Journal of Ayurvedic and Herbal Medicine 2019; 5(3): 103-105

Research Article

ISSN: 2454-5023
J. Ayu. Herb. Med.
2019; 5(3): 103-105

www.ayurvedjournal.com
Received: 24-05-2019
Accepted: 03-08-2019

Gas chromatography – Mass spectrometric analysis of *Haritakyadi* eye drops: A poly herbal compound for ophthalmia neonatorum

Srikantha K V[1], Chethan Kumar VK[2], Nagaratna S J[3], Sunil Kumar KN[4], Suchitra N Prabhul[5]

1 *PG Scholar, Department of PG Studies In Kaumarabhritya, Sri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, Kuthpady, Udupi, Karnataka, India*

2 *Professor & HOD, Department of Kaumarabhritya, Taranath Govt Ayurvedic Medical College, Ballari, Karnataka, India*

3 *Assistant Professor, Department of PG Studies In Kaumarabhritya, Sri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, Kuthpady, Udupi, Karnataka, India*

4 *Research Officer (Pharmacognosy), Siddha Central Research Institute, Central Council for Research in Siddha, Ministry of AYUSH-Govt. of India, Chennai, India*

5 *Research Officer, Pharmaceutical Chemistry and Pharmacognosy, SDM Centre for Research in Ayurveda and Allied Sciences, Udupi, Karnataka, India*

ABSTRACT

Haritakyadi eye drops are prepared by hydro-distillation (*Arka kalpana*) of ingredients like *Terminalia chebula* Retz. (*Haritaki*), *Emblica officinalis* Linn. (*Amalaki*), *Berberis aristata* Dc. (*Daruharidra*) and *Glycyrrhiza glabra* Linn. (*Yastimadhu*). The research was designed to explore the volatile constituents by Gas Chromatography-Mass Spectrometry (GC-MS). Secondary metabolite screening using GC-MS revealed the presence of fifteen constituents. Out of fifteen constituents 8 could not be identified and remaining 7 compound identified as 1,3,5,7-Cyclooctatetraene; Benzene, 1-ethyl-4-methyl; Benzene, 1,2,3-trymethyl; 1,3-Xylyl-15-crown-4, 2,3-pinanedioxyboryl; Silane,[[9-[[(dimethyl-2-propenylsily)oxy]methyl]6a,7,10,10a-tetrahydro-6,6-dimethyl-3-pentyl-6H-dibenzo[b,d]; Silane, dimethyl (3-phenylprop-2-enyloxy)hexyloxy; Eicosanoic acid and tetradecyl ester at different retention time and peak area percentage. The compounds were identified using NIST library, two of them were silane were artifacts. This article reveals that *Haritakyadi* eye drops can be used for pharmacological activities.

Keywords: *Haritakyadi* eye drops, *Arka Kalpana*, Gas Chromatography-Mass Spectrometry, NIST library.

INTRODUCTION

Health is a most important fundamental necessity of human life. Most of the cultures around the word uses their traditional healing practices. Both developed both developed and developing countries, traditional approaches and alternative remedies to overcome from that necessity and modern medicines exits side by side. About 80% of the population in the Asia and African countries use traditional healing practices to overcome their primary healthcare needs. Traditional system of medicine uses natural products which are derived from the medicinal plants having many important medicinal biological active compounds, which leads the development in the modern pharmaceuticals like development of new leads. Due to increased resistance of therapeutic agents for common microorganisms which led to renewed interest in the discovery of potent new lead compounds.[1] Most of the medicinal plant derivatives contains phenolics which acts as antioxidants has considerable importance due to their potential health benefits. The extraction of these antioxidants requires different complex procedure and techniquesform the medicinal plants due to uneven distribution in plant matrix.[2] For the conformation of the phytoconstituents suitable analytical tools are required. To determine theses phytochemical constituents requires largely performed by relatively expensive and hyphenated meta-analysis technique such as gas chromatography coupled mass spectrometry (GC-MS). GC-MS analysis can identify the pure compounds even at lower concentration in a polyherbal formulation.[3]

In *Ayuvereda* Acharya *Sarangadhara* in *Sarangadhara* Samhita mentioned the concept of polyherbalism to achieve greater therapeutic efficacy. Particular herbs if used in definite ratio it increases the potency of the drugs when compared to individual herbs, which gives better therapeutic effect and reduce the toxicity.[4] Henceforth, different parts of four medicinal plants were used to prepare a polyherbal formulation i.e. *Haritakyadi Eye drops* in the form of *Arka* (distillate). This research work was aimed to explore the bioactive constituents of *Haritakyadi Eye drops* using gas chromatography mass spectrometry.

***Corresponding author:**
Dr. Chethan Kumar V.K.
Professor & HOD, Department of Kaumarabhritya, Taranath Govt Ayurvedic Medical College, Ballari, Karnataka, India
Email: drchethankumar[at]gmail.com

MATERIALS AND METHODS

Plant materials

Terminalia chebula Retz. (*Haritaki - kernel*), *Emblica officinalis* Linn. (*Amalaki - fruit*), *Berberis aristate* Dc. (Daruharidra - stem) and *Glycyrrhiza glabra* Linn. (Yashtimadhu - root) were collected from Sri Dharmasthala Manjunatheshwara Pharmacy, Udupi, Karnataka, India.

Method of preparation

Haritakyadi eye drops were prepared by using different parts of medicinally important plant such as *Haritaki* (kernel), *Amalaki* (fruit), *Daruharidra* (stem) and *Yastimadhu* (root) each taken in equal quantity. The plant materials were identified morphologically on the basis of Ayurvedic Pharmacopoeia of India and quality standards for Indian Medicinal Plants. The above-mentioned plant materials were taken and made into coarse powder and soaked overnight in distilled water. Next morning, the soaked drugs were subjected for the distillation process. The vapours are condensed and collected in a reciever. In the beginning, the vapours consist of only steam and may not contain the essential principles of the drugs. It should therefore be discarded. The last portion also may not contain therapeutically essential substance and should be discarded. The final product was in the form of drops. This method of preparation of Arka is followed according to The Ayurvedic Formulary of India.[5] The prepared *Haritakyadi* eye drops were analysed for the active constituents of drug using Gas Chromatography and Mass Spectroscopy (GC-MS).

Mass spectrometric fingerprinting by GC-MS:

Gas chromatography mass spectrometry (GC-MS) of n-hexane soluble portion of Arka was performed using 7890 A, MS 5975 [Agilent]. The capillary column [HP-5Ms Ultra Inert (length: 30.0 m; diameter: 0.25 mm), with a film thickness of 0.25 mm] and carrier gas (helium) at a flow rate of 1.0 ml/min was used. 2ul sample with 36.445 cm/sec average velocity was utilized. The inlet temperature was maintained as 250°C. The oven temperature was programmed at 50°C for 1 min and then increase to 300°C at a rate of 10°C. Total run time was 35 min and there was solvent delay for 4 minutes. The mass transfer line was maintained at a temperature of 230°C. Mass spectrum was recorded using -70eV electron energy. Fragmented compounds were evaluated using total ion count for compound identification and quantification. The spectra of the unknown components were identified and compared using spectral database NIST-11 library.

RESULTS

Gas Chromatography Mass Spectrometry

Compound identification using (GC-MS) validated the presence of pharmacologically active compounds. Presence of phytoactive components in *Haritakyadi* eye drops with different peak area %, retention time, molecular formula and weight are detailed in Table 1. Identified compounds are 1,3,5,7-Cyclooctatetraene; Benzene, 1-ethyl-4-methyl; Benzene, 1,2,3-trymethyl; 1,3-Xylyl-15-crown-4, 2,3-pinanedioxyboryl; Silane,[[9-[[(dimethyl-2-propenylsily)oxy]methyl]6a,7,10,10a-tetrahydro-6,6-dimethyl-3-pentyl-6H-dibenzo[b,d]; Silane, dimethyl(3-phenylprop-2-enyloxy)hexyloxy; Eicosanoic acid, tetradecyl ester. Identified compounds were matched with NIST library for confirmation of compounds. The compounds were identified on the basis of mass charge ration (*m/z*) and retention time. Molecular formula, weight and nature of compound were identified on the basis literature available in databases like NIST, NCBI, PubChem and Google scholar. GLC chromatogram represented the peaks of fragmented compounds at different retention indices (Figure 1).

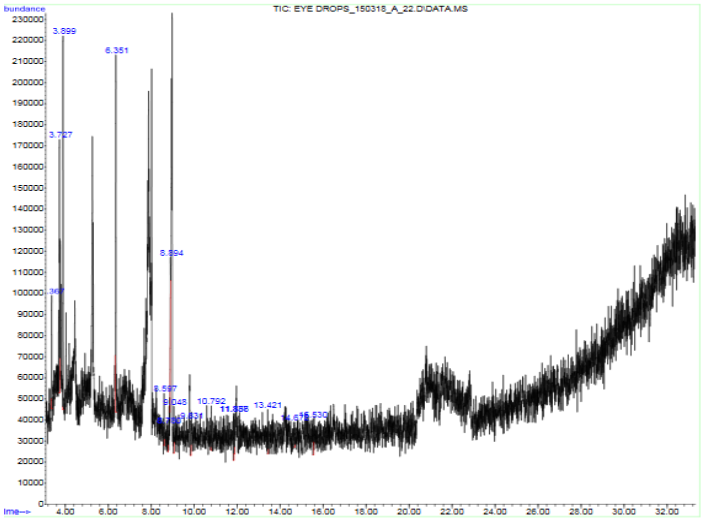

Figure 1: GLC of *Haritakyadi* eye drops

Fifteen low polar constituents were eluted by GCMS from the Haritakyadi eye drops n-hexane as solvents. Out of fifteen low polar constituents 8 constituents could not be identified because mass fragmentation showed low matching in NIST-11 library. A major compound eluted at RT 6.351 mins could not be identified though it accounted for 28.40%. Out of 7 identified constituents, 4 compounds such as Benzene, 1-ethyl-4-methyl(20.42%), Benzene, 1,2,3-trymethyl(18.43), 1,3,5,7-Cyclooctatetraene(5.57%),Silane,[[9-[[(dimethyl-2 propenylsily)oxy]methyl]6a,7,10,10a-tetrahydro-6,6-dimethyl-3-pentyl-6H-dibenzo[b,d](4.60) were major. The remaining 3 constituents were minor. Two identified silanes were artifacts.

DISCUSSION

1,2,3-trimethyl benzene which is Hemimellitene at RT 3.899 with pecentage area of 18.43 and 1-ethyl-4-methyl benzene at RT 3.727 with percentage area of 20.42 were the two benzene compounds acts as antiseptic, anti-oxidant and anti-microbial.[6] Hemimellitene known to cause eye irritation [7] might helped in lacrimation. Tetradecyl ester of eicosanoic acid which serves also as arachidonic acid pathway initiator possible inflammatory mediator but on the contrary *Terminalia chebula* and *Embelica officinalis* both found to contain vitamin C and gallic acid which serves as antioxidant will have lubricant and serves to protect corneal epithelium from ROS (reactive oxygen species) induced injury.[8] The arifacts of silane exhibits analgesic and anti-microbial activity[9]. GC-MS data shows majorly as unidentified and might be responsible for the protective effect in case of eye conditions. Thus, *Haritakyadi* eye drops helps in controlling the growth of micro-organisms and prevents the diseases of eye related to inflammation.

Table 1: GC-MS identification of constituents of Haritakyadi eye drops

Peak	Retention time (RT)	Area %	Name of the compound	Molecular formula	Molecular weight (g/mol)
1	3.367	5.57	1,3,5,7-Cyclooctatetraene	C_8H_8	104.1491
2	3.727	20.42	Benzene, 1-ethyl-4-methyl	C_9H_{12}	120.1916
3	3.899	18.43	Benzene, 1,2,3-trymethyl	C_9H_{12}	120.1916
4	6.351	28.40	Unidentified	-	-
5	8.597	1.89	Unidentified	-	-
6	8.780	1.44	1,3-Xylyl-15-crown-4, 2,3-pinanedioxyboryl	$C_{24}H_{35}BO_6$	430.348
7	8.894	4.60	Silane,[[9-[[(dimethyl-2-propenylsily)oxy]methyl]6a,7,10,10a-tetrahydro-6,6-dimethyl-3-pentyl-6H-dibenzo[b,d]	$C_{26}H_{40}O_2Si$	412.6801
8	9.048	2.46	Silane, dimethyl(3-phenylprop-2-enyloxy)hexyloxy	$C_{17}H_{28}O_2Si$	292.4885
9	9.831	3.26	Unidentified	-	-
10	10.792	2.36	Unidentified	-	-
11	11.837	3.08	Unidentified	-	-
12	11.866	1.87	Unidentified	-	-
13	13.421	1.98	Unidentified	-	-
14	14.672	1.57	Unidentified	-	-
15	15.530	2.69	Eicosanoic acid, tetradecyl ester	$C_{34}H_{68}O_2$	508.9025

CONCLUSIONS

In the present study result showed presence of various bioactive compounds which known to process antimicrobial, antioxidant and lacrimation effect on the eyes. Hence it can be conclude, *Haritakyadi* eye drops prevents the growth of microbes, due to antioxidant activity it lubricates the eye and finally lacrimation scrapes the eye. Thus, it prevents the eye from disease.

HOW TO CITE THIS ARTICLE
Srikantha KV, Chethan Kumar VK, Nagaratna SJ, Sunil Kumar KN, Suchitra N Prabhul. Gas chromatography – Mass spectrometric analysis of *Haritakyadi* eye drops: A poly herbal compound for ophthalmia neonatorum. J Ayu Herb Med 2019;5(3):103-105.

REFERENCES

1. Enzo A. Palombo. Traditional Medicinal Plant Extracts and Natural Products with Activity against Oral Bacteria: Potential Application in the Prevention and Treatment of Oral Diseases. Evidence-Based Complementary and Alternative Medicine, vol. 2011, Article ID 680354, 15 pages, 2011.
2. Sultana B, Anwar F, Ashraf M. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. Molecules. 2009;14(6):2167-80.
3. Sudhakar M, Balliah R. Phytochemical determination of a polyherbal extract using FTIR and GC-MS analysis. European Journal of Pharmaceutical and Medical Research. 2015; 2(7): 173-8.
4. Parasuraman S, Thing G S, Dhanaraj S A. Polyherbal formulation: concept of Ayurveda. Pharmacognosy Review. 2014; 8(16): 73–80.
5. Rathore S, VK Chethan Kumar, R Sharaschandra. Preparation of Amruta abheervadi drops: An ayurvedic formulation for neonatal jaundice. The Pharma Innovation 2018; 7(4): 323-26.
6. Salwa MEK, Bdalla EMA, Mohamed SA, Barajob AEA. Novel Compounds in Lyophilized Female Camel Urine: Journal of Infectious Diseases and Therapy. 2016:4(5): 1-5.
7. National Center for Biotechnology Information. PubChem Database. 1,2,3-Trimethylbenzene, CID=10686, https://pubchem.ncbi.nlm.nih.gov/compound/1_2_3-Trimethylbenzene (accessed on Oct. 24, 2019)
8. Shukla V, Vashistha M, Singh SN. Evaluation of antioxidant profile and activity of amalaki (*Emblica officinalis*), spirulina and wheat grass. Indian Journal of Clinical Biochemistry. 2009; 24(1): 70-5.
9. LeVier RR, Chandler ML, Wendel SR. The pharmacology of silanes and siloxanes. InBiochemistry of silicon and related problems 1978 (pp. 473-514). Springer, Boston, MA.