Open Access Journal

Indian Journal of Medical Research and Pharmaceutical Sciences

June 2016; 3(6) ISSN: ISSN: 2349-5340

Impact Factor: 3.052

GC-MS ANALYSIS OF PHYTOCHEMICALS OF METHANOLIC EXTRACT OF LEAVES OF *LAWSONIA INERMIS LINN*

Swami Narsingh Chandra Dev, Kantishree De and Mohd. Washid Khan

Dept. of Post-Graduate Studies and Research in Biological Sciences, Rani Durgawati Vishwavidyalaya, Jabalpur (M.P.)

Keywords:

Lawsonia inermis Methanol extract; GC-MS analysis; Phytochemical compounds.

Abstract

L. inermis leaves powder extracted with methanol by Soxhlet, the presence of phytochemical compounds determined by using Gas Chromatography-Mass Spectrometry (GC-MS) analysis. It showed different peaks with low and high molecular weight determining the presence of 51 compounds. Among them predominantly Squalene (19.77%) 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (14.90%); 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (11.52%); Pentadecanoic acid (10.54%); Vitamin E (6.82); Hexadecanoic acid, methyl ester (5.85%); 9,12-Octadecadienoicc acid (Z,Z)-, Methyl ester (4.98%); Stigmast-5-En-3-Ol, (3.Beta.)- (5.67), Phytol (1.77%) were present. These are mostly reported for the different pharmacological efficacy.

Introduction

Screening of active components from plants has direct to the development of new medicinal drugs which have efficient protection and treatment role against various diseases (Mukhrjee, 2007). Gas Chromatography-Mass Spectroscopy, a hyphenated system which is a very compatible technique and the most commonly used for the identification and quantification purpose. The unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra **(**Ronald, 1997).

Lawsonia inermis is an important medicinal plant in the Indian system of medicine. It is commonly called as henna or mehandi which grows in warm and arid regions. The dye derived from green leaves of henna is used to decorate the body with intricate designs and the principle coloring matter is lawsone, 2-hydroxy-1, 4- naphthoqunone (Prosen *et al*., 2005).

Materials and Methods

Collection and Identification of Plant materials

Fresh leaves of *L. inermis* were collected from Panagar block of Jabalpur district, Madhya Pradesh, India. This plant was identified in Forest Botany Division, State Forest Research Institue (SFRI). The leaves were shade dried and ground into fine powder and stored in air tight containers till further use.

Preparation of extracts

The dried samples were extracted with methanol by using soxhlet extraction method (Ramkumar *et al*., 2007). The extract was concentrated in a water bath till dry powder was obtained. This was then analyzed by using GC-MS with dilution of 1mg/ml in methanol.

Gas Chromatography-Mass spectrometry (GC-MS) analysis

The GC-MS was performed by using Simadzu QP2010Ultra Model and the software used is Turbomass ver 5.2. The fused silica column was packed with Elite -5MS(5% Phenyl 95% dimethylpolysiloxane,30m x 250µm)The oven temperature was set up from 50ºC with an increase of 8 oC/min to 220 o C for 5 min and 7ºC /min to 280 ºC for **15** mins. Helium gas (99.999%) was used as the carrier gas at constant flow rate of 1 ml/min. An aliquot of 2µl of sample was injected into the column with the injector at 280ºC of the split ratio of 10:1. The ionizing energy of 70

eV was used and the electron ionization is involved. The mass range is 40-600amu.The Inlet line temperature was 200 ºC and source temperature was 150 ºC Total GC running time was 60 minutes.

Identification of compounds

Interpretation of mass spectrum of GC-MS was conducted using the database of National Institute Standard and Technique (NIST Version-Year 2005) having more patterns. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The spectrum of the unknown component was compared with the spectrum of the known component stored in the NIST data library. The molecular weight, molecular formula, structure and name of the components of the test material were determined.

Results and Discussion

GC–MS chromatogram of the methanol extract of leaves of *Lawsonia inermis* clearly showed fifty one peaks indicating the presence of fifty one compounds (Fig. 1). The identification of phytochemical compounds was based on the peak area, retention time and molecular formula (Table-1). The results reveal the presence of total 51 compounds, among them Squalene was maximum (19.77%) followed by 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (14.90%); 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (11.52%); Pentadecanoic acid (10.54%); Vitamin E (6.82); Hexadecanoic acid, methyl ester (5.85%); 9,12-Octadecadienoicc acid (Z,Z)-, Methyl ester (4.98%); Stigmast-5-En-3-Ol, (3.Beta.)- (5.67); 5-Hydroxymethylfurfural (1.86); Phytol (1.77); Methyl stearate (1.26); Phytol (1.77%); Phenol, 2,6-Bis (1,1-Dimethylethyl)- (0.19); etc.

Predominantly compounds exhibited from the methanolic extract i.e Squalene by peak at tR 36.92 min, it is a terpenoid and a biological precursor of steroids with activities against colon, lung and skin cancers. It is also protet human skin surfaces from lipid peroxidation due to exposure to UV light and other sources of oxidative damage (Huang *et al*., 2009).

Hexadecanoic acid methyl ester, also known as Methyl palmitate, in the methanol fraction is an aliphatic acid ester reported to cause growth inhibition and apoptosis induction in human gastric cancer cells (Yu *et al*., 2005). Phytol was also present which is a diterpene alcohol which functions as a precursor for Vitamins E and K1 and an antioxidant and a preventive agent against epoxide-induced breast cancer (Yu *et al*., 2005). It's also an effective vaccine adjuvant with no adverse auto-immune effects (Lim *et al*., 2006).

Some other studieds on *L. inermis*, six compounds were identified by **Hema *et al*., 2010** in *L.inermis* by GC-MS analysis. Similarily Jacob and **Saral (2013)** identified five major compounds in seed oil which were trans-fatty acid in nature may be important in pharmaceutical applications. **Rajeswari and Rani, (**2015) reported 41 phyto compounds in ethanol extract of roots of *L. inermis* with their biological activity.

Almost similar 7 compounds had been also reported from the methanolic extract of *G.sylvestre* by Parimala Devi (2010**)** and Subhashini *et al.* **(2015**) where terpenes, saturated and unsaturated fatty acids such as squalene , 9-Octadecenoic acid (Z)-, methyl ester were found.

Most of these bioactive compounds were also reported from different plant extracts by **Daniel *et al* (2011**) from *Vernonia amygdalina* extract; Rajeswari *et al*., (2012**)** from *Hugonia mystax* L.; **Sivakumar and Dhivya (2015**) from *Cordia monoica* Roxb.

Indian Journal of Medical Research and Pharmaceutical Sciences
June 2016; 3(6) ISSN: ISSN: 2349-5340
Impact Factor: 3.052

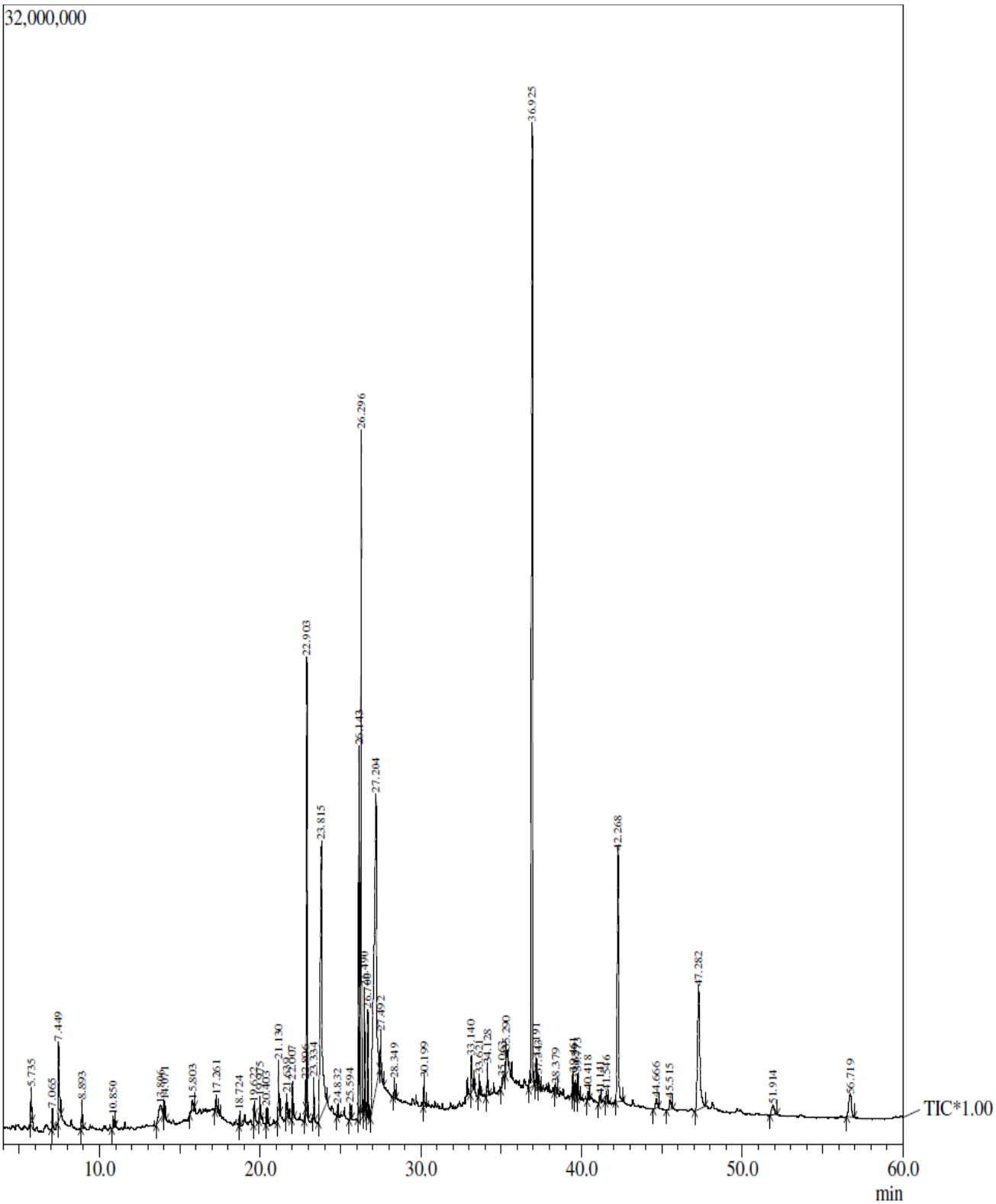

Fig. 1 Chromatogram of methanolic extract of L. Inermis leaves (Li-M1, Crude)

Open Access Journal

Indian Journal of Medical Research and Pharmaceutical Sciences

June 2016; 3(6) ISSN: ISSN: 2349-5340

Impact Factor: 3.052

Table 1: Phytochemical compounds identified in methanol extract of roots of *Lawsonia inermis*

Peak No.	Compound name	Chemical structure	Mol.wt.	tR (min)	Area%
1	2,3-DIHYDRO-3,5-DIHYDROXY-6-METHYL-4H-PYRAN-4-ONE	$C_6H_8O_4$	144	5.735	0.54
2	Dianhydromannitol	$C_6H_{10}O_4$	146	7.065	0.31
3	5-Hydroxymethylfurfural	$C_6H_6O_3$	126	7.449	1.86
4	5-(Hydroxymethyl)-2-(dimethoxymethyl)furan	:$C_8H_{12}O_4$	172	8.893	0.36
5	5-OXO-PYRROLIDINE-2-CARBOXYLIC ACID METHYL ESTER	$C_6H_9NO_3$	143	10.850	0.22
6	beta.-D-Glucopyranose, 1,6-anhydro-	$C_6H_{10}O_5$	162	13.806	0.92 .
7	PHENOL, 2,6-BIS(1,1-DIMETHYLETHYL)-	$C_{14}H_{22}O$	206	14.071	0.19
8	3-HEXENE, 2-(3-DEUTERO-2,2-DIMETHYLCYCLOPROPYL)-2-METHYL-	$C_{12}H_{21}D$	167	15.803	0.21
9	BENZENEACETIC ACID, 4-HYDROXY-3-METHOXY-	:$C_9H_{10}O_4$	182	17.261	0.32
10	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242	18.724	0.15
11	TETRADECANOIC ACID	$C_{14}H_{28}O_2$	228	19.622	0.19
12	CYCLOTETRADECANOL, 1,7,11-TRIMETHYL-4-(1-METHYLETHYL)-, 13	:$C_{20}H_{40}O$	296	19.975	0.33
13	2-Cyclohexen-1-one, 4-hydroxy-3,5,6-trimethyl-4-(3-oxo-1-butenyl)-	$C_{13}H_{18}O_3$	222	20.403	0.23
14	2,6,10-TRIMETHYL,14-ETHYLENE-14-PENTADECNE	$C_{20}H_{38}$	278	21.130	0.86
15	2,6,10-TRIMETHYL,14-ETHYLENE-14-PENTADECNE	$C_{20}H_{38}$	278	21.639	0.34
16	2-HEXADECEN-1-OL, 3,7,11,15-TETRAMETHYL-, [R-[R*,R*-(E)]]-	$C_{20}H_{40}O$	296	22.007	0.54
17	Oxirane, hexadecyl-	$C_{18}H_{36}O$	268	22.806	0.36
18	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	22.903	5.85
19	1-HEXADECEN-3-OL, 3,5,11,15-TETRAMETHYL-	$C_{20}H_{40}O$	296	23.334	0.50
20	PENTADECANOIC ACID	$C_{15}H_{30}O_2$	242	23.815	10.54
21	HEPTADECANOIC ACID, METHYL ESTER	$C_{18}H_{36}O_2$	284	24.832	0.14
22	2(3H)-FURANONE, 5-HEPTYLDIHYDRO-	$C_{11}H_{20}O_2$	184	25.594	0.23
23	9,12-OCTADECADIENOIC ACID (Z,Z)-, METHYL ESTER	$C_{19}H_{34}O_2$	294	26.143	4.98
24	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292	26.296	11.52
25	Phytol	$C_{20}H_{40}O$	296	26.490	1.77
26	Methyl stearate	$C_{19}H_{38}O_2$	298	26.700	1.26
27	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	:$C_{18}H_{30}O_2$	278	27.204	14.90
28	Octadecanoic acid	$C_{18}H_{36}O_2$	284	27.492	0.67
29	Phytol, acetate	$C_{22}H_{42}O_2$	338	28.349	0.27
30	Methyl 18-methylnonadecanoate	$C_{21}H_{42}O_2$	326	30.199	0.40
31	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	: $C_{19}H_{38}O_4$	330	33.140	0.78

32	1,2-BENZENEDICARBOXYLIC ACID	$C_{24}H_{38}O_4$	390	33.621	0.23
33	PHENOL, 4,4'-METHYLENEBIS[2,6-BIS(1,1-DIMETHYLETHYL)-	$C_{24}H_{38}O_4$	424	34.128	0.47
34	1,1'-Biphenyl-3,4,4'-trimethoxy-6'-formyl-	$C_{16}H_{16}O_4$	272	35.063	0.26
35	TRIACONTANE	$C_{30}H_{62}$	422	35.290	0.17
36	Squalene	$C_{30}H_{50}$	410	36.925	19.67
37	(E,E,E)-3,7,11,15-Tetramethylhexadeca-1,3,6,10,14-pentaene	$C_{20}H_{32}$	272	37.191	0.42
38	Triacontyl pentafluoropropionate	$C_{33}H_{61}F_5O_2$	584	37.343	0.23
39	4-METHYL-6-(2,6,6-TRIMETHYL-1-CYCLOHEXEN-1-YL)-4-HEXEN-1-40	:$C_{16}H_{28}O$	236	38.379	0.20
40	(6E,10E,14E,18E)-2,6,10,15,19,23-HEXAMETHYL-1,6,10,14,18,22-	$C_{30}H_{50}O$	426	39.461	0.37
41	Hexadeca-2,6,10,14-tetraen-1-ol, 3,7,11,16-tetramethyl-	$C_{20}H_{34}O$	290	39.629	0.30
42	SOLANESOL	:$C_{45}H_{74}O$	630	39.773	0.52
43	Stigmasta-5,22-dien-3-ol, acetate, (3.beta.)-	:$C_{31}H_{50}O_2$	454	40.418	0.17
44	DOCOSANE	$C_{22}H_{46}$	310	41.141	0.17
45	Stigmast-5-en-3-ol, oleate	$C_{47}H_{82}O_2$	678	41.546	0.22
46	Vitamin E	$C_{29}H_{50}O_2$	430	42.268	6.82
47	ERGOST-5-EN-3-OL, (3.BETA.,24R)-	$C_{28}H_{48}O$	400	44.666	0.30
48	Stigmasterol	$C_{29}H_{48}O$	412	45.515	0.35
49	STIGMAST-5-EN-3-OL, (3.BETA.)-	$C_{29}H_{50}O$	414	47.282	5.67
50	Stigmast-4-en-3-one	$C_{29}H_{48}O$	412	51.914	0.48
51	Phytol, acetate	:$C_{22}H_{42}O_2$	338	56.719	1.21

Therefore by this investigation it is clear that methanol differentially extracted active compounds from *L.inermis* plant leaves which may be useful for further pharmacological studies.

Conclusion

GC-MS analysis revealed presence of fifty one phytochemical constituents in methanol extract of leaves of *L.inermis*. The presence of phytochemicals *viz*. squalene, phytol and sterols justifies the uses of this plant in various ailments by traditional practitioners. Therefore *L.inermis* may be used as a source of these pharmacologically important compounds. However further studies will need to be evaluate biological activities of the compounds.

Acknowledgement

The authors express their deep sense of gratitude to the Dr. Ajai Kumar, AIRF Laboratory, Jawaharlal University (JNU), New Delhi, for providing GC-MS equipment facilities.

References

1. Mukherjee P K, Kumar V, Houghton P J,. 2007. Screening of Indian medicinal plants for acetyl cholinesterase inhibitory activity. Phytother.Res.; 21: 1142-5.
2 Ronald Hites A, Gas Chromatography Mass Spectroscopy: Handbook of Instrumental Techniques for Analytical Chemistry, 1997. pg. 609-611.
3 Prosen H, Antonic J, Klobcar A, Determination of some organochlorine compounds in herbal colouring agent henna (*Lawsonia inermis*) and in tea (*Thea sinensis*), Arh Hig Rada Toksikol 2005; 56, 1-7.

4 Parimala Devi B and Ramasubramaniaraja R. Pharmacognostical and antimicrobial screening of *Gymnema sylvestre* R.Br, and evaluation of gurmar herbal tooth paste and powder, composed of *Gymnema sylvestre* R.Br, extracts in dental caries. International Journal of pharma and Bio sciences. 2010 ;s 1(3): 1-16.
5 Zih-Rou Huang 1, Yin-Ku Lin 2,3 and Jia-You Fang, 2009. Biological and Pharmacological Activities of Squalene and Related Compounds: Potential Uses in Cosmetic Dermatology. *Molecules, 14,* 540-554
6 M.S. Subashini, P. Rajendran, G.Ashok and B.M. Kanthesh, 2015.TLC, FTIR and GCMS analysis of leaves of *Gymnema sylvestre* R.Br from Kolli Hills, Tamil Nadu, India. *Int.J.Curr.Microbiol. App.Sci* (2015) 4(7): 757-764.
7 Senthilkumar, S., Devaki, T., Manohar, B. M., Babu, M. S., *Clinica Chimica Acta*. **2005**, 364, 335-342.
8 Yu, F., Gapor, A., Bender, W., *Cancer Detection and Prevention.* **2005**, 29, 383-388.
9 Lim, S., Meyer, M., Kjonaas, R. A., Ghosh, S. K., *Journal of Immune Based Therapies and Vaccines*. **2006**, 6, 1476-8518.
10 Yu, F., Lian, X., Guo, H., Mc Guire, P., Li, R., Wang, R., Yu, F., *J. Pharm. Pharmaceut. Sci.* **2005**, 3, 528-535.
11 G Rajeswari, 1 M Murugan2 and VR Mohan2*, 2012, GC-MS analysis of bioactive components of *Hugonia mystax L*. (Linaceae) , Research Journal of Pharmaceutical, Biological and Chemical Sciences. RJPBCS Vol. 3 Issue 4 pp. 301-308.
12 R. Sivakumar*, A. Dhivya,2015, GC-MS analysis of bioactive compounds on ethyl acetate extract of *Cordia monica* Roxb. leaves. *International Journal of Research and Development in Pharmacy and Life Sciences*, Vol. 4, No.1, pp 1328-1333.
13 P. P. Jacob and A. M. Saral, 2013. GC-MS analysis of *Lawsonia inermis* seed oil. *Int J Pharm Pharm Sci, Vol 5, Suppl 2, 617-618.*
14 Ramkumar *K.M ., P. Rajaguru and 2R. Ananthan (2007).* Antimicrobial Properties and Phytochemical Constituents of an Antidiabetic Plant *Gymnema montanum.* Advances in Biological Research 1 (1-2): 67-71.