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NATURAL COMPOUNDS PROFILE OF *AERIDES ODORATA* USING THE GC-MS/MS TECHNIQUE

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ABSTRACT

Aerides odorata is epiphytic and commercially available as one of the potential orchids with great medicinal value. The investigation that we conducted yielded alkaloids. The alkaloids found in *Aerides odorata* seedlings were analysed for the very first time. The majority of alkaloids were extracted using methanol. In addition, the levels of total alkaloids in *Aerides odorata* and *Trichoderma longibrachiatum* VKPMGU#1 were determined. The results of the GC-MS/MS analysis of *Aerides odorata* and *Trichoderma longibrachiatum* are presented. These tables and figures indicate the peaks, retention time (RT), Compounds/ID, Reference number, CAS, and Quality. GC-MS/MS The identification of alkaloids was carried out by GC-MS/MS without the need for any derivatization, as was discovered by conducting an analysis on samples of *Aerides odorata* to determine the presence of total alkaloids. The seedling of *Aerides odorata* had a total of 57 different alkaloid peaks. GC-MS/MS examination of co-cultured *Aerides odorata* samples for the presence of total alkaloids indicated that a total of 65 alkaloid peaks were found in the seedling of *Aerides odorata* that had been co-cultured with *Trichoderma longibrachiatum* VKPMGU#1. In addition, we introduced the latest complete development of the biostrategies for the cultivation of endophytes from *A. odorata* and the integration of bioengineered methods by emphasizing the combined omics as a powerful way to obtain novel metabolite novel chemicals.

Keywords: *Aerides odorata*; Endophytes; Natural compounds; *Trichoderma longibrachiatum*; GC-MS/MS

1. INTRODUCTION

Recent ethnobotanical research on orchids show that they have enormous promise for treating a variety of ailments, and the Chinese were the first to describe their medicinal use. India is home to 1331 species and 186 genera of orchids **[1]**. There is a species of vanda known as *Aerides Odorata*, which is renowned for producing fragrant flowers. The flowers are similar to those of the foxtail *Aerides odorata*, and the shrub blooms frequently. This orchid plant thrives in just one habitat, a form of lowland woods with high light intensity and low humidity, yet it is highly adaptable to various settings. The plants may reach a length of one metre, have stout, branching stems, and their natural leaves are oblong and lobed. The flowers range in colour from white to purple and range in length from 1.5 to 2.5 cm. Ethnobotanically, *A. odorata* was used to treat a variety of ailments, including chest pain and stomach ailments, boils in the ears and nose, pneumonia, and inflammations **[2]**. Endophytes were shown to be tissue selective, with just a small amount of overlap occurring between the leaf and root tissues at the junction. There were several species of Xylaria, each with its own unique roots and leaves, which distinguished them from the *Aerides odorata* species **[3]**.

Important in influencing the quality of the ethnobotanical documentation generated is the researcher's ability to work effectively with the indigenous informants. *Aerides odorata* is an epiphytic species of orchid. Endophytes may also reduce biotic and abiotic stress in plants that contain them. Both types of fungi's hyphae entered the seed's general surface and encircled it as they grew. The development of Orchidaceae can be encouraged by the presence of fungal endophytes, which work to activate soil nutrients in the rhizosphere. In addition to that, the impact that alterations in the total number of SMs have is discussed. Orchidaceae should be safe from soil bacteria in general if they have access to a living chemical source. A prominent and ubiquitous method of seed propagation, sustainable symbiotic orchid germination has proven particularly important in situations in which particular species are needed for the continuation of the co-planting system **[4]**.

World-wide, Traditional Medicine (TM) has embraced Natural Products (NPs) as a type of therapy for a number of ailments. Today's practise of Modern Medicine (MM) is based on the Traditional Medical System (TMS). The contribution of Medicinal Plants (MPs) to the development of new medications and medical materials was crucial. Several traditional medical systems, among them, make reference to various MPs. It is common knowledge that

plants have medicinal value, and humans have utilised plants for therapeutic purposes since the time of the Vedas. Ayurveda is an ancient Indian medical system that prescribes numerous plant extracts to treat a broad variety of illnesses. In modern medicine, phytochemicals are utilised to treat a broad spectrum of diseases. Recent developmental study on *Aerides odorata*, including its dynamics, new knowledge of endophytes, and the synthesis of secondary metabolites (SMs) **[5]**. This study is one-of-a-kind since it focuses on the naturally occurring chemicals linked with *Aerides odorata*, an endophytic fungus. Natural compounds originating from *Aerides odorata* and endophytic fungus should be researched in order to meet the local and international demand for therapeutic substances. Investigate natural compounds obtained from *Aerides odorata* and endophytic fungus with the goal of meeting the domestic and worldwide demand for medical chemicals. It is widely known that *Aerides odorata* present a broad variety of floral forms and attract several species of foragers via the use of specialised signals. Despite this, not enough study has been conducted on *Aerides odorata* to understand how floral antagonists impact the interaction between plants and pollinators. However phytochemical research on orchids has not yet been undertaken, so existing orchid species that are currently utilised as herbal remedies for a range of health issues have been chosen for phytochemical investigation. If successful, this research might lead to the discovery of new sources of medications of herbal origin in the near future **[6]**.

When *A. odorata* was investigated with Hager's reagent (H), it was discovered that a greater quantity of alkaloids was present. The purity of the alkaloids that were obtained from *A. odorata* was determined with the use of Wagner's reagent (W). An examination of the tannin, terpenoids, steroids, quinine, and cumarin content of *A. odorata* revealed that it possessed the most potent orchid medications **[6]**. Phytocompounds derived from *A. odorata* have been shown to possess anti-cancer activity. It inhibits the growth of cancer cells, fights infections and inflammation, lowers blood pressure, and acts as an antioxidant **[7]**.

This paper aims to highlight the findings of recent developmental studies on *Aerides odorata*, including its dynamics, new insights on endophytes, and SM formation. This study is unique since it focuses on the naturally occurring chemicals linked with *Aerides odorata*, an endophytic fungus. Please offer facts and an explanation in support of your assertion that *Aerides odorata* and endophytic fungus are responsible for the production of naturally occurring chemicals.

2. MATERIALS AND METHODS

2.1 Coculturing endophytic fungi on Aerides odorata seedlings in test bottles

The detected endophytic fungal mycelium of *Trichoderma longibrachiatum* VKPMGU#1 was employed for the purpose of quantifying natural alkaloids. In order to isolate the mycelium suspension, the endophytic cultures were filtered before being added to *Aerides odorata* seedlings. After being concentrated, the *Trichoderma longibrachiatum* VKPMGU#1 mycelium was injected into cut marks made on terminal leaves at the point where they connect to the stem. In the trials concerning the co-culturing of seedlings, the sterile cotton plug was used as a control. The control was consisting of three independent replicates performed in the identical environment but without the addition of any endophytic fungal cultures. Following the inoculation, the test co-culturing bottles were moved into the plant growth chamber instrument, where they were kept at a temperature of 28 degrees Celsius and subjected to a light/dark cycle with a duration of 12 hours and a light intensity of 1500 lux. At the 15-day mark, each test vial was scrutinised for results.

2.2 Extraction and detection of natural compounds from cocultured plant samples

2.2.1 Analysis for the detection of natural compounds

In order to extract the natural alkaloids, a powder made from the baked-and-dried stem of *Aerides odorata* were employed. The powder was combined with methanol, then subjected to a two-hour boil at a temperature of ninety degrees Celsius. Upon the completion of the extraction process, the contents were filtered using whatman filter paper#42, and the resulting filtrate was placed in sample tubes prior to being analysed by chromatography.

2.2.2 Plant natural compounds extraction

For the purpose of extracting natural chemicals from *Aerides odorata* plant samples (Stem, acquired), 150 mg of dry plant parts were pulverised in a mortar and pestle after they have been freeze-dried, and then they were soaked in 50 ml of methanol for 12 hours while being rotated at 180 rpm. The liquid component, measuring 40 ml, were extracted from the mixture. After separating the aqueous phase from the methanol phase, the methanol phase was evaporated at 35 degrees Celsius using a rotary evaporator. Before doing the analysis, the residual residue was re-dissolved in 5 ml of methanol and filtered using a filter with a pore size of 0.45 millimetres.

2.2.3 Detection of natural compounds by GC-MS/MS

In order to identify alkaloids using the GC-MS/MS, the following conditions were kept constant at all times. They are going to be: The multi residue pesticide analysis performed using UHPLC Vanquish Core and, TSQ QUANTIS with the help of TRACEFINDER 5.2 EFS Quan software was used. A summary of all instrument conditions and gradient conditions are given in the **Table 1 and 2**.

Table 1: LC-MS/MS Instrument conditions.

	Liquid chromatography method
	Instrumentation: Vanquish UHPLC
Column:	**Thermo Scientific TM Accucore TM aQ (100 mm × 2.1 mm × 2.6 µm) (P/N 17326-102130)**
	Sample Compartment temperature: 10° C
	Column oven temperature: 25° C
Mobile phase:	**A: 5mM ammonium formate + 0.1% formic acid in water**
	B: 5mM ammonium formate + 0.1% formic acid in methanol
	Total run time: 15.0min

Table 2: Gradient method for GC-MSMS.

Retention time (min)	Rate (°C/min)	Target value (°C)	Hold time (min)
0.000	0.00	40.0	1.50
1.500	25.000	90.0	1.50
8.600	25.00	180.0	0.00
28.60	5.00	280.0	0.00
35.60	10.00	300.00	5.00
35.60	StopRun		

3 RESULTS AND DISCUSSION

3.1 Extraction and detection of natural compounds from cocultured plant samples

In the course of our research, the extraction of alkaloids was carried out. It is the very first time that a comprehensive investigation of the alkaloids that may be found in the seedling of *Aerides odorata* has been described. The methanol extraction procedure was used to get most of the alkaloids out of the plant. In a similar manner, the total alkaloid presence of *Aerides odorata* and *Trichoderma longibrachiatum* VKPMGU#1 was investigated. The findings of the GC-MS/MS analysis are presented in **Tables 3-4 and Figure 1-2**. These tables and figures show the peaks, retention time (RT), and Compounds/ID, Reference number, and CAS from *Aerides odorata* and *Trichoderma longibrachiatum*, respectively.

3.2 GC-MS/MS Analysis of *Aerides odorata* samples for the presence of total alkaloids

The identification of alkaloids was made by GC-MS/MS without any derivatization. Total of 57 alkaloid peaks were identified **(Table 3 and Figure 1)** in the seedling of *Aerides odorata* of which diverse compounds namely (1) 3-Heptene, (2) Benzene, (3) Ethane, (4) Benzene, (5) Benzene, (6) tetramethyl, (7) Tetramethylbenzene, (8) Dodecane, (9) Benzoic acid, (10) Dodecane, (11) Dodecane, (12) dimethylethyl, (13)

Benzene, 1,3-bis(1,1-dimethylethyl)-, (14) Eicosane, (15) Decene, (16) Hexane, (17) 1-Hexene, (18) Tetradecane (19) Ethanone, (20) Dodecane, (21) Heptacosane, (22) 4,6-dimethyl, (23) Propofol, (24) Octadecane, (25) Phenol, (26) Nonane, (27) Cyclohexane, (28) Eicosane, (29) 3-Heptafluorobytyryloxy-6-ethyldecane, (30) Pyridine-3-carboxamide, (31) Hexadecane (32) O-Acetylcitric acid triethyl ester, (33) Heptadecane, (34) Pentacosane, (35) Pyridine-3-carboxamide, (36) Pyridine-3-carboxamide, (37) Heptafluorobutanoic acid, (38) Undecene, (39) Octacosane, (40) Cyclohexane, (41) Octadecane, (42) Cyclohexane, (43) Nonadecane (44) Hexadecanoic acid, (45) Ascorbic acid 2,6-dihexadecanoate, (46) Phthalic acid, (47) Dendroban, (48) 1-Docosene, (49) 3-METHYL-1-PHENYL-1H-PYRAZOLO(3,4-B)QUINOLINE (50) 9-Octadecenoic acid (51) Octadecanoic acid (52) Eicosane, (53) 4-Isothiazolecarboxamide, (54) Tetradecanoic acid, (55) 9-Octadecenamide, (56) Dodecane, (57) Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl.

Table 3: Total compounds at different retention time in the seedling of *Aerides odorata*

Peak	Retention Time	Area Pct	Compounds/ID	Ref no.	CAS	Qual
1	4.3462	0.7589	3-Heptene	6500	004485-16-9	41
2	4.3948	0.9921	Benzene	14405	001758-88-9	59
3	4.4326	0.5184	Ethane	414745	000067-72-1	91
4	4.4921	0.8277	Benzene	358315	000099-87-6	94
5	4.8648	0.5389	Benzene	358385	000488-23-3	95
6	4.9188	0.8549	Tetramethyl	358383	000488-23-3	97
7	5.3294	0.26	Tetramethylbenzene;	358396	000095-93-2	87
8	5.7723	2.1442	Dodecane	382614	000112-40-3	95
9	5.8534	0.451	Benzoic acid	369585	000119-36-8	95
10	6.2477	0.3383	Dodecane	66080	031295-56-4	72
11	6.3557	0.5974	Dodecane	398678	061141-72-8	87
12	6.4476	0.533	dimethyl-	398678	061141-72-8	92
13	6.5502	7.9078	Benzene	393902	001014-60-4	95
14	6.8095	0.3789	Eicosane	430875	000112-95-8	94
15	7.1552	0.9903	Decene	25942	074630-23-2	52
16	7.2633	1.5119	Hexane	12319	000921-47-1	46
17	7.3713	1.1166	1-Hexene	6551	003404-77-1	38
18	8.3167	2.2465	Tetradecane	398653	000629-59-4	96
19	8.8677	1.2437	Ethanone	376964	001009-61-6	93
20	9.1107	0.5369	Dodecane	55993	061141-72-8	64
21	9.2134	0.6105	Heptacosane	165301	000593-49-7	64
22	9.3376	0.3708	4,6-dimethyl-	55993	061141-72-8	74
23	9.3809	1.4903	Propofol	41614	002078-54-8	59
24	9.5321	0.8622	Octadecane	422273	000593-45-3	91
25	9.7482	7.9477	Phenol	402567	000096-76-4	95
26	9.8724	0.5404	Nonane	27253	017302-23-7	35
27	9.9859	0.5332	Cyclohexane	11235	002234-75-5	43
28	10.0615	0.6838	Eicosane	113489	000112-95-8	64
29	10.1966	0.6266	3-Heptafluorobytyryloxy-6-ethyldecane	165607	1000215-97-2	43
30	10.3208	0.3153	Pyridine-3-carboxamide, oxime	112295	288246-53-7	53
31	10.7314	1.4407	Hexadecane	412222	000544-76-3	98
32	11.466	0.5965	O-Acetylcitric acid triethyl ester	135411	000077-89-4	78

33	11.855	0.6261	Heptadecane	417585	000629-78-7	98
34	12.0062	0.4303	Pentacosane	446033	000629-99-2	90
35	12.3357	0.5886	Pyridine-3-carboxamide, oxime	112295	288246-53-7	59
36	12.4222	0.484	Pyridine-3-carboxamide, oxime	112295	288246-53-7	90
37	12.5194	0.6744	Heptafluorobutanoic acid, heptadecyl ester	180157	1000282-97-3	78
38	12.6274	0.6475	Undecene	44652	055170-93-9	46
39	12.7247	0.4595	Octacosane	169721	000630-02-4	38
40	12.8651	0.3067	Cyclohexane	183899	055521-27-2	10
41	12.9354	0.8049	Octadecane	422272	000593-45-3	96
42	12.984	0.3528	Cyclohexane	25967	062238-33-9	43
43	14.2426	0.3003	Nonadecane	426701	000629-92-5	86
44	14.5668	2.3781	Hexadecanoic acid	422834	000057-10-3	98
45	14.6316	0.4666	Ascorbic acid	189413	028474-90-0	46
46	14.6694	1.2915	Phthalic acid	175178	1000308-91-3	72
47	14.9881	38.216	Dendroban	424944	002115-91-5	94
48	15.1069	0.3585	Docosene	129888	001599-67-3	83
49	15.2312	0.2684	3-METHYL-1-PHENYL-1H-PYRAZOLO(3,4-B)QUINOLINE	138667	000894-88-2	86
50	16.2522	4.2712	9-Octadecenoic acid	430800	000112-80-1	99
51	16.4466	1.945	Octadecanoic acid	431420	000057-11-4	97
52	16.7708	0.5284	Eicosane	430880	000112-95-8	91
53	17.3164	0.3938	4-Isothiazolecarboxamide	123345	037572-35-3	90
54	17.6999	1.33	Tetradecanoic acid	277643	002040-64-4	89
55	18.3967	0.7836	9-Octadecenamide	112657	000301-02-0	70
56	18.7317	0.6452	Dodecane	382625	000112-40-3	92
57	19.153	0.6831	Phenol, 2,2'-methylenebis [6-(1,1-dimethylethyl)-4-methyl	444204	000119-47-1	98

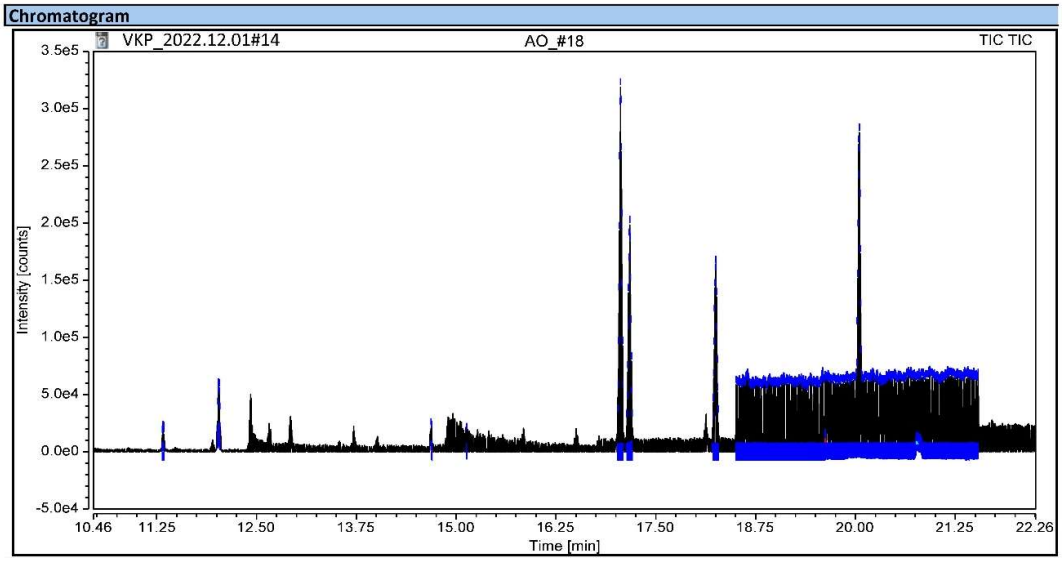

Figure 1: GC-MS chromatogram of *Aerides odorata* samples for the presence of total alkaloids

3.3 GC-MS/MS Analysis of co-cultured *Aerides odorata* samples for the presence of total alkaloids

Total of 65 alkaloid peaks were identified **(Table 4 and Figure 2)** in the cocultured seedling of *Aerides odorata with Trichoderma longibrachiatum* VKPMGU#1 of which diverse compounds namely (1) Ergosterol, (2) Acetic acid, (3) Ethane, (4) Benzene, (5) Benzene, (6) Dodecane (7)

Undecane, (8) Nonane, (9) Benzene, (10) Nonane, (11) 2-Decene, (12) Hexane, (13) Cyclohexane, (14) Tetradecane, (15) Ethanone, (16) Eicosane, (17) 3-methyl-5-propyl, (18) Hexadecane, (19) Phenol, (20) Pentadecane, (21) Phenol, (22) 4,5-dimethyl-, (23) Cyclohexane, (24) 10-Methylnonadecane, (25) 1-Hexene, (26) Pyridine-3-carboxamide, (27) Hexadecane, (28) Propanetricarboxylic acid, (29) dihydro-2'-(morpholin- 4-yl)-5',7'-dinitrospiro, (30) Nonacosane, (31) Tetradecene, (32) 5-Undecene, (33) Muscimol, (34) Oxalic acid, (35) Octadecane, (36) NEOPHYTADIENE; (37) Benzenedicarboxylic acid, (38) Tricosane (39) Benzyl (dideuterated) methyl ether, (40) Hexadecanoic acid (41) Palmitic acid; (42) n-Hexadecoic acid, (43) n-Hexadecanoic acid, (44) Dendroban-12-one, (45) Heptadecane, (46) Heptadecane, (47) 9-Hexacosene, (48) Oleic Acid, (49) Octadecanoic acid, (50) Stearic acid, (51) 9-Octadecenoic acid, (52) 9-Octadecenoic acid, (53) Cyclopropaneoctanal, (54) Carbonic acid, (55) Heptadecene, (56) Hexadecane, (57) Octadec-9-enoic acid, (58) Pentatriacontene, (59) Octadecenamide, (60) Nonadecatriene, (61) Octadecenal, (62) Dodecanedioic acid, (63) Octadecenal, (64) Trimethyltritriacontane, (65) Cyclohexanecarboxylic acid.

Table 4: Total compounds at different retention time.

PK	RT	Area Pct	Library/ID	Ref	CAS	Qual
1	4.3461	0.1199	Ergosterol	170280	000057-87-4	53
2	4.3894	0.1547	Acetic acid	116808	065611-32-7	47
3	4.4326	0.2	Ethane	81288	000067-72-1	91
4	4.8701	0.1159	Benzene	358395	000095-93-2	90
5	4.9188	0.2018	Benzene	358385	000488-23-3	90
6	5.7722	0.429	Dodecane	382630	000112-40-3	87
7	6.3557	0.1345	Undecane	47346	017312-82-2	90
8	6.4475	0.1189	Nonane	27252	017302-28-2	64
9	6.5555	2.6814	Benzene	393902	001014-60-4	95
10	6.8148	0.134	Nonane	46170	062185-53-9	80
11	7.1551	0.2672	2-Decene	25942	074630-23-2	49
12	7.2686	0.3341	Hexane	12319	000921-47-1	50
13	7.3766	0.3154	Cyclohexane	25982	081983-71-3	49
14	8.3166	0.8004	Tetradecane	398647	000629-59-4	98
15	8.8676	0.2954	Ethanone	376962	001009-61-6	94
16	9.1107	0.1835	Eicosane	430875	000112-95-8	87
17	9.2133	0.1914	3-methyl-5-propyl	46168	031081-18-2	53
18	9.343	0.1118	Hexadecane	113507	000638-36-8	72
19	9.3808	0.3857	Phenol	41646	002934-05-6	53
20	9.5482	0.4321	Pentadecane	405601	000629-62-9	97
21	9.7535	2.6756	Phenol	402563	000096-76-4	96
22	9.8724	0.137	4,5-dimethyl	27253	017302-23-7	46
23	9.9858	0.1615	Cyclohexane	25967	062238-33-9	43
24	10.0668	0.1491	10-Methylnonadecane	113493	056862-62-5	72
25	10.2019	0.1588	1-Hexene	6551	003404-77-1	45
26	10.3261	0.1369	Pyridine-3-carboxamide	112295	288246-53-7	59
27	10.7313	0.6155	Hexadecane	412222	000544-76-3	98
28	11.4713	0.2008	Propanetricarboxylic acid	428966	000077-93-0	86
29	11.8603	0.192	dihydro-2'-(morpholin-4-yl)-5',7'-dinitrospiro	252160	130138-26-0	87
30	12.0061	0.1233	Nonacosane	452986	000630-03-5	87

31	12.4275	0.123	Tetradecene	54533	041446-60-0	58
32	12.6382	0.1721	5-Undecene	34990	143185-91-5	35
33	12.73	0.1325	Muscimol	6883	002763-96-4	14
34	12.865	0.1169	Oxalic acid	154534	1000309-24-4	45
35	12.9407	0.3119	Octadecane	422268	000593-45-3	98
36	13.3728	0.3483	NEOPHYTADIENE	429805	000000-00-0	98
37	13.7348	0.212	Benzenedicarboxylic acid	429636	000084-69-5	86
38	14.2479	0.2603	Tricosane (CAS); n-Tricosane	441203	000638-67-5	95
39	14.2966	0.1124	Benzyl (dideuterated) methyl ether	5235	000000-00-0	89
40	14.3884	3.7901	Hexadecanoic acid	422834	000057-10-3	99
41	14.4208	4.037	Palmitic acid	422835	000057-10-3	99
42	14.5127	10.4515	n-Hexadecoic acid	422834	000057-10-3	99
43	14.5667	7.8091	n-Hexadecanoic acid	422834	000057-10-3	99
44	14.9934	10.9861	Dendroban-12-one	424944	002115-91-5	93
45	15.6201	0.8128	Heptadecane	85525	000629-78-7	64
46	15.6633	0.2679	Heptadecane	85525	000629-78-7	60
47	15.8848	0.2586	9-Hexacosene	255379	071502-22-2	90
48	16.2629	22.4998	Oleic Acid	430796	000112-80-1	99
49	16.4412	8.3246	Octadecanoic acid	431424	000057-11-4	98
50	16.5438	0.8649	Stearic acid	431420	000057-11-4	90
51	16.5816	0.9036	9-Octadecenoic acid	430795	000112-80-1	93
52	16.6248	1.4121	9-Octadecenoic acid	430800	000112-80-1	95
53	16.9868	1.5059	Cyclopropaneoctanal	165614	056196-06-6	98
54	17.2136	1.5599	Carbonic acid	156874	1000314-55-8	52
55	17.2406	1.4993	Heptadecene	84041	006765-39-5	83
56	17.4567	0.8052	Hexadecane	181759	004113-12-6	49
57	17.6728	2.1705	Octadec-9-enoic acid	113356	1000190-13-7	15
58	17.8133	0.2582	Pentatriacontene	183897	006971-40-0	44
59	18.3912	0.4156	Octadecenamide, (Z)-	112657	000301-02-0	78
60	19.0665	0.2456	Nonadecatriene	100228	1000131-11-3	38
61	19.1151	1.645	Octadecenal	148195	002423-10-1	92
62	19.4176	0.3131	Dodecanedioic acid	78353	000693-23-2	27
63	19.7525	2.4053	Octadecenal	426018	058594-45-9	90
64	20.0065	0.4386	Trimethyltritriacontane	309322	081469-02-5	89
65	20.0767	0.3728	Cyclohexanecarboxylic acid	65800	027948-10-3	38

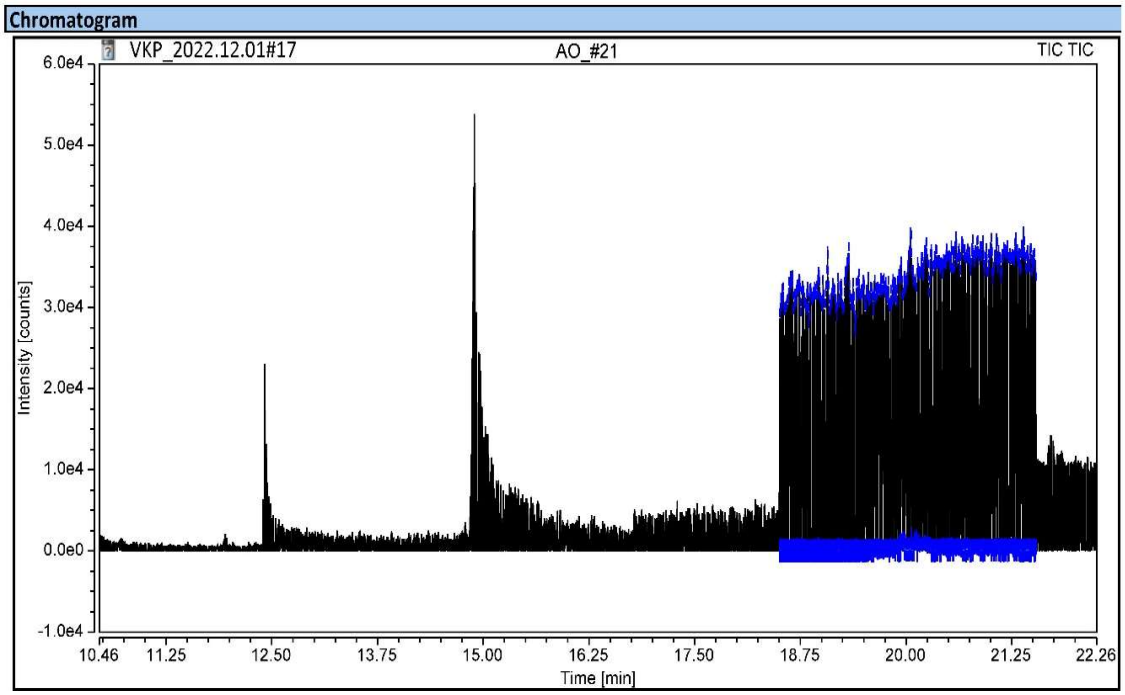

Figure 2: GC-MS chromatogram of co-cultured *Aerides odorata* samples for the presence of total alkaloids

The current experiment was carried out by Paul *et al*. **[8]**, and their focus was on the presence of a variety of alkaloids in orchid tissue. Some of them ought to have applications that are useful. On the other hand, the effectiveness of creating antibacterial activity may differ from species to species. In a similar manner, the active principle may likewise be different from one species to another. Phytochemical studies and bioactivity testing for anti-inflammatory and anticancer activity indicated the intriguing compounds' potential usefulness in these areas **[9]**. *Aerides odorata* Lour. This was determined by comparing the amount of total phenolic compounds found in each of the two extracts. A total of 52 different endophytes were identified in the leaves, stems, and roots of the *Aerides* plant. It was discovered that the plant's leaves contained 27 times more fungal endophytes than either the roots (17) or the stem tissue (8) **[10]**. Phytocompounds derived from *A. odorata* have been shown to possess anti-cancer activity. It inhibits the growth of cancer cells, fights infections and inflammation, lowers blood pressure, and acts as an antioxidant. There are a number of other chemicals in addition to those that have already been mentioned. In addition to having anticancer capabilities. Squalene has been shown to inhibit the growth of cancer cells and is effective in warding off a variety of diseases that can affect both humans and animals. For example, 1,3 propanediol may be utilised in a variety of settings. In addition to its many other applications, it is utilised in the manufacture of adhesives, grease, antifreeze, and medicines. Because of its oily nature, hexadecan-1-ol is frequently utilised in the cosmetic industry as an emulsifier in skin creams and lotions **[1]**. It has been discovered that *A. odorata* includes naturally occurring alkaloids and compounds that have antibacterial, antioxidant, and anticancer properties.

4 CONCLUSIONS

GC-MS/MS Analysis of *Aerides odorata* samples for the presence of total alkaloids revealed that the identification of alkaloids was made by GC-MS/MS without any derivatization. Total of 57 alkaloid peaks were identified **(Table 3 and Figure 1)** in the seedling of Aerides odorata. GC-MS/MS Analysis of co-cultured *Aerides odorata* samples for the presence of total alkaloids revealed that total of 65 alkaloid peaks were identified **(Table 4 and Figure 2)** in the cocultured seedling of *Aerides odorata* with *Trichoderma longibrachiatum* VKPMGU#1.

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6 REFERENCES

[1] Katta J, Rampilla V and Khasim SM (2019). A Study on Phytochemical and Anticancer Activities of Epiphytic Orchid Aerides odorata Lour. European Journal of Medicinal Plants. 28(3): 1-21.

[2] Jhansi K, Khasim SM (2018). Antimicrobial and in vitrocytotoxic studies of *Acampe praemorsa* and *Aeridis odarata* of Orchidaceae. Annals of Plant Sciences 7.2(2018) pp. 2088-2095.

[3] Govinda RM, Suryanarayanan TS, Tangjang S. Endophytic fungi of orchids of Arunachal Pradesh, North Eastern India. Curr Res Environ Appl Mycol 2016;6:293-9.

[4] Hasnu S, Deka K, Saikia D, Lahkar L, Tanti B (2022). Morpho-taxonomical and phytochemical analysis of Vanilla borneensis Rolfe a rare, endemic and threatened orchid of Assam, India. Vegetos 2022; 35: 381-91.

[5] Uppala PK, Gudhanti SNKR, Alavala RR, Prasanth D, Vangoori1 Y (2022). Antioxidant and Hepatoprotective Activity of Aerides odorata Lour. on Alcohol Induced Liver Damage in Rats. Indian J Pharm Sci 2022;84(5):1257-1268.

[6] Akter M, Huda MK and Hoque MM (2018). Investigation of secondary metabolites of nine medicinally important orchids of Bangladesh. Journal of Pharmacognosy and Phytochemistry 2018; 7(5): 602-606.

[7] Jhansi K, Khasim SM (2020). Anticancer Property in *Acampe praemorsa* and *Aerides odorata* (Orchidaceae), Orchid Biology: Recent Trends & Challenges. Springer, Singapore. https://doi.org/10.1007/978-981-32-9456-1_27. pp 519-530.

[8] Paul P, Chowdhury A, Nath D and Bhattacharjee MK (2013). Antimicrobial efficacy of orchid extracts as potential inhibitors of antibiotic resistant strains of *Escherichia coli*. Asian Journal of Pharmaceutical and Clinical Research, Vol 6, Issue 3, 108-111.

[9] Huda MK (2015). Conservation of Medicinal Orchids. Conservation of two medicinal orchids in Bangladesh. Orchid Conservation News, The Newsletter of the Orchid Specialist Group of the IUCN Species Survival Commission. Issue 1, 3-5.

[10] Sopalun K, Iamtham S (2020). Isolation and screening of extracellular enzymatic activity of endophytic fungi isolated from Thai orchids. South African Journal of Botany. doi:10.1016/j.sajb.2020.02.005.