PHYSICO – CHEMICAL ANALYSIS AND HEAVY METAL TOXICITY STUDIES OF MAM GRANULES -A SIDDHA DRUG

Manickavasagam R[1]*, Shree Devi M[2]*,

1. *Siddha Clinical Research Unit, Govt.Sri Jaya Chama Rajendra Institute of Indian Medicine, Bengaluru, Karnataka 560 009, India*
2. *Siddha Central Research Institute, Arumbakkam, Chennai, Tamil Nadu – 600106, India*

**Corresponding Authors:*

1. Dr. R. Manickavasagam,

Siddha Clinical Research Unit

A unit under Central Council for Research in Siddha,

Govt.Sri Jaya Chama Rajendra Institute of Indian Medicine,

Bengaluru, Karnataka 560 009, India

2. Dr. Shree Devi. M

Siddha Central Research Institute,

Arumbakkam, Chennai,

Tamil Nadu – 600106, India

ABSTRACT:

Standardization of raw drugs which are used in the manufacturing of AYUSH medicine, is a need of the hour to ensure efficacy and safety of the medicine. Quality standards of AYUSH products must be documented Prior to the Pharmacological studies since awareness and utilization of herbal products among the public are increasing day by day globally. MAM Granules is a Siddha Poly herbal medicine which is made up of ***Manjal – Turmeric*** (Curcuma

longa L.), ***Amukkara - Ashwagandha*** (Withania somnifera, fam.) and ***Milagu – Black Pepper*** (Piper nigrum L.). MAM Granule is used as a preventive and add-on drug for immunity restoration against viral infections. This study is aimed to standardize the MAM Granule as per the guidelines of PCIM for the standard testing protocol for AYUSH drugs. **The main objective of the study is to** evaluate the quality control standards for MAM Granules - a Siddha Poly herbal medicine. **It was derived by using** Macroscopic Identification, Physico-chemical analysis, and heavy metal analysis were performed. **Result:** The result of the performed tests gave diagnostic characteristics to standardize the MAM Granule formulation and proven the safety profile. In addition to that we had derived a monograph of the same.

KEYWORDS: MAM Granules, Monograph, Quality standards, Siddha Medicine, Standardization

1. INTRODUCTION:

Siddha system of Medicine is one of the traditional systems of Indian Medicine (AYUSH) which also uses herbal drugs to manufacture medicines as like as Ayurveda (1). Standardization of raw drugs which are used in the manufacturing of AYUSH medicine, is a need of the hour since the awareness and utilization of herbal products among the public are increasing day by day globally. Quality standards of herbal products must be documented Prior to the Pharmacological studies (2). This is due to a lot of factors involving environmental pollution (Air, Water, and soil) of the growing of the plants. Adulteration of identical herbal products instead of the original ones is also a major threat to the AYUSH industry to make quality medicine. World Health Organization (WHO) also promotes herbal-based medicines in community health care (3). In this regard, WHO has already published exclusive guidelines for the validation of traditional plant-origin drugs for developing countries (4).

MAM Granules is a Siddha Poly herbal medicine which is made up of ***Manjal – Turmeric*** (Curcuma longa L.), ***Amukkara - Ashwagandha*** (Withania somnifera, fam.) and ***Milagu – Black Pepper*** (Piper nigrum L.). MAM Granule is used as a preventive and add-on drug for immunity restoration against viral infections. (Withania Article)

In view of this point, the Standardization of the MAM granule was done by using various tests like Macroscopic Identification, Physico-chemical analysis, and heavy metal analysis based on standard testing protocols for AYUSH drugs (5).

This Article explains the standardization methods for MAM Granules for authentication and gives a clear picture of the same as a monograph.

2. AIM:

This study is aimed to standardize the MAM Granule as per the guidelines of PCIM for the standard testing protocol for AYUSH drugs.

3. OBJECTIVES:

3.1. Primary:

To evaluate the quality control standards (Physico-Chemical Analysis) and Safety standards (Heavy Metal Analysis) for MAM Granules - a Siddha Poly herbal medicine.

2.2. Secondary:

To document and develop a monograph on the MAM Granules - a Siddha Poly herbal medicine

3. MATERIALS AND METHODS:

4.1 Raw Drugs:

The Components of the MAM Granules were obtained and purchased from the local country drug shop in Chennai, Tamil Nadu, India. The Purchased raw drugs were identified and authenticated by the officials of the Department of Pharmacognosy, Siddha Central Research Institute (SCRI), Arumbakkam, Chennai, Tamil Nadu, India. **Certificate of Authentication No. 214.06021001 dated 06.01.2021.**

3.2. Purification of raw drugs:

Purification of raw drugs for the preparation of Siddha medicines is strictly adhered as per the Siddha Classical Literatures for ensure the implementation of traditional Siddha Gunapadam practices

4.2.1 Purification of *Manjal – Turmeric* (Curcuma longa L): Remove the outer skin of route tuber and made into small, small round pieces. Then ground them in to powder and dried in sunlight. (7)

4.2.2. Purification of *Amukkara - Ashwagandha* (Withania somnifera, fam.): route tubers dried in sunlight and made into powder. Then went under the Pitaviyal process with milk and again dried in Sun light. (7)

4.2.3. Purification of *Milagu – Black Pepper* (Piper nigrum L.): Dried fruits soaked with butter milk for 3 hours. Then dried and made into the powder. (7)

4.3. **Preparation of MAM Granule:**

All Purified raw drugs are ground and made into Chooranam (Powder form) as per the ratio mentioned in Table 1 and then made into granules form.

Table 1: components of MAM Granule:

S. No	Siddha Name	Common Name	Botanical Name	Proportion
1	*Manjal*	Turmeric	Curcuma longa L.	1
2	*Amukkara*	Ashwagandha	Withania somnifera, fam.	4
3	*Milagu*	Pepper	Piper nigrum L.	1

Figure 1: **Components of MAM Granule**

4. Analysis of MAM Granules:

5.1. Macroscopic examination:

MAM Granules is found to be brownish yellow in colour with a characteristic odor and bitter taste.

5.2. Physico – Chemical Analysis:

5.2.1. Percentage Loss on Drying:

2gm of the test medication were precisely weighed in an evaporating dish. After being dried for five hours at 105°C, the sample was weighed.

5.2.2. Determination of Total Ash:

2gm of test medication were precisely weighed in a silicon dish and burned in a furnace set to 400 degrees Celsius until no carbon remained. After cooling, the sample was weighed.

5.2.3. Percentage of total ash

Percentage of total ash will be calculated with reference to the weight of air-dried drug.

5.2.4. Determination of Acid Insoluble Ash:

The ash obtained by total ash was boiled with 25 ml of dilute hydrochloric acid for 6 mins. Then the insoluble matter is collected in crucible, washed with hot water and ignited to constant weight. Percentage of acid insoluble ash calculated with reference to the weight of air-dried ash.

5.2.5. Determination of Alcohol Soluble Extractive:

5gm of test sample was macerated with 100 ml of Alcohol in a closed flask for twenty-four hours, shaking frequently during six hours and allowing it to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105oC, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

5.2.6. Determination of Water-Soluble Extractive:

5gm of test sample was macerated with 100 ml of chloroform water in a closed flask for twenty-four hours, shaking frequently during six hours and allowing it to stand and for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105oC, to constant weight and weigh. Calculate the percentage of water-soluble extractive with reference to the air-dried drug.

5.2.7. pH determination:

pH determination Required quantity of test sample was admixed with distilled water and the subjected to screening using pH meter.

The observations were documented and presented in Table 2.

Table 2: Physico-Chemical Analysis of MAM Granules

S. No	Physico-Chemical Parameters	
1	Loss on drying	13.363 %
2	Total ash	3.636 %
3	Water soluble ash	2.380 %
4	Acid insoluble ash	0 %
5	Water soluble extractives	14.27 %
6	Alcohol soluble extractives	6.49 %
7	pH (10% solution)	6.0

5.3. Heavy Metal Analysis of MAM Granules:

The Heavy metal analysis of MAM Granules was performed as per the standard protocols by using an Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) Instrument.100gm of the Sample was taken in the Teflon microwave digestion vessel and 1.0 mL of ultra-pure nitric acid was added and digested about 45 minutes in a closed vessel microwave digestion unit. Then the sample was made up to 50 mL in a standard measuring flask. The calibration standard was prepared to elucidate the linearity of the analyte ranging from 0.25µg/mL to 10 µg/Ml. Agilent 5100 VDV ICP-OES instrument was used with the following operation conditions View: Axial view, RF Power: 1.2Kw, Plasma gas flow Rate: 12L min^{-1}, and nebulizer gas flow rate: 0.70 L min^{-1}. The samples are introduced into the plasma using a nebulizer and spray chamber. The observations were documented and presented in Table 3.

Table 3: Heavy Metal Analysis of MAM Granules:

S.No	Element	MAM Sample -1 (µg/g)	MAM Sample -2 (µg/g)
1	Arsenic (As)	BDL	BDL
2	Cadmium (Cd)	BDL	BDL
3	Lead (Pb)	BDL	BDL
4	Mercury (Hg)	BDL	BDL

* BDL – Below Detection Limit

Standard Linearity:

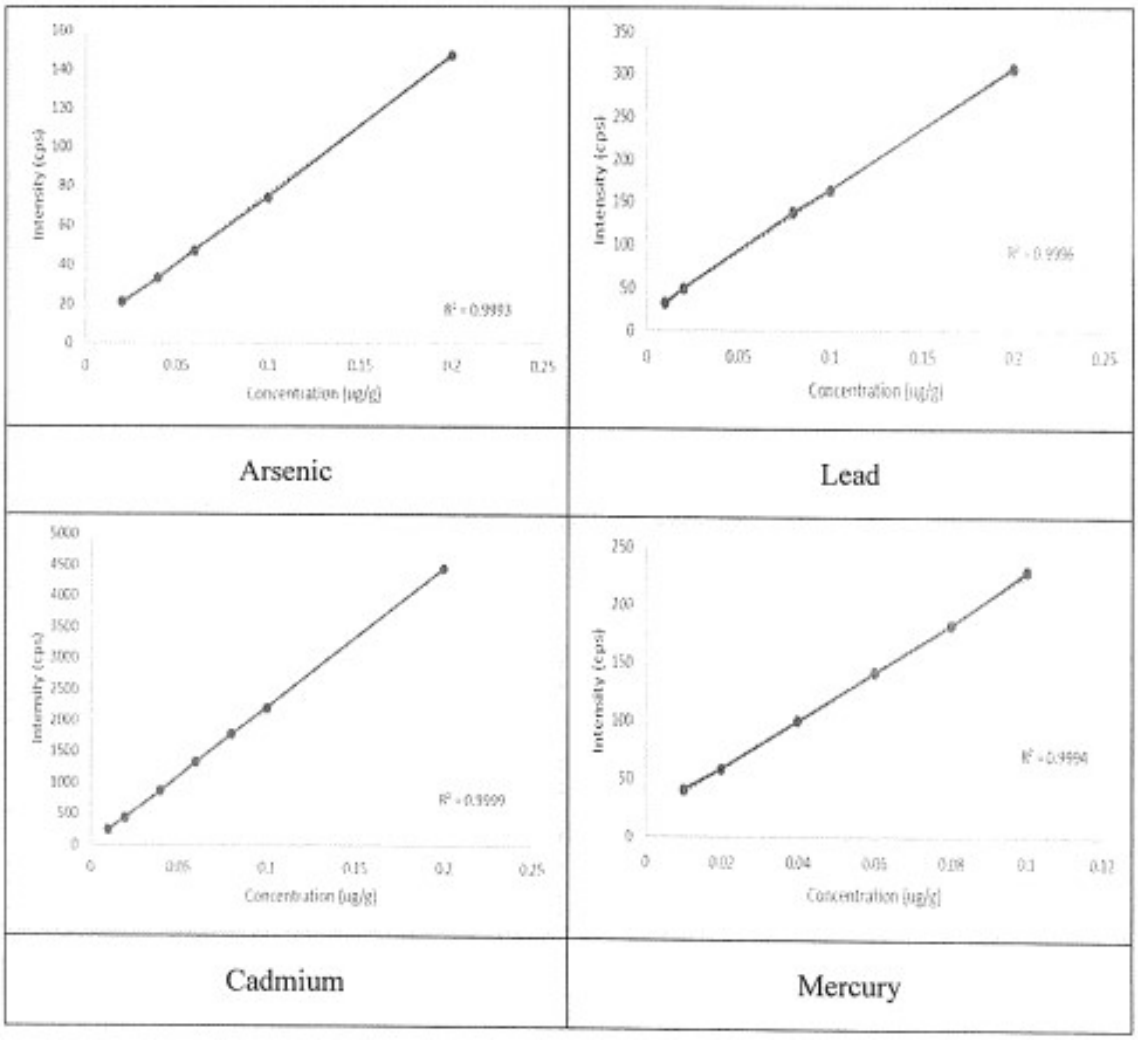
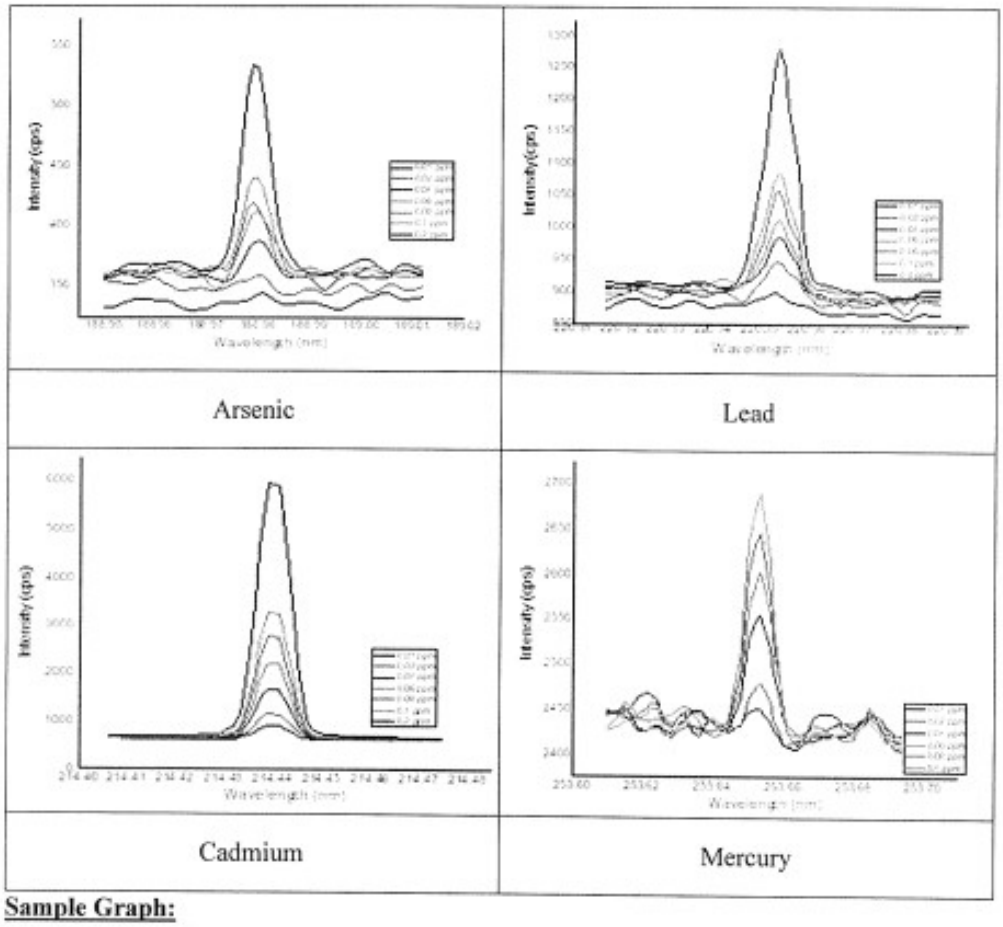


Figure 2: Heavy Metal Analysis

Standard Overlay:



Sample Graph:

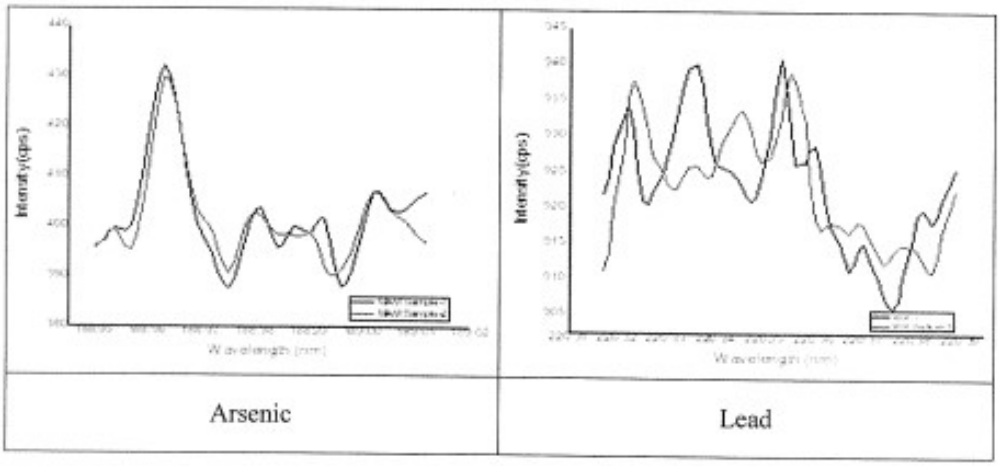
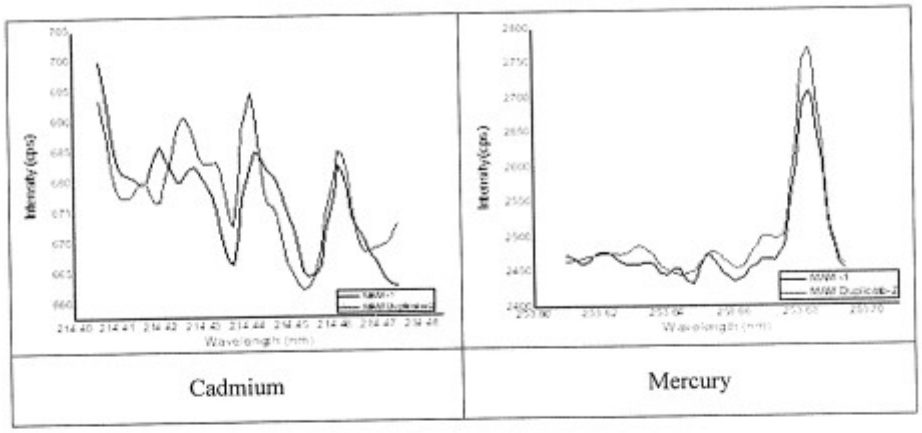
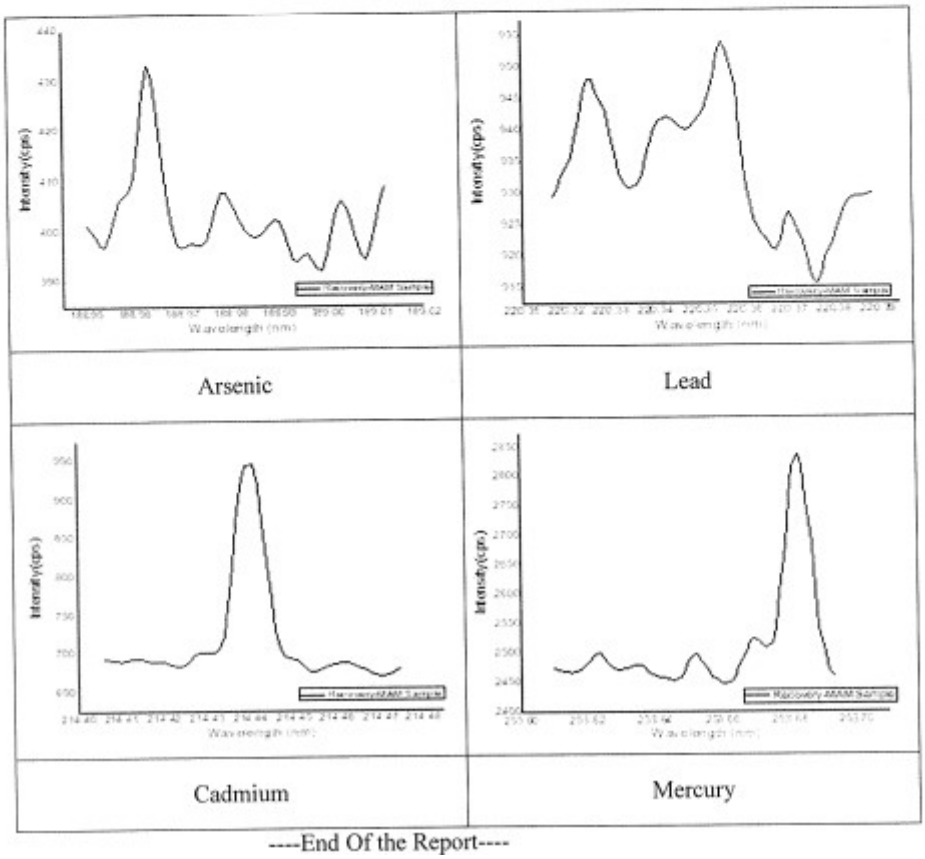


Figure 3: Heavy Metal Analysis



Recovery:



----End Of the Report----

Figure 4: Heavy Metal Analysis

5. RESULTS:

Standardization tests were performed for MAM Granules as per standard testing protocol. Macroscopic features of the drug and powder respectively confirmed the authenticity of the raw drugs in MAM Granules (Figures 1). MAM Granules is found to be brownish yellow in color with a characteristic odor and bitter taste. The Physicochemical characteristics are presented in Table 2.

It is evident that there are no heavy metal particles found during the analysis by using an Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) Instrument. The result of the heavy metal analysis showed in Table 3 & Figure 2,3,4.

6. DISCUSSION:

The safety profile of AYUSH medicines is an essential need for global acceptance and wide-range utilization. Standardization of the quality of raw drugs (Herbal/Herbo-Mineral and Animal origins) is a big challenge to AYUSH systems. Developing authenticated quality standards for those raw drugs will enhance and ensure the safety and efficacy of the AYUSH medicines manufactured from them. This study aimed to standardize the quality standards of the components of MAM Granule.(9)

The pharmacognosy study shows the authentication of herbal components used for the MAM Granule preparation.

Moisture contents of MAM Granule denoted by loss on drying was found to be 13.36%. The total ash value of MAM Granule discloses the salts content of the drug which was identified as 3.636%. The water-soluble ash in the MAM Granule predicts the inorganic content as 2.380%. The acid-insoluble ash was found to be 0%. The water-soluble extractives of the formulation are 14.27%. The Acid-soluble extractives of the formulation are 6.49%. The PH value of the MAM Granule was estimated as 6.0 which indicates the acidic nature of the MAM Granule. (Physico-Chemical Parameters were listed in Table.2).

Based on Heavy metal analysis, in 2 various samples, it was found MAM granules are free from heavy metal toxicity which ensures the safety of the medicine.

7. CONCLUSION:

The results from the powder microscopy study reveal the authenticity of the raw drugs of MAM Granules. It gives a clear picture of the quality standards of individual raw drugs as

well as combined medicine.. Heavy metal analysis shows the safety parameters of the drug. The results of this study will help to arrive a complete monograph of MAM Granule.

8. ACKNOWLEDGMENT:

1. Prof.Dr. Bhushan Patwardhan, Chairmen, Publication Management Unit (PMU) for COVID-19, Min. of Ayush, Govt. of India.
2. Prof. Dr S. P. Thiagarajan, Chancellor, Avinashilingam Institute for Home Science and Higher Education for Women (Deemed to be University), (Former Vice-Chancellor, University of Madras)
3. Prof. Dr Jugal Kishore, Director, HOD- Department of Community Medicine, VMMCH & Safdarjung Hospital, New Delhi.

9. CONFLICT OF INTEREST:

The authors declare no conflict of interest**.**

10. REFERENCE:

1. Quality Control Methods for Medicinal Plant Materials, WHO, Geneva. Delhi: AITBS Publishers and Distributors, 2002; p.65-67.
2. Pulok MM. Quality Control of Herbal Drugs of Inda. 2005; p.273-4.
3. Houghton PJ, Raman A. Laboratory Handbook for the Fractionation of natural extracts. London: Chapman and Hall, 1998; p.154-62.
4. Sethi PD. High-Performance Thin Layer Chromatography. New Delhi: CBS Publishers and Distributors; 1996.
5. Wallis TE. Analytical Microscopy – Its aims and methods in relation to foods, water, spices, and drug, Third edition. Boston: Little, Brown and company:1965
6. Wallis TE.Text Book of Pharmacognosy,5th Edition,CBS Publishers & Distributors,New Delhi ;2005
7. Kannusamy Pillai, Chikicha Rathna Deepam, B.Rathina Nayakar and Sons, Chennai, Edition-2011; Pages 27,28,312
8. Dileep Kumar Verma, Abdul Hasan, Manickavasagam Rengaraju, Shree Devi, Geetika Sharma, Vimal Narayanan, Sathiyarajeswaran Parameswaran, Thirumal Kumar D, Kanakavalli Kadarkarai, Sujatha Sunil,
9. Evaluation of Withania somnifera based supplement for immunomodulatory and antiviral properties against viral infection,Journal of Ayurveda and Integrative Medicine,Volume 15, Issue 5,2024,100955,ISSN 0975-9476,https://doi.org/10.1016/j.jaim.2024.100955. (https://www.sciencedirect.com/science/article/pii/S0975947624000706)

10. Srivastava, A., Rengaraju, M., Srivastava, S. 2021 A double-blinded placebo-controlled comparative clinical trial to evaluate the effectiveness of Siddha medicines, Kaba Sura Kudineer (KSK) & Nilavembu Kudineer (NVK) along with standard Allopathy treatment in the management of symptomatic COVID 19 patients - a structured summary of a study protocol for a randomized controlled trial. Trials 22, 130 (2021). https://doi.org/10.1186/s13063-021-05041-x

11. Manickavasagam Rengaraju et.al.(2021), An Open labelled RCT to Evaluate the Efficacy and Safety of Siddha Supplement (MAM Granules) along with Standard Allopathy Treatment in the management of RT PCR Positive Pre Symptomatic COVID-19 patients – A Structured Summary of A Study Protocol. Int J Clinical & Case. 5:4, 49-53