Chemical Investigations of *Polygala Chinensis* L. by GC-MS

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ABSTRACT

In the present study, the bioactive components of *Polygala chinensis* whole plant have been evaluated using Gas Chromatography-Mass Spectrum (GC-MS). Fourteen compounds in ethanolic extract were identified. 1, 5–Anhydro-d-mannitol (92.30%) was the prevailing compound in ethanolic extract, which is suggested to be an anticancer compound. This is the first report of identification of active constituents from whole plant of *Polygala chinensis.*

Key words: 1, 5–Anhydro-d-mannitol, phytol, GC-MS, *Polygala chinensis*, anti-cancer.

INTRODUCTION

Polygala chinensis L. belongs to Polygalaceae family. It is commonly known as "Siriyanangai". Genus *Polygala* is an annual, diffuse herb, 10-25cm tall. Flowers are papilionaceous, primary root orange, stems woody at base, branches terate, crisped pubescent. Leaf blade green, obovate, elliptic or lanceolate, 2.6-10x1-1.5cm, papery, pubescent, inflorescence raceme, super-axillary, rarely axillary, shorter than leaves, densely few flowered. Flowers 4-5mm long, sepals 5, persistent, green, ciliate, outer sepals 3, ovate-lanceolate, apex acuminate; inner sepals 2, petaloid, falcate, petals 3, connate at base, yellowish or white with pink, lateral petals shorter than keel, inside with fascicled white hairs at base. Stamens 8, ovary compressed- orbicular.

Polygala was traditionally used by Americans to treat snake bites (McGuffin *et al*. 1997) and as an expectorant to treat cough and bronchitis. *Polygala* is considered as a powerful tonic herb (Teeguarden 1998) that can help to develop the mind and aid in creative thinking. Taking into consideration of the medicinal importance of *Polygala chinensis*, the ethanolic extract of whole plant of *Polygala chinensis* were analyzed for the first time using GC-MS. This work will help to identify the compounds of therapeutic value.

MATERIALS AND METHODS

Collection of plant sample

Whole plant of *Polygala chinensis* were collected from Vadavalli, Coimbatore, Tamilnadu.

Plant sample extraction

Leaves were cleaned, shade dried and pulverized to powder in a mechanical grinder. Required quantity of powder was weighed and transferred to Stoppard flask, and treated with ethanol until the powder is fully immersed. The flask was shaken every hour for the first 6 hrs and then it was kept aside and again shaken after 24 hrs. This process was repeated for 3 days and then the extract was filtered. The extract was collected and evaporated to dryness by using vacuum distillation unit. The final residue thus obtained was then subjected to GC-MS analysis.

GC-MS analysis

GC-MS analysis of these extracts was carried out by following the method of Hema *et al.* (2010). GC-MS analysis were performed using a Perkin-Elmer GC clauses 500 system and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with a Elite-I, fused silica capillary column (30m x 0.25 mm ID x 1 µ df), composed of 100% Dimethyl poly siloxane). For GC/MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1ml/min and an injection volume of 2 µl was employed split ratio of 10:1) injector temperature 250^0C; ion-source temperature 280^0C. The oven temperature was programmed from 110^0C (isothermal for 2 min) with an increase of 10^0C / min to 2000^0C, then 5^0C/min to 280^0C, ending with a 9 min isothermal at 280^0C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 minutes. The relative % amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was a Turbo mass.

Identification of components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the

known components stored in the NIST library. The Name, Molecular weight and Structure of the components of the test materials were ascertained.

RESULTS AND DISCUSSION

The compounds present in the ethanolic extracts of *Polygala chinensis* were identified by GC-MS analysis (Figure 1). The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) in the ethanolic extracts of *Polygala chinensis* are presented in Table 1. Fourteen compounds were identified in ethanol extract by GC-MS. The major components present in the whole plant of *Polygala chinensis* were 1, 5–Anhydro-d-mannitol (92.30%), 9H-Foro [2,3-H] chromene-2,8-dione,4-methyl-9-(3,4,5-trimethoxybenzylidene)(2.11%), propane, 1,1,3-triethoxy (1.80%), 9,12, octadecadienoic acid (2,2)- (0.89%), Myristin, 1,3-diaceto-2- (0.84%) and squalene (0.56%). Figure 2 and 3 shows the mass spectrum and structures of medicinally important phytochemical constituents which contribute to the medicinal activity of the ethanolic extracts of *Polygala chinensis*. 1, 5–Anhydro-d-fructose is a metabolite of 1, 5–Anhydro-d-mannitol, is an useful anticarcinogenic agent as it inhibits the growth of the oral pathogen *Streptococcus mutans*. It also shows anti-inflammatory and anticancer effects (Fiskesund *et al*. 2010). 1, 5–Anhydro-d-mannitol is the major component found in the whole plant of *Polygala chinensis* which is being used for the pharmacological work.

In the present study, fourteen compounds have been identified from ethanolic extract of the whole plant of *Polygala chinensis* by Gas chromatography – Mass spectrometry (GC-MS) analysis. The presence of various bioactive compounds justifies the use of the whole plant for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents and subjecting it to pharmacological activity will definitely give fruitful results.

Table 1: Components detected in the whole plant ethanol extract of *Polygala chinensis*.

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1.	2.07	Propane, 1,1,3-triethoxy-	$C_9H_{20}O_3$	176	1.80
2.	11.31	1,5-Anhydro-d-mannitol	$C_6H_{12}O_5$	164	92.30
3.	11.66	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	0.89
4.	13.49	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	0.09
5.	15.00	Phytol	$C_{20}H_{40}O$	296	0.22
6.	19.17	Myristic anhydride	$C_{28}H_{54}O_3$	438	0.14
7.	20.87	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	0.31
8.	23.17	Oleic Acid	$C_{18}H_{34}O_2$	282	0.14
9.	24.71	Squalene	$C_{30}H_{50}$	410	0.56
10.	26.32	9H-Furo[2,3-H]chromene-2,8-dione, 4-methyl-9-(3,4,5-trimethoxybenzylidene)-	$C_{22}H_{18}O_7$	394	2.11
11.	27.18	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	0.06
12.	28.86	6,7-Epoxypregn-4-ene-9,11,18-triol-3,20-dione, 11,18-diacetate	$C_{25}H_{32}O_8$	460	0.22
13.	32.31	1-Heptatriacotanol	$C_{37}H_{76}O$	536	0.32
14.	35.02	Myristin, 1,3-diaceto-2-	$C_{21}H_{38}O_6$	386	0.84

Table. 2 Activity of phyto-components identified in the ethanolic extracts of the whole plant of *Polygala chinensis* by GC-MS.

RT	Name of the compound	Molecular Formula	Nature of Compound	**Activity
11.31	1,5-Anhydro-d-mannitol	$C_6H_{12}O_5$	Sugar alcohol	Anticancer compound
11.66	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	Linoleic acid	Hepatoprotective Nematicide, Insectifuge Antihistaminic, Antieczemic Antiacne, Alpha reductase inhibitor, Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge Cancer preventive Antiinflammatory, Hypocholesterolemic
13.49	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	Palmitic acid ester	Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic 5-Alpha reductase inhibitor
15.00	Phytol	$C_{20}H_{40}O$	Diterpene	Antimicrobial Antiinflammatory Anticancer Diuretic
20.87	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	Plasticizer compound	Antimicrobial Antifouling
23.17	Oleic Acid	$C_{18}H_{34}O_2$	Oleic acid	Antiinflammatory, Antiandrogenic Cancer preventive, Dermatitigenic Hypocholesterolemic, 5-Alpha reductase inhibitor, Anemiagenic Insectifuge, Flavor
24.71	Squalene	$C_{30}H_{50}$	Triterpene	Antibacterial, Antioxidant, Antitumor, Cancer preventive, Immunostimulant, Chemo preventive, Lipoxygenase-inhibitor, Pesticide Diuretic
26.32	9H-Furo[2,3-H]chromene-2,8-dione, 4-methyl-9-(3,4,5-trimethoxybenzylidene)-	$C_{22}H_{18}O_7$	Chromene compound	Yellow Color pigment
27.18	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	Steroid	Antimicrobial Antiinflammatory Anticancer Diuretic Antiasthma Antiarthritic
28.86	6,7-Epoxypregn-4-ene-9,11,18-triol-3,20-dione, 11,18-diacetate	$C_{25}H_{32}O_8$	Steroid	Antimicrobial Antiinflammatory Anticancer Diuretic Antiasthma Antiarthritic
32.31	1-Heptatriacotanol	$C_{37}H_{76}O$	Alcoholic compound	Antimicrobial

****Source: Dr.Duke's:** Phytochemical and Ethnobotanical Databases

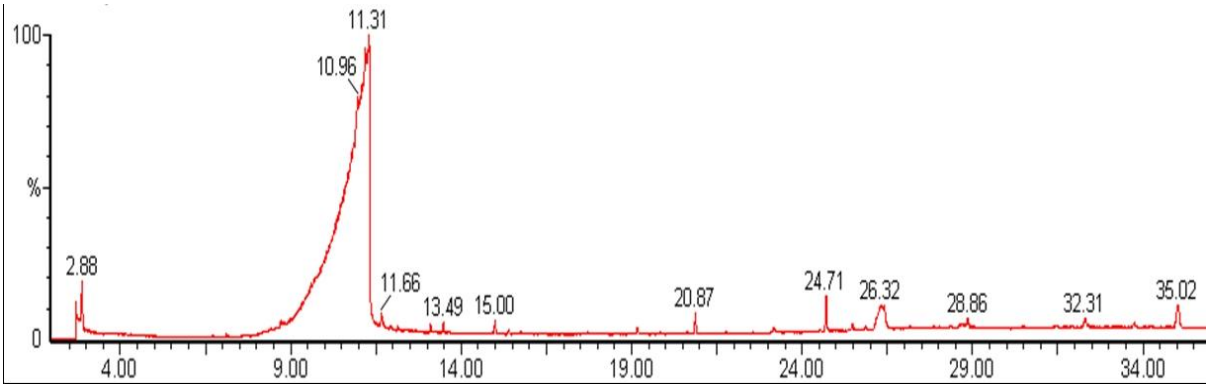

Fig. 1 GC-Ms chromatogram of the ethanolic extract of the whole plant of *Polygala chinensis*

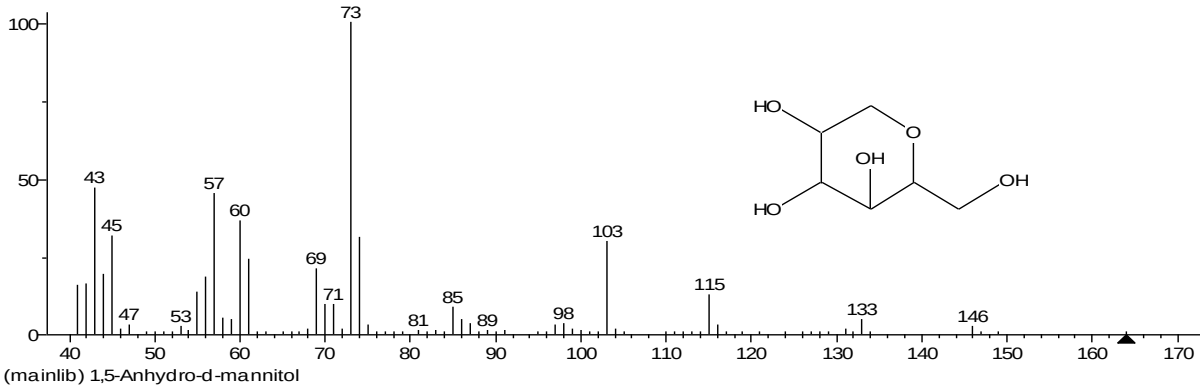

Fig. 2 Mass spectrum of 1,5-Anhydro-d-mannitol

Fig. 3 Mass spectrum of Phytol

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