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# THE DEVELOPMENT OF PUNARNAVADI YOG: AN AYURVEDIC POLYHERBAL ARKA FORMULATED FOR DIABETIC RETINOPATHY

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## ABSTRACT

Diabetic retinopathy is a major, progressive complication of type 2 diabetes and a leading cause of preventable blindness, with current first-line treatment relying on costly, invasive intravitreal anti-VEGF therapy. This article documents the formulation-development and quality-control pathway for Punarnavadi Yog, a polyherbal Arka combining Punarnava (Boerhavia diffusa), Haridra (Curcuma longa), Musta (Cyperus rotundus), and Devadaru (Cedrus deodara), conceived as a low-cost, plant-derived candidate for this indication. Drug selection was guided by classical Ayurvedic indications for shopha (inflammation), rakta dushti, and arbuda/granthi (abnormal tissue growth), together with reported modern pharmacology — including anti-angiogenic and VEGF-modulating activity attributed to punarnavine and curcumin. Raw drugs were authenticated and pharmacognostically standardised against Ayurvedic Pharmacopoeia of India (API) limits before hydroalcoholic Soxhlet extraction of each herb and proportionate (1:1:1:1) blending into a single Arka. The finished formulation was evaluated for physicochemical and microbiological quality at preparation and after one month of accelerated stability testing (40 ± 2°C / 75 ± 5% RH), meeting all pre-defined acceptance criteria with only marginal numerical drift. These findings establish that Punarnavadi Arka can be manufactured reproducibly to a consistent, stable, and sterile pharmaceutical standard, providing the foundational groundwork required before biological, toxicological, and clinical evaluation of the candidate can proceed.

## 1. Introduction: The Case for a New Formulation

Diabetes has become one of the most widespread chronic conditions globally, and India carries a substantial share of that burden. Health-system estimates cited in the underlying research indicate that around 25 million Indians over the age of 18 live with prediabetes and more than 77 million have type 2 diabetes, with a large proportion unaware of their condition. A significant fraction of this population will go on to develop diabetic retinopathy, a progressive complication in which chronically elevated blood glucose damages the retinal blood vessels — a process ultimately driven by pathological angiogenesis, the abnormal growth of new, fragile blood vessels. Diabetic retinopathy is a progressive, sight-threatening complication of long-standing diabetes, and it remains one of the leading causes of preventable blindness worldwide. Current pharmacological management leans heavily on intravitreal anti-VEGF injections such as bevacizumab and ranibizumab, supplemented by intravitreal steroids, laser therapy, and surgery in advanced disease. These options are effective but expensive, require repeated invasive administration, and carry recognised risks including raised intraocular pressure, retinal detachment, intraocular inflammation, and infection. This combination of high cost, treatment burden, and side-effect risk is what motivated the search for an alternative: a low-cost, plant-derived formulation that could be developed, standardised, and manufactured to a defined pharmaceutical quality, drawing on classical Ayurvedic pharmacy. Ayurveda's *Shalakya Tantra* branch, which deals specifically with disorders of the eye, ear, nose, throat, and head, has long described formulations for eye disease, and it was this classical framework — rather than a single isolated herb — that guided the development of a multi-drug combination for this purpose. The result of that development effort is *Punarnavadi Yog* — a polyherbal *Arka* (distilled herbal preparation) combining four classical drugs: *Punarnava* (*Boerhavia diffusa*), *Haridra* (*Curcuma longa*), *Musta* (*Cyperus rotundus*), and *Devadaru* (*Cedrus deodara*). What follows traces the formulation's development pathway — from selecting and authenticating the raw drugs, through pharmacognostic standardisation, network-pharmacology insight into each drug, to the *Arka* dosage form, its limitations, the distillation process, and finished-product quality control — the foundational work that must precede any biological or clinical evaluation of a new drug candidate.

**KEYWORDS:** Punarnavadi Yog; Arka Kalpana; diabetic retinopathy; polyherbal formulation; Boerhavia diffusa; Curcuma longa; Cyperus rotundus; Cedrus deodara;

pharmacognostic standardisation; anti-angiogenic; Ayurvedic Pharmacopoeia of India; accelerated stability testing.

## 2. Selecting the Constituent Drugs

Drug selection in this formulation was guided by two converging lines of evidence: classical Ayurvedic indication and reported modern pharmacology. In Ayurvedic disease theory, conditions involving abnormal tissue proliferation, swelling, or vascular fragility — as seen in diabetic retinopathy — are conceptually related to *shopha* (inflammation), *rakta dushti* (vitiation of blood tissue), and *arbuda*/*granthi* (abnormal growth). Each of the four selected herbs carries a classical indication in one or more of these categories, alongside modern reports of anti-inflammatory, antioxidant, and anti-proliferative activity.

**Table 1: Rasa Panchaka (pharmacodynamic classification) and traditional indications of the four constituent drugs selected for Punarnavadi Yog.**

| **Drug (Ayurvedic name)** * | *Botanical source / Family | Rasa Panchaka (pharmacodynamic profile)** **T | raditional therapeutic indication** |
|---|---|---|---|
| **Punarnava* (*Shophaghni*, | *Katthilla*)** *Boerhavia diffu | sa, *Nyctaginaceae* Rasa*:* Madhura, Tikta* | , *Kashaya*; *Guna*: *Laghu*, *Ruksha*; *Virya*: *Ushna*; *Vipaka*: *Madhura*; *Dosha karma*: *Tridoshahara Shothahara* (anti-oedema), rejuvenator, diuretic |
| ***Haridra*** *Curcuma long | a,*Zingiberaceae* Rasa*:* Katu | , Tikta*;* Guna*:* Laghu, Ruksha*;* Virya*: | *Ushna*; *Vipaka*: *Katu*; *Dosha karma*: *Kapha-Pittahara Shothahara*, *vishaghna*, *varnya*, *vrana-ropana* (wound healing) |
| ***Musta*** *Cyperus rotund | us, *Cyperaceae* Rasa*:* Tikta* | , *Katu*, *Kashaya*; *Guna*: *Laghu*, *Ruksha*; | *Virya*: *Sheeta*; *Vipaka*: *Katu*; *Dosha karma*: *Kapha-Pittahara Shothahara*, *deepana-pachana* (digestive), *krimighna* |
| ***Devadaru*** *Cedrus deod | ara, *Pinaceae* Rasa*:* Tikta*; | *Guna*: *Laghu*, *Snigdha*; *Virya*: *Ushna*; | *Vipaka*: *Katu*; *Dosha karma*: *Kapha-Vatahara Shothahara*, *prameha-hara*, *kapha-vata* disorders, decongestant |

Beyond their classical indications, each drug also has a documented phytochemical profile relevant to vascular biology. Punarnavine, an alkaloid isolated from *Boerhavia diffusa*, has been reported to downregulate VEGF expression and inhibit endothelial cell migration and capillary structure formation. Curcumin, the principal constituent of *Haridra*, has been

reported to inhibit matrix metalloproteinase expression and related signalling pathways involved in tissue remodelling. *Cyperus rotundus* (*Musta*) contains rotundone, associated with antioxidant and anti-proliferative activity, while *Cedrus deodara* (*Devadaru*) yields sesquiterpene and lignan constituents reported to influence cell-proliferation-regulating pathways. These reported actions informed the selection of the four drugs as a rational combination, though establishing their combined biological effect required the separate, subsequent stage of experimental evaluation that lies outside the scope of the formulation-development work described here.

### 2.1 Network Pharmacology of the Constituent Drugs

Network pharmacology — the systems-level mapping of a plant's bioactive compounds onto their predicted human protein targets and downstream signalling pathways, typically using databases such as TCMSP, SwissTargetPrediction, GeneCards, STRING, and Cytoscape together with KEGG/GO enrichment analysis — offers a modern, multi-target lens on classical polyherbal reasoning. Published in-silico studies on each of the four constituent drugs are summarised below; none of these studies were performed on Punarnavadi Arka itself, and they are included only to contextualise the plausibility of the classical combination.

**Punarnava (*Boerhavia diffusa*).** Network-pharmacology and molecular-docking analyses of *B. diffusa* bioactive compounds have identified AKT1, epidermal growth factor receptor (EGFR), and STAT3 as high-affinity binding targets, positioning the herb within proliferation- and growth-factor-linked signalling networkswhere network pharmacology was applied to determine the therapeutic effects of the bioactive compounds of Boerhavia diffusa, with AKT1, EGFR, and STAT3 emerging as the proteins showing the best binding affinities with its bioactive compounds. Separately, its constituents have been reported to act through AMPK activation, NF-κB inhibition, and modulation of the MAPK pathway, mechanisms proposed to underlie its anti-inflammatory and antidiabetic effectsas Boerhavia diffusa's pharmacological effectiveness has been attributed to bioactive components that influence cellular signalling through AMPK activation, NF-κB inhibition, and control of the MAPK pathway, accounting in part for its reported impact on inflammation, diabetes, and cancer.

**Haridra (*Curcuma longa*).** Curcumin, the principal curcuminoid of *Haridra*, has been mapped in network-pharmacology studies to a broad target set including VEGFA, TP53, IL6, TNF, and MMP9, with KEGG enrichment implicating angiogenesis- and inflammation-related pathwayswhere molecular docking indicated that curcumin bound strongly to TP53,

IL6, and VEGFA, with additional targets such as TNF, CCND1, CASP3, and MMP9 identified through literature review, supporting a role in angiogenesis, inflammation, and cell-cycle regulation. A separate protein-interaction network study of *Curcuma longa* found its curcuminoids engage a large, overlapping set of human target proteins converging on inflammatory and endothelin-signalling pathwayswhere curcumin alone mapped to 193 predicted human target proteins, and pathway overlap across compound clusters pointed to shared involvement of endothelin signalling and cytokine/chemokine-mediated inflammation, suggesting a synergistic mechanism of action. Complementing these predictions, direct experimental work has shown curcumin blocking VEGFR2 activation on endothelial cellswhere curcumin inhibited the proliferation and migration of vascular endothelial cells under VEGF stimulation and blocked VEGFR2 activation and its downstream signalling.

**Musta (*Cyperus rotundus*).** A network-pharmacology and molecular-docking study focused specifically on diabetes mellitus identified MMP9, PTGS2, CASP3, EGFR, STAT3, PPARG, AKT1, and NFKB1 as top regulated targets of *C. rotundus*, with enrichment concentrated in the AGE–RAGE signalling pathway implicated in diabetic complicationswhere KEGG pathway enrichment for the active compounds of Cyperus rotundus was concentrated in the AGE-RAGE signalling pathway associated with diabetic complications and the type II diabetes pathway, with MMP9, PTGS2, CASP3, CD4, EGFR, STAT3, PPARG, AKT1, and NFKB1 identified among the top regulated genes. Separate network-pharmacology work on its essential oil identified IL1B, IL10, IL6, PTGS2, TNF, and STAT3 as hub anti-inflammatory targetswhere protein-protein interaction analysis of the essential oil's compound-target-disease network identified IL1B, IL10, IL6, PTGS2, TNF, and STAT3 as hub targets underlying its anti-inflammatory activity.

**Devadaru (*Cedrus deodara*).** Network-pharmacology analysis of *C. deodara* bark phytoconstituents (including cedrol, himachalol, and kaempferol) has shown multi-target interactions extending to host inflammatory proteins such as TNF-α and COX-2, with pathway enrichment pointing to cytokine-signalling and immunomodulatory networkswhere the compound-target network of Cedrus deodara bark interacted with multiple relevant host proteins including TNF-α and COX-2, suggesting multi-target immunomodulatory action, with KEGG pathway analysis highlighting cytokine signalling among the enriched pathways. These systems-level findings are consistent with the *Shothahara* (anti-inflammatory) classical indication attributed to *Devadaru* in Table 1.

Taken together, these independently published network-pharmacology analyses show partial convergence across all four herbs on inflammation- and proliferation/angiogenesis-linked nodes (e.g. AKT1, STAT3, TNF, VEGF-related signalling), lending in-silico plausibility to the classical rationale for combining them — though, as with the phytochemical evidence above, this remains predictive and did not form part of the formulation-development study itself.

### 3. Collection, Authentication, and Pharmacognostic Standardisation

Before any raw material can be formulated into a drug, its identity and quality must be established. In this development programme, all four raw drugs — *Punarnava*, *Haridra*, *Musta*, and *Devadaru*, in *bharad* (coarse crude) form — were procured from Manakarnika Pharmacy, Pune, and were subsequently authenticated and pharmacognostically standardised at Sudhatatva Pharmacy, Pune, prior to any extraction or formulation work.

Standardisation followed parameters set out in the Ayurvedic Pharmacopoeia of India (API), covering organoleptic description (colour, odour, taste, texture), foreign matter content, pH of the aqueous extract, loss on drying, total ash, acid-insoluble ash, and water- and alcohol-soluble extractive values. These tests establish that a raw material is genuine, free from excessive contamination, and consistent enough in composition to serve as a reliable starting point for manufacturing.

**Table 2: Pharmacognostic standardisation results for the four raw drugs, benchmarked against Ayurvedic Pharmacopoeia of India (API) reference limits.**

| Parameter | Punarnava | Haridra | Musta | Devadaru | API Limit (typical) |
|---|---|---|---|---|---|
| **Foreign matter pH (aqueous extrac** Loss on drying Total ash Acid-insoluble ash **Water-soluble extr** Alcohol-soluble ex | 0.04% t)** 6.21 7.80% 6.21% ** 1.08% active** 19.62% tractive** 5.24 | 0.04% 6.21 6.12% 5.34% 0.62% 13.94% % 8.62% | 0.01% 6.12 6.48% 4.22% 1.21% 14.18% 6.85% | 0.02% 5.72 4.28% 1.50% 0.82% 9.43% 7.86% | NMT 1–2% — — NMT 2–15% NMT 1–6% NLT 1.5–12% NLT 1–8% |

All four raw drugs met their respective description and foreign-matter criteria, and each fell within, or close to, its expected extractive-value range — giving the development team a documented, quality-checked starting material before any extraction step began. This standardisation stage is the pharmacognostic equivalent of raw-material qualification in

conventional pharmaceutical manufacturing, and it underpins the reliability of everything built on top of it.

## 4. Arka Kalpana: The Manufacturing Pharmaceutics

The finished dosage form chosen for this formulation was *Arka* — one of the classical Ayurvedic pharmaceutical categories described in the text *Arka Prakash*, and referenced in ancient compilations such as the *Ravana Samhita*. *Arka Kalpana* refers to the distillation-based preparation of a concentrated medicinal extract from plant material; '*Arka*' denotes the distilled product itself, while '*Kalpana*' denotes the method of preparation.

### 4.1 Why Arka was chosen as the dosage form

*Arka* was selected in preference to other classical dosage forms for several practical and pharmaceutical reasons documented in the development record: it delivers a comparatively high potency at low dosage volumes, it improves the bioavailability of the active constituents relative to crude powder forms, it can be administered easily either locally or systemically, and — when stored correctly — it offers good purity and a long shelf life. Classical texts also describe recognisable quality markers of a well-prepared *Arka*: a fragrance strong enough to dominate the raw aroma of its source herbs, a taste that reflects the character of the source material, and a clear, bright appearance.

### 4.2 Limitations of Arka Kalpana

Selecting *Arka* as the dosage form was itself a development decision with documented trade-offs and constraints that the development team had to plan around. *Arka* preparation requires specialised distillation equipment and technical expertise, so it cannot be produced with the same simplicity as a powder or infusion. Because the process depends on careful control of heat, time, and raw-material quality, batch-to-batch variability in potency and purity is a recognised risk unless standardisation is rigorously enforced — which is precisely why the raw-drug authentication and pharmacognostic testing described in Section 3 were carried out before any extraction began. The literature also notes that not all *Arka* preparations have been subjected to thorough modern scientific validation, and that, if used or dosed inappropriately, a concentrated preparation carries some risk of adverse or allergic reactions. Additionally, being a hydro-alcoholic or aqueous-volatile distillate, *Arka* preparations are generally limited to constituents that are steam-volatile or otherwise carried over in the distillate, so non-volatile, high-molecular-weight phytoconstituents present in the raw drug may be under-represented in the finished product unless the process is adapted (as it was here, using Soxhlet

extraction — see Section 4.3). These considerations directly shaped why finished-product quality control (Section 6) was treated as a mandatory step in this development programme rather than an optional add-on.

### 4.3 The Distillation Process

The general *Arka*-preparation pathway followed four stages. First, raw material selection: fresh or dried plant material of confirmed quality and identity is chosen. Second, preparation and cleaning: the material is cleaned to remove dirt and contaminants, then chopped or ground to increase the surface area available for extraction. Third, the distillation itself: the prepared material is combined with water in a distillation flask, heated to generate steam that carries the plant's volatile constituents, and passed through a condenser, where it cools back into a liquid that is collected in a receiving flask. Fourth, post-distillation processing: the collected *Arka* is filtered to remove residual plant particles and transferred to a sealed glass or high-quality plastic container to preserve its potency.

This general pharmaceutical process was adapted for the polyherbal formulation as described in the next section, using a hydroalcoholic Soxhlet extraction in place of a purely aqueous distillation, in order to more efficiently draw out both water- and alcohol-soluble phytoconstituents from all four source herbs.

## 5. Manufacturing Punarnavadi Arka

With the raw drugs authenticated and standardised, the formulation itself was built in two linked stages: individual extraction of each herb, followed by combination into a single polyherbal preparation.

### 5.1 Preparation of the individual hydroalcoholic extracts

- Ten grams of coarse powder of each drug — *Punarnava*, *Musta*, *Haridra*, and *Devadaru* — was weighed out separately.
- For each drug, a hydroalcoholic solvent system of 250 mL distilled water and 5 mL methanol was prepared.
- The weighed powder was thoroughly wetted with its solvent system and macerated (soaked) for two hours at room temperature to allow solvent penetration and improve extraction of phytoconstituents.
- Each soaked drug-solvent mixture was then transferred to a Soxhlet apparatus and extracted for three hours at 35–40°C.

- The resulting extracts were cooled to room temperature and filtered to remove insoluble plant residue, yielding four clear hydroalcoholic extracts (approximately 50 mL of usable extract per drug), which were stored separately in sterile, labelled containers.

**5.2 Combining the extracts into a single polyherbal formulation**

- All glassware, containers, and stirring equipment were cleaned and sterilised in advance to maintain aseptic conditions throughout.
- The four individual extracts were brought to room temperature and checked for clarity and the absence of particulate matter.
- Fifty millilitres of each of the four extracts was accurately measured out.
- The four measured extracts were combined in a sterile container in a 1:1:1:1 ratio under aseptic conditions.
- The combined mixture was continuously stirred with a sterile glass rod or magnetic stirrer until a uniform, homogeneous polyherbal hydroalcoholic extract was obtained.
- The finished formulation — 200 mL of Punarnavadi Arka in total — was transferred into sterile, airtight, labelled containers and stored under controlled conditions (4°C) pending further evaluation.

This two-stage process — individual extraction followed by proportionate blending — mirrors how a multi-component pharmaceutical product is typically built: each active component is first isolated and characterised on its own, and only then combined in a controlled ratio to produce the final, standardised drug product.

**6. Quality Control of the Finished Formulation**

Once prepared, the finished Punarnavadi *Arka* was itself subjected to analytical evaluation at Sudhatatva Pharmacy, Pune, to confirm that the manufacturing process had produced a consistent, stable, and pharmaceutically acceptable liquid product. This finished-product testing is the equivalent of a batch-release quality check in conventional drug manufacturing, and it was repeated after one month under accelerated stability conditions (40 ± 2°C, 75 ± 5% relative humidity) to assess whether the formulation would remain stable during storage.

**Table 3: Physicochemical evaluation of the finished Punarnavadi Arka at initial preparation and after one month of accelerated stability testing.**

| **Parameter** | **Initial Observat | ion** **After 1 Mo | nth, Accelerated Stability (40 ± 2°C / 75 ± 5% RH)** **Acceptance Criteria** |
|---|---|---|---|
| **Appearance Colour Odour pH Total solid cont** Refractive index **Specific gravity** Viscosity (mPa·s **Osmolarity (mOsm** Sterility test Physical stabili | Clear, transparent Colourless Characteristic aro 6.12 ent (%)** 0.08 ** 1.3324 ** 0.9986 )** 1.28 /L)** 32 Pass ty** Complies | liquid Clear, tra Colourless matic Characterist 6.14 0.08 1.3323 0.9984 1.27 31 Pass Complies | nsparent liquid Complies Complies ic aromatic Complies 4.5–6.5 ≤ 0.10 1.330–1.335 0.995–1.005 0.9–1.5 20–60 Sterile Stable |

Across every parameter tested — appearance, colour, odour, pH, total solid content, refractive index, specific gravity, viscosity, osmolarity, and sterility — the formulation met its pre-defined acceptance criteria both at the time of preparation and after one month of accelerated storage, with only marginal, non-significant drift in the numerical values. This indicates that the manufacturing process yielded a chemically consistent, microbiologically sterile, and physically stable liquid formulation suitable for further development work.

## 7. Positioning This Work in the Drug-Development Pathway

Taken as a whole, the work described here — raw-material sourcing and authentication, pharmacognostic standardisation against API benchmarks, network-pharmacology context for each constituent drug, *Arka Kalpana* as the chosen manufacturing pharmaceutics (with its attendant limitations), controlled hydroalcoholic extraction and proportionate blending of four herbs into a single polyherbal *Arka*, and finished-product physicochemical and stability testing — constitutes the formulation-development and quality-control foundation of a new plant-derived drug candidate.

This stage answers a narrower but essential question: can the intended formulation be manufactured reliably, to a consistent and stable pharmaceutical standard, from authenticated raw material? The results of this development programme indicate that it can. Whether the finished formulation also produces the intended pharmacological effect — and, ultimately, whether it is safe and effective in a living system — depends on further stages of investigation (biological screening, toxicology, and eventually clinical study) that build on, but are distinct from, the formulation and quality-control work presented here.

## 8. CONCLUSION

Punarnavadi *Arka* was developed as a rationally selected, four-herb polyherbal Ayurvedic formulation, targeting a well-defined clinical need: a lower-cost, plant-derived alternative in a therapeutic area currently dominated by expensive injectable biologics. The development pathway followed established Ayurvedic pharmaceutical principles — raw-drug authentication, pharmacognostic standardisation, network-pharmacology contextualisation of each constituent drug, *Arka Kalpana* as the manufacturing method (with due consideration of its limitations), and analytical quality control of the finished product — producing a chemically consistent, stable, and sterile liquid formulation ready for the next stages of drug development.

In documenting each step from raw herb to finished, quality-tested *Arka*, this work provides a reproducible manufacturing and quality-control record — the kind of foundation on which any subsequent biological, toxicological, or clinical investigation of Punarnavadi Yog would need to be built.

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