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Extract Activity, Compound Activity, and Target Activity Are Different Claims: An Evidence-Translation Model for Preventing Mechanistic Overstatement in Pharmacognosy

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ABSTRACT

Pharmacognosy frequently moves across materials and levels of inference: a botanical extract produces a phenotype, chemical analysis detects candidate constituents, an isolated compound shows activity, and computational or experimental work nominates a molecular target. These observations are scientifically valuable but do not represent equivalent claims. Mechanistic overstatement arises when evidence supporting one level is silently transferred to another—for example, when extract activity is attributed to a detected constituent, a compound–activity association is treated as causal, or biochemical inhibition is described as proof that a target mediates a cellular phenotype. This article develops an original evidence-translation model for keeping these claims analytically separate while still allowing evidence to accumulate across levels. The model treats material definition, chemical identity, constituent attribution, compound activity, target-specific activity, target engagement, and causal mechanistic interpretation as related but non-interchangeable evidential states. Translation between states is proposed to require claim-matched bridging evidence rather than rhetorical continuity. The framework also treats assay interference, mixture interactions, analytical uncertainty, exposure, off-target behavior, and biological context as active constraints on interpretation rather than secondary caveats. Its purpose is not to impose a single experimental sequence or universal proof threshold, but to make the evidential basis of mechanistic language more explicit. Applied consistently, the model can support clearer pharmacognostic reporting and better identification of where additional validation is required. Its boundaries remain important: the framework is conceptual, context-dependent, and not prospectively validated as a scoring or decision system.

Keywords: Pharmacognosy, Natural products, Bioactivity attribution, Target engagement, Mechanistic inference, Evidence translation

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Introduction

Natural-product pharmacology begins with a material whose identity, composition, and preparation may be substantially more complex than those of a single synthetic reference compound. This matters because the interpretability of an activity result depends not only on the assay but also on whether the tested material is sufficiently defined for another investigator to understand what produced the observation. Best-practice analyses in phytopharmacology have therefore emphasized rigorous botanical, chemical, pharmacological, and methodological description as a condition for reproducible interpretation [1]. The ConPhyMP guidance similarly makes chemical characterization of extracts central to pharmacological and toxicological research, while allowing the depth of characterization to remain proportionate to the scientific question [2]. These principles establish an important starting boundary: evidence that an extract has been well characterized improves confidence about what

material was tested, but it does not by itself identify which constituent caused an observed biological effect or which molecular target mediated it.

The attribution problem becomes more difficult when activity emerges from chemically complex mixtures. Contemporary strategies increasingly combine high-resolution mass spectrometry, NMR, chemometrics, molecular networking, and biological testing to connect mixture composition with candidate bioactive components, while broader natural-product discovery has similarly moved toward integrated chemical and biological approaches [3, 4]. Such integration can increase resolution, but it does not erase the logical distance between detecting a feature, assigning a structure, associating that structure with activity, reproducing activity with an isolated compound, and establishing a target-dependent mechanism. A natural-product paper may therefore contain several valid observations at different inferential levels while using language that inadvertently makes them appear equivalent.

This distinction also protects the scientific value of ethnopharmacological observations. A reproducible phenotype caused by a well-defined preparation does not become uninformative merely because its active principle or molecular target remains unresolved. Conversely, increasingly sophisticated downstream analyses cannot retrospectively repair uncertainty about the starting material or justify attributing its activity to a molecule that was only detected or predicted. Evidence quality is therefore not represented adequately by a single scale from "weak" extract evidence to "strong" molecular evidence. The evidential question is instead whether the method used is appropriate for the particular proposition being made. Material definition, bioactivity attribution, structural identification, exposure, target interaction, and causal mediation each require different forms of support.

This article addresses the resulting problem as one of evidence translation. The central proposition is that an activity claim should remain at the level directly supported by the experiment unless additional evidence specifically bridges it to a stronger claim. The purpose is not to diminish extract-level pharmacology, demand target identification from every ethnopharmacological study, or privilege reductionist mechanisms over meaningful mixture effects. Rather, the proposed model provides a vocabulary for distinguishing what has been observed from what has been chemically attributed, molecularly engaged, or causally explained. This distinction is especially important in pharmacognosy because botanical variability, mixture interactions, analytical annotation, fractionation, exposure, assay behavior, and target context can each interrupt apparently simple transitions from "active extract" to "active compound" to "validated target." Mechanistic strength is consequently treated here as claim-specific and evidence-bounded rather than as an automatic consequence of moving downstream in a discovery workflow.

Different levels of activity claims in pharmacognosy

An extract-level claim is fundamentally a claim about the tested preparation under defined experimental conditions. The extract may contain multiple active, inactive, antagonistic, or mutually modifying constituents, and the observed response can reflect combinations that are not reducible to simple addition. Evidence for synergy and antagonism in natural-product mixtures shows why whole-extract activity cannot automatically be assigned to the most abundant, best-known, or computationally favored constituent [5]. An extract can therefore be pharmacologically active even when no single compound has yet been shown to account for that activity, and an isolated constituent can be active without explaining the magnitude or direction of the original extract response.

Constituent-level attribution requires a different evidential step. Integrated chemical profiling and bioactivity-guided analyses can associate changing metabolite patterns with changing activity and prioritize compounds for isolation [6]. Bioassay-guided fractionation followed by structural characterization and retesting of isolated metabolites can strengthen the inference that particular constituents contribute to a measured effect [7]. Yet even this stronger design can leave unresolved whether activity was altered by fractionation, whether correlated compounds traveled together, whether an isolated compound acts at the concentration present in the parent extract, or whether lost interactions contributed to the original phenotype. "Compound activity" should therefore describe what a sufficiently identified compound does when tested, whereas "compound contribution to extract activity" requires a bridge back to the parent material.

This distinction prevents another common compression of evidence. A fraction can retain activity without demonstrating that every molecule enriched within it contributes to that activity. Conversely, loss of activity after fractionation does not necessarily show that the original observation was spurious; disruption of concentration ratios or chemical interactions can change the pharmacological behavior of the preparation. The proposed framework therefore treats extract, fraction, and isolated-compound experiments as related evidence states rather

than interchangeable repetitions of the same test. Their inferential value depends on what changed chemically between preparations and on whether the biological response changed in a manner capable of discriminating among competing explanations.

Exposure constitutes another distinct level. A constituent can be chemically present and biologically active in vitro yet fail to reach a relevant systemic or tissue concentration after administration of the extract. Conversely, measurable exposure does not demonstrate efficacy or causal mechanism. Human pharmacokinetic measurement of constituents after administration of a standardized *Withania* extract illustrates that exposure can be investigated directly rather than inferred from composition or in vitro potency [8]. Extract response, constituent attribution, purified-compound activity, and exposure therefore answer connected but different questions. Each can be scientifically informative without being translated automatically into the others.

What evidence supports each level

The first evidential requirement is correspondence between the name used in the claim and the chemical certainty actually achieved. In mass-spectrometry-based metabolomics, feature detection, annotation, identification, and quantification carry different confidence requirements; best-practice guidance therefore recommends explicit reporting of how identities were assigned and where authentic standards or orthogonal evidence were available [9]. Computational metabolite annotation adds another layer of uncertainty because performance depends on reference libraries, representations, algorithms, and benchmark design [10]. A database match, predicted formula, molecular-network neighbor, or ranked candidate can support prioritization, but it should not silently become a confirmed molecular identity when the analytical evidence does not establish that level.

Chemical certainty and pharmacological certainty must also remain separate. Definitive structural identification establishes which molecule was examined; it does not establish that the molecule accounts for a correlated extract phenotype or that a nominated protein is its biologically relevant target. Similarly, reproducibility of an activity measurement strengthens confidence that a response exists under the tested conditions but does not determine why it occurred. The proposed model therefore asks two questions at every transition: what proposition was directly tested, and what additional proposition is being inferred? Bridging evidence is required when those answers differ. This formulation avoids treating sophisticated instrumentation as a general substitute for biological validation or treating biological response as a substitute for chemical identity.

The second requirement is correspondence between observed bioactivity and assay specificity. A positive signal can result from genuine pharmacology, but it can also arise from optical interference, reactive chemistry, membrane perturbation, aggregation, nonspecific binding, or other assay-dependent behavior. A positive small-molecule assay signal is not by itself evidence of specific target activity because assay interference, nuisance behavior, and colloidal or other nonspecific interactions can generate convincing positives [11–13]. Orthogonal assay formats, counter-screens, concentration-response behavior, detergent sensitivity where relevant, and other context-appropriate controls do not guarantee mechanism, but they can remove alternative explanations that would otherwise weaken the translation from “signal observed” to “pharmacological activity supported.”

The appropriate bridge is therefore determined by the inferential gap rather than by a universal hierarchy of experimental prestige. Chemical characterization supports the identity and reproducibility of the tested preparation; analytical confirmation strengthens constituent identity; fractionation, depletion, reconstitution, and isolated-compound testing can strengthen constituent attribution; interference controls strengthen interpretation of bioactivity; and direct molecular experiments become necessary when wording moves toward target-specific action. No single method substitutes for all others, and not every project needs to reach every level. The principal requirement is that the strength and object of the claim remain aligned with the evidence actually obtained. The proposed classification in **Table 1** organizes these distinctions and identifies where an additional evidential bridge becomes necessary.

Table 1. Proposed claim-and-evidence classification for early pharmacognostic activity interpretation

Claim level	What the evidence establishes	Typical bridge to a stronger claim	What should not yet be inferred
Defined extract	Identity and composition of the tested preparation	Reproducible bioassay plus constituent-resolved analysis	Active molecule or target
Extract activity	Preparation changes a measured biological endpoint	Fractionation, depletion, reconstitution, or linked profiling	One constituent caused the response

Constituent attribution	A constituent is associated with or contributes to activity	Isolation and retesting under relevant conditions	Full explanation of parent-extract activity
Compound activity	An identified compound produces a reproducible effect	Specificity controls and target-directed experiments	Direct target engagement
Target-specific activity	Effect is consistent with action on a nominated target	Orthogonal physical or cellular engagement evidence	Target causally mediates the whole phenotype

Note: The classification is proposed as an evidence-translation aid, not as a universal scoring system or mandatory experimental sequence.

Where evidence is commonly over-transferred

One common over-transfer occurs when the nominal identity of a pharmacological tool is treated as proof of the mechanism attributed to it. Chemical-biology guidance has repeatedly emphasized that potency, selectivity, cellular activity, concentration, inactive controls, and orthogonal tools influence whether a small molecule can support target-level inference. Mechanistic attribution to a named protein requires fit-for-purpose chemical tools, suitable experimental use, and appropriate negative or orthogonal controls rather than reliance on nominal inhibitor identity alone [14–16]. The relevance to pharmacognosy is direct: an isolated natural product does not become a target-validating probe merely because prior databases, docking, pathway annotations, or commercial descriptions associate it with that target.

Probe quality is also question-dependent. A compound may be adequate for demonstrating a reproducible biochemical effect but inadequate for assigning a cellular phenotype to one protein if its selectivity, permeability, stability, concentration, or competing targets are unresolved. Guidance on small-molecule selection for target validation therefore stresses fitness for the biological question rather than a single universal definition of a "good" probe [17]. In an evidence-translation framework, target-directed language should consequently be strengthened only to the degree justified by the tool and experimental context.

Other over-transfers occur earlier. Correlation between a metabolite and an active fraction can be mistaken for constituent causality; a confidently identified compound can be mistaken for the active compound merely because it is present; and in vitro potency can be interpreted as in vivo relevance without exposure evidence. A further error runs in the opposite direction: failure to reach a target-level claim may be treated as evidence that an extract-level observation lacks value. The problem is not that lower-level evidence is weak by definition. It is that each level answers a different scientific question, and confidence in one answer cannot simply be borrowed to answer another.

Mechanistic overstatement is especially likely when several individually reasonable observations are connected by an untested causal conjunction. For example, an extract may alter a phenotype, a constituent may be detected, that constituent may dock favorably to a protein, and the associated pathway may change after treatment. Taken together, these findings can motivate a mechanistic hypothesis, but the untested transitions remain between constituent presence and responsibility for extract activity, between computational nomination and physical engagement, and between target-associated change and causal mediation of the phenotype. The proposed model treats those transitions as locations where evidence must be added rather than where certainty can be inferred from narrative coherence.

The required discipline is therefore symmetrical. Authors should neither inflate a claim beyond its bridge evidence nor downgrade well-supported descriptive pharmacology because a molecular target remains unknown. Mechanistic language becomes most defensible when each transition is explicit: material to activity, activity to constituent, constituent to compound effect, compound effect to target-specific action, and target action to causal explanation. The next section develops that translation logic as a formal conceptual model rather than treating these boundaries as isolated reporting cautions.

An evidence-translation model

Target nomination and target validation answer different questions. A protein may be plausible because of pathway biology, computational prediction, prior literature, or association with a responsive phenotype, yet those observations do not establish that the tested natural product directly engages that protein or that the protein is required for the observed effect. Structured target-assessment recommendations emphasize assembling evidence appropriate to the specific target hypothesis rather than treating nomination itself as validation [18]. Building on that distinction, the model proposed here treats evidence translation as a sequence of claim-specific bridges rather than a hierarchy in which later-sounding terminology is automatically stronger.

The model contains six claim states: defined material, observed activity, constituent attribution, compound activity, target-specific activity, and causal mechanistic interpretation. These states are connected conditionally. A study may legitimately stop at any state, and some investigations may move between them nonlinearly. The governing rule is that a claim should advance only when new evidence tests the additional proposition introduced by the stronger wording. For example, moving from compound activity to target-specific activity requires evidence that distinguishes the nominated target from alternative explanations. Moving from target-specific activity to causal mechanism requires evidence that the engaged target materially contributes to the phenotype rather than merely changing alongside it.

Target engagement forms a particularly important bridge because it directly asks whether a compound interacts with its proposed target under the relevant experimental context. Available approaches include biophysical, biochemical, thermal, proteomic, and cellular strategies, each with method-specific strengths and limitations [19]. Engagement is therefore neither synonymous with biochemical potency nor the final mechanistic endpoint. It strengthens the inference that a compound reaches and interacts with the proposed target, but causal mediation still depends on how that interaction relates to the biological response. The appropriate bridge also varies with pharmacological modality: covalent ligands and degraders, for example, require quality criteria that account for their distinctive mechanisms rather than importing assumptions from reversible inhibitors [20].

The model consequently rejects a single mandatory ladder. Extracts may remain valuable at the phenotype level; fractions may be used to localize activity without immediate structural resolution; isolated compounds may establish reproducible pharmacology without a validated target; and target engagement may be demonstrated before complete phenotypic causality is resolved. Uncertainty is carried forward rather than erased. Alternative explanations—mixture interactions, assay artefacts, incomplete identity, inadequate exposure, off-target binding, indirect pathway effects, or context-specific target availability—function as constraints on translation.

Importantly, failure at a bridge does not automatically invalidate the preceding claim. If a compound fails to show direct engagement with a nominated target, the compound-level phenotype may remain reproducible while that target hypothesis is weakened. Likewise, failure to isolate a single constituent that recreates an extract response may redirect interpretation toward multiple contributors, unstable constituents, or mixture-dependent behavior rather than negating the extract observation. The model therefore distinguishes evidential containment from evidential rejection: unsupported translation is blocked, but evidence already secured at a lower claim state is retained unless new experiments directly challenge it.

Figure 1 summarizes the proposed architecture by separating claim states, bridging evidence, common over-transfer routes, application to different material states, and the reporting boundary that keeps unresolved uncertainty visible.

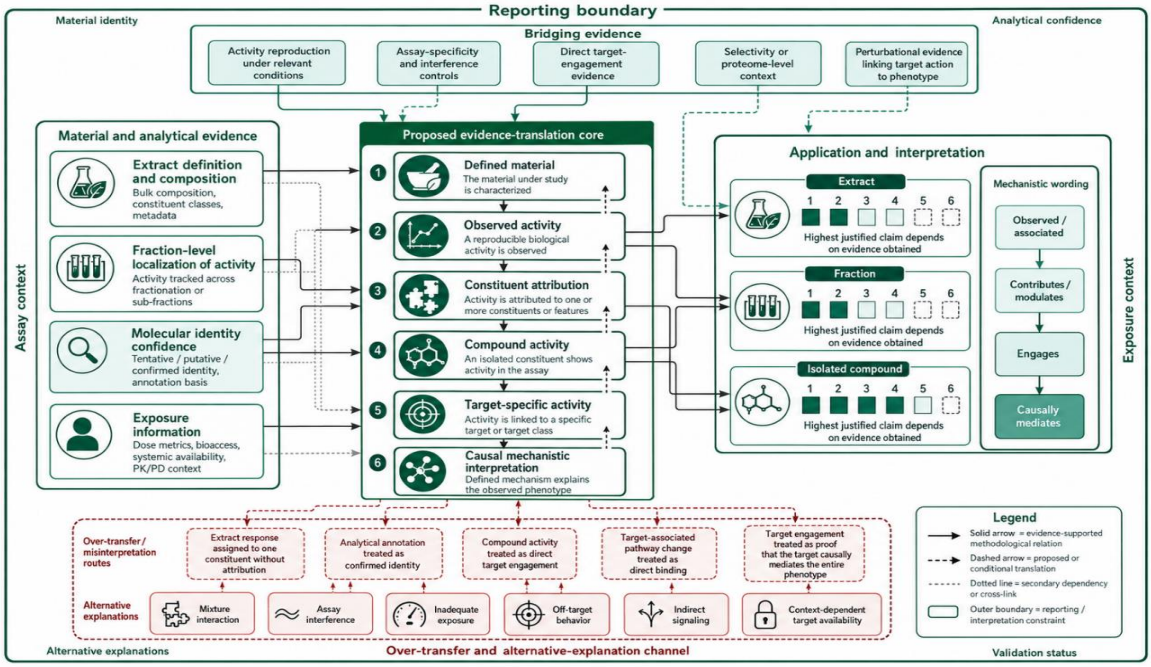

Figure 1. Evidence translation across pharmacognostic activity claims

Applying the model to extracts, fractions, and compounds

Application begins by asking what the tested material permits the investigator to claim. For an extract, the most defensible initial statement concerns the preparation and its measured response. If activity is localized through fractionation, the evidence becomes more specific about where activity travels chemically, but fraction activity still does not identify every active constituent. Chemoproteomics adds a different type of resolution after a compound has been sufficiently defined: it can map or prioritize interacting proteins across a broader proteomic context and thereby support target deconvolution beyond prior nomination [21]. Such a result is a bridge toward target attribution, not an automatic endpoint, because enriched or interacting proteins may still require orthogonal confirmation and functional interpretation.

For fractions, the central question is whether compositional change and biological change are linked in a discriminating way. Activity that tracks with enrichment can prioritize candidates, while loss of activity can suggest removal of necessary components; neither pattern uniquely establishes causality when correlated constituents, instability, concentration shifts, or mixture interactions remain plausible. The proposed model therefore favors designs that preserve provenance between parent extract, fraction, and isolated compound. Depletion, spike-back, reconstitution, and retesting at chemically relevant concentrations are especially informative when they distinguish a candidate constituent from competing explanations rather than merely reproducing activity in another format.

For isolated compounds, the model separates three questions: does the compound reproduce a relevant biological effect, does it interact with a nominated target, and does that target explain the phenotype? Natural-product chemoproteomic work on salvianolic acid A illustrates how target assignment can be strengthened by convergence between chemoproteomic and phosphoproteomic evidence rather than by prediction alone [22]. A separate study of caesaldekarin e combined docking with CETSA, microscale thermophoresis, pull-down/mass spectrometry, and biological models to support a glucocorticoid-receptor mechanism more strongly than any single method would permit [23]. These examples are useful because they show evidence accumulation across levels without implying that the same experimental package is universally required.

The practical rule is therefore material-sensitive but claim-centered. Extracts require attention to preparation identity and mixture behavior; fractions require compositional provenance and discrimination among co-varying constituents; isolated compounds permit more direct specificity and engagement experiments but still require evidence connecting molecular interaction to the phenotype. A chemically simpler material does not automatically justify a stronger mechanistic verb. Conversely, a complex extract need not be forced into single-target language to count as pharmacologically informative.

Table 2 applies the model comparatively to the three material states and shows what must remain unresolved when the relevant bridge has not been crossed.

Table 2. Comparative application of the proposed evidence-translation model to extracts, fractions, and isolated compounds

Material state	Primary defensible claim	Most informative next bridge	Residual interpretive risk
Extract	Defined preparation produces a measured response	Constituent-resolved profiling, perturbational fractionation, exposure evidence	Mixture interaction; unidentified actives; preparation dependence
Fraction	Activity localizes with a changed chemical subset	Isolation, depletion, spike-back, or reconstitution	Co-variation; altered concentration ratios; fractionation artefacts
Isolated compound	Identified compound reproduces a defined effect	Specificity controls, direct engagement, selectivity testing	Off-target action; context mismatch; inadequate exposure
Target-engaged compound	Compound interacts with a proposed target	Functional perturbation linking engagement to phenotype	Engagement without causal mediation; parallel targets

Note: Categories describe proposed interpretive states, not mandatory stages or quantitative grades of evidence.

Consequences for mechanistic interpretation

The model changes how mechanistic language is selected. “Associated with” can describe covariation without assigning causality; “contributes to” requires evidence that changing the constituent or process changes the response; “engages” requires direct evidence of compound–target interaction; and “mediates” is strongest when

the target is shown to be necessary or materially explanatory for the relevant phenotype. These verbs should not be treated as rigid universal definitions, but as reminders that linguistic strength should track evidential strength.

Selectivity complicates this translation because a nominal target can coexist with broader cellular binding. Large-scale chemoproteomic profiling has shown that ligand behavior and interaction patterns can be mapped across cellular proteomes rather than inferred solely from a preferred biochemical target [24]. This does not mean that every additional interaction is functionally important. It means that target-specific interpretation becomes less secure when broader engagement is unknown, especially if the phenotype could plausibly arise through several proteins or pathways. A direct binder may therefore be real without being the exclusive or dominant mediator of the observed response.

The reverse problem occurs when pathway-level biological change is described as direct target evidence. An extract can alter a phenotype together with markers associated with a signaling pathway, as demonstrated in a study linking *Morus alba* root extract with reduced fibroblast senescence and PI3K/Akt-associated changes [25]. Such evidence supports a relationship between treatment, phenotype, and pathway state in that experimental system; it does not by itself show that a constituent physically binds PI3K, Akt, or another pathway component. Indirect upstream regulation, downstream feedback, metabolic effects, or multi-target action remain possible.

This distinction is not semantic caution for its own sake. Over-transfer can distort candidate prioritization, make follow-up experiments appear confirmatory when they are actually exploratory, and obscure why apparently contradictory replication results occur. Under-transfer is also undesirable: insisting on a validated molecular target before reporting a reproducible extract phenotype would discard legitimate pharmacology. The proposed model therefore treats mechanistic interpretation as calibrated translation. Claims can become stronger when appropriate bridges are crossed, remain deliberately bounded when they are not, and be revised when new evidence exposes an alternative route.

Reporting boundaries

Transparent reporting is the mechanism by which readers can reconstruct these evidential transitions. Natural-product pharmacology guidance emphasizes documenting the identity and characterization of the tested material, experimental conditions, and pharmacological methods sufficiently for interpretation and reproducibility [26]. Within the proposed model, reporting should additionally make the claim boundary explicit: whether a molecule was detected, annotated, confirmed, isolated, quantified, shown to reproduce activity, demonstrated to engage a target, or linked causally to a phenotype.

A useful report therefore separates observation from inference. For extract studies, material provenance, preparation, compositional characterization, batch information where relevant, and assay conditions define the response being claimed. For constituent-level attribution, authors should state how candidates were prioritized and whether isolation, depletion, reconstitution, or concentration matching was performed. For target claims, the report should distinguish computational nomination, biochemical activity, direct binding or engagement, selectivity evidence, and functional perturbation. Contemporary perspectives in natural-product pharmacology likewise emphasize the value of rigorous methodological practice and emerging mechanistic tools, including chemical-proteomic approaches [27]. The presence of such tools, however, should not itself be treated as proof beyond what their measurements establish.

Reporting boundaries also include what remains unknown. Alternative explanations, analytical confidence, absence of exposure data, unresolved off-targets, dependence on a specific preparation, or lack of causal perturbation should be stated when they materially constrain interpretation. This does not weaken a manuscript; it identifies the evidential state accurately. The proposed model is therefore intended as a reporting discipline as much as an interpretive framework: every stronger claim should make visible the bridge that supports it and the uncertainty that remains.

Conclusion

Extract activity, compound activity, and target activity are scientifically connected but logically distinct claims. Pharmacognosy becomes vulnerable to mechanistic overstatement when evidence is allowed to travel between those claims without testing the additional proposition introduced at each transition. The evidence-translation model proposed here addresses that problem by treating defined material, observed activity, constituent

attribution, compound activity, target-specific activity, target engagement, and causal mechanistic interpretation as related but non-interchangeable evidential states.

Its central rule is deliberately simple: claims should advance only when evidence bridges the new inference. This rule preserves the value of extract- and fraction-level pharmacology while preventing chemical detection, statistical association, computational nomination, pathway response, or nominal inhibitor identity from being mistaken for stronger molecular evidence. It also prevents direct target engagement from being treated automatically as proof that the engaged target explains the entire phenotype.

The framework is not a universal scoring system, mandatory experimental ladder, or validated predictor of research quality. Different natural products, assay systems, targets, routes of administration, and biological contexts require different bridging experiments, and some questions are appropriately answered without identifying a molecular target. Its practical value lies instead in making the evidential object of each claim explicit, carrying unresolved uncertainty forward, and aligning mechanistic language with what was actually measured.

Used in that bounded way, evidence translation can help distinguish productive mechanistic hypotheses from prematurely closed explanations. It provides a structured basis for deciding what has been demonstrated, what remains inferred, and which experiment would most directly test the next claim.

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