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Research Article

Phytochemical Screening and GC-MS Analysis of Methanol Extract of the Leaves of Nerium oleander Linn

Debashisa Mishra[1]*, Susanta Kumar Rout[2] and Alaka Priyadarshani Kar[1]

[1]*IMT Pharmacy College, Odisha, India*

[2]*Patent Information Centre, Science and Technology Department, Secretariat, Odisha, India*

***Corresponding Author:** Debashisa Mishra, IMT Pharmacy College, Odisha, India.

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Abstract

Nerium oleander Linn is one of the medicinally important plants belonging to the family Apocynaceae has been studied for different activities with special reference to Central Nervous System (CNS) disorder along with wound healing, anti-oxidant effect. The experimental results showed that N. oleander Linn has effective in various disorders. The plant's methanol extract at 300 mg/kg showed highly significant result towards different animal models mimicking the different CNS disorder. Similarly the sub maximal dose i.e. 150 mg/kg showed significant effect in few animal models. The wound healing and antioxidant study showed also showed significant effect in different tests. Different literature study revealed that the plant extract has antibacterial activity, antifungal activity, antibiotic activity anticancer property. Since the plant's producing different activities. GC-MS analysis in the leaf powder's methanol extract was done by using National Institute Standard and Technology (NIST) database 2005 to identify the compounds present. The spectra of unknown compounds were compared with that of known compounds stored in the NIST library by matching the molecular weight and retention time. Sixteen numbers of phytochemical constituents those are bioactive in nature were identified in N. oleander Linn methanol leaf extract. The compounds predominantly Phenolic compounds, Flavonoids derivatives, Carbohydrate, Glycoside, Saponin, Phytosterols on basis of the molecular formula, molecular weight, peak area percentage etc. The phytochemicals present in the methanol extract might have produced different biological activities which have already been studied. It may suggest that, the phyto constituents may help to give protection from different kind of diseases.

Keywords: Phytochemicals; GCMS Analysis; Spectra; Antibacterial Activity

Introduction

Plants have been utilized as a wide source for discovering novel drug or compounds. Now a day's medicines obtained from different parts of the plant have made a huge contributions towards human health and well-being [1]. From different literatures it has been reported that most of the medicinal plants possesses antimicrobial and antioxidants activity [1]. Traditional medicines obtained from plant materials are easily available in rural areas. Due to readily available traditional medicines in rural belt traditional medicines are cheaper than the modern medicines. Medical plants and plant products are the oldest and tried health-care products. Their importance is growing not only in developing countries but in many developed countries. The allopathic drugs are good in onset and have good therapeutic activity but side effects associated are poignant. Thus, the herbal medicines from natural sources with least or no side effect having similar or better therapeutic activity are best. The herbal medicines have wide therapeutic actions and safety profile [2] from different survey it has been estimated that around 80% of the world populations used traditional medicine for cure as primary health care. It is also reported that traditional medicines played an important role in the primary health care system [3]. The role of World Health Organization (WHO) is to encourage, promote and facilitate the effective herbal medicine for the primary use in developing countries for different health programs. Different biological activities like anti-microbial, anti-oxidant, sedative and anxiolytic effects of the plant extracts may be due to presence of the active compounds. Consequently, due to some other biological activities on the same time make excellent leads for new drug development [4]. *N. oleander* Linn is an important medicinal plant belongs to family Apocynaceae commonly name as Kaner is a large glabrous evergreen shrub having milky juice. This habitat of the plant is India and Mediterranean region up to Iran. Leaves of the plants are stalked, coriaceous and having length 10 - 15 cm, The leaves are linear lanceolate with dark green in colour. The plants having salver-shaped pink flower with white scentless and having no fragrance. *N. oleander* Linn is one of the evergreen shrub (or small tree) the peculiarity of the shrub is it is grow up to 6 m. Due to its ornamental characteristics it has been cultivated in warm-temperate and dry subtropical regions. Active cardiac glycosides are the main active constituents which is used in the treatment of cardiac diseases. Leiomyosarcoma and prostate/breast cancer patients are getting positive effect when treated with N. oleander Linn. Some of the other use of *N. oleander* Linn is it used against inflammation, neurological disorders, cardiac abnormalities and disease caused by parasite [3,5]. Ayurvedic medicine for the treatment of ailments there are no reports on the constituents that are responsible for the therapeutic effect. With this background the present study was aimed to identify the phytoconstituents present in *N. oleander* Linn.

Materials and Methods

Experimental

Nerium oleander Linn leaves were collected from Anandapur, Keonjhar district of Odisha, India. Department of Biosciences, Sardar Patel University, Gujarat has authenticated the *Nerium oleander* Linn. Collected materials i.e. leaves were collect in bulk quantity. After collection the plant materials are washed properly by using running tap water to remove the adhering soil and dirt

particles and then shade dried. Voucher specimen of the plant was deposited at the school of pharmaceutical science, SOA University, Bhubaneswar, Odisha for further reference. The dried plant materials were coarsely powered and stored in airtight, non-toxic polyethylene bags until used [3].

Preparation of extract and fractions

The powdered leaves of *Nerium oleander* Linn was extracted with different solvents like petroleum ether (60 - 80°C) for 72h to de-fat it and then the remaining plant materials were extracted by maceration process by using methanol as solvent. Filtered was occurred with the extractives where the solvents were incorporate and subsequently the marc is pressed to squeeze out residual extractives. This process was repeated thrice to achieve complete extraction. The extracts obtained during the three cycles were combined and reduced to 1/8th of its original volume in a rotary evaporator at 45°C and then lyophilized in a freeze dryer to obtain the yield. The extracts were again dissolved in distilled water and then successively extracted by the solvents with increasing polarities with the following solvents; chloroform, ethyl acetate and methanol. Different fractions were obtained in different quantity. After obtaining the product the product is concentrated by drying method and then preserved for further study. Phytochemical screening of the plant extracts and fractions revels alkaloids, glycosides, saponins, flavonoids, carbohydrates, tannins, phenolic compounds, protein, and fats. All the extracts/fractions of plant were prepared by 10% w/v in normal saline consisting of 0.1% propylene glycol [3].

Gas Chromatography- Mass Spectrum Analysis (GC-MS)

The GC-MS analysis for identification of compounds present in different fractions prepared from crude methanol extract of *N. oleander* Linn had been carried to identify the compounds. GC-MS analysis was done by using GC Clarus 500 Perkin Elmer system and gas chromatograph interfaced with mass detector Turbo mass gold-Perkin Elmer (GC-MS) [6]. Column: Elite-5MS (5% Diphenyl/95% Dimethyl poly siloxane), 30 x 0.25 mm x 0.25m df, Carrier gas: Helium (99.999%) with constant flow rate of 1 ml per min, (Split ratio: 10:1), Sample Injection volume 2 µl, Software: Turbomass 5.2, Oven operating in electron impact mode at 70 eV, oven temperature was fixed from 110°C (isothermal for 2 minutes), with an increase rate of 10°C/min up to 200°C with no hold and then at rate of 5°C/min upto 280°C ending with a 9 min hold. Injector temperature was 2500C, Ion-source temperature 280°C and total GC running time was 36 minutes.

MS programme: For GC-MS, NIST Library having 2005- Year Version is used, 200°C temperature was maintained for Inlet line temperature and Source temperature, 70 eV energy and 45 - 450 Mass scan (m/z) with 0 - 2 minutes Solvent Delay were considered as different parameters. Total running time for MS: 36 minutes.

Identification of compounds

Interpretation on mass spectrum generated during GC-MS analysis was done by using National Institute Standard and Technology (NIST) database 2005 to identify the compounds present. To identify the compounds well established known compounds are considered as a standard whereas Unknown compounds of spectra were kept as test in the NIST library by considering different parameters like retention time, molecular weight and structure. The Structure of the compound and the Molecular weight of the compound with name of the test materials were confirmed [6]. The major compounds identified were searched in Dr. Duke's Phytochemical and Ethnobotanical Database [7].

Result

The GC-MS analysis revealed presence of sixteen compounds in crude methanol extract of *N. oleander* Linn. The compounds with their Retention Time (RT), Molecular Formula, Molecular Weight (MW) and Peak Area (%) have been presented in different tables. The major compounds had been identified on basis of the percentage Peak Area in the Chromatograph. The compounds identified in the crude methanol extract (Table 1) are; 1-Dodecanol (RT - 5.09, Peak Percentage - 0.88%); 1-beta-d-Ribofuranosyl-3-[5-tetraazolyl]-1,2,4-triazole (RT - 12.796, Peak Percentage - 11.79%); N-Acetyl-N'-butyrylurea (RT-14.891, Peak Percentage - 7.12%); 2-Chloroethyl vinyl sulfide (RT - 15.129, Peak Percentage - 19.47%); Hexadecanoic acid, methyl ester (RT - 18.538, Peak Percentage - 1.16%); 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (RT - 20.329, Peak Percentage - 2.37%); Phenol, 4,4'-(1-methylethylidene)bis- (RT - 21.153, Peak Percentage - 3.21%); 1,8,9-Anthracenetriol, 3-methyl (RT - 22.505, Peak Percentage - 2.11%); 9,10-Anthracenedione, 1,8-dihydroxy-3-methyl (RT - 22.736, Peak Percentage - 2.23%); Danthron methyl derivative (RT - 24.065, Peak Percentage - 5.52%); P-(Adamantyl-1)-ethylthiobenzene (RT - 24.556, Peak Percentage - 2.96%); 4-[(2-Methyl-5-nitro-phenylimino)-methyl]-benzene-1,3-diol (RT - 25.603, Peak Percentage - 5.56%); beta-Tocopherol (RT - 28.552, Peak Percentage - 4.08%); dl-alpha-Tocopherol (RT - 32.541, Peak Percentage - 14.18%); Ethanone, 2-(2-benzothiazolylthio)-1-(3,5-dimethylpyrazolyl)- (RT - 34.235, Peak Percentage - 12.31%). The molecular formulae of the compounds had been mentioned in table 1 whereas the chromatograph has been represented in figure 1. Search for any reported hypoglycemic or anti-hyperglycemic potential of major compounds in Dr. Dukes Phytochemical and Ethno botanical Database revealed no result.

Discussion

Drugs obtained from plant materials have a long history in both traditional and modern societies. The present study revealed that the methanol extract possess different active constituents. Since the crude extracts and some fractions produced antimicrobial, antioxidant, sedative, anxiolytic, antiepileptic and wound healing activity with dose dependent manner it indicates the major identified compound may produce different activities. From different literatures it was reported that antimicrobial, antioxidant, sedative, anxiolytic, antiepileptic and wound healing activities of leave extract might be due to the secondary metabolites such as flavonoids and phenolic compounds. For anti-oxidant activity the leaves can be used to prevent oxidative damage caused by free radicals and for anti-microbial activity the plant extract may be treat infections caused by pathogenic bacteria not to fungus. Further studies may be required to purify the constituents and needed to understand the complete mechanism of wound healing activity of the test plants. Further, it also needs more evaluation in clinical settings before consideration for the treatment of different disorders. The GC-MS analysis of crude methanol extract of N. oleander Linn showed presence sixteen (16) different compounds in it. On basis of the peak area percentage compounds like; 1-beta-d-Ribofuranosyl-3-[5-tetraazolyl]-1,2,4-triazole, 2-Chloroethyl vinyl sulfide, N-Acetyl-N'-butyrylurea, 9,12,15-Octadecatrienoic

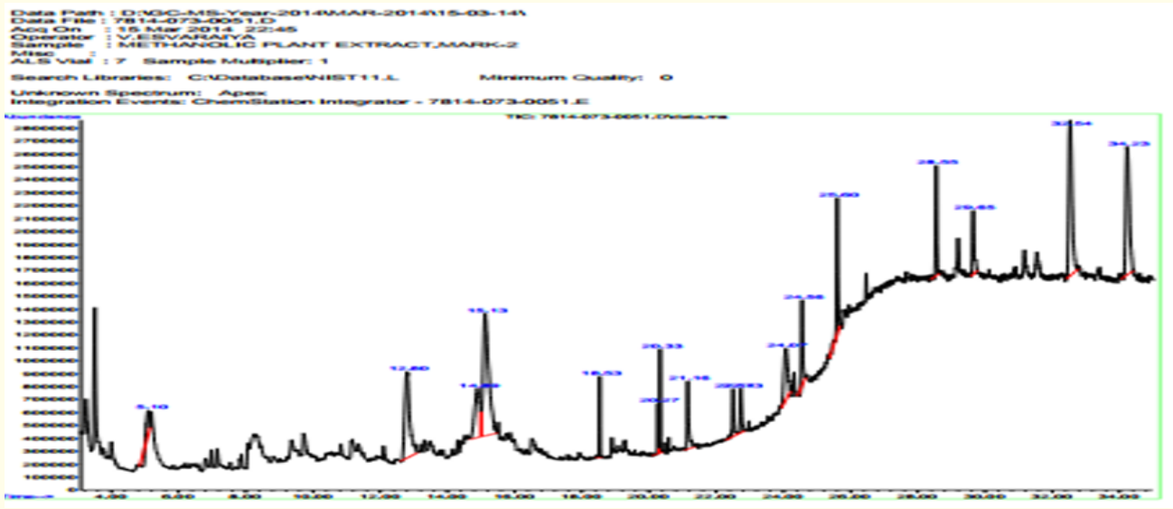

Figure 1: GC-MS analysis of crude methanol extract of *N. oleander* Linn.

S. No.	RT	Compound	Molecular Formula	MW	Peak Area %
1.	5.093	D-Arabinitol	$C_5H_{12}O_5$	152.068	0.88
2.	12.796	1-.beta.-d-Ribofuranosyl-3-[5-tetraazolyl]-1,2,4-triazole	$C_8H_{12}N_4O_5$	269.087	11.79
3.	14.891	N-Acetyl-N'-butyrylurea	$C_8H_{15}NO_6$	172.085	7.12
4.	15.129	2-Chloroethyl vinyl sulfide	$C_{13}H_{24}O_2$	121.996	19.47
5.	18.538	Hexadecanoic acid, methyl ester	C_4H_7CIS	270.256	1.83
6.	20.269	9,15-Octadecadienoic acid, methyl ester, (Z,Z)-	$C_{19}H_{34}O_2$	294.256	1.16
7.	20.329	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292.24	2.37
8.	21.153	Phenol, 4,4'-(1-methylethylidene)bis-	$C_{28}H_{29}O_5P$	228.115	3.21
9.	22.505	1,8,9-Anthracenetriol, 3-methyl-	$C_{15}H_{12}O_3$	240.079	2.11
10.	22.736	9,10-Anthracenedione, 1,8-dihydroxy-3-methyl-	$C_{15}H_{10}O_4$	254.058	2.23
11.	24.065	Danthron methyl derivative	$C_{15}H_{10}O_4$	254.058	5.52
12.	24.556	P-(Adamantyl-1)-ethylthiobenzene	$C_{12}H_{17}O_2$	272.16	2.96
13.	25.603	4-[(2-Methyl-5-nitro-phenylimino)-methyl]-benzene-1,3-diol	$C_4H_5N_3O_2$	272.08	5.56
14.	28.552	beta.-Tocopherol	$C_{28}H_{48}O_2$	416.365	4.08
15.	32.541	dl-.alpha.-Tocopherol	$C_{29}H_{50}O_2$	430.381	14.18
16.	34.235	Ethanone, 2-(2-benzothiazolylthio)-1-(3,5-dimethylpyrazolyl)-	$C_{14}H_{13}N_3OS_2$	303.05	12.31

Table 1: Compounds Identified from crude methanol extract of *N. oleander* Linn.

acid, methyl ester, Phenol, 4,4'-(1-methylethylidene)bis, Danthron methyl derivative, 4-[(2-Methyl-5-nitro-phenylimino)-methyl]-benzene-1,3-diol, Ethanone, 2-(2-benzothiazolylthio)-1-(3,5-dimethylpyrazolyl), and Tocopherol can be deliberated as the major compounds present in crude methanol extract. The compound 1-beta-d-Ribofuranosyl-3-[5-tetraazolyl]-1,2,4-triazol may produce analgesics, antipyretics, anti-convulsants, anti-inflammatory, immune modulatory activity [8]. N-Acetyl-N'-butyrylurea - is urea derivatives possess valuable anti-tuberculosis, antibacterial and anticonvulsant properties [9-11]. Hexadecanoic acid, methyl ester have been found to show the biological activity like instance, the anti-inflammatory, antioxidant, hypocholesterolemic, antibacterial etc [12-14]. Danthron methyl is an anthraquinones derivative used as antimicrobial, anticancer, antioxidant and immunomodulatory effect [15,16]. 4-[(2-Methyl-5-nitro-phenylimino)-methyl]-benzene-1,3-diol play vital role in biological fields such as, Antimicrobial, Anticonvulsant, Anticancer, Anti-inflammatory activity [17]. Biological activity of dl-.alpha.-Tocopherol has producing Antioxidant activity, Immune Activity, Anticancer activity, Anti-inflammatory activity etc. Ethanone, 2-(2-benzothiazolylthio)-1-(3, 5-dimethylpyrazolyl) has producing antimicrobial activity [18]. So, presence of these compounds may be responsible for the observed anti-hyperglycemic effect of the fraction. The search for any reported anti-hyperglycemic or hypoglycemic property of the major compounds in Dr. Duke's Phytochemical and Ethnobotanical Database yielded no result, and hence are subjected to further investigation.

The different parts of the plant such as root, bark and leaves of *N. oleander* Linn has been used for thousands of years for its medicinal properties [19]. It is rich in a wide variety of secondary metabolites such as glycosides, phytosterols, proteins, saponins and phytosterols. In this connection the present study on the methanolic extract and the different fractions of crude meth-

anolic extract was conducted to evaluate the antimicrobial activity of leaves. Phytomedicines can be used for the treatment of diseases as is done in case of Unani and ayurvedic system of medicines, a natural blue print for the development of new drugs [20]. The phytochemical screening (Table 1) of the crude methanolic extract of *N. oleander* Linn revealed the presence of glycosides, steroids, flavonoids, terpenoids, saponins and reducing sugar.

Conclusion

In the present study sixteen chemical constituents have been identified from methanolic extract by Gas Chromatogram Mass spectrometry (GC-MS) analysis. The presence of various bioactive compounds justifies the use of plant in various ailments by traditional practitioners. The GC-MS analysis of methanol extract revealed out of the sixteen identified compounds; 3,7-Dimethyl-6-nonen-1-ol acetate (33.58%), 9-Octadecenamide,(Z)- (20.45%), Ethyl iso-allocholate (10.27%), 9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3α,5Z,7E)- (4.74%), 5-Octadecene, (E)- (3.86%), 2H-Oxecin-2-one,3,4,7,8,9,10-hexahydro-4-hydroxy-10-methyl-,[4S-(4R*,5E,10S*)]- (3.64%), and 1-Eicosanol (3.56%) were considered as major compounds on basis of the percentage peak area shown on the chromatograph.

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