

GC-MS ANALYSIS AND ANTIMICROBIAL ACTIVITY OF SUDANESE *Solanum incanum* L. (SOLANACEAE) FIXED OIL

Dr. Abdel Karim M.*¹, Inas A.² and Dalia O.

¹Sudan University of Science and Technology, Faculty of Science, Dept. of Chemistry.

²Omdurman Islamic University, Faculty of Pharmacy.

*Corresponding Author: Dr. Abdel Karim M.

Sudan University of Science and Technology, Faculty of Science, Dept. of Chemistry.

Article Received on 17/10/2017

Article Revised on 07/11/2017

Article Accepted on 28/11/2017

ABSTRACT

The present study was designed to investigate the chemical constituents of *Solanum incanum* seed oil and to evaluate its potential antimicrobial activity. GC-MS showed the presence of 27 components. Major constituents are: 9,12-Z,Z-octadecadienoic acid methyl ester (31.65%), 9-Z-octadecenoic acid methyl ester (19.46%), hexadecanoic acid methyl ester (12.39%), campesterol (10.22%), 2,6-bis-(2,4-methylenedioxyphenyl)-3,7-dioxabicyclo(3.3.0) Octane (6.49%), methyl stearate (3.90%), gamma sitosterol (3.02%). The antimicrobial activity of the oil was evaluated via cup plate agar diffusion assay against five standard human pathogens (Gram positive: *Staphylococcus aureus* and *Bacillus subtilis*; Gram negative: *Escherichia coli* and *Pseudomonas aeruginosa* and the yeast *Candida albicans*). The oil gave significant activity against *Pseudomonas aeruginosa*. It also showed excellent activity against *Staphylococcus aureus* and *Escherichia coli*. However, it exhibited weak anticandidal activity.

KEYWORDS: *Solanum incanum*, Fixed oil, GC-MS, Antimicrobial activity.

INTRODUCTION

The genus *Solanum* is widespread in temperate and tropical regions. It is a large genus comprising more than 1700 species.^[1,2] This genus contains a biologically important steroidal alkaloids.^[3-6]

Solanum incanum (thorn apple) is used traditionally against some diseases including: dyspepsia, earache and haemorrhoids.^[7] This species is considered as a potential source for the synthesis of steroids. The plant contains two important glycosides – solamargine and solasonine which are structurally related to steroidal hormones^[8,9] and they are proposed as leads for contraceptives and steroidal antiinflammatory chemotherapeutics.^[2,10] Solamargine and solasonine have been investigated for their antifungal, antibacterial, antidiabetic, antiparacetacetic, antiviral and anticancer activities.^[10,11]

Some reports have indicated that different fruit and leaf extracts exhibited significant antimicrobial activity.^[12-15] Root extract showed marked antipyretic effect.^[16] Also the analgesic efficacy of root extract has been documented.^[17] The hypoglycemic effect of *Solanum imncaum* has been studied.^[18] In one study the spasmolytic properties of root extract has been assessed.^[19]

Solanum species has been used traditionally for centuries for the treatment of cancer. Some studies showed that *Solanum imncaum* extract inhibited sarcoma in model animals. An important constituent of *Solanum imncaum* – solamargine- is capable of disrupting phosphatidylcholine or cholesterol liposomes.^[20]

MATERIALS AND METHODS

Materials

Plant material

Fruits of *Solanum incanum* were collected from Khartoum (Sudan) and authenticated by the Department of Phytochemistry and Taxonomy, National Research Center, Khartoum-Sudan.

Instruments

GC-MS analysis was conducted on a Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m, length; 0.25mm diameter; 0.25 μm, thickness).

Test organisms

Solanum incanum oil was screened for antibacterial and antifungal activities using the standard microorganisms shown in table(1).

Table 1: Test organisms.

Ser. No	Micro organism	Type
1	<i>Bacillus subtilis</i>	G+ve
2	<i>Staphylococcus aureus</i>	G+ve
3	<i>Pseudomonas aeruginosa</i>	G-ve
4	<i>Escherichia coli</i>	G-ve
5	<i>Candida albicans</i>	fungi

Methods**Extraction of oil**

Powdered fruits of *Solanum incanum* (500g) were exhaustively extracted with n-hexane (soxhlet). The solvent was removed under reduced pressure and the oil was kept in the fridge at 4°C for further manipulation.

GC-MS analysis

The oil of *Solanum incanum* was analyzed by gas chromatography – mass spectrometry. A Shimadzo GC-MS-QP2010 Ultra instrument was used. Helium was used as carrier gas. Oven temperature program is given in Table 2, while other chromatographic conditions are depicted in Table 3.

Table 2: Oven temperature program.

Rate	Temperature(°C)	Hold Time (min. ⁻¹)
-	150.0	1.00
4.00	300.0	0.00

Table 3: Chromatographic conditions.

Column oven temperature	150.0°C
Injection temperature	300.0°C
Injection mode	Split
Flow control mode	Linear velocity
Pressure	139.3KPa
Total flow	50.0ml/ min
Column flow	1.54ml/sec.
Linear velocity	47.2cm/sec.
Purge flow	3.0ml/min.
Spilt ratio	- 1.0

Antimicrobial suceptability

The oil was evaluated for its antimicrobial activity using the disc diffusion bioassay.^[21] The antibacterial activity was accomplished on Mueller Hinton agar using four bacterial species, Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*), Gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*). Antifungal activity was assessed on Sabouraud dextrose agar using the fungal species (*Aspergillus niger*, *Candida albicans*). Bacteria test strains were cultured on Mueller –Hinton agar for 24h at 37°C; fungal strains were cultured on Sabouraud dextrose agar at 35°C for four days. A standard suspension(0.5 McFarland) was prepared on normal saline. These suspensions were uniformly spread on the media(Moeller-Hinton agar for bacteria and Sabouraud dextrose agar for fungi). Paper discs (6mm in diameter) were impregnated with 10µl of the test sample, dried and aseptically placed onto the surface of the inoculated

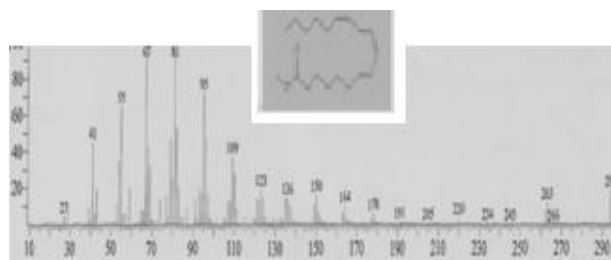
media. The plates were incubated at 37°C for 24h (for bacteria) and at 35° for 72h (for fungi). After incubation the inhibition zones were measured in two replicates and averaged as indicators of activities.

RESULTS AND DISCUSSION

The GC-MS analysis of *Solanum incanum* fruit oil revealed 26 constituents (Table 4). Major constituents are briefly discussed below:

9,12-Z,Z-Octadecadienoic acid methyl ester (31.65%)

The mass spectrum of 9,12-octadecadienoic acid methyl ester is displayed in Fig.2. The peak at m/z294 (R.T. 17.324 -in total ion chromatogram) corresponds to M⁺[C₁₉H₃₄O₂]⁺. The signal at m/z263 corresponds to loss of a methoxyl function.



9-Z-Octadecenoic acid methyl ester(19.46%%)

Fig. 3 shows the EI mass spectrum of 9-octadecenoic acid methyl ester. The peak at m/z 296, which appeared at

R.T. 17.368 in total ion chromatogram, corresponds $M^+[C_{19}H_{36}O_2]^+$ while the peak at m/z 266 accounts for loss of a methoxyl.

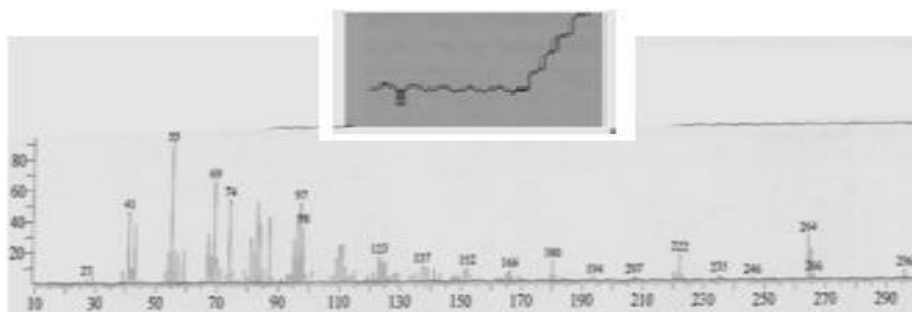


Fig. 3: Mass spectrum of 9-octadecenoic acid methyl ester

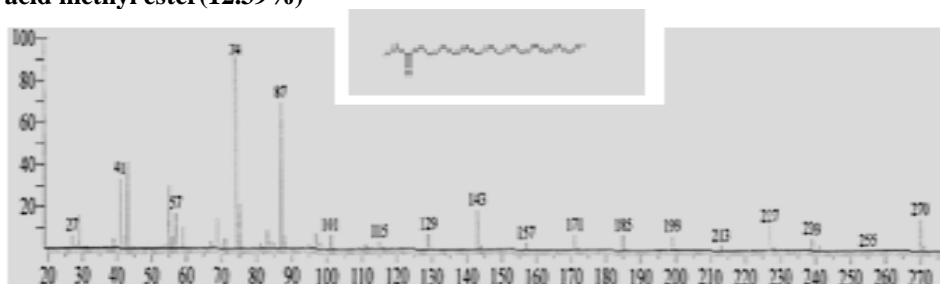
Hexadecanoic acid methyl ester(12.39%)

Fig. 4: Mass spectrum of hexadecanoic acid methyl ester.

The mass spectrum of hexadecanoic acid methyl ester is depicted in Fig.4. The peak at m/z 270 (R.T.15.672) corresponds $M^+[C_{17}H_{34}O_2]^+$. The signal at m/z 239 corresponds to loss of a methoxyl.

Campesterol(10.22%)

Campesterol ($C_{28}H_{48}O$) appeared at retention time 23.362 which corresponds m/z 400 (Fig. 5).

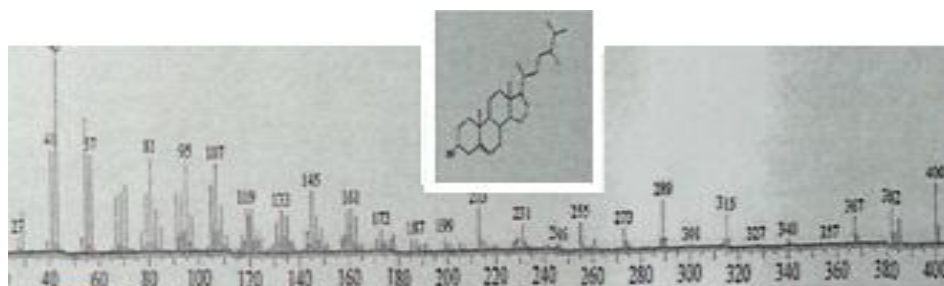


Fig. 5: Mass spectrum of campesterol.

2,6-bis(2,4-methylenedioxyphenyl)-3,7-dioxabicyclo(3.3.0)octane(6.49%)

The mass spectrum of 2,6-bis(2,4-methylenedioxyphenyl)-3,7-dioxabicyclo(3.3.0)octane is

displayed in Fig. 6. The molecular ion (m/z 354) with retention time 22.364 corresponds ($C_{20}H_{18}O_6$).

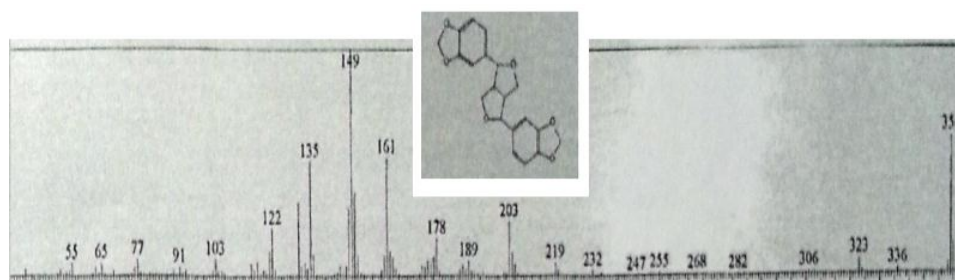


Fig. 6: Mass spectrum of 2,6-bis(2,4-methylenedioxyphenyl)-3,7-dioxabicyclo(3.3.0)octane.

Methyl stearate(3.90%)

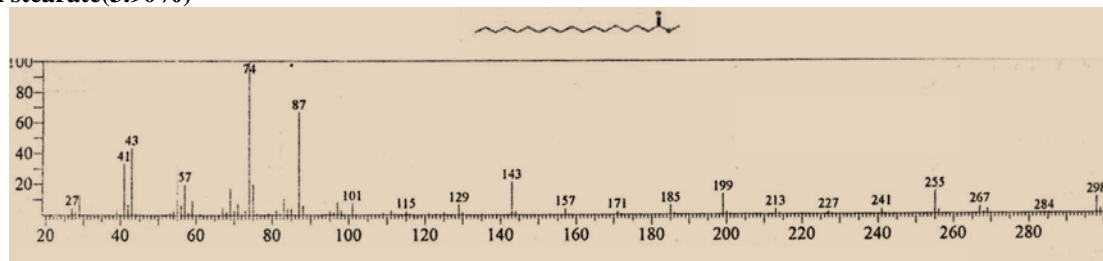


Fig. 7: Mass spectrum of methyl stearate.

Fig.7 shows the mass spectrum of methyl stearate.The signal at m/z 298(R.T.17.585) corresponds $M^+[C_{19}H_{38}O_2]^+$ while the peak at m/z267 corresponds to loss of a methoxyl group.

Gamma sitosterol(3.02%)

The mass spectrum of gamma sitosterol is depicted in Fig.8.The peak at m/z 414 (R.T,18.751) corresponds $M^+[C_{29}H_{50}O]^+$ The signal at m/z400 corresponds to loss of a methyl.

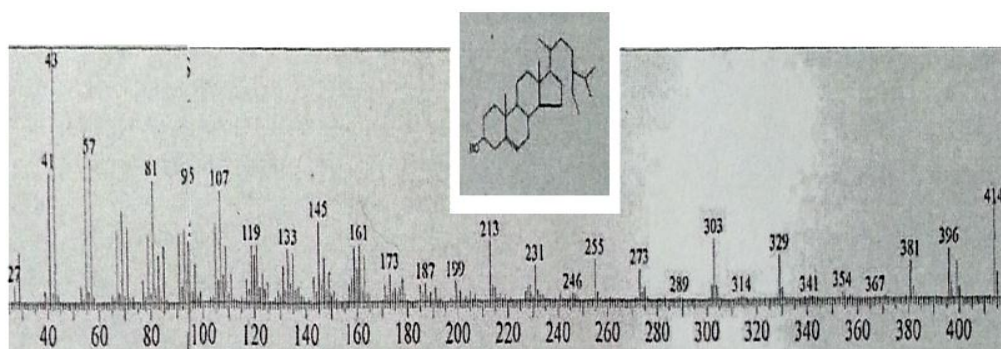


Fig. 8: Mass spectrum of gamma sitosterol.

Antibacterial activity

Solanum incanum oil was assessed for antimicrobial activity against five standard human pathogens. The diameters of the growth of inhibition zones are shown in Table (5). Results were interpreted as follows: (<9mm: inative;9-12mm:partially active;13-18mm: active;>18mm:very active). Tables (6) and (7) show the antifungal and antibacterial activities of standard drugs respectively.

Table 5: Antibacterial activity of *Solanum incanum* oil.

Type	Conc.(mg/ml)	Sa	Bs	Ec	Ps	Ca
Oil	100	18	16	17	25	10

Table 6: Antifungal activity of standard chemotherapeutic agent.

Drug	Conc.(mg/ml)	An	Ca
Clotrimazole	30	22	38
	15	17	31
	7.5	16	29

Table 7: Antibacterial activity of standard drugs.

Drug	Conc.(mg/ml)	Bs	Sa	Ec	Ps
Ampicilin	40	15	30	-	-
	20	14	25	-	-
	10	11	15	-	-
Gentamycin	40	25	19	22	21
	20	22	18	18	15
	10	17	14	15	12

Sa.: *Staphylococcus aureus*
 Ec.: *Escherichia coli*
 Pa.: *Pseudomonas aeruginosa*
 An.: *Aspergillus niger* *Escherichia coli*
 Ca.: *Candida albicans*
 Bs.: *Bacillus subtilis*

The oil gave significant activity against *Pseudomonas aeruginosa*,It also showed excellent activity against *Staphylococcus aureus* and *Escherichia coli*. However, it exhibited weak anticandidal activity.

REFERENCES

- Bhat, M.A., Ahmad,S., Aslam,J., Mujib,B., Mahmood U., zzzfar, *Chem. Pharm. Bull.*, 2008; 56: 17-21.
- Maurya,A., Manika,N., Verma,R.K., Singh,S.C., Sri vasta,S.K., *Phytochem. Anal.*, 2013; 24: 87-9.

3. Carman,A.S., Kuan,S.S., Ware,G.M., Francis,O.J., G. P. Kirsch, G.P., *J. Agric. Food. Chem. Am. Chem. Soc.*, 1986; 279-282.
4. Friedman,M., L. Dao, L., *J. Agric. Food. Chem.*, 1992; 40: 419-423.
5. Milner, S.E., Brunton,N.P., Jones,P.W., O'Brien, N.M., Collins,S.G., Maguire,A. *J. Agric. Food Chem.*, 2011; 59: 3454-3484.
6. Manase,M., Mitaine,A.C.,Offer, D. Pertuit, T. Miyamoto, C.,Tanaka, S. Delemasure, P.,Dutartre, J.F. Mirjolet, O. Duchamp, M.A. Lacaille,D., *Fitoterapia*, 2012; 83: 1115-1119.
7. Ghazanfar,S.,Al-Sabahi,A., *Econ. Bot.*, 1993; 47: 89-98.
8. Dinan,L.,Harmatha,J.,Lafont,R., *J Chromatogr. A*, 2001; 935: 105-123.
9. Trivedi,P.,K.,*Chromatographia*, 2007; 65: 239-243.
10. Tioosi,R.F., Miranda,M.A., Sousa,,B.D., Praca,F.S., Bentley,M.V., Mcchesney,J.D., J.K. Bastos,J.K., *J. Anal. Methods Chem.*, 2012; 20: 1-8.
11. Lee,M.H.,Cheng,J.J., Lin,C.Y., Chen,Y.J.,Lu,M.K., *Process Biochem.*, 2007; 42: 899-903.
12. Mbaya B and Muhammed S., *Anti-microb. Agents Chemother.*, 1976; 6: 920-927.
13. Alamri SA and Moustafa.F., *Saudi Med. J.*, 2012; 33(3): 272-277.
14. Britto, S.J. and Senthinkumar, S., *Asian J. Microbio. Biotechn. Environ. Sci.*, 2001; 3(1-2): 65-66.
15. Taye B, Giday M, Animum A, Seid A., *Asian Pacific J. Trop. Bio Med.*, 2011; 370-375.
16. Mwonjoria, J.K, Kariuki, H. and Waweru, F.N., *Intern. J. Phytopharm.*, 2011; 2(1): 22-26.
17. Le Bars, D., Gozariu, M., *Pharmacol. Rev.*, 2001; 53: 597-652.
18. Musabayane, C.T., Bwititi, P.T. and Ojewole, J.A., *Methods find Exper. Clin Pharmacol.*, 2006; 28(4): 223-228.
19. Assefa, A., Urga, K., and Guta,A., *Ethiop J Biol Sci.*, 2006; 5: 2.
20. Roddick, J.G., Rijneberg, A.L.,Weissenberg, M., *Phytochemistry*, 1990; 29(5): 1513-1518.
21. Vlientink,J., Van-Hoof,L., Lasure, A.,VandenBerghe,D., Rwangabo,P.C., Mwkiumwani,J., *J.Ethnopharmacol.*, 1995; 46: 31.