# THE GC-MS DETERMINATION OF CHEMICAL CONSTITUENTS FROM HARPAGOPHYTUM PROCUMBENS DC ROOTS

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**Abstract:** The present work is devoted to the study of the component composition of the volatile fraction of the heptanoic extract from Harpagophytum procumbens roots ("Starwest Botanicals", USA).

For this purpose the use of gas chromatography-mass spectrometry (GC-MS) was proposed. The GC-MS analysis was carried out on an Agilent 6890N/5973 inert gas chromatograph/mass selective detector (Agilent Technologies, USA). The quantitative content of the components was calculated by the method of the internal standard. The components with the probability of coincidence for mass spectra of more than 90 % were taken into account.

As a result of the study more than 31 substances have been identified. Among the main groups of compounds the presence of aromatic hydrocarbons, esters and fatty acids, as well as alkanes and alkenes should be mentioned. Hexadecanoic acid (29.92 mg/kg), Octadec-9-enoic acid (21.39 mg/kg), 8-[4-[N-Aziridyl]butyl]amino-2,6-dimethyl-2,6-octadiene (6.46 mg/kg), Pentacosane (5.85 mg/kg), Hexadecanoic acid, trimethylsilyl ester (4.53 mg/kg) prevail in terms of quantity.

Based on the results obtained the conclusion can be made that Harpagophytum procumbens DC is a promising herbal source for obtaining active ingredients with the anti-inflammatory, regenerative, analgesic, immunostimulating properties.

**Keywords**: Harpagophytum procumbens DC, chemical constituents, chemical structure, gas chromatography combined with mass spectrometry.

## 1. Introduction

The modern stage of using herbal medicinal products is based not only on the rational use of the current experience, but also on achievements of pharmacy in the field of studying the composition and peculiarities of the biological activity of medicinal plants [1]. As a result of the numerous studies conducted it has been proven that medicinal plant raw material (MPRM) and drugs based on them contain unique combinations of biologically active substances providing a wide range of the therapeutic action [2].

Harpagophytum procumbens DC (Devil's claw) belongs to the Pedaliaceae family and is also known as grapple plant, wood spider, and harpago. It is native to the southern part of the African continent and may be found in the Kalahari Sands of Namibia, South Africa, Angola, Zambia, and Zimbabwe [3]. It has historically been used to treat several symptoms such as fever, malaria, indigestion and pain [4]. In addition, it was demonstrated that H. procumbens extracts have beneficial effects in the case of rheumatic diseases according to animal and clinical studies [5]. There have been reports verifying the anti-inflammatory effects of H. procumbens extracts on acute or sub-chronic inflammation in a rat mode [6].

The correct identity of the crude herbal material, or the botanical quality, is of prime importance in establishing the quality control of herbal drugs [7]. Today gas chromatography-mass spectrometry (GC-MS) is widely used to study the chemical composition and standardization of MPRM [8]. The high selectivity of capillary columns enables separation of many volatile compounds simultaneously within comparatively short times [9]. Due to the powerful separation efficiency and the sensitive detection, GC-MS has become a popular and useful analytical tool in the research field of herbal medicines [10].

The aim of the work presented was identification and the quantitative determination of major chemical compounds extracted by heptane from the raw material of Harpagophytum procumbens roots.

## 2. Materials and Methods

The object of the study was the sample of MPRM from Harpagophytum procumbens roots ("Starwest Botanicals", USA). The GC-MS analysis was carried out on an Agilent 6890N/5973 inert gas chromatograph/mass selective detector (Agilent Technologies, USA).

*Preparation of the analytical test*

Grind the accurate weight (approximately 0.500 g) to the powder (355) and place in a 20 ml vial with addition of the internal standard – tridecane. Take the standard in the amount of 50 μg per the accurate weight with the subsequent determination of the resulting concentration of the internal standard used for the final calculations. Dissolve the sample in 20 ml of water R and distill volatile compounds under reflux at the temperature of 100 °C for 4 h. Extract the resulting aqueous extract with heptane R with the subsequent concentration in the flow of nitrogen gas (100 ml/min) to the residual volume of the solution of 10 μl. Carry out further sample concentration to the volume of 2 μl directly in the syringe.

*Gas Chromatography – Mass Spectroscopy Analysis*

The GC-MS analysis was carried out on an Agilent 6890N/5973 inert gas chromatograph/mass selective detector (Agilent Technologies, USA). The column used was HP-5MS – the capillary column with the size of 30 m × × 0.25 mm and the layer thickness of 0.25 mm.

Helium gas (99.999 %) was used as a carrier gas at a constant flow rate of 1ml/min, and the injection volume of 2μl (the split ratio – 1:50). The temperature of the evaporator detector was 300 °C. The injector temperature was 250 °C; the ion-source temperature was 280 °C. The oven temperature was programmed at 50 °C (isothermal for 5 min), with an increase to 220 °C at the rate of 4 c/min, then to 300 °C at the rate of 10 C/min, ending with a 10 min isothermal at 300 °C. The total GC-MS running time was 50 min.

*Identification of components*

Interpretation of GC-MS mass-spectra was conducted using the database of the National Institute of Standards and Technology having more than 62.000 patterns.

The quantitative content of the components was calculated by the method of the internal standard. The components with the probability of coincidence for mass spectra of more than 90 % were taken into account. Determination was conducted in triplicate.

## 3. Results

Thirty one compounds were identified in Harpagophytum procombens root in the chromatogram (Fig. 1) obtained from the GC/MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in Table 1.

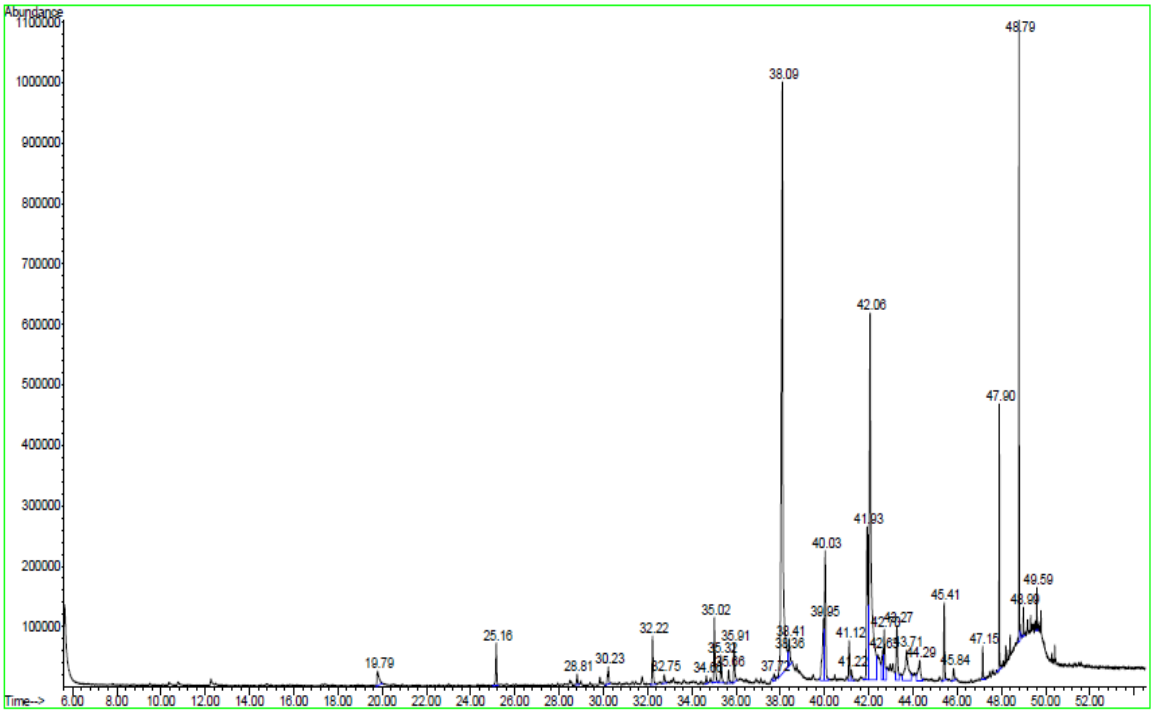


**Fig. 1.** The chromatogram of Harpagophytum procumbens obtained from the GC/MS analysis

**Table 1**
The GC-MS analysis of Harpagophytum procumbens DC roots

| No. | RT | Peak area, mV/s | MW | MF | Name | Content, mg/kg |
|---|---|---|---|---|---|---|
| 1 | 19.79 | 23329 | 150.1745 | $C_9H_{10}O_2$ | 2-Methoxy-4-vinylphenol | 0.89 |
| 2 | 25.16 | 58865 | 204.3511 | $C_{15}H_{24}$ | 1,4-Methanoazulene, decahydro-4,8,8-trimethyl-9-methylene | 1.20 |
| 3 | 28.81 | 58760 | 204.352 | $C_{15}H_{24}$ | 1S,2S,5R-1,4,4-Trimethyltricyclo[6.3.1.0(2,5)]dodec-8(9)-ene | 0.30 |
| 4 | 30.22 | 23767 | 151.165 | $C_8H_9NO_2$ | 2,6-Dimethyl-3-aminobenzoquinone | 0.63 |
| 6 | 32.75 | 60823 | 207.273 | $C_{12}H_{17}NO_2$ | Cyclohexanecarboxamide, N-furfuryl) | 0.33 |
| 7 | 34.65 | 100682 | 270.4507 | $C_{17}H_{34}O_2$ | Isopropyl Myristate | 0.26 |
| 8 | 35.02 | 25176 | 167.164 | $C_8H_9NO_3$ | 6-Methoxynicotinic acid | 2.12 |
| 9 | 35.32 | 117607 | 300.5520 | $C_{17}H_{36}O_2Si$ | Tetradecanoic acid, trimethylsilyl ester | 0.96 |
| 10 | 35.66 | 105073 | 278.3435 | $C_{16}H_{22}O_4$ | 1,2-Benzenedicarboxylic acid, bis( 2-methylpropyl) ester | 0.43 |
| 11 | 35.91 | 77434 | 232.275 | C14H16 | 2-(2,5-Dimethoxyphenyl)cyclohex-2-enone | 1.34 |
| 12 | 37.72 | 13577 | 132.2023 | C10H12 | Benzene, 2-butenyl | 0.27 |
| 13 | 38.09 | 92228 | 256.4241 | $C_{16}H_{32}O_2$ | n-Hexadecanoic acid | 28.86 |
| 14 | 38.36 | 83686 | 256.4241 | $C_{16}H_{32}O_2$ | Pentadecanoic acid | 0.20 |
| 15 | 38.41 | 92226 | 256.4241 | $C_{16}H_{32}O_2$ | n-Hexadecanoic acid | 1.06 |
| 16 | 39.95 | 100859 | 270.4522 | $C_{20}H_{30}$ | 7-Isopropyl-1,1,4a-trimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene | 2.96 |
| 17 | 40.03 | 131777 | 328.6052 | $C_{19}H_{40}O_2Si$ | Hexadecanoic acid, trimethylsilyl ester | 4.53 |
| 18 | 41.11 | 115570 | 278.3435 | $C_{16}H_{22}O_4$ | 1,2-Benzenedicarboxylic acid, bis( 2-methylpropyl) ester | 1.21 |
| 19 | 41.22 | 88561 | 296.5741 | $C_{21}H_{44}$ | Heneicosane | 0.38 |
| 21 | 42.06 | 107520 | 280.4455 | $C_{18}H_{32}O_2$ | 9,12-Octadecadienoic acid (Z,Z) | 21.39 |
| 22 | 42.63 | 95828 | 282.4614 | $C_{18}H_{34}O_2$ | Octadec-9-enoic acid | 1.18 |
| 23 | 42.70 | 76880 | 262.473 | $C_{19}H_{34}$ | 1,3,12-Nonadecatriene alkene | 2.03 |
| 25 | 43.71 | 142786 | 270.3661 | $C_{18}H_{22}O_2$ | Gona-1,3,5(10)-trien-17-one, 3-methoxy-, (13 alpha) | 2.99 |
| 26 | 44.29 | 84601 | 354.6425 | $C_{21}H_{42}O_2Si$ | Oleic acid, trimethylsilyl ester | 1.54 |
| 27 | 45.41 | 115570 | 244.4457 | $C_{13}H_{28}O_2Si$ | Decanoic acid, trimethylsilyl ester | 2.15 |
| 28 | 45.84 | 110097 | 244.4457 | $C_{21}H_{44}$ | Heneicosane | 0.42 |
| 29 | 47.15 | 148383 | 286.4516 | $C_{20}H_{30}O$ | Ferruginol | 0.67 |
| 30 | 47.90 | 142112 | 370.5665 | $C_{22}H_{42}O$ | Hexanedioic acid, bis(2-ethylhexyl) ester | 2.82 |
| 31 | 48.79 | 151556 | 286.4516 | $C_{25}H_{52}$ | Pentacosane | 5.85 |

After analyzing the chromatogram it has been found that Hexadecanoic acid (29.92 mg/kg), Octadec-9-enoic acid (21.39 mg/kg), 8-[4-[N-Aziridyl]butyl]amino-2,6-dimethyl-2,6-octadiene (6.46 mg/kg) prevail in terms of quantity. The chemical compounds found can be divided into aromatic hydrocarbons (1,4-Methanoazulene, decahydro-4,8,8-trimethyl-9-methylene; Cyclohexanecarboxamide, N-furfuryl; 6-Methoxynicotinic acid; 2-(2,5-Dimethoxyphenyl)cyclohex-2-enone; Benzene, 2-butenyl; 7-Isopropyl-1,1,4a-trimethyl-1,2,3,4,4a, 9,10,10a-octahydrophenanthrene, ), esters (Isopropyl Myristate; Tetradecanoic acid, trimethylsilyl ester; 1,2-Benzenedicarboxylic acid; bis(2-methylpropyl) ester; Hexadecanoic acid, trimethylsilyl ester; 1,2-Benzenedicarboxylic acid, bis( 2-methylpropyl) ester; Oleic acid, trimethylsilyl ester; Oleic acid, trimethylsilyl ester; Decanoic acid, trimethylsilyl ester; Hexanedioic acid, bis(2-ethylhexyl) ester), fatty acids (n-Hexadecanoic acid; Pentadecanoic acid; 9,12-Octadecadienoic acid (Z,Z); Octadec-9-enoic acid), alkanes and alkenes (1,3,12-Nonadecatriene, Heneicosane, Pentacosane).

It should be noted that linoleic acid (6.46) related to polyunsaturated fatty acids of n-6 (omega-6) family is a structural element of cell membranes, regulates the cholesterol metabolism; it is involved in formation of tissue hormones – prostaglandins, and is the biochemical precursor of linolenic and arachidonic acids. Under the action of trace elements, enzymes and vitamins in the body it is converted into gamma-linolenic acid, from which prostaglandin E1 is synthesized; in its turn, it improves immunity, reduces inflammatory processes, support sthe blood vessel tone, and accelerates metabolism. Elaidic acid (21.39 mg/kg) is a geometric transisomer of oleic acid. Myristic acid is used by the body to stabilize different proteins, including proteins of the immune system.

## 4. Discussion

As a result of the study of the volatile fraction of Harpagophytum procumbens DC roots conducted using the GC-MS method 31 biologically active substances with different chemical structures have been identified. Among the compounds identified aromatic hydrocarbons, esters and fatty acids are found in the greatest amount. Hexadecanoic acid (29.92 mg/kg), Octadec-9-enoic acid (21.39 mg/kg), 8-[4-[N-Aziridyl]butyl]amino-2,6-dimethyl-2,6-octadiene (6.46 mg/kg) prevail in terms of quantity.

The obtained data confirms the use of Harpagophytum procumbens DC for the treatment of rheumatic diseases. The isolated compounds have anti-inflammatory, regenerative, analgesic, immunostimulating properties. Taking into account the data obtained it should be noted that Harpagophytum procumbens DC is a promising source for obtaining new active ingredients and herbal drugs.

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