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Gas chromatographic mass spectroscopic evaluation of *Punarnavadi Kwatha*

Resmi Vijayan [1] , P. Y. Ansary [2] , Sara Moncy Oommen [3] , V. V. Shincymol [4]

[1]PG Scholar, Department of Dravyaguna Vijnanam, GAVC, Tripunithura, Kerala
[2]HOD and Professor, Department of Dravyaguna Vijnanam, GAVC, Tripunithura
[3]Professor and HOD, Department of Dravyaguna Vijnana, Government Ayurveda College, Kannur
[4]Associate Professor, Department of Dravyaguna Vijnanam, GAVC, Tripunithura

*Email: resmivijaybams@gmail.com

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Abstract

Punarnavadi kwatha is a most commonly used polyherbal formulation in Ayurvedic medical practice. It is also called as *Punarnavashtaka kwatham*. For the purpose of quality assurance and uniformity, scientific verification of Ayurvedic medications is important. Usage of modern analytical techniques helps in the proper authentication of medicines. Gas chromatographic mass spectroscopic evaluation is one among the modern analytical tool to assess the bio molecules in the formulation. *Punarnavadi kwatha* was prepared according to the classical method and Gas Chromatographic Mass Spectroscopic evaluation was done using DB5MS column. 27 compounds were identified in the formulation. The compound 1,2,3-Benzenetriol was identified with the maximum percentage area 17.189%. On comparing the phytoconstituents of individual ingredient drugs, showed the presence of chemical compounds from ingredients. From the analysis the specific compounds from ingredients can be used as a marker compound so that we can analyse the presence of all ingredients in the polyherbal formulation. So, gas chromatographic mass spectroscopic evaluation can be used as a tool in quality control and standardisation.

Introduction

Punarnavadi kwatha is a most commonly used polyherbal formulation in Ayurvedic medical practice. The ingredients of this polyherbal formulation as per Ayurvedic Formulary of India are *Punarnava* (*Boerhaavia diffusa* Linn.), *Nimba* (*Azadirachta indica* A. Juss), *Patola* (*Trichosanthes dioica* Roxb.) *Sundi* (*Zingiber officinale* Rosc.), *Tiktha* (*Picrorhiza kurroa* auct Non-Royle.), *Amrita* (*Tinospora cordifolia* (Wild). Miers ex Hook. f & Thoms) *Daru*, (*Cedrus deodara* Roxb.) and *Abhaya* (*Terminalia chebula* Retz.).[1] Main indications of *Punarnavadi kwatha* are *Sarvanga sopha* (generalised oedema), *Udara* (ascites), *Kasa* (cough), *Soolam* (colicky pain), *Swasa* (dyspnoea) associated with *Pandu* (anaemia).[2]

In a poly herbal formulation, pharmacological actions are mainly contributed by the combination of ingredient drugs. The scientific validation of Ayurvedic medicines is a need of hour for the quality control and standardization. Usage of modern analytical techniques helps in the proper authentication of medicines. Gas chromatographic mass spectroscopic evaluation is one among the modern analytical tool to assess the bio molecules in the formulation. In GCMS evaluation, compounds within a specific boiling point range can be detected and identified. In gas chromatography mass spectroscopy, the

compounds are separated based on the boiling point. The present study deals with the comparison of phytoconstituents present in the finished formulation with that of individual ingredients and how this method can be used in the standardization technique of poly herbal formulations.

Materials and methods

a. Preparation of *Punarnavadi kwatha*

i. Collection of raw drugs

Root of *Punarnava* (*Boerhaavia diffusa* Linn.), bark of *Nimba* (*Azadirachta indica* A. Juss) and stem of *Amritha* (*Tinospora cordifolia* (Wild). Miers ex Hook. f & Thoms) was collected from the natural habitat of Kuruppankulangara, Cherthala in the month of June, March and August respectively. Rhizome of *Sundi* (*Zingiber officinale* Rosc.) was collected from cultivation fields of Murikkassery, Idukki in the month of December. Whole plant of *Patola* (*Trichosanthes dioica* Roxb.), rhizome of *Tiktha* (*Picrorhiza kurrooa* auct non-Royle), heartwood of *Daru* (*Cedrus deodara* Roxb.), fruit rind of *Abhaya* (*Terminalia chebula* Retz.) were supplied by Ambuja Institute of Ayurvedic Research and Documentation, Udayamperoor Ernakulam. The collected plants and raw drugs of *Punarnavadi kwatha* was identified by the faculty in the Department of Dravyaguna vijnanam, Government Ayurveda College, Tripunithura. Pharmacognostical identification of all the drugs was done in Pharmacognosy lab, Department of Dravyagunavijnana Government Ayurveda College, Tripunithura.

ii. Preparation of *kwatha*

The ingredients above mentioned are crushed individually using the instrument disintegrator. *Kwatha* (decoction) was prepared according to classical method described by Sarngadhara Samhita. 48gms of crushed raw drugs of ingredients were taken in an earthen vessel. 16 times of water (768ml) was added and boiled on gas stove with low flame. It was then reduced to 1/8th quantity (96ml). The prepared *Kwatha* (decoction) was filtered through the 3 layered muslin cloth in to a glass jar and allowed to cool.

b. Preparation of Sample for GCMS evaluation

10ml of sample was taken and evaporated to dryness using a water bath at 80^{0}C. Residue from evaporated sample was reconstituted in 10 ml of methanol continuously stirring with a glass rod of 20 minutes, filtering is done through a syringe filter (Nylon 13 mm 0.2um) into vials and the filtered clear solution was used for GCMS analysis. Instrument Model used was 7890 A GC with 5975C with triple axis detector. Column used in GCMS machine is DB 5MS with dimension 30 m x 0.250mm diameter x 0.25 µm thickness, DB-5MS is a gas chromatography (GC) Column with non-polar phenyl arylene polymer, equivalent to (5%-phenyl)-methyl polysiloxane

c. Procedure

1 µl of the sample was injected to injection port of GC machine. The oven program that has selected has an initial temperature of 50^{0}C for 10 minutes, which then increased to 100^{0}C at a rate of 10^{0}C per minute, then increased to 150^{0}C finally the temperature is maintained for 280^{0}C for 15 minutes Analysis was done by injecting 1 µl of the sample with a split ratio of 5:1. The gas used as carrier was Helium gas (99.9995%) at a flow rate of 1 mL/min . EI (electron impact) mode was used for analysis with 70 eV of ionization energy. The injector temperature was maintained at 280°C. The sample in liquid state is converted in to gaseous state and converted in to ions. These ions are having specific mass to charge ratio. These ions are detected by the photon multiplier detector. Identification of compounds were done after comparing the obtained spectral configurations with that of available mass spectral database (National Institute of Standards and Technology -08 spectral data)

Results

Gas Chromatographic Mass Spectroscopic analysis of *Punarnavadi Kwatha* identified 27 compounds. The compound 1,2,3-Benzenetriol was identified with the maximum percentage area 17.189%. Decanoic acid, 1,2,3-propanetriyl ester with the percentage area 10.997%, Trans-Cinnamic acid with the percentage area 10.069%.3-Hydroxy-4-methoxybenzoic acid with the percentage area 8.088%, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl with the percentage area 6.284%, Ethanone, 1-(2-hydroxy-5-methylphenyl)- with the percentage area 3.279%, 2-Methoxy-4 -vinyl phenol with the percentage area 3.141%,2-Furancarboxaldehyde, 5-(hydroxymethyl)- with percentage area 2.439% , Benzofuran,2,3-dihydro with percentage area Tetra decanoic acid with percentage area 1.267%, furfural with percentage area 1.328% were the major identified compounds.

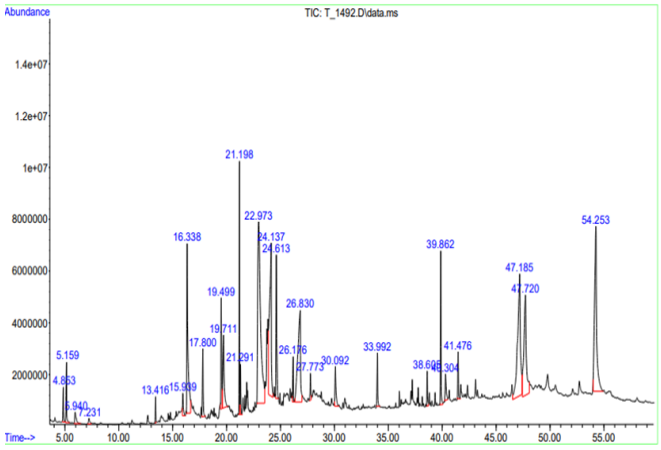

Figure No.1.GCMS Chromatogram of *Punarnavadi Kwatha*

Table No:1 GCMS Evaluation of *Punarnavadi Kwatha*

Sl. No.	Retention time	Name of compounds	Molecular formula	Percentage Area
1	4.853	2,3-Butanediol	C4H10O2	0.572%
2	5.159	Furfural	C5H4O2	1.328%
3	5.940	2-Furanmethanol	C5H4O2	0.555%
4	7.231	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	C5H6O2	0.261%
5	13.416	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	C6H8O4	0.384%
6	15.939	Phenol, 2-methoxy	C6H8O3	0.568%
7	16.338	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	C7H8O2	6.284%
8	17.800	Benzofuran,2,3-dihydro	C6H8O4	1.580%
9	19.499	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	C8H8O	2.439%
10	19.711	2-Methoxy-4-vinyl phenol	C6H6O3	3.141%
11	21.198	Ethanone, 1-(2-hydroxy-5-methylphenyl)-	C9H10O2	3.279%
12	21.291	Cyclohexane methanol, 4-hydroxy-α, α,4-trimethyl	C10H20O2	0.743%
13	22.973	1,2,3-Benzenetriol	C6H6O3	17.189%
14	24.137	Trans-Cinnamic acid	C9H8O2	10.069%
15	24.613	Ethanone, 1-(4-hydroxy-3-methoxyphenyl)	C9H10O3	3.592%
16	26.176	Dodecanoic acid	C12H24O2	0.874%
17	26.830	3-Hydroxy-4-methoxybenzoic acid	C8H8O4	8.088%
18	27.773	Butan-2-one, 4-(3-hydroxy-2-methoxyphenyl)-	C11H14O3	0.741%
19	30.092	Tetra decanoic acid	C14H28O2	1.267%
20	33.922	n-Hexadecanoic acid	C16H32O2	1.054%
21	38.605	Gingerol	C17H26O4	0.677%
22	39.862	4'-Methoxy-2-hydroxystilbene	C15H14O2	2.698%
23	40.304	Acridin-9-amine, 1,2,3,4-tetrahydro-5,8-dimethyl	C15H18N2	0.902%
24	41.476	Gingerol	C17H26O4	0.933%
25	47.185	Decanoic acid, 1,2,3-propanetriyl ester	C33H62O6	10.997%
26	47.720	Dodecanoic acid, 1,2,3-propanetriyl ester	C39H74O6	8.306%
27	54.253	(-)-Nortrachelogenin	C20H22O7	11.467%

Discussions

Among the identified compounds in *Punarnavadi Kwatha*, as per the previous GCMS study of root of *Boerhaavia diffusa* Linn,[3] n-hexa decanoic acid and tetra decanoic acid are the compounds from the root of *Boerhaavia diffusa* Linn. Gingerol from the ingredient rhizome of *Zingiber officinale* Rosc. Benzofuran,2,3-dihydro is the compound from the ingredient *Trichosanthes dioica.* Roxb. as per the previous gcms study.[4].1.2,3 benzene triol is the compound from the ingredient of *Terminalia chebula* Retz as per the previous GCMS work.[5] 3-Hydroxy-4-methoxybenzoic acid from the ingredient *Picrorhiza kurroa* auct non-Royle**.** As per the previous gcms study the compound Tetradecanoic acid was present in stem of *Tinospora cordifolia* (Wild). Miers ex Hook. f & Thoms.[6] The gas chromatographic mass spectroscopic evaluation can be used as a tool for the evaluation of quality in the industrial preparation of polyherbal formulation for the detection of presence of all ingredients in the finished product.

Conclusion

Gas Chromatographic Mass Spectroscopic analysis of *Punarnavadi kwatha* identified 27 compounds. The compound 1,2,3-Benzenetriol was identified with the maximum percentage area. On comparing the phytoconstituents of individual ingredient drugs, showed the presence of chemical compounds from ingredients. From the analysis the specific compounds from ingredients can be used as a marker compound so that we can analyse the presence of all ingredients in the polyherbal formulation. So, gas chromatographic mass spectroscopic evaluation can be used in quality control and standardisation. Further research works are required for better understanding of biomolecules present in *Punarnavadi kwatha.*

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