World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 4.223

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.

[1]Sudan University of Science and Technology, Faculty of Science, Dept. of Chemistry.
[2]Omdurman 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 *Pseudomonasa aeruginos*a 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, antiparacetic, antiviral and anticancer activities.[10,11]

Some reports have indicated that different fruit and leave 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 imncanum* 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 imncanum* e*xtract* inhibited sarcoma in model animals. An important constituent of *Solanum imncanum* – 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	*Bacillus subtilis*	G+ve
2	*Staphylococcus aureus*	G+ve
3	*Pseudomonas aeroginosa*	G-ve
4	*Escherichia coli*	G-ve
5	*Candida albicans*	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^{o}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(^{o}C) Hold Time (min.$^{-1}$)		
-	150.0	1.00
4.00	300.0	0.00

Table 3: Chromatographic conditions.

Column oven temperature	150.0^{o}C
Injection temperature	300.0^{o}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 (*Staphylococus aureus* and *Bacillus subtilis*), Gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*).Antifungal activity was assessed on Sabouuradu dextrose agar using the fungal species (*Aspergillus niger*, *Candida albicans*). Bacteria test strains were cultured on Mueller –Hinton agar for 24h at 37^{o}C; fungal strains were cultured on Sabouraud dextrose agar at 35^{o}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^{o}C for 24h (for bacteria) and at 35^{o} for 72h (for fungi). After incubation the inhibiton 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 $M^{+}[C_{19}H_{34}O_2]^{+\cdot}$The signal at m/z263 corresponds to loss of a methoxyl function.

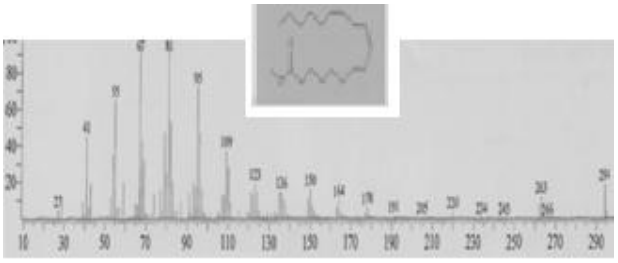

Fig. 2: Mass spectrum of 9,12-octadecadienoic acid methyl ester.

Table 4: Consituents of Solanum incanum oil.

	R.Time	Area	Area%	Name
k#	11.266	57347	0.20	Dodecanoic acid, methyl ester
1	12.129	96694	0.35	(-)-Spathulenol
2	12.599	87735	0.31	Apiol
3	13.576	192725	0.69	Methyl tetradecanoate
4	14.490	20206	0.07	6-Octadecenoic acid, methyl ester, (Z)-
5	14.646	32426	0.12	Pentadecanoic acid, methyl ester
6	14.865	62777	0.22	2-Pentadecanone, 6,10,14-trimethyl-
7	15.480	64247	0.23	9-Hexadecenoic acid, methyl ester, (Z)-
8	15.672	3471833	12.39	Hexadecanoic acid, methyl ester
9	16.332	134363	0.48	Hexadecanoic acid, ethyl ester
10	16.649	60349	0.22	Hexadecanoic acid, 14-methyl-, methyl es
11	17.324	8865857	31.65	9,12-Octadecadienoic acid (Z,Z)-, methyl
12	17.368	5452189	19.46	9-Octadecenoic acid, methyl ester, (E)-
13	17.499	649553	2.32	Phytol
14	17.585	1091272	3.90	Methyl stearate
15	17.930	137261	0.49	Linoleic acid ethyl ester
16	17.970	97705	0.35	Ethyl Oleate
17	18.751	847484	3.02	.gamma.-Sitosterol
18	19.113	202508	0.72	cis-11,14-Eicosadienoic acid, methyl ester
19	19.344	197810	0.71	Eicosanoic acid, methyl ester
20	20.260	148063	0.53	Phenol, 2,2'-methylenebis[6-(1,1-dimethy
21	20.963	451559	1.61	Docosanoic acid, methyl ester
22	21.726	134095	0.48	Tricosanoic acid, methyl ester
23	22.364	1817801	6.49	2,6-Bis(3,4-methylenedioxyphenyl)-3,7-di
24	22.414	298128	1.06	1,3-Benzodioxole, 5,5'-(tetrahydro-1H,3H
25	23.362	2862704	10.22	Campesterol
26	23.793	481320	1.72	Stigmasterol
27		28016011	100.00	

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 $M^{+}[C_{19}H_{36}O_2]^{+\cdot}$ while the peak at m/z266 accounts for loss of a methoxyl.

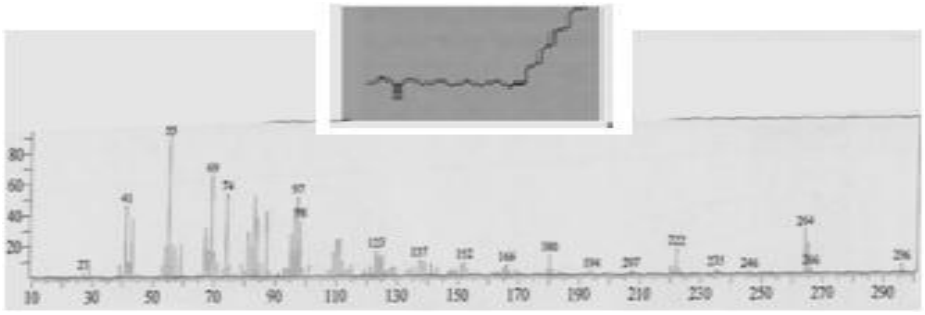

Fig. 3: Mass spectrum of 9-octadecenoic acid methyl ester

Hexadecanoic acid methyl ester(12.39%)

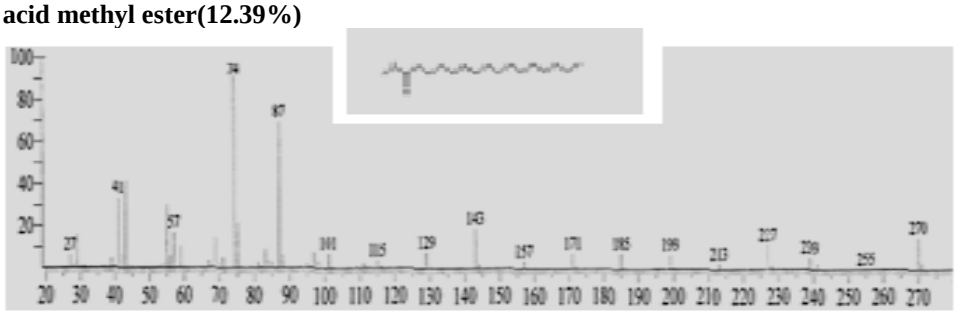

Fig. 4: Mass spectrum of hexadecanoic acid methyl ester.

The mass spectrum of hexadecanoic acid methyl ester is depicted in Fig.4.The peak at m/z 270 (R.T.15.672) corresponds $M^{+}[C_{17}H_{34}O_2]^{+\cdot}$The signal at m/z239 corresponds to loss of a methoxyl.

Campesterol(10.22%)

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

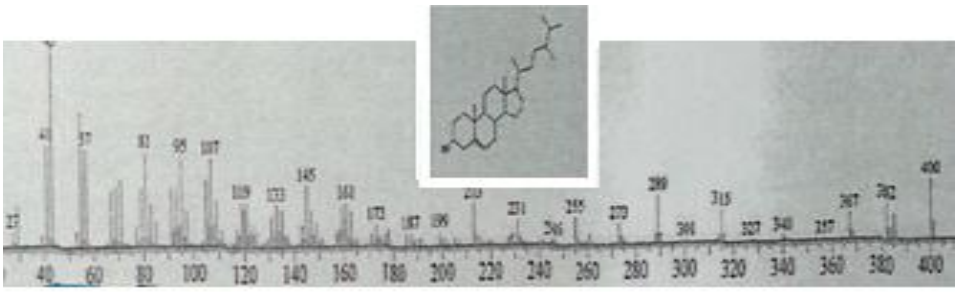

Fig. 5: Mass spectrum of campesterol.

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/z354) with retention time 22.364 corresponds ($C_{20}H_{18}O_6$).

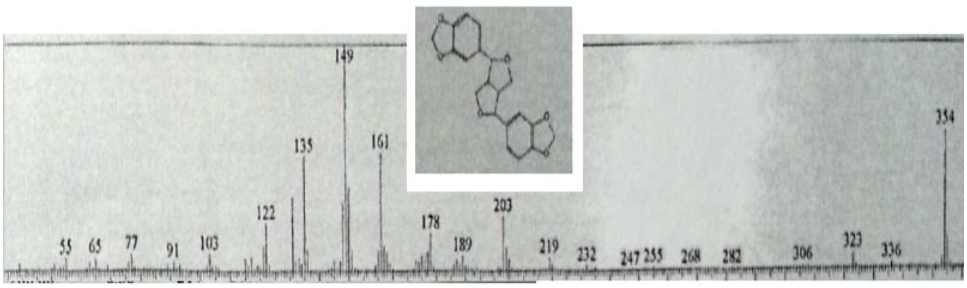

Fig. 6: Mass spectrum of 2,6-bis(2,4-methylenedioxyphenyl)-3,7-dioxabicyclo(3.3.0)octane.

Methyl stearate(3.90%)

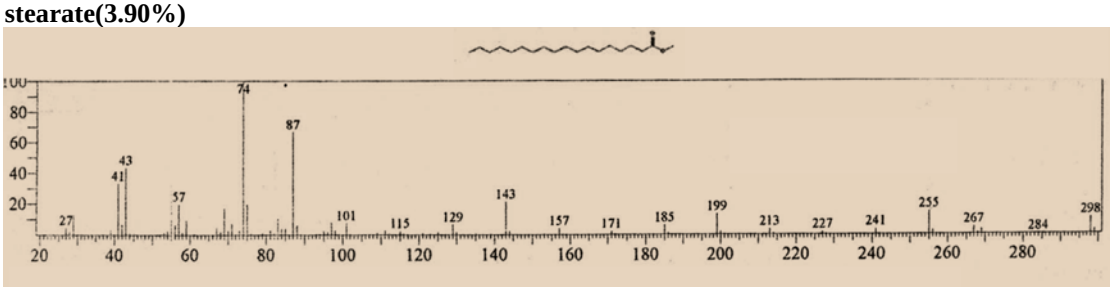

Fig. 7: Mass spectrum of methyl stearate.

Fig.7 shows the mass spectrum of methyl stearate.The signal at m/z 298(R.T.17.585) corresponds $M^{+}[C_{19}H_{38}O_2]^{+\cdot}$ 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 $M^{+}[C_{29}H_{50}O]^{+\cdot}$The signal at m/z400 corresponds to loss of a methyl.

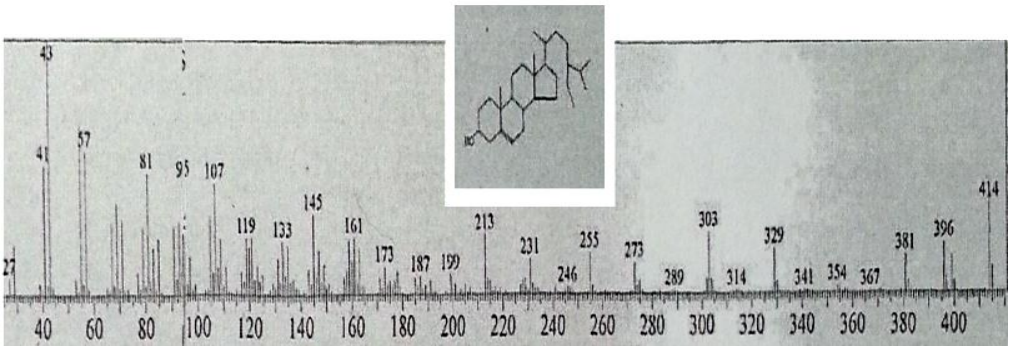

Fig. 8: Mass spectrum of gamma sitosterol.

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

1. Bhat, M.A., Ahmad,S., Aslam,J., Mujib,B., Mahmood U., zzfar, *Chem. Pharm. Bull*., 2008; 56: 17-21.
2. 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. Miya moto, C.,Tanaka, S. Delemasure, P.,Dutartre, J.F. M irjolet, 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. Tiossi,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. 14-Britto, S.J. and Senthinkumar, S., *Asian J. Microbio. Biotechn. Environ. Sci.,* 2001; 3(1-2): 65-66.
15. Taye B, Giday M, Animut 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.,Vanden-Berghe,D., Rwangabo,P.C., Mwkiyumwani,J., *J.Ethnopharmacol*., 1995; 46: 31.