

GC-MS Analysis of Bioactive Compounds on Ethanolic Leaf Extract of *Phyllodium pulchellum* L. Desv.

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ABSTRACT

The present investigation was carried out to characterize the bioactive compounds present in leaf extract of *Phyllodium pulchellum* using Gas Chromatography-Mass Spectrum (GC-MS). The results of the GC-MS analysis provide different peaks determining the presence of 10 phytochemical compounds with different therapeutic activities. The major phytocompounds 9-Octadecenamide, (Z) (18.89), 9-Cyclohexylnonadecane (15.93), 2,2,2-trifluoroethyl 2-methyltetrahydro-5-oxo-3-furancarboxylate (10.47) and minor compounds were also present. From the results, it could be concluded that *P.pulchellum* contains various bioactive compounds have various biological activities. Therefore, it is recommended as a plant of phytopharmaceutical importance.

Keywords: GC-MS analysis, *Phyllodium pulchellum*, Ethanol leaf extract, Bioactive compounds

INTRODUCTION

Medicinal plants are of great importance to the health of individuals and communities in general. About 3.4 billion people in the developing world depend on plant-based traditional medicines. This represents about 88 per cent of the world's inhabitants, who rely mainly on traditional medicine for their primary health care. The World Health Organization (WHO) supports the use traditional medicine provided they are proven to be efficacious and safe (WHO 1985). Hence there is a need to validate the ethnomedicinal use of herbal medicine and subsequently isolate and characterize the compounds which are likely to be added to the potential list of drugs¹. Over the last few decades, use of herbal drugs has been emphasized due to their easy availability, therapeutic potential, least side effects and minimum cost. At present nearly 80% of the world populations rely on plant based drugs for their health care need². GC-MS is the best technique to identify the bioactive constituents of long chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino and nitro compounds etc. Hence, Gas chromatography (GC) and Mass spectroscopy (MS) associated with particular detection techniques have become a sophisticated means for analysis of various compounds³. *Phyllodium pulchellum* L. Desv. belongs to the family Fabaceae, it is found in the Bangladesh, India, Srilanka and Southern China⁴. The plant is widely used to treat various diseases such as anti-inflammatory, analgesic, antioxidant, haemorrhage, diarrhoea, poisoning and eye diseases. Our research is therefore being directed towards elucidating potential sources of ethnomedicinal plants using modern scientific analysis like Gas Chromatography-Mass Spectrometry because developments in biotechnology have enhanced investigation of natural compounds faster

with more precision than before, leading to isolation of bioactive compounds with health benefits. In the last few years, gas chromatography mass spectrometry (GC-MS) has become firmly established as a key technological platform for secondary metabolite profiling in both plant and non-plant species^{4,6}. A detailed literature review on the plant in investigation has shown that so far there are no published reports worldwide, related to the possible chemical components of "*Phyllodium pulchellum*". So, the present study was aimed to investigate the possible chemical components by first preparing the ethanolic leaf extract and separation and identification of the compounds by subjecting it to GC-MS analysis.

MATERIALS AND METHODS

Collection of plant material

The leaf parts of *Phyllodium pulchellum* were collected from the *jumbudhu* hamlet of bodamalai hill, Namakkal District, Tamil Nadu, India. The authenticity of the plant was confirmed in Botanical Survey of India, Southern Regional Centre, Coimbatore, by referring in the direction of the deposited specimen (Voucher specimen number: BSI/SRC/5/23/2012-13/Tech-1795 & Serial No. 2).

Preparation of Extract

30 g of powdered leaf parts of *Phyllodium pulchellum* was separately extracted with 250 mL ethanol at the temperature between 60 and 65°C for 24 h by using soxhlet extractor. The solvent was evaporated by rotary vacuum evaporator to obtain viscous semisolid masses. This semidry ethanolic crude extract was subjected to GC-MS analysis.

GC-MS (Gas chromatography- Mass Spectrometry) Analysis

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Sample ID: EM-654 Low Mass(m/z): 50 Sample Name: PLANT EXTRACT_ETHANOL
Operator: RD High Mass(m/z): 650 Comments:
Run Time(min): 37.52 Instrument Name: DSQ Acquisition Date: 01/06/16 11:53:00 AM

EQUIPMENT : THERMO GC - TRACE ULTRA VER: 5.0,
THERMO MS DSQ II
COLUMN : DB 5 - MS CAPILLARY STANDARD NON - POLAR COLUMN
DIMENSION : 30 Mts, ID : 0.25 mm, FILM : 0.25 µm
CARRIER GAS : He, FLOW : 1.0 ML/Min
TEMP PROG : OVEN TEMP 70 C RAISED TO 260 C AT 6 C /MIN

INJECTION
VOLUME : 1 MICRO LITER

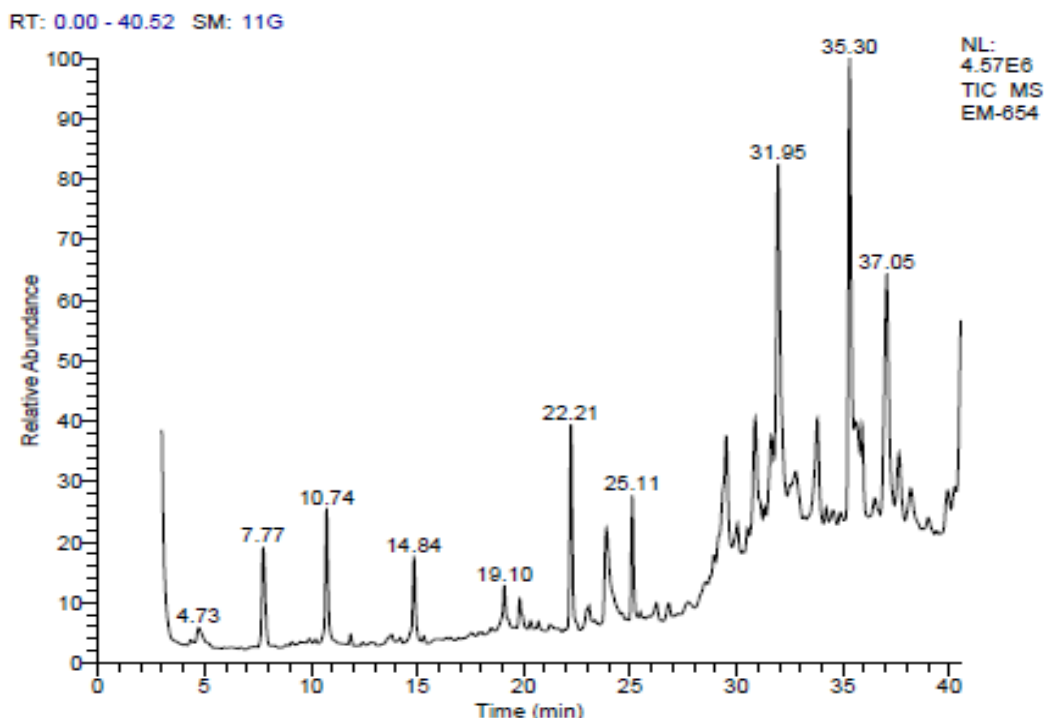


Figure 1: GC-MS analysis of *Phyllodium pulchellum* leaf extract.

The phytochemical investigation of ethanolic extract was performed on a GC-MS equipment (Thermo Scientific Co.) Thermo GC-TRACE ultra ver.: 5.0, Thermo MS DSQ II. Experimental conditions of GC-MS system were as follows: DB 5 - MS Capillary Standard Non - Polar Column, dimension: 30Mts, ID: 0.25 mm, Film thickness: 0.25µm. Flow rate of mobile phase (carrier gas: He) was set at 1.0 ml/min. In the gas chromatography part, temperature programme (oven temperature) was 70°C raised to 260°C at 6°C/min and injection volume was 1 µl. Samples dissolved in ethanol were run fully at a range of 50-650 m/z and the results were compared by using Wiley Spectral library search programme.

RESULTS

Interpretation of mass spectrum of GC-MS was done using database of National Institute Standard and Technology (NIST). The mass spectrum of unknown component was compared with the spectrum of the known component stored in the NIST library. Major components were identified by with authentic standards and by with recorded from computerized libraries. The compound name, probability, molecular formula, molecular weight, peak area and biological activity of the test materials were ascertained. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas.

The results of the GC-MS analysis of the ethanolic extract of the leaf of *P. pulchellum* are listed in Figure 1. The list of constituents is given in Table 1. The major components

Table 1: The chemical composition of ethanolic leaf extract of *P. pulchellum*.

S.N O.	R/T	Name of the compound	Probability	Molecular Formula	MW	Peak area %	Biological Activity
1.	4.73	2,3,5,6-tetramethyl-benzenesulfonamide	10.19	C ₂₂ H ₃₃ N	391	0.65	Anti-inflammatory activity and Antifungal activity
2.	7.77	Tridecane, 6-methyl	11.34	C ₁₄ H ₃₀	198	3.33	Antimicrobial activity, Antipyretic activity and Anticoagulant
3.	10.74	Tetradecane	40.66	C ₁₄ H ₃₀	198	3.56	Antimicrobial Activity, Wound healing activity, Anti-viral and Antitumor activities
4.	14.84	Hexadecane	31.70	C ₁₆ H ₃₄	226	2.37	Antibacterial and Antioxidant activities
5.	19.04	Octadecane	17.98	C ₁₈ H ₃₈	254	1.46	Hypoglycaemic activity, Antimicrobial activity and Antioxidant activity
6.	22.21	Phthalic acid, butyl hept-2-yl ester	9.57	C ₁₆ H ₂₂ O ₄	278	5.27	Antioxidant activity and Larvicidal activities
7.	25.11	Phytol	66.11	C ₂₀ H ₄₀ O	296	2.94	Antinociceptive, Antioxidant activities, Antioxidant, Antimicrobial, Antinociceptive and Cytotoxic activities
8.	31.95	9-Cyclohexylnonadecane	3.73	C ₂₄ H ₃₄ O ₄	350	15.93	Antimicrobial activity, Anticancer activity, Analgesic and Anti-inflammatory
9.	35.30	9-Octadecenamide, (Z)	9.78	C ₁₈ H ₃₅ NO	281	18.89	Anti-inflammatory activity, antibacterial activity and Antioxidant Activities
10.	37.05	2,2,2-trifluoroethyl 2-methyltetrahydro-5-oxo-3-furancarboxylate	61.63	C ₂₃ H ₃₃ N ₆ O ₆	419	10.47	Anti-malarial activities, Antimicrobials, Antitumourals and Antifungal

were 2,3,5,6-tetramethyl-benzenesulfonamide (0.65), Tridecane, 6-methyl (3.33), Tetradecane (3.56), Hexadecane (2.37), Octadecane (1.46), Phthalic acid, butyl hept-2-yl ester (5.27), Phytol (2.94), 9-Cyclohexylnonadecane (15.93), 9-Octadecenamide, (Z) (18.89), 2,2,2-trifluoroethyl 2-methyltetrahydro-5-oxo-3-furancarboxylate(10.47) (Fig 2).

The GC-MS spectrum confirmed the presence of various components with different retention times as illustrated in Figure 1. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios. These mass spectra are fingerprint of that compound which can be identified from the data library. The GC-MS study of the ethanolic extract of the leaf of *Phyllodium pulchellum* had shown the presence of lots of phytochemicals which strength contribute to the medicinal bioactive of that plant.

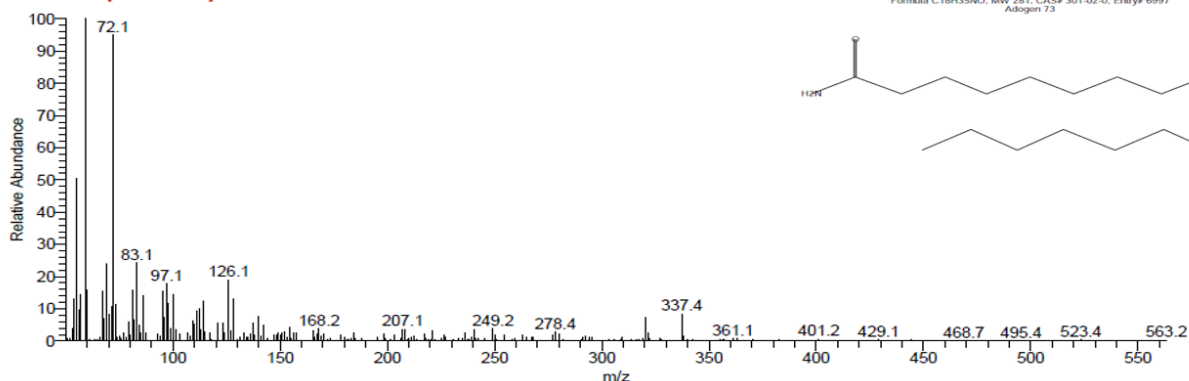
DISCUSSION

The identified major compounds possess some important biological potential for future drug development. There is growing awareness in correlating the phytochemical compounds and their biological activities. Similar to this study, five major compounds were characterized through

GC-MS analysis in *Polygonum chinense*⁷. Eighteen phytochemical constituents have been identified from the ethanolic extract of the leaves of *Desmodium gyrans* by Gas chromatogram Mass spectrometry (GC-MS)⁸. Nanadagopalan reported the presence of Phytol in the leaves of *Kirganelia reticulata* aerial parts, which was found to be effective in different stages of arthritis⁹. Aja et al., on GC/MS analysis of *Moringa oleifera* leaf and seed which revealed that 9 - octadecenoic acid (20.89%) constitutes the major constituent of the leaf extract while oleic acid (84%) is the major component of the seed extract¹⁰. In spite of the advantage of modern high drug discovery and screening techniques, traditional medicinal knowledge have also given clues to the discovery of valuable drugs¹¹. There is growing awareness in correlating the phytochemical compounds with their biological activities¹². GC-MS analysis of ethanol extract has led to identification of twenty-eight compounds from *Macrotyloma uniflorum* Linn. by comparison of their retention indices and mass spectra fragmentation¹³. The ethanolic leaf extract obtained from *P. pulchellum* were subjected to chemical analysis by GC-MS method which confirmed the presence of phytochemicals which are responsible for pharmacological activities.

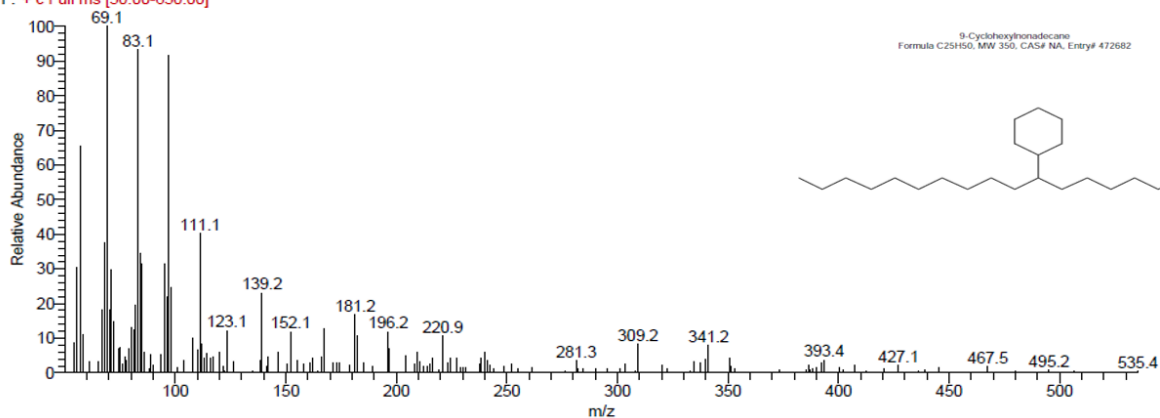
CONCLUSION

EM-654 #1586 RT: 35.30 AV: 1 RF: 6.00, 5 NL: 2.46E5
F: + c Full ms [50.00-650.00]



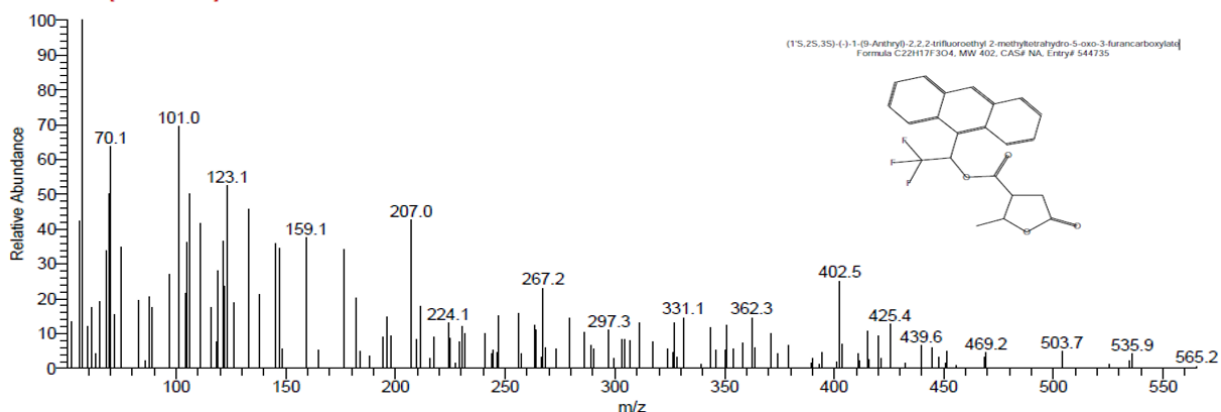
Spectra of compounds identified by GC-MS
9-Octadecenamide, (Z)

EM-654 #1422 RT: 31.95 AV: 1 RF: 6.00, 5 NL: 4.47E4
F: + c Full ms [50.00-650.00]



Spectra of compounds identified by GC-MS
9-Cyclohexylnonadecane

EM-654 #1670 RT: 37.05 AV: 1 RF: 6.00, 5 NL: 1.09E4
F: + c Full ms [50.00-650.00]



Spectra of compounds identified by GC-MS
2,2,2-trifluoroethyl 2-methyltetrahydro- 5-oxo-3-furancarboxylate

Figure 2: Mass spectrum of Major compounds of *Phyllodium pulchellum* leaf extract.

The presence of bioactive compounds justifies the use of the leaf part for various ailments by traditional practitioners. The present study aimed at identifying the nature of the components responsible for their antioxidant activity. This study clearly shows that GC-MS is a powerful technique enabling fast separation and characterization of bioactive metabolites. The high sensitivity of this technique helps in

characterization of active compounds in *phyllodium pulchellum*.

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