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Gas Chromatography Mass Spectrometry (GC-MS) Profiling of Arjunarishta

Research Article

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Abstract

Gas chromatography with mass spectrometric detectors are used to separate different analytes present in a mixture and identify the same. It can also be used for quantification of the analytes. Sample preparation is essential for GC-MS analysis to obtain desired analytes. Aim of the study: The present study is pursued to profile the phytochemicals present in *Arjunarishta*, a medicated alcoholic preparation through Dichloromethane (DCM) and Ethyl acetate (EA). Materials and Methods: A sample obtained from a GMP-certified pharmacy was subjected to liquid-liquid extraction using DCM and EA, which was examined with GC-MS. Results: The analysis yielded 19 and 20 phytochemicals in DCM and EA respectively. Presumably, due to a small difference in the polarity of the solvents used, nine phytochemicals such as Ethyl gallate, Catechols etc., were found in both extracts. Conclusion: 30 unique phytochemicals were established in two different solvent extracts of the same sample.

Key Words: GC-MS, *Arjunarishta, Parthadyarishta,* Dichloromethane, Ethyl acetate.

Introduction

Gas chromatography - Mass spectrometry is a technique ideal for metabolomic profiling of vaporized single or a blend of various plant samples(1). A wide range of phytochemicals can be sought with a single run of the sample based on its nature. The obtained metabolites are identified through mass spectrometry based on their mass to charge ratio(1). It can be utilized to identify various compounds such as amino acids, sterols, sugars, catecholamines etc.(2)

Arjunarishta, also known by the name *Parthadyarishta*, is a polyherbal self-generated alcoholic preparation commonly prescribed for cardiac disorders(3). Alcohol present in the *asavārishta* (medicinal alcohols) acts as a natural preservative for the formulation(4,5).

The study is aimed at determining the different metabolites present in Arjunarista through two different solvents, namely- Dichloromethane (DCM) and Ethyl acetate (EA) through Gas chromatography-Mass spectrometry.

Materials And Methods (Experimental)

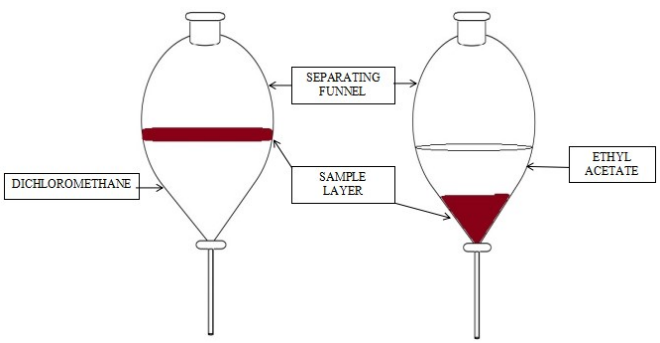
Arjunarishta was acquired from an *Ayurvedic* Pharmacy certified by GMP standards. A quantity of 10 g of the sample was combined with 50 ml of DCM in a conical flask and subjected to agitation using an orbital flask shaker at a temperature of 21°C and 60 rpm for four hours. Similar procedure was carried out with EA.

Afterwards, the mixture was permitted to settle in separating funnels, and the solvent layer was collected. The solvent layer was evaporated and sent to Sophisticated Analytical Instrument Facility (SAIF) of IIT-Madras, Chennai for GC-MS analysis.

Table 1: Ingredients of Arjunarishta with their proportions

Sl.no	Ingredients	Latin/English name	Part used	Qty
1	*Arjuna*	*Terminalia arjuna* Roxb.	Stem bark	4.800 kg
2	*Mridvika*	*Vitis vinifera* Linn.	Dried fruit	2.400 kg
3	*Madhuka*	*Madhuca indica* J.F. Gmel	Flower	0.960 kg
4	*Jala*	Water	-	49.152 L
5	*Dhataki*	*Woodfordia fruticosa* (L.) Kurz	Flower	0.960 kg
6	*Guda*	Jaggery	-	4.800 kg

Figure 1: Pictorial representation of *Arjunarishta* in DCM and EA settled in separating funnel



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GC-MS was carried out with the help of Agilent 8890 with Single quadrupole Agilent 5977 MSD with column of dimensions 30m X 250 µm X 0.25 µm. One microliter of sample was injected through the 250°C injection port with the split ratio of 15:1. The column oven temperature program is given in table 2. The ionizing energy of the EI was 70 eV. The mass analyzer temperature was noted to be 150 ^{0}C. The peaks obtained were referred to the NIST17 Library.

Table 2: Column oven temperature program

	Rate	Value	Hold time
Initial temperature		75^{0}C	0.5 min
Boost	5^{o}C/min	180^{0}C	3.0 min
Boost	5^{o}C/min	300^{0}C	5.0 min

Results

The obtained peaks are represented with their compounds in tables 3 and 4.

Table 3: Phytochemicals present in DCM extracted *Arjunarishta*

Sl.No	Time	Compound	Area %
1	5.392	2-Furancarboxylic acid	1.23
2	7.903	Ethyl hydrogen succinate	18.01
3	8.362	Catechol	0.97
4	9.231	5-Hydroxymethylfurfural	51.23
5	9.239	5-Hydroxymethylfurfural	8.86
6	10.215	1,5-Anhydro-2-O-acetyl-3,4,6-tri-O-methyl-d-mannitol	0.63
7	12.210	(+)-Diethyl L-tartrate	0.78
8	13.907	Benzeneethanol, 4-hydroxy-	1.92
9	14.318	L-Pyroglutamic acid	1.98
10	15.308	1,1-Diethoxynon-2-yne	2.24
11	16.217	1H-Pyrazole-4-carboxylic acid, 1-methyl-	1.37
12	16.310	Ethylparaben	0.78
13	16.532	Homovanillyl alcohol	1.22
14	17.171	3-Hydroxy-4-methoxybenzoic acid	0.98
15	18.494	2-Butyl-3-methylcyclopent-2-en-1-one	1.25
16	19.509	Triethyl citrate	1.71
17	21.385	2-Amino-3-(4-hydroxyphenyl)-propanoic acid	0.60
18	22.780	Benzoic acid, 4-hydroxy-3,5-dimethoxy-	1.40
19	26.247	Ethyl gallate	2.26
20	37.793	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	0.59

Table 4: Phytochemicals present in EA extracted *Arjunarishta*

Sl.No	Time	Compound	Area %
1	4.034	Glycerin	5.43
2	6.791	Methylmalonic acid	1.91
3	7.772	Ethyl hydrogen succinate	8.18
4	8.346	Catechol	0.94
5	9.152	5-Hydroxymethylfurfural	26.59
6	9.828	Lactic acid	2.36
7	11.751	1-Deoxy-d-mannitol	8.08
8	12.682	1,2,3-Benzenetriol	20.26
9	13.920	Benzeneethanol, 4-hydroxy-	1.00
10	14.368	2-Cyclohexylpiperidine	0.85
11	15.303	2-Nonyl-1-ol, diethyl acetal	1.05
12	15.911	Benzoic acid, 4-hydroxy-	2.26
13	16.231	1H-Pyrazole-4-carboxylic acid, 1-methyl-	4.15
14	17.156	3-Hydroxy-4-methoxybenzoic acid	1.00
15	18.539	2-Butyl-3-methylcyclopent-2-en-1-one	1.01
16	19.691	12,15-Octadecadiynoic acid, methyl ester	1.62
17	19.784	1-Isobutyl-7,7-dimethyl-octahydro-isobenzofuran-3a-ol	3.07
18	20.395	1-Isobutyl-7,7-dimethyl-octahydro-isobenzofuran-3a-ol	1.51
19	21.973	N-Acetyltyramine	1.46
20	22.774	Benzoic acid, 4-hydroxy-3,5-dimethoxy-	1.02
21	26.279	Ethyl gallate	6.25

Figure 2: Peaks obtained in DCM extracted *Arjunarishta*

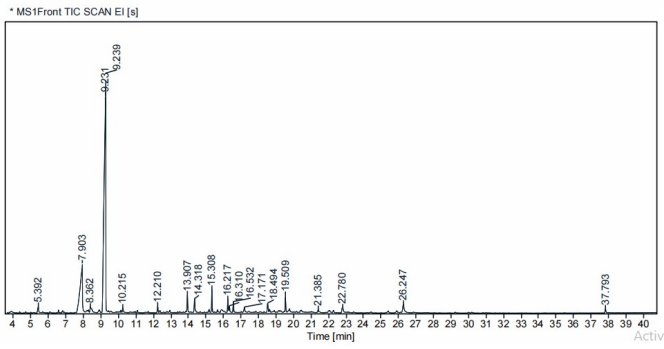
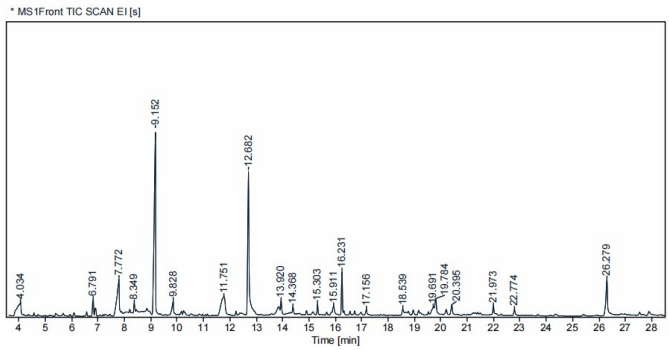


Figure 3: Peaks obtained in EA extracted *Arjunarishta*



Discussion

Selection of Solvents

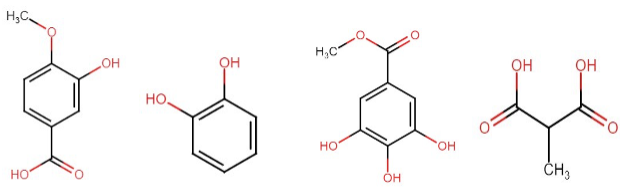
The hydrophilic compounds are extracted using ethyl acetate (EA), whereas the lipophilic components are extracted using dichloromethane (DCM).(6) The polarity of the solvent employed significantly affects the nature of the constituents extracted. When a polar solvent is used, a greater quantity of polar constituents is extracted, while a non-polar solvent results in the extraction of compounds with similar characteristics.(7)

Phytochemicals (15)

Arjunarishta was found to contain a range of acids, esters, phenols, and alcohols. The majority of the identified compounds, such as pyroglutamic acid(8), 4-hydroxy benzeneethanol(9), and ethyl hydrogen succinate(10), also being present in grape wines.

To better understand the compounds present in *Arjunarishta*, it's important to consider the fermentation process that it undergoes. During fermentation, certain compounds undergo biotransformation, including catechin and gallic acid(11), which are converted into catechol and ethyl gallate, respectively. Additionally, the presence of 4-hydroxybenzoic acid in grapes(12) leads to the production of ethyl paraben, an antifungal agent, when it undergoes condensation with ethanol(13). Malic acid, a common component of grapes, is transformed during malolactic fermentation to produce mannitol, a sugar alcohol and lactic acid(14). Furthermore, as a byproduct of the fermentation process, glycerin, a tasteless and odourless liquid, is produced, which aids in the balance of osmotic pressure in the yeast.

Figure 4: Structure of (a) 3-Hydroxy-4-methoxybenzoic acid, (b) Catechol, (c) Ethyl gallate and (d) Methylmalonic acid



Conclusion

Thirty distinct phytochemicals, including alcohols, phenols, and others, were identified through GC-MS analysis, with nine of these phytochemicals overlapping. A more complete picture of *Arjunarishta*'s medicinal properties can be better understood with the help of these findings, which could aid in further research and comprehension of its benefits.

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Conflict of Interest: None

Sources of Funding: None

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