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Research Article

Evaluation of phytochemical compound in leaf extract of calanthe masuca (d.don) lindl., using UV-VIS, FTIR and GCMS analysis - An orchidaceae member

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Abstract

The present investigation was focused on the UV-VIS spectrum, FT-IR and GC-MS Analysis of *Calanthe masuca* (D.Don) Lindl. The organic solvent methanol was taken for extraction. The leaf part of *Calanthe masuca* which belongs to the Orchidaceae family. The UV-VIS profile showed different peaks ranging from 220-970 nm with different absorption respectively. The FTIR spectrum confirmed the presence of compound such as alcohol, alkane, carboxylic acid, aldehyde, allene, cyclic alkene, alky halide, aromatic compound, isothiocyanate and alkenes in leaf methanol extract. The results of the GC-MS analysis provide for leaf extract which have dissolved to shows different peaks. The major peak of Leaf extract are 3-Tetradecanynoic acid, 9,12- Octadecadienoic acid (Z,Z)-, n-Hexadecanoic acid, L-proline, 1-acetyl, Neophytodiene. Therefore this plant had potential bioactive compounds.

Keywords: Bioactive compound, *Calanthe masuca*, UV-VIS, FTIR and GC- MS analysis.

Introduction

Orchids are generally to development of the flowering plant ant it belongs to the angiosperm species and are widely known for their economic importance medicinal therapeutic use. The first documentary evidence to summit the Chenese and to medicinal uses of orchids (Paudel et al., 2018). The earliest reported that use of the medicinal orchids at 28th century B.C. When Shen-nung described as the *Bletilla striata* and *Dendrobium* species of *Materia Medica*. The literal meaning of the term orchid (*órkhis*), in Greek words is testicles and it was Theophrastus who first coined the term as the anatomy of plant resembles testicles. Orchids have been subjected for phytochemical and pharmacological studies (Thomas et al., 2013).

The India is the richest habitats of orchids and was comprise of about 2500 species in 167 genera (Haridas et al., 2016). The orchid's species certain constituent such as alkaloids, flavonoids etc suggest medicinal properties **(**Jyoti et al., 2018). In India, some orchid's plants like *Eulophia campestris, Orchis latifolia, and Vanda roxburgii* were drawn the attention of scientific community and because of the medicinal properties. Some orchids have deal with the phytochemistry and medicinal uses of orchids. Biological activity naturally present in this plants. They act as natural system of plants aroma colour and flavor (Dong Chen et al., 2015).

The important role in cure treating human disease such as cancer, coronary heart diseases, diabetes and infectious diseases. Therapeutic phytochemical compounds and various secondary metabolites such as flavonoids, carotenoids, alkaloids, anthocyanidins, phenolics, tannins, carboxylic acids, terpenoids, amino acids and inorganic acids has a different bio-potential of medicinal plants. The identification of bioactive compound from the medicinal plants using various instrumentation techniques such as UV-VIS, FTIR and GC-MS analysis (Theng and Korpenwar, 2015; Antony Sandosh et al., 2103; Phukan et al., 2017).

The plant *Calanthe masuca (D.Don) Lindl* is belongs into family Orchidaceae. The genus *Calanthe* was described in 1821 by Robert Brown in Botanist's Repository. *Calanthe masuca* is an endangered terrestrial orchid. There is no report on compound isolation in the *Calanthe masuca* leaf extract. The present studies into identify the bioactive compound by the Spectral analysis such as UV-VIS, FTIR and GCMS analysis. This study was carried out in order to secure some standards for standardization of these crude drugs.

Materails and Methods

Plant material collection & authentication

The plant leaf materials of *Calanthe masuca* were collected from the Kolli Hills of Namakkal District, Tamil Nadu, and

India. The leaf parts of the plant were washed thoroughly 2-3 times with running water and once sterile with distilled water. The fresh plant material were shade into two month dried and coarsely powdered separately and stored in air tight glass bottles for further analysis in laboratory. The plant was authenticated at Botanical survey of India (BIS), Southern circle, Coimbatore, India. The Voucher number is BSI/SRC/5/23/2019 Tech/252. The Herbarium was stored in the National College, Tiruchirappalli.

Plant sample extraction

100g of dried leaf powder was soxhlet extracted with Methanol solvents for about 12-24h the crude extract of methanol solvent were evaporated by the vacuum rotary evaporator pressure reduced. The sample extract were collected in air tight container and refrigerator at 4°C until further analysis.

UV-VIS

The plant extract were centrifuged into 3000 rpm for 10 min and after filtered that through the Whatmann No.1 filter paper. In this sample were diluted into 1:10 with the same solvent. The plant extract have been scanned for the wave length ranging at 200nm to 1100 nm using Perkin Elmer spectrophotometer and the characteristic peaks were detected. The peak values at the UV-VIS were recorded (Theng & Korpenwar, 2015).

FTIR analysis

The FTIR analyses have been performed using Perkin Elmer spectrophotometer system. This was used to deck the characteristic peaks in ranging from 400- 4000 cm^{-1} and their functional groups. The peak values of the FTIR were recorded and were as each and every analysis was repeated twice for the spectrum confirmation (Jain et al., 2016).

GC-MS analysis

The analysis at unidentified constituents of GC-MS plays major role in plant origin. The leaf methanol extract containing different compounds of Calanthe masuca was subjected for (GC-MS) analysis. Instruments and chromatographic circumstance GC-MS examination was carried out on GC clarus 500 Perkin Elmer system containing a Aoc-2oi auto analyst and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument retaining the subsequent condition; column Elite-1 attached silica capillary column 30x0.25mmxIDx1µm of capillary column, composed of (100% dimethyl poly siloxane), the operational electron impact mode at 70 eV; and the helium (99.99%) have been as transporter gas at a persistent flow of 1 ml/minute and an injection capacity of 0.5 EI was employed (split ratio of 10:1) inject or temperature 250°C; ion –source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/ minutes, to 200°C, minutes, then 5°C/ minutes to 280°C/ min, finish with a 9 minutes isothermal at 280°C. Mass spectra have been occupied at 70 eV; and the scan intermission of 0.5 seconds and the fragments from 45 to 450 Da. To evaluate constituent is identified in the mass detector. The spectrum of the recognized constituents stored in NIST library and concludes the name and molecular weight (Sathish et al., 2012; Antony Sandosh et al., 2103).

RESULT AND DISCUSSION

UV-VIS analysis

The UV-VIS analysis preformed for identification of phytocontituents present in methanol leaf extract of Calanthe masuca. The UV- visible spectra have been performed to identify the compounds containing σ-bonds, π-bonds and lone pair of electrons, chromophores and aromatic rings. The qualitative UV-VIS profile of methanol leaf extract of Calanthe masuca was taken at the wavelength of 200 nm to 1100 nm due to the sharpness of the peaks and proper baseline. The methanol leaf extract were showed the peaks at (Table 1) 220.55, 364.10, 397.55, 531.70 and 664.50 nm with the absorption 4.000, 1.0848, 1.3319, 0.1693 and 0.2762 nm respectively (Figure 1) (Theng & Korpenwar, 2015; Jain et al., 2016;

Sathish et al., 2012; Elufioye & Mada, 2018).

FTIR analysis

The FTIR spectrums have been used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The results of FTIR peak values and functional groups were represented in (Table 2 and Figure 2). The FTIR spectrum confirmed the presence of alcohols, alkane, carboxylic acid, aldehyde, allene, cyclic alkene, aliphatic ether, amine, flurocompound, alkyl halide and isothiocyanate in leaf methanol extracts. Hence, the methanol extracts subjected to FTIR analysis is used for the identification of chemical constituents' presents in *Calanthe masuca* is proved to be a reliable and sanative method for detection of biomolecular composition (Raphael Marandi et al., 2018; John Peter Paul and Amster Regain Lawrence, 2017; Perraudin et al., 2006).

Table 1: UV-VIS Peak values of methanol leaf extract of *Calanthe masuca.*

S.NO	Wavelength (nm)	Absorption Peak(O.D)
1	220.55	4.0000
2	364.10	1.0848
3	397.55	1.3319
4	531.70	0.1693
5	664.50	0.2762

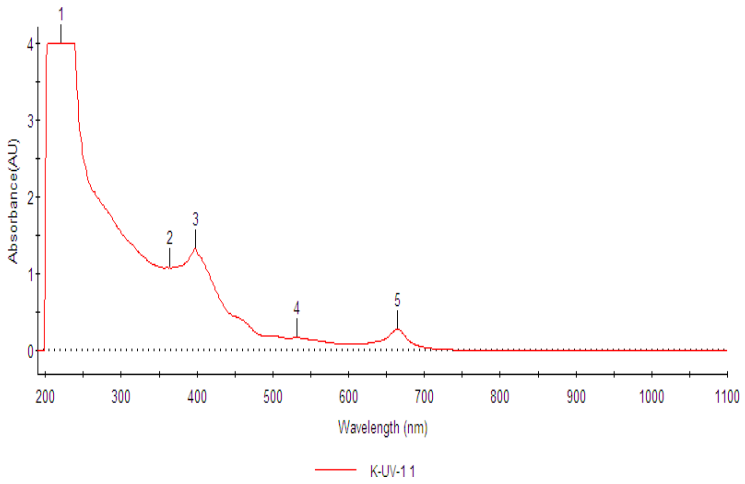

Figure 1. UV-VIS spectra of methanol leaf extract of *Calanthe masuca*.

Table 2: FTIR Peak values functional groups of methanol leaf extract of *Calanthe masuca.*

S.NO	Peak Value	Functional group	Functional group Name	Vibrations
1	656.33	C-Br	Alkyl Halide	Stretching
2	1018.94	C-F	Flurocompound	Stretching
3	1030.62	C-N	amine	Bending
4	1049.62	C-O	esters	Stretching
5	1112.96	C-O	Aliphatic ether	Stretching
6	1413.32	O-H	Carboxylic acid	Stretching
7	1452.98	C-H	Alkane	Bending
8	1646.13	C=C	Cyclic alkene	Stretching
9	2050.20	C=C=C	allene	Stretching
10	2523.60	C-H	aldehyde	Stretching
11	2837.87	O-H	Carboxylic acids	Stretching
12	2864.13	O-H	Carboxylic acids	Stretching
13	2947.58	C-H	Alkane	Stretching
14	3401.49	O-H	alcohol	Stretching

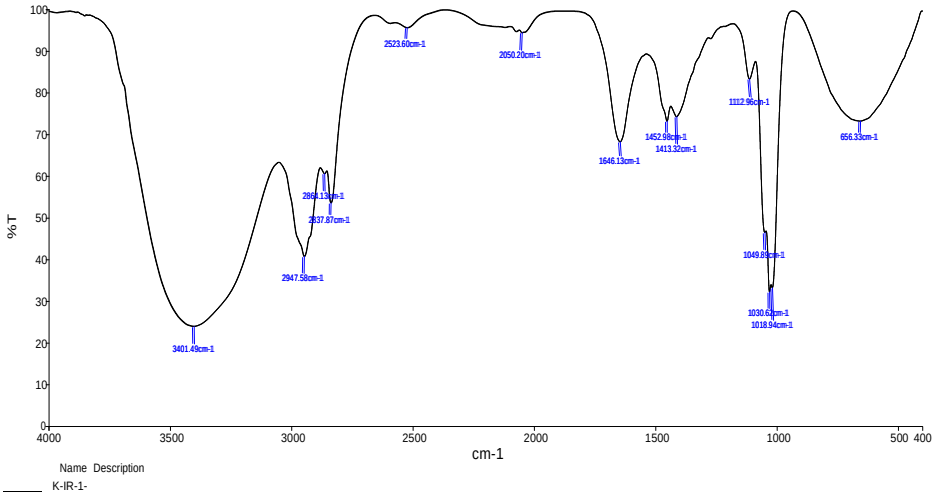

Figure 2. FTIR spectra of methanol leaf extract of *Calanthe masuca.*

GC-MS analysis

The chemical constituents identified by the GC-MS analysis of the methanol leaf extract of *Calanthe masuca* was enumerated along with their molecular formula, retention time and peak area (Table 3 and Figure 3) (Perraudin et al., 2006; Haridas et al., 2016). The GC-MS analysis of *Calanthe masuca* revealed the presence of methanol leaf extract 20 compounds identified in the methanol extract (Perraudin et al., 2006). The chromatogram of methanol plant leaf extract shows 5 prominent peaks as L-Proline, 1-Acety- ($C_7H_{11}NO_3$)

Table 3: Phytochemical Compounds identified in the methanol leaf extract of *Calanthe masuca* by GC-MS Analysis.

S.NO	R/ T	Peak Area %	Name of the compound	Molecular Formula	Molecular Weight
1	9.415	1.14	1-pyrazolecarboxamide	$C_4H_5N_3O$	111
2	12.038	8.91	L-Proline, 1-Acetyl	$C_7H_{11}NO_3$	157
3	19.042	0.63	Ethanone, 1-(3,4-dimethoxyphenyl)-	$C_{10}H_{12}O_3$	180
4	24.405	0.14	Cyclopentane,1,1,3,3-tetramethyl-	C_9H_{18}	126
5	24.515	6.04	Neophytadiene	$C_{20}H_{38}$	278
6	24.621	0.67	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-	$C_{20}H_{40}$	280
7	24.937	1.32	3,7,11,15-Tetramethyl-2-hexadecen-1-	$C_{20}H_{40}O$	296
8	25.250	1.71	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296
9	26.007	0.61	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270
10	26.666	15.85	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256
11	28.222	0.33	Hexadecanoic acid	$C_{16}H_{32}O_2$	294
12	28.680	1.86	8,11-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	256
13	28.780	1.06	3,6-octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294
14	28.954	0.34	Dodecnal	$C_{12}H_{24}O$	184
15	29.192	1.94	2-hexadecan-1-ol,3,7,11,15-tetramethyl-,[R-R*,R*-(E)]]-	$C_{20}H_{40}O$	296
16	29.369	24.92	9,12-Octadecadienoic acid, methyl ester	$C_{18}H_{32}O_2$	280
17	29.455	26.16	3-Tetradecanynoic acid	$C_{14}H_{24}O_2$	224
18	29.780	3.35	Octadecanoic acid	$C_{18}H_{36}O_2$	284
19	33.827	0.42	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	$C_{12}H_{23}O_2$	213
20	38.301	2.61	Squalene	$C_{30}H_{50}$	410

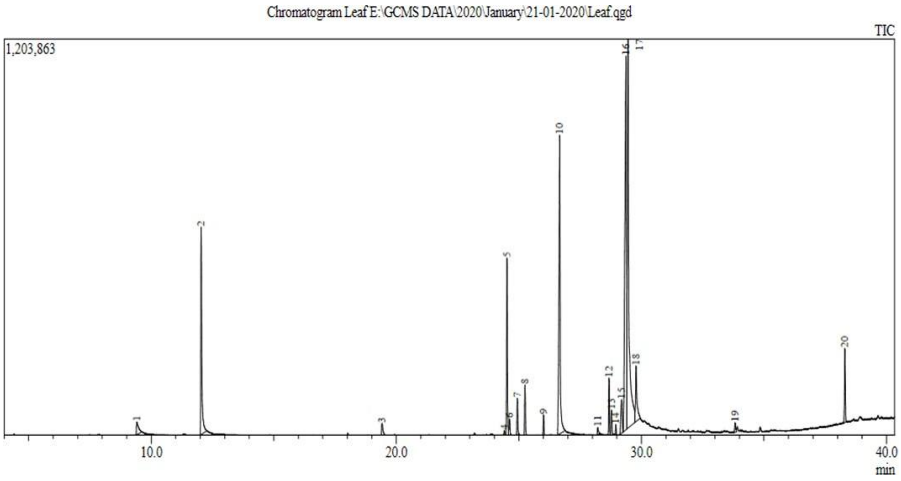

Figure 3. GC-MS Chromatogram of methanol leaf extract of *Calanthe masuca.*

with retention time of 12.038 and peak area of (8.91%), Neophytadiene ($C_{20}H_{38}$) with retention time of 24.515 and peak area of (6.04 %), n-Hexadecanoic acid ($C_{16}H_{32}O_2$) with retention time of 26.666 and peak area of (15.85%), 9,12-Octadecadienoic acid, methyl ester ($C_{18}H_{32}O_2$) with retention time of 29.369 and peak area of (24.92%) and 3-Tetradecanynoic acid ($C_{14}H_{24}O_2$) with retention time of 29.455 and peak area of (26.16) (Thomas et al., 2013; Katta et al., 2020).

It contains five major peaks along with many small peaks indicating presence of major compounds (Table 3). Structural assignment of GC retention data of compounds have been based on spectral matching with NIST library (National Institute of Standards and Technology). The small peaks have been attributed to the compounds present in small quantities as well as disintegrated major compounds. The peaks related to low retention times are mainly low polar plant compounds (Keerthiga and Anand, 2015; Swathi Krishna et al., 2017; Mistra et al., 2018). The GCMS analysis of the methanol leaf extract resulted many compounds which have diverse use in anti-diabetic, antibacterial, antifungal, antioxidant, anti-inflammatory Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Anliandrogenic, Flavor, Hemolytic, 5 Alpha reductase inhibitor, anti-tumor and anticancer properties have been identified. In addition to these, the plant is extensively used as a fracture medicine by tribal people of the area.

Conclusion

This investigation has given preliminary information to determine the chemical composition of *Calanthe masuca*

using spectral of UV-VIS, FTIR and GC-MS techniques. UV spectrophotometric analysis is a simple, rapid and accurate method for the determination of bioactive compound present in crude drug powder of medicinal plants. The FTIR spectral analysis has been shows the presence of characteristic functional groups in methanol leaf extracts of *Calanthe masuca*. In the present study 20 bioactive compounds have been identified from methanol leaf extract of *Calanthe masuca* by Gas Chromatogram-Mass spectrometry (GC-MS) analysis. Whereas *Calanthe masuca* plant extract contains various bioactive compounds 3-Tetradecanynoic acid, 9,12-Octadecadienoic acid (Z,Z)-, n-Hexadecanoic acid, L-proline, 1-acetyl, Neophytodiene and it has identify pharmaceutical importance. The presence of these bioactive compounds in *Calanthe masuca* plant cure specific diseases such as anticarcinogenic, antidiabetic, antimicrobial, antioxidant and anti-inflammatory properties.

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