S.S Gololo et al /J. Pharm. Sci. & Res. Vol. 8(10), 2016, 1141-1143

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Identification of Phytochemical Constituents within the *N*-Hexane Leaf Extract of *Senna italica* (Mill) using Gas Chromatography-Mass Spectrometry (GC-MS) analysis

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Abstract

Senna italica is widely used in traditional medicine for treatment of intestinal tumours and urinary tract infections. Both its leaves and roots were reported to possess antioxidant, antibacterial and anti-inflammatory activities. The leaves of *Senna italica* were extracted with *n*-hexane by cold maceration extraction procedure and the resultant extract was subjected to phytochemical analyses using GC-MS technique. Several compounds with known biological activities were detected and identified based on GC-MS data as Phytol (3,7,11,15-Tetramethyl-2-hexadecen-1-ol); 1,2-Benzenedicarboxylic acid, mono (2-ethylheptyl) ester; n-Tetracontane; 13-Docosenamide; Squalene (2,6,10,14,18,22-Hexamethyltetracosane), 1-Heptacosanol; 1,2-Epoxynonadecane; α-Tocopherol-β-D-mannoside; Stigmasterol; γ-Sitosterol and Lupeol. The findings of the study support the usage of *Senna italica* in traditional medicinal as most of the identified compounds are known to possess biological activities.

Keywords: *Senna italica*, *n*-hexane extract, phytochemical constituents, GC-MS, compound identification

INTRODUCTION

Senna italica belongs to the family Fabaceae with abundant distribution in the Capricorn district of Limpopo province, South Africa. *Senna italica* is of medicinal interest due to its wide usage in traditional medicine that is supported by a number of reported biological activities. The leaves of *S. italica* are used for skin problems such as burns and ulcers, whereas the roots are used for many ailments such as liver complications, gallbladder disorders, nausea, dysmenorrhoea and urinary tract infections [1, 2]. The leaves are also reported to possess antioxidant and antibacterial activities [3]. Leaves and pods of medicinal plants from the Senna genus are well known for their laxative properties [4].

Generally, medicinal plants are reported to possess many biological activities such as antimicrobial, anti-inflammatory, anti-diabetic, anti-cancerous and anti-atherosclerosis properties [5]. The nature of biological activities possessed by medicinal plants depends on the types of their inherent phytochemicals. Plants possess a wide range of phytochemical compounds with important applications in human and veterinary medicine [6]. As such, knowledge regarding the identity of chemical constituents of plants is of importance in the discovery of therapeutic agents [7].

The detection and analyses of phytochemical constituents within medicinal plants extracts have relied on a number of chromatographic and spectrometric techniques such as TLC, UV, IR and NMR [8]. Of recent, phytochemical analysis of medicinal plant extracts involve sophisticated techniques that include Gas chromatography instrument coupled with mass spectrometer (GC-MS). GC-MS affords direct analysis of the phytochemicals present in medicinal plant extracts [7]. Based on available literature, the identification of phytochemicals within the *n*-hexane leaf extract of *S. italica* using GC-MS analysis is reported for the first time.

MATERIAL AND METHOD

Plant Material and Extraction

The leaves of *S. italica* were collected from Bolahlakgomo village in the Zebediela sub-region of Limpopo province, South Africa. The identity of the medicinal plant was authenticated and voucher specimen was deposited in the Department of Botany Herbarium, School of Molecular and Life Sciences, University of Limpopo. The leaves were dried at room temperature and ground to powder. The ground leaves were extracted with *n*-hexane by cold maceration technique and the filtered extract was then concentrated by evaporating the solvent using rotary vapour. The concentrated extract was dried under a stream of air and the residue was subjected to phytochemical analysis using GC-MS.

Gas chromatography-Mass spectrometry analysis

GC-MS analysis of the n-hexane leaf extract of *S. italica* was done using a SHIMADZU QP2010 SE gas chromatograph-mass spectrometer (GC-MS) with an Inert cap 5MS/SIL, silica capillary column (30 mm X 0.25 mm ID x 1 µmdf, composed of 100% Dimethyl-poly-siloxane). An electron ionization system with ionizing energy of 70 eV was used for detection. Helium gas (99.99%) was used as the carrier gas at constant flow rate of 1 ml/min with an injection volume of 2 µl; injector temperature of 290 ^{o}C and ion-source temperature of 230 ^{o}C. The oven temperature was set from 50 ^{o}C (isothermal for 1 min), with an increase of 20 ^{o}C/min to 180 ^{o}C (isothermal for 5 min), then increased to 240 ^{o}C at 20 ^{o}C/min, ending with an increase of 20 ^{o}C/min to 280 ^{o}C (isothermal for 5 min). Mass spectra were taken at 70eV; scan interval of 0.3 sec and fragments from 50 to 700 m/z. Total GC running time

was 33.00 min. The relative % amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatogram was a GC-MS SOLUTIONS version 2.6.

Compound Identification

Identification of compounds based on the mass spectra from GC-MS was done using the compound database of National Institute of Standard and Technology (NIST08 library). The mass spectra of unknown compounds were compared with those of the known components stored in the National Institute of Standard and Technology (NIST 08) library with the hit of 90% and above regarded as positive match. The name, molecular weight, molecular formula and amounts of identified compounds were recorded.

RESULTS AND DISCUSSION

The phytochemical constituents within the n-hexane extract of the leaves of *S. italica* were separated according to the GC chromatogram presented in Figure 1. Compounds were eluted between 16 and 28.45 minutes. The detected phytochemicals characterized by their retention time (RT), name, molecular weight (MW), molecular formula and peak area (%) are presented in Table 1. In total, eleven compounds were detected which were identified by comparison of their mass spectra with those of compounds stored in the NIST 08 library. Identified compounds were Phytol (3,7,11,15-Tetramethyl-2-Hexadecen-1-ol) (RT: 16 min); 1,2-Benzenedicarboxylic acid, mono (2-ethylheptyl) ester (RT: 18.72 min); n-Tetracontane (RT: 19.92 min); 13-Docosenamide (RT: 20.72 min); Squalene (2,6,10,14,18,22-Hexamethyltetracosane) (RT: 21 min); Oxirane (1,2-Epoxynonadecane) (RT: 23.61 min); 1-Heptacosanol (RT: 24.3 min); α-Tocopherol-β-D-mannoside (RT: 24.54 min); Stigmasterol (RT: 26.45 min); γ-Sitosterol (RT: 27.06 min) and Lupeol (RT: 28.45). The relative amounts (%) of detected compounds were described on the basis of their calculated peak areas. Compounds in higher amounts were 1-Heptacosanol (7.54 %), Squalene (6.62 %), α-Tocopherol-β-D-mannoside (6.31 %) and Orixane (5.40 %), whereas Stigmasterol (0.42 %), γ-Sitosterol (0.88 %) and Lupeol (0.33 %) were in lower amounts. 1-Heptacosanol (peak no.: 6,8 and12) and Oxirane (peak no.: 7 and10) were represented by more than one peak in the chromatogram at different retention times.

Table 1: Phytochemical constituents identified within the leaf n-hexane extract of *Senna italica* by GC-MS analysis.

Peak no.	Retention time (min)	Compound name	Molecular weight	Molecular formula	Peak area (%)
1	16.01	Phytol (3,7,11,15-Tetramethyl-hexadecen-1-ol)	296	$C_{20}H_{40}O$	1.13
2	18.72	1,2-Benzenedicarboxylic acid, mono (2-ethylheptyl) ester	278	$C_{16}H_{22}O_4$	2.26
3	19.92	n-Tetracontane	618	$C_{44}H_{90}$	4.18
4	20.72	13-Docosenamide	337	$C_{22}H_{43}NO$	3.76
5	20.99	Squalene (2,6,10,14,18,22-Hexamethyltetracosane)	410	$C_{30}H_{50}$	6.62
6 **8** **12**	21.96 24.30 26.79	1-Heptacosanol	396	$C_{27}H_{56}O$	2.31 7.54 3.26
7 **10**	23.61 25.90	Oxirane (1,2-Epoxynonadecane)	282	$C_{19}H_{38}O$	5.40 2.45
9	24.54	(α)-Tocopherol-β-D-mannoside	592	$C_{35}H_{60}O_7$	6.31
11	26.14	Stigmasterol	412	$C_{29}H_{40}O$	0.42
13	27.05	(γ)-Sitosterol	414	$C_{29}H_{50}O$	0.88
14	28.45	Lupeol	426	$C_{30}H_{50}O$	0.33

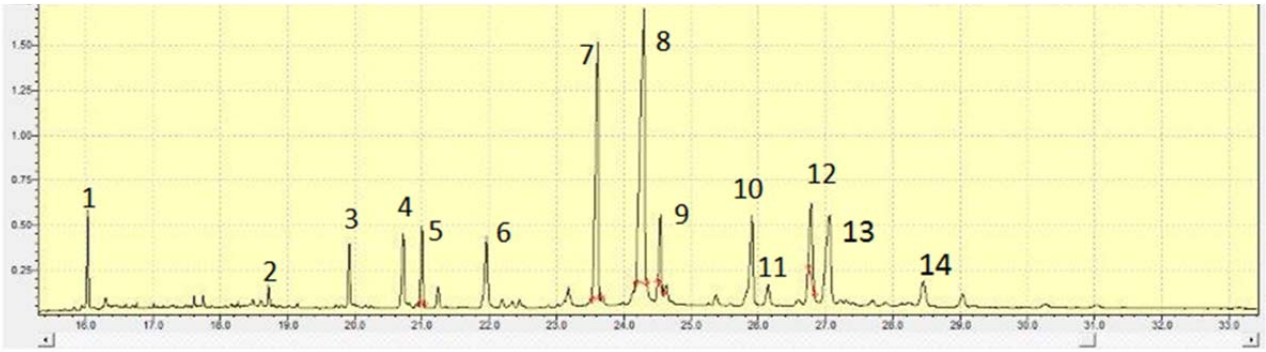

Figure 1: GC-MS chromatogram of the n-hexane extract of the leaves of *Senna italica* (numbers on the chromatogram indicate the peak numbers of identified compounds).

Most of the identified phytochemical constituents are known compounds with biological activities. In this regard, Phytol was reported to have antioxidant activity [9], as well as therapeutic effects against arthritis [10]. Squalene was reported to possess antioxidant and chemopreventive activities [11, 12]. 1, 2-Benzenedicarboxylic acid, mono (2-ethylheptyl) ester is a known plasticiser compound with antimicrobial activity [13]. 13-Docosenamide is a fatty acid amide known for enhancing neovascularization in regenerating skeletal muscles [14] and modulation of water balance in the visceral organs and cerebrospinal fluid [15]. α-Tocopherol-β-D-mannoside is a vitamin E compound with many biological activities that include antioxidant, antimicrobial, anticancerous and enzyme inhibitory properties [7]. Among the detected phytochemical constituents were steroid compounds; Stigmasterol, γ-Sitosterol and Lupeol. Steroids are important class of natural products occurring widely in the plant kingdom with several biological properties that include anticancer and anti-HIV activities [16, 17]. The identification of compounds done in the current study highlight the potential therapeutic properties of the leaves of *Senna italica*.

CONCLUSION

GC-MS analysis enabled identification of eleven phytochemical constituents within the n-hexane extract of the leaves of *Senna italica*. The identified phytochemical constituents are known compounds with biological activities whose presence in *Senna italica* justifies its usage in traditional medicine.

ACKNOWLEDGEMENT

The authors would like to acknowledge the Departments of Chemistry and Biochemistry, at Sefako Makgatho Health Sciences University for GC-MS and research facilities, respectively. Dr Eagan Bronwyn of the Department of Botany, University of Limpopo is also acknowledged for the identification of the plant species.

REFERENCES

[1]. Debela, H., Zemode, A., Ensermu, K. *Ethiopian J. Health Sci*. 2006, 16,141-156
[2]. Masoko, P., Gololo, S.S., Mokgotho, M.P., Eloff, J.N., Howard, R.L., Mampuru, L.J. *Afr. J. Trad. CAM*. 2010, 7, 138-148
[3]. Lekganyane, M.A., Matsebatlela, T.M., Howard, R.L., Shai, L.J., Masoko, P. *Afri. J. Biotechnol*. 2012, 11, 13210-13219
[4]. Rajesham, C., Narasinga rao, N., Venkateswarlu, M., Sammaiah, D., Anitha, U., Ugandhar, T. *Biosci. Discovery* 2013, 4, 81-88
[5]. Gurib-Fakim, A. *Mol. Aspects Med*. 2006, 27, 1-93
[6]. McGaw, L.J., Eloff, J.N. *J. Ethnopharmacol*. 2008, 119, 559-574
[7]. Shunmuga jothi, R., Uthayakumari, F., Bharathy, V. *Biosci. Discovery* 2015, 6, 106-111
[8]. Oyugi, D.A., Ayorinde, F.O., Gugssa, A., Allen, A., Izevbigie, E.B., Eribo, B., Anderson, W.A. *J. Biosci. Tech.* 2011, 2, 287-304
[9]. Yuenyongsawad, S., Tewtrakul, S. *Songklanakarin J. Sci. Tech*. 2005, 27, 497-502
[10]. Ogunlesi, M., Okiei, W., Ofor, E., Osibote, A.E. *Afri. J. Biotech*. 2009, 8, 7042-7050
[11]. Rao, C.V., Newmark, H.L., Reddy, B.S. *Carcinogen.* 1998, 19, 287-297
[12]. Kala, S.M.J., Balasubramanian, T., Tresina soris, P., Mohan, V.R. *Int. J. Chem. Tech. Res*. 2011, 3, 1534-1537
[13]. Sermakkani, M., Thangapandian, V. *Asian J. Pharmac. Clin. Res*. 2012, 5, 90-94
[14]. Mitchel, C., Davies, M., Grounds, M., McGeachie, J., Crawford, G., Hong, Y., Chirila, T. J. *Biomaterials Appl*. 1996, 3, 230-249
[15]. Hamberger, A., Stenhagen, G. *Neurochem. Res*. 2003, 28, 177-185
[16]. Janeczko, A. *Steroids* 2012, 77, 169-173
[17]. Patocka, J. *J. Appl. Biomed*. 2013, 1, 7-12