**# Methodology**

- \*\*Disease\*\*: Type 2 Diabetes Mellitus.

- \*\*Approach\*\*: Multi-target phytochemical/polyherbal strategy.

\*\*End-product\*\*: A \*\*standardized herbal capsule or sachet\*\* with reproducible potency (multi-target enzyme inhibition).

**# Step 1. Collection of Phytochemical Compounds**

\*\*Literature mining\*\*

PubMed, Scopus, IMPPAT, PhytoHub, PubChem for anti-diabetic phytochemicals.

- Extract compound name, PubChem CID/SMILES, plant source, reported IC₅₀ against α-glucosidase, α-amylase, DPP-4, or PTP1B.

\*\*Build your database\*\*

- Spreadsheet: Plant → Compound → CID → SMILES → Reported Target(s) → IC₅₀ → Reference.

\*\*Outcome → \*\*Curated phytochemical library\*\*.

**# Step 2. In-silico Screening (Machine Learning + Docking)**

\*\*Descriptor calculation\*\*

- Convert SMILES → 2D/3D structures (ChemAxon Marvin, RDKit).

- Compute molecular descriptors (PaDEL) → MW, XLogP, TPSA, H-bond donors/acceptors, ring counts, fingerprints.

\*\*Machine Learning\*\*

- Train QSAR models (Random Forest, Gradient Boosting) using literature IC₅₀ data.

- Predict activity of untested phytochemicals against each target.

- Rank top 20–30 candidates per target.

\*\*Docking support\*\*

- Dock top-ranked compounds to α-glucosidase, DPP-4, PTP1B PDB structures.

- Validate binding interactions with catalytic residues.

\*\*Outcome→ \*\*Ranked list of phytochemicals with predicted potency and docking scores\*\*.

**# Step 3. Select Plants & Extracts**

- Choose \*\*3–5 plants\*\* rich in the prioritized phytochemicals (anchors = morin, catechin, rosmarinic acid, caffeic acid).

- Source authenticated raw material (farm/herbal suppliers).

- Prepare \*\*hydro-ethanolic extracts\*\* (50–70%).

- Standardize each extract with \*\*HPLC/UPLC marker quantification\*\*.

\*\*Outcome → \*\*Standardized plant extracts with defined marker content\*\*.

**# Step 4. In-vitro Validation (Wet Lab)**

\*\*Primary assays (multi-target)\*\*

- α-Glucosidase (pNPG, A405 nm).

- α-Amylase (starch/DNS).

- DPP-4 (AMC fluorogenic substrate).

- PTP1B (pNPP colorimetric).

\*\*Controls\*\*: acarbose (α-glucosidase/α-amylase), sitagliptin (DPP-4), sodium orthovanadate (PTP1B).

\*\*IC₅₀ determination\*\*: dose-response curves, triplicates.

\*\*Classify potency\*\*: High (<50 µM), Moderate (50–100 µM), Low (>100 µM).

\*\*Outcome → \*\*Experimental IC₅₀ table for candidate compounds/extracts\*\*.

**# Step 5. Combine Literature + Experimental Data**

- Add your new IC₅₀ results to the \*\*literature dataset\*\*.

- Retrain ML models → improve accuracy.

- Predict again → refine candidate list.

- This is the \*\*active learning loop\*\* (each experiment improves the model).

\*\*Outcome → \*\*Updated model + improved predictions\*\*.

**# Step 6. Synergy Testing for Polyherbal blend**

1. Select \*\*2–3 top compounds/extracts\*\* per target.

2. Perform \*\*checkerboard assays\*\* (pairwise + 3-component).

3. Calculate \*\*Combination Index (CI)\*\* or Fractional Inhibitory Concentration (FIC).

- CI < 1 = synergy.

- CI ≈ 1 = additive.

- CI > 1 = antagonism.

4. Identify best synergistic mixtures across multiple targets.

\*\*Outcome → \*\*Synergistic multi-target blend

**# Step 7. Safety & ADMET Filtering**

\*\*In-silico ADMET\*\*: SwissADME, pkCSM.

- Screen for oral absorption, hepatotoxicity, CYP inhibition, hERG risk.

\*\*In-vitro safety\*\*: MTT cytotoxicity in HepG2/3T3-L1.

- SI = CC₅₀ / IC₅₀ ≥ 10 is acceptable.

\*\*Outcome → \*\*Shortlisted phytochemicals extracts\*\*.

**# Step 8. Prototype Formulation**

1. \*\*Formulation type\*\*: capsule/powder blend of standardized extracts.

2. \*\*Composition\*\*: optimized % ratios based on synergy + potency.

3. \*\*Excipients\*\*: microcrystalline cellulose, magnesium stearate, silica.

4. \*\*Quality testing\*\*:

- Marker assay (HPLC).

- Microbial load.

- Heavy metals/pesticides.

- Functional potency test (fixed-dose α-glucosidase inhibition).

\*\*Outcome → \*\*Pilot capsule\*\*.

**# Step 9. Stability & Scale-up**

\*\*Stability testing\*\* (ICH Q1A):

- Accelerated (40 °C/75% RH, 3–6 months).

- Long-term (25 °C/60% RH, 12 months).

- Monitor marker content + potency.

\*\*Scale-up extraction & blending\*\* under GMP/AYUSH norms.

\*\*Outcome → \*\*Stable, standardized pilot-scale product\*\*.

**# Step 10. Tech Transfer → Market**

- Prepare \*\*tech dossier\*\*: SOPs, batch records, assays, stability, safety.

- Regulatory: AYUSH nutraceutical filing (India) or dietary supplement path (US/EU).

- Branding, packaging, commercialization.

\*\*Expected outcome\*\*: A reproducible, standardized, multi-target \*\*polyherbal anti-diabetic capsule\*\*, validated by AI screening and wet lab validation.