



Research Article

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STANDARDIZATION OF POLYHERBAL SIDDHA FORMULATION ASWATHY CHOORANAM

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ABSTRACT

Standardization of a siddha drug is required to set a standard for definitive quantitative and qualitative value, inherent characteristics, constant parameters to ensure quality, safety and efficacy and reproducibility by agreeing upon certain technical standards. It enables us to understand and develop specific standards for a particular drug Since the drugs are expected to exert desired outcomes at particular concentrations, there is an increasing need for their validation, scientific standards and uniformity for its Globalization. Also, with the increasing demand for traditional medicine among patients, there is a curiosity among the modern medicine practitioners to explore traditional medicine through a scientific approach. The poly herbal drug Aswathy chooranam from Agasthiyar vaithiya kaviyam has been subjected to various physicochemical analysis as per PLIM guidelines for standardization of chooranam such as, sterility test, test for pathogens and pesticides, HPTLC, TLC, Particle size determination, Heavy metal analysis and Aflatoxin assays and the results revealed that the formulation Aswathy chooranam has met the criteria of PLIM guidelines. This article might provide valuable insights for further usage and preparation of the drug.

Keywords: Aswathy chooranam, Standardization, Siddha drug, Polyherbal drug, Anaemia

INTRODUCTION



The ultimate objective of standardization is to have uniformity across manufacturers with respect to its chemical and biological properties. Standardization is mandatory to confirm the quality and reliability of traditional medicines¹. In this study the parameters for quality assessment have been followed as per guidelines for analytical specifications of chooranam It enables us to provide standards based on compatible technologies and processes inside a particular industry. Plants have been extensively used in all native medicines of which Siddha is the most prominent in South India. The drug Aswathy chooranam is obtained from the sastric siddha text “Agasthiyar vaithiya

kaaviyam 1500⁴. The drug is a polyherbal medicine and is indicated for the treatment of various diseases like PCOD, menorrhagia, anaemia, infertility, Arthritis, Scabies, Cough and Various toxins like scorpion bite². The ingredients of the drug have potent anti-inflammatory, antioxidant, anti-microbial and hematinic activities validated through various research articles.

MATERIALS AND METHODS

The drugs were procured from a country medical shop at Chennai and the authentication of drugs were obtained from the Botanist, National Institute of Siddha, Tambaram.

Table 1: Ingredients and preparation of Aswathy chooranam

Traditional name	Botanical name	Parts used	Quantity
 Chukku	<i>Zingiber officinale</i> Rosc.	Tuber (Dried)	15 grams
 Milagu	<i>Piper nigrum</i> Linn.	Seeds	15 grams

Thippili 	<i>Piper longum</i> Linn	Dried fruit spikes	15 grams
Saathipathiri 	<i>Myristica fragrens</i> Houtt.	Aril	15 grams
Saathikaai 	<i>Myristica fragrens</i> Houtt	Nut	15 grams
Omam 	<i>Carum copticum</i> Benth & Hook	Seeds	15 grams
Kurosani omam 	<i>Hyoscyamus niger</i> Linn	Seeds	15 grams
Katukurohani 	<i>Piccorhiza kurroea</i> Royle ex Benth	Root	15 grams
Athimathuram 	<i>Glycyrrhiza glabra</i> Linn	Root	15 grams
Kirambu 	<i>Syzygium aromaticum</i> Linn.	Dried flower buds	15 grams
Amukkara 	<i>Withania somnifera</i> Linn	Tuber	300 grams
Sarkarai 	<i>Saccharum officinarum</i> Linn	Stem	150 grams

The raw drugs were purified, and the medicine was prepared as per SOP in the Gunapadam Laboratory of NIS, Chennai. The raw drugs were purified separately through de weeding, drying, removing the outer skin, and washing in clean water. They were further dried in the sunlight until there was no moisture content. Then they were fried in a pan individually until they were dry. Further the medicinal drugs were powdered in required amounts and mixed along with powdered fine sugar. The homogenized mixture was stored in an airtight container and was sent for further analysis ².

Physicochemical Evaluation

The physicochemical evaluation was carried out through Sample Description study, Solubility Profile, Percentage Loss on Drying, Determination of Total Ash, Determination of Acid Insoluble Ash, Determination of Alcohol Soluble Extractive, Determination of Water-Soluble Extractive and pH determination,

The Percentage Loss on Drying was determined by precisely weighing the experimental medication in an evaporating dish. The sample was then dried at 105 °C for 5 hours and subsequently re-weighed.

The Total Ash content was assessed by accurately weighing the test drug in a silica dish and incinerating it in a furnace at a temperature of 400 °C until it reached a white color, indicating the absence of carbon. The percentage of total ash was then calculated based on the weight of the air-dried drug.

Determination of Acid Insoluble Ash: The ash from the total ash test underwent boiling with 25 ml of dilute hydrochloric acid for 6 minutes. The resulting insoluble matter was gathered in a crucible, washed with hot water, and then heated until a constant weight was achieved. The percentage of acid insoluble ash was then determined based on the weight of the air-dried ash.

Determination of Alcohol Soluble Extractive: The test sample was mashed with 100 ml of alcohol in a sealed flask for twenty-four hours, with frequent shaking for six hours and then allowing it to settle for eighteen hours. Filter the mixture quickly, while being careful to avoid losing any of the solvent. Then, evaporate 25 ml of the filtered liquid until it dries in a weighed flat-bottomed shallow dish, and dry it at 105°C until a constant weight is achieved, then weigh it. Finally, calculate the percentage of alcohol-soluble extractive in relation to the air-dried drug

Determination of Water-Soluble Extractive: The test sample was mixed with 100 ml of chloroform water in a sealed flask for twenty-four hours, with frequent shaking for six hours and then allowing it to settle for eighteen hours. Quickly filter the mixture, making sure not to lose any solvent, then evaporate 25 ml of the filtered liquid in a tared flat-bottomed shallow dish until it is completely dry, and then dry it at 105°C until the weight remains constant. Finally, calculate the percentage of water-soluble extractive in relation to the air-dried drug.

pH determination: The specified amount of test sample was mixed with distilled water and then tested using a pH meter.

Particle size determination by microscopic method: Optical microscopic analysis was used to determine the particle size. wherein the sample was diluted at a 1/100th dilution in sterile distilled water. Mounted on the slide and secured with a suitable stage was a diluted sample. To determine the average particle size, scale micrometers were used to make light microscopic images. A minimum of thirty observations were conducted in order to determine the sample's mean average particle size.

Heavy metal analysis by Atomic Absorption Spectrometry

Standard: Hg, As, Pb and Cd – Sigma Methodology.

One of the most popular and trustworthy methods for identifying metals and metalloids in environmental materials is atomic absorption spectrometry (AAS). The total heavy metal content of the sample was performed by Atomic Absorption Spectrometry (AAS) Model AA 240 Series. in order to ascertain the test item's quantities of heavy metals like lead, cadmium, arsenic, and mercury. The test sample was digested using 1mol/L HCl in order to measure the levels of mercury and arsenic. Similarly, 1mol/L of HNO₃ was used to digest the sample in order to determine the levels of lead and cadmium.

Sterility test by pour plate method: The product's sterility was established using the pour plate techniques. When a contaminated or non-sterile sample (formulation) comes into touch with a medium rich in nutrients, it encourages the growth of the organism. After the required amount of time, the organism's growth is recognized by a distinctive pattern of colonies. The colonies are known as CFUs, or colony forming units.

Test for specific pathogen: Test sample was infected in sterile petri plate to which roughly 15 mL of molten agar 45°C were added. The dish was tilted and swirled to properly mix the agar and sample. The agar was left undisturbed until it fully gelled. (about ten minutes). After that, plates were turned over and incubated for a further 48 hours at 37 °C before being left open for a further 72 hours to observe fungal development. The number of grown organism colonies was then determined for CFU. Using the pour plate method, the test sample was directly inoculated into the designated pathogen medium (EMB, DCC, Mannitol,

Cetrimide). For observation, the plates were incubated for 24 to 72 hours at 37°C. Existence of a particular pathogen recognized by its unique color in relation to the pattern of colony formation in every type of media.

TLC Analysis

Test sample was subjected to thin layer chromatography (TLC) as per conventional one-dimensional ascending method using silica gel 60F254, 7X6 cm (Merck) were cut with ordinary household scissors. Soft pencil was used to mark plates. A 10-micro liter sample was applied using a micro pipette, which was positioned five tracks apart at a distance of one centimeter each. Following the run plates' drying and observation using visible light, they were placed in the twin trough chamber equipped with the designated solvent system. Light long-wave UV light (365 nm) and short-wave UV light (254 nm)⁵

High Performance Thin Layer Chromatography Analysis

The HPTLC technique is a contemporary, advanced, and automated form of chromatography that evolved from traditional thin layer chromatography (TLC). It utilizes pre-coated HPTLC plates and an automatic sampler to ensure accuracy, sensitivity, and significant separation, both in terms of quality and quantity, in both qualitative and quantitative aspects. High performance thin layer chromatography (HPTLC) serves as an effective tool for assessing the quality of botanical substances in a cost-efficient and efficient manner. This technique offers a high level of selectivity, sensitivity, and speed, along with a straightforward method for sample preparation, making it an ideal choice for regular quality control testing. It provides a chromatographic profile of phytochemicals, which is useful for verifying the identity and purity of plant-based therapeutics.⁵

Chromatogram Development: Regular samples were mixed with a solution of chloroform and acetonitrile in a 9.8:0.2 ratio to create a solution with concentrations of 0.5 µg per ml for both aflatoxin B1 and aflatoxin G1, and 0.1 µg per ml for both aflatoxin B2 and aflatoxin G2

Scanning: Plates were scanned under UV at 366nm. The data obtained from scanning were brought into integration through CAMAG software. Chromatographic fingerprint was developed for the detection of phytoconstituents present in each sample and their respective R_f values were tabulated.

Aflatoxin assay by TLC (B1, B2, G1, G2)

Standard: Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2

Solvent: Regular samples were mixed with a solution of chloroform and acetonitrile in a 9.8:0.2 ratio to create a solution with concentrations of 0.5 µg per ml for both aflatoxin B1 and aflatoxin G1, and 0.1 µg per ml for both aflatoxin B2 and aflatoxin G2.

Procedure: Aflatoxin standard solution was evenly spread across the TLC plate's surface using volumes of 2.5 µL, 5 µL, 7.5 µL, and 10 µL. In a similar manner, the test sample was positioned, and the spots were left to dry before the chromatogram was developed in a chamber that was not saturated. This was achieved by immersing the plate in a solvent mixture, which included a combination of chloroform, acetone, and isopropyl alcohol in a ratio of 85: 10: 5. The solvent front had to advance at least 15 cm from the starting point. After this, the plate was taken out of the developing chamber, the solvent was marked, and the plate was left to air dry. The positions of the spots were identified by observing them under UV light at 365 nm.⁶

RESULTS AND OBSERVATION

Aswathy chooranam – Gross appearance



Table 2: Sample description

State	Solid
Nature	Fine powder
Odour	Strongly Aromatic
Touch	Soft
Flow Property	Free flowing
Appearance	Pale Brownish

Table 3: Solubility profile

Solvent Used	Solubility / Dispersibility
Chloroform	Insoluble
Ethanol	Soluble
Water	Soluble
Ethyl acetate	Insoluble
DMSO	Soluble

Table 4: Physiochemical evaluation of Aswathy chooranam

Parameter	Mean (n=3) SD
Loss on Drying at 105 °C (%)	11.3 ± 1.2
Total Ash (%)	8.133 ± 1.595
Acid insoluble Ash (%)	0 %
Water soluble Extractive (%)	16.37 ± 2.46
Alcohol Soluble Extractive (%)	8.2 ± 1.58
pH	6.5

Table 5: Results on acid and basic radicals

Experiment	Observation	Inference
Test for Acid radicals		
Test for sulphate : 2ml of the above prepared extract was taken in a test tube , to this was added 2ml of 4% ammonium oxalate solution	No cloudy appearance was noted	Absence of sulphate
Test for chloride : 2ml of the above prepared extract was added with diluted HNO ₃ till the effervescence ceases. Then 2ml of silver nitrate solution was added .	No cloudy appearance was noted	Absence of chloride
Test for carbonate : 2ml of the extract was treated with 2ml of magnesium sulphate solution	No cloudy appearance was noted	Absence of carbonate
Test for Basic radicals		
Test for calcium : 2ml of the extract was added with 2ml of 4% ammonium oxalate solution	No cloudy appearance seen	Absence of calcium
Test for magnesium : To 2ml of the extract sodium hydroxide solution was added in drops to excess	No white precipitate was seen	Absence of magnesium
Test for ammonium : To 2ml of the extract few ml of Nessler's reagent and excess of sodium hydroxide solution were added	No brown colour appeared	Absence of ammonium
Test for mercury : 2ml of the extract was treated with 2ml of sodium hydroxide solution	No yellow precipitate appeared	Absence of mercury
Test for arsenic : 2ml of the extract was treated with 2 ml of sodium hydroxide solution	No brownish red precipitate appeared	Absence of arsenic
Test for iron : To the 2ml of extract 2ml of ammonium thiocyanate solution was added To the 2ml of extract 2ml ammonium thiocyanate solution and 2ml of Conc HNO ₃ was added	Mild red colour appeared Blood red colour appeared	Presence of Iron Presence of Iron
Test for Zinc : To 2ml of the extract sodium hydroxide solution was added in drops to excess	No white precipitate appeared	Absence of zinc

Miscellaneous		
Test for reducing sugars : 5ml of Benedict's qualitative solution was taken in a test tube and allowed to boil for 2 minutes and added 8 to 10 drops of the extract and again boil it for 2 minutes. The colour changes were noted	Brick red colour appeared	Presence of reducing sugars
Test for alkaloids : 2ml of the extract was treated with 2ml of picric acid	Yellow colour developed	Presence of alkaloids
Test for amino acids : 2 ml of the extract was placed on a filter paper and dried well	No violet colour appeared	Absence of amino acids

Heavy metal analysis of the contents of Aswathy chooranam: Standard preparation As & Hg- 100 ppm sample in 1mol/L HCl Cd & Pb- 100 ppm sample in 1mol/L HNO₃ Test Report Name of the Heavy Metal Absorption Max Λ max Result Analysis Maximum Limit Lead 217.0 nm 1.846 10 ppm Arsenic 193.7 nm BDL 3 ppm Cadmium 228.8 nm BDL 0.3 ppm Mercury 253.7 nm 0.274 1 ppm.

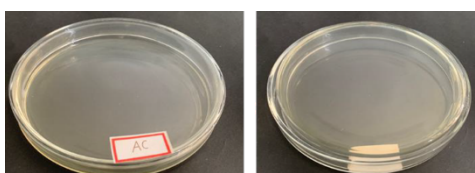
Table 6: Heavy metal analysis of the contents of Aswathy chooranam

Name of the Heavy Metal	Absorption Max Λ max	Result Analysis	Maximum Limit
Lead	217.0 nm	1.846	10 ppm
Arsenic	193.7 nm	BDL	3 ppm
Cadmium	228.8 nm	BDL	0.3 ppm
Mercury	253.7 nm	0.274	1 ppm

BDL- Below Detection Limit

Report and Inference: Results of the present investigation have clearly shown that the sample has no traces of heavy metal such as Arsenic and Cadmium were as the sample evident the presence of Lead and Mercury at 1.846 and 0.274 ppm as listed in the table.

Sterility test by pour plate method



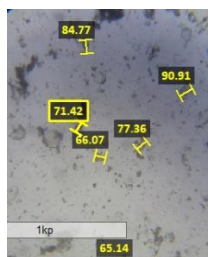
Observation: No growth was observed after incubation period reveals the absence of specific pathogen

Result: No growth / colonies was observed in any of the plates inoculates with the test sample.

Table 7: Sterility test - Results

Test	Result	Specification	As per AYUSH/WHO
Total Bacterial Count	Absent	NMT 10 ⁵ CFU/g	As per AYUSH specification
Total Fungal Count	Absent	NMT 10 ³ CFU/g	

Particle size



Microscopic observation of the particle size analysis reveals that the average particle size of the sample was found to be $76.29 \pm 14.26 \mu\text{m}$ ⁷

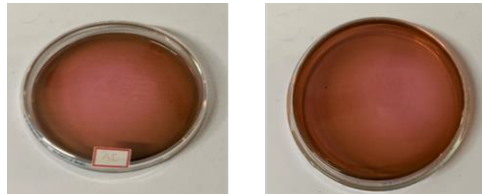
Test for specific pathogen

Table 8: Detail of Specific Medium and their abbreviation

Organism	Abbreviation	Medium
<i>E-coli</i>	EC	EMB Agar
<i>Salmonella</i>	SA	Deoxycholate agar
<i>Staphylococcus Aureus</i>	ST	Mannitol salt agar
<i>Pseudomonas Aeruginosa</i>	PS	Cetrimide Agar

Organism	Specification	Result	Method
<i>E-coli</i>	Absent	Absent	As per AYUSH specification
<i>Salmonella</i>	Absent	Absent	
<i>Staphylococcus Aureus</i>	Absent	Absent	
<i>Pseudomonas Aeruginosa</i>	Absent	Absent	

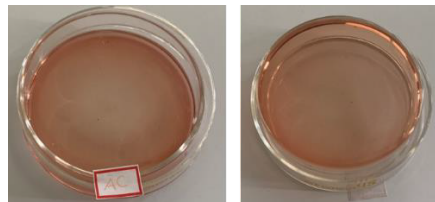
Culture plate with E-coli (EC) specific medium



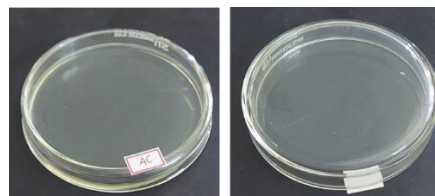
Culture plate with Salmonella (SA) specific medium



Culture plate with Staphylococcus Aureus (ST) specific medium



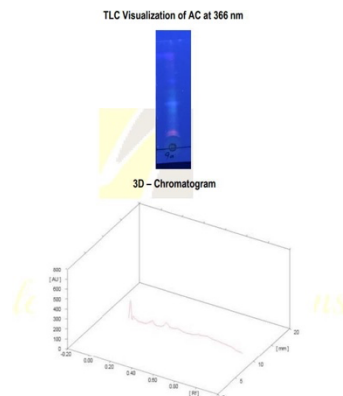
Culture plate with Pseudomonas Aeruginosa (PS) specific medium



Observation: No growth was observed after incubation period. Reveals the absence of specific pathogen

Result: No growth / colonies were observed in any of the plates inoculated with the test sample.

HPTLC Analysis



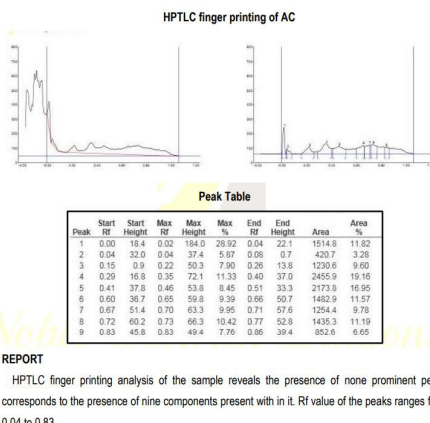


Table 9: Test for pesticides residue in Aswathy chooranam

Organo Chlorine Pesticides	Sample AC	Ayush limit (mg/Kg)
Alpha BHC	BQL	0.1mg/kg
Beta BHC	BQL	0.1mg/kg
Gamma BHC	BQL	0.1mg/kg
Delta BHC	BQL	0.1mg/kg
DDT	BQL	1mg/kg
Endosulphan	BQL	3mg/kg
Organo Phosphorus Pesticides		
Malathion	50 µg/kg	1mg/kg
Chlorpyrifos	BQL	0.2 mg/kg
Dichlorvos	BQL	1mg/kg
Organo carbamates		
Carbofuran	BQL	0.1mg/kg
Pyrethroid		
Cypermethrin	BQL	1mg/kg

BQL- Below Quantification Limit

Result: The results showed that there were no traces of pesticides residues such as Organo chlorine, Organo phosphorus, Organo carbamates and pyrethroids in the sample provided for analysis. Whereas the sample reveals the presence of mild traces of Malathion at 50 µg/kg which belongs to the category of Organo Phosphorus Pesticide^{8,9}

Table 10: Aflatoxin assay by TLC (B1, B2, G1, G2) in Aswathy chooranam

Aflatoxin	Sample AC	AYUSH Specification Limit
B1	Not Detected - Absent	0.5 ppm (0.5mg/kg)
B2	Not Detected - Absent	0.1 ppm (0.1mg/kg)
G1	Not Detected - Absent	0.5 ppm (0.5mg/kg)
G2	Not Detected - Absent	0.1 ppm (0.1mg/kg)

Result: The results shown that there were no spots were being identified in the test sample loaded on TLC plates when compared to the standard which indicates that the sample were free from Aflatoxin B1, Aflatoxin B2, Aflatoxin G1 and Aflatoxin G2.

DISCUSSION

Chooranam occupies a major part of Siddha formulations due to its easy preparation and dispensing factors. Aswathy chooranam is a polyherbal drug obtained from the Siddha sastric text Agasthiyar vaithiya kaviyam 1500 in which the major ingredient is Amukkara (*Withania somnifera*) a highly potent herb with antioxidant, anti-inflammatory, anti-cancerous and haematinic activity¹⁰. The chooranam is rich in alkaloids such as Withaferin A, Glycyrrhizin, Gingerone A, Piperine, Grandisin, Myristicin, Thymol, Hyoscyamide and vitexin^{11,12,13}. These alkaloids are antioxidants and have haematinic activity, anti-cancerous activity and anti-inflammatory activities. The drug Aswathy chooranam is strongly aromatic and did not contain any heavy metals such as arsenic, cadmium and mercury and pathogenic organisms such as *E. coli*, *Salmonella*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. There were no lethal levels of pesticide residues such as

Organophosphorus, Organo chlorine, Organo carbonates and Pyrethroids. The drug Aswathy chooranam was free from Aflatoxins A, B1, B2, G1 and G2. The HPTLC analysis shows the presence of nine phytochemicals Rf value of the peaks are between 0.04 to 0.83. Biochemical analysis shows the presence of Iron, alkaloids and reducing sugar and absence of arsenic, sulphide, chloride, carbonate, magnesium, ammonia, mercury, arsenic and zinc. These observations validate that the Siddha polyherbal formulation Aswathy chooranam is a formulation prepared as per PLIM guidelines.

CONCLUSION

The polyherbal formulation Aswathy chooranam has been prepared according to sastric methodologies. The standardization was carried in accordance with the PLIM criteria for traditional medicine standard procedures. Standardization of the test drug is the preliminary step for assessing and validating the pharmacological activities. The results and observations have exhibited certain diagnostic characters that will help to identify and prepare the Chooranam and add a valuable data to the evolving scientific standards for Siddha. Further clinical trials may add more significance to this work and bioassays.

REFERENCES

1. VethaMerlin Kumari H *et al*, Standardization of a Siddha poly herbal formulation Adathodai chooranam, International journal of Research In Ayurveda And Pharmacy 6(5) :609-615
2. Agasthiyar, Agasthiyar vaithiya kaviyam 1500, Edition 2001, Pg no 293, Thamarai noolagam, Chennai
3. India Pharmacopeia I Volume I, Government of India, Ministry of Health and Family welfare, Indian Pharmacopeia commission, 2014.
4. Pharmacopoeial Laboratory for Indian Medicine (PLIM) Guideline for standardization and evaluation of indian medicine which include drugs of Ayurveda, Unani and Siddha systems. Department AYUSH. Ministry of Health & Family Welfare, Govt. of India
5. Wagner H. Plant Drug Analysis. A thin Layer chromatography Atlas. 2nd ed. Heidelberg: Springer-Verlag Belgium; 2002:305, 227.
6. Luciana de CASTRO. Determining Aflatoxins B1, B2, G1 and G2 in Maize Using Florisil Clean Up with Thin Layer Chromatography and Visual and Densitometric Quantification. Ciênc. Tecnol. Aliment. vol.21 no.1 Campinas. 2001
7. Xu Z. Particle and Size Distribution. Fundamentals of Air Cleaning Technology and Its Application in Cleanrooms. 2013;1-46. Published 2013 Aug 7. DOI:10.1007/978-3-642-39374-7_1
8. WHO guideline for assessing the quality of herbal medicines with reference to contaminants and residues. WHO Geneva. 2007.
9. Lohar. D.R. Protocol for testing of ASU medicines. Pharmacopoeial Laboratory for Indian Medicines. Ministry of AYUSH. 2007
10. G.Singh, PK Sharma ,Biological activities of *Withania somnifera*, Annuals of biological research, Scholars biological research, 2010;1(3) :56-63
11. Pastorino G, Cornara L, Soares S, Rodrigues F, Oliveira MBPP. Liquorice (*Glycyrrhiza glabra*): A phytochemical and pharmacological review. Phytoter Res. 2018;32(12)::2323-2339
12. Arya Kadukkattil Ramanunni, Sheetu Wadhwa, Sachin Kumar Singh. *Myristica fragrans* (Houtt.). Herbs, Spices and Their Roles in Nutraceuticals and Functional Foods, 2023: 279- 307
13. Boskabady MH, Alitaneh S and Alavinezhad A. *Carum copticum* L.: A herbal medicine with various pharmacological effects. BioMed research international, 2014;569087.

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