carbonate (200 mM, 130 μl) was added to stop the reaction. Changes in absorbance of the solution were determined at 405 nm. Acarbose was employed as a reference standard. The inhibitory activity was presented as mg acarbose equivalents per g of extract (mg ACAE/g).[22] The calibration curve plotted for acarbose against concentrations was $y = 0.003x + 0.0346$, with $R^2 = 0.999$.
Statistical analysis
All the measurements were conducted three times and the results were presented as mean ± standard deviation. Data analysis was performed using one-way ANOVA with Tukey's HSD test at a significance level of 0.05. Statistical analyses were implemented using XLSTAT ver. 2016 (Addinsoft, Paris, France).

Results and Discussion

Phenolics
Using an HPLC-DAD system, six phenolic acids and two flavonoids were identified and quantified. A summary of the results on concentrations of the compounds in the five extracts is presented in Table 1. Figure 1 displays a representative HPLC-DAD chromatogram of the compounds detected in an extract of *S. calendulacea*. Among the extracts, MET comprised the greatest amount of phenolics (22.8.10 μg/ml). The average concentration of chlorogenic acid in this extract (115.16 μg/ml) was about 2 – 8 times as high as compared to the other extracts. Gallic acid was detected only in MET and ETH, with the level in the former (5.13 μg/ml) fourfold higher than in the latter. Along the lines of these two phenolic acids, caffeic acid and ferulic acid were found to be much more abundant in MET compared to the other extracts. Methanol and ethanol appeared to have much higher extractability on salicylic acid than acetone. In general, seven out of eight compounds monitored in the study were found to be present at the highest levels in MET.
As stated earlier, the use of enzyme and ultrasound in extraction of phenolics was evaluated. The results revealed that p-coumaric acid, ferulic acid, syringic acid, and quercetin had higher concentrations in the extracts obtained with enzyme and ultrasound in comparison with ACW. Notably, EE consisted of about seven times as much of p-coumaric acid (10.49 μg/ml) as ACW (1.58 μg/ml). This concentration value was also considerably higher compared to the other extracts. The results also demonstrated the effectiveness of the application of ultrasound to the extraction of phenolics in *S. calendulacea* leaves as six out of eight compounds were found to have higher levels in UE compared to ACW. As seen in Table 1, the sum of the phenolics in the former was 50% higher than that in the latter. Previous research showed 3% cellulase solution effectively assisted in extraction of walnut phenolics.[23] This can be explained that the enzyme had a capacity to hydrolyse cell walls, facilitating the release of phytochemicals attached to carbohydrate molecules within the cells.

Table 1: Phenolic contents of the *S. calendulacea* extracts obtained from various methods

Phenolics	Concentrations, μg/ml				
	MET	ETH	ACW	UE	EE
Gallic acid	5.13	0.97	nd	nd	nd
Chlorogenic acid	115.16	14.08	39.39	56.41	15.32
Caffeic acid	13.88	6.10	2.26	2.16	2.51
p-Coumaric acid	3.60	1.40	1.58	2.58	10.49
Ferulic acid	70.23	13.27	21.48	37.10	33.96
Salicylic acid	10.47	9.01	0.73	1.53	1.53
Rutin	9.01	8.31	4.31	5.32	3.92
Quercetin	0.61	nd	0.24	0.31	0.28
Sum of the phenolics	228.10	53.15	69.99	105.41	68.02

Concentrations of phenolics were shown as μg/ml. The results are presented as means of duplicate measurements. MET, ETH, and ACW stand for methanol, ethanol and 80% acetone extracts while UE and EE represent the extracts from ultrasound and enzyme assisted extractions, respectively.
nd: not detected

Antioxidant activity

In this study, the capacity of the *S. calendulacea* extracts to remove free radicals was evaluated by ABTS assay as described earlier. As shown in Figure 2, UE may exhibit the greatest antioxidant activity (IC_{50} = 443.90 ± 2.66 µg/ml), followed by ACW (IC_{50} = 566.69 ± 1.74 µg/ml). It is noted that EE, with IC_{50} > 1000 µg/ml, was not as strong to neutralize ABTS radicals as UE and ACW, implying the use of enzyme did not enhance the antioxidant activity of the extract. On the other side of the graph, MET and ETH with the higher IC_{50} values (> 1500 and 2000 µg/ml, respectively) may exert much lower abilities to scavenge ABTS radicals compared to the other extracts.

The activity of these extracts was not so strong as ascorbic acid (IC_{50} = 60.52 µg/mL). One study reported that extract of *S. calendulacea* leaves obtained from a 7-day extraction with methanol was about 36 µg/ml.[24] The differences in extraction time and sample collection site between the studies could explain the variation in the ABTS antioxidant activity.

Inhibition of α-glucosidase

α-Glucosidase is an enzyme responsible for breaking down carbohydrates into simple sugars in the small intestine. In people with diabetes, the activity of α-glucosidase can have significant impacts on blood sugar levels. α-Glucosidase inhibitors work by blocking the activity of this enzyme, which slows down the breakdown of carbohydrates into simple sugars. In the present study, extracts from *S. calendulacea* leaves (4 mg/ml) were tested for α-glucosidase inhibition. ETH may be the extract that displayed the most potent inhibition of the enzyme (Figure 3). The activity of ETH was about 7 – 30 times as high as those of ACW and MET. The results also indicated EE and UE had significantly higher inhibitory effect compared to ACW. This may imply that cellulase and ultrasound was able to improve the capacity to inhibit α-glucosidase. Previous research revealed several phytochemicals isolated from the plant showed strong inhibition of α-glucosidase.[13] Of these, jaceosidin exerted a comparable activity compared to acarbose (an antidiabetic drug). In an in vivo study, a 21-day treatment of methanol extract of the plant was found to reduce blood glucose level and exhibit ameliorative effect on triglyceride, total cholesterol, high density lipoprotein, and low density lipoprotein in diabetic mice.[8] It also decreased the activity of serum alanine aminotransferase, serum aspartate aminotransferase, and C reactive protein level. All these findings demonstrate that this plant species may exert a powerful action on blood glucose levels and diabetes management.

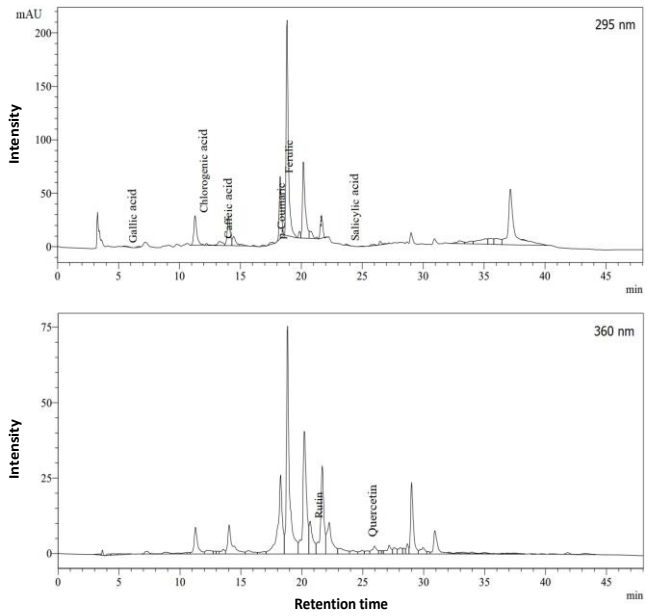

Figure 1: A representative HPLC-DAD chromatogram shows the presence of eight phenolics in an *S. calendulacea* diluted extract at the wavelengths of 295 and 360 nm.

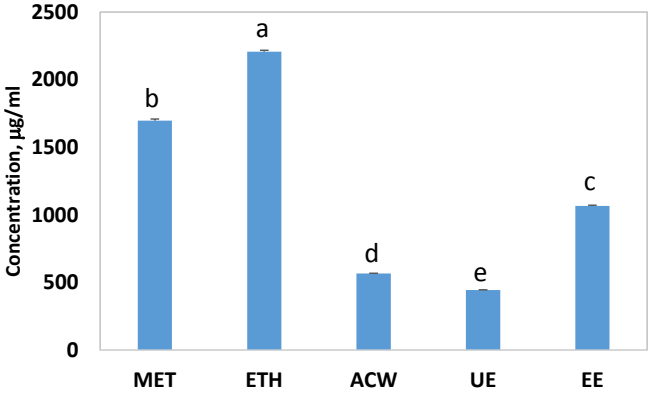

Figure 2: Antioxidant activity (IC_{50}, µg/ml) of the *S. calendulacea* extracts. Different letters show significant differences in the activity among the extracts ($p < 0.05$).

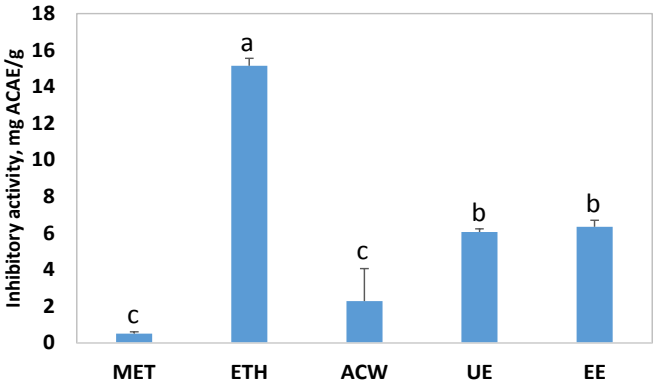

Figure 3: α-Glucosidase inhibitory activity (mg ACAE/g) of the *S. calendulacea* extracts. Different letters show significant differences in the activity among the extracts ($p < 0.05$).

Conclusion

In summary, phenolic compounds, antioxidant activity, and inhibition of α-glucosidase of *S. calendulacea* dried leaf extracts were analyzed. The use of ultrasound and enzyme to assist extraction affected the concentration of phenolics and bioactivities of the resulting extracts. The investigation gives a deeper insight into the plant's phenolic composition and health endorsing potential, which could pave the ways for development of plant-derived drugs for disease prevention and treatment.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. The World Flora Online. *Sphagneticola calendulacea* (L.) Pruski. 2023; Available from: http://www.worldfloraonline.org/taxon/wfo-0000072506. Accessed 2023 January 30.
2. Meena AK, Rao MM, Meena RP, and Panda P. Pharmacological and phytochemical evidences for the plants of *Wedelia* Genus–A Review. Asian J Pharm Res. 2011; 1(1):7-12.

3. Darah I, Lim SH, and Nithianantham K. Effects of methanol extract of *Wedelia chinensis* Osbeck (Asteraceae) leaves against pathogenic bacteria with emphasise on *Bacillus cereus*. Indian J Pharm Sci. 2013; 75(5):533.
4. Kumar MR, Teelavath M, and Yellanki SK. Development and evaluation of polyherbal emulgel formulation (A preventive hair care preparation). Int J Herb Med. 2019; 7(1):08-10.
5. Koul S, Pandurangan A, and Khosa RL. *Wedelia chinenis* (Asteraceae)–an overview. Asian Pac J Trop Biomed. 2012; 2(2):S1169-S1175
6. Manjamalai A and Grace VM. Antioxidant activity of essential oils from *Wedelia chinensis* (Osbeck) in vitro and in vivo lung cancer bearing C57BL/6 mice. Asian Pac J Cancer Prev. 2012; 13(7):3065-3071.
7. Huang Y-T, Wen C-C, Chen Y-H, Huang W-C, Huang L-T, Lin W-C, Arulselvan P, Liao J-W, Lin S-H, and Hsiao P-W. Dietary uptake of *Wedelia chinensis* extract attenuates dextran sulfate sodium-induced colitis in mice. PLoS One. 2013; 8(5):e64152.
8. Bari M, Islam M, Khatun M, Sultana MJ, Ahmed R, Islam A, Hossain M, Rahman M, and Islam MA. Antidiabetic effect of *Wedelia chinensis* leaf extract in alloxan induced Swiss albino diabetic mice. Clin Phytosci. 2020; 6(1):1-8.
9. Chen L-J, Hsu T-C, Yeh P-J, Yow JL, Chang C-L, Lin C-H, and Tzang B-S. Differential Effects of *Wedelia chinensis* on Human Glioblastoma Multiforme Cells. Integr Cancer Ther. 2021; 2015347354211000119.
10. Tsai Y-T, Chen P-Y, and To K-Y. Induction of polyploidy and metabolic profiling in the medicinal herb *Wedelia chinensis.* Plants. 2021; 10(6):1232.
11. Lin F-M, Chen L-R, Lin E-H, Ke F-C, Chen H-Y, Tsai M-J, and Hsiao P-W. Compounds from *Wedelia chinensis* synergistically suppress androgen activity and growth in prostate cancer cells. Carcinogenesis. 2007; 28(12):2521-2529.
12. Islam MA, Zaman S, Biswas K, Al-Amin MY, Hasan MK, Alam A, Tanaka T, and Sadik G. Evaluation of cholinesterase inhibitory and antioxidant activity of *Wedelia chinensis* and isolation of apigenin as an active compound. BMC Complement Altern Med. 2021; 21:1-12.
13. Thao NP, Binh PT, Luyen NT, Hung TM, Dang NH, and Dat NT. α-Amylase and α-glucosidase inhibitory activities of chemical constituents from *Wedelia chinensis* (Osbeck.) Merr. leaves. J Anal Methods Chem. 2018; 2018: 2794904.
14. Okolie NP, Falodun A, and Davids O. Evaluation of the antioxidant activity of root extract of pepper fruit (*Dennetia tripetala*), and it's potential for the inhibition of lipid peroxidation. Afr J Tradit Complement Altern Med. 2014; 11(3):221-227.
15. Egharevba E, Chukwuemeke-Nwani P, Eboh U, Okoye E, Bolanle IO, Oseghale IO, Imieje VO, Erharuyi O, and Falodun A. Evaluation of the antioxidant and hypoglycaemic potentials of the leaf extracts of *Stachytarphyta jamaicensis* (Verbenaceae). Trop J Nat Prod Res. 2019; 3(5):170-174.
16. Verma A, Singh D, Anwar F, Bhatt PC, Al-Abbasi F, and Kumar V. Triterpenoids principle of *Wedelia calendulacea* attenuated diethynitrosamine-induced hepatocellular carcinoma via down-regulating oxidative stress, inflammation and pathology via NF-kB pathway. Inflammopharmacology. 2018; 26:133-146.
17. Prakash T and Janadri S. Anti-inflammatory effect of wedelolactone on DSS induced colitis in rats: IL-6/STAT3 signaling pathway. J Ayurveda Integr Med. 2022; 100544. In press. https://doi.org/10.1016/j.jaim.2022.100544.
18. Institute of Traditional Medicine Research and Development (Viện Y Dược Cổ Truyền Dân Tộc). *Cây Sài Đất*. 2023; Available from: https://vienyduocdantoc.org.vn/duoc-lieu/cay-sai-dat. Accessed 2023 January 30.
19. Van Hung P, Yen Nhi NH, Ting LY, and Lan Phi NT. Chemical composition and biological activities of extracts from pomelo peel by-products under enzyme and ultrasound-assisted extractions. J Chem. 2020; 2020:1-7.
20. Vu D. Assessing the Differences in Phenolics, Antioxidant and Anti-Tyrosinase Activities of Spent Coffee Ground Fractions. Trop J Nat Prod Res. 2022; 6(4):558-562.
21. Vu D. Effects of extraction solvents on phytochemicals and bioactivities of *Ganoderma lucidum.* Egypt J Chem. 2023. In press. https://doi.org/10.21608/EJCHEM.2023.172356.7142.
22. Vu D, Nguyen T, and Vo XT. Influence of Green Solvent Extraction on phytochemicals, Potential Antidiabetic and In Vitro Anti-Inflammatory Activities of *Pseuderanthemum palatiferum* (Nees.) Radlk. Leaves. Trop J Nat Prod Res. 2023; 7(1):2215-2219.
23. Zhang Y-G, Kan H, Chen S-X, Thakur K, Wang S, Zhang J-G, Shang Y-F, and Wei Z-J. Comparison of phenolic compounds extracted from Diaphragma juglandis fructus, walnut pellicle, and flowers of *Juglans regia* using methanol, ultrasonic wave, and enzyme assisted-extraction. Food Chem. 2020; 321:126672.
24. Bari MW, Islam A, Islam MM, Sultana MJ, Afroz R, Khan MMR, Parul SS, Swaraz AM, Hossain MI, and Islam MA. Determination of in vitro antioxidant activity and in vivo antineoplastic effects against Ehrlich ascites carcinoma of methanolic extract of *Sphagneticola calendulacea* (L.) Pruski. Heliyon. 2021; 7(6):e07228.