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Short communication

Dendrobium nobile orchid in traditional medicine - A phytochemical analysis

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ABSTRACT

The qualitative and quantitative analysis of the native orchid *Dendrobium nobile* was taken into consideration to validate the uses in traditional medicine. Methanol extracts of different parts of *Dendrobium nobile* reveal the presence of bioactive compounds such as, Vitamin A Aldehyde; Longifolene; 1-Heptatriacotanol; Z,Z-6,28-Heptatriactontadien-2-One and Dendroban-12-One.The pharmaceutical properties would draw attention of the modern medical science.

Keywords: Sikkim Himalaya, polar compounds, GC-MS.

Orchidaceae is the second largest angiosperm family having 800 genera with more than 25,000 species. Orchids occupy important space in the life of people in Sikkim Himalaya. Rich Orchid diversity and its cultural association in this area have led to adoption of *Dendrobium nobile* Lindl. as the state flower of Sikkim. Sikkim harbours 557 orchids under 133 genera [ENVIS, Forest Department, GoS], out of 1331 reported taxa in India. Apart from the majestic ornamental importance, orchids also occupy a very important part in the traditional folklore medicine. The Indian and Chinese systems of medicine mention about the use of orchids since ancient times. *Dendrobium nobile* Lindl.(Fig. 1) is an epiphytic orchid species distributed in the North-Eastern part of India and extended upto other south-east Asian countries like Bhutan, China, Myanmar, Nepal, Thailand, Laos and Vietnam. The flowers are light to deep purple-pink and white with a characteristic deep purple-pink silky center(Lucksom,8). The medicinal importance of this species has been reported by Ngilyang *et al.* (11). According to a worldwide report, in 42 *Dendrobium* species, 100 phytochemical compounds comprising of 32 alkaloids, 15 bibenzyls, 6 coumarins, 4 fluorenones, 22 phenanthrenes and 7 sesquiterpenoids were found (Zhang *et al*., 16).

Dendrobium nobile Lindl. has been reported to used in different traditional medicines in various parts of Asia. The stem has been used in curing eye infection and to appease burnt injury. Fresh and dried stems are used for anti-ageing, antimicrobial, as aphrodisiac, fever, stomachic, tongue dryness and analgesic (Kong *et al*., 5). In Chinese Materia Medica, the plant has been regarded as a vital source from which many important drugs are mentioned to be derived. Moreover, whole plant is being used in nervous disorder (Ngilyang *et al*., 11). In Vietnam, the plant is used in the treatment of pulmonary tuberculosis, weakness, flatulence, dyspepsia, reduce salivation, parched and thirsty mouth, night sweats, fever and anorexia.

But no phytochemical reports have been made from the native *Dendrobium nobile* of Sikkim Himalaya. The current work is a part of phytochemical analysis of medicinal orchids, in which qualitative and quantitative screening work was conducted.

The plant samples were collected from the *Dendrobium nobile* polyhouse of ICAR-National Research Centre for Orchids, Sikkim during 2018. Proper tagging and coding were made before collection and during preparation of samples. Following qualitative tests were done following the protocols described by Raaman (13).

Fig. 1. *Dendrobium nobile* Lindl.

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Test for reducing sugar: About 2ml of Benedict's reagent was added in 2ml of plant (leaf, stem and roots) aqueous extracts separately and were shaken for thorough mixing. The leaf and stem mixture form dark yellow red precipitate where the root mixture form dark yellow orange precipitate, showing the test was positive.

Test for alkaloid: Few drops of Wagner's reagent was added in 1 ml of aqueous extract of the sample, wherein red brown precipitate was formed in both leaf and stem samples. A dark brown precipitate was also seen in the roots samples showing positive test for all the samples.

Test for flavonoid: About 2 ml of crude acid extract of leaf, stem and roots were mixed separately with 2ml of NaOH in a reagent tube. A yellow coloration was observed at the lower layer of the leaf mixture. On the other hand, the stem mixture shows yellow brown coloration while yellow coloration at the bottom layer was also observed in root mixture. All the mentioned observations indicated positive test for flavonoids.

Test for Tannin: For the test, 2 ml of the aqueous extracts of the samples 4 drop of 5 % $FeCl_3$ was added. Light green to dark green precipitate was observed in all the samples. The leaf sample shows light to dark coloration precipitate, the stem forms green precipitate while the root sample form dark green precipitate. The observation shows the presence of condensed tannins in all the samples.

Test for steroid:2ml of acetic anhydride was added to 2ml of acid extracts of leaf, stem and roots samples mixed with 2ml of H_2SO_4 separately. The color changed to green in stem and root samples which indicates the presence of steroids, except in the leaf sample.

Test for Glycoside: In 0.5 ml of aqueous extract 0.25 ml aqueous NaOH (40%) was added. Yellow coloration was observed with variable intensity. The leaf extract shows yellow, stem extract with light yellow and root extract was observed to give dark yellow. All the samples show positive observations for the test.

Test for phytosterol: To the acid extract of the samples 2 drops of concentrated H_2SO_4 was added slowly along sides of test tube. On the upper layer green coloration was observed whereas on the bottom layer red brown ring was formed in all the samples which show presence of phytosterol.

Test for Saponin: The Honeycomb test was conducted in two different approaches where in the first approach 4 drops of 5 % $NaOHCO_3$ was added in 2ml of aqueous extract of the samples. In another approach 4 drops of 5 % $NaOHCO_3$ was added in 0.5 mg powdered sample. The test gave no evidence for the test of the leaf, stem and roots samples.

GC-MS analysis

The air dried samples (8 gm each of stem, leaf and root) were subjected to Soxhlet extraction for 8 hrs using methanol (250 ml) as the solvent. The solvent were extracted using Buchi Rotavapor R-200 Rotary Evaporator after which 1 ml of optimized samples in GC vials were loaded on sample loader of Perkin Elmer (USA): laurus 680 GC/Claurus 600C MS [GC Liquid Autosampler] for analysis.

Different ethnobotanical reports suggest there are almost 110 species of orchids in Sikkim which have medicinal importance. Medicinal use of *Dendrobium nobile* has been known in Chinese medicine, folklore healing system of the North-eastern India. In Sikkim, the tribal population uses this species to cure ailments, diseases, injuries and in maintaining sexual health (Jalal *et al.*, 4, Panda and Mandal, 12).

On preliminary test of the plant samples *Dendrobium nobile* contents different group of potential compounds with potential bioactivity (Table 4) *viz*. alkaloids, flavonoid, glycosides, phytosterols, reducing sugar, steroids, tannin (Table 1) (Xiaolin *et al.*, 15). Further analysis of methanol extract of the plant parts *via.* GCMS approach, the compounds were identified as γ-Himachalene; Vitamin A Aldehyde; 2,6-DI-tert-butylphenol; Longifolene; Bis-1,6-(4-Cyclohepta-1,3,6-Trienyl-Phenoxy)-Hexane;7R,8R-8-Hydroxy-4-Isopropylidene-7-Methylbicyclo [5.3.1]

Table 1. Qualitative analysis of *Dendrobium nobile.*

S. N.	Plant Metabolites	Test	Leaf	Stem	Root
1.	Reducing Sugar	Benedict's Test	+	++	+++
2.	Flavonoid	Flavanoids Test	+	++	+++
3.	Tannin	Tannin test (Condensed tannins)	+	++	+++
4.	Glycosides	Glycosides Test	+	++	+++
5.	Alkaloids	Wagner Test	+	++	+++
6.	Steroids	Salkowski test	-	++	+++
7.	Phytosterols	Libermann Burchard Test	+	++	+++
8.	Saponins	Honeycomb Test	-	-	-

[*"-":Absent; "+": Presence in leaf; "++":Presence in stem* and *"+++": Presence in roots*]

undec-1-en-8-ol; 2-(4A,8-Dimethyl-1,2,3,4,4A,5,6,7-Octahydro-Naphthalen; 1-Heptatriacotanol; Z,Z-6,28-Heptatriactontadien-2-One; Dendroban-12-One [Dendrobine] (Table 2 & 3); 2-Pentacosanone; N-Octyl Trimethyl Ammonium Bromide; 4-Ethyl-5-octyl-2,2-bis (trifluoromethyl)-1,3-dioxolane and Methyl 2-Hydroxy-Eicosanoate from comparison of the spectrometric data with NIST databases and literature values.

Table 2. GCMS analysis of *Dendrobium nobile* methanol extract (leave).

S. N.	RT	Compound Name	MF	MW	Peak area %
1.	26.991	γ-Himachalene	$C_{15}H_{24}$	204	3.497
2.	27.031	Vitamin A Aldehyde	$C_{20}H_{28}O$	284	0.249
3.	27.546	2,6-DI-Tert-butylphenol	$C_{14}H_{22}O$	206	3.892
4.	31.478	Longifolene	$C_{15}H_{26}O$	222	1.810
5.	31.683	Bis-1,6-(4-Cyclohepta-1,3,6-Trienyl-Phenoxy)-Hexane	$C_{32}H_{34}O_2$	450	2.771
6.	32.308	7R,8R-8-Hydroxy-4-Isopropylidene-7-Methylbicyclo [5.3.1] undec-1-en-8-ol	$C_{15}H_{24}O$	220	4.219
7.	33.469	2-(4A,8-Dimethyl-1,2,3,4,4A,5,6,7-Octahydro-Naphthalen	$C_{15}H_{24}O$	220	6.680
8.	35.004	1-Heptatriacotanol	$C_{37}H_{76}O$	536	4.191
9.	36.165	Z,Z-6,28-Heptatriactontadien-2-One	$C_{37}H_{70}O$	530	0.885
10.	39.181	Dendroban-12-One	$C_{16}H_{25}O_2N$	263	9.545
11.	40.256	2-Pentacosanone	$C_{25}H_{50}O$	366	1.311

Table 3. GCMS analysis of *Dendrobium nobile* methanol extract (stem & root).

S. N.	RT	Compound Name	Molecular formula	MW	Peak area %
Stem					
1.	39.191	Dendroban-12-One	$C_{16}H_{25}O_2N$	263	9.921
2.	40.237	N-Octyl Trimethyl Ammonium Bromide	$C_{11}H_{26}BrN$	252	3.441
Root					
1.	39.701	Dendroban-12-One	$C_{16}H_{25}O_2N$	263	5.221
2.	47.674	4-Ethyl-5-octyl-2,2-bis(trifluoromethyl)-1,3-dioxolane	$C_{15}H_{24}O_2F_6$	350	0.658
3.	50.265	Methyl 2-Hydroxy-Eicosanoate	$C_{21}H_{42}O_3$	342	1.493

Table 4. Bioactive compounds found in *Dendrobium nobile*.

S. N.	Compound Name	Activity	Reference
1.	Vitamin A Aldehyde	• Carotenoid constituent of visual pigments. • Diagnosis and treatment of disease e.g Celiac disease	• Lindsay and Tadao, (7)
2.	Longifolene	• Antifungal, antibacterial and antioxidant potential.	• Bourgou *et al*. (1) • Hassanien *et al*. (2) • Mukai *et al.* (10)
3.	1-Heptatriacotanol	• Anti-hypercholesterolemic effects. • Antimicrobial activity.	• Junweia *et al*. (3)
4.	Z,Z-6,28-Heptatriactontadien-2-One	• Vasodilatory effect.	• Manjari *et al*. (9)
5.	Dendroban-12-One	• Antiviral activity against influenza A viruses. • Anti-cancer (Lungs cancer treatment). • Analgesic and antipyretic action. • Hyperglycemia activity.	• Li *et al*. (6) • Song *et al*. (14)

From all the compounds identified only Dendroban-12-One (Fig. 2) was found to present in all the plant parts of *Dendrobium nobile* with highest peak area percentage ranging from 5.221 to 9.921, which indirectly reveals the quantity in the plant.

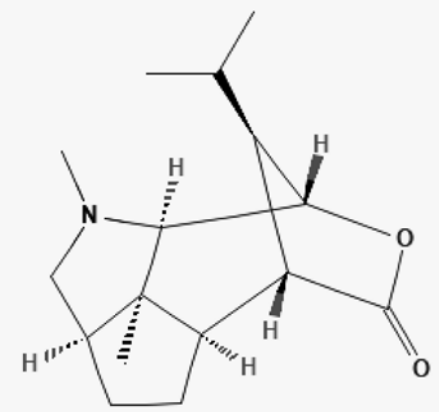

Fig. 2. Dendroban-12-One

The study reveals the compounds present in the native *Dendrobium nobile* of North-East India *w.r.t.* methanol solvent extract. The results also show the presence of important bioactive compounds. In conclusion, the above work projects the importance to screen reported medicinal orchids for potential biological activities.

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