



### RESEARCH ARTICLE

#### GC-MS PROFILING OF PHYTOCHEMICALS OF AN AYURVEDIC ANTI-HYPOTHYROID POLYHERBAL FORMULATION

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#### Abstract

The therapeutic efficacy of Ayurveda medicine is time tested. Exploring the chemical composition of the formulations not only throws light into probable mode of action but may also lead the way to further drug discovery. Hence with the above objective an Ayurveda herbal composition effective in managing hypothyroidism was selected for GCMS to elucidate the phytochemicals present in it the formulation to gain insight into the probable bioactive principles. The methanolic extract of the Ayurveda formulation was subjected to mass spectrometry and the compounds were identified after comparing the spectral configurations obtained with that of available mass spectral database (NIST -08 SPECTRAL DATA). The results of GCMS revealed compounds that could have direct relation to medicinal properties attributed to the formulation. There lies a future scope for studies to explore the molecular mechanisms of these compounds.

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#### Introduction:-

The phytochemicals present in an herb or raw drug are responsible for the exhibition of multitude of medicinal actions. When made into compound formulations the chemical profile is altered wherein some phytochemicals may be lost while new compounds may be produced. Thus to understand the mechanism of action of a compound formulation, its vital to explore in depth the phytochemical profile of the formulation. The herbal formulation selected in the present study is a combination of herbs popularly used in Ayurveda for the management of clinical symptoms of hypothyroidism- a quite common endocrine disorder. The clinical symptoms of hypothyroidism may persist even in individuals under Thyroxine supplementation. In such cases this Ayurveda formulation is observed to improve the Quality of Life in the individuals. The composition of the formulation is as shown below (Table 1):

**Table 1:-** Composition of the polyherbal Anti-hypothyroid formulation.

| Sl no. | Drug                    | Botanical name            | Family     | Part used |
|--------|-------------------------|---------------------------|------------|-----------|
| 1      | Aswagandha <sup>1</sup> | Withania somnifera Dunal. | Solanaceae | Root      |

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|    |                        |                                      |                  |                 |
|----|------------------------|--------------------------------------|------------------|-----------------|
| 2  | Brahmi <sup>2</sup>    | Bacopa monnieri (Linn.) Wettst.      | Scrophulariaceae | Whole plant     |
| 3  | Makandi <sup>3</sup>   | Coleus forskohlii (Willd.) Briq.     | Lamiaceae        | Arial parts     |
| 4  | Guggulu <sup>4</sup>   | Commiphora wightii (Arn.) Bhand.     | Bursaraceae      | Exudate         |
| 5  | Punarnava <sup>5</sup> | Boerhaavia diffusa Linn.             | Nyctaginaceae    | Root            |
| 6  | Madhuka <sup>6</sup>   | Glycyrrhiza glabra Linn              | Fabaceae         | Stolon and root |
| 7  | Vasaka <sup>7</sup>    | Adhatoda zeylanica Medic.            | Acanthaceae      | Roots           |
| 8  | Pippali <sup>8</sup>   | Piper longum Linn.                   | Piperaceae       | Fruits          |
| 9  | Guduchi <sup>9</sup>   | Tinospora cordifolia (Willd.) Miers. | Menispermaceae   | Stem            |
| 10 | Triparni <sup>10</sup> | Desmodium triflorum                  | Fabaceae         | Root            |
| 11 | Nimba <sup>11</sup>    | Azadirachta indica A. Juss.          | Meliaceae        | Leaf            |

The inclusion of the herbs was based on their properties and actions suitable to the management of clinical symptoms of Hypothyroidism as emphasised in Ayurveda treatises.

#### **Ashwagandha:**

The root of Ashwagandha is said to be effective in curing oedema (sopha), muscle wasting and acts as potent rejuvenative (Rasayana)<sup>12</sup>. There are numerous clinical and experimental results that support the use of Ashwagandha in hypothyroidism. A study shows that Ashwagandha improves thyroid function by ameliorating thyroid hormones and by preventing oxidative stress<sup>13</sup>. A clinical trial confirms the same wherein treatment with Ashwagandha was shown to normalise thyroid function test in subclinical hypothyroid patients<sup>14</sup>.

#### **Brahmi:**

Brahmi is praised for its ability to rejuvenate (rasayana), acclaimed as a brain tonic (medhya) and effective in managing anaemia, oedema, and insomnia<sup>15</sup>. Experimental studies prove that Brahmi could be used as thyroid stimulating drug for management of Hypothyroidism as it increases T(4) concentration by 41% without enhancing hepatic lipid peroxidation (LPO)<sup>16</sup>.

#### **Makandi:**

Makandi corrects the metabolism (deepana-pachana ) and is useful in managing anaemia (pandu), oedema (sopha) and heart diseases<sup>3</sup>.

#### **Guggulu:**

It is an appetiser, promotes strength, alleviates excess fat and is useful in treating oedema, cervical lymphadenitis, thyroid swellings, carbuncles etc<sup>17</sup>. Experimentally Guggulu has shown to ameliorate hypothyroidism in female mice<sup>18</sup>. Clinically Purified (Suddha) Guggulu is effective in managing inflammatory process and is effective in managing hypothyroidism, hyperlipidaemia, and oedema. Compound formulations of Guggulu like Vyoshadiguggulu<sup>19</sup>, KanchanaraGuggulu, Triphaladi Guggulu<sup>20</sup> are used clinically in managing hypothyroidism.

#### **Punarnava:**

Punarnava is a diffuse herb that is used in managing oedema, hepato-biliary disorders, and anaemia.<sup>21</sup> Punarnavadikasayam is an important formulation used in managing clinical symptoms of hypothyroidism.

#### **Madhuka:**

it is used in promoting strength, managing debility, increasing vitality and metabolism<sup>22</sup>.

#### **Vasaka:**

The herb is bitter and astringent in taste and is highly recommended in the management of haematemesis, haemoptysis, dyspnoea, cough, fever, nausea, skin diseases, emaciation etc<sup>23</sup>. It has proven anti-inflammatory, antioxidant, bronchodilator and expectorant activity.<sup>24</sup>

**Pippali:**

Piper longum is used as a single drug remedy and in formulations like Vyoshadi Guggulu<sup>19</sup>, Shadushnachurna, Triphaladi Guggulu<sup>20</sup> etc. for the management of hypothyroidism. It is appraised in Ayurveda as vitaliser, mild laxative, cures skin diseases, anaemia, gastrointestinal diseases, cough etc.<sup>25</sup> There are various clinical studies where the effect of Pippali individually and as Vardhamanapipalli (Pippali in increasing and decreasing dose) in hypothyroidism has been explored<sup>26</sup>.

**Guduchi:**

One of the most potent rejuvenative agent (rasayana) in Ayurveda, Guduchi also exhibits effect as appetiser, antioxidant, anti-aging, antihyperlipidemic, antidiabetic, anti-ulcerative, antipyretic, anti-inflammatory agent etc<sup>27</sup>.

**Triparni:**

Desmodium triflorum is used as Tripadi, Triparnika etc. in Kerala. Triparni is therapeutically included in treating conditions like diarrhoea, poisonous bites, skin diseases, ulcers etc<sup>28</sup>.

**Nimba:**

Nimba is useful in skin diseases, allergy, vomiting, ulcers, urinary disorders, fever etc.<sup>29</sup> Multitude of studies show that Neem is useful in management of PCOS, Diabetes and hyperlipidemia.<sup>31,32</sup>

The chemical profile of this unique compound was yet to be determined and this warranted further scope for studies that could reveal the mechanism of action of the medicine. Thus, the present study was taken up with the objective to analyse the phytochemical profile by Gas Chromatography Mass Spectrometry (GCMS) techniques.

**Materials And Methods:-****Study drug:**

The compound Ayurveda formulation was prepared in tablet form at the pharmacy Vaidyaratnam Ayurveda College. The herbal drugs in the composition (given in Table no. 1) were included in the preparation of the study drug. The ingredients 1-6 of the composition were pulverised and sieved separately to produce fine powder individually. The powdered drugs were added into a mass mixer and mixed thoroughly to obtain a uniform combination. The last five ingredients of the compound (ingredients 7-11) were made into decoction by adding 4 times water and was reduced to one fourth. This decoction was used to thoroughly grind the powdered mixture to get a wet mass. The compound was then converted into tablets of average weight of 650 mg.

**Methodology:-**

The Ayurveda compound so prepared was subjected to GCMS as per standard procedure

**Preparation of Extracts**

1g of sample was extracted with 10mL of methanol and was filtered through a syringe filter (Nylon 13 mm 0.2um) and injected to GCMS.

Instrument Model -7890 A GC with 5975C with triple axis detector

Column - DB 5MS 30 m x 0.250mm Diameter x 0.25 Micro Meter Thickness

Analysis was performed by injecting 1 µL of the sample with a split ratio of 50:1. Helium gas (99.9995%) was used as the carrier gas at a flow rate of 1 mL/min. The analysis was performed in the EI (electron impact) mode with 70 eV of ionization energy. The injector temperature was maintained at 280°C (constant). The column oven temperature program is shown in Table 2

**Table 2:-** GC-MS operating conditions.

| Oven    | Rate °C/min | Value °C/min | Hold time |
|---------|-------------|--------------|-----------|
| Initial | 40          | 5            |           |
| Ramp 1  | 5           | 100          | 10        |
| Ramp 2  | 7           | 150          | 10        |
| Ramp 3  | 5           | 280          | 5         |

The compounds were identified after comparing the spectral configurations obtained with that of available mass spectral database (NIST -08 SPECTRAL DATA)

### Results:-

The Chromatogram of GC-MS spectra analysis showing peaks of the number of compounds from the GC fractions of the

**Figure 1:-** Chromatogram Of Gc-Ms Analysis.

File :D:\GCMSD\2022\FEBRUARY\07.02.2022\U2006.D  
Operator :  
Acquired : 7 Feb 2022 19:00 using AcqMethod NUTMEG OIL.M  
Instrument : GCMS  
Sample Name: Trial Drug  
Misc Info :  
Vial Number: 2



**Figure 2:-** Area Percent Report Of GCMS.

#### Area Percent Report

Data Path : D:\GCMSD\2022\FEBRUARY\07.02.2022\  
Data File : U2006.D  
Acq On : 7 Feb 2022 19:00  
Sample : Trial Drug  
Method : D:\RESEARCH\GC-MS PROFILING\_A .M

| peak # | R.T. min | first scan | max scan | last scan | PK TY | peak height | corr. area | corr. % max. | % of total |
|--------|----------|------------|----------|-----------|-------|-------------|------------|--------------|------------|
| 1      | 53.645   | 5636       | 5645     | 5662      | rVB2  | 152226      | 573588     | 100.00%      | 45.264%    |
| 2      | 54.041   | 5685       | 5691     | 5700      | rBV6  | 18279       | 71199      | 12.41%       | 5.619%     |
| 3      | 54.471   | 5733       | 5741     | 5752      | rBV5  | 60351       | 213797     | 37.27%       | 16.872%    |
| 4      | 56.673   | 5989       | 5997     | 6013      | rBV7  | 100200      | 408619     | 71.24%       | 32.246%    |

Sum of corrected areas: 1267203

GC-MS PROFILING\_A .M Thu Feb 10 12:24:26 2022

GCMS Analysis revealed the presence of the following compounds:

| Peak name                                                                                                                            | Formula                           | Retention time | Structure |
|--------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------|----------------|-----------|
| 1,3,6,10-Cyclotetradecatetraene, 3,7,11-trimethyl-14-(1-methylethyl)-, [S-(E,Z,E,E)]-                                                | C <sub>20</sub> H <sub>32</sub>   | 53.645         |           |
| Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1 $\alpha$ ,4 $\alpha\alpha$ ,8 $\alpha\alpha$ )- | C <sub>15</sub> H <sub>24</sub>   | 54.041         |           |
| 1,5,9-Cyclotetradecatriene, 1,5,9-trimethyl-12-(1-methylethenyl)-                                                                    | C <sub>20</sub> H <sub>32</sub>   | 54.471         |           |
| Bicyclo[9.3.1]pentadeca-3,7-dien-12-ol, 4,8,12,15,15-pentamethyl-, [1R-(1R*,3E,7E,11R*,12R*)]-                                       | C <sub>20</sub> H <sub>34</sub> O | 54.471         |           |
| Thunbergol                                                                                                                           | C <sub>20</sub> H <sub>34</sub> O | 56.673         |           |
| 1-Heptatriacotanol                                                                                                                   | C <sub>37</sub> H <sub>76</sub> O | 56.673         |           |
| Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1 $\alpha$ ,2 $\beta$ ,4 $\beta$ )]-                                 | C <sub>15</sub> H <sub>24</sub>   | 54.041         |           |

### Discussion:-

GCMS analysis reveals the presence of numerous bioactive compounds and opens the scope for analysis of their role in therapeutics. Literature review shows that many of these compounds have antioxidant properties,<sup>32,33,34</sup> antimicrobial,<sup>33</sup> antiinflammatory,<sup>34</sup> and hypolipidemic<sup>34</sup> properties. The combined effect of these activities may be responsible for alleviating the clinical symptoms of hypothyroidism. Furthermore, studies are needed to elucidate the pharmacological properties of each of these compounds in specific conditions like hypothyroidism where the formulation is presently being prescribed. .

**Conclusion:-**

The GCMS analysis of methanolic extract from the Ayurvedic Formulation demonstrates that the formulation contains various bioactive phytochemical constituents that may grant the biological applications of the formulation in some clinical symptoms of hypothyroidism. Further ADMET analysis and in vivo studies may throw light on the molecular mechanisms of the compound

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