

**GC-MS ANALYSIS OF PHYTOCHEMICAL COMPOUNDS PRESENT IN THE RHIZOMES OF *Nervilia aragoana* GAUD**ELIZABETH THOMAS<sup>1</sup>, ANEESH T. P<sup>1\*</sup>, DELLA GRACE THOMAS<sup>2</sup>, R. ANANDAN<sup>3</sup>

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**ABSTRACT**

Medicinal plants have had a crucial role in human culture and civilization. The rhizomes of the plant *Nervilia aragoana* were collected, washed, shade dried and powdered. Ethanol extract ether extract and methanol extract (from the marc of ether extract) were prepared by simple maceration process and soxhalation method. All the extracts were concentrated and analyzed using Gas Chromatography Mass Spectroscopy for the identification of biochemical components present in the rhizome of *N. aragoana*. A wide range of fatty acids, heterocyclic compound which are having anti fungal anti inflammatory antibiotic activity, skin conditioning property were identified so that it can be recommended as a plant of phytopharmaceutical importance.

**Keywords:** GC-MS, *Nervilia aragoana*, Phytochemical compounds, medicinal plants,

**INTRODUCTION**

For millennia, people around the world have healed the sick with herbal derived remedies, and handed down through generations. Traditional medicine is the sum total of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve or treat physical and mental illness [1]. Various types of traditional medicine and other medical practices referred to as complementary or alternative medicine are increasingly used in both developing and developed countries.

Ayurveda stresses the use of plant-based medicines and treatments. But when compared the Chinese medicine is more established than Ayurvedic 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 [2]. 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 [3].

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 [4].

*Nervilia aragoana* GAUD is a terrestrial orchid belongs to the family Orchidaceae. The parts of the plant mainly used are underground rhizome and leaf [5]. The French botanist Charles Gaudichaud Beaupre (1789-1854) gave the plant the name *Nervilia aragoana* in 1829 [6]. The Orchidaceae family is considered to be the most evolved species and the largest and highly advanced botanical family in higher plant [7]. It is a widely distributed monocotyledonous family with a large number of terrestrial, saprophytic and epiphytic

species. The family comprises more than 30,000 species. *N. aragoana* Gaudichaud is a terrestrial orchid found mainly in hilly humid shady areas of dense forests in India [5], [8]. *N. aragoana* is a herb which grows up to 15 cm in height [5]. The plant is perennating by underground subglobose white tubers. The leaves are bright green and the drooping flowers are attractive yellowish green in colour. Leaf appears usually after the withering of inflorescence [9].



**Figure 1: The plant *Nervilia aragoana***

The white round fleshy rhizomes of the plant *N. aragoana* is having a very diverse use. The rhizomes are traditionally used for the treatment of epilepsy, in urinary complaints, diarrhoea and asthma [10]. The dried powdered rhizome along with milk is used as aphrodisiac, galactagogue and also increase sperm count. The plant contains alkaloids, flavonoids, triterpenoids, mineral elements, amino acids glycosides and sterols [9], [11]. This indigenous plant has been a very integral part of the life of many tribes in India as they had identified and has been using it for past many centuries. The rhizome paste is being used as a remedy for headache by Bhilla tribe of Maharashtra [12]. And also the rhizome is reported to be used for the treatment of blood dysentery by tribal rehabitants of Amarkantak plateau, Madhya Pradesh, India [13].



Figure 2: The rhizome of *Nervilia aragoana*

### Collection of plant material

The rhizomes of *Nervilia aragoana* were collected from Wadakkanchery, Palakkad district, Kerala, India. The Herbarium of the plant *N. aragoana* was prepared and preserved in the laboratory of Amrita School of Pharmacy

### Preparation of plant material

Fresh rhizomes of the plant *N. aragoana* were collected and washed thoroughly under running tap water first and then brushed gently under tap water to be freed fully from silica. Then the rhizomes were cut into small pieces and shade dried. The dried rhizomes were then pulverized to powder using a mechanical grinder. And the powder was preserved in air sealed polythene cover.

### Preparation of samples

Dried rhizome powder was macerated in ether and ethanol separately for 10 days by occasional stirring. After 10 days both the extracts were filtered using Whatman No. 1 filter paper. The residue obtained after the filtration of ether extract was again extracted in methanol by soxhlation method. The extract was taken and was filtered. The crude extracts obtained were concentrated by rotary evaporator at 40°C. A part of all the concentrated extracts were kept aside.

The rest of ethanol and methanol extracts were separated into ether layer and water layer using a separating funnel by adding 5% HCl and ether. These layers were filtered. The ether layers were then concentrated by rotary evaporator at 40°C and the water layers at 60°C. The crude extracts and the separated layers were analyzed by GC-MS.

### Gas Chromatography- Mass Spectroscopy Analysis

*Derivatization procedure:* Two procedures were followed. For the crude ethanol ether and methanol extracts, a small amount of

concentrated sample was taken in a separating funnel and shaken by adding water and ethyl acetate in the ratio of 1:4. The upper layer was collected and concentrated in rotary evaporator to about 1.5 ml. Added 100µl N, O-Bis(trimethylsilyl)trifluoroacetamide and trimethyl chlorosilane (BSTFA+TMCS) and 20µl pyridine and heated at 60°C for 30 minutes.

For the layers which are separated from the crude extracts, a small amount of extract was taken and evaporated out totally. To this added acetonitrile and filtered into a conical flask. To the filtrate added 50µl BSTFA+TMCS and heated at 60°C in a water bath for 30 minutes. Filtered using 0.45µm membrane filter to a vial.

*GC-MS Analysis:* GC-MS analysis was carried out on a Perkin Elmer Turbo Mass Spectrophotometer (Norwalk, CT06859, and USA) which includes a Perkin Elmer Auto sampler XLGC. The column used was Perkin Elmer Elite - 5 capillary column measuring 30m × 0.25mm with a film thickness of 0.25µm composed of 95% Dimethyl polysiloxane. The carrier gas used was Helium at a flow rate of 0.5ml/min. 1µl sample injection volume was utilized. The inlet temperature was maintained as 250°C. The oven temperature was programmed initially at 110°C for 4 min, then an increase to 240°C. And then programmed to increase to 280°C at a rate of 20°C ending with a 5 min. Total run time was 90 min. The MS transfer line was maintained at a temperature of 200°C. The source temperature was maintained at 180°C. GCMS was analyzed using electron impact ionization at 70eV and data was evaluated using total ion count (TIC) for compound identification and quantification. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS library. Measurement of peak areas and data processing were carried out by Turbo-Mass-OCPTVS-Demo SPL software.

### RESULTS AND OBSERVATIONS

Gas chromatography mass spectroscopy analysis was carried out in crude extracts of the rhizome such as ethanol extract, methanol extract, ether extract and also the separated layers such as water layer separated from ethanol extract and water layer separated from methanol extract.

The total ion chromatogram (TIC) of ethanol extract, ether extract, methanol extract, water layer separated from ethanol extract and water layer separated from methanol extract of *N. aragoana* rhizome showing the GC-MS profile of the compounds identified is given in the figures 3, 4, 5, 6 and 7 respectively. The peaks in the chromatogram were integrated and were compared with the database of spectrum of known components stored in the GC-MS library. The detailed tabulations of GC-MS analysis of the extracts are given in Table 1, Table 2, Table 3, Table 4 and Table 5 respectively. Phytochemical analysis by GCMS analysis of the plant rhizomes revealed the presence of different fatty acids, heterocyclic compounds etc.

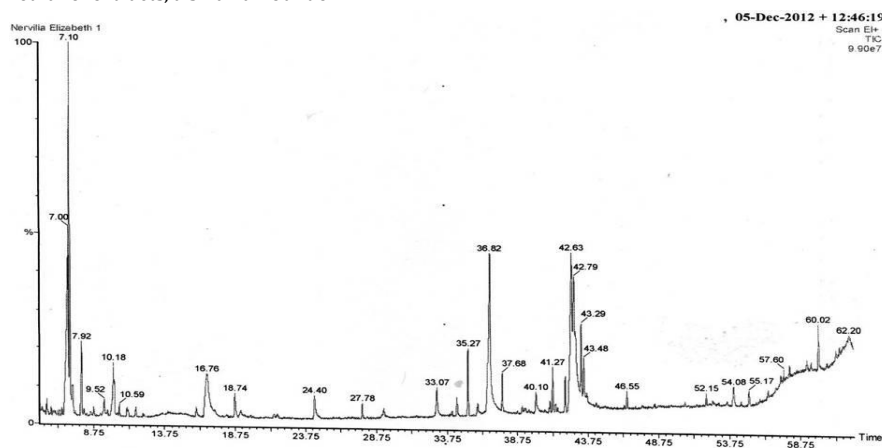


Figure 3. Total Ion Chromatogram (TIC) of ethanol extract of rhizomes of *N. aragoana*.

**Table 1: Phytochemicals identified in the ethanol extract of the rhizomes of *N. aragoana* by GC-MS Peak Report TIC.**

Peak	R. time	IUPAC Name; chemical formula; common name	Chemical structure	Nature and uses
1	7.004	5-Nonanol; C <sub>9</sub> H <sub>20</sub> O; dibutylcarbinol		Fatty alcohol; pheromone, surfactant
2	7.924	5-hydroxy-2-(hydroxyl methyl)-4H-pyran-4-one; C <sub>6</sub> H <sub>6</sub> O <sub>4</sub> ; Kojic acid		White crystalline powder, Inhibits skin melanin formation, antibacterial, antifungal
3	18.738	2-octenoic acid; C <sub>8</sub> H <sub>14</sub> O <sub>2</sub>		Powerful odour; Food flavoring agent
4	24.405	1-hydroxy-2,2,6,6-tetramethyl-3-(4-nitroso-1-(piperazinylmethyl)-piperidin-4-one) C <sub>14</sub> H <sub>26</sub> N <sub>4</sub> O <sub>3</sub>		Intermediate of plastic stabilizer
5	33.06	Pentadecanoic acid; C <sub>15</sub> H <sub>30</sub> O <sub>2</sub>		Rare Fatty acid in nature; white powder; flavoring agent
6	35.269	Hexadecanoic acid; C <sub>16</sub> H <sub>32</sub> O <sub>2</sub> ; Palmitic acid		Fatty acid; white crystals; Anti inflammatory
7	36.821	11-bromoundecanoic acid; C <sub>11</sub> H <sub>21</sub> BrO <sub>2</sub>		Used for nylon plastic preparation; Off-white to beige crystalline powder;

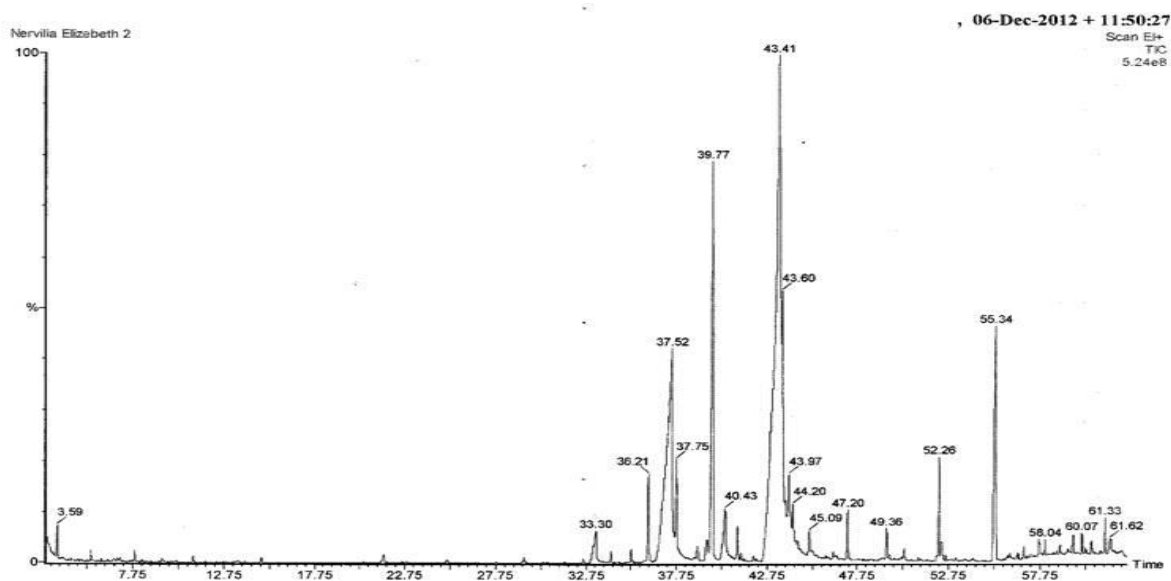
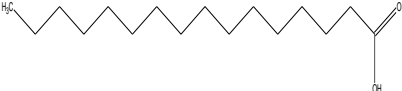
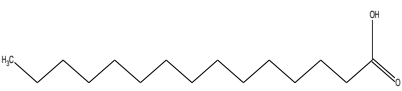

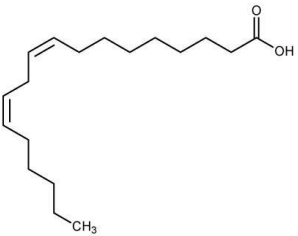
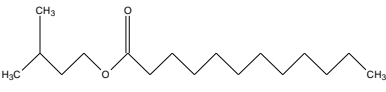
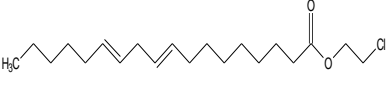
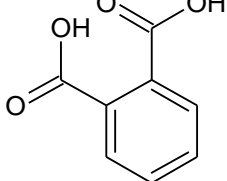
**Figure 4: Total Ion Chromatogram (TIC) of ether extract of rhizomes of *N. aragoana*.**

Table 2: Phytochemicals identified in the ether extract of the rhizomes of *N. aragoana* by GC-MS Peak Report TIC.

Peak	R. time	IUPAC Name; chemical formula; common name	Chemical structure	Nature and uses
1	33.296	Hexadecanoic acid; C <sub>16</sub> H <sub>32</sub> O <sub>2</sub> ; Palmitic acid		Fatty acid; Anti inflammatory
2	36.21	Pentadecanoic acid C <sub>15</sub> H <sub>30</sub> O <sub>2</sub>		Rare Fatty acid in nature; white powder; flavoring agent
3	40.434	Heptadecanoic acid; Margaric acid		saturated fatty acid; marker
4	43.409	9,12-Octadecadienoic acid C <sub>18</sub> H <sub>32</sub> O <sub>2</sub> ; Linoleic acid		Unsaturated fatty acid, EFA
5	47.203	Isoamyl laureate C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>		Fragrance ingredient, skin conditioning agent
6	49.359	2-chloroethyl linoleate		Terpinene; flavouring agent
7	55.344	1,2-benzenedicarboxylic acid; Phthalic acid		Colourless crystalline acid used in synthesis of dyes and perfumes; neurodegenerative disorders

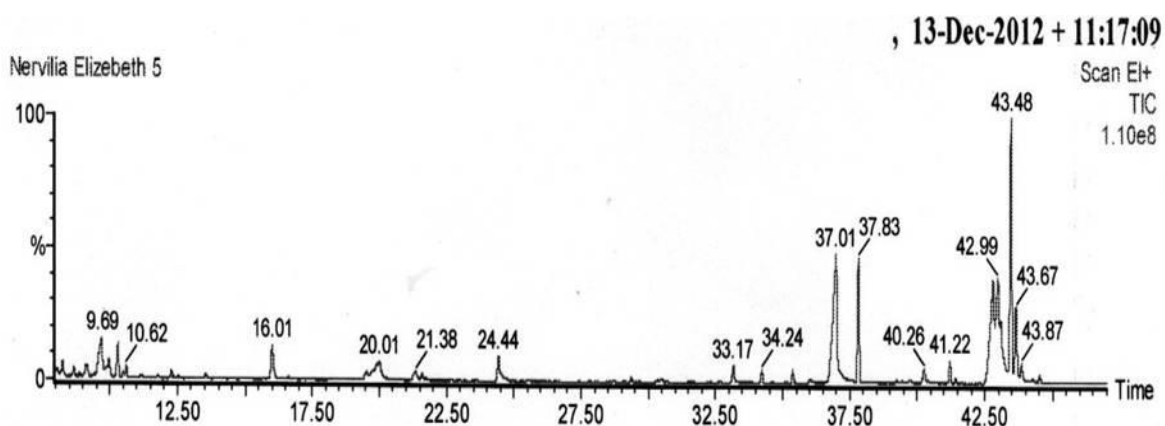
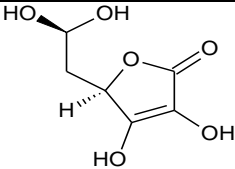
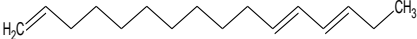

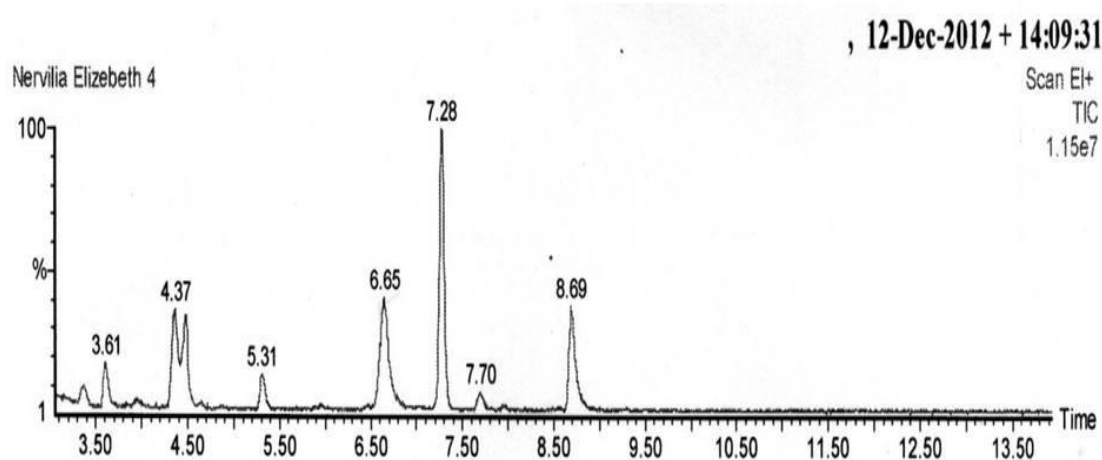
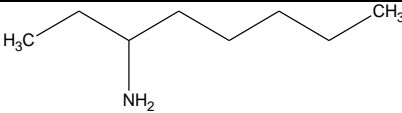
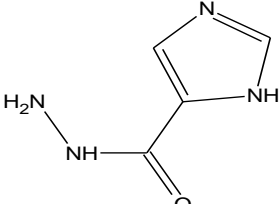
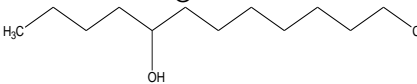
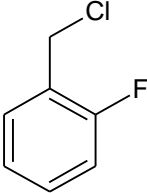
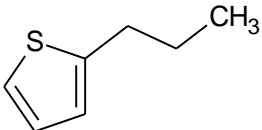
Figure 5: Total Ion Chromatogram (TIC) of methanol extract of rhizomes of *N. aragoana*.

Table 3: Phytocomponents identified in the methanol extract of the rhizomes of *N. aragoana* by GC-MS Peak Report TIC.

Peak	R. time	IUPAC Name; chemical formula; common name	Chemical structure	Nature and uses
1	37.005	L-(+)-ascorbic acid; Vitamin C		white or slightly yellow crystal or powder with a light acidic taste; antiscorbutic, antioxidant
2	42.808	1,E-11,Z-13-hexadecatriene; C <sub>16</sub> H <sub>28</sub>		Not intended for therapeutic and diagnostic purpose
3	43.481	6,8-dodecadien-1-ol		Colorless liquid; Pheromone

Figure 6. TIC of the water layer separated from ethanol extract of rhizomes of *N. aragoana*.Table 4: Phytocomponents identified in the water layer separated from ethanol extract of the rhizomes of *N. aragoana* by GC-MS Peak Report TIC.

Peak	R. time	IUPAC Name; chemical formula; common name	Chemical structure	Nature and uses
1	4.367	3-octanamine		Not intended for therapeutic and diagnostic purpose
2	5.311	5-hydrazinocarbonyl imidazole		Not intended for therapeutic and diagnostic purpose
3	6.653	5-tridecanol		White crystal powder; Lubricant, detergent, antifoaming agent

4	7.278	1-chloromethyl-2-fluoro-benzene; C <sub>7</sub> H <sub>6</sub> ClF		Clear colorless liquid; Not intended for therapeutic and diagnostic purpose
5	8.691	2-propylthiophene; C <sub>7</sub> H <sub>10</sub> S		Colorless liquid, Flavoring agent

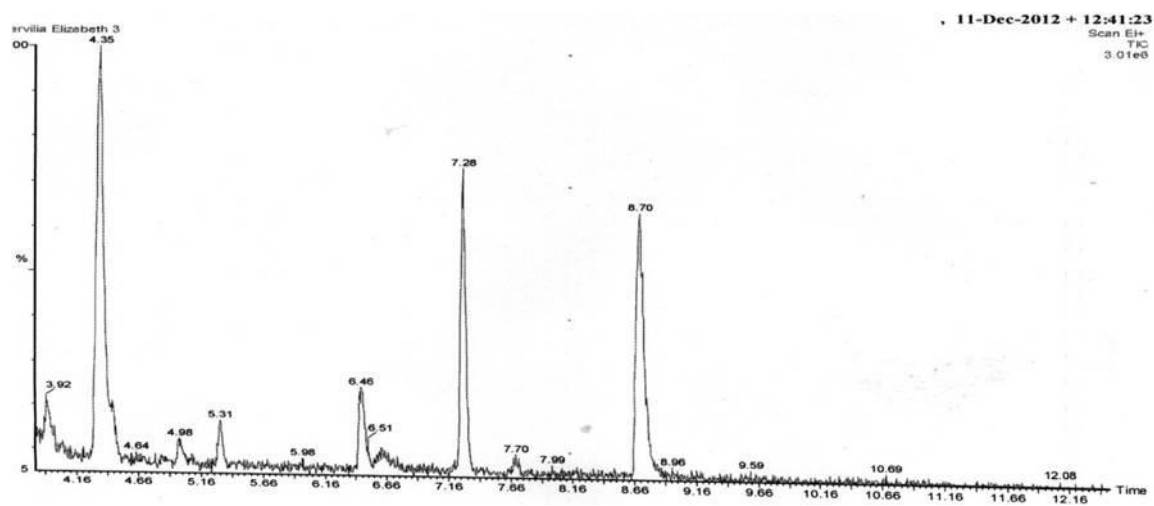
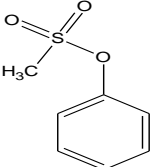
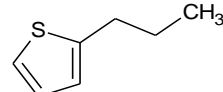


Figure 7. TIC of the water layer separated from methanol extract of rhizomes of *N. aragoana*.

Table 5: Phytocomponents identified in the water layer separated from methanol extract of the rhizomes of *N. aragoana* by GC-MS Peak

Report TIC.						
Peak	R. time	IUPAC Name; chemical formula; common name	Chemical structure	Nature and uses		
1	7.279	P-mesyloxyphenol		Not intended for therapeutic and diagnostic purpose		
2	8.701	2-propyl-thiophene; C <sub>7</sub> H <sub>10</sub> S		Colorless liquid, Flavoring agent		

## DISCUSSION

The GCMS analysis of various compounds from *N. aragoana* rhizome extracts was performed using Perkin Elmer Elite - 5 capillary column and typical total ion chromatograms (TIC) of each sample were given in fig. 3, 4, 5 and 6 respectively. The comparison of the mass spectrums with the data base gave more than 90% match as well as confirmatory compound structure match. The GCMS analysis of the concentrated ethanol extract, ether extract, methanol extract resulted many compounds which have diverse use. Compounds having anti-inflammatory, antibacterial, antifungal, skin conditioning properties have been identified. The plant is extensively used as a diuretic traditionally in India.

The concentrated ethanol extract contains a variety of fatty acids.

The compound 5-hydroxy-2-(hydroxyl methyl)-4H-pyran-4-one is having antibacterial antifungal properties and it inhibits melanin production. Anti inflammatory compounds like Hexadecanoic acid, fragrance and flavoring agents such as 2-octenoic acid, pentadecanoic acid etc are identified (Table 1). The ether extract contains hexadecanoic acid which has anti inflammatory activity, flavouring agents like pentadecanoic acid, 2-chloroethyl linoleate, isoamyl laureate which is a skin conditioning agent, phthalic acid which is used in neurodegenerative disorders (Table 2). The methanol extract contains vitamin C having antioxidant activity (Table 3). The water layers separated from ethanol extract contain 2-propyl thiophene which is a flavouring agent (Table 4 and 5). This study explores the goodness of the rhizome of the plant *Nervilia aragoana* which has a commendable sense of purpose and can be advised as a plant of phytopharmaceutical importance.

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**REFERENCE**

1. Home page on the internet, World Health Organization, available from url <http://www.who.int/medicines/areas/traditional/definitions/en/>.
2. Aneesh TP, Mohamed Hisham, Sonal Sekhar M, Manjusree Madhu Deepa TV. International Market Scenario of Traditional Indian Herbal Drugs. *Int. J. Green Pharm.* 2009; 3 suppl 3: 184-190.
3. Kirtikar KR, Basu BD. *Indian Medicinal Plants*. Indian Press. 1918. p. 34-44.
4. Ronald Hites A. *Gas Chromatography Mass Spectroscopy: Handbook of Instrumental Techniques for Analytical Chemistry*, 1997. p. 609-611.
5. Ayurvedic medicinal plants [http://ayurvedicmedicinalplants.com/index.php?option=com\\_zoom&Itemid=26&page=view&catid=14&key=2](http://ayurvedicmedicinalplants.com/index.php?option=com_zoom&Itemid=26&page=view&catid=14&key=2)
6. Prof. Summer's Web Garden [http://www7a.biglobe.ne.jp/~flower\\_world/Orchids/Nervilia%20aragoana.htm](http://www7a.biglobe.ne.jp/~flower_world/Orchids/Nervilia%20aragoana.htm)
7. Rajendra Yonzone, Lama D, Bhujel RB, Samuel Rai. Orchid species diversity of Darjeeling Himalaya of India. *Int. J. of Pharm. & Life Scie* 2012; 3 suppl 3: 1533-1550.
8. Envis centre on medicinal plants. Available from url <http://envis.frlht.org/>
9. Bhogaonkar PY, and Devankar VD. 2006. Pharmacognostic Studies on Padmacarini. *Aryavaidyan* 2006; 20 suppl 2: 74-79.
10. NeMedPlant A Database of medicinal plants from northeast india, available from url <http://bif.uohyd.ac.in/nemedplant/search/RightsearchResult.php?g=Nagaland>.
11. Beena C, Radhakrishnan VV. Bioprospecting on the endangered medicinal plant *nervilia aragoana* gaud. *Journal of Progressive Agriculture* 2011; 2 suppl 1: 75-78.
12. Kamble S Y, Patil SR, Sawant PS, Sangita Sawant, Pawar SG, Singh EA. Studies on Plants used in traditional medicine by Bhilla tribe of Maharashtra, *Indian J of Traditional Knowledge*. 2010; 9 suppl 3: 591-598.
13. Arjun Prasad Tiwari, Bhavana Joshi, Ansari AA. Less Known Ethnomedicinal Uses of Some Orchids by the Tribal inhabitants of Amarkantak Plateau Madhya Pradesh, India. *Nature and Science*. 2012; 10 suppl 12: 33-37.