



**PHYTOCHEMICAL ANALYSIS OF BIOACTIVE COMPOUNDS IN
CALANTHE TRICARINATA LINDL. PSEUDOBULBS EXTRACT
(ORCHIDACEAE) BY GCMS METHOD**

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ABSTRACT

During the present study, an attempt was made to screen for bioactive phytocompounds present in methanolic extracts of *Calanthe tricarinata* Lindl. pseudobulbs using highly sophisticated biological and chemical characterization technique– Gas chromatography-mass spectrometry (GC-MS). The pseudobulbs were collected from Kufri hills (latitude: 31.097858°N, longitude: 77.267815°E), Shimla, Himachal Pradesh (India). The GC-MS chromatogram revealed 69 bioactive compounds with the following as the most abundant, 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (7.10%), Acetic acid, 1-(2-methyltetrazol-5-yl) ethenyl ester (6.76%), 3-Hydroxy-4-methoxybenzyl alcohol (5.31%), 9,12-Octadecadienoic acid (Z,Z)-methyl ester (5.09%). These phytocompounds and many others present in the extract have been reported to possess multiple therapeutic activities including antioxidant,

antimicrobial, and antiinflammatory. The present investigation therefore explains the use of *C. tricarinata* pseudobulbs in ancient medicine to treat wounds and eczema and further implies that it could be exploited for the formulation of therapeutic molecules. This present study is the first report made by GCMS analysis for the presence of bioactive components of phytopharmaceutical importance.

KEYWORDS: Orchid, Pseudobulbs, Methanol, GCMS, Phytocompounds, Biological activity.

INTRODUCTION

Calanthe tricarinata Lindl., (Orchidaceae) commonly referred to as *Monkey Orchid* is distributed in the India, Nepal and China at the elevations between 2000-3000 m.^[1] It is known to be used in the traditional system of medicine to cure various ailments around the world. In China, its roots are used to stimulate blood circulation, relax muscles and joints, and stop bleeding. Leaves and pseudobulbs are valued as aphrodisiacs; and are used to treat wounds and eczema in Nepal. In Uttarakhand, roots and leaves are used to treat jaundice and typhoid.^[2,3]

The plants owing to its medicinal value have continued to play a prevailing role in the maintenance of human health since ancient times. Currently, a large number of peoples around the world are using the plants and plant based preparations to cure various diseases because they believe the herbal medicines are safer than synthetic medicines as these phytochemicals in the plant extracts target the biochemical pathway.^[4] However, without screening the active compounds from particular species, the researchers may not be able to invent a new medicinal drug to cure a particular disease. Gas Chromatography (GC) and Mass Spectrometry (MS) serve as potent methodologies for gaining profound insights by identifying the various compounds (amino acids, alkaloids, esters, long-chain hydrocarbons, steroids, organic acids, nitro compounds, and alcohols) contained in a sample. Though some chemical compounds have earlier been reported earlier from the other different species of the genus *Calanthe*, such as 2, 5-dihydro-1H-pyrrole, and 5-Methoxy-4-methyl-2,1,3-benzothiadiazole isolated from *C. triplicata*,^[5] Squalene, Ergosta-7,22-dien-3 β -ol, N-benzoyl-L-phenylalalinol and Glyceryl-5,7,24-triene-3 β -ol, n-hexadecane, Dotriacont-1-ene, 1H-indole-2,3-dione derivatives, calaliukiunenoside, and calaphenanthrenol from *C. discolor*,^[6,7] Eudesmol, 4-Methoxyphenol, 4-Ethoxyphenol, Geranial, Myrtenol, 1-Phenyl-2-methylpropanol, 2,3-Dehydrotheaspirane and 2-Hydroxypinan-3-one from *C. sieboldii*,^[8,6] Pentyl cyclopropane(-), Glycerol Linoleate, Linolenic acid, 3-butadiene-1'-based cyclobutene isolated from *C. alpina*,^[9] no information is available on detailed bioactive compounds detected from *C. tricarinata*. Hence, in order to better understand its therapeutic effects, the present investigation was focused on the identification of chemicals utilizing the GC-MS method from the *C. tricarinata* Lindl. methanolic pseudobulbs extract. This present study is the first report made by GCMS analysis for the presence of bioactive components of phytopharmaceutical importance.

MATERIAL AND METHODOLOGY

Plant extract preparation

The plant material was collected from Kufri hills (latitude: 31.097858°N, longitude: 77.267815°E), Shimla, Himachal Pradesh (India). The pseudobulbs of *C. tricarinata* were washed with fresh tap water followed by washing with distilled water and then drying in the shade at room temperature. After drying, the plant material was powdered well by using grinder and placed into a well closed container. Plant part powder (10g) mixed with methanol (100ml) in conical flask was kept on an orbital shaker at room temperature for 48 hours. The mixture was then filtered through muslin cloth and resultant filtrate was again filtered through Whatman's Filter No.1. The final extracts were collected in vials and stored in refrigerator till further use.

Gas Chromatography (GC) and Mass Spectrometry (MS)

[GC-2010]

Column Oven Temperature. : 70.0 °C
Injection Temp. : 250.00 °C
Injection Mode : Splitless
Sampling Time : 1.00 minute
Pressure : 61.3kPa

Oven temperature program

Rate	Temperature (°C)	Hold Time (minutes)
-	70.0	5.00
5.00	310.0	10.00

[MS Table]

Start Time : 5.00minutes
End Time : 63.00minutes
Event Time : 0.50seconds
Scan Speed : 1666
Start m/z : 40.00
End m/z : 850.00

Identification of bioactive compounds

Bioactive compounds from the pseudobulb extract were identified based on retention time. Interpretation of mass spectrum of GC-MS was done using the database of National Institute

Standard and Technology (NIST). The peak in GC-MS spectrum represents the presence of the secondary phytochemical compounds. The compound name, molecular formula, molecular weight, molecular structure, retention time (RT), peak area percent (area %) and biological activity of the test materials were ascertained and listed in Table 1.

RESULTS AND DISCUSSION

The methanolic extract of *C. tricarinata* pseudobulbs was analyzed by GCMS analysis so as to detect various compounds with the help of NIST library. A total of 69 compounds were identified which have been listed in Table 1; the pharmacologically active compounds isolated from species belong to various classes of phytochemicals (alkaloids, fatty acids, steroids, terpenes, terpenoids, and phenanthrenes). The major constituents were identified in the extract were 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (7.10%), Acetic acid, 1-(2-methyltetrazol-5-yl) ethenyl ester (6.76%), 3-Hydroxy-4-methoxybenzyl alcohol (5.31%), 9,12-Octadecadienoic acid (Z,Z)-methyl ester (5.09%), 2-Hydroxy-gamma-butyrolactone (4.07%) and other compounds were found in minimal amount. Regardless of the quantity or concentration (high or low) of these chemicals, nearly all have been documented to exhibit certain pharmacological or biological activities such as antibacterial, anti-fungal, antioxidant and antiviral (Table 2). Antioxidant property is one of the crucial properties possessed by plant, amongst the presently detected, compounds Tetradecanoic acid, Benzoic acid, 4-hydroxy-3,5-dimethoxy-, hydrazide, Phytol, 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione have been reported to possess potential antioxidant activity.^[10,11] Hexadecanoic acid, 2-hydroxy-1- (hydroxymethyl) ester, a fatty acid ethyl ester exhibits antioxidant, hypocholesterolemic, antiandrogenic, hemolytic and alpha reductase inhibitor activity. It has been reported that Hexadecanoic acid also works as one of the potent volatile flavour components.^[12] Octadecanoic acid, 2, 3-dihydroxypropyl ester belonging to the family of monoacylglycerols and used as a food additive also have been reported to show antimicrobial and anticancer activities.^[13] Phytol (PYT) is a diterpene member of the long-chain unsaturated acyclic alcohols which has been indicated to show anti-inflammatory, anticancer, antioxidant, diuretic, antiallergic, immunostimulant, anti-trypanosomal, antimicrobial activity against *Mycobacterium tuberculosis* and *Staphylococcus aureus*.^[14] Methyl stearate is used as a solvent and lipid carrier in agriculture. Other chemicals such as 9,12-octadecadienoic acid, methyl ester (Z, Z) has been reported to possess strong anticancer activity against human lung cancer cell line A549 with an IC₅₀ value of 10.60 ± 1.12.^[15] Other activities such as

antitumour promoting activity, dermatologic, antiacne, anti-inflammatory, antiprotozoal (Leishmania), and antisecretoric activities have been reported earlier by Stigmasterol.^[16]

During the present investigation, some detected compounds such as Tetradecanoic acid (*Calanthe alpina* leaves).^[8] Methyl anthranilate and Dibutyl phthalate (*C. discolor* whole plant)^[7], Benzyl benzoate (*C. izu-insularis*, *C. sylvatica* aerial part).^[8,17] Benzyl benzoate, Hexadecanoic acid, Pentadecanoic acid, Tetradecanoic acid, Vanillin (*C. sieboldii* flowers).^[8] Phytol acetate (*C. triplicata* whole plant)^[5] have been found to be common with other earlier studied species of *Calanthe*. Generally, it is known that family Orchidaceae contain a wide variety of phytochemicals that are thought to show biological activity. The GCMS analysis is the first step towards understanding the nature of active principles in medicinal plants. GC-MS techniques has been successfully used earlier to detect bioconstituents of a few other orchid species including *Aerides multiflora*,^[18] *Nervilia aragoana*,^[19] *Rhynchostylis retusa*,^[2] *Vanda cristata*.^[2,20] The presence of various bioactive compounds in the present species opens new vistas in the field of medicinal applications, further detailed investigation need to be taken up for establishment of medical potencies, particularly pharmaceutical standardization and toxicology.

Currently it is observed that anthropogenic activities (overharvesting, indiscriminate collection, habitat loss), natural calamities (landslides, wild fires, earthquakes, global climate change) have deteriorated and destroyed natural orchid habitats, which has impacted the distribution of wild orchid populations, especially *Calanthe* species. The genus *C. tricarinata* is listed in endangered category of the International Union for Conservation of Nature (IUCN) Red List, highlighting the urgency of conserving this species. In this context, the use of *in vitro* tissue culture remains a feasible strategy for the production of structurally complex and high-value natural products, especially if the plant source material is an overexploited, slow-growing or low-yielding plant. A few attempts have been made earlier to propagate and conserve a few other medicinal orchid species including other species of *Calanthe* using *in vitro* asymbiotic seed germination and regeneration techniques.^[21, 22, 23,24,25,26,27,28,29,30] thereby reducing the collection pressure on wild populations. The implementation of plant culture systems for pharmaceutical production confers several advantages including cost reduction, rapid production and scalability. Therefore, future research directions should focus on further elucidating and refining these technologies, facilitating revolutionary breakthroughs in secondary metabolite production.

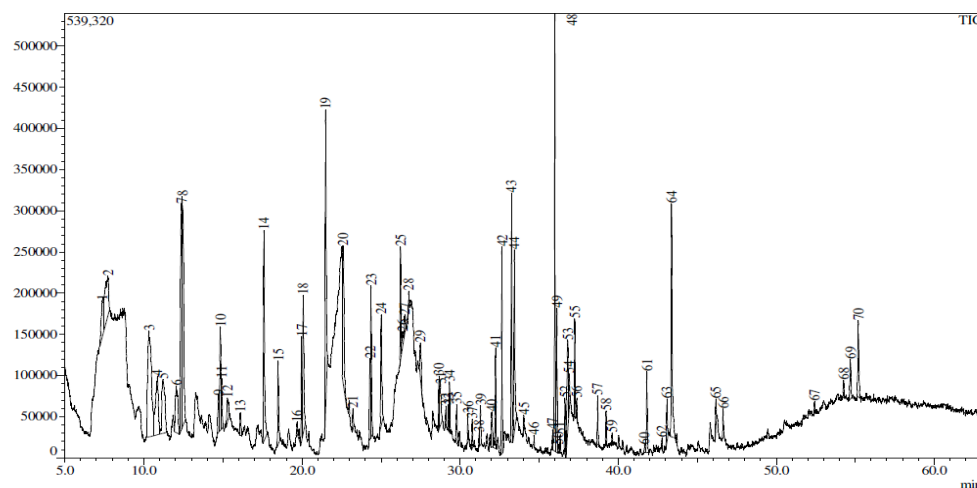


Fig. 1: Chromatogram of GC-MS analysis in methanolic extract of *Calanthe tricarinata* pseudobulbs.

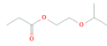
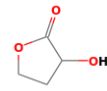
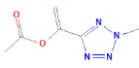
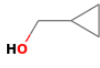
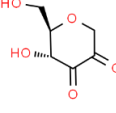
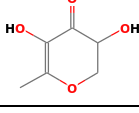
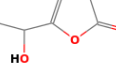
Table 1: Bioactive compounds detected in methanolic extract of <i>Calanthe tricarinata</i> pseudobulbs.					
Compound	Molecular formula	Molecular weight	Chemical structure	Retention time	Area %
2-Isopropoxyethyl propionate	$C_8H_{16}O_3$	160.21		7.367	1.75
2-Hydroxy-gamma-butyrolactone	$C_4H_6O_3$	102.0886		7.714	2.50
Acetic acid, 1-(2-methyltetrazol-5-yl)ethenyl ester	$C_6H_8N_4O_2$	168.15		10.317	6.76
Cyclopropylcarbinol	C_4H_8O	72.1057		10.857	2.95
1H-Pyrrole, 2,5-dihydro-	C_4H_7N	69.1051		11.204	2.65
2-Acetyl-2-hydroxy-gamma.-butyrolactone	$C_6H_8O_4$	144.13		12.055	2.07
1,5-Anhydro-6-deoxyhexo-2,3-diulose	$C_6H_8O_5$	160.125		12.367	4.21
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144.1253		12.403	7.10
5-(1-Hydroxyethyl)-2(3H)-furanone, solerol isomer	$C_6H_8O_3$	128.1259		14.708	0.92
L-Proline, 1-acetyl-	$C_7H_{10}NO_3$	156.1592		14.827	2.11

Table 1: Bioactive compounds detected in methanolic extract of *Calanthe tricarinata* pseudobulbs.

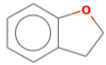
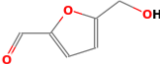
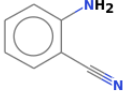
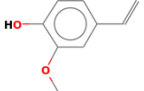
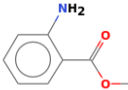
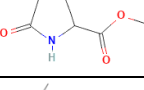
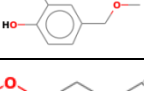
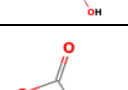
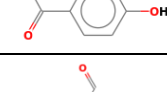
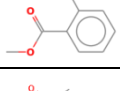
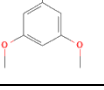
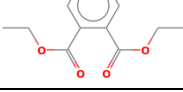
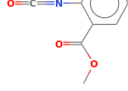
Compound	Molecular formula	Molecular weight	Chemical structure	Retention time	Area %
Benzofuran, 2,3-dihydro-	C ₈ H ₈ O	120.1485		14.917	0.60
5-Hydroxymethylfurfural	C ₆ H ₆ O ₃	126.1100		15.288	0.64
Benzonitrile, 2-amino-	C ₇ H ₆ N ₂	118.1359		16.092	0.43
2-Methoxy-4-vinylphenol	C ₉ H ₁₀ O ₂	150.1745		17.594	3.82
Methyl anthranilate	C ₈ H ₉ NO ₂	151.1626		18.483	1.43
Methyl 5-oxopyrrolidine-2-carboxylate	C ₆ H ₉ NO ₃	143.14		19.683	0.35
Phenol, 2-methoxy-4-(methoxymethyl)-	C ₉ H ₁₂ O ₃	168.1898		19.966	1.24
Vanillin	C ₈ H ₈ O ₃	152.1473		20.070	2.46
3-Hydroxy-4-methoxybenzyl alcohol	C ₈ H ₁₀ O ₃	154.1632		21.492	5.31
2-Hydroxy-gamma-butyrolactone	C ₄ H ₆ O ₃	102.0886		22.564	4.07
Benzoic acid, 4-hydroxy-3-methoxy-, methyl ester	C ₉ H ₁₀ O ₄	182.1733		23.217	0.27
Methyl N-formylanthranilate	C ₉ H ₉ NO ₃	179.1727		24.292	0.90
3',5'-Dimethoxyacetophenone	C ₁₀ H ₁₂ O ₃	180.20		24.361	2.34
Diethyl Phthalate	C ₁₂ H ₁₄ O ₄	222.2372		25.008	2.15
2-Carbomethoxyphenyl isocyanate	C ₉ H ₇ NO ₃	177.1568		26.228	1.55

Table 1: Bioactive compounds detected in methanolic extract of *Calanthe tricarinata* pseudobulbs.

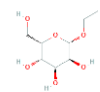
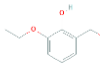
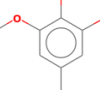
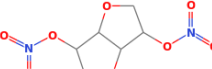
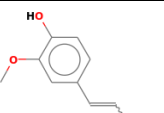
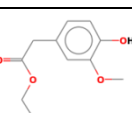
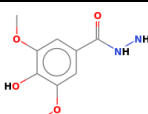
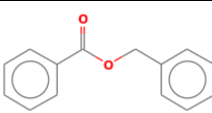
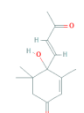
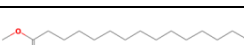
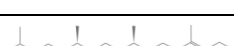
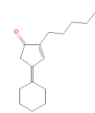
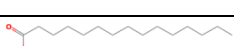
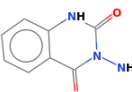
Compound	Molecular formula	Molecular weight	Chemical structure	Retention time	Area %
Ethyl alpha-d-glucopyranoside	$C_8H_{16}O_6$	208.21		26.342	0.38
Methyl-(2-hydroxy-3-ethoxy-benzyl)ether	$C_{10}H_{14}O_3$	182.22		26.492	0.16
Benzaldehyde, 4-hydroxy-3,5-dimethoxy-	$C_9H_{10}O_4$	182.1733		26.752	0.14
IsosorbideDinitrate	$C_6H_8N_2O_8$	236.1363		27.489	0.82
Phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy-	$C_{10}H_{12}O_3$	180.2005		28.670	0.83
Ethyl homovanillate	$C_{11}H_{14}O_4$	210.2265		28.758	0.90
Tetradecanoic acid	$C_{14}H_{28}O_2$	228.3709		29.095	0.23
Benzoic acid, 4-hydroxy-3,5-dimethoxy-, hydrazide	$C_9H_{12}N_2O_4$	212.2026		29.257	0.26
Benzyl Benzoate	$C_{14}H_{12}O_2$	212.2439		29.331	0.63
4-Hydroxy-3,5,5-trimethyl-4-[(1e)-3-oxo-1-butenyl]-2-cyclohexen-1-one	$C_{13}H_{18}O_3$	222.28		29.780	0.70
Pentadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	256.4241		30.490	0.34
Phytol	$C_{20}H_{40}O$	296.5310		30.771	0.34
2-Pentyl 2-cyclopentene-1-one 1	$C_{16}H_{24}O$	232.36		31.208	0.23
Pentadecanoic acid	$C_{15}H_{30}O_2$	242.3975		31.289	0.53
3-Amino-1H-quinazoline-2,4-dione	$C_8H_7N_3O_2$	177.1601		32.005	0.67

Table 1: Bioactive compounds detected in methanolic extract of *Calanthe tricarinata* pseudobulbs.

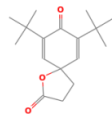
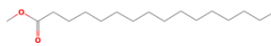
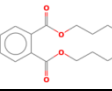
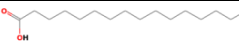
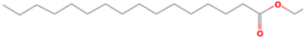
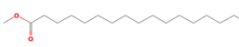
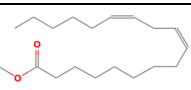
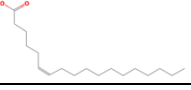
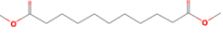
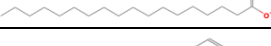
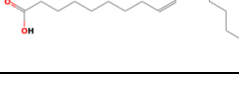
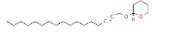
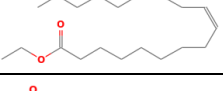
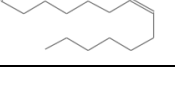
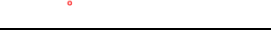
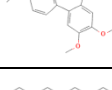
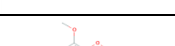
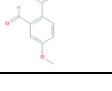
Compound	Molecular formula	Molecular weight	Chemical structure	Retention time	Area %
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	C ₁₇ H ₂₄ O ₃	276.3707		32.241	1.35
Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270.4507		32.639	2.30
Dibutyl phthalate	C ₁₆ H ₂₂ O ₄	278.3435		33.254	2.99
n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.4241		33.434	3.26
Hexadecanoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	284.4772		34.029	0.18
Heptadecanoic acid, methyl ester	C ₁₈ H ₃₆ O ₂	284.4772		34.677	0.09
1-Heneicosanol	C ₂₁ H ₄₄ O	312.5735		35.847	0.23
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C ₁₉ H ₃₄ O ₂	294.4721		35.992	5.09
6-Octadecenoic acid, methyl ester, (Z)-	C ₁₉ H ₃₆ O ₂	296.4879		36.119	1.88
Undecanedioic acid, dimethyl ester	C ₁₃ H ₂₄ O ₄	244.3273		36.225	0.12
Methyl stearate	C ₁₉ H ₃₈ O ₂	298.5038		36.633	0.60
9,12-Octadecadienoic acid (Z,Z)-	C ₁₈ H ₃₂ O ₂	280.4455		36.803	1.91
2H-Pyran, 2-(2-heptadecyloxy)tetrahydro-	C ₂₂ H ₄₀ O ₂	336.6		36.903	0.52
Linoleic acid ethyl ester	C ₂₀ H ₃₆ O ₂	308.4986		37.261	1.03
7-Tetradecenal, (Z)-	C ₁₄ H ₂₆ O	210.3556		37.376	0.18
Hexadecanoic acid, 2-hydroxyethyl ester	C ₁₈ H ₃₆ O ₃	300.4766		38.700	0.59
Dibenz[b,d]cycloheptanone, 2,3,9-trimethoxy-	C ₁₈ H ₁₈ O ₄	298.3		39.233	0.50
1-Eicosanol	C ₂₀ H ₄₂ O	298.5469		39.622	0.12
Glyceryl 2-pentadecanoate	C ₁₈ H ₃₆ O ₄	316.5		41.658	0.13
1,1'-Biphenyl-3,4,4'-trimethoxy-6'-formyl-	C ₁₆ H ₁₆ O ₄	272.29		41.812	1.22

Table 1: Bioactive compounds detected in methanolic extract of *Calanthe tricarinata* pseudobulbs.

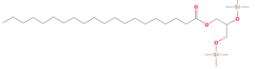
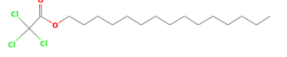
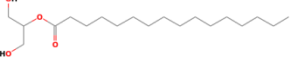
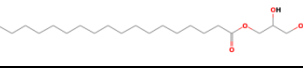
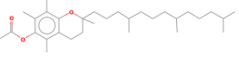
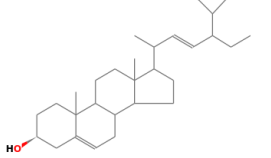
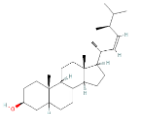
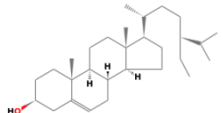
Compound	Molecular formula	Molecular weight	Chemical structure	Retention time	Area %
2,3-Dihydroxypropyl icosanoate, 2TMS derivative	$C_{29}H_{62}O_4Si_2$	530.9712		42.766	0.13
Trichloroacetic acid, pentadecyl ester	$C_{17}H_{31}Cl_3O_2$	373.786		43.087	0.64
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330.5026		43.379	3.86
9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{38}O_4$	354.5240		46.183	0.34
Octadecanoic acid, 2,3-dihydroxypropyl ester	$C_{21}H_{42}O_4$	358.5558		46.632	0.39
(.+/-.)- α -Tocopherol acetate	$C_{31}H_{52}O_3$	472.7428		52.422	0.12
Stigmasterol	$C_{29}H_{48}O$	412.6908		54.260	0.27
Ergosta-7,22-dien-3-ol, (3 β ,22E)-	$C_{28}H_{46}O$	398.7		54.690	0.69
γ -Sitosterol	$C_{29}H_{50}O$	414.7067		55.191	1.33

Table 2: Detected Phytochemicals and Their biological activities.

Compound	Biological activity
Acetic acid, 1-(2-methyltetrazol-5-yl)ethenyl ester	Antifungal, carminative, and antispasmodic
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	Anti-microbial, anti-inflammatory, and antioxidant
Benzofuran, 2,3-dihydro-	Anticancer and anti-inflammatory
5-Hydroxymethylfurfural	Antioxidant
2-Methoxy-4-vinylphenol	Antimicrobial
Phenol, 2-methoxy-4-(methoxymethyl)-	Anti-microbial and anti-inflammatory
Vanillin	Allergenic, antianemic, anticancer, antimutagenic, antioxidant, antipolio, antiradicular, antitumor, antiviral, and candidicide
3-Hydroxy-4-methoxybenzyl alcohol	Anti-asthmatic
3',5'-Dimethoxyacetophenone	Antioxidant
Diethyl Phthalate	Antimicrobial, acetylcholinesterase, and

Compound	Biological activity
	neurotoxic activity
Ethyl alpha-d-glucopyranoside	Antioxidant, alpha amylase inhibitor, and anticonvulsant
Benzaldehyde, 4-hydroxy-3,5-dimethoxy-	Antimicrobial
IsosorbideDinitrate	Antianginal and circulatory
Tetradecanoic acid	Antioxidant cancer-preventive, cosmetic, hypercholesterolemic, and nematocide
Benzoic acid, 4-hydroxy-3,5-dimethoxy-, hydrazide	Antioxidant
Benzyl Benzoate	Antibacterial
Pentadecanoic acid, methyl ester	Antibacterial and antifungal
Phytol	Anti-inflammatory, anticancer, antioxidant, diuretic, antiallergic, immunostimulant, anti-trypanosomal, antimicrobial activity against <i>Mycobacterium tuberculosis</i> and <i>Staphylococcus aureus</i>
Pentadecanoic acid	Antimicrobial
3-Amino-1H-quinazoline-2,4-dione	Antibacterial
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	Antioxidant, antimicrobial and antiviral
Hexadecanoic acid, methyl ester	Antioxidant and anti-inflammatory
Dibutyl phthalate	Antibacterial and antifouling
n-Hexadecanoic acid	Antioxidant, hypocholesterolemic nematocide, pesticide, and antiandrogenic
Hexadecanoic acid, ethyl ester	Antioxidant, hypocholesterolemic, nematocide, pesticide, antiandrogenic, antioxidant, and nematocide
Heptadecanoic acid, methyl ester	Mosquito repellent
1-Heneicosanol	Antifungal and antimicrobial
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Anti-inflammatory, cancer preventive, insectifuge, antiandrogenic, nematocide, and antihistaminic
6-Octadecenoic acid, methyl ester, (Z)-	Anti-inflammatory and antimicrobial
Undecanedioic acid, dimethyl ester	Cytotoxic effect against breast, ovarian, prostate and liver cancer cell lines
Methyl stearate	Antioxidant, antiviral (Influenza A, Rhinovirus, Picornavirus, Adenovirus, Herpes, Hepatitis B, Poxvirus and Parainfluenza)
9,12-Octadecadienoic acid (Z,Z)-	Anticancer, anti-inflammatory, antimicrobial, diuretic, and anti-arthritic
Linoleic acid ethyl ester	Antimicrobial
Hexadecanoic acid, 2-hydroxyethyl ester	Antioxidant and anti-inflammatory
1-Eicosanol	Antioxidant
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	Anticancer, anti-inflammatory, antimicrobial, diuretic, hepatoprotective,

Compound	Biological activity
	anti-arthritic, and antiasthma
9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	Hypocholesterolemic, nematocide, antiarthritic, hepatoprotective, antiandrogenic, hypocholesterolemic 5-alpha reductaseinhibitor, Antihistaminic, anticoronary, insectifuge, antieczemic and antiacne
Octadecanoic acid, 2,3-dihydroxypropyl ester	Arachidonic acid inhibitor, increase aromatic amino acid decarboxylase activity, inhibit uric acid production
(.+/-)- α -Tocopherol acetate	Anti-oxidant
Stigmasterol	Antitumour promoting activity, dermatologic, antiacne, anti-inflammatory, antiprotozoal (Leishmania), and antisecretoric
γ -Sitosterol	Anticancerous, hepatoprotective, antihyperglycemic, antidiabetic, antihypercholesterolemic, antiviral (influenza), anti-inflammatory, antiacne, antiprotozoal (Leishmania), and antibacterial

CONCLUSION

From the present study, it may be concluded that *Calanthe tricarinata* is a rich source of novel and biologically active metabolites which may be of great interest for the pharmaceutical industry and medicinal research. Considering the biological activities of compounds present in its pseudobulbs extract indicates the medicinal application of this species and predicts the possibility of discovery of new drugs. However, isolation of individual phytochemical constituent and subjecting these to other biological activity may lead to new research insights.

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