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SOXHLET EXTRACTION OF ACHILLEA MILLEFOLIUM STEM, FLOWERS AND LEAVES AND GC-MS ANALYSIS

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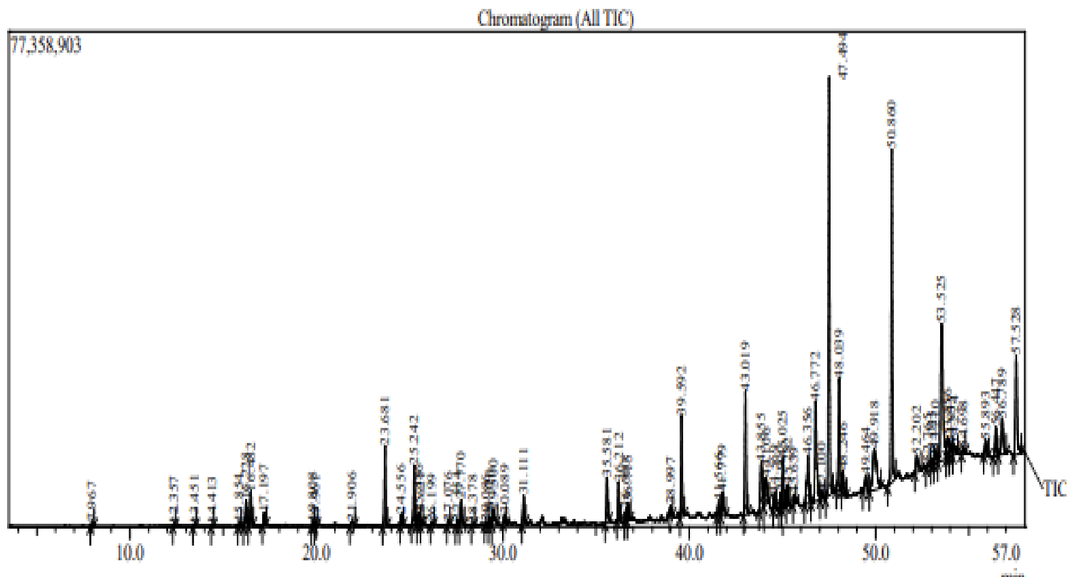
• Introduction

Plant extracts have been used for thousands of years due to the bioactive components responsible for physiological activities. Numerous phytochemicals exist in the fruits, flowers, seeds and leaves of plants, highlighting the importance of investigating herbal preparations for health benefits. In direct proportion to the development of technology, the understanding of the mode of action of bioactive molecules increases and requires the approach of concrete studies to verify a cause-effect relationship. As a result, accurate studies are needed to demonstrate the potency of plant extracts against various conditions.

• Material and method

Leaves, stems and flowers of the genus Achilleamillefolium were used for the study. The plant material was dried and ground into a fine powder. The resulting powder was placed in a soxhlet apparatus. The extraction involved continuously washing of sample with hexane, through 16 cycles of boiling and condensation, allowing for the efficient extraction of the desired compounds into the solvent. Gas chromatography coupled with mass spectrometry (GC-MS) was chosen to identify the extracted compounds. The initial temperature of the chromatographic column was set at 60°C for 3 minutes, followed by heating at a rate of 5°C/min to 300°C with a dwell time of 3 minutes. The injector temperature was set at 250°C, with a helium gas flow rate of 1ml/min, and a split ratio of 1/20. The mass detector parameters were set as follows: solvent delay at 3 minutes, scan range 40-350 a.m.u. (atomic mass unit), with a scan rate of 1500 a.m.u./sec. The ion source temperature was set at 230°C, and the transfer line temperature at 280°C. The analysis time was 58 min. The identification of the extracted compounds was achieved by comparing the mass spectra obtained with the NIST mass spectrum library.



Correlogram of maternal parameters

• Results and discussions

Among the biologically active compounds identified in the hexane extract of Achillea millefolium, there are sesquiterpene lactones. They are known to exhibit a wide range of biological activities, including antimicrobial, anti-inflammatory, antifungal, and healing. Geranyl linalool, which belongs to the class of diterpenoids, and which possess a wide spectrum of biological activities including anti-inflammatory and antimicrobial ones, was identified in a percentage of 4.53%.

Chemical composition of Achillea millefolium in hexane extract by GC-MS analysis

Retention time [min.]	Compounds name	% of total
16.525	Endo-borneol	0.70
17.240	α-terpineol	0.29
23.723	caryophyllene	2.03
24.600	humulene	0.34
25.285	Germacrene D	1.80
31.157	chamazulene	2.40
35.630	β- carotene	2.52
36.252	Geranyl-α-terpinene	1.69
38.917	Linoleic acid	2.22
39.633	Geranyl linallol	4.53
41.823	1-bromo-triacontane	1.51
43.057	8,9-dehydro-9-vinyl-cycloisolonigifolene	2.92
43.925	2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-phenol	3.13
46.395	3a,8-dihydroxy-3,5a,9-trimethyl-4,5,6,7,8,9b-hexahydro-3H-benzo[g][1]bezofuran-2-one Ac - sesquiterpene lactone	5.54
46.808	(9a hydroxy 3,8a dimethyl 5 methylidene 2 oxo 1,4a,6,7,8,9 hexahydrobenzo[f][1]benzofuran-8-yl) acetate – sesquiterpene lactone	12.61
48.283	(3S,3aR,4S,9aS,9bR)-3,6,9-trimethyl-2,7-dioxo-2,3,3a,4,57,9a,9b-octahydroazuleno[4,5-b]furan-4-yl angelate	2.28
50.898	hexacontane	14.52
53.563	tetrapentacontane	6.53
53.905	β-sitosterol acetate	0.61
54.270	dl-α-tocopherol	0.32
54.757	24-norursa-3,12-diene	0.35

Conclusion

The plant extract consists of a mixture of numerous compounds and their phytochemical investigation occupies an important place in establishing the bioactivity. In addition, the characteristics of the compounds together with their structural conformations and molecular size are factors that influence the activity of the constituents. Therefore, the presented study includes phytochemical analysis and elucidation of volatile components obtained from different parts of the plant, i.e. stems, leaves and flowers. In addition to other identified compounds, O-acetyl-(9a-hydroxy-3,8a-dimethyl-5-methylidene-2-oxo-4,4a,6,7,8,9-hexahydrobenzo[f][1]benzofuran-8-yl) acetate, a sesquiterpene lactone was found in a percentage of 12.61%. Due to the fact that there are many factors of both intrinsic and extrinsic nature that can influence the activity of these compounds, future studies aim to perform *in vitro* biological investigations, as part of the current work.

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