Development and Standardization of Narayana Churna—A Polyherbal Ayurvedic Formulation

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ABSTRACT

Aim: The present study aims to develop pharmacognostical standards, standard operating procedure (SOP), and analytical profiling including physicochemical analysis of Narayana Churna, a polyherbal Ayurvedic formulation.

Materials and methods: The pharmacognostical (macroscopy, microscopy and powder drug analysis), thin layer chromatography (TLC), and quantitative physicochemical analysis including loss on drying, alcohol and water-soluble extractive values, total and acid-insoluble ash, and pH value were performed as per the standard procedures described in the Ayurvedic Pharmacopoeia of India (API). The microbial limit, aflatoxins, heavy metals and pesticide residues were also analyzed.

Results and discussion: Narayana Churna is of grayish-brown color with a slightly pungent taste. The powder microscopy revealed the presence of pentagonal/hexagonal/polygonal cork cells, polygonal pale green endosperm, oval/polygonal/irregular shaped stone cells, elongated and flat-shaped bordered pitted vessels, pitted tracheids, annular vessels, unicellular covering trichomes, prismatic crystals of calcium oxalate, starch grains and oil globules. The TLC fingerprint was developed using toluene:ethyl acetate:formic acid (6:4:1) as the solvent system. The standardized limits of the physicochemical parameters, microbial count, aflatoxins, heavy metals and pesticide residues were also laid down.

Conclusion: This is the first ever attempt made in order to develop the SOP and standardized parameters of Narayana Churna. Thus, the present study would be useful as the standardized reference protocol for the identification and standardization of this formulation.

Keywords: Narayana Churna, Pharmacognosy, Standard operating procedure, Standardization, Thin-layer chromatography.

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INTRODUCTION

Standardization of herbal formulation is the method of prescribing a set of standards/inherent characteristics, constant parameters, definitive qualitative and quantitative values that leads to the assurance of quality, efficacy, safety, and reproducibility. The quality of a finished product can only be assessed by some basic standard parameters and its method of preparation. The specific standard parameters are laid down to carry out the experimentation, which would further lead to the development of a set of characteristics possessed by the particular herbal formulation. Hence, standardization is an essential tool in the quality control process.

As far as the synthetic preparations are concerned, several sets of parameters are involved for testing the quality and safety. But the standardization of the herbal/Ayurvedic formulations is a big challenge for any researcher to assess the quality of a finished product due to its complexity of the ingredients. Thus, for the global acceptance of the Ayurvedic formulations, standardization is the need of hour.

One of the commonly used Ayurvedic formulations “Narayana Churna” mentioned in Bhaisajyaratnavali and further included in Ayurvedic Formulary of India (AFI) has been targeted for the present study. The formulation basically comprises Guduchi, Vrddhadara, Kutajaphala, Bilva, Ativisha, Bhringaraja, Narga, Shakrashana shuddha and Kutajatvak as therapeutically active ingredients. It is widely used in inflammation (Shotha), fever (Uvra) and cough (Kasa). It is also useful in gastro-intestinal disorders such as impaired digestion (Agnimandya), thirst (Trishna), diarrhoea associated with blood (Raktatisaara), and hemorrhoids (Arsha). Besides, the formulation also finds its therapeutic applications in chronic obstructive jaundice/chlorosis/advanced stages of jaundice (Halimoka), pallor/anemia (Pandu) and diabetes mellitus (Prameha).

Till date, no standards are available for this formulation even in API. Therefore, to assess the quality parameters, standard operating procedure (SOP) of the formulation and its standardization parameters have been laid down in the present study.

MATERIALS AND METHODS

Collection and Authentication of Raw Drugs

The ingredients of different batches of Narayana Churna were procured from four different local markets of Punjab state. Each ingredient of all the batches was identified/authenticated from...
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Method of Preparation of Narayana Churna

On a laboratory scale, different batches of the formulation were manufactured and labeled as N1, N2, N3 and N4. All the ingredients (Table 1) of pharmacopeial quality were taken and Shakrashana (Vijaya) was treated to prepare Vijaya-Shuddha. The ingredients were properly cleaned, dried and powdered separately and then, passed through IS Sieve no. 44. Each ingredient was weighed separately and mixed thoroughly in specified ratio (as mentioned in AFI) to obtain a homogeneous blend. Then, it was stored in air-tight containers to protect from light and moisture.

Pharmacognostical Evaluation of the Formulation

Macroscopical Characters

The powder was studied by organoleptic and morphological characters such as Rupa (color), Rasa (taste) and texture.

Powder Microscopy

The powder microscopy was carried out through Magnus MLM microscope using 50% glycerin. Micro photographs were taken with the help of Deno Capture 2.0 version 142D, the versatile digital microscope. The prominent/distinguished characters were drawn through camera lucida line drawings.

Determination of Physicochemical Parameters

Physicochemical parameters, namely loss on drying, ash values, acid-insoluble ash, extractive values and pH were measured as per the standard procedures.

Table 1: Ingredients of Narayana churna

| S. no. | Name of ingredient | Botanical name/English name | Part used | Quantity (g) |
|-------|--------------------|----------------------------|-----------|--------------|
| 1     | Guduchi API        | Tinospora sinensis (Lour.) Merr. | Stem      | 100          |
| 2     | Vriddhadara (Vriddhadaruka API) | Argyreia nervosa (Burm. f.) Bojer | Root      | 100          |
| 3     | Kutajaphala (Indrayava API) | Holarrhena antidysenterica (Roth) Wall. ex A.DC. | Seed      | 100          |
| 4     | Bilva API         | Aegle marmelos (L.) Corrêa | Fruit pulp | 100          |
| 5     | Ativisha API      | Aconitum heterophyllum Wall. ex Royle | Root      | 100          |
| 6     | Bhringaraja API   | Eclipta prostrata (L.) L. | Whole plant | 100          |
| 7     | Nagara (Shunthi API) | Zingiber officinale Roscoe | Rhizome   | 100          |
| 8     | Shakrashana (Vijaya API) shuddha | Cannabis sativa L. | Leaf      | 100          |
| 9     | Kutajatvak (Kutaja API) | Holarrhena antidysenterica (Roth) Wall. ex A.DC. | Stem bark | 800          |

Thin-layer Chromatography

The TLC of ethanolic extract of powdered material was tried using different ratios of the solvent systems. The sample was loaded on precoated silica gel 60 F254 TLC plate (Merck) and run in solvent system by using twin-trough chamber of Camag make. Plates were observed under ultra violet light at 254 and 366 nm and derivatized using anisaldehyde sulphuric acid reagent. Rf was calculated, band colors were recorded and photographs were snapped using the digital SLR Canon Camera.

Microbial Limit Test

The total viable aerobic count analysis was outsourced from NABL accredited Punjab Biotechnology Incubator, SAS Nagar, Mohali, Punjab.

Tests for Aflatoxins

The test for aflatoxins (B1, B2, G1, G2) was carried out according to AOAC (outsourced from NABL accredited Punjab Biotechnology Incubator).

Determination of Heavy Metals

The heavy metal analysis for lead, cadmium, mercury and arsenic was outsourced from NABL accredited Punjab Biotechnology Incubator using inductively coupled plasma mass spectrometry (ICPMS).

Test for Pesticide Residues

The pesticide residues were determined using QuEChERS extraction method and its quantification was done by gas chromatography-tandem mass spectrometry (outsourced from NABL accredited Punjab Biotechnology Incubator).

Results

Pharmacognostical Evaluation of the Formulation

Macroscopic Characters

The grayish-brown powder preparation possesses slightly pungent taste (Fig. 1). The powder completely passed through 355 μm IS Sieve (sieve number 44) and not less than 50% passed through 180 μm IS Sieve (sieve number 85).

Powder Microscopy

The powder microscopy (Figs 2 and 3) of Narayana Churna showed non-uniform cork cells which are pentagonal and few...
Figs 2A to N: Powder microscopy of Narayana Churna; (A) Cork in sectional view; (B) Endosperm of seed; (C to E) Stone cell; (F and G) Bordered pitted vessel; (H) Pitted tracheid; (I) Annular vessels; (J) Fragments of trichomes; (K) Fragments of fibers; (L) Cortex; (M) Thin fiber; (N) Prism crystal, oil globules and starch grain
are hexagonal/polygonal in shape (Kutaja stem bark); pale green polygonal endosperm (Kutaja seed); oval, polygonal and irregular-shaped stone cells (Bilva fruit pulp and Kutaja stem bark), wherein the irregular-shaped stone cells were enclosed with brown matter. The elongated and flat-shaped bordered pitted vessels (Bhringaraja whole plant and Vrddhadara root, respectively) were also observed, wherein the elongated bordered pitted vessels were enclosed with brown matter. It also showed the presence of pitted tracheids (Vrddhadara root) that were rectangular in shape having a width and length ratio of 2:7; non-lignified annular type vessels and fragments of unicellular covering trichomes (Shakrashana leaves); fragments of fibers (Guduchi stem); cortex; prismatic crystals (Kutaja stem bark); and oil globules (Bilva fruit pulp) and starch grains (Shunthi rhizome and Ativisha root).

Physicochemical Parameters
The physicochemical parameters, namely foreign matter, loss on drying, ash values, acid-insoluble ash, extractive values and pH were measured and the results are given in Table 2.

Table 2: Physicochemical parameters of Narayana Churna

| S. no. | Parameter                           | Batch-I | Batch-II | Batch-III | Batch-IV | Developed limits |
|--------|-------------------------------------|---------|----------|-----------|-----------|-----------------|
| 1      | Loss on drying                      | 7.29    | 6.90     | 7.04      | 6.57      | NMT 8%          |
| 2      | Total ash value                     | 7.10    | 9.97     | 7.11      | 9.90      | NMT 10%         |
| 3      | Acid insoluble ash value            | 1.29    | 3.53     | 1.40      | 3.63      | NMT 4%          |
| 4      | Alcohol soluble extractive value    | 18.72   | 10.57    | 18.26     | 10.43     | NLT 10%         |
| 5      | Water soluble extractive value      | 17.66   | 15.09    | 18.07     | 15.84     | NLT 15%         |
| 6      | pH                                  | 6.42    | 6.59     | 6.20      | 6.11      | 6.10–6.60       |

NMT, not more than; NLT, not less than
Thin-layer Chromatography
Different solvent systems for developing the TLC fingerprint of the ethanolic extract of the formulation were tried and toluene:ethyl acetate:formic acid (6:4:1) was selected as the solvent system. It showed four bands under 254 nm and thirteen band under 366 nm. On spraying with vanillin sulfuric acid reagent, eight bands were observed. Details of the results are given in Table 3 and Figure 4.

Microbial Limit Test
The total viable aerobic count was measured and given in Table 4.

Tests for Aflatoxins
The test for aflatoxins (B1, B2, G1, G2) was carried out and given in Table 5.

Table 3: Rf and corresponding band color observed under short UV (254 nm), long UV (366 nm) and after derivatization

| Rf values | At short UV (254 nm) | At long UV (366 nm) | After derivatization |
|-----------|---------------------|---------------------|---------------------|
| 0.10      | –                   | –                   | Blue                |
| 0.14      | –                   | Blue                | –                   |
| 0.16      | Blue                | Light yellow        | –                   |
| 0.33      | –                   | Purple              | –                   |
| 0.40      | Purple              | Light pink          | –                   |
| 0.44      | –                   | Light orange        | Yellow              |
| 0.51      | –                   | Bluish purple       | –                   |
| 0.56      | Light black         | Light blue          | –                   |
| 0.61      | Light black         | –                   | –                   |
| 0.70      | Light black         | Blue                | Blue                |
| 0.77      | –                   | Purple              | Violet              |
| 0.83      | –                   | Blue                | –                   |
| 0.86      | –                   | Bluish purple       | –                   |
| 0.90      | –                   | Yellow              | –                   |
| 0.93      | Light black         | Bluish purple       | Light purple        |

Fig. 4: Thin layer chromatography of Narayana Churna in short UV (254 nm), long UV (366 nm) and after derivatization

Determinaton of Heavy Metals
The heavy metals such as lead, cadmium, mercury and arsenic was determined and given in Table 6.

Test for Pesticide Residues
The different pesticides residues such as organochlorine, organophosphorus, and pyrethroids were measured and given in Table 7.

Discussion
The grayish-brown Narayana Churna has slightly pungent taste. The powder microscopy revealed the presence of important diagnostic characters such as pentagonal/hexagonal/polygonal cork cells, polygonal pale green endosperm, oval/polygonal/irregular-shaped stone cells, elongated and flat-shaped bordered pitted vessels, pitted tracheids, annular vessels, unicellular covering trichomes, prismatic crystals of calcium oxalate, starch grains and oil globules.

The TLC showed the presence of UV-active compounds under short (254 nm) wavelength at 0.56, 0.61, 0.70 and 0.93 (light black); and under long (366 nm) wavelength at 0.14 (blue), 0.16 (blue), 0.33 (purple), 0.40 (purple), 0.44 (light orange), 0.51 (bluish purple), 0.56 (light blue), 0.70 (blue), 0.77 (purple), 0.83 (blue), 0.86 (bluish purple), 0.90 (yellow), and 0.93 (bluish purple); and some colored prominent bands at 0.10 (blue), 0.16 (light yellow), 0.40 (light pink), 0.44 (yellow), 0.70 (blue), 0.77 (violet), 0.83 (blue), and 0.93 (light purple) after the derivatization with anisaldehyde sulphuric acid reagent.

The physicochemical parameters revealed the presence of more polar compounds through alcohol [Not less than (NLT) 10%] and water-soluble extractive values (NLT 15%).

Table 4: Microbial limit test

| S. no. | Parameter | Observed value (cfu/g) | Permissible limits as per API (cfu/g) |
|--------|-----------|------------------------|-------------------------------------|
| 1      | Total viable aerobic count | $9.2 \times 10^3$ | $10^5$ |

Table 5: Tests for aflatoxins

| S. no. | Parameter | Observed value (ppm) | Permissible limits as per API (ppm) |
|--------|-----------|----------------------|-------------------------------------|
| 1      | Aflatoxin B1 | Not detected | 0.5 |
| 2      | Aflatoxin B2 | Not detected | 0.1 |
| 3      | Aflatoxin G1 | Not detected | 0.5 |
| 4      | Aflatoxin G2 | Not detected | 0.1 |

Method detection limit, MDL = 5.0 ppb

Table 6: Determination of heavy metals

| S. no. | Parameter | Observed value (ppm) | Permissible limits as per API (ppm) |
|--------|-----------|----------------------|-------------------------------------|
| 1      | Lead (Pb) | 1.40                 | 10                                  |
| 2      | Cadmium (Cd) | Not detected | 0.3 |
| 3      | Mercury (Hg) | Not detected | 1 |
| 4      | Arsenic (As) | 0.34                 | 3                                   |

Method detection limit, MDL = 0.2 ppb
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Table 7: Test for pesticide residues

| S. no. | Parameter                               | Observed value | Permissible limits as per API (ppm) |
|--------|-----------------------------------------|----------------|-----------------------------------|
| 1.     | Organochlorine pesticide residues       |                |                                   |
| i      | Aldrin and dieldrin                     | Not detected   | 0.05 (sum of)                     |
| ii     | BHC (α, β, δ isomers)                   | Not detected   | 0.3 (sum of)                      |
| iii    | BHC (γ-isomer)                          | Not detected   | 0.6                               |
| iv     | Endosulfan (α, β isomers and endosulfan sulfate) | Not detected | 3.0 (sum of)                     |
| v      | DDT (p,p′-DDT, o,p′-DDT, p,p′-DDE, p,p′-DDD and o,p′-DDD) | Not detected | 1.0 (sum of)                      |
| vi     | Heptachlor and heptachlor epoxide       | Not detected   | 0.05 (sum of)                     |
| vii    | Methoxychlor                            | Not detected   | 0.05                              |
| 2.     | Organophosphorus pesticide residues     |                |                                   |
| i      | Diazinon                                | Not detected   | 0.5                               |
| ii     | Chlorpyrifos                            | Not detected   | 0.2                               |
| iii    | Chlorfenvinphos                         | Not detected   | 0.5                               |
| iv     | Phorate                                 | Not detected   | Not mentioned in API              |
| v      | Phorate sulfones                        | Not detected   | Not mentioned in API              |
| vi     | Phorate sulfoxides                      | Not detected   | Not mentioned in API              |
| vii    | Fenthion, its oxygen analogs, their sulfoxides and sulfones | Not detected | 0.05 (sum of)                     |
| viii   | Malathion                               | Not detected   | 1.0                               |
| ix     | Malaoxon                                | Not detected   | Not mentioned in API              |
| x      | Parathion-methyl                        | Not detected   | 0.2                               |
| xi     | Methyl paraoxon                         | Not detected   | Not mentioned in API              |
| xii    | Dichlorvos                              | Not detected   | 1.0                               |
| xiii   | Ethion                                  | Not detected   | 2.0                               |
| 3.     | Pyrethroids pesticide residues          |                |                                   |
| i      | Cypermethrin                            | Not detected   | 1.0                               |
| ii     | Permethrin                              | Not detected   | 1.0                               |

Method detection limit, MDL = 10 ppb

The total viable aerobic count in the formulation was found to be 9.2 \times 10^2 \text{cfu/g}. The aflatoxins (B1, B2, G1 and G2) and pesticide residues (organochlorine, organophosphorus and pyrethroids) were not detected. The heavy metal analysis were also within the API limits indicating the safety of the formulation.

### Conclusion

The SOP of Narayana Churna, an Ayurvedic polyherbal formulation consisting of nine ingredients was developed and analyzed. The pharmacognostical data, TLC fingerprint, and quantitative physicochemical data developed from the study can be considered for laying down standards and used as the diagnostic tools for the quality assessment of Narayana Churna.

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हिंदी सारांश

नारायण चूर्ण- बहुऔषधीय आयुर्वैदिक औषधयोग का विकास और मानकीकरण

उद्देश्य: वर्तमान अध्ययन का उद्देश्य नारायण चूर्ण, एक बहुऔषधीय आयुर्वैदिक औषधयोग के भौतिकरासायनिक विश्लेषण को सम्मिलित कर एसओपी (मानक संचालन प्रक्रिया), भाष्जगुणविज्ञानीय मानकों और विश्लेषणात्मक प्रोफाइलिङ को विकसित करना है।

सामग्री और विधियाँ: भारतीय आयुर्वैदिक औषधकोष (एनीआई) में वर्णित मानक प्रक्रिया के अनुसार सूखने पर क्षतिग्रस्त, एन्कोहोल और जल में चुलनशील सारत्व मूल्य, कुल और अम्ल-अघुलनशील राख तथा pH मूल्य को शामिल करते हुए क्षेत्रज्ञाविज्ञानीय (मैक्रोकोषी, माइक्रोकोषी और पाउडर औषध विश्लेषण), थिन लेयर कोमेटोग्राफी (टीएलसी) और मात्रात्मक भौतिकरासायनिक विश्लेषण किया गया। एनएबीएल मान्यता प्राप्त प्रयोगशाला से सूचमात्रेय सीमा, एफलाटोक्सिङ्स, हेवी एमेटल्स और कीटनाशक अवशेषों का विश्लेषण आउटसोर्स किया गया।

परिणाम व विचार-विवरण: नारायण चूर्ण में थोड़े तीखे स्वाद के साथ बेखूरा रंग होता है। पाउडर माइक्रोकोषी अध्ययन के महत्वपूर्ण लक्षणों यथा पॅंटोग्लोन/एक्साग्लोन/पॉलीग्लोन कॉर्क नेस्ट, पौलीग्लोन पेल शीन-कल्लर एंडोस्पर्म, ऑवल/पॉलीग्लोन/इरेस्कुलर टॉट स्टॉन सेल्स, एलिमेटेड एंड फेलेट-शेड बॉर्ड पिटिंग वेल्स, पिटिंग ट्राइक्स, एंजुलर वेस्लस, ज्यूनिस्लोजर क्वार्टर ट्राइक्स्स, कैल्सियम ओक्सीनेट के प्रिज्मेटिक क्रिस्टल्स, स्टार्व ग्रेस और ऑइल ग्लोब्यूल्स की उपस्थिति दर्शाती है। टीएलसी फिगरप्रिज्न को दर्शाता है: इथाइल एसिटेट: फॉमिक एसिड (6:4:1) की चुलनशील प्रणाली के रूप में विकसित किया गया था। भौतिकरासायनिक मापदंड, सूक्ष्मजीवी गणना, एफलाटोक्सिङ्स, हेवी एमेटल्स और कीटनाशक अवशेषों के मानकीकरण की सीमा भी विकसित की गई।

निष्कर्ष: नारायण चूर्ण के एसओपी और मानकीकृत मापदंडों को विकसित करने के क्रम में यह पहला प्रयास है। अतः इस औषधयोग की पहचान और मानकीकरण हेतु वर्तमान अध्ययन मानकीकृत संदर्भ प्रोटोकॉल के रूप में उपयोगी होगा।

मुख्य शब्द: नारायण चूर्ण, एसओपी, भाष्जगुण-विज्ञान, टीएलसी, भौतिकरासायनिक, मानकीकरण।