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Phytochemical Constituents of *Securigera securidaca* Seed Extract Using GS-MS and HPLC

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ABSTRACT

Securigera securidaca (L.) seeds are used in Jordanian folk medicine to treat several disorders like diabetes and hyperlipidemia. The aim of this study was to evaluate the chemical composition of *S. securidaca* methanol extract. The methanol extract of *S. securidaca* seeds obtained from local herbal shop in Jordan was investigated for its phytochemical composition using GC-MS and HPLC instruments. Results showed that the methanol extract has twenty-seven natural compounds which made up 99.5% of the total composition. The HPLC-PDA results of *S. securidaca* showed many phytochemicals that are mainly composed of aromatics and oxygenated hydrocarbons. Dodecanedioic acid and its derivatives, as well as *β*-Sitosterol are pharmacologically active components that have not been reported before in this plant. Three unidentified peaks originated in the GC-MS. A further detailed study is needed to investigate their identity. These results provide fine evidence for the ethno-pharmacological use of this plant to treat diabetes and hyperlipidemia.

Keywords: *Securigera securidaca*, Phytochemical, Dodecanedioic acid, *β*-Sitosterol.

Introduction

Securigera securidaca (L.) Degen & Dörfl., Fabaceae, is an annual herb occurring wild in Asia Africa, and Europe. This plant grows in Jordan in Humid habitats, specifically in Irbid, Madaba, and Upper Jordan Valley, with a common name Hatchet Vetch.[1] It is commonly used in Jordan as edible and flavoring food as well as a folk drink.[2] Moreover, it is widely used in the Middle-East, Indian, and Europe folk medicine as an antidiabetic and anti-hyperlipidemic remedy.[2-4] The methanol extract of *S. securidaca* is believed to decrease the serum glucose level.[5] The aqueous extract of the seeds also showed a marked decrease in blood glucose levels in mice.[6] Besides, *S. securidaca* seed powder suspension has shown a protective effect against alloxan-induced hyperglycemia and oxidative stress in rats which is related to its flavonoid content.[7,8]
It has been reported that the Middle East and Northern Africa have the highest prevalence of diabetes with more than 34 million diabetic persons according to international diabetes federation. [9-12]

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The naturally occurring flavonoids are believed to possess the ideal chemical structures for scavenging free radicals. Saponins create considerable hypolipidemic effects via two pathways, enhancement of cholesterol secretion and suppression of its luminal absorption. Saponins are known to be isolated from several natural resources.[13,14] Nowadays, there is a growing interest in the analysis and identification of medicinal plant's phenolic constituents with a view to finding new compounds of the phenolic class and to establish their structure-activity relationship.[15,16]
With this background, the present study aims to identify the chemical composition of the methanol extract of *S. securidaca* seeds. The total polyphenols and flavonoids were determined and the phenolic composition of the extract was identified using HPLC (high-performance liquid chromatography) and GC-MS (gas chromatography-mass spectrometry) analysis.

Materials and Methods

Plant material collection and extraction

S. securidaca seeds were bought from a local herbal shop (Karak-Jordan) in the spring of 2019 and identified according to Al-Eisawi.[17] A voucher specimen was deposited in a drug discovery laboratory along with a given specimen number R003. Plant seeds dried in the shade was then ground into a fine powder by a coffee blender. The obtained fine powder was stored in a special container until use. A 25 g of the seed powder was macerated in 250 mL of methanol (put in a shaker device) at room temperature for 4 days. The extract was filtered, centrifuged at 3000 rpm for 15 minutes and concentrated *in vacuo* at 50°C using a rotary evaporator. The resulting crude extract

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was air-dried in the fume hood and stored in the refrigerator at 4°C in a glass container until further use.[18,19]

GC-MS system and analysis

GC-MS analysis was carried out on an Agilent technology type 6890 GC equipped with a Split- splitless injector and capillary column type of HP-5MS coated with a film 5% of phenylmethyl polysiloxane (30 m x 0.25 mm, 0.25 µm film thickness). The Agilent 6890 GC is equipped with a mass spectrometer type of 5973C Inert MSD (Mass Spec, Mass Spectrometer, Mass Selective Detector, MS, and GC/MS). The temperature of the column oven was programmed as follows: initial temperature was 60°C, increased to 300°C with a ramp of 15 ºC/min, the temperature was held at 300°C for 7 min until all of the elution was completed. The split valves were opened for 3 minutes after 15 seconds to purge the injector. All injections of 1µL volume were made with a 10 µL syringe. Helium was used as the carrier gas with a purity of 99.999% and a flow rate of 1.0 mL/min.[18, 20]

HPLC system and chromatographic conditions

The HPLC is a Waters Alliance (e2695 separations module), equipped with 2998 Photodiode Array detector (PDA). Data acquisition and control was carried out using Empower 3 chromatography data software (Waters, Germany). The HPLC analytical experiments of the crude extracts of the three samples was run on the ODS column of Waters (XBridge, 4.6 ID x 150 mm, 5 µm) with a guard column of Xbridge ODS, 20 mm x 4.6 mm ID, 5 µm. The mobile phase composition is a mixture of acetic acid in water (0.5%) (Solvent A) and acetonitrile (solvent B) ran in a linear gradient mode.

Solvent A descended to 70% (from 100%) in 40 minutes, then to 40% (solvent A) in 20 minutes and finally to 10% (solvent A) in 2 minutes. After that the mobile phase composition stayed constant for 6 minutes and then returned back to the initial conditions in 2 minutes. Before injecting the next sample, system equilibrates for 7 minutes with solvent A. The PDA wavelength range was 210-500 nm. The flow rate was 1 mL/min. Injection volume was 20 mL and the column temperature was set at 25°C.

Results and Discussion

The methanol extract of *S. securidaca* seeds was investigated using GC-MS as analytical technique. Each constituent in the extract was quantified and identified by matching mass fragmentation patterns against standards such as NIST and Wiley 9 library spectral data. The chromatogram showed twenty-seven peaks with retention time between 5.56 and 24.52 (Figure 1).

The twenty-four components representing 99.46% of the total composition were identified, while three peaks representing 0.54% of total composition were unknown, as presented in Table 1. The analysis revealed that the extract is mainly composed of oxygenated hydrocarbons and aromatics. The major identified compounds were 2-Dodecenedioic acid (43.27%), Unsaturated 3-OH- sebaceous (14.64%), 6-Dodecenedioic acid (14.08%), L-Ascorbic acid (5.61%), Acyl Glucuronides (3.4%), α-D- Glucopyranose (3.34%) N-Butylglycine (2.39%) and 1,3-Propanediol (2.29%).

Figure 2 shows the chromatogram of the crude extract of *S. securidaca* at 284 nm. The eluted compounds detected in the range of 2-26 minutes with the main peak eluted at 5.6 minutes. This indicates the polar and non-polar characteristics of the eluted compounds. The UV-Vis ranges of these compounds were in the range of 210-350 nm.

The major compounds shared two similar wavelength maxima: 280 and 320 nm. Phenolic compounds and flavonoids have this typical absorption range.

Previous studies discovered the presence of various classes of compounds in the seeds of *S. securidaca* using several extraction techniques.[13,17,23] It is well-known that origin; geographic area; collection date; part used as well as extraction method affect the phytochemical composition for plant extracts.[26]

Garani *et al*. investigated the chemical composition of the hydroalcoholic extract of *S. securidaca* seeds.[23] Their study revealed the presence of saponins, flavonoids, tannins, and alkaloids in *S. securidaca*. The present study used a more specific technique for separation and identification than the general tests used in such studies. 2-Dodecanedioic acid is present with a high percentage (43.3%) as well as 6-Dodecenedioic acid (14.08%) and 3-OH-Sapienic acid acid available in a percentage of about 14%, as shown in Table 1. These acids are C-12 dicarboxylic acid, Salinari *et al*. demonstrated that these components may reduce muscle fatigue and considered as a suitable energy substrate during exercise, as they are rapidly oxidized, and do not stimulate insulin secretion in Type 2 diabetic patients.[24] The current results justify and support the use of *S. securidaca* seeds extract for diabetic patients. No previous studies have reported the presence of 2-Dodecanedioic acids as well as 6-Dodecenedioic acid in *S. securidaca* seeds.

Other important component reported here is β-Sitosterol/Sitosterol that is a plant sterol ester. Kaffarnik *et al*., revealed the use of β-Sitosterol in the treatment of hypercholesterolemia.[25] It lowers cholesterol levels via limiting the amount of cholesterol that enters the body and improves the symptoms in case of benign prostatic hyperplasia. In a previous study, there was a report about the beneficial effect of the hydroalcoholic extract on serum lipid profiles in rats, without reporting the presence of □-Sitosterol.[23] This component supports and explains the use of S. securidaca in folk medicine in addition to these studies.

Conclusion

In this study, the methanol extract of *S. securidaca* seeds was analyzed by GC-MS and HPLC. The results showed that the crude extract contains numerous phytochemicals. About 27 peaks were identified. The major component is 2-Dodecanedioic acid with a percentage of 43.3, whereas 6-Dodecenedioic acid and 3-OH-Sapienic acid has about 14%. Dodecanedioic acid and its derivatives, as well as β-Sitosterol, were identified as components of seeds extract for the first time. This clarifies the use of *S. securidaca* to treat hypercholesterolemia and some other uses in folk medicine. Other identified constituents are nutrients and micronutrients. Further studies is needed to elucidate the structures of the chemical constituents.

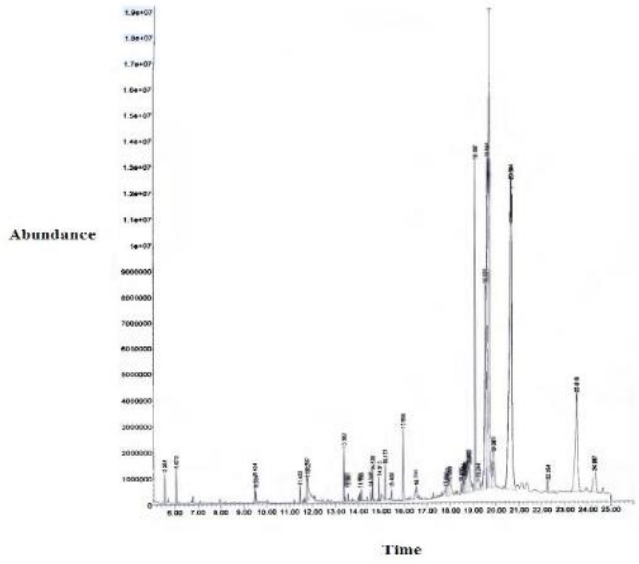

Figure1: GC/MS chromatogram of Methanol extract of *Securigera securidaca*

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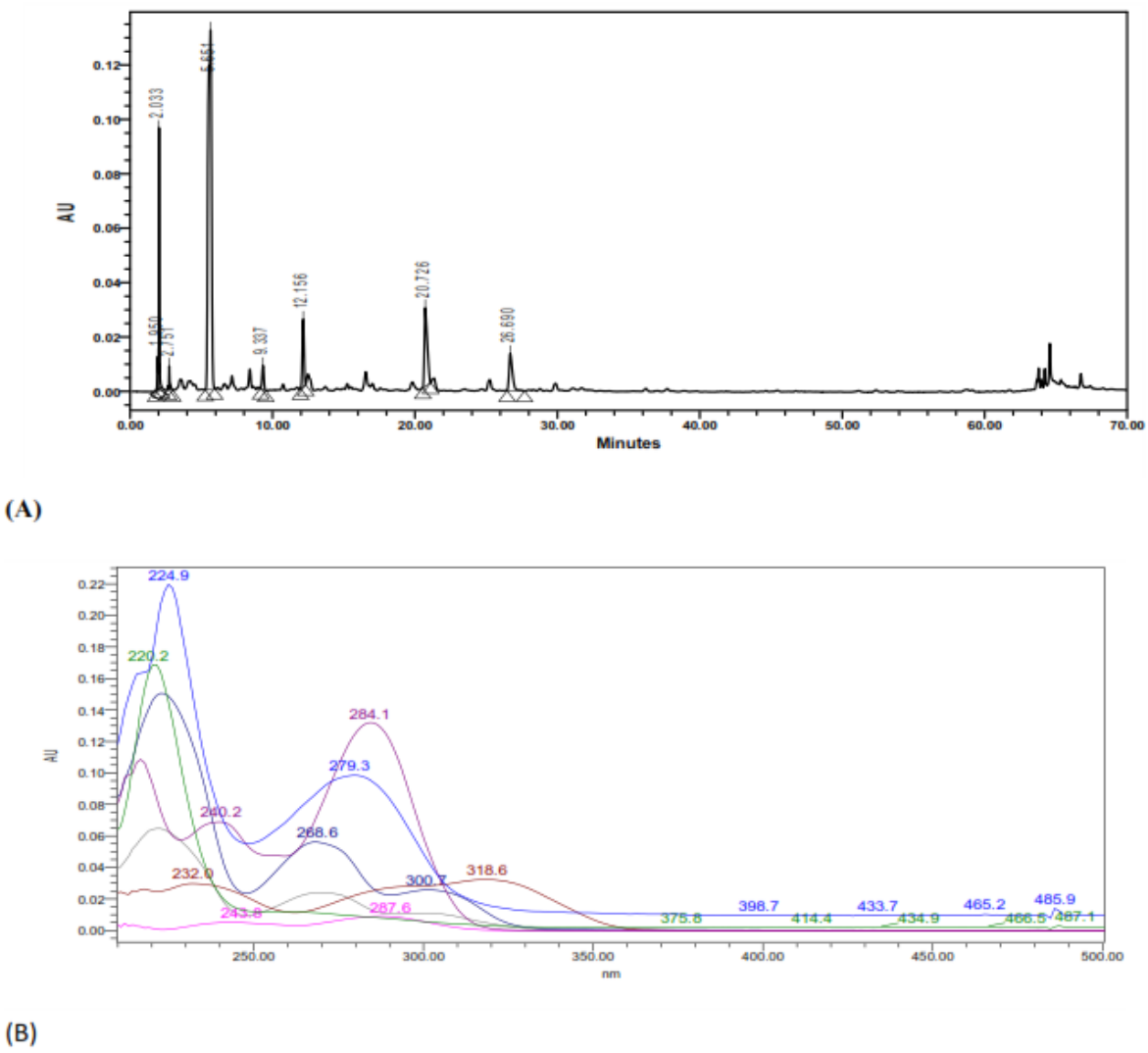

Figure 2: HPLC-PDA chromatogram of crude extract of *Securigera securidaca* at 284 nm (A), Their overlaid UV-Vis spectra (B)

Table1: List of chemical components of *S securidaca*

Compound name	Retention Time	Composition Percentage %	Molecular Formula	Molecular Weight g/mol
Ethylamine TMS	5.56	0.21	$C_5H_{15}NSi$	117.26
Unknown	6.88	0.11	-	-
1,3-Propanediol	9.49	2.29	$C_3H_8O_2$	76.09
Androstan-3-one	11.45	0.13	$C_{19}H_{30}O$	274.4
N-Butylglycine	13.38	2.39	$C_6H_{13}NO_2$	131.17
Acyl Glucuronide	14.00	3.4	$C_6H_9O_7$	193.14
L-Ascorbic acid	14.14	5.61	$C_6H_8O_6$	176.12
Unknown	14.26	0.10	-	-
2-Dodecenedioic acid	14.63	43.27	$C_{12}H_{20}O_4$	228.28

3-OH- Sapienic acid acid	14.84	14.64	$C_{16}H_{30}O_3$	270.41
Undecanedioic acid	14.91	0.69	$C_{11}H_{20}O_4$	216.27
6-Dodecenedioic acid	15.19	14.08	$C_{12}H_{20}O_4$	228.28
Palmitic Acid	15.45	1.47	$C_{16}H_{32}O_2$	256.42
D-Glucuronic acid	15.96	0.87	$C_6H_{10}O_7$	194.14
Isobutyric acid	16.54	1.93	$C_4H_8O_2$	88.11
3-Hydroxy-tetradecanedioic	17.66	0.61	$C_{14}H_{26}O_5$	274.35
Tetradecanedioic acid	18.28	0.24	$C_{14}H_{26}O_4$	258.35
Hexadecanoic acid	18.58	0.80	$C_{16}H_{32}O_2$	256.42
Phenobarbital	18.88	0.94	$C_{12}H_{12}N_2O_3$	232.23
α-D-Glucopyranose	19.14	3.34	$C_6H_{12}O_6$	180.16
Unknown	19.64	0.33	-	-
2,6,10,14,18,22-Tetracosahexaene	19.85	0.14	$C_{24}H_{36}O_2$	356.5
D-glucose	20.30	0.22	$C_6H_{12}O_6$	180.16
Maltose	21.09	0.12	$C_{12}H_{22}O_{11}$	342.3
α -Tocopherol	22.27	1.26	$C_{29}H_{50}O_2$	430.7
β-Sitosterol	24.52	0.36	$C_{29}H_{50}O$	414.7
Total identified components	99.46%			
Unknown components	0.54%			

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.

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References

1. Jordan plant red list, Publisher: Royal Botanic Garden, Jordan ©2017 Royal Botanic Garden. 2017. 416 p.
2. Yoona K, Keogh J, Clifton P. Polyphenols and Glycemic Control. Nutrients. 2016; 8 (1):17-44.
3. Porchezhian E and Ansari S. Effect of Securigera securidaca on Blood Glucose Levels of Normal and Alloxan-Induced Diabetic Rats. Pharm Biol. 2008; 39(1):62–64.
4. Azarmiy Y, Zakheri A, Alaf A, Fathiazad F, Fakhrjou A, Andali S, Maaleki D, Babaei, Garjani A. The effect of total extract of *Securigera securidaca* L. seed on thoracic aorta function in nigh-fat fed rats. Pharm Sci. 2009; 15:83–92.
5. Zahedi-Asl S, Marahel H, Zaree B. Study of the effect of chloroformic extract of *Securigera securidaca* on serum glucose level and liver glycogen content of mice. J.Kerman Univ Med Sci. 2005; 12:32–38.
6. Ali A, Mohamed M, Kamel M, Fouad M, Spring O. Studies on *Securigera securidacea* (L.) Fabaceae) seeds, an antidiabetic Egyptian folk medicine. Pharmazie. 1998; 53(10):710-715.
7. Mahdi P, Mohammad E, Ali-Akbar M,Karim P. Tostudythe effects of *Securigera securidaca* (L.) seed against alloxan-induced hyperglycemia. J Med Plants Res. 2011; 5(14):3188–3191.
8. Rehman K, Al-Gubory K, Laher I, Akash M. Dietary Polyphenols in the Prevention and Treatment of Diabetes Mellitus. Nutrit. Antioxid. Ther Treat Perspec. 2017;1: 377-395.
9. Kim J, Ju B, Choi W, Kim S. Hypoglycemic and anti-hyperlipidemic effect of four Korean medicinal plants in alloxan-induced diabetic rats. Am J Biochem Biotechnol. 2006; 2:154-160.
10. Xiao J, Hogger P. Dietary polyphenols and type 2 diabetes: current insights and future perspectives. Curr Med Chem. 2015; 22(1):23-38.
11. Lin D, Xiao M, Zhao J, Li Z, Xing B, Li X, Kong M, Li L, Zhang Q, Liu YChen H, Qin W, Wu H, Chen S. An Overview of Plant Phenolic Compounds and Their Importance in Human Nutrition and Management of Type 2 Diabetes. Mol. 2016; 21(10):E1374.
12. Bahadoran Z, Mirmiran P, Azizi F. Dietary polyphenols as potential nutraceuticals in management of diabetes: a review. J Diabet Metab Disord. 2013; 12:43-51.
13. Garjani A, Fathiazad F, Zakheri A, Akbari N,Azarmie Y, Fakhrjoo A, And alib S, Maleki-Dizaji N. The effect of total extract of *Securigera securidaca* L. seeds on serum lipid profiles, antioxidant status, and vascular function in hypercholesterolemic rats. J Ethnopharmacol. 2009; 126(3):525-532.
14. Diabetes Atlas IDF. 5thed. International Diabetes Federation, Brussels, Belgium, 2011.

15. Odhiambo R, Kareru P, Mwangi E, Onyango W. Antioxidant Activity, Total Phenols, Flavonoids and LC-MS Profile of *Chamaecri stahildebrandtii* (Vatke) Lock and *Clerodendrum rotundifolium* (Oliv.). Eur J Med Plants. 2018; 26(3):1-11.
16. Al-Zereini W, Al-Rimawi F, Abu-Lafi S, Alakhras F, Al-Mazaideh G, Ayyal-Salman H, Jamhour R. Identification and Antibacterial Evaluation of Selected Jordanian Medicinal Plants. Orient J Chem. 2018; 34(5):1-8.
17. Alizadeh-Fanalou S, Babaei M, Hoseini A, Azadi N, Nazarizadeh A, Shojaii A, Borji M, Malekinejad H, Bahraini E. Effects of *Securigera securidaca* seed extract in combination with glibenclamide on antioxidant capacity, fibroblast growth factor 21 and insulin resistance in hyperglycemic rats. J Ethnopharmacol. 2019;11(2):331
18. Aldal'in H K, Wedian F, Al-Mazaideh G M, Ayyal-Salman H, AL-Rimawi F, Alakhras F, Abu-Lafi S, AL-Nadaf A H. Comparative Analysis of Phytochemical Composition of Ethanolic Extract of Jordanian *Silvia officinalis*. PJBS. 2020: 23(8):989-994.
19. Al-Nadaf A H , Seder N J and Abu-Rayyan W. Wound healing; Antimicrobial and Anti-oxidant activity for Jordanian *Juglans regia* L. Unripe Fruits. JIPBS. 2018; 5(3):26-34.
20. Falodun A, Siraj R and Choudhary M I . GC-MS Insecticidal Leaf essential oil of *Pyrenacantha staudtii* Hutch and Dalz (Icacinaceae). Trop J Pharm Res. 2009; 82:139-143.
21. Tofighi Z, Molazem M, Doostdar B, Taban P, Shahverdi A, Samadi N, Yassa N. Antimicrobial Activities of Three Medicinal Plants and Investigation of Flavonoids of *Tripleuro spermumdisciforme*. Iran J Pharm Res. 2015; 14(1):225-231.
22. Jaradat N, Abualhasan M, Ali I. Comparison invitro of Antioxidant Activity between Fifteen Campanula Species (Bellflower) from Palestinian Flora. J Appl Pharm Sci. 2015; 5(4):101-106.
23. Garjani A, Fatemeh F, Arezoo Z, Akbari N A Yadollah A, Ashraf F, Sina A, Nasrin M. The effect of total extract of *Securigera securidaca* L. seeds on serum lipid profiles, antioxidant status, and vascular function in hypercholesterolemic rats. J Ethnopharmacol. 2009; 126:525–532.
24. Salinari S, Bertuzzi A, Gandolfi A, Greco A V, Scarfone A, Manco M, Mingrone G. Dodecanedioic Acid Overcomes Metabolic Inflexibility in Type 2 Diabetic Subjects. Am J Physiol Endocrinol Metab. 2006; 291(5):1051-1058.
25. Kaffarnik H, Mühlfellner G, Mühlfellner O, Schneider J, Hausmann L, Zöfel P, Schubotz R, F Fuchs. Beta-sitosterin in the Treatment of Essential Type II Hyperlipoproteinemias. Fortschr Med. 1977; 95(46):2785-2787.
26. Al-Nadaf A H., Bastoni H M and Hamdan D F. Microwave-assisted efficient extraction of phenolics from *Juglans regia* L.: pellicle; kernel unripe fruits; and leaves in different solvents. Int. J. Green Pharm . 2018; 12(3):182.