



## Identification of Active Biomolecules in Saraswatarishtam (An Ayurvedic Preparation) by GC-MS Analysis

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### ABSTRACT

Scientific validation of Ayurvedic, Siddha and other types of alternative medicines is very important to bring them to the main stream of medical practice. One step in this direction is to identify the active biomolecules and their roles as medicines. Bioactive molecules in standard ayurvedic medical formulations play a vital role in curing diseases. The present study deals with the GC MS analysis of one such ayurvedic nerve tonic, namely, Sarswatarishtam. It was observed that two important biomolecules, namely, eugenol and serine were predominantly present in Saraswatarishtam. These two biomolecules are well known for their neuroprotective and neuroplastic activities. This finding clearly is one step forward in the much needed scientific validation of ayurvedic medicines.

**Keywords:** Saraswatarishtam, Eugenol, Serine, Neuroprotective, Ayurvedic.

### INTRODUCTION

Rigveda, one among the four Vedas, was presumably written between 4500-1600 B.C. Ayurveda, the science of health and well being is a part of Rigveda. It deals with specific properties of drugs and various aspects of science of life and the art of healing.<sup>1</sup> Ayurveda, and other forms of complementary and alternative medicinal practices like Siddha, Unnani, etc, are gaining importance and many herbal drugs are clinically tested and accepted for manufacturing.<sup>2</sup> Ayurvedic formulations are prepared by traditional processing methods which involve the use of several herbs and minerals. They are available in different forms such as decoction, fresh juice, oil, vati, powder, clarified butter preparation and alcoholic preparation. Among the 7,000 species of medicinal plants recognized all over the world, about 1500 plants are systematically used in indigenous system of medicine, like Ayurveda, Unani and Siddha.

They can be used to provide synergistic therapeutic effect and help to minimize the adverse effect of major drugs (Bhattacharya, 1994) and are validated by doing biological screening programs.

The use of modern drugs is beset with safety concerns.<sup>3</sup> It was estimated that 2.22 million hospitalized patients had serious Adverse Drug Reactions (ADR) and 106,000 died in a single year in the USA. The herbal and natural products used as folk medicine have lesser incidences of reactions as compared to modern conventional pharmaceuticals. Coupled with their reduced cost and easy availability, the use of herbal medicines is

encouraging for both the consuming public and national health care institutions.<sup>4</sup>

With the increasing acceptance of herbal medicine as an alternative form of health care, the screening of medicinal plants for active compounds has become very interesting to understand the novel mechanism of action.<sup>5-7</sup>

The present study deals with the analysis of one such multi ingredient plant based herbo-mineral formulation, Saraswatarishtam. Saraswatarishtam consists of many plants and also some metals like gold. It is also used to treat acute anxiety, fatigue, insomnia, partial loss of memory, low grasping power, slurred speech and dementia and for certain neuro degenerative disease like Alzhemier disease. Some of the basic ingredient plants like Aswangandha, Brahmi, and Shatavari are known as medhayarasayana which means "chemicals pertaining to brain". Medhyarasayanans are used to improve memory and cognitive deficits.<sup>8-9</sup>

The present study envisages the following goals:

- a. Finding out the active biomolecules present in ayurvedic formulation, namely, Saraswatarishtam, a drug used as nerve tonic.
- b. Correlating the biological and medicinal functions of such biomolecules and
- c. pointing towards establishing the medicinal efficacy of the drug.

### MATERIALS AND METHODS

**A.** Saraswatarishtam was procured from standard ayurvedic medicine vendors at Chennai. This consisted of the following ingredients:



1. *Bacopa Moniera* (Bramhi)
2. *Zinziber Offininalis* (Adraka, Ginger),
3. *Anethum Sowa* (Anne seed, Sounf)
4. *Operculina ipomoea* (Harenuka, Trivrita)
5. *Piper Longum* (Pippali)
6. *Syzygium aromaticum* (Lavanga)
7. *Acorus calamus* (Vekhand)
8. *Saussurea Luppa* (Kushtham)
9. *Withania somnifera* (Aswagandha)
10. *Terminalia chebula* (Hirda)
11. *Tinospora cordifolia* (Guduchi)
12. *Elettania cardmomum* (Cardamom, Elaichi)
13. *Terminalica embelica* (vidanga)
14. *Cinamomum zelonica* (Dalchini)
15. *Asparagus racemoses* (Sathavari)
16. *Pueraria tuberosa* (Vidarikand)
17. *Terminalia belerica* (Behada) and
18. Pure Metallic gold.

This preparation was done as per the Ayurvedic treatise "Atha Rasayanaprakaranam".

## B. GC MS analysis of the drug

### Procedure

The GC-MS analyses for the sample was carried out in a Shimadzu GC-MS\_P 2010 gas chromatograph fitted with DBI (methylphenylsiloxane, 30m\*0.25 mm i.d) capillary column. Carrier gas, helium with a flow rate of

1ml/min initial temperature 50c ramp 10 c/min to 150c, hold 10min ramp 10c/min to 280c hold 10min injector temperature 200c volume detector temperature 290c volume injected 2 L: split ratio 25:1. The MS operating parameters were as follows: ionization potential 70 eV ion source temperature 180c solvent delay 3.00mm scan speed amu/s and scan range 30-600 amu eV voltage volts.

The concentrated extract form of the drug is injected into the GC MS instrument (Hewlett Packard 5890 GC/MS with mass selective detector with turbo mass gold perkin elmer. With increasing the temperature the sample is volatilized at the injection port and eluted through a capillary column. When the sample moves through the column, due to their affinity for the stationary phase, the various components are separated and can be identified by their retention time. The retention time is defined as time taken for the compound to pass through the column in the gas chromatograph system. Each chemical compound in the sample has a specific retention time measured in minutes which are shown as peaks in a graph and measures abundance on the ordinate against retention time on the abscissa and the peak is correlated to the concentration of the chemical compound.

The computer search of the mass spectra which corresponds to the peaks for the sample should yield a statistical match for nicotine at a 12.9 min retention time value and there is a scan mode which looks at all the constituents of a sample, listing whatever chemical components are present.

## C. Compound Identification

The molecules of the ethnolic extracts of the drug were identified by comparing their mass spectra and retention indices with those published in the literature and contained in the NIST 2005 MS computer library (Wiley).

## RESULTS AND DISCUSSION

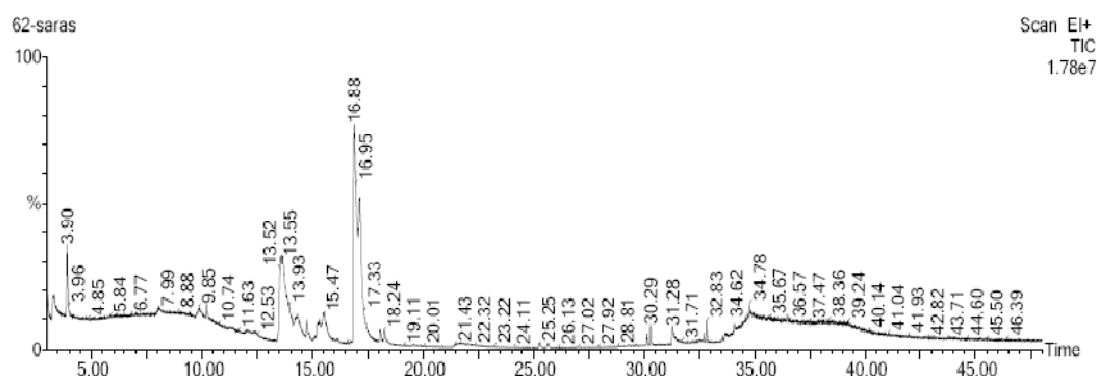


Figure 1: Chromatogram of Saraswatarishtam, ethanolic extract.

Table 1: Indicating the results of GCMS analysis of Saraswatarishtam.

No	Retention Time	Name of the compound	Molecular formula	MW
1.	16.88/17.8	Eugenol	C <sub>10</sub> H <sub>12</sub> O <sub>2</sub>	164
2.	3.90	Serine	C <sub>3</sub> H <sub>7</sub> NO <sub>3</sub>	105

GC/MS chromatogram of Saraswatarishtam extract gives three prominent peaks with retention time 16.88 and 17.8 suggesting the presence of Eugenol and Serine (Figure 1). In the books published by Wiley HPLC methods for recently approved pharmaceuticals synthetic Eugenol has RT 18.8 which closely matches with natural analog seen in Sarawatarishtam (Table 1).

The role of Ayurvedic and Sidha medicines in curing diseases from common cold through cancer is a time tested fact. But the advent of allopathic treatment and its far reaching technological advancements has left the traditional medicines in the back bench. The basic reason for this are, to mention a few;

- a. The lack of knowledge of the chemical composition and mechanism of action of the ayurvedic drugs at the molecular level;
- b. The lack of knowledge of side effects of such medicines
- c. the lack of dosage specifications,
- d. the long term and short term benefits and/or disadvantages,
- e. lack of standardized manufacturing methodology
- f. lack of standardized packing, sealing and transport methods
- g. lack of information of contraindications etc.
- h. some ayurvedic and sidha drugs contain heavy metals like, gold, silver, copper, zinc etc. which are considered to be harmful for health. This fact is a cause of major concern as to what is the role of these metals as medicines, what the role of their assimilation is and how much is excreted and at which time, what is the retention time inside the body, what is the chance of its bioaccumulation and consequent health hazards thereof.
- i. lack of proper statistical (clinical) data about the efficacy of the medicines as well as the side effects and harmful effects (both short term and long term).

All the above facts must be addressed to bring the alternative therapies into the main stream of medical practice.

A number of workers in complementary and alternative medicine have done fairly substantial work to prove the validity, efficacy and safety of such medicines although a lot more is needed to be done in this regard.<sup>10-19</sup>

In the present study two major compounds were identified from Saraswatarishtam, namely Eugenol and Serine. Both these bimoelcules are present in a variety of plants and they are also produced synthetically.

Eugenol – methoxy phenol with a short hydrocarbon chain is the major component of Saraswatarishtam. Synthetic Eugenol has been reported to have many important medicinal properties as is described by many reporters. It is an antifungal agent particularly against *Candida albicans*.<sup>20,21</sup> Eugenol is a powerful fat soluble

antioxidant and maintains the activities of the body antioxidant enzymes.<sup>22</sup> Pharmacologically eugenol has been reported as anticonvulsant and local anaesthetic, antistress and bacteriostatic and bactericidal.<sup>23,45,25</sup> Rompelberg 1996, have demonstrated the effect of eugenol on the genotoxicity of established mutagens in liver.<sup>26</sup> Anticarcinogenic potential of Eugenol was reported by Zheng.<sup>27</sup> It also depresses the activity of central nervous and neuro muscular function.<sup>28</sup> It also prevents radiation induced chemical oxidative damage in cell membranes and modify the membrane associated signaling process after radiation exposure.<sup>29</sup>

Eugenol being a major components in saraswatarishtam, thus contributes in the management of neuro degenerative diseases. The plants like cloves, cinnamon and cardamom present in Saraswatarishtam are known to contain eugenol in them.

Due to its nonmutagenic and noncarcinogenic properties eugenol is considered as safe by FAO with an acceptable daily intake of up to 2.5mg/kg body weight. (FAO report, 1982)<sup>30</sup> Eugenol is hepatotoxic and overdose cause convulsions, diarrhea, nausea, unconsciousness, dizziness, rapid heartbeat and it also cause allergic reactions. The amounts of eugenol present in Saraswatarishtam is well within the dose limit and thus very safe and effective.

Another neuroprotective agent seen in Saraswatarishtam is serine, an amino acid, which have vasodilation action which contributes to the neuroprotective effect and also increase the cerebral blood flow of the ischemic brain and reduce the neurological deficit, infarct volume and cortical neuronal loss.<sup>31</sup> The herbs of Saraswatarishtam rich in serine are *Zinziber* and *Piper longum*.

D-Serine has been reported to have a cardinal modulatory role in major N-methyl-D-Aspartate Receptor (NMDAR) dependent processes including NMDAR neurotransmission, Neurotoxicity, synaptic plasticity and cell migration. Abnormalities of NMDAR neurotransmission may cause neurotoxicity leading to behavioral and cognitive dysfunction. The hyperactivity of NMDAR can cause cell death mediated by excitotoxic calcium overload in stroke and neurodegenerative disorders such as Alzheimer's disease. D-serine modulation appears to play a critical role in achievement of balancing NMDAR activity and may be involved in rectifying the patho-physiology of neuropsychiatric disorders.<sup>32</sup>

## CONCLUSION

The acceptance of herbal formulations is beset with criticism that these medicines are prepared by age old practices and there is not much scientific background about the mechanism of action of such medicines.

The present study which involves the scientific analysis of Saraswatarishtam clearly indicates the medicinal efficacy of this drug as a nerve tonic due to the presence of bioactive molecules, which are known to work on nervous



system. Further work is warranted in this regard to prove the validity of such and similar Ayurvedic formulations.

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