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Identification Of Phytoconstituents In N-Hexane Extract Of *Nerium indicum* Leaves By GC-MS Analysis.

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ABSTRACT

The aim of this study was to prepare and identify the phytoconstituents in n-Hexane extract of *Nerium indicum* leaves by GC-MS analysis. The n-hexane extract was prepared by soxhlet extraction method. GC-MS analysis of n-hexane extract was performed by standard protocol using the equipment 6890 GC with 5973 I MSD. The GC-MS analysis revealed the presence of 11 compounds belonging to higher acyclic alkanes, alkene, organohalogen, fatty acid, aromatic and cyclohexane compounds. Out of eleven compounds the following four such as Hexacosane, n-Hexadecanoic acid, Phenol 2, 4-bis (1, 1-dimethylethyl), and 4-Isopropyl-1,3-cyclohexanedione derivatives have been reported to possess medicinally important properties. From the results, it could be concluded that n-Hexane extract of *Nerium indicum* leaves contain bioactive compounds which may possibly be utilized for developing drugs to manage various diseases.

Keywords: GC-MS, *Nerium indicum*, Phytoconstituents, n-Hexane extract.

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INTRODUCTION

Nerium indicum L. is a small tree belonging to dogbane family Apocynaceae which grows in Mediterranean, tropical and subtropical regions [1,2]. It is one of the most important drug in Indian traditional system of medicine to treat various ailments and is called as Karavira [3]. The plant is reported to possess antibacterial [4], antimicrobial [5], anti-inflammatory, antinociceptive [6] and antitumor [7] activity. The roots, bark, stem, leaves and flowers of *Nerium oleander* are also reported to possess insecticidal, antifeedant, Larvicidal, insect growth regulatory and insect repellent activity [8-16].

MATERIALS

The *Nerium indicum* fresh leaves were collected from the medicinal garden of Vignan Institute of Pharmaceutical Sciences, Vignan hills, Deshmuki, Pochampally, Yadadri Bhuvangiri District, Telangana State, India in the month of February 2017 by the authors. Plant sample was identified by botanist and the voucher specimen has been deposited in the department of pharmacognosy of our college.

All the chemicals, glass ware and equipments used in this study were obtained from the central store house of the institution. The chemicals were of analytical grade and glass ware of borosilicate type. Detailed phytoconstituents screening of n-Hexane extract was performed with GC-MS equipment 6890 GC with 5973 I MSD.

METHODS

Preparation of the n-hexane extract

The extraction was performed using a Soxhlet apparatus in the normal way at the boiling point of the solvent used. The fresh leaves of *Nerium indicum* (50 g) was extracted with 500 ml of n-Hexane solvent on a heating mantle until the solvent became colorless. The extract was concentrated under reduced pressure on a rotary evaporator. The extract was stored in sealed container at 4°C until further analysis [17].

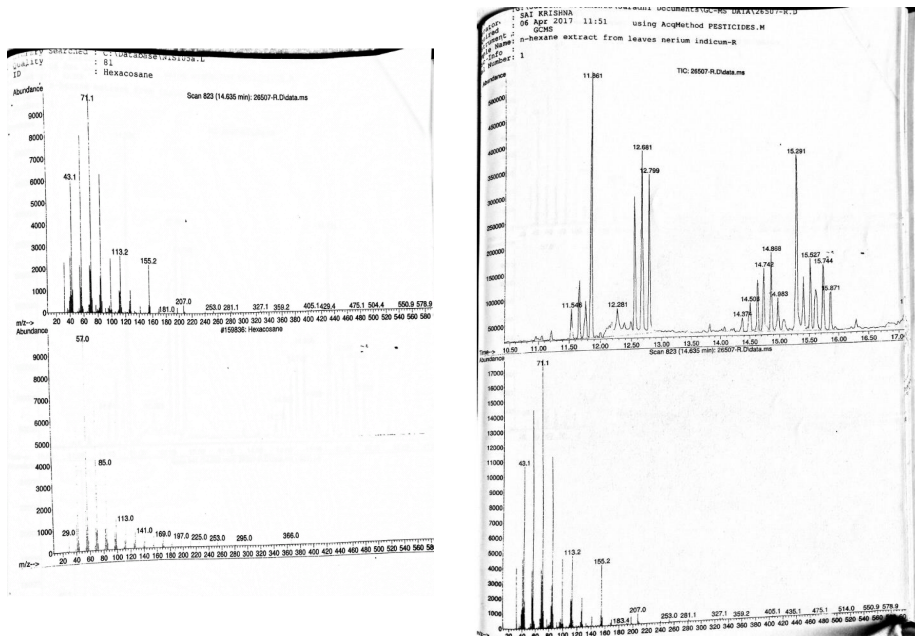
Gas Chromatography-Mass Spectrometry Analysis

The sample preparation, instrument, operating conditions and identification of compounds were same as procedures reported by ashok et al[18].

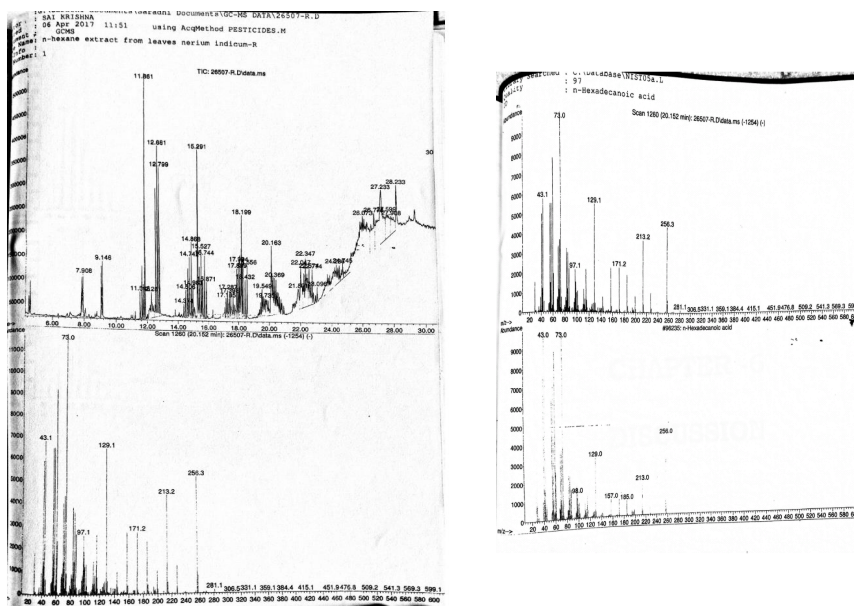
RESULTS

The results of GC-MS analysis of n-Hexane extract of *Nerium indicum* leaves is tabulated in Table 1. The Graphical representation of GC-MS Data of FOUR compounds namely Hexacosane, n-Hexadecanoic acid, Phenol 2, 4-bis (1, 1-dimethylethyl), and 4-Isopropyl-1,3-cyclohexanedione in graphs with numbers 2,3,4,5 respectively.

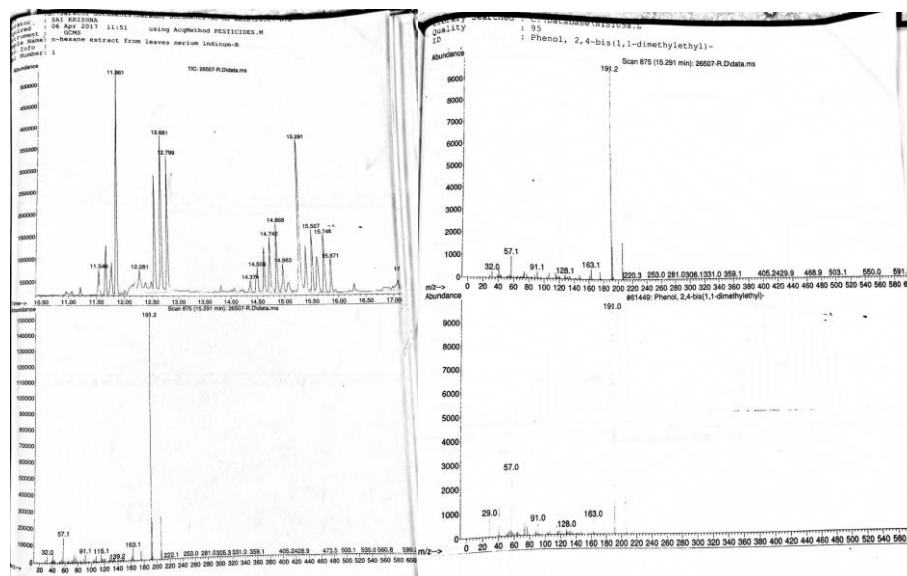
2. Graphical representation of GC-MS Data of Hexacosane obtained from nerium indicum leaves extract.



3. Graphical representation of GC-MS Data of n-Hexadecanoic acid obtained from nerium indicum leaves extract.



4. Graphical representation of GC-MS Data of Phenol, 2,4-bis(5,1-dimethylethyl) obtained from nerium indicum leaves extract.



5. Graphical representation of GC-MS Data 4-Isopropyl-1,3-cyclohexanedione obtained from nerium indicum leaves extract.

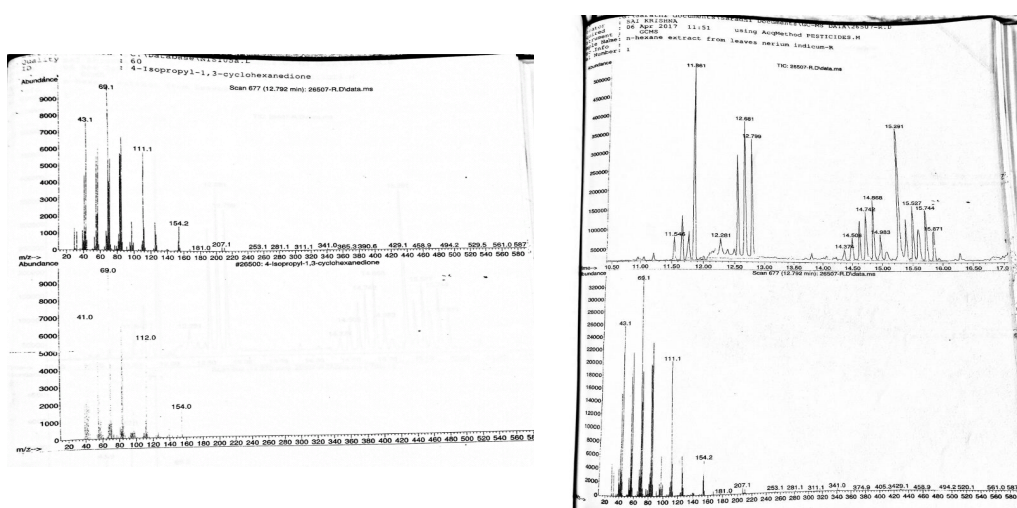


Table 1: Phytochemicals identified in the n-Hexane extract of Nerium indicum by GC-MS

S. No.	Retention time	Chemical name	Nature of the compound	Molecular formula	Molecular weight	Activity reported
1	9.143	5-Dodecene	Alkene	C ₁₂ H ₂₄	168.32	-----
2	11.542	Dodecane 4,6-dimethyl	Alkane	C ₁₄ H ₃₀	198.38	-----
3	11.857	Benzene 1,3-bis(1,1-dimethyl ethyl)	Aromatic compound	C ₁₄ H ₂₂	190.32	-----
4	12.792	4-Isopropyl-1,3-cyclohexanedione	cyclohexane	C ₉ H ₁₄ O ₂	154.21	-----
5	14.370	Hexadecane 2,6,10,14 tetramethyl	Higher acyclic alkane	C ₂₀ H ₄₂	282.5475	-----
6	14.509	Dodecane	Higher acyclic alkane	C ₁₂ H ₂₆	170.33	-----
7	14.635	Hexacosane	Higher acyclic alkane	C ₂₆ H ₅₄	366.71	Anti-inflammatory

8	14.862	Pentacosane	Higher acyclic alkane	C ₂₅ H ₅₂	352.69	-----
9	15.291	Phenol 2,4-bis(1,1-dimethylethyl)	Phenol	C ₁₄ H ₂₂ O	206.17	Anti-quorum, Biofilm activity
10	15.405	Heptafluorobutanic acid, heptadecyl ester	Organohalogen compound	C ₂₀ H ₃₃ F ₇ O ₂	438.46	-----
11	20.152	n-Hexadecanoic acid	Fatty acid	C ₁₆ H ₃₂ O ₂	256.42	Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Lubricant,

DISCUSSION

The GC-MS analysis of n-hexane extract revealed presence of eleven compounds. Few of these compounds such as n-Hexadecanoic acid, Phenol, 2, 4-bis (1, 1-dimethylethyl) - and 4-Isopropyl-1, 3-cyclohexanedione have been reported to possess useful pharmacological actions.

The compound n-Hexadecanoic acid has been reported to have important role in treating various inflammation conditions [19]. Phenol, 2, 4-bis (1, 1-dimethylethyl) - derivative is one of the compound in n-hexane extract and is also present in various plants and is known for its antibacterial and anti-inflammatory activities [20, 21]. It has also been reported to have anti-quorum sensing and anti-biofilm efficacy. These properties increase the susceptibility of microorganisms to antibiotics when administered synergistically. Hence such compounds open another avenue for combinatorial therapy [22]. Thomas N. Wheeler of Union Carbide Corporation has US patent on 2-Aryl-1,3-cyclohexanedione compounds and their alkali metal and ammonium salts which exhibit outstanding herbicidal, miticidal and mite ovicidal activity. 4-Isopropyl-1, 3-cyclohexanedione which is detected in n-hexane extract may be responsible for herbicidal activity of *Nerium indicum* leaves which has been reported by several researchers [23]. Hence it could be concluded that n-hexane extract of *Nerium indicum* leaves contain bioactive compounds and in further studies these compounds should be isolated and tested for activities reported for *Nerium indicum* leaves in traditional literature.

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