International Journal of Advanced Research in Engineering Technology & Science
ISSN: 2349-2819
Email: editor@ijarets.org Volume-9, Issue-8 August– 2022 www.ijarets.org

Analysis of *Polypodium Vulgare* by Gas Chromatography-Mass Spectrometry Method

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ABSTRACT

The main type of the compound identified was fatty acids derivatives. The two compounds which are similar to the *Polypodium leucotomas* are Polypodoside A and Polypodoside B which is used as marker. Sample volumes of 1 µl were injected and analyzed by GC-MS. The following compounds were identified for the first time in the propolis sample: 3,4-dihydroxybenzoic acid, Linoleic acid, Chlorogenic acid, 4-hydroxybenzoic acid and Ferulic acid. The carrier gas used was helium at a flow rate of 1 ml/min. The oven temperature was 100°C to 280°C with a constant increase of 10°C. *Polypodium* extract were analyzed with the column held initially at 60°C for 2 min and then increased to 230°C with a 2°C/ min heating ramp and then kept at 230°C for 3 min. Finally, the temperature was increased to 280°C with a 3°C/min heating ramp. The injection was performed in split mode at 220°C.A gas chromatography-mass spectrometry was carried out on a Agilent GC-MS 5975 under electron impact ionization (70 eV). The chromatographic column for the analysis was done by the HP5MS capillary column (30 m x 0.25 mm internal diameter).

Key Words- *GC-MS, Polypodium Vulgare, Samambia, Ethanol*

INTRODUCTION

The name *Polypodium* is derived from word *poly*, meaning "many," and *podus*, meaning "foot," for the many foot-like divisions of the root or rhizomes of polypody ferns and popularly known as Samambaia[1]. Samambaia contains flavonoids, alkaloids and lipids. It is a rich source of lipids and fatty acids. The main plant chemicals identified in Samambaia include adenosine, alkaloids, arachidonic acid, arabinopyranosides, calagualine, ecdysone, ecdysterone, eicosapentaenoic acid, elaidic acid[2,5,6] etc. *Polypodium* is a genus of between 75-100 species of true ferns, widely distributed throughout the world, with the highest species diversity in the tropics. Polypodies have some use in herbalism, but are today most important in horticulture where several species, hybrids, and their cultivars like *Polypodium* 'Green Wave' are commonly used as ornamental plants for shady locations. The Latin America use the

rhizome and leaves for many different diseases treatment like cancer, psoriasis, peptic ulcers, kidney problems, diarrhoea, arthritis, pains in joints and tendons.

MATERIAL AND METHODS

Chemicals

Bis-(trimethyl-silyl)trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) (Merck-2333) were used as silylation reagents with spectrophotometric grade pyridine (Merck-7460).

Instrument

Gas chromatography-mass spectrometry was carried out on a Agilent GC-MS 5975, under electron impact ionization (70 eV). The interface temperature 230 ˚C, and the MS scan range was 35-450 atomic mass units (AMU). The chromatographic column for the analysis was done by HP5MS capillary column (30 m x 0.25 mm internal diameter). The carrier gas used was helium at a flow rate of 1 ml/min[4]. the oven temperature was 100˚C to 280˚C with a constant increase of 10˚C. Polypodium samples were analyzed with the column held initially at 60˚C for 2 min and then increased to 230˚C with a 2˚C/ min heating ramp and then kept at 230˚C for 3 min. Finally, temperature was increased to 280˚C with a 3˚C/min heating ramp. The injection was performed in split mode at 220˚C.

Sample preparation

Polypodium leaves were extracted with ethanol using soxhalet apparatus, for 8 hrs. The extracts were filtered and after filtration; the extracts were combined and evaporated to dryness under vacuum at 50˚C. 1 mg of dry extract was reacted with 50µl pyridine + 100µl
bis-(trimethylsilyl) trifluoroacetamide (BSTFA) including 1% trimethylchlorosilane (TMCS) in a sealed glass tube for 30 min at 100˚C to prepare samples for gas chromatography. Sample volumes of 1µl were injected and analyzed by GC-MS.

Identification of compounds

Peaks were identified by Mr. M. Prabhakara Reddy in Bangalore Test House, Bangalore. Good spectral matches for some compounds could be found in the Wiley and National Bureau of Standards (NBS) mass spectral library. The result is tabulated in the Table No.1**.**

Chromatographic Condition

1. Column : HP5MS (30m x 0.25 i.d.)
2. Carrier gas : Helium
3. Oven temperature : 100 - 280˚C
4. Inlet temperature : 280˚C
5. Detector temperature (MSD) : 230˚C

6. Carrier gas flow rate : 1 ml/min
7. Injection quantity : 1 µl
8. Scan mode : 50 – 550 mod range

RESULTS

The chemical composition of *Polypodium Vulgare*, which was collected from the Sikkim, was investigated by GC-MS after silylation. More than 13 individual compounds were identified [Table 1] and [Figure 1]. The phytochemical analysis showed the presence of terpenoids, saponins and flavonoids[3,8]. The following compounds were identified for the first time in the propolis sample: 3,4-dihydroxybenzoic acid, Linoleic acid, Chlorogenic acid, 4-hydroxybenzoic acid and Ferulic acid. The two compounds which are similar to the *Polypodium leucotomas* are Polypodoside A and Polypodoside B[7]. However there may be variation in the chemical composition based of topography. The main compound identified was long chain fatty acids along with the flavanoids.

Table 1: GC-MS analysed compounds of ethanolic extract of *Polypodium Vulgare*

Sl. No.	Retention time (RT)	Compounds
1	10.33	4-vinylphenol
2	11.21	Lauric acid
3	15.439	Myristic acid
4	19.088	Chlorogenic acid
5	19.527	Palmitic acid
6	20.820	Chlorogenic acid
7	22.834	3,4-dihydroxybenzoic acid
8	22.688	Linoleic acid
9	23.281	Stearic acid
10	23.996	Caffeic acid
11	26.744	Polypodoside A
12	23.996	4-hydroxybenzoic acid
13	26.253	Polypodoside B
14	23.883	Ferulic acid

CONCLUSION

The finding shows that *Polypodium Vulgare* contains long chain fatty acid, terpenoids, saponins and flavonoids. The literature revealed the rhizome and leaves are used for treatment of disease like cancer, psoriasis, peptic ulcers, kidney problems, diarrhoea, arthritis, and pains in joints and tendons. In the further studies the compounds will be isolated by coloumn chromatography and will be checked for various activities.

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