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PHARMACEUTICAL AND ANALYTICAL STANDARDIZATION OF AGASTYA PUSPA THROUGH MODERN PARAMETERS - PHYSICO-CHEMICAL PARAMETERS, HPTLC, ICP-MS METHODS

Renu Dixit[1], B. Lizitha[2]*, K. V. Vijaya Bhaskara Reddy[3], Aryan Surendran[4]

[1]Professor & H.O.D, Department of Dravyaguna, S.V. Ayurvedic College and Hospital, T. T. D, Tirupati, Andhra Pradesh, India.

[2]P. G Scholar, Department of Dravyaguna, S.V. Ayurvedic College and Hospital, T. T. D, Tirupati, Andhra Pradesh, India.

[3]Professor, Department of Salya Tantra, S.V. Ayurvedic College and Hospital, T. T. D, Tirupati, Andhra Pradesh, India.

[4]B.A.M.S, Dharma Ayurveda Medical College & Hospital, The Tamilnadu Dr. M.G.R Medical University, Chennai.

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***Corresponding Author**
Dr. B. Lizitha
P. G Scholar, Department of Dravyaguna, S.V. Ayurvedic College and Hospital, T. T. D, Tirupati, Andhra Pradesh, India.

ABSTRACT

Flowers/Puspa have special place in Ayurveda and Indian society. From beautification to spiritual connect the flowers are used for various purposes in the treatment of many diseases. The Agastya Puspa has been mentioned in various references in Ayurveda treatises was studied for its Physico-chemical studies, HPTLC and ICP-MS Method. The results of the Agastya Puspa were noted and it was observed that Quercetin a chemical compound was identified in HPTLC. In ICP-MS Method metals like Calcium, Copper, Iron, Potassium, Magnesium, Manganese, Sodium and Zinc were identified. Here an attempt has been made to study Agastya Puspa Curna Analytically and to develop fingerprints by Physico-Chemical parameters, High-Performance Thin Layer chromatography study (HPTLC) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

KEYWORDS: Dravyaguna, Pharmaceutical preparation, HPTLC, Physico-chemical parameters, ICP-MS.

INTRODUCTION

Analytical studies have an important role in the standardization of drugs. It provides information about the safety, efficacy, stability, and contraindication of any formulation. The quality of the drugs, that is, the profile of the constituents in the final product, has implications for efficacy and safety.

For the purpose of establishing biological activity, maintaining a constant chemical profile, or ensuring product quality, standardising Ayurvedic drugs is important. Physico- chemical parameters and quantitative/qualitative tests like HPTLC and ICP-MS are conducted to assess the standards of the drugs.

AIMS AND OBJECTIVES

This study is aimed to develop Pharmaceutical and Analytical profile of Agastya Puspa.

METHODOLOGY

Two stages were taken to complete the process.

1. Pharmaceutical study
2. Analytical study

Pharmaceutical study

Pharmaceutical study was carried out in several stages to obtain the contents in the desired form for the preparation of Agastya Puspa Vati.

1. The fresh Agastya Puṣpa were collected from the natural sources of Tirupati surroundings and thoroughly washed under water to remove the impurities.
2. Later, they were dried under shade. After proper drying the Puspa is Pulverized. The obtained coarse powder was sieved with mesh no. 100 to obtain fine powder.
3. To the fine powder Binding agent is added to turn the powder into thick consistency. Here the binding agent was Rice starch.
4. After adding the binding agent, the trays were kept in drier which are arranged in layers separately.
5. From the trays, the drug was transferred into a granulation machine.
6. The collected granules were made into 500 mg tablets in tablet making machine.

The above mentioned process was carried out in Srinivasa Ayurveda pharmacy, Srinivasa Mangapuram. Because of the convenience and palatability, tablet form is preferred.

Analytical studies

Physico-Chemical parameters

Physico-chemical testing examines a sample's physical and chemical characteristics which can provide clues about the molecular behaviours inside under various natural settings. When the drug is stored for a long time, these tests assist in understanding the stability and Potency of the drug.

Results of standardization parameters of Agastya Puspa (*Sesbania grandiflora* (L) Poiret) Curna

Parameter	Results n = 3 (%w/w) (Avg ± SD)
Loss on drying	9.01 ± 0.00
Total Ash	7.84 ± 0.15
Acid Insoluble Ash	0.28 ± 0.01
Water soluble Ash	5.96 ± 0.01
Alcohol soluble extractive value	13.73 ± 0.01
Water soluble extractive value	69.62 ± 0.01

High-performance thin-layer chromatography (HPTLC)

A more advanced type of thin layer chromatography is high performance thin layer chromatography (HPTLC). The fundamental thin layer chromatography technique can be improved in a number of ways to automate the various procedures, boost the attained resolution, and enable more precise quantitative measurements.

The procedure begins with development of sample loaded plate with first solvent. After removing it, the plate is rotated 90° and developed with a second solvent.

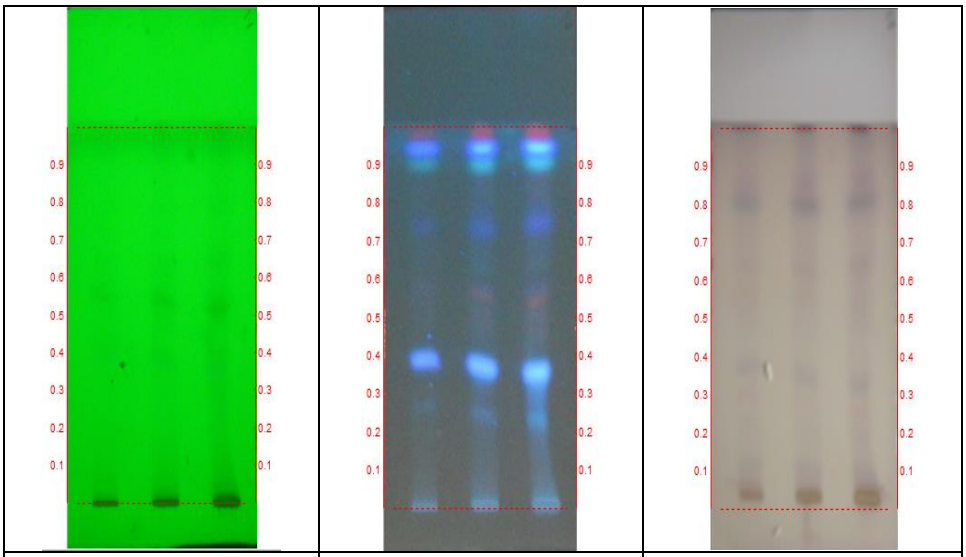
General steps involved in HPTLC method developments are as follow

Basic steps

- Selection of the Stationary phase
- Mobile phase selection and Optimization
- Sample Preparation and Application
- Chromatogram Stationary phase, Physical and Chemical properties of Analyte.

RESULTS

HPTLC photo documentation of ethanol extract of Flower of Agastya Puspa (*Sesbania grandiflora* (L) Poiret).



Short UV	Long UV	Post derivatisation

Track 1 - **Flower of Agastya Puspa** – 3µl

Track 2 - **Flower of Agastya Puspa** – 6µl

Track 3 - **Flower of Agastya Puspa** – 9µl

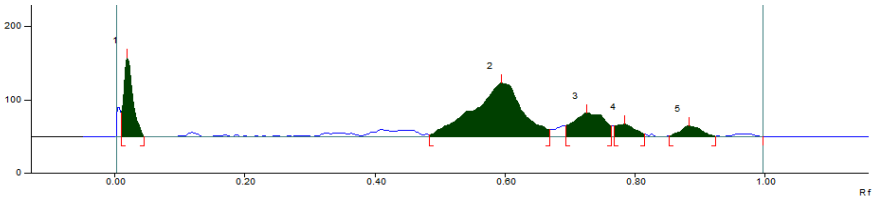
Solvent system – Toluene: Ethyl acetate: Formic acid: Methanol (5.5: 3.0: 1.0: 0.5)

R_f values of sample of Flower of Agastya Puspa (*Sesbania grandiflora* (L) Poiret) Curna

Short UV	Long UV	Post derivatisation
-	0.23 (F. blue)	-
-	-	0.26 (Purple)
-	-	0.34 (Purple)
0.37 (Green)	0.36	-
0.52 (Green)	0.54 (F. red)	-
0.64 (Green)	0.63 (F. green)	0.64 (Purple)
-	0.75 (F. blue)	-
-	-	0.80 (Purple)
-	-	0.86 (Purple)
-	0.90 (F. blue)	-
-	0.94 (F. blue)	-

*F – Fluorescent; L –Light; D – Dark

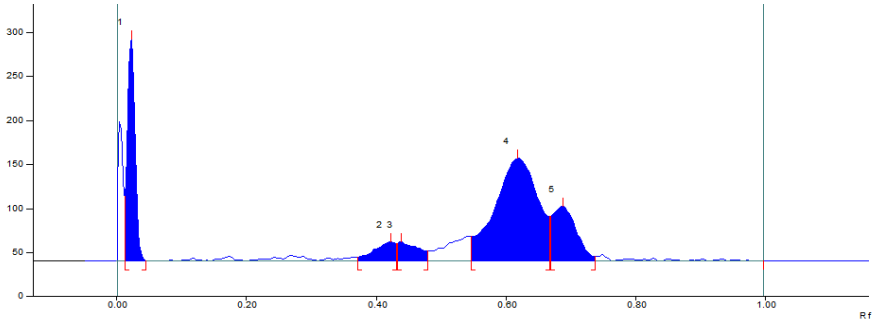
Densitometric scan of agastya puspa (*Sesbania grandiflora* (L) Poiret) Curna



Track 3, ID: Agasthya pushpa (Sesbania grandiflora)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	32.8 AU	0.02 Rf	107.6 AU	43.98 %	0.05 Rf	0.4 AU	1064.9 AU	15.67 %
2	0.48 Rf	2.6 AU	0.60 Rf	73.2 AU	29.91 %	0.67 Rf	9.5 AU	3926.2 AU	57.78 %
3	0.70 Rf	15.1 AU	0.73 Rf	32.4 AU	13.26 %	0.76 Rf	14.4 AU	1096.4 AU	16.13 %
4	0.77 Rf	14.3 AU	0.79 Rf	16.9 AU	6.91 %	0.82 Rf	3.8 AU	364.9 AU	5.37 %
5	0.85 Rf	0.8 AU	0.88 Rf	14.5 AU	5.93 %	0.93 Rf	0.6 AU	343.2 AU	5.05 %

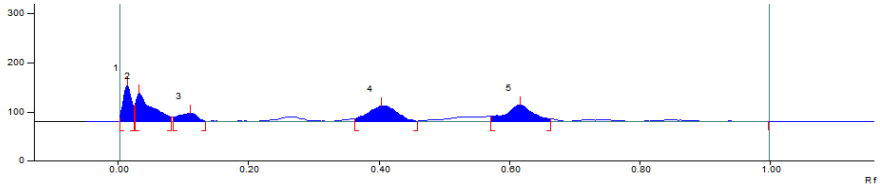
At 254nm. 0.71



Track 3, ID: Agasthya pushpa (Sesbania grandiflora)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	72.9 AU	0.02 Rf	252.3 AU	53.22 %	0.05 Rf	0.2 AU	2126.6 AU	20.61 %
2	0.37 Rf	4.3 AU	0.42 Rf	21.4 AU	4.51 %	0.43 Rf	19.8 AU	519.3 AU	5.03 %
3	0.43 Rf	20.1 AU	0.44 Rf	21.9 AU	4.61 %	0.48 Rf	10.9 AU	490.2 AU	4.75 %
4	0.55 Rf	27.6 AU	0.62 Rf	116.4 AU	24.55 %	0.67 Rf	50.7 AU	5525.7 AU	53.54 %
5	0.67 Rf	50.7 AU	0.69 Rf	62.1 AU	13.10 %	0.74 Rf	4.8 AU	1658.6 AU	16.07 %

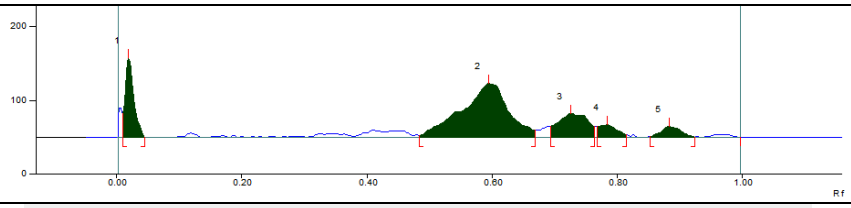
Fig 25: At 386nm, ab, Rf 0.69



Track 3, ID: Agasthya pushpa (Sesbania grandiflora)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	8.1 AU	0.01 Rf	73.6 AU	34.42 %	0.02 Rf	29.8 AU	669.1 AU	16.30 %
2	0.03 Rf	33.6 AU	0.03 Rf	57.6 AU	26.96 %	0.08 Rf	9.3 AU	1041.3 AU	25.37 %
3	0.08 Rf	9.4 AU	0.11 Rf	17.1 AU	8.02 %	0.13 Rf	0.3 AU	356.3 AU	8.68 %
4	0.36 Rf	5.9 AU	0.40 Rf	31.5 AU	14.72 %	0.46 Rf	0.8 AU	1020.5 AU	24.87 %
5	0.57 Rf	11.3 AU	0.62 Rf	34.0 AU	15.89 %	0.66 Rf	5.0 AU	1016.9 AU	24.78 %

Fig 26: At 386nm, flu



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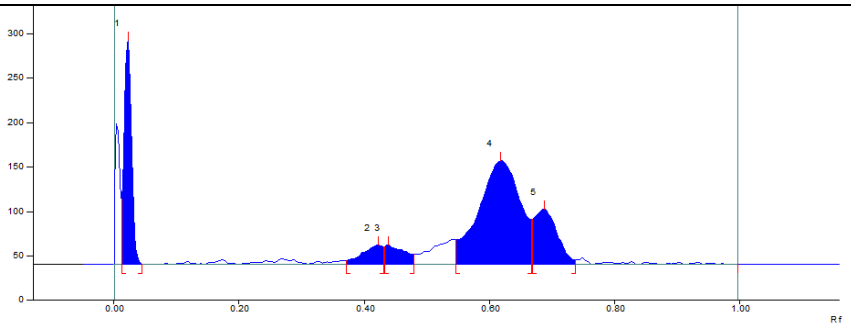
3	0.70 Rf	15.1 AU	0.73 Rf	32.4 AU	13.26 %	0.76 Rf	14.4 AU	1096.4 AU	16.13 %
4	0.77 Rf	14.3 AU	0.79 Rf	16.9 AU	6.91 %	0.82 Rf	3.8 AU	364.9 AU	5.37 %
5	0.85 Rf	0.8 AU	0.88 Rf	14.5 AU	5.93 %	0.93 Rf	0.6 AU	343.2 AU	5.05 %

At 254nm. 0.71



Track 3, ID: Agasthya pushpa (Sesbania grandiflora)

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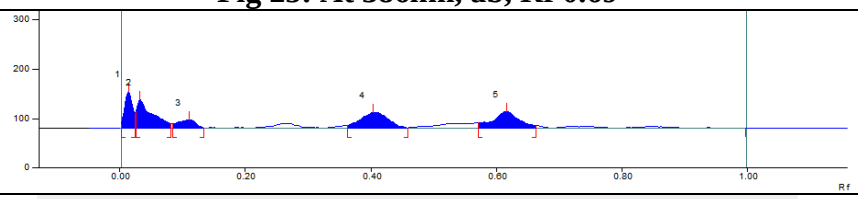
3	0.43 Rf	20.1 AU	0.44 Rf	21.9 AU	4.61 %	0.48 Rf	10.9 AU	490.2 AU	4.75 %
4	0.55 Rf	27.6 AU	0.62 Rf	116.4 AU	24.55 %	0.67 Rf	50.7 AU	5525.7 AU	53.54 %
5	0.67 Rf	50.7 AU	0.69 Rf	62.1 AU	13.10 %	0.74 Rf	4.8 AU	1658.6 AU	16.07 %

Fig 25: At 386nm, ab, Rf 0.69



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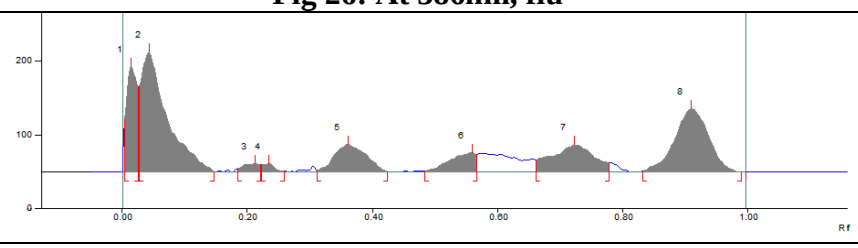
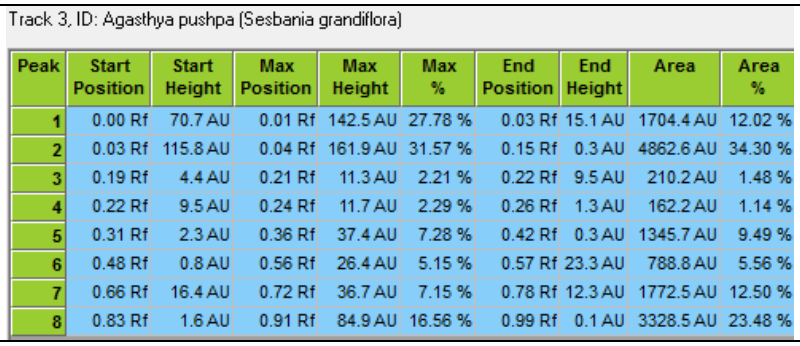
2	0.03 Rf	33.6 AU	0.03 Rf	57.6 AU	26.96 %	0.08 Rf	9.3 AU	1041.3 AU	25.37 %
3	0.08 Rf	9.4 AU	0.11 Rf	17.1 AU	8.02 %	0.13 Rf	0.3 AU	356.3 AU	8.68 %
4	0.36 Rf	5.9 AU	0.40 Rf	31.5 AU	14.72 %	0.46 Rf	0.8 AU	1020.5 AU	24.87 %
5	0.57 Rf	11.3 AU	0.62 Rf	34.0 AU	15.89 %	0.66 Rf	5.0 AU	1016.9 AU	24.78 %

Fig 26: At 386nm, flu





Track 3, ID: Agasthya pushpa (Sesbania grandiflora)

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	70.7 AU	0.01 Rf	142.5 AU	27.78 %	0.03 Rf	15.1 AU	1704.4 AU	12.02 %
2	0.03 Rf	115.8 AU	0.04 Rf	161.9 AU	31.57 %	0.15 Rf	0.3 AU	4862.6 AU	34.30 %
3	0.19 Rf	4.4 AU	0.21 Rf	11.3 AU	2.21 %	0.22 Rf	9.5 AU	210.2 AU	1.48 %
4	0.22 Rf	9.5 AU	0.24 Rf	11.7 AU	2.29 %	0.26 Rf	1.3 AU	162.2 AU	1.14 %
5	0.31 Rf	2.3 AU	0.36 Rf	37.4 AU	7.28 %	0.42 Rf	0.3 AU	1345.7 AU	9.49 %
6	0.48 Rf	0.8 AU	0.56 Rf	26.4 AU	5.15 %	0.57 Rf	23.3 AU	788.8 AU	5.56 %
7	0.66 Rf	16.4 AU	0.72 Rf	36.7 AU	7.15 %	0.78 Rf	12.3 AU	1772.5 AU	12.50 %
8	0.83 Rf	1.6 AU	0.91 Rf	84.9 AU	16.56 %	0.99 Rf	0.1 AU	3328.5 AU	23.48 %

Fig 27: At 620nm (Post derivatisation with VSA), Rf 0.71

Rf 0.71-Quercetin

Inductively coupled plasma – Mass spectrometry

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is a type of Mass Spectroscopy that is highly sensitive and capable of the determination of a range of Metals and several Non-metals at concentrations below one part in 1012 (part per trillion).

Principle

ICP (Inductively Coupled Plasma) Spectroscopy is an analytical technique used to measure and identify elements within a sample matrix based on the ionization of the elements within the sample. Mass Spectrometer (MS) separates the ions out by their mass-to-charge ratio after going through the ICP, and the detector counts the number of selected ions per second which allows the instrument to determine the concentration of each chosen element.

Details of instrumentation

Name of the Instrument	**Microwave digestion** • Model: MARS 6 • Make: CEM Corporation **ICP-MS** • Model: NexION 2000 • Make: Perkin Elmer

OBSERVATIONS

The ICP-MS analysis of **Agastya Puspa (***Sesbania grandiflora* Linn.) Curna showed the following elements present in sample in % - Percentage w/w

Elements present in **Agastya Puspa** (*Sesbania grandiflora* Linn.) Curna by ICP-OES

Heavy metal analysis		
S. No.	**Parameters**	**Result**
1	Calcium (Ca)	1657.8ppm
2	Copper (Cu)	12.3ppm

3	Iron (Fe)	0387.7ppm
4	Potassium (K)	7092.0ppm
5	Magnesium (Mg)	489.4ppm
6	Manganese (Mn)	32.1ppm
7	Sodium (Na)	375.4ppm
8	Zinc (Zn)	21.7ppm
Kew.-Word: ppm- Parts per million		

DISCUSSION

Physico-chemical studies of Agastya Puspa Curna shows Loss on drying 9.01**(%w/w), this means the flower had lot of moisture content.** Total Ash 7.84 **(%w/w) this may be due to the presence of metallic elements like Copper, Calcium and Zinc as present in elemental form,** Acid Insoluble Ash 0.28**(%w/w),** Water soluble Ash 5.96**(%w/w),** Alcohol soluble extractive value 13.73 **(%w/w),** Water soluble extractive value 69.62 **(%w/w).** Both Alcohol soluble Extractive value and Water- soluble Extractive value indicates that the chemical compositions of Agastya Puspa Curna are more water soluble than Alcohol soluble and Foreign - matter was (Nil).

This is the reason why it is seen that Agastya Puspa is mostly formulated in the form of Svarasa in Ayurveda and used as Bhavana Dravya for many preparations in Rasasastra. It indicates that the Knowledge of Ancient seems was so advanced that every preparation prepared was based on its Physico-chemical understanding.

HPTLC study of Agastya Puspa Curna at **short UV 254nm 3 bands** are observed at Rf values of **0.37, 0.52, 0.64** with Green colour intensity. **At long UV 366nm**, **7 bands** are observed at Rf values of **0.23, 0.36** with colour intensities of Fluorescent Blue, Rf value of **0.54** with Fluorescent Red colour, Rf value of **0.63** with Fluorescent Green colour, Rf value of **0.75, 0.90, 0.94** with colour intensities of Fluorescent Blue. After derivatisation with Vanillin Sulphuric Acid, at **UV 254 nm, 5 bands** are spotted with Rf values of **0.26, 0.34, 0.64, 0.80, 0.86** with Purple colour intensity.

From the HPTLC studies **"Quercetin"** an important chemical compound was identified.

Quercetin is a Polyphenolic compound with Anti-oxidant, Anti-coagulant, Anti-bacterial and Immune enhancing properties. It also helps to stabilize the cells that release histamine in the body and thereby have an Anti-histamine effect. It reduces eosinophils in the blood, acts as a Potent Bronchodilator, Eosinophil and Neutrophil recruitment, Bronchial epithelial cell

activation, Mucus and Collagen production and Airway hyperactivity.

ICP-MS Metal Analysis of the powdered **Agastya Puspa** shows the presence of all these elements. **Calcium**, **Copper**, **Iron**, **Potassium**, **Magnesium** and **Zinc**.

CONCLUSION

Ayurveda is a vast science which can be corelated to many Multi-disciplines of Modern sciences. The Pharmaceutical and Analytical study of Agastya Puspa redefines the values of Ayurvedic Dravyaguna. The Agastya Puspa which is of Tikta-Kashaya Rasa, Katu Vipaka, Natisitosna Virya, Vatakara and Kpaha-Pittahara. The presence of "Quercetin" compound proves that it acts as Kaphahara, Shothahara, Visahara and Krimighna property. The Presence of Calicum, Copper, Iron, Potassium, Magnesium and Zinc elements.

Finally, the Analytical studies which gives fingerprint assessment for future studies is very helpful for standardization of Agastya Puspa.

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