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Research Article

Phytochemical Studies on *Nerium oleander L.* Using GC-MS

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ABSTRACT
Phytoconstituents are chemical compounds often referred to as secondary metabolites. Fifty eight bioactive phytochemical compounds were identified in the solvent free extract of *Nerium Oleander L.* root *using Keshav Narean Apparatus*[3] [IP no. 2343/MUM/2013A] .The identification of phytochemical compounds is based on the peak area, retention time, molecular weight and molecular formula. A wide range of fatty acids, heterocyclic compound which are having anticancer, anti-fungal, anti-inflammatory and antibiotic activity were identified so that it can be recommended as a plant of phytopharmaceutical importance. The GCMS analyse Fifty eight phytochemical compounds in *Nerium oleander L.* root extract. The major phytoconstituents were 2-methoxy-5-methyl phenol and isomers (2.92%), 2, 6-dimethoxyphenyl ester (4.47%), trans-Isogenol (4.89%),5-ter-butylpyrogallol(2.25%),2-hydroxy-4-isopropyl-7 methoxytropone (3.72%),l-(+)-ascorbic-acid-2,6-dihexadecanoate,(4.47%),trans-Isogenol(4.89%),2,3,5-trimetho oxyamphetamine (2.25%), 2-hydroxy-4-isopropyl-7-methoxytropone (3.72 %), Oleic acid(1.09 %), 2-(9,12- octadecadienyloxy)-,(Z,Z)-ethanol (9.48 %), 9-hexadecenoic acid (2.24%), 9-octadecenamide (3.13%), α-amyrin (10.85 %).

Keywords: Phytoconstituents, GC-MS, phytopharmaceutical, *Nerium Oleander L.*

INTRODUCTION

Medicinal plants form the backbone of traditional medicine in the last few decades with intense pharmacological studies. They are regarded as potential sources of new compounds of therapeutic value and as sources of lead compounds in drug development. In developing countries, it is estimated that about 80% of the population really depends on traditional medicine for their primary healthcare. There arises a need to screen medicinal plants for bioactive compounds as a basis for further pharmacological studies. Plants are rich sources of secondary metabolites with interesting biological activities. In general, these secondary metabolites are an important source with a variety of structural arrangements and properties. Natural products from microbial sources have been the primary source of antibiotics, but with the increasing recognition of herbal medicine as an alternative form of health care, the screening of medicinal plants for active compounds has become very significant. The family *Apocynaceae* consists of several important medicinal plants with wide range of biological activities and interesting phytochemical constituents[1]. *Nerium Oleander l.* commonly known as the kaner is a genus of tropical shrubs and vines belonging to the family *Apocynaceae.*

Ayurveda stresses the use of plant-based medicines and treatments. But when compared the Chinese medicine is more established than Ayurveda medicine. This is due to even after Chinese people migrating to other countries they still follow their own culture. And also the Chinese people wherever in the world are actively participating in export and import of their medical system . It is a sad fact that nowadays we are moving away from nature and due to our undisciplined life style new diseases are being identified. But the fact is that our rich nature contains remedy for all diseases. Potentially valuable treasures in medicinal plants remain unexplored. By considering the scope of these medicinal plants we have to use more amounts of time and resources into developing medicines by medicinal plants. If we can come back to our nature, culture and tradition on use of medicinal plants it can bring up a bright and healthy new generation. Gas Chromatography-Mass Spectroscopy, a hyphenated system which is a very compatible technique and the most commonly used technique 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[2]. The volatile compounds in plant were determined by gas chromatography (GC) with mass spectrometry (MS).

MATERIALS AND METHODS

Collection and Preparation of plant materials

The roots of plant were collected at Igatpuri region, Akole, Maharashtra. The taxonomic identification was made by BSI pune.(Voucher no. BSI/WRC/Cert./2015/121). The roots were washed thoroughly in running tap water to remove soil particles and adhered debris and finally washed with sterile distilled water. The cleaned roots are chopped and stored at cold condition.

Plant sample extraction

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Table 1: Phytoconstituents identified in root extract of *Nerium oleander l.* by GCMS.

Sr. No.	RT	Name of the compound	Molecular Formula	MW	Peak area (%)	Compound Name	Reported Activity
1	7.55	P-cresol	C_7H_8O	108	0.91	Phenol	Cytotoxic[4], antioxidant[4], antimicrobial
2	7.33	2-methoxy-phenol,Mequinol	$C_7H_8O_2$	120	1.09	phenol	Anticancer[4]
3	7.33	2-methoxy phenyl ester	$C_8H_8O_3$	152		ester	Namaticide[1], pesticide[1]
4	8.07	Androsta-1,4-diene-3-one,17-hydroxy-(17α'-)	$C_{20}H_{28}O_2$	300	0.70	stereoid	Mild anticancer[7], pain killer , jaundice
5	8.30	[5,9-Dimethyl-1-(3-phenyl-oxiran-2-yl)-deca-4,8-dienylidene]-(2-phenyl-aziridin-1-yl) –amine	$C_{28}H_{34}N_2O$	414	0.95	alkaloid	***
6	8.30	4-hexyl-(7-methoxycarbonylheptyl)bicycle[4.4.0] deca-2,5,7-triene	$C_{25}H_{40}O_2$	372	0.95	ester	Namaticide[1]
7	9.13	1H-cyclopropa[3,4]benz91,2-e]azulene-5,7b.9,9a-tetrol	$C_{24}H_{34}O_7$	434	1.29	phenol, ester ,azulene	Anticancer[4], Antimycrobial, Antifungal[5]
8	9.13	Cyclopropene ,1-(3-acetoxy-1,1-dimethylhexan-5-onyl)- 2-isopropenyl	$C_{16}H_{24}O_3$	264	1.29	ester	Antimycrobial[5], Antifungal[5]
9	9.34	2-methoxy-5-methyl phenol and isomers	$C_8H_{10}O_2$	138	2.92	phenol	Cytotoxic[4]
10	10.01	2H-Benzo[f]oxireno[2,3-E] benzofuran8-(9H)-one,octahydro-9-[[[(2-methoxyphenyl) methyl]amino]-methyl]-2,5a-dimethyl-	$C_{23}H_{31}NO_4$	385	0.42	Lactone, epoxide	Antimycrobial[8]

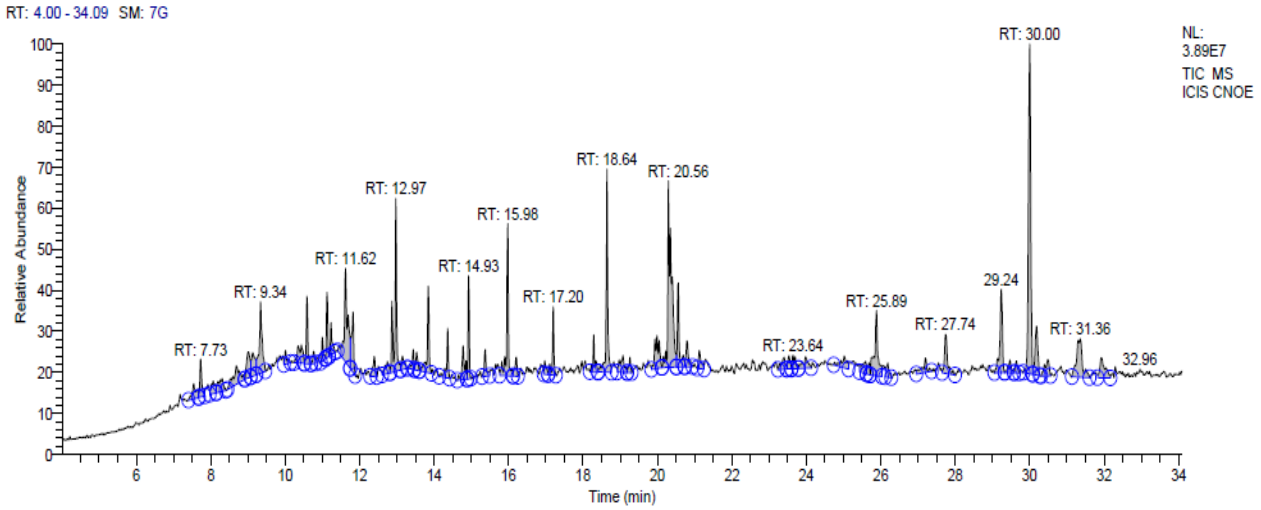

Figure 1: GCMS chromatograph of solvent free extract of *Nerium Oleander l.*

8 kg chopped root sample was successively add to Keshav Narean apparatus[3]. The sample was filled in container without solvent and constant heat for 8 hrs. Then extract was collected. The concentrated extract was analysed by

Table 2: Phytoconstituents identified in root extract of *Nerium oleander l.* by GCMS.

Sr. No.	RT	Name of the compound	Molecular Formula	MW	Peak area (%)	Compound Name	Reported Activity
11	10.44	2-(1,2,3,4-tetrahydronapthalene-1-ylidene)hydrazine-1-carbothioamide	$C_{11}H_{13}N_3S$	225	1.26	thioamide	Cytotoxic, antimicrobial
12	10.44	α-D-Glucofuranose	$C_6H_{12}O$	180	1.26	Sugar moiety	***
13	10.59	4- ethyl-2-methoxy-phenol	$C_9H_{12}O_2$	162	1.37	phenol	Anticancer[4], antioxidant[4], antimicrobial
14	10.59	5-Isopropyl-3,3-dimethyl-2-methylene-2,3-dihydrofuran	$C_{10}H_{16}O$	152	1.37	furan	Cytotoxic[9], antimycrobial
15	10.76	3-methyl -1H-Indazole	$C_8H_8N_2$	132	0.33	Indazole	Anti-inflammatory, anti-microbial, anticancer
16	10.76	4-(1H-1,2,3,4 – tetrazolr-1-yl)-benzeneaceticacid	$C_9H_8N_4O_2$	204	0.33	tetrazole	Antifungal[10], antimicrobial[10], Analgesic[10], Antinociceptive[10]
17	11.13	2-methoxy-4-vinyl phenol	$C_9H_{10}O_2$	150	1.18	Conjugated phenol	Anti-oxidant , Cytotoxic
18	11.62	2,6-dimethoxyphenyl Ester	$C_9H_{10}O_4$	172	4.47	ester	Pesticide[1], antifungal
19	11.82	A-ethyl-4-methoxy-benzenemethanol	$C_{10}H_{14}O_2$	166	1.98	alcohol	Antiinflammatory [11],analgesic
20	11.82	1H-1,2,3,4-Tetrazole1,5-diamine, N(1)[(2-ethoxy3methoxyphenyl) methyl]	$C_{11}H_{16}N_6O_2$	264	1.98	tetrazole	Antifungal[10]

Table 3: Phytoconstituents identified in root extract of *Nerium oleander l.* by GCMS.

Sr. No.	RT	Name of the compound	Molecular Formula	MW	Peak area (%)	Compound Name	Reported Activity
21	12.39	[5,9-Dimethyl1(3-phenyloxiran2yl) Deca-4,8-dienylidene](2-phenylaziridin1yl) amine	$C_{28}H_{34}N_2O$	430	0.65	Epoxide	Antimicrobial
22	12.75	Anobin[6]	$C_{15}H_{20}O_5$	280	0.66	Sesqiterepene lactone	Antitumor[12]
23	12.97	trans- Isogenol	$C_{10}H_{12}O_2$	164.2	4.89	Conjugated phenol	Antifungal[5]
24	13.15	6,7-epoxypregn-4-ene-9,11,18-triol-3,20-dione, 11,18-diacetate	$C_{25}H_{32}O_8$	460	0.57	stereoid	***
25	13.44	Oleic acid, eicosyl ester	$C_{38}H_{74}O_2$	562	0.48	Fatty acid	5-α- reductase inhibitor, allergenic ,anti-inflammatory, anticancer, insectifuge
26	13.44	Decanoic acid(Z), tetradecyl ester	$C_{32}H_{62}O_2$	550	0.48	ester	Namaticide[1] , pesticide[1]

27	13.85	5-ter-butylpyrogallol	$C_{10}H_{14}O_3$	182	2.25	phenol	Anti-inflammatory, anti-oxidant, cytotoxic[4]
28	13.85	2,3,5-trimethoxyamphetamine	$C_{12}H_{19}NO_3$	225	2.25	amine	***
29	14.78	2,6-dimethoxy-4-(-2-propenyl)-phenol	$C_{11}H_{14}O_3$	194	1.32	phenol	antioxidant
30	14.93	1,15-pentadecanediol	$C_{15}H_{32}O_2$	224.4	1.72	diol	Antibacterial[15], antifungal, Antiviral

Table 4: Phytoconstituents identified in root extract of *Nerium oleander l.* by GCMS.

Sr. No.	RT	Name of the compound	Molecular Formula	MW	Peak area (%)	Compound Name	Reported Activity
31	15.98	2-hydroxy-4-isopropyl-7-methoxytropone	$C_{11}H_{14}O_3$	194	3.72	tropone	***
32	16.21	17-chloro-7-heptadecene	$C_{17}H_{33}Cl$	272.9	0.56	alkene	***
33	16.97	2-methyl-Z,Z-3,13-octadecadienol	$C_{19}H_{36}O$	280.4	0.38	enol	anticancer
34	16.97	Tridecanedial	$C_{13}H_{24}O_2$	212	0.38	aldehyde	***
35	18.29	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.4	1.00	ester	Flavor
36	18.64	l-(+)-ascorbic-acid-2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652	4.67	Vitamin-C	Anticancer[19]
37	19.07	Oleic acid	$C_{38}H_{74}O_2$	282	1.09	Fatty acid	5-α-reductase inhibitor, anticancer
38	19.27	Z-10- methyl-11-tetradecen-1-ol-propionate	$C_{18}H_{34}O_2$	294	0.38	ester	***
39	19.99	E,E,Z-1,3,12-nonadecatriene-5,14-diol	$C_{18}H_{34}O_2$	294	2.00	alcohol	***
40	20.30	2-(9,12- octadecadienyloxy)-,(Z,Z)- ethanol	$C_{20}H_{38}O_2$	310	9.48	alcohol	Antibacterial,anti-inflammatory[11]
41	20.56	9-hexadecenoic acid	$C_{16}H_{30}O_2$	254	2.24	acid	Non-cytotoxic[16]
42	20.56	Octadecanoic acid	$C_{18}H_{36}O_2$	284	2.24	acid	Non-cytotoxic[16]
43	20.80	6,9,12,15-docosatetraenoic acid(Z,Z), methyl ester	$C_{23}H_{38}O_2$	346	0.90	fatty acid	***
44	21.12	9,12-octadecadienoic acid(Z,Z)	$C_{18}H_{32}O_2$	280	0.69	fatty acid	***
45	23.40	3-(tetradecyloxy)-1,2-propanediol	$C_{17}H_{36}O_3$	288	0.67	diol	antifungal[15]
46	23.53	1H-cyclopropa[3,4]benz[1,2-e]azulene-4a,5,7b,9,9a(1aH)-pentol	$C_{28}H_{38}O_{10}$	518.2	0.44	azulene	***
47	23.64	5,16,20-pregnatriene-3 beta, 20-diol diacetate hydrocortisone acetate	$C_{25}H_{34}O_4$	404.5	0.56	stereoid	Pain Killer, jaundice
48	25.69	2-[1-hydroxy-2-(3-methyl phenyl)ethyl- Cholestan-3-one	$C_{36}H_{56}O_2$	386.3	0.77	stereoid	***

thermo scientific TSQ 8000GC-MS, CIL, Punjab National University, and Chandigarh, India.

GC-MS analysis

The TSQ 8000 GC-MS used for analysis employed column packed with silica with elite -1 (100% dimethyl polysiloxane) 35 nm to 0.30 nm and injection volume 1 µl and component are separated as helium gas are mobile phase with continous flow 1 ml/min. The number scans were 3583 with separated components.The MS part consists of Triple Quadrupole. This mass spectrometer comes paired with the TRACE 1300 GC along with auto-sampler for automated sample handling. The mass range used for ion detection used in the range of 50 to 700 Da. The programmable EI ion source having temperature 350 ^{o} C used for detection. The polar as well as non-polar column used for seperation and temperature of column was 400 ^{o} C.

Identification of compounds

Table 5: Phytoconstituents identified in root extract of *Nerium oleander l.* by GCMS.

Sr. No.	RT	Name of the compound	Molecular Formula	MW	Peak area (%)	Compound Name	Reported Activity
49	25.69	5-[(acetyloxy)methyl-3a,4,6a,7,9,10,10a,10b-octahydro-3a,10a-dihydroxy-2,10-dimethyl-,(3aa',6aa',10a'10aa',10ba')	$C_{19}H_{24}O_6$	348	0.77	Aromatic compound	***
50	25.69	2,2-difluroheptacosanoic acid	$C_{14}H_{26}F_2O_2$	226	0.77	floroacid	***
51	25.89	13-docosenamide(Z)	$C_{22}H_{43}NO$	337	0.36	amide	Anti-inflammatory
52	25.89	9-octadecenamide	$C_{18}H_{35}NO$	281	3.13	amide	anti-inflammatory activity ,antibacterial activity
53	26.19	6,10,14,18,22-tetracosapenaen-2-ol,3-bromo-2,6,10,15,19,23-hexamethyl-,(all-E)	$C_{30}H_{51}BrO$	506.3	0.59	alcohol	***
54	27.21	Azafrin	$C_{27}H_{38}O_4$	426.2	0.85	acid	***
55	27.74	A-Neooleana-3(5),12-diene	$C_{30}H_{48}$	408	1.65	stereoid	***
56	30.00	α – amyrin	$C_{30}H_{50}O$	426.7	10.85	triterpenoid	Anticancer[14], anti-inflammatory[13]
57	31.36	Acetic acid, 3- hydroxy-6-isopropenyl-4,8a- dimethyl-1,2,3,5,6,7,8,8a-octahydronapthalen-2-yl-ester	$C_{17}H_{26}O_3$	278	3.20	ester	***
58	31.93	Astaxanthin	$C_{40}H_{52}O_4$	596.8	1.48	terpene	antioxidant

*** = no activity reported

Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technique, WILEY8 and FAME having more than 65,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST08S, WILEY8 and FAME library. The name, molecular weight, molecular formula and structure of the component of the test material were ascertained. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a GC-MS solution ver. 2.53[1].

RESULTS

Gas chromatography-mass spectrometry (GC-MS) analysis of *Nerium Oleander L.* revealed the existence of the P-cresol, L-arginine, 2- methoxy phenol, mequinol, 2-methoxy phenyl ester, 2,4,6-decatrienoic acid , 1,4-diene-3-one,17-hydroxy-17-methyl-17(α')-androsta, 4-Hexyl1(7-methoxycarbonylheptyl)- bicycle [4.4.0] - deca 2,5,7-triene , 2,3-dimethyl- Phenol, 3,4-dimethyl- Phenol, 2-(3,3-dimethyl-but-1-ynyl)-2,6,6-trimethyl-cyclohexane,1,4-dione, 3,4-dimethyl,methyl carbamate , 1H-Cyclopropa [3,4] benz [1,2 e] azulene-5,7 b, 9,9 α -tetrol, 1(3-acetoxy1,1dimethy l-hexan-5-onyl)2-isopropenyl, Cresol, 2H-Benzo[f] oxireno[2,3E] benzofuran-8 (9H)one,octahydro9[[[(2-methoxyphenyl) methyl]amino] methyl] 2,5α- dimethyl, 2(1,2,3,4-Tetrahydronaphthalen1yliden) hydrazine,1-carbothioamide, N(2-Phenylethyl) undeca (2Z,4E) diene 8,10-diynamide, 4-ethyl-2-methoxy phenol, 5-Isopropyl-3,3dimethyl-2-methylene-2,3-dihydrofuran ,3-methyl-1H-Indazole, 4 (1H-1,2,3,4 tetrazol-1yl)- Benzeneacetic acid, 2-Methoxy-4-vinylphenol, 1H-1,2,3,4-Tetrazole1,5-diamine, [5,9-Dimethyl1(3-phenyloxiran 2-yl) Deca-4,8-dienylidene](2-phenylaziridin1-yl)-amine,Podocarpa1,12dieneë14,à acetic acid,7-hydroxy8,13dimethyl-3-oxo,ë-lactone, anobin, trans-isogenol, 2-methoxy-4-(1- propenyl) phenol, 2-methoxy-4-(Z-1- propenyl) phenol, 6,7-Epoxypregn-4-ene-9,11,18-triol-3,20-dione, eicosyl ester, oleic acid, hydrocortisone acetate, 1H-Cyclopropa[3,4]- [1,2e]benzazulene-4a,5,7b,9,9a(1aH)pentol,Benz[e]azulene3,8dione,5[(acetyloxy)methyl]3a,4,6a,7,9,10,10a,10boctahydro3a,10adihydroxy2,10dimethyl, (3aà,6aà,10á,10aá,10bá)(+) , Decanoic acid(Z), tetradecyl ester, 5-ter-butylpyrogallol, 2,3,5-trimethoxyamphetamine, 1,15-pentadecanediol, 2-hydroxy-4-isopropyl-7-methoxytropone, 2-methyl-Z,Z-3,13-octadecadienol, Hexadecanoic acid, methyl ester, l-(+)-ascorbic-acid-2,6-dihexadecanoate, 2-(9,12-octadecadienyloxy)-,(Z,Z)- ethanol, 3-(tetradecyloxy)-1,2-propanediol, 9-octadecenamide, α -amyrin, astaxanthin. The GC-MS spectrum confirmed various phytoconstituents with different retention time illustrated in figure 1a to 1e. The biological activities listed are based on Dr. Duke's phytochemical and botanical

databases by Dr. Jim Duke of the Agricultural Research Service/USDA.

DISCUSSION

The bioactive phytoconstituents were identified by GCMS having different retention time. The comparison of the mass spectrums with the NIST data base gave more than 95% match as well as confirmatory compound structure match. The GCMS analysis of the solvent free extract of *Nerium Oleander L*. root extract have potent anticancer, anti-inflammatory, antimicrobial, 5-α-reductase inhibitor, namaticide, pesticide, analgesic, antioxidant activity due to presence of several phytoconstituents. The reported activity illustrated in table 1a to 1e with different retention times, peak areas, molecular weight , name of the compounds and in figure 1 shows GC-MS spectrum of *Nerium Oleander L*. root extract with different relative abundance.

CONFLICT OF INTEREST STATEMENT

No conflict of interest.

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