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Amplifying the Worth of Traditional Pharmacopeias by Utilizing Integrative Technologies

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What is an herbal remedy?

- Traditional or serendipitous
- Varies in formulation & preparation
- May contain one or more plants and other components
- Unreliable as to plant identification
- Appropriate clinical validation
- Varies in dosage and treatment regimens
- Usually used for acute not chronic conditions
- Potency and toxicity frequently moderate
- Rarely evolved, pharmaceutically
- If one plant may be “standardized” through chemical fingerprinting of bioreactive compounds as a phytopharmaceutical.
New therapeutics from herbal remedies

- Is there a local, regional, global need?
- Does anecdotal information suggest that there are remedies worthy of study?
- Is there scientific evidence to suggest that regional plants have a therapeutic potential?
- Have therapeutic agents already been derived from local taxa?
- Is it more logical to evaluate these in the context of use or to look for new commercial entities?
How can integrative technologies be utilized to understand and optimize the worth of a traditional pharmacopeia

- By conducting appropriate ethnobotanical/medical surveys to identify those of primary interest to a population.
- By understanding the relative value of plant selections by identifying those of presumptive merit “targeted” for specific disease syndromes or whose traditional uses suggest such a linkage.
- By more carefully matching screening methodologies with known uses.
- By applying dereplication methods through eliciting data available in oral, written and electronic data bases in order to identify what is already known about the medicinal flora and its relationship to others elsewhere.
- By identifying useful remedies within a population whenever a critical need arises by a method called ethnomedical/dental focusing.
- By applying phylogenetic amplification techniques to identify new sources of bioreactive compounds or new treatment targets.
- By utilizing new high throughput and computer based technologies.
Discovery of New Therapeutics: A team approach

- Intellectual Property Lawyers, National regulatory bodies
- Botanists in: Ethnobotany, Taxonomy, Ecology
- Ethnolinguists, Epidemiologists
- Practitioners of allopathic and alternative medicine
- Biologists: Targeted screening specialists
- Data base analysts
- Pharmacologists and Natural Products Chemists
- Toxicologists
- Medicinal Chemists
- Formulation Chemists
- Commercial Partners: Nutritional Supplement (Botanical), Phytopharmaceutical, Pharmaceutical
What methods are applied to identifying phytomedicines of worth?

- **Primary data**: Identifies known leads targeted to a specific disorder e.g., malaria. These data are particularly valuable if knowledge is still being applied and consensus of value and can be correlated with conventional parameters of bioreactivity and efficacy.

- **Doctrine of Signatures**: are not always invalid criteria e.g., as evident by the shape or color of a plant part.

- **Secondary data**: Identifies possible leads from information in the public domain as cited orally or in written and electronic data base form.

- **Dereplications** identify taxa, chemical, and bioreactivity links or used retrospectively following isolation of a bioreactive compound to provide information on parameters of safety and efficacy, alternative sourcing of taxa or its compounds, or identify compounds of equal or greater worth.

- **Preliminary bioreactivity screening**: identifies functional or mechanistic links to traditional uses.
Types of Assays to Detect Bioreactivity

In medical ethnobotany, the human is the first, not the last functional screen - a fact that has rarely been appreciated in recent pharmaceutical drug research studies.

- **Functional**
  - humans
  - Animals*
  - Cells*
  - Mechanistic*

- provides value data
- limited, complex
- general bioreactivity
- specific
- toxicity and use parameters unknown
- may not match any parameters of traditional use
- High throughput technologies for purifying, identifying and characterizing DNA, RNA, proteins and other bioreactive molecules.
The incidence of bioreactivity associated with the type of specimen.

<table>
<thead>
<tr>
<th>Type</th>
<th>Hit rate</th>
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</thead>
<tbody>
<tr>
<td>Indiscriminate screening</td>
<td>&lt; 5%</td>
</tr>
<tr>
<td>Medicinal Plants</td>
<td>15-25%</td>
</tr>
<tr>
<td>Targeted Medicinal Plants (secondary)</td>
<td>40-50%</td>
</tr>
<tr>
<td>Targeted Medicinal Plants (primary)</td>
<td>&gt; 75%</td>
</tr>
<tr>
<td>Focused data</td>
<td>&gt; 90%</td>
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Recent Advances in Screening for bioreactivities associated with toxicity drug discovery & therapy

“Omics” High throughput technologies used to analyze various kinds of micro- & macromolecules simultaneously. This technique is useful in toxicity testing, and in the discovery of novel biomarkers for use in translational and clinical studies

- **Proteomics**: Measures proteins
- **Metabolomics**: comprises the comprehensive and simultaneous profiling of multiple metabolites in a biological system as well as their temporal changes caused by genetic, environmental and pharmaceutically induced effects.
- **Genomics**: multivariant analyses of the genome e.g, defining therapeutic tests for a drug or as *theranostics* useful for identifying personalized therapies for cancer.
- **Transcriptomics**: Measures transcripts

- **ADMET** (absorption, distribution, metabolism, excretion, toxicity)
  - Herbal substances identification.
  - Major and minor herbal constituents affecting enzyme metabolism.
  - Subcellular organelles (microsomes) for identification and quantification of metabolites.
  - Cells for toxicity testing
  - Volunteers to test pharmacokinetics of herbal components.
First Commercialized Antimalarial from the Amazonian Rainforest:
Useful compounds derived from primary data.

- From Jivaro and neighboring tribal pharmacopeias; popularized by the Jesuits
- Cultivated worldwide in tropics
- Bark contains quinine, isolated in 1823.
- Synthesis of primaquine and chloroquine occurred in the 1930’s
- Chloroquine is used as mainstay for treatment until resistance occurred.
- Primaquine is used for relapsing cases.
- Isomer quinidine has been used for arrythmia since 1918.

*Cinchona officinalis* (Rubiaceae)
Named after the Countess of Cinchon, wife of the Viceroy of Peru in 1638 who was cured of malaria using a bark extract.
The Value of Primary Data to Modern Medicine and How Integrative Approaches Amplified these Discoveries

- **Brazilian Amerindians** used the leaves for salivation and sweating.

- **Pilocarpine** was isolated in 1877.

- It is a parasympathomimetic alkaloid obtained from the leaves of the shrubs from the genus *Pilocarpus*.

- **Pilocarpine** is used to treat xerostomia (dry mouth) in cases of radiation treatment or Sjogren’s syndrome.

- It is also strongly miotic causing pupil constriction and has been used for over one hundred years to treat glaucoma.
Traditional use with a medicinal potential:

*Blow gun darts to muscle relaxant in surgery*

*Chondrodendron tomentosum & Curarea toxifera* (Menispermaceae)

- Curare: used by Amazonian tribes for immobilizing animals during hunting.
- Made from the stem & root bark of *Strychnos* (Loganaceae) and Menispermaceae such as *C. tomentosum* and *C. toxifera*.
- Chondrodendron was once a cottage industry in Amazonia with harvesting of the large lianas from the wild without consideration for managing this resource as a renewable crop. The taxa was becoming endangered hence synthesis of similar compounds.
- (+) tubocurarine isolated & developed in 1950’s
- The taxa became endangered hence synthesis of similar compounds in the 1980’s.

*Aturcurium*

*Vercuronium*

These compounds are used as reversible skeletal muscle relaxants in surgical procedures with deep anesthesia.
Antimalarial with an Anticancer & anti-Trypanosomal Potential: 
**Cryptolepis sanguinolenta (Periplocaceae)**


Yellow roots of this twining liana known as nibima, kadze or gangamau are used as antiinfectives in West African traditional medicines. The Twi and Ewe of Ghana use it for malaria. It is considered a threatened species.

The herbal division of Phyto-Riker Pharmaceuticals has made a medicinal tea for malaria called Phyto-laria®. The tea is effective against chloroquine resistant *Plasmodium* strains and is claimed to be effective over 90% of individuals treated.

- It contains indolquinolene alkaloids such as cryptolepene which has been synthesized. It is antimycobacterial and has potent cytotoxicity and antiinflammatory activity suggesting it and its analogues are potential anticancer agents affecting many cancer cell lines. Its activity is related to intercalating with DNA at cytosine-cytosine sites eliciting Topoisomeriae II and microtubule inhibition.

- Its analogues are potent antimalarials and are effective against trypanosomes. Some are hypoglycemic thus lowering lower blood sugar.
A group of related plants in the Celastraceae.
Maytansines: From traditional African medicine to future cancer treatments.

- In East African traditional medicines, *Maytenus* roots are used to treat cancers and antinfective conditions.
- In the 1970’s *maytansine* was isolated from the Ethiopian *M. serrata* but deemed too toxic to develop.
- Maytansinoids were isolated from other species and genera in the Celastraceae such as Puterlickia and Triptergium.
- The macrolide, *maytansine* inhibits the assembly of microtubules by binding to tubuline at the rhixin binding site thus arresting metaphase and cell division.
- To reduce toxicity, immunotargeting strategies have evolved. One analogue, *DM4* linked to human mesothelin antibody (anetumab raxtansine) has promise in the treatment of mesothelioma, ovarian, pancreatic and lung adenocarcinomas.
**Digitalis lanata** a Bioreactive Plant called Foxglove Identified from a Polyherbal

- Present in pharmacopeias of England and Europe of the 18th century.
- Its leaves were identified by Withering in 1775 from a complex polyherbal as the primary source for the treatment of dropsy (congestive heart failure) and angina pectoris.
- Digitoxin was extracted from its leaves in 1875. Its affects are longer lasting than digoxin and in elderly patients it has lower rate of toxicity than digoxin.
- Digoxin (Lanoxin©) is a purified cardiac glycoside, which until recently was favored for the treatment of atrial fibrillation, atrial flutter and sometimes heart failure. Beta blockers are now preferred because digoxin does not confer any mortality benefit in patients with congestive heart failure. It may still be used when ACE inhibitor treatment and diuretics do not reduce symptoms.

*D. lanata*

*Digitoxin*

*D. purpurea* is the current source and is grown in plantations.
In-silico technology: Merging Data Bases
Barlow, DJ 2012. In-silico studies in Chinese herbal medicines research: Evaluation of in-silico methodologies and phytochemical data sources, and a review of research to date. J. Ethnopharmacol. 140: 526-534

- **Data bases related to the:**
  - Nature of the remedy
  - Ethnopharmacological data
  - The compounds it contains and their structures.
  - Known phytochemicals
  - Therapeutic targets
  - Potential therapeutic targets
  - Protein data bank

- **Types of software for use in computational studies:**
  - Ligand based screening programs.
  - Pharmacophore programs either ligand- or target-based
  - Docking programs used to dock potential molecule ligands into protein active sites and scoring their interaction.
  - Pattern recognition e.g., principle component analysis, multi-dimensional scaling, self organizing maps and cluster analysis.
  - Proteomics and/or genomics data visualization and analysis tools.
“In silico” discoveries in TCM

- Fingerprint and docking
- Biofilm inhibition by docking to transcription activation factor TraR.
- Protein based pharmacophores to screen for acetylcholinesterase (AChE) and cyclooxygenase (COX) inhibitors.
- Pharmacophores, docking and QSAR methods to mimic phosphodiesterase 5 (PDE5)
- Aurantiamide acetate from *Artemesia annua.*
- Baicalein
- Scopoletin AChE inhibitor, Sanggenons Cox inhibitor
- Several inhibitors identified from *Epimedium* a tonic used to invigorate Yang.
Doctrine of Signatures: Integrative Approaches Like Semisynthesis Amplified its Medicinal Value

- **Podophyllum peltatum**
  - Penobscot Indians of Maine used its rhizomes to treat cancers and venereal warts or Condyloma acuminata.

- Podophyllin resin
  - As the resin, podophyllin it appeared in the 19th century Materia Medica for the treatment of cancerous tumors, polyps and unhealthy granulations.

- Podophyllotoxin
  - Because of toxicity its lignans podophyllotoxin and α and β peltatins were semisynthesized.

- Both teniposide and etoposide are topoisomerase II inhibitors used for leukemias and lymphomas, brain and bladder tumors and acute refractory leukemias.
A novel survey derived from secondary data. Conducted in 1975 to determine relevant values of chewing stick/sponge use in Ghana.


- 173 species identified from secondary data
- 870 individuals in all ages from 12 tribal/linguistic groups were surveyed to determine which of these species were favored and if these were linked to patterns of efficacy and bioreactivity against dental pathogens by:
  - The types of plants that were used, their ages, the plant part, the time of year collected, its acquisition, preparation and frequency of use.
  - If the nature of the chewing stick influenced their choice e.g., flavor, texture, foaminess etc.
  - How social parameters of age, gender, religion, family or tribe influenced the selection.
  - How dental health was influenced by their use as regards to incidence of apparent dental decay, gum disease and tooth loss.
  - If data sorted by a SAS program according to numerous parameters of interest elicited meaningful correlations.
Favored Ghanaian Chewing-sticks and Sponges

- *Garcinia afzelii*, *G. epunctata* (Clusiaceae) are most favored (60%) throughout all tribes of Southern Ghana because of their soft, fibrous and sweet bristles. They confer excellent dental health. *G. afzelii* contains antimicrobial benzoid compounds such as 2,6-dimethoxy-p-benzopquinone and 2,4,6-trimethoxybenzaldehyde. *G. kola* is used by 20% of the population except for the Krobo, it is hard and bitter and is favored by males (88%) because it is considered an aphrodisiac. It contains the bioflavonoid (GKE-1). These species contain 1-2 ppm fluoride (5% W/V) and are broadly antimicrobial against both cariopathogens and periopathogens.

- *Techlea verdoorniana* (Rutaceae) favored by 9 tribes. Cleans efficiently is mild to gums. Active against *Candida albicans*. Contains Nkolbeine, a furoquinoline alkaloid, 7-deacetlyazdione, phenolic furoquinoline alkaloids tecleaverdoornine, tecleaverdine, and tecleine, lupeol, evoxanthine, and tecleanone, tecleanthine, skimmianine, and halfordinine. Fluoride 1.6 ppm (5%w/V)

- Ashanti children apply charcoal with the pedicel of *Musa* (Banana] (Musaceae). *Musa* contains a lectin that prevents adherence of plaque bacteria. Considered to confer excellent dental health to users. Pedicel makes a soft bristle.

- *Acacia kamerunensis* (Fabaceae:Mimosoideae) is used as chewing sponge with other species used in Africa, Asia and by the Aztecs. The source of antimicrobial activity is not specifically identified but dalbergions, acidic gums or ethyl gallate are possible candidates. The species also contains 1 ppm fluoride. Used by all Southern Ghanians except the Northern tribe and mostly by women (93%). Bedouin women prefer chewing gum arabic, a resin from *Acacia senegal* known to have antiplaque properties.

- *Terminalia glaucescens* and *T. ivornensis* are only used by the Northern tribe as a chewing sponge. These species are considered sweet, bitter and fibrous and are preferred by women (93%). Yellow tooth staining is a problem. They contain euflorotic concentrations of fluoride and are known to contain tannins which affect adherence of plaque bacteria. Triterpene antibiotics have been isolated from other species. These are active against both cariogenic and periopathogens.
Evaluating Hepatitis Remedies in the Context of Use: Results of an Ethnobotanical Focusing Study


- One quarter (523/2015) of the populations of 14 communities on 4 river systems were surveyed to understand their plant uses to treat hepatitis.

- Twenty seven species in fourteen plant families were identified as being used. The majority were trees with only a few herbaceous plants represented. 10 fit the "doctrine of signatures" by virtue of their yellow roots, flowers, fruits etc.

- Roots and rhizomes contained ethereal oils, alkaloids, flavanoids and saponins that could be antiviral, diuretic & antiinflammatory; fruits and nuts could be antiinflammatory, as well as being nutritious, young leaves and root buds, antiviral; flowers anthroquinones with purging qualities.

- Mestizo and indigenous remedies were quite distinct, with the former also including post Colombian plant introductions such as turmeric.

- Plants were used alone, several together, or sequentially but use was not always the same within a family unit.

- A pattern of favored plants emerged for both populations.

- Substitutions were made when the favored plant/s were unavailable.

- Of 358 individuals tested for their acute carrier status, 34 were shown to have recently been infected, and 4 others were identified as chronic carriers. Rates of infection on 4 river systems varied from 0.01-0.08%.

- In mestizo villages infections were related to proximity of an army base (tattooing, prostitutes), biting river flies, carriers with history of disease etc., nearby medical facilities at oil camps, traffic on river systems.

- In indigenous villages infections were related to family and neighbor related, returning former military personnel, drinking masato contaminated with HBV/HDV spittle, immunizations with improperly sterilized needles (once common practice among missionaries).

- Overall when favored bioreactive treatments were applied appropriately, and for an adequate period of time, the duration and severity of disease was significantly reduced as determined by clinical, serological, and physiological parameters.

- Co-infections with HDV were prevented when appropriate treatments were applied at the onset of disease.

The chronic carrier status was reversed with reduction of liver size and reduced viral load.
Where other possibilities lie.

Phylogenetic Amplification: A way of optimizing the therapeutic potential of medicinal plants.

- By looking for similar compounds in related plant species or families particularly as related to their phylogenetic groupings.
  
  *In flowering plants, certain related orders are known to share similar bioreactivities, suggesting a commonality of certain compounds. These are often found in the same phylogenetic group (10 in all) made up of subclasses and/or monophyletic clades. The same can be true for fungi.*

- discover similar bioreactivities and thus medical uses.
- discover related compounds with greater potencies and value.
- to address resource management issues.

- By looking for cross-sensitivity among related groups of microorganisms, viruses, fungi, parasites.

  *Similar cross sensitivities are known to genera and families of infectious organisms.*

- Identify sensitivity spectra of bioreactive phytochemical
- Identify needed therapeutic among allied diseases that are known ethnomedically.
Endophytic parasitic fungi containing Ergot Alkaloids
Amazonian and European Connections in the
Clavicipitaceae used in Partuision


*Balansia cyperi* sclerotia on the species *Cyperus prolixus* (known as piri piri) is commonly used in Amazonia to aid in childbirth. It contains the compound, ergobalansine.

*Claviceps purpurea* on Secale cereale (rye) is the source of ergot alkaloids such as ergometrine, and methylergometrine. These are strong uterotonics. Their use as parturition aids evolved from European herbalism. In conventional obstetrics they may be used in the 3rd stage of labor or to reduce blood loss in 1st trimester abortion currettage.
A Retrospective Ethnobotanical Linkage: The West Coast Yew and Other Sources of Taxol (Paclitaxel) and its Derivatives

As a mitotic inhibitor that stabilizes microtubules and prevents depolymerization they are used to treat metastatic breast, ovarian and non-small cell lung cancers and infrequently bladder, prostate, oesophageal cancers and melanomas.

- Wood teas of *Taxus brevifolia* used by Upper NorthWest Coastal & Alaskan Tsimshian for cancer (ethnobotanical linkage unknown at time of taxol discovery in 1977 of the isoprenoic compound, *paclitaxol* or *taxol*). This grows slowly in the cool coastal forests in the land of the “spirit bear” and was not a suitable source of the compound.

- *T. wallachiana*, valued for cancer in Himalayan medicine, source of baccatins e.g., *baccatin III*. The ethnobotanical linkage (The Bower Manuscript, 1893) was ignored by NCI at time of taxol discovery.

- *T. baccata* which grows in Europe, SW Asia, NW Africa and Iran is used for other medical, homeopathic and horticultural purposes. Its cultivar (*Taxus X media “Hicksii”*) is the primary source of baccatins such as *Baccatin III* used in the semisynthesis of *docetaxel*, used in the treatment of advanced or metastatic breast and non-small cell lung cancers.

Taxol is also elicited by certain endophytic fungi such as *Taxomyces*, *Pestalotiopsis*, *Sporormia*, *Trichothecium*, & *Aspergillus*. Other unrelated taxa similarly infected with endophytic fungi elicit other taxol derivatives and include temperate species such as *Corylus* and *Ilex* and the tropical Guyanian Rubiaceae *Maguireothamnus speciosus* infected with *Seimatoantherium tepuiense*.

Strategies to produce taxol from fungi include fermentative culture, and as a source for genetic engineering.
Testing phylogenetic amplification for possible sources of antimalarial compounds. With the advent of resistance the need to find alternatives is critical!


The Indian medicinal species L. camara is used to treat malaria and its extracts are effective against both chloroquine-sensitive and chloroquine resistant strains of Plasmodium falciparum.

Lantana cujabensis is a common Amazonian and Andean shrub, collected in Aguaruna territory on the Nieva River.

Its crushed leaves are sniffed to treat head colds in Bolivia and elsewhere Lantana spp. are valued for their rubafacient and andodynal properties.

Its antiinfective uses are possibly linked to the discovery of a new triterpenoid oleanene3β,25-epoxy-3α-hydroxy-22β-isobutanoxyloxyolean-12-ene-28-oic acid (1) and two known triterpenoids lantanilic acid (2) and camaric acid (3) all possessing anti-TB activities.

Also like its Indian counterpart extracts of L. cujabensis possessed low activity against P. falciparum.

Landenbergia oblongifolia (Rubiaceae) a non medicinal species growing in Kunchin, an OCCAMM Aguaruna village was studied to determine, if like other Rubiaceae , it might be a new source of antimalarial compounds. While its bark yielded three novel cinchoncine-derived alkaloids namely: Epicinchonicinol (1), cinchonidicinol (2) and dihydrocinchonicinol/dihydrocinchonidicinol mixture (3) none of these proved bioreactive in antimalarial assays using the chloroquine-sensitive strain HB-3 of Plasmodium falciparum.
Phylogenetic Applification:

Effects of Malaria Remedies against Apicoplexan protozoa.


- These 8 reactive plants were tested against related Apicoplexan protozoa (Cryptosporidium, Cyclospora, Isospora) Toxoplasma gondii protozoa and a fungus Pneumocystis (carinii) jerovici known to cause serious AIDS related infections elicited promising leads from 8 taxa. This was particularly true for those antimalarial plants also used to treat diarrhea. Toxoplasmosis is also the leading cause of death due to a food borne illness in the US.

  - **Cryptosporidium**: All 7/8 extracts proved positive, with 5 being highly positive. One of these plants was also used to treat diarrhea.
  
  - **Toxoplasma**: 7/8 extracts were cytotoxic however 1 extract proved active & with toxicity equal to the standard treatment of the nathoquinone, atavaquone also present with proguanil in the antimalarial composition Malarone. Alone atavaquone has antiparasitic and therapeutic effects when used to treat toxoplasmosis and is effective for mild cases of Pneumocystis pneumonia. While P. falciparum resistance to atavaquone is known, the bioreactivity of this Amazonian plant had proved otherwise, and thus it is possible the compounds are not similar and might serve as an alternative.
In Conclusion

- These examples have shown that integrative techniques when applied to traditional knowledge of medicinal plants can not only optimize their worth to the populations that use them, but can be a source of pharmaceuticals valued globally.
With Thanks

- To my husband, Dr. Walter Lewis who collaborated with me in understanding the worth of medicinal plants
- To peoples of the world that shared with us the worth of their traditional knowledge.
- To the scientists that collaborated with us in all aspects of our research.
- To the many countries that allowed us to work within their boundaries.
**Duboisia myoporoides** (Solanaceae): The Corkwood Tree

A tree or shrub native to high-rainfall areas of wet Eucalypt rainforests of New South Wales and Queensland in Eastern Australia.

- Considered poisonous, psychoactive, sedative and hypnotic because of its alkaloids e.g., *scopolamine* (hyoscine) atropine and nicotine.
- Water poured into trunk, drunk next day for stimulant, euphoric, antispasmodic and analgesic effects.
- Used to stupify fish and Emus by aboriginal people.
- Leaves are pharmaceutical source of tropane alkaloids, *scopolamine* and *atropine*.

- **Scopolamine** was used for motion sickness as in WW2 D Day, and also now to treat side effects of cancer chemotherapy.
- **Atropine** as a parasympopatholytic is used to dilate eye pupils and as a muscarinic receptor antagonist is used in resuscitation to speed up the slowed action of the heart and prevent death rattle in dying patients. It also inhibits excessive salivation, tearing, urination, sweating and GI motility and vomiting in organophosphate poisoning.

A derivative of *scopolamine* is the main active ingredient of the drug *butylscopalamine*, a potent abdominal-specific antispasmodic and analgesic.
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