



In Vitro Evaluation of Quality Control Standards and Antioxidant Activity of a Compound Ayurveda Formulation: Agastya Haritaki Rasayana

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KEYWORDS

DPPH, Agastya Rasayana, Reactive Oxygen Species, anti-oxidants.

ABSTRACT:

Introduction: Agastya Rasayana is one of the unique formulations which are said to have a property of rejuvenation apart from its other wide range of therapeutic applications. Agastya Rasayana comprises a blend of herbal ingredients believed to support health and wellbeing through various mechanisms like; anti-oxidant, anti-inflammatory, immune-modulatory, tissue repair and regeneration which are essential to maintain physiological functioning. The present study was planned to understand its antioxidant activity to establish its mode of action scientifically and also to provide scientific evidence for the traditional use of *Agastya Haritaki Rasayana*. One of the recognized methods for the evaluation of antioxidant activity of plant extracts is DPPH free radical scavenging.

Materials & Methods: The formulation Agastya Rasayana was analysed for its antioxidant activity using in vitro assays, that is, DPPH free radical scavenging. Additionally, it was also subjected to quality control measures such as physicochemical and microbiological tests to assess its purity and potency, ensuring compliance with established standards for herbal products.

Results: The findings indicate that Agastya Rasayana exhibits significant antioxidant capacity, demonstrating effective free radical scavenging activity. The antioxidant activity is likely attributed to the presence of specific phytochemicals within the formulation, which may mitigate oxidative damage to the body tissues.

Conclusions: These results suggest that Agastya Rasayana could serve as a natural source of antioxidants, offering protective effects against oxidative stress and potentially reducing the risk of diseases.

1. Introduction

The ancient Indian medical system of Ayurveda offers a variety of herbal, mineral and herbo-mineral formulations that could be effectively used as a medicine to treat a wide range of conditions. Due to its wider range of application in treating diseases and near to nil adverse

effects, it is being recognized globally. *Avaleha* (~medicated jam) is one of the dose forms employed in Ayurveda and is well known for its palatability. *Avaleha* is a semisolid medicine preparation and is made by infusing drugs with recommended decoctions, sugar, and jaggery^[1]



Sage Agastya explained the *Avaleha* preparation known as *Agastya Haritaki Rasayana*, which is widely utilized by Ayurvedic practitioners to treat a range of ailments including allergic reactions and as a *Rasayana*. It is particularly effective in disorders of respiratory system such as cough (~*Kasa*), asthma (~*Swasa*), and hiccup (~*Hikka*). Regular consumption of *Agastya haritaki rasayana* is in the recommended dose and duration by an Ayurveda physician is believed to prevent signs of ageing, delay premature greying of hair, enhance complexion, longevity and physical strength. This potent formulation is also said to be beneficial as a complementary therapy in various conditions such as tuberculosis (~*Kshaya*), chronic fever (~*Vishama Jwara*) and heart disease (~*Hrdroga*).^[2] One of its key ingredients is *Haritaki* (*Terminalia Chebula*) possesses *Ushna veerya* (~hot potency), *Ruksha guna* (~Drying nature) as well as an ability to pacify the ill effects of toxins. *Haritaki* has been scientifically proven for its immune modulatory activities^[3]. *Rasayanas* are specifically widely known for its anti-oxidant properties, primarily due to their high content of tannins, flavonoids, phenols and other compounds. These constituents help neutralize free radicals, chelate metal ions, and protect cellular components from oxidative damage.^[4]

A significant contributor to the aetiology of various clinical disorders is oxidative stress, which is brought by highly reactive free radicals that form long-lasting complexes with biological components^[5]. Excessive build-up of reactive free radicals is due to an imbalance between the production and elimination of Reactive Oxygen Species (ROS) and reactive Nitrogen species (RNS). Although at physiological concentrations ROS and RNS function as signalling molecules, when oxidation conditions arise, excess ROS and RNS cause cell damage through their harmful interactions with proteins, lipids, and DNA. The human body has an innate antioxidant enzyme defence system to combat free radicals. Antioxidants mainly act in three key ways: by stimulating the body's own antioxidant defences, preventing oxidative stress formation, and supporting the repair of damage caused by reactive oxygen species (ROS)^[6] and it plays a vital role in minimizing oxidative damage in disorders where oxidative stress is a major contributor. The current polyherbal formulation includes ingredients that possess anti-oxidant action^[7].

There is no available literature or studies that have assessed the antioxidant effect of the poly-herbal formulation *Agastya Rasayana* as a whole. Hence, the present study was taken up to document its physicochemical characters, microbiological limits and antioxidant activity. These evaluations provide scientific evidence for the traditional use of *Agastya Haritaki Rasayana*, ensuring that it is both safe and effective for consumers, and also supporting its therapeutic claims with data.

2. Materials & Methods:

For the present study, the formulation *Agastya Rasayana* was procured from B.V Pundit, Savaidyasala Pvt Limited, Bengaluru which was prepared as per the reference of *Ashtanga Hridayam*. The ingredients are mentioned in the Table 1. Thus the obtained *Agastya Rasayana* was analysed for various analytical parameters such as Organoleptic, physicochemical B.V Pundit, Savaidyasala Pvt Limited, Bengaluru and Phytochemical tests in AYUSH approved ASU drug testing Laboratory, Central Research Faculty of KAHER's Shri BMK Ayurveda Mahavidyalaya, Shahpur, Belagavi, Karnataka, India

Organoleptic Characters:

Methods:

Colour and form were determined by examining the untreated sample under day light. Odour was determined by slowly keeping sample on the hand then smelling it. Taste was determined by chewing a pinch of polyherbal sample.

Physicochemical Characters:^[8]

Physicochemical analysis such as determination of total ash, acid insoluble ash, loss on drying, fat content, total sugars (%), pH (10% aqueous solution), water soluble extractive value and alcohol soluble extractive value were performed.

Determination of Total Ash:

2 to 3g of sample and crucible weight was obtained on digital weighing machine. The sample was incinerated at 450 c in muffle furnace for 30 minutes, after 30 minutes carbon free ash was obtained. Later it was transferred to desiccator at cooled at room temperature and then percentage of total ash was calculated



$$\text{Percentage of total ash} = \frac{W_z - W_x}{W_y - W_x} \times 100$$

where,

W_x-Empty silica crucible weight.

W_y-Weight of the crucible with the sample.

W_z-Crucible weight with the sample after desiccation.

Determination of Acid-Insoluble Ash:

Ash was prepared from sample by placing it in a muffle furnace, 25 ml of 2 ml dilute hydrochloric acid was added to ash mixture. Mixture was heated in water bath and was filtered through ashless filter paper, followed by washing of filter paper by hot boiling water. ashless filter paper (Whatman 41) was collected in a crucible and weighed. Later it was incinerated in muffle furnace till ash was obtained. Then it was cooled in desiccator and weight on machine and percentage was calculated.

$$\text{Percentage of acid insoluble ash} = \frac{W_a - W_x}{W_y - W_x} \times 100$$

where,

W_x-Empty crucible's weight.

W_y-Weight of the crucible with the sample.

W_a-Crucible weight with filterpaper ash after desiccation.

Determination of Moisture Content (Loss on Drying):

Sample was taken in a watch glass and weighed. It was kept in hot air oven for 45 minutes at 110 c temperature, after 45 minutes again weight was taken and checked until a constant weight is obtained after intervals. It is marked as W₂ and then percentage of loss on drying was calculated.

Determination of Alcohol Soluble Extractive:

5 gm of sample was taken with 100 ml of ethanol and closed in flask for 24 hours. Solution is shaken for first 6 hours in flask shaker and was kept stable for next 18 hours, 25 ml of filtrate was evaporated to dryness in tared flattened dish by boiling in a water bath and the percentage of alcohol- soluble extractive was calculated with reference to the air-dried drug.

$$\text{Percentage of alcohol soluble extract} = \frac{W_m - W_k}{W_l - W_k} \times 100$$

where,

W_k-Weight of the empty petri dish.

W_l-Weight of the petri dish with the sample.

W_m-Weight of the petri dish with the sample after evaporation

Determination of water-soluble extractive:

5 g of the air-dried sample was taken with 100 ml of chloroform and was closed in a conical flask for twenty-four hours, it was shaken frequently during the first 6 hours and allowed to stand for 18 hours. After 24 hours, the extract was filtered rapidly by filter paper, 25 ml of filtrate was taken and transferred to dried and weighed evaporating dish. Solution was evaporated till we get constant weight. Later extractive value was calculated.

$$\text{Percentage of alcohol soluble extract} = \frac{W_c - W_a}{W_b - W_a} \times 100$$

where,

W_a-Weight of the empty petri dish.

W_b-Weight of the petri dish with the sample.

W_c-Weight of the petri dish with the sample after evaporation

Determination of PH [9]:

pH value represents acidity or alkalinity of an aqueous solution.

Determination of pH was carried out at a temperature of 25±2°C. The electrodes were dipped in the solvent being examined and the pH was measured.

Sugar estimation (Mont Gomery, 1957) [Spectrophotometric method]^[10]:

A 10 percent homogenate of sample was prepared in 80 percent ethanol. It was then centrifuged at 2000 rpm for 15 minutes. The resulting supernatant was made up to a known volume (typically 10 ml or adjusted according to the expected sugar concentration). A 0.1 ml aliquot was taken, followed by the addition of 0.1 ml of 80 percent phenol and 5 ml of concentrated sulfuric acid. After cooling, the absorbance was measured at 490 nm.

Microbiological Analysis:

Microbiological analysis was performed following the standard procedures mentioned in Ayurvedic Pharmacopoeia of India^[9-10]. This included determining the total microbial count, total yeast & mould and



presence of pathogens like *Escherichia coli*, *Salmonella ebony*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*.

Antioxidant Activity of Agastya Rasayana:

DPPH Activity ^[11]

Chemicals required: Ascorbic acid, methanol, DPPH chemical and aluminium foil.

Principle:

DPPH is organic nitrogen radical capable of accepting the hydrogen from the sample(s). DPPH is purple in color and converts to yellow by formation of the DPPH (upon conversion from radical to the compound). The anti-oxidant is measured by the disappearance of UV absorption in the DPPH in the test sample and the control samples.

Brief protocol:

Ascorbic acid was used to make the calibration curve by dissolving in methanol and then diluted to 6.25- 200 µg/ml of serial concentrations. Stock solution of extracts (1mg/ml) was prepared with methanol and further diluted to serial concentrations Eg. (6.25- 200 µg/ml).

Reaction solutions of 5 ml contained: Sample extract serial concentrations / (Ascorbic acid standard serial concentrations) to which DPPH dissolved in methanol is added and mixed well. Blank contained only DPPH dissolved in methanol. Incubate the tubes for 30mins in the darkroom.

Absorbance of blank, sample and standard Solutions were measured at 517 nm with a Shimadzu UV-1800 spectrophotometer. Calibration curve using absorbance vs concentration of sample extract serial concentrations and Ascorbic acid standard serial concentrations were prepared and the percentage (%) of radical scavenging activity in the sample was determined by using following equation.

Percentage (%) of radical scavenging activity =

$(\text{Abs of the blank} - \text{Abs of the sample}) / \text{Abs of the blank} * 100$

3. Results:

Table No 2: Shows Organoleptic characters of Agastya Rasayana sample

Sr. no.	Parameters	Agastya Haritaki Rasayana
1.	Odour	Fragrant
2.	Colour	Brown
3.	Taste	Sweet and a tinge of bitterness
4.	Consistency	Semisolid

Table No 3: Shows Physicochemical characters of Agastya Haritaki Rasayana sample

Test Parameters	Specifications	Results
Loss on drying	NMT 14% W/W	8.82% W/W
Total Sugars %	40-50% W/W	49.40% W/W
Fat content	2.5-5.0% W/W	4.40 % W/W
Total ash	NMT 3% W/W	1.95 % W/W
Acid Insoluble ash	NMT 1% W/W	0 % W/W
Ph (10% aqueous solution)	5-6.5	6.09
Alcohol soluble extract	NLT 50%W/W	73.43 % W/W
Water soluble extractive	NLT 60% W/W	76.68 % W/W

Table No 4: Shows Microbiological Report of Agastya Rasayana

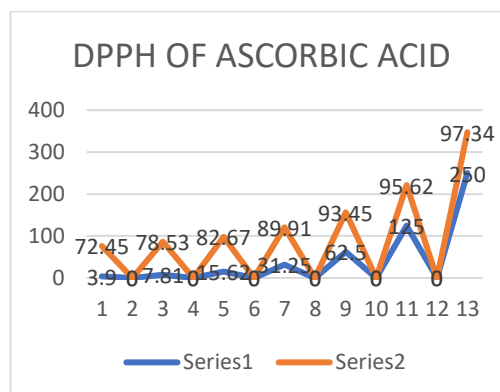
Test Parameters	Specifications	Results
Total microbial count	1,00,000cfu/g	70cfu/g
Total yeast and mould	1000cfu/g	20cfu/g
E.coli	Absent in 1g	Absent
Salmonella Typhi	Absent in 10g	Absent
Staphylococcus Aureus	Absent in 1g	Absent



Pseudomonas Aeruginosa	Absent in 1g	Absent
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DPPH Activity:**Table No:5 Showing the results of DPPH scavenging activity of Agastya Rasayana**

ALCOHOL EXTRACT		
Concentration µg/ml	% Radical scavenging activity	IC50 Value
1.90	37.22	2.89 µg/ml
3.90	67.58	
7.81	68.78	
15.62	71.94	
31.25	83.30	
62.5	88.86	
125	88.97	
250	89.08	
Ascorbic Acid		
3.90	72.45	4.13 µg/ml
7.81	78.53	
15.62	82.67	
31.25	89.91	
62.5	93.45	
125	95.62	
250	97.34	

**DISCUSSION:**

It is essential to ensure that polyherbal formulations like *Agastya Haritaki Rasayana* are free from impurities and harmful substances, making quality control a critical aspect. This study aimed to establish analytical data for the formulation, including macroscopic, physicochemical, microbiological parameters to support its safety, efficacy, and potency for therapeutic use, evidence-based practice and to generate data that can serve as a reference for future quality control measures.

Our investigation found that the amount of foreign matter in the sample was within the permissible limits set by the Ayurvedic Pharmacopoeia of India (API).

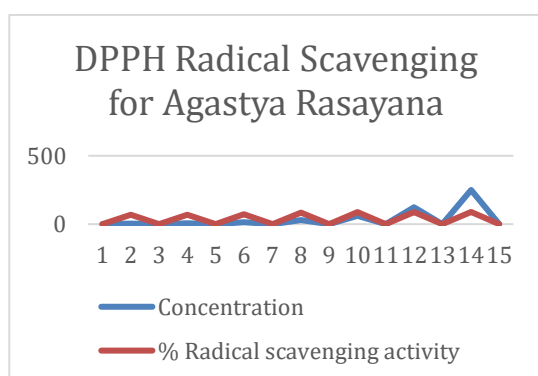
Organoleptic Characters:

Organoleptic characters showed that the AH sample was reddish brown in colour, bitter with the tinge of sweetness in taste, fragrant in odour and semisolid in consistency. Astringent and bitter taste of sample may be due to presence of *Terminalia Chebula* in the formulation.

Physicochemical tests:

The preliminary and physicochemical criteria established offer information for future investigations and assist in identification of formulations. Extractive values play a key role in evaluating the consistency of both the type and quantity of chemical constituents in a medicinal product.

Physico-chemical tests such as loss on drying (LOD), total ash, and extractive values are essential for evaluating the quality, purity, and safety of *Agastya Haritaki Rasayana*. LOD was found to be 8.82%, indicating low moisture content, which inhibits microbial growth and prevent product degradation^[12]. The pH was





6.09, indicating slight acidity. The total ash content was 1.95%, with 0% acid-insoluble ash and is in accepted limits confirming the absence of unwanted siliceous materials [13]. The water-soluble and alcohol-soluble extractive values were 73.43% and 76.68%, respectively, indicating a significant concentration of active constituents. Additionally, total sugars were measured at 49.40%, providing further insight into the formulation's nutritional and therapeutic properties.

The microbiological load in *Agastya Haritaki Rasayana* was found to be within the permissible limits established by the Ayurvedic Pharmacopoeia of India (API). Pathogens such as *E. coli*, *S. aureus*, *P. aeruginosa*, and *S. Typhi* were absent, confirming the microbiological safety of the formulation for human consumption.

DPPH Activity:

The concentrations of the alcohol extract tested ranged from 3.90 µg/ml to 250 µg/ml and the corresponding percentage of radical scavenging activity increases with higher concentrations indicating a dose-dependent antioxidant effect. At the lowest concentration (3.90 µg/ml), the scavenging activity is 67.58%, and as the concentration increases, the activity continues to rise:

- At 7.81 µg/ml, the activity is 68.78%.
- At 15.62 µg/ml, the activity reaches 71.94%.
- At 31.25 µg/ml, the activity rises to 83.30%.
- The activity plateaus around 88-89% from 62.5 µg/ml to 250 µg/ml.

This suggests that as the concentration of the *Agastya Rasayana* extract increases, the percentage of free radical scavenging activity also increases, suggesting strong antioxidant capacity of the formulation. IC₅₀ is approximately 2.89 µg/ml, indicating the concentration at which 50% of the DPPH radicals are neutralized.

Lifestyle disorders have become increasingly prevalent due to improper diet, sedentary habits, oxidative stress, which plays a central role in the pathogenesis of many diseases. *Agastya Rasayana*, mentioned under in Ayurvedic classics, has also demonstrated potential benefits in managing cardiac conditions. While direct studies on the antioxidant potential of *Agastya Rasayana* are lacking, its individual plant ingredients, such as *Terminalia Chebula* (the primary ingredient), *Mucuna*

prurins and *Sida Cardifolia* have been scientifically proven to possess antioxidant properties. Furthermore, the DPPH assay of *Agastya Rasayana* has shown significant results in reducing oxidative stress. Research underscores the role of anti-oxidants in slowing the onset and progression of atherosclerosis by preventing LDL oxidation, inhibiting platelet aggregation, enhancing HDL levels, and delaying the proliferation of vascular smooth muscle cells.

The DPPH antioxidant activity of *Agastya Rasayana* provides scientific support for its potential as an antioxidant in preventing oxidative stress in various diseases.^[14]

Conclusion:

Based on the study conducted, the validation and quality control criteria for *Agastya Haritaki Rasayana* adhere to established standards set by the API, confirming the purity and potency of this formulation. In vitro-antioxidant activity showed it had an anti-oxidant activity which lays a further scope to explore its bio-molecules in depth and understand its mechanism of action.

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Table No:1: Ingredients of Agastya Rasayana

S.No	SANSKRIT NAME	Botanical Name	Family	Part used	QTY
1	Bilva	Aegle marmelos Linn.	Rutaceae	Stem bark	2 pala
2	Agnimantha	Premna mucronata	Verbenaceae	Stem bark	2 pala
3	Syonaka	Oroxylum indicum(L)	Bignoniaceae	Stem bark	2 pala
4	Patala	Stereospermum Suaveolans	Bignoniaceae	Stem bark	2 pala
5	Gambhari	Gmelina arborea	Verbenaceae	Stem bark	2 pala
6	Brihati	Solanum indicum	Solanaceae	Stem bark	2 pala
7	Kantakari	Solanum surattense Burm.F	Solanaceae	Stem bark	2 pala
8	Gokshura	Tirbulus terrestris	Zygophyllaceae	Stem bark	2 pala
9	Shalaparni	Desmodium ganeticum	Leguminosae	Stem bark	2 pala
10	Prishnaparni	Uraria Picta	Leguminosae	Stem bark	2 pala
11	Kapikachu	Mucuna pruriens	Fabaceae	Seed	2 pala



12	Shankhapushpi	Convolvulus pluricaulis	Convolvulaceae	Whole plant	2 pala
13	Shati	Hedychium Spicatum			
14	Bala	Sida Cardifolia	Malvaceae	Whole plant	2 pala
15	Hastippali	Scindapsus officianalis	Piperaceae	Fruit	1 kudava
16	Apamarga	Achyranthes Aspera	Amaranthaceae	Whole palnt	2 pala
17	Pippalimula	Piper longum	Piperaceae	Root	2 pala
18	Chitraka	Plumbago Zeylanica	Plumbagianaceae	Root	2 pala
19	Pushkaramula	Inula Racemosa	Asteraceae	Root	2 pala
20	Yava	Hordeum Vulgare	-	Seed	1 adhaka
21	Haritaki	Terminalia Chebula	Combretaceae	Fruit	100 fruits in numbering
22	Taila	Sesamum orientale	-	Seed oil	1 kudava
23	Ghrta	Ghee	-	-	1 kudava
24	Madhu	Honey	-	-	1 Kudava
25	Guda	Jaggery	-	-	5 tula