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Changing rules re the use of botanicals in NIH-funded research

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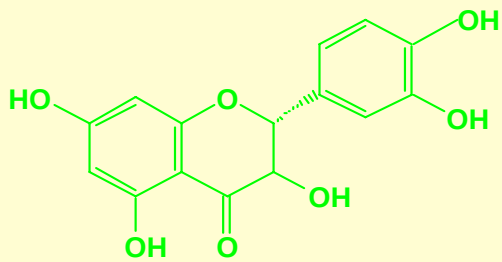
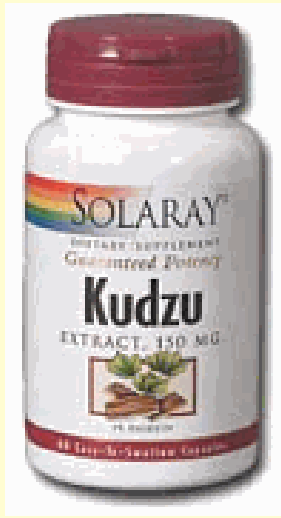
The composition of botanicals

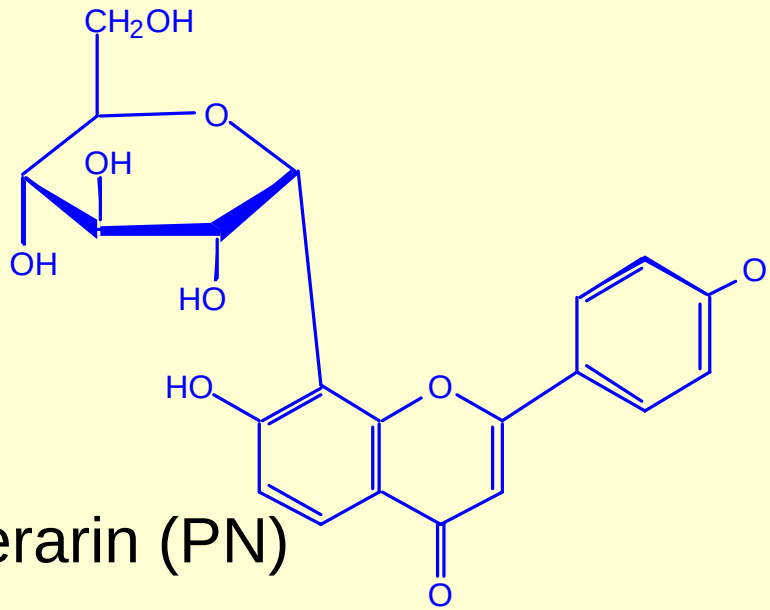
Botanicals can be the specific parts of a plant, a simple extract of the plant part, or a purified component

- **e.g., green tea (powdered dry leaves of the *Camellia sinensis*)**
- **Green tea polyphenols (a dried, concentrated water extract of green tea enriched in tea catechins)**
- **Epigallocatechin-3-gallate (purified green tea catechin with alleged highest antioxidant activity)**



catechin





Puerarin (PN)

Study reproducibility

- **Systematic science predicts that a single compound or even a complex matrix with a defined and constant composition will yield the same biological response assuming that all other experimental conditions are carefully controlled**
- **If the botanical preparation is defined and has a constant composition, it will behave just like a pharmaceutical agent**

Regulations re composition of botanicals

- **The 1994 DSHEA act did not legislate a standard for the composition of botanicals**
- **The ODS initiated a program in 2002 to define validated methods for the analysis of the bioactive components in the most popular botanicals/dietary supplements (interagency agreement with the FDA which led to a contract to AOAC International)**
- **A second contract was established with NIST to prepare standardized botanicals/dietary supplements in specific matrices to be reference materials for analyses.**

AOAC Methods

On the PTFDS List	Constituents
Hawthorn (Crataegus spp.)	Chlorogenic acid, epicatechin, hyperoside, isoquercitrin
Biotin	
Feverfew	Parthenolide(s)
Comfrey	Pyrrolizidine alkaloids (PA's)
Colostrum	
Creatine	
Podophyllotoxins	
Valerian	Valerenic acid(s)
Baical skullcap	Baicalin
Cardiac glycosides	
Goldenseal	Hydrastine, berberine, palmatine
Chaste tree	aucubin, agnuside, casticin, isovitexin, orientin
Choline	
Pine bark extract	Polyphenols

AOAC Methods

Prioritized Methods (IRS)	Constituents
Red clover	Isoflavones
L-Carnitine	
B Vitamins	
Black cohosh	Triterpene glycosides
Ω-3 Fatty acids	
Soy isoflavones	
Green tea catechins	EGc, EGCG
Lutein	
Turmeric	Curcumin
Ginger	Gingerol, shoagol
Milk thistle	silymarin
African plum	Fatty acids, phytosterols
Flax seed	Secoisolariciresinol-diglucoside

AOAC Methods

In Progress (ERP)	Constituents
Ephedra*	Ephedrine, pseudoephedrine, 4 other alkaloids
Aristolochic acids	Aristolochic acids A and B
SAMe	
ß-Carotene*	
Chondroitin sulfate	
Glucosamine*	
St. John's wort	Rutin, quercitrin, quercetin, isoquercitrin, hyperoside, pseudohypericin, hypericin, hyperforin
Ginkgo flavonols*	Quercetin, kaempferol, isorhamnetin
Ginkgoterpenes	Bilobalide, Ginkgolides A, B, C, J
Saw palmetto phytosterols	Campesterol, stigmasterol, Beta-sitosterol
Saw palmetto fatty acids	Free fatty acids
Bitter Orange	p-synephrine, tyramine, N-methyltyramine, octopamine, hordenine

Botanicals versus therapeutics

- **Although we might think of classical therapeutics as pure compounds, many are not.**
  - **Many agents used to treat cancer are derived from plants (vinblastine, vincristine, taxol, etc.)**
  - **Premarin™ is an extract of horse urine and contains 8-10 estrogen-like compounds as well as many phenolic acids - its composition is regulated with specific ranges of its components defined**
- **Botanicals/supplements are rarely pure compounds (an exception is vitamin c)**
  - **Not always clear that the presumed bioactive is present**

The Echinacea saga

- **NCCAM funded a study of Echinacea and clinical immunity in children - this was published in JAMA - the study conclusion was that “Echinacea had no significant effect”**
- **Criticism of this study has included discussion about whether the correct part of the *Echinacea purpurea* was used, to the late intervention with this supplement**
- **Interestingly, there was a significant reduction in the number of second and third event colds in children treated with *E. purpurea* compared to controls. This was not reported by the media.**

Reaction to the Echinacea saga

- **NCCAM has created a set of new regulations that govern its funding of experiments in which botanicals or dietary supplements are used.**
- **It is therefore crucial to properly characterize the botanical you're going to use in a study.**
- **You may wish to use a pure component derived from a botanical - NCCAM has rules for that, too.**

NCCAM rules for botanicals

- **See NOT-AT-05-004**
- **Evidence that a reproducible product is available**
- **Reserve test and control diets for later analysis**
  - **Provide a plan for sampling scheme, storing and analyzing samples, establishing variances and making results available to NCCAM**
- **The dose to be used should be justified**

How NCCAM views the botanical

- **The complex natural product (the botanical) is the purpose of studies**
- **Isolated constituents can be used if the intent is to**
  - **characterize or standardize the whole botanical**
  - **compare their activity to the whole botanical**
  - **determine the mechanism(s) of action**
  - **improve the preparation of the whole product**
- **NCCAM will not support development of isolated constituents as drugs**
  - **However, if a pure compound is already available as a dietary supplement, it qualifies for support (e.g., vit C)**

In the text of the application

Descriptors

- **Name of the product (species, strain, as applicable);** *Vitis vinifera*
- **Parts to be used (e.g., root, stem, leaf) as applicable;** *seed of the grape; purified to 90% purity (as polyphenols)*
- **Description of placebo or vehicle control group;** *the grape seed extract was added to AIN-76A diet. Control diets had no added grape seed extract*
- **Doses or concentrations to be used.** *Previous toxicity studies in Dr. Kim's lab had suggested that 5% GSE in the diet was non-toxic. The goal of the proposed study was to examine GSE's effects down to a dose of 0.1% (by wt.) in diet.*

More on Botanicals

In the Background and Significance section, provide the following:

- **Justification/rationale for studying the chosen product;**
- **Justification for the chosen form of the product (extract, powder, etc.);**
- **Justification for the proposed doses/concentrations;**
- **Description of the pharmacokinetics of the product (if known);**
- **Source of the product (and if not using a commercial source, an explanation of why a product generally available to the public is not being used).**

Just-in-time issues

- **If the application has a priority score that gives it a likelihood of being funded, NCCAM will notify the PI to provide product quality information**
- **“Unsatisfactory” information may prevent funding**
  - **Applicant may have to re-apply and submit additional information for the next review cycle**

Approaching this in a recent R21 application

The potential for plant polyphenols to selectively mimic hormone replacement therapy in maintaining cognition

- ***What has been the standard hormone replacement therapy (Premarin™) for 40-50 years, has been proved to be ineffective in preventing heart disease***
- ***Experiments with diets supplemented with other proanthocyanidins in fruits and berries have shown improvements in cognitive function induced by ovariectomy***

Use of grape seed extract

Grape seed extract (GSE) is a widely available dietary supplement marketed for anti-oxidant health benefits

The GSE material forming Gravinol™ was supplied by Kikkoman Corp. It was 90% by weight of oligomeric PACNs and 7% by weight of other polyphenols including catechin monomers

GSE and health

- **Documented history of health benefits of GSE and related PACNs in cognitive dysfunction**
  - **Peng (2005) found that 0.5% GSE enhanced cognitive function in OVEX spontaneously hypertensive rats**
  - **Deshane et al. (2004) showed effects on brain protein expression from 5% GSE in the diet**

- **Role for PACNs in other models of human chronic diseases:**
  - **Prevention of gastric erosions due to stress (Iwasaki, 2004)**
  - **Lens cataract disease in ICR/f rats (Yamasoki, 2002) and diabetic rats (Osakabe, 2004; El-Alfy, 2005)**
  - **Prevention of colonic cancer (Nomoto, 2004; Gosse, 2005**

Dose selection of GSE

- **Rationale for systematic analysis of dose response of GSE in animal studies, and dose selection (0, 0.05, 0.1, 0.25, 1.0 and 2.5%)**
  - **Previous studies had shown that when 5% GSE was given to young adult rats, brain proteins were systematically changed (Deshane, 2004)**
  - **0.5% GSE improves cognitive function in OVEX SHR (Peng, 2005)**
  - **0.2% GSE slows onset of lens cataract disease in ICR/f rats (Yamasoki, 2002)**
  - **0.1% GSE is equivalent to human dosage from supplements on a per kg body weight basis**

Safety and Toxicity

- **Safety and toxicity of GSE PACN**
  - **Kim (2004) found that there were no systematic changes in organ weights up to 10% GSE in the diet in rats**
  - **Yamasoki (2002) observed a no-observed-adverse-effect level (NOAEL) of 2% in the diet**
  - **Bentivegna (2002) proposed a NOAEL of 2.5%**

Known pharmacokinetics of the product to be used

- **Current knowledge of the pharmacokinetics of GSE PACN**
  - **The flavonols present are quickly absorbed, methylated, sulfated and glucuronidated**
  - **Half lives are ~ 4 h, ring-opened metabolites have longer half lives (Takizawa, 2003)**
  - **The oligomeric PACNs are poorly absorbed; small amount of catechin dimers from chocolate (Holt, 2002), and higher oligomers (Shoji, 2006) from apple PACNs; their tetramethyl ethers (Garcia-Ramirez, 2006) found in blood following administration of synthetic PACNs**

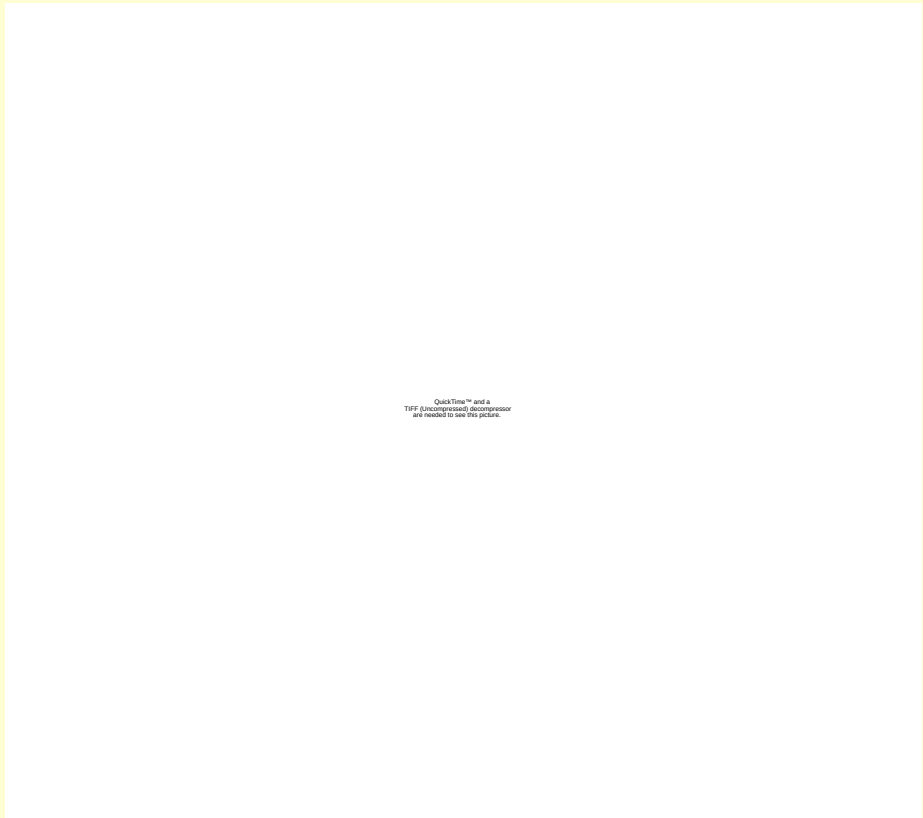
Choice of GSE and the model

- **Rationale for the study of actions of GSE polyphenols in the spontaneously hypertensive rat (SHR)**
  - **The SHR model is well-established**
  - **Peng (2005) found that 0.5% GSE improved cognitive function in OVEX SHR rats**
  - **Others have reported that PACNs improve cognitive function**

What is in the diet in the experiment

- **Analysis, validation and monitoring of GSE and diet composition over the course of the study**
  - **This will be covered by Dr. Prasain in his section**

Making and storing the diet



Diet mixing and storage

- **Both liquids and solids can be blended into the diet - must be well mixed - batch lots of ~9 kg**
- **Diets stored both in the dark and at -20^{o}C**
- **Normally diet used over a 4-6 week period**
- **New NCCAM rules require tests of the stability of the added compounds in the diet both at room temperature and after cold storage**
- **Diet must be retained for post study analysis**

Acknowledgements

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