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Formulation and Standardization of Proprietary Ayurvedic Medicine - Manas Satva Tablet

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ABSTRACT: Manas Satva tablet is a proprietary Ayurvedic poly-herbal formulation widely used in depression and stress. Its contains polyherbs. This study aims to standardize the formulation using advanced analytical techniques and pharmacopeia standards to evaluate raw materials including physicochemical studies and safety profile including heavy metals analysis and microbial limit test evolutions, were carried out by Ayurvedic Pharmacopoeia of India. The Preformulation parameters and parameters for the finished product include uniformity of weight, disintegration time, moisture content, pH, phytochemical estimation, and microbial load assay. Regarding bacteria, it was discovered that the microbial load was safe for human consumption. The present study revealed that there was no significant variation in analytical values. These methods will assist drug manufacturers in adhering to regulations and substantiating their goods' stability, safety, and therapeutic efficacy.

KEYWORDS: Mental Depression, Manas Satva tablet, Formulation, Quality control, Standardization, Heavy metals, Microbial limit test, Accelerated Stability testing

INTRODUCTION

Depression is a prevalent, crippling, and sometimes fatal illness. Depression is thought to affect about 300 million people worldwide, and the World Health Organization lists it as the primary cause of disability worldwide. Additionally, there is proof that throughout the past ten years, depression rates have gone up (WHO, 2012, 2014). [1]

Plants or plant parts that are transformed into Phytopharmaceuticals by straight forward procedures like harvesting, drying, and storing are referred to as "herbal drugs." As such, they can vary. [2] Herbal medicine standardization refers to establishing a set of unchanging criteria, unchanging standards, and unambiguous qualitative and quantitative values that guarantee safety, effectiveness, and quality.

Manas Satva tablet is a proprietary Ayurvedic polyherbal formulation comprising variant proportions of herbs such as *Celastrus Painculatus- Seed, Acorus Calamus- Rhizome, Anacyclus pyrethrum- Root, Bacopa monnieri* - whole plant, *Mucuna pruriens* - seeds. Manas Satva tablet is useful for depression and stress.

This research aims to use pharmacopoeial standards and sophisticated analytical methods to standardize the Manas Satva tablet. A series of tests will be carried out utilizing both traditional and contemporary methods in order to evaluate the quality of the standardized formulation. These tests will include physicochemical parameters, heavy metal analysis, microbial testing, and stability.

MATERIAL AND METHODS

1. Selection of plant material: The selected plant materials viz *Celastrus Painculatus, Acorus Calamus, Anacyclus pyrethrum, Bacopa monnieri,* and *Mucuna pruriens*, and the process material was *Bacopa Monnieri*.

2. Standardization of raw material: The Ayurvedic Pharmacopoeia of India is followed in the organoleptic evaluation and determination of foreign organic matter in raw materials.

3. Physicochemical studies: The ash values, extractive values, and loss on drying were performed according to the official methods prescribed in Ayurvedic Pharmacopoeia of India.

4. Preparation of polyherbal formulation by wet granulation method: Prepare Ghan of Herbs Using the traditional method (boiling with DM water, filtering, and boiling the filtrate until it reaches the desired dryness level). Brahmi Ghan and Kaucha Ghan were then Combined with all ingredients in kharal. Give Bhavana of Brahmi svaras liquid after the mixing process, thoroughly mardan it, then dry it and give it another dose of Bhavana of Brahmi svaras liquid. Repeat this process six times. Dry and combine gum acacia with a small amount of DM water to bind this bulk. Form moist clumps. Use a tray dryer to dry it. After that, run through a multimill to obtain granules of the right size. Notify the QC department about the Granules IPQC tests. Utilizing a tablet punching machine, create tablets once the QC Department has given its approval. Weigh tablets often while they're operating. Place the tablets in a coating pan and apply the designated color. After drying, gather in a storage container. Send a sample to Q.C. Laboratories for bulk analysis.

5. Drugs and Chemicals: Manas Satva tablet is a proprietary Ayurvedic poly-herbal formulation of Namo Nakshatra Health Care Pvt. Ltd., Halol. All other chemicals and solvents of analytical grade were purchased at Loba Chemical Pvt. Ltd.

6. Physicochemical Parameters:

Description: Various parameters such as colour, taste, shape, and odour of the samples of the Manas Satva tablet were observed and recorded.

Average weight: From each test product, twenty tablets were chosen at random and weighed both separately and collectively. A percentage deviation was discovered and recorded. [3]

Friability: Ten tablets from each test product were chosen at random. The starting weight was established. A Roche friabilator was filled with tablets and rotated 100 times. After dusting, the final weight was established, and the percentage of loss was computed and recorded. [4]

Disintegration Time (D.T.): The disintegration test apparatus machine IP standard (Campbell Electronics, Mumbai) was used for D.T. studies. The experiment was run at 37± 2. [5]

Hardness: The pressure required to break six randomly selected tablets along their diametrical axis was measured using a Monsanto-style tablet hardness tester to ascertain the tablets' level of hardness. The result was expressed in terms of kg/cm^2 as the average of six readings. [5]

pH (5% Aqueous Solution): A glass electrode, a reference electrode, and a digital or analog pH meter can be used to potentiometrically measure the pH of a liquid. Measure the pH at the same temperature as the standard solutions after submerging the electrodes in the solution under investigation. Once a series of measurements is completed, note the pH of the solution that was used to calibrate the electrodes and meter. The measurements need to be repeated if there is a difference of more than 0.05 between this reading and the initial value. Make sure the glass electrode is appropriate for use in alkaline conditions and make any necessary corrections when measuring pH values higher than 10.0. All solutions and suspensions of substances being examined must be prepared using carbon dioxide-free water. [6]

Determination of Total Ash: In a tared platinum or silica dish, incinerate 2 to 3 g precisely weighed of the ground drug at a temperature not to exceed 450º until it is carbon-free. Then, chill the mixture and weigh it. If this method is not able to produce carbon-free ash, then use hot water to extinguish the burned mass, gather the residue on ashless filter paper, burn the residue and filter paper together, add the filtrate, evaporate until dry, and ignite at a temperature not higher than 450º. Determine the ash percentage in relation to the medication that has been air-dried. [7]

Determination of Acid Insoluble Ash: Boil the ash obtained in (7) for 5 minutes with 25 ml of dilute hydrochloric acid; collect the insoluble matter in a Gooch crucible, or Wash with hot water on ashless filter paper, then ignite to constant weight. Calculate the percentage of acid-insoluble ash concerning the air-dried drug. [8]

Determining the Water Soluble Extractive Weigh an appropriate amount (based on the fixed oil content) of the dried and crushed drug, then place it in an extraction thimble. Utilize a Soxhlet extractor for continuous extraction with a mixture of chloroform and water for a duration of 24 hours. After extraction, quantitatively filter the extract into a pre-weighed evaporating dish and evaporate the solvent in a water bath. Proceed to dry the residue at 105º until a constant weight is achieved. Finally, calculate the percentage of chloroform water extractive in relation to the air-dried drug. [9]

Determination of Moisture Content (Loss on Drying): Procedure set forth here determines the amount of volatile matter (i.e., water drying off from the drug). For substances appearing to contain water as the only volatile constituent, the procedure given below is appropriately used. Place about 10 g of the drug (without preliminary drying) after accurately weighing (accurately weighed to within 0.01 g) it in a tared evaporating dish. For example, for underground or unpowdered drug, prepare about 10 g of the sample by cutting shredding so that the parts are about 3 mm in thickness. Seeds and fruits, smaller than 3 mm should be cracked. Avoid the use of high speed mills in preparing the samples, and exercise care that no appreciable amount of moisture is lost during preparation and that the portion taken is representative of the official sample. After placing the above said amount of the drug in the tared evaporating dish dry at 105º for 5 hours, and weigh. Continue the drying and weighing at one-hour interval until difference between two successive weighing corresponds to not more than 0.25 per cent. Constant weight is reached when two consecutive weighing after drying for 30 minutes and cooling for 30 minutes in a desiccator, show not more than 0.01 g difference. [10]

7. Heavy metal analysis:

An atomic absorption spectrophotometer (EC Electronics Corporation of India Limited AAS Element AS AAS4141) equipped with a hydride generator was used to measure trace elements and heavy metals. Hollow cathode lamps were utilized as the radiation sources for Ca, As, and Pb (Photron) and Al, Cu, Mg, Zn, Cd, and Hg (ECIL). The fuel that was used was air/acetylene. Nitrogen serves as the carrier gas. Chemicals: As and

Hg instrument standards were supplied, along with CPA chem standards for Ca, Zn, Cu, Mg, Cd, and Pb. E. Merck is the manufacturer of sodium borohydride, stannous chloride, nitric acid (HNO_3), hydrochloric acid (HCl), hydrogen peroxide (H_2O_2), sulfuric acid (H_2So_4), and hydrogen peroxide (H_2O_2). The company Reverse Osmosis WaterRions.

Manas Satva tablet preparation for the sample The wet digestion method was used to process the samples. In summary, 2 g of precisely weighed dried Manas Satva tablet sample was placed in a 100 ml beaker, 10 ml of nitric acid was added, and the mixture was heated on a hot plate at 95°C for 15 minutes. After the digest had cooled, 5 ml of concentrated nitric acid was added, and it was heated to 95°C for a further 30 minutes. The solution was reduced to roughly 5 ml without boiling by repeating the previous step. After cooling the sample once more, 3 ml of 30% hydrogen peroxide and 2 ml of deionized water were added. The sample was gradually heated in a covered beaker to initiate the peroxide reaction. The sample was taken off the hot plate and 30% hydrogen peroxide was added in 1 ml increments if the effervescence became too intense. The mixture was then slowly heated until the effervescence subsided. After adding 10 milliliters of deionized water and 5 milliliters of concentrated hydrochloric acid, the sample was heated for a further 15 minutes without boiling. After cooling, the sample was diluted to 50 ml with deionized water and filtered through the Whatman No. 42 filter paper. Sample analysis: Using a flame atomic absorption spectrophotometer, the digested samples were examined for Pb , Cd, Ca, Zn, Mg, Cu, and Al. For As , Hg, a hydride generation technique was employed. Hg was examined using an atomic absorption spectrophotometer in cold vapor. To create the calibration curve, standard dilutions of each metal were made in five different concentrations from the corresponding stock solution (1000 ppm). For both the standard solutions and the samples, every measurement was performed three times. The instrumental parameters used in the trace analysis. Recovery study: The standard addition method was used to validate the AAS method. A recovery study was conducted to show that our method is legitimate. A synthetic solution containing Ca, Mg, Zn, Cu, Al, Cd, Pb, and Hg was prepared to perform the recovery test. Samples were diluted to 50 milliliters and then portions were combined with the synthetic solution to determine the elements. [11]

8. Microbial Limit Test:

To count the number of bacteria on each plate, use 9–10 cm diameter Petri dishes. Add 1 ml of the pretreatment solution and up to 15 ml of liquefied casein soyabean digest agar (maximum volume: 450 per dish). Alternatively, apply the prepared preparation to the surface of the solidified medium in a Petri dish with the same diameter. If necessary, dilute the pretreatment preparation by the guidelines above to enable a maximum estimated colony count of 300. Prepare at least two of these Petri plates with the same dilution and incubate at 300 to 350 for five days, or until a more precise count is obtained in less time. Number the colonies that have been founded. When computing the results, use the plates with the most colonies, bearing in mind that the maximum number that is consistent with a complete evaluation is 300 colonies per plate. [12]

No. of colonies seen on SCDA plate:

$$\text{Therefore, Total Plate Count} = \frac{\text{No. of colonies x dilution facto}}{\text{Weight of sample plated}}$$

For fungi – Follow the instructions for the bacteria test, but instead of using casein soyabean digest agar, use Sabouraud dextrose agar with antibiotics. Incubate the plates at 20° to 25° for 5 days, or until a more trustworthy count is reached sooner. Utilizing plates with no more than 100 colonies, compute the findings.

$$\text{Therefore, Total Plate Count} = \frac{\text{No. of colonies x dilution factor}}{\text{Weight of sample plated}}$$

Tests for Specified Microorganisms

Sample preparation:

Dissolve the 5 gm Manas Satva tablet sample in 50 ml of buffered NaCl-peptone solution (pH -7.0) or any other suitable medium devoid of antimicrobial activity if testing in Lactose Broth, which has a pH of 7.2. Suppose the Manas Satva tablet is not soluble in the buffer. In that case, a surface active agent such as 0.1% w/v polysorbate 80 may be added to aid in the suspension of poorly wet table substances. They are kept at 37°C for two to four hours of incubation. 5 ml of the pH 7.2 Lactose Broth are pipetted into the Nutrient Broth (NB) and Soyabean Casein Digest broth (SCDB). Following that, both flasks are incubated at 37°C for 18 to 24 hours.

Escherichia coli: sample prepared in accordance with the guidelines. After pipetting out 1 milliliter of the SCDB sample, it was placed in a Durham's tube along with five milliliters of MacConkey Broth. At 36° to 44°C, incubate for 24 to 48 hours. After the incubation period, keep an eye out for gas and acid production, as these signify the early presence of *E. coli.*

Proceed to confirmatory testing in the event that the results are positive. On a MacConkey Agar plate, cultivate a subculture and incubate it at 30 to 35 degrees Celsius for 18 to 72 hours. The appearance of pink, non-mucoidal colonies raises the possibility of the presence of E. coli. Locate the aforementioned colonies, if any, and place them individually on the Levin eosin-methylene blue agar medium's surface before plating the mixture on a petri dish. After covering and flipping the plates, allow them to incubate. Upon examination, the sample meets the test's requirements for the absence of *E. coli* if the colonies don't exhibit both the characteristic blue-black appearance under transmitted light and a green metallic sheen under reflected light.

Salmonella: Sample in compliance with the protocol for sample preparation. Pipette out 1 milliliter of the NB sample and add it to 5 milliliters of Tetrathionate Broth Medium. The tube is then incubated at 37 °C for 18 to 24 hours. After that, they are checked to see if their color has changed from the previously mentioned subcultures of bismuth sulphate agar and xylose-lysine deoxycholate agar. Incubate the plate at 36 to 38 degrees for 24 hours. If, after inspection, none of the colonies match the description given below, the sample satisfies the test's requirements for the genus Salmonella's absence.

Staphylococcus aureus: sample prepared by the guidelines. Pipette out one milliliter of the NB sample and add it to five milliliters of Tetrathionate Broth Medium. The tube is then incubated at 37 °C for 18 to 24 hours. After that, they are scrutinized to check if their color has altered. from the previously stated subcultures of bismuth sulphate agar and xylose-lysine deoxycholate agar. Incubate the plate at 36 to 38 degrees for 24 hours. The sample meets the test criteria for the absence of the *Salmonella* genus if, upon observation, no colony matches the description provided below.

Pseudomonas aeruginosa: sample by the "Sample preparation method." To detect *Pseudomonas aeruginosa*, 0.1 ml of the solution is pipetted out of SCDB and streaked onto Cetrimide agar plates. Following the "Sample preparation method," the plates are inverted and incubated at 37 °C for 18 to 24 hours. Colonies that exhibit fluorescence when viewed under a UV light are then looked for. Pipetting 0.1 ml of the solution out of SCDB and streaking it onto

Cetrimide agar plates is how *Pseudomonas aeruginosa* is found. The plates are then incubated at 37 °C for 18 to 24 hours after being turned over. Subsequently, colonies on the plates that glow when exposed to UV light are discovered. [12]

4]. Accelerated Stability testing

The prepared tablet's Accelerated Stability study was conducted by ICH guideline Q1. A (R2). (11) The tablet was stored in milky white HDPE bottles and maintained at 40°C ±2°C &75% RH ± 5%. Hardness, pH (5% aqueous solution), total ash, acid insoluble ash, alcohol soluble extractive, water soluble extractive, and

moisture content were the parameters assessed every three months. Both at the start and the conclusion of the study period, the microbial load and qualitative estimation were completed. [13]

OBSERVATION AND RESULTS

Table 1: Physicochemical parameters of raw material used in the preparation of Manas-Satva Tablets.

Physicochemical parameters	***Celastrus Painculatus***	***Acorus Calamus***	***Anacyclus pyrethrum***	***Bacopa monnieri***	***Mucuna pruriens***
Foreign matter	0.90 %	0.85 %	0.68 %	0.86 %	0.82 %
Total Ash	3.68 %	6.92 %	8.27 %	11.35 %	3.05 %
Acid insoluble Ash	0.85 %	0.89 %	1.43 %	3.02 %	0.41 %
Water soluble Extractive	54.64 %	33.39 %	36.29 %	23.60 %	32.82 %
Loss on Drying	7.89 %	6.90 %	9.65 %	6.40 %	9.33 %
Alcohol Soluble Extractive	26.70 %	25.27 %	12.27 %	8.55 %	11.49 %

Table 2: Microbial load Analysis

Parameter	***Celastrus Painculatus***	***Acorus Calamus***	***Anacyclus pyrethrum***	***Bacopa monnieri***	***Mucuna pruriens***
Staphylococcus aureus/g	Absent	Absent	Absent	Absent	Absent
Salmonella spp. /g	Absent	Absent	Absent	Absent	Absent
Pseudomonas aeruginosa/g	Absent	Absent	Absent	Absent	Absent
E.coli /g	Absent	Absent	Absent	Absent	Absent
Total Plate Count (TPC)	12 cfu / g	20 cfu / g	24 cfu / g	32 cfu / g	Nil
Total Yeast & Mould Count	Nil	Nil	Nil	Nil	Nil

Table 3: Organoleptic parameters of Manas Satva Tablet

Parameters	**Tablet**
Color	Pink
Taste	Characteristic
Odor	Characteristic
Other	Round biconvex tablet

Table 4: Quantitative parameters of Manas Satva Tablet

Parameter	**Result**	**Specification**
Average Weight	535.19 mg	525 mg ± 5 %
Hardness	7.0 kg/sq.cm	Not less than 2.5 kg/sq.cm
Disintegration time	46 minutes.	Not more than 60 minutes.
Friability	0.16 %	Not more than 1 %

Table 5: Physicochemical parameters of Manas Satva Tablet

Parameter	Result	Specification
pH	4.95	4 to 6
Total Ash	17.80 %	Not more than 18 %
Acid insoluble Ash	11.41 %	Not more than 12 %
Water Soluble Extractive	38.11 %	Not less than 35 %
Loss on dry	6.19 %	Not more than 7 %

Table 6: Heavy metal determination of Manas Satva Tablet

Sr No.	Heavy metal	Standard limit (ppm)	Observation value (ppm)
1.	lead	Not more than 10 ppm	ND
2.	Arsenic	Not more than 3 ppm	0.07 ppm
3.	Cadmium	Not more than 0.3 ppm	ND
4.	Mercury	Not more than 1 ppm	ND

(ND - Not detected, ppm - parts per million)

Table 7: Microbial limit test of Manas Satva Tablet

Parameters	Ref.	Specification	Result
Staphylococcus aureus/g	API	Should be Absent	Absent
Salmonella spp. /g	API	Should be Absent	Absent
Pseudomonas aeruginosa/g	API	Should be Absent	Absent
E.coli /g	API	Should be Absent	Absent
Total Plate Count (TPC)	API	NMT 10^5 cfu /g	210 cfu / g
Total Yeast & Mould Count	API	NMT 10^3 cfu /g	Nil

(cfu – colony forming units)

Table 8: Accelerated stability testing of Manas Satva Tablet

Parameter	Initial	Three month	Six month
pH	4.95	5.02	4.98
Total Ash	17.80 %	13.18 %	17.18 %
Acid insoluble Ash	11.41 %	8.34 %	11.05 %
Water Soluble Extractive	38.11%	36.01 %	40.30 %
Loss on drying	6.19 %	6.51 %	6.15 %
Staphylococcus aureus/g	Absent	Absent	Absent
Salmonella spp. /g	Absent	Absent	Absent
Pseudomonas aeruginosa/g	Absent	Absent	Absent
E.coli /g	Absent	Absent	Absent
Total Plate Count (TPC)	210 cfu / g	300 cfu / g	420 cfu / g
Total Yeast & Mould Count	Nil	Nil	Nil

DISCUSSION

In the Manas Satva tab. Made up of the selected plant materials viz *Celastrus Painculatus, Acorus Calamus, Anacyclus pyrethrum* and *Mucuna pruriens*, and the process material was *Bacopa Monnieri*. The pre-formulation and formulation investigations of the formed polyherbal tablets were reviewed since it was first

developed and then assessed for quality herbal goods, which is crucial regardless of their medicinal content and therapeutic states. A physico-chemical analysis is necessary to verify the product's biological activity and quality. The Physicochemical Parameters are fundamental guidelines for product conformance and raw material selection. The tablet's pH of 4.95 (Table-5) indicated that it was somewhat acidic, according to the pH scale, which measures acidity and alkalinity. Measuring physical attributes such as pH helped prevent stomach discomfort, and measuring moisture content helped spot any weight gain caused by absorbing moisture. It was concluded that the obtained value fell within the permissible range.

The amount of inorganic substances a drug contains determines its ash value; this parameter is crucial for drug standardization and quality control. A drug's ash content increases with the amount of inorganic substances it contains. the higher its ash in this instance, the ash value was 17.80 % (Table-5). The higher its ash During the ash process, product components oxidize; an increase in ash value indicates contamination, adulteration, and substitution. The acid insoluble ash value indicates the presence of silicate impurities that may have developed as a result of improperly cleaning crude drugs, while the total ash value indicates the total amount of inorganic material that remains after incineration is completed. The two ash values found show that premium raw ingredients were used in the formulation process.

Different components are soluble in different kinds of media. There is exhausted material present when the value is less than the standard value. The extractive values—more especially, water-soluble values—show how much active ingredient, when extracted with the appropriate solvents, is present in a given amount of plant material. In the samples used in this study, the proportion of soluble principles was 38.11 (Table-5) percent in water. Because the tablet's sample was derived from a water extractive, it was more soluble in the media. Increases in water-soluble extractives were found during the solubility test, which suggests a higher bioavailability in a water medium.

"Tablet hardness" is defined as the force necessary to break a tablet in a test device that applies tension or bending stress. It is also an essential part of tablet quality control. This sample had an average hardness of 7.0 kg/sq.cm (Table-4). The physical characteristic known as "tablet friability" refers to a tablet's propensity to fracture into smaller pieces or to separate a portion of powder or powder loss from the tablet's exterior when subjected to mechanical and physical stress. The main purpose of disintegration testers is to gauge how long it takes for a sample or tablet to totally dissolve in a liquid medium. The average friability was 0.16 % (Table-4). 46 (Table-4) minutes were allotted to the disintegration process.

Renal toxicity is the result of heavy metals' detrimental effects on the body's organs, particularly the kidneys when they are present in formulations. Therefore, the evaluation of heavy metals is essential. Heavy metals include arsenic, cadmium, lead, and mercury. In the current study, heavy metals were evaluated using spectrophotometry, and it was found that all of the metals were within acceptable ranges. The absence of heavy metals is a sign of the purity of the final product and raw materials (Table-6).

Many populations of soil-dwelling bacteria and molds are commonly found in medicinal plant materials. The current formulation's microbiological count fell within permissible bounds, indicating that proper hygienic procedures were followed during formulation and packing.

To maintain the proper quality, safety, and efficacy of natural goods, manufacturers must ensure the lowest possible levels of microorganisms in the raw material, completed dosage forms, and packaging components.

The microbiological load is the total number of living microorganisms (bacteria and fungi) in one Manas Satva tablet. Generally speaking, the allowable limit is less than 1000 colony-forming units (CFU) per gram. Yeast and mold growth indicate contamination. Tablets of Manas Satva; these should be absent. Furthermore, no infections have been found at all for *Salmonella* species, *P. aeruginosa*, *E. coli*, and *S. aureus.* In terms of microbes, this suggested that it was safe for humans to consume (Table-7).

CONCLUSION

The present work was carried out for the formulation and standardization of Manas Satva tablet and raw materials. The various physico-chemical parameters was studied which was helpful for the detection of raw drug as well as the original formulation of Manas Satva Tablet. This evaluation could help establish the legitimacy of the medication and offer relevant information for the safer and more efficient application of this formulation in treatments.

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