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Identification of bioactive compounds using different solvents through FTIR studies and GC-MS analysis

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Abstract

Andrographis paniculata belonging to the family Acanthaceae and the genus *Andrographis* Nees comprises of herbs and shrubs distributed in tropical Asia. GC-MS and FTIR analysis was carried out using different solvent extracts done after soxhlation. FTIR analysis was carried out with dried *Andrographis paniculata* leaf powder and GC-MS analysis was done with chloroform, ethyl acetate, ethanol and methanolic extracts of *Andrographis paniculata*. The detailed investigation done with these processes helps in screening active compounds from plants which lead to further discovery of new drugs.

Keywords: Acanthaceae, *Andrographis paniculata*, soxhlation, screening

1. Introduction

Andrographis paniculata is an annual herbaceous plant in the family Acanthaceae, native to India and Srilanka. It is an erect annual herb extremely bitter in taste in all parts of the plant body. The plant is known as King of bitters and also it is known by various vernacular names. It grows erect to a height of 30-110 cm (12-43 inch) in moist shady places. The lance shaped leaves have hairless blades measuring upto 8cm (3.1 inch) long by 2.5cm (0.98 inch). The small flowers were borne in spreading racemes. The fruit is a capsule around 2 cm (0.79 inch) long and a few millimeters wide. *Andrographis paniculata* derive compounds which show less side effects. Phytoconstituents found in the plant are responsible for the medical activity of plant species, in that various secondary metabolites like alkaloids, flavanoids, phenolic compounds, steroids, saponnins and tannins play a major role. The present observations were based on the study of phytochemicals through GC-MS and FTIR analysis.

Mass spectrometry, coupled with chromatographic separations such as Gas chromatography (GC/MS) is normally used for direct analysis of components existing in traditional medicines and medicinal plants. In recent years GC-MS studies have been increasingly applied for the analysis of medicinal plants as this technique has proved to be a valuable method for the analysis of nonpolar components and volatile essential oil, fatty acids, lipids and alkaloids.

2. Materials and Method

2.1 Sample collection

Leaf samples of *Andrographis paniculata* were collected from Kuniamuthur Village, (Coimbatore). Collected samples were washed with tap water shade dried and powdered using blender were stored in air tight container.

The samples were soxhlated using different solvents based on polarity and the extracts were collected and stored in brown bottles which was subjected to GC-MS and FTIR analysis.

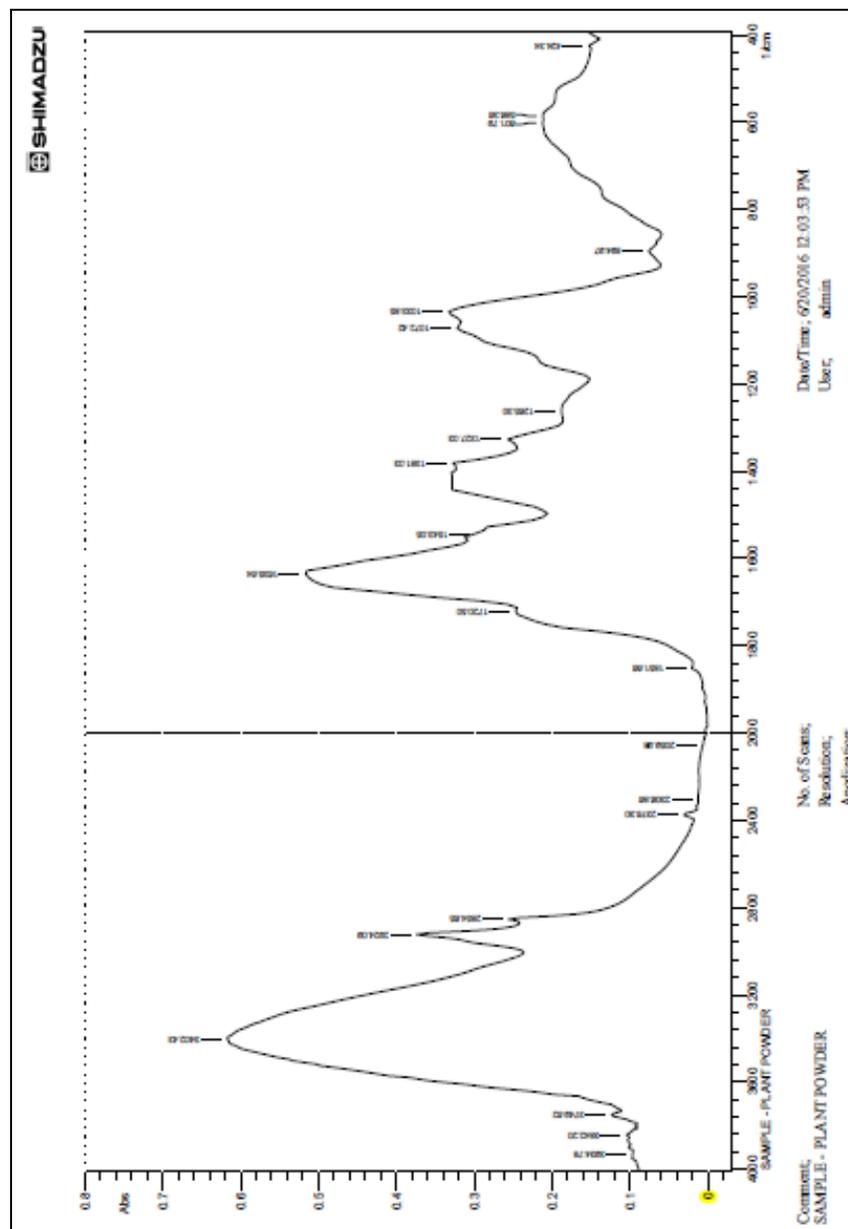
2.2 Extraction and Testing

The *Andrographis paniculata* leaf dried powder were subjected to IR spectroscopy which showed the presence of functional groups. The functional groups were identified by the transverse frequencies of infra-red waves in wave number cm^{-1} .

The transverse frequencies of each of the functional groups varied from one another. The identified functional groups are showed in the table.

Table 1: Fourier- Transform Infrared Spectroscopy (FTIR) analysis for *Andrographis paniculata* leaf powder

S. No	Functional Group	Absorption Frequency
1.	Phenol	3749.62
2.	Amines	3402.43
3.	Nitriles	2942.09
4.	Alkanes	2854.65
5.	Alkynes	2376.86
6.	Esters	1851.66
7.	Carbonyls	1720.50
8.	Alkenes	1635.64
9.	Nitrocompounds	1543.05
10.	Alcohols, carboxylic acids, esters and ethers	1327.03
11.	Aliphatic amines	1072.42
12.	Alkenes	1033.85
13.	Aromatic amines	894.97
14.	Alkyl halides	424.3

**Graph 1:** FTIR of leaf powder of *Andrographis paniculata*

2.3 Gas Chromatography and Mass spectrometry (GC-MS) Studies

The soxhlet extracts of *Andrographis paniculata* with solvents such as chloroform, ethyl acetate, ethanol and

methanol were subjected to GC-MS studies. After comparison of all the four solvents were compared and a final table of compounds were made as shown below:

Table 2: Major Common Compounds in Different Solvent Extracts of *Andrographis Paniculata* Leaf Sheath

S.NO	COMPOUND	Chloroform	Ethyl acetate	Ethanol	Methanol
1	3,7,11,15-tetramethyl-2-hexadecen-1-ol	Present	Absent	Absent	Absent
2	Eicosanoic acid	Present	Absent	Present	Absent
3	1-hexyl-2-nitrocyclohexane	present	Absent	present	Absent
4	2,4,4-trimethyl-3-hydroxymethyl-5a-(3-methyl-but-2-enyl)-cyclohexen	Present	Absent	Absent	Absent
5	aromadendrene oxide-(2)	Present	Absent	Absent	Absent
6	dibenzof[a,h]cyclohexadecene, 2,3,11,12-tetraethenyl-1,2,3,4,5,6,7,8,9,10	Present	Absent	Absent	Absent
7	cis-5,8,11,14,17-eicosapentaenoic acid, tert-butyl dimethylsilyl ester	Present	Absent	Absent	Absent
8	3-butoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane	Present	Absent	Absent	Absent
9	carbamidithioic acid, 1-methylethyl ester	Absent	Present	Present	Present
10	pentadecanal	Present	Present	Present	Present
11	bicyclo[3.2.1]oct-3-en-2-one, 3,8-dihydroxy-1-methoxy-7-(7-methoxy-1,3-	Absent	Present	Present	Present
12	tetrahydropyrrole-3-ol-5-carboxylic acid, 1-acetyl-, methyl ester	Absent	Absent	Present	Absent
13	oleic acid	Absent	Absent	Present	Absent
14	1-hexyl-2-nitrocyclohexane	Present	Absent	Present	Absent
15	carbamic acid, 3-(1-propylbutylidene)-, ethyl ester	Absent	Absent	Present	Present
16	propanoic acid, 2-(aminoxy)-	Absent	Present	Present	Present
17	1,3-bis-t-butylperoxy-phthalan	Absent	Absent	Absent	Present
18	14-heptadecenal	Absent	Absent	Absent	Present

3. Results and Discussion

3.1 Fourier-Transform infrared spectroscopy (FTIR)

The FTIR spectrum confirms the presence of maximum absorbance (λ_{max}) of the active compound was found to be 224 nm. From the FTIR graph the compound was found to possess an asymmetric $-CH$ stretch of $-CH_3$, an asymmetric $-CH$, an asymmetric $=CH_2$ stretch, a free OH group attached to the lactone ring demonstrating a peak, $C=C$ bond of the diterpene ring demonstrating a peak, a $O-C=O$ group in the lactone ring, when compared with $C-O$ of the diterpene ring.

3.2 Gas Chromatography and Mass spectrometry (GC-MS) Studies

GC-MS analysis of different solvent extracts that is Chloroform, Ethyl acetate, Ethanol and Methanol of *Andrographis paniculata* leaf sheath showed the presence of various compounds. A comparative study was conducted in order to know the compounds in each solvent extracts. A total of compounds were characterized in chloroform, ethyl acetate, ethanol and methanol extracts respectively.

In the present study, Pentadecanal was the compound present in all the four extracts as compared to the other compounds present in the extracts. Bicyclo[3.2.1]oct-3-en-2-one, 3,8-dihydroxy-1-methoxy-7- compound is present in all the three extracts such as ethylacetate, ethanol and methanol. Propionic acid, 2-(aminoxy)- compound was also present in all the three extracts except chloroform extract. More number of compounds were found in ethanol as when compared to other solvent extracts. (Eddy *et al.*, 2011).

More than nineteen compounds were identified in the ethanolic extract. The prevailing compounds were 2,5-Octadecadienoic acid, methyl ester (27.83%), 1,2,3,5-Cyclohexanetetrol, 10-12-Pentacosadienoic acid, 2H-Pyran, 2-(7-heptadecyloxy) tetrahydro-9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyloxy)-1-[[[(trimethylsilyloxy)methyl]ethyl ester, 1,3-Propanediol, 2-(hydroxymethyl)- 2-nitro-3-O-Methyl-d-glucose, Phytol. (Kalaiselvan *et al.*, 2012).

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have supported me and encouraged me for carrying out my project work.

5. Summary

FTIR revealed better peak for the active compounds. The GC-MS analysis reported the presence of Pentadecanal in all the solvent extracts. Other active compounds were detected in each solvent extracts.

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