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Phytochemical and Antimicrobial Screening of Proprietary *Ayurvedic* Medicine - N-Dopa Tablet

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ABSTRACT

The N-Dopa tablet is a proprietary *Ayurvedic* poly-herbal formulation widely used in Parkinson's disease, chronic fatigue syndrome, Myalgia and Muscular dystrophy. This study aims to standardize the formulation using advanced analytical techniques and pharmacopoeia standards. The physicochemical testing, heavy metal analysis and microbiological limit test evaluations were carried out in accordance with *Ayurvedic* pharmacopoeia of India. All the heavy metals were determined to be within the acceptable ranges. The extractive value revealed increases in water soluble extractive, which suggests higher bioavailability in a water medium. In addition, the microbial load was found to be safe for ingestion by humans in terms of microbes. This assessment could aid in determining the drug's authenticity and provide pertinent data for the safer and more effective use of this formulation in therapeutics. These methods will assist drug manufacturers in adhering to regulations and substantiating the stability, safety, and therapeutic efficacy of their goods.

Key words: Parkinson's disease, N-Dopa tablet, Quality control, Standardization, Heavy metals, Microbial limit test, Accelerated Stability testing

INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra region of the brain, leading to motor symptoms such as tremors, bradykinesia, and rigidity. The primary treatment for PD involves the use of levodopa (L-Dopa), a precursor of dopamine, which helps alleviate these symptoms by replenishing the depleted dopamine

levels in the brain.^[1]

The effectiveness of formulation is influenced by several factors, including the quality and standardization of the formulation. Standardization and quality control of herbal as well as the *Ayurvedic* products is most essential for the acceptance on the modern parameters. *Ayurveda* emphasizes the importance of standardization of medicinal herbs as well as the finished products on the basis of physical and chemical parameters.^[2]

Standardization of the N-Dopa tablet formulation is essential to ensure its quality, efficacy, and safety. This study will contribute to the establishment of standardized protocols for quality control of N-Dopa tablets, thereby enhancing the reliability and effectiveness of formulation.

This is the first report on the standardization of N-Dopa tablet; a polyherbal formulation comprises variant proportion of herbs such as *Mucuna pruriens*, *Sida cordifolia* and *Bhavana* of *Mashbaladi Kwath*. N-Dopa tablet is a proprietary *Ayurvedic* poly-herbal

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formulation widely used in Parkinson's disease, Chronic fatigue syndrome, Myalgia and Muscular dystrophy.

This research aims to standardize the N-Dopa tablet using advanced analytical techniques and pharmacopeial standards. The standardized formulation will undergo rigorous testing to evaluate its quality, including the Physicochemical parameters, Heavy metal analysis, Microbial testing, and stability by using standard and modern techniques.

MATERIAL AND METHODS

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

1] Physicochemical Parameters

Description

Various parameters such as colour, taste, and odour of the samples of N-Dopa tablet were observed and recorded.

Average weight: Twenty tablets were selected at random from each of the test products and weighed individually and all together. Percent deviation was found and documented.^[4]

Friability: 10 tablets were selected at random from each test product. Initial weight was determined. Tablets were put in Roche friabilator and run for 100 revolutions. Final weight was determined after dusting and % loss was calculated and documented.^[5]

Disintegration Time (D.T.): D.T. studies were carried out on disintegration test apparatus machine IP standard (Campbell Electronics, Mumbai). The test was carried out at 37 ± 2 .^[3]

Hardness: Using a Monsanto-style tablet hardness tester, the pressure needed to break six randomly chosen tablets along its diametrical axis was measured to determine the tablets' hardness. The average of six readings in terms of kg/cm² was used to express the outcome.^[3]

pH (5% Aqueous Solution): The pH value of a liquid can be determined potentiometrically by means of the glass electrode, a reference electrode and pH meter either of the digital or analogue type. Immerse the electrodes in the solution being examined and measure the pH at the same temperature as for the standard solutions. At the end of a set of measurements, record the pH of the solution used to standardize the meter and electrodes. If the difference between this reading and the original value is greater than 0.05, the set of measurement must be repeated.

When measuring pH values above 10.0 ensure that the glass electrode is suitable for use under alkaline conditions and apply any correction that is necessary. All solutions and suspension of substances being examined must be prepared using carbon dioxide-free water.^[6]

Determination of Total Ash: Burn approximately 2 to 3 g precisely weighed, of the ground medicate in a tared platinum or silica dish at a temperature not surpassing 450° until free from carbon, cool and weigh. On the off chance that a carbon free cinder cannot be gotten in this way, deplete the charred mass with hot water, collect the buildup on a fiery debris less channel paper, burn the buildup and channel paper, include the filtrate, dissipate to dryness, and touch off at a temperature not surpassing 450°. Calculate the rate of fiery debris with reference to the air-dried sedate.^[7]

Determination of Acid Insoluble Ash: Heat the ash from step (6) in boiling dilute hydrochloric acid for a duration of 5 minutes; gather the residue in a Gooch crucible or on a filter paper with minimal ash content, rinse with hot water, and heat until a constant weight is achieved. Determine the percentage of ash insoluble in acid in relation to the air-dried plant material.^[8]

Determination of 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 on a water

bath. Proceed to dry the residue at 105° 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):

The method outlined in this document determines the quantity of volatile matter, specifically the water that evaporates from the drug. When dealing with substances that seem to only contain water as the volatile component, the following procedure is suitable. Take approximately 10g of the drug (without prior drying) and place it in a tared evaporating dish after accurately weighing it (with an accuracy of 0.01 g). For instance, if dealing with illicit or unprocessed drugs, prepare a 10g sample by cutting and shredding it into approximately 3 mm thick parts. It is important to avoid using high-speed mills when preparing the samples and to ensure that no significant amount of moisture is lost during the preparation process. Additionally, the portion taken should be representative of the official sample. After depositing the specified quantity of the substance in the pre-weighed evaporating dish, heat at 105° for 5 hours, and measure the weight. Proceed with the drying and weighing every hour until the variance between two consecutive weighing is no greater than 0.25 percent. The equilibrium weight is achieved when two successive weighing following a 30-minute drying period and a 30-minute cooling period in a desiccator exhibit a difference of not more than 0.01g.^[10]

2] Heavy metal analysis

For the purpose of determining trace elements and heavy metals, an atomic absorption spectrophotometer (EC Electronics Corporation of India Limited AAS Element AS AAS4141) fitted with a hydride generator was employed. The radiation sources used were hollow cathode lamps for Al, Cu, Mg, Zn, Cd, Hg (ECIL) and Ca, As, and Pb (Photron). Air/acetylene was the fuel utilized. The carrier gas was nitrogen. Chemicals: Instrument standards for As and Hg were provided, along with standards for Ca, Zn, Cu, Mg, Cd, and Pb (CPA chem). E. Merck is the manufacturer of sodium borohydride, stannous

chloride, nitric acid (HNO₃), hydrochloric acid (HCl), hydrogen peroxide (H₂O₂), sulfuric acid (H₂SO₄), and hydrogen peroxide (H₂O₂). The company Reverse Osmosis Water Rions.

N-Dopa tablet preparation for the sample the wet digestion method was used to process the samples. In summary, 2g of precisely weighed dried N-Dopa 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 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. To perform the recovery test, a synthetic solution containing Ca, Mg, Zn, Cu, Al, Cd, Pb, and Hg was prepared. Samples were diluted to 50 milliliters and

then portions were combined with the synthetic solution to determine the elements.^[11]

3] Microbial Limit Test

Using Petri dishes with a diameter of 9 to 10 cm, count the number of bacteria on each plate by adding 1 ml of the pretreatment solution and up to 15 ml of liquefied casein soyabean digest agar, with a maximum volume of 450 per dish. As an alternative, cover the surface of the solidified medium in a Petri dish with the same diameter with the prepared preparation. If required, dilute the pretreatment preparation according to the preceding instructions to allow for an estimated colony count of no more than 300. Unless a more accurate count is obtained in less time, prepare at least two of these Petri plates using the same dilution and incubate at 300 to 350 for five days. Count how many colonies are established. Utilize the plates with the most colonies when calculating the findings, keeping in mind that 300 colonies per plate is the highest number that is consistent with a thorough assessment.^[12]

No. of colonies seen on SCDA plate

Therefore,

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

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.

Therefore,

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

Tests for Specified Microorganisms

Sample preparation:

If testing in Lactose Broth with a pH of 7.2, dissolve the 5 gm N-Dopa tablet sample in 50 ml of buffered NaCl-peptone solution (pH -7.0) or any other appropriate medium with no antimicrobial activity. To help with the suspension of poorly wet table substances, a surface

active agent such as 0.1% w/v polysorbate 80 may be added if the N-Dopa tablet is not soluble in the buffer. They are incubating for two to four hours at 37°C. Using a pipette, 5 ml of the pH 7.2 Lactose Broth is transferred to the Soyabean Casein Digest broth (SCDB) and Nutrient Broth (NB). After that, both flasks are incubated for 18 to 24 hours at 37°C.

Escherichia coli: sample in accordance with the sample preparation protocol. 1 milliliter of the SCDB sample was pipetted out and then transferred to 5 milliliters of MacConkey Broth in a Duram's tube. Incubate for 24 to 48 hours at 36° to 44°C. Observe for acid and gas production following the incubation period, as these indicate the early presence of *E. coli*.

If results are positive, move on to confirmatory testing. Grow a subculture on a MacConkey Agar plate and incubate it for 18 to 72 hours at 30 to 35 degrees Celsius. The development of pink, non-mucoidal colonies suggests that *E. coli* may be present. If the aforementioned colonies are located, place each one separately on the surface of the Levin eosin-methylene blue agar medium and plate it on a petri dish. Once the plates are covered and inverted, incubate. After inspection, if the colonies don't show both the distinctive blue-black look under transmitted light and a green metallic sheen under reflected light, the sample satisfies the test's requirements for the absence of *E. coli*.

Salmonella: Sample in accordance with the sample preparation protocol. 1 milliliter of the NB sample should be pipetted out and put into 5 milliliters of Tetrathionate Broth Medium. After that, the tube is incubated for 18 to 24 hours at 37 °C. They are then examined to see if their color has changed. From the subcultures on xylose-lysine dextrocholate agar and bismuth sulphate agar mentioned above. For 24 hours, incubate the plate at 36 to 38 degrees. 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 in accordance with the sample preparation protocol. 1 milliliter of the NB sample should be pipetted out and put into 5 milliliters

of Tetrathionate Broth Medium. After that, the tube is incubated for 18 to 24 hours at 37 °C. They are then examined to see if their color has changed. From the subcultures on xylose-lysine dexycolate agar and bismuth sulphate agar mentioned above. For 24 hours, incubate the plate at 36 to 38 degrees. 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.

Pseudomonas aeruginosa: sample in accordance with the "Sample preparation method." To detect *Pseudomonas aeruginosa*, 0.1 ml of the solution is pipetted out of SCDB and streaked onto Centrimide 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 Centrimide 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 Accelerated Stability study of prepared tablet was carried out as per ICH guideline Q1. A (R2).^[11] The tablet was kept at 40°C ±2°C/75% RH ± 5% and tablet was stored in Milky white HDPE bottles. The parameters evaluated every three months were Hardness, pH (5% aqueous Solution), determination of total Ash, Acid insoluble Ash, Alcohol Soluble Extractive, Water Soluble Extractive, Determination of Moisture content. The qualitative estimation, and microbial load was done at the beginning and at end study period.^[13]

OBSERVATION AND RESULTS

Table 1: Organoleptic parameters of N-Dopa Tablet

Parameters	Tablet
Color	Dark Black
Taste	Bitter

Odor	Characteristic
Other	Round biconvex tablet

Table 2: Quantitative parameters of N-Dopa Tablet

Parameter	Result	Specification
Average Weight	531.83 mg	525 mg ± 5 %
Hardness	8 kg/sq.cm	Not less than 2.5 kg/sq.cm
Disintegration time	42 minutes.	Not more than 60 minutes.
Friability	0.12 %	Not more than 1 %

Table 3: Physicochemical parameters of N-Dopa Tablet

Parameter	Result	Specification
pH	5.60	5 to 7
Total Ash	4.26 %	Not more than 8 %
Acid insoluble Ash	2.26 %	Not more than 4 %
Water Soluble Extractive	35.35 %	Not less than 32 %
Loss on dry	5.07 %	Not more than 7 %

Table 4: Heavy metal determination of N-Dopa Tablet

SN	Heavy metal	Standard limit (ppm)	Observation value (ppm)
1.	lead	Not more than 10 ppm	ND
2.	Arsenic	Not more than 3 ppm	0.91 ppm
3.	Cadmium	Not more than 0.3 ppm	0.19 ppm
4.	Mercury	Not more than 1 ppm	ND

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

Table 5: Microbial limit test of N-Dopa Tablet

Parameters	Ref.	Specification	Result
<i>Staphylococcus aureus</i> /g	API	Should be Absent	Absent
<i>Salmonella</i> spp. /g	API	Should be Absent	Absent
<i>Pseudomonas aeruginosa</i> /g	API	Should be Absent	Absent
<i>E.coli</i> /g	API	Should be Absent	Absent
Total Plate Count (TPC)	API	NMT 10 ⁵ cfu /g	300 cfu / g
Total Yeast & Mould Count	API	NMT 10 ³ cfu /g	Nil

(cfu – colony forming units)

Table 6: Accelerated stability testing of N-Dopa Tablet

Parameter	Initial	Three month	Six month
pH	5.60	5.65	5.75
Total Ash	4.26 %	6.95 %	7.79 %
Acid insoluble Ash	2.26 %	3.25 %	3.60 %
Water Soluble Extractive	35.35 %	34.79 %	36.23 %
Loss on dry	5.07 %	5.06 %	4.49 %
<i>Staphylococcus aureus</i> /g	Absent	Absent	Absent
<i>Salmonella</i> spp. /g	Absent	Absent	Absent
<i>Pseudomonas aeruginosa</i> /g	Absent	Absent	Absent
<i>E.coli</i> /g	Absent	Absent	Absent
Total Plate Count (TPC)	300 cfu/g	260 cfu/g	300 cfu/g
Total Yeast & Mould Count	Nil	Nil	Nil

DISCUSSION

All pharmaceutical steps were processed in the prescribed order, with any modifications duly documented. To confirm the biological activity and quality of the product, a physico-chemical analysis is required. The foundational guidelines for raw material selection and product conformance are the Physicochemical Parameters. The pH scale indicates the degree of acidity and alkalinity; the tablet's pH revealed that it was somewhat acidic. Which were 5.6 in this sample.

Physical characteristics like pH were measured to prevent stomach discomfort, and moisture content was measured to identify any weight gain brought on by moisture absorption. The obtained value was determined to be within the acceptable range. The ash value of a drug is determined by the quantity of inorganic substances it contains; this parameter is essential for drug standardization and quality control. The more inorganic substances that a drug contains, the higher its ash value. In this instance, the ash value was 4.26 %.

Product components oxidize during the ash process; an increase in ash value denotes adulteration, substitution, and contamination. The total ash value indicates the total amount of inorganic material that remains after incineration is finished, and the acid insoluble ash value indicates the presence of silicate impurities that may have developed as a result of improperly cleaning crude drugs. The two ash values obtained indicate that high-quality raw materials were utilized in the formulation process.^[14]

Distinct constituents exhibit solubility in varying media types. A lower value than the standard value indicates the presence of exhausted material. The extractive values, specifically water-soluble, indicate the amount of active constituent in a given amount of plant material when extracted with respective solvents. The percentage of soluble principles in the samples in this investigation was 35.35 percent in water. The tablet's sample had a higher solubility in the media because it came from a water extractive. The solubility test

revealed increases in water soluble extractive, which suggests higher bioavailability in a water medium.

The amount of force required to shatter a tablet in a test apparatus that applies a tension or bending stress is how "tablet hardness" is determined. It is also a crucial component of quality control for tablets. The average hardness of 8 kg/sq.cm was found in this sample. "Tablet friability" is a physical property that describes a tablet's tendency, under mechanical and physical stress, to break into smaller fragments or to separate a portion of powder or powder loss from the tablet's exterior. Disintegration testers are primarily used to measure how long it takes a tablet or sample to completely disintegrate within a liquid medium. Friability was 0.12% on average. There were 42 minutes for the disintegration.

If heavy metals are present in formulations, they will negatively impact the body's organs, especially the kidneys, and cause renal toxicity. Thus, the assessment of heavy metals plays a crucial role. Mercury, cadmium, lead, and arsenic are examples of heavy metals. Using spectrophotometry, heavy metals were assessed in the current study and all of the metals were determined to be within the acceptable ranges. The fact that there are no heavy metals indicates how pure the finished product and raw materials are.

Medicinal plant materials typically contain large populations of bacteria and molds that originate in the soil. The microbiological count in the current formulation was within allowable bounds, demonstrating that appropriate hygienic practices were adhered to throughout formulation and packing. *Salmonella species*, *Pseudomonas Aeruginosa*, *Staphylococcus Aureus*, and *Escherichia coli* were discovered by microbial testing. These findings suggest a lack of hygiene and faecal contamination during the production and storage of herbal formulations. The high degree of bacterial contamination seen in herbal medications may be caused by poor quality water utilized in the production process.

Manufacturers must guarantee the lowest feasible levels of microorganisms in the raw material, completed dosage forms, and packaging components

in order to preserve the appropriate quality, safety, and efficacy of natural goods. The total amount of living microorganisms (fungi and bacteria) contained in the N-Dopa tablet is known as the microbiological load. Generally, less than 1000 colony-forming units (CFU) per gram is the permitted limit. Mold and yeast growth are signs of contamination. N-Dopa tablets; these ought to be missing. Additionally, infections including *Salmonella species*, *P. aeruginosa*, *E. coli*, and *S. aureus* are entirely undetected. This indicated that it was safe for ingestion by humans in terms of microbes.

CONCLUSION

The formulation N-Dopa tablet was examined in this study for its physicochemical screening, heavy metal analysis, and microbiological limit test evaluations in accordance with standard pharmacopoeia procedures. In addition to helping to create a quality control profile for future use, this study can be used to qualitatively assess N-Dopa tablet using contemporary metrics. This assessment could aid in determining the drug's authenticity and provide pertinent data for the safer and more effective use of this formulation in therapeutics. More sophisticated methods are undoubtedly required as the use of *Ayurvedic* medications grows. These methods will assist drug manufacturers in adhering to regulations and substantiating the stability, safety, and therapeutic efficacy of their goods. In addition, evaluating the quality of *Ayurvedic* medications requires an integrated approach that incorporates both conventional and contemporary analytical methods. This will contribute to the validation of the age-old knowledge of *Ayurveda*, which has been utilized for numerous centuries.

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