The Effectiveness of Natural Products for Women's Health

8th Annual NHRI Scientific Symposium

Presented by: UIC COLLEGE OF PHARMACY

Safety and Efficacy of Botanical Dietary Supplements As Alternatives to Hormone Replacement Therapy

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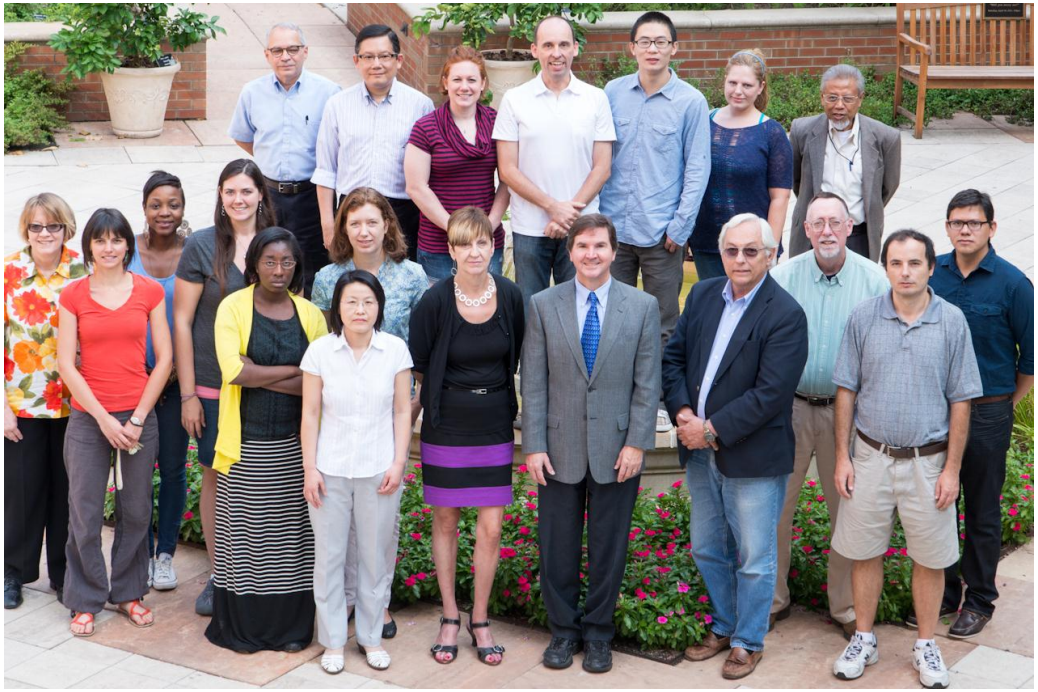
University of Illinois College of Pharmacy



UIC/NIH Botanical Center for Dietary Supplements Research

- 1998 – US Congress appropriated funds for the NIH Office of Dietary Supplements to establish botanical research centers
- 1999 – UIC Botanical Center founded
- First Director, Norman R. Farnsworth

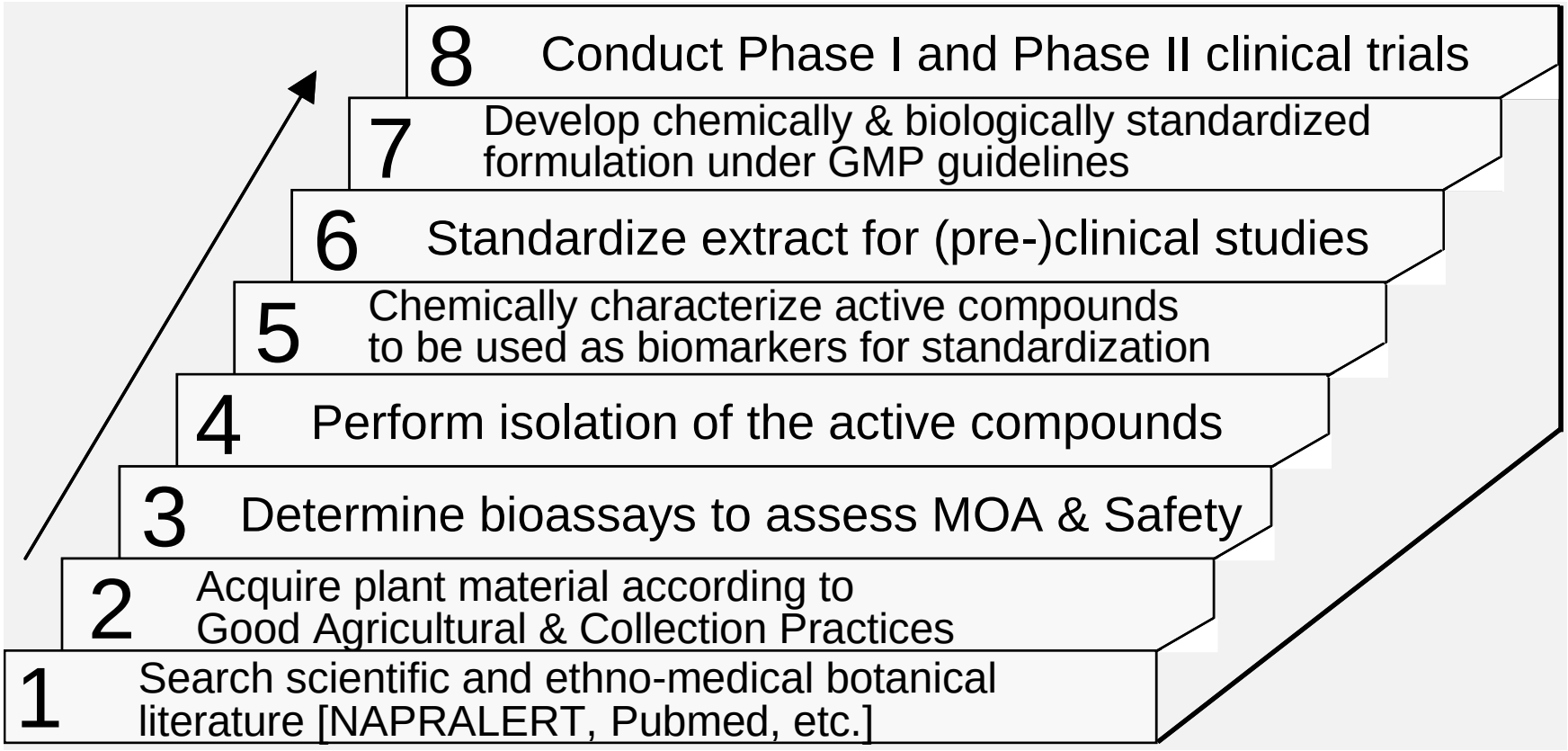
First meeting of the UIC/NIH Botanical Center External Advisory Board 2000

UIC Botanical Dietary Supplement Research Philosophy

Any botanical dietary supplement to be studied in humans must be:

- Botanically authenticated
- Biologically standardized
- Chemically standardized (based on active constituents)
- Manufactured under GMP
- Have a [proposed] mechanism of action
- Safe

Steps in Developing a Botanical Dietary Supplement for Clinical Evaluation



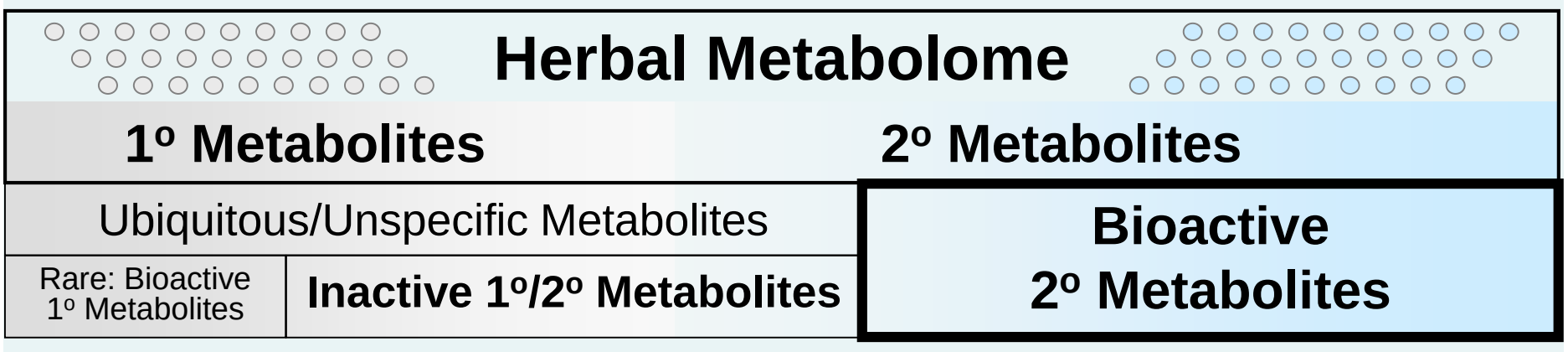
UIC Botanicals for Women's Health

- ***Angelica sinensis***
  Estrogenic, serotonergic, QR Induction, antioxidant, anti-inflammatory
- ***Cimicifuga (Actea) racemosa*** (black cohosh)
  Serotonergic, antioxidant, anti-inflammatory
- ***Vitex agnus-castus***
  Serotonergic, anti-inflammatory, QR induction, antioxidant
- ***Valeriana officinalis***
  Serotonergic, anti-inflammatory
- ***Trifolium pratense*** (red clover)
  Estrogenic, progestogenic, anti-inflammatory, QR induction, antioxidant
- ***Humulus lupulus*** (hops)
  Estrogenic, anti-inflammatory, QR induction, antioxidant
- ***Viburnum prunifolium***
  Serotonergic, anti-inflammatory, QR induction
- ***Glycyrrhiza glabra*** (licorice)
  Estrogenic, anti-inflammatory, QR induction
- ***Glycyrrhiza uralensis***
  Estrogenic, anti-inflammatory

Project 1: Metabolomics: Characterization of Botanical Chemistry and Synergy

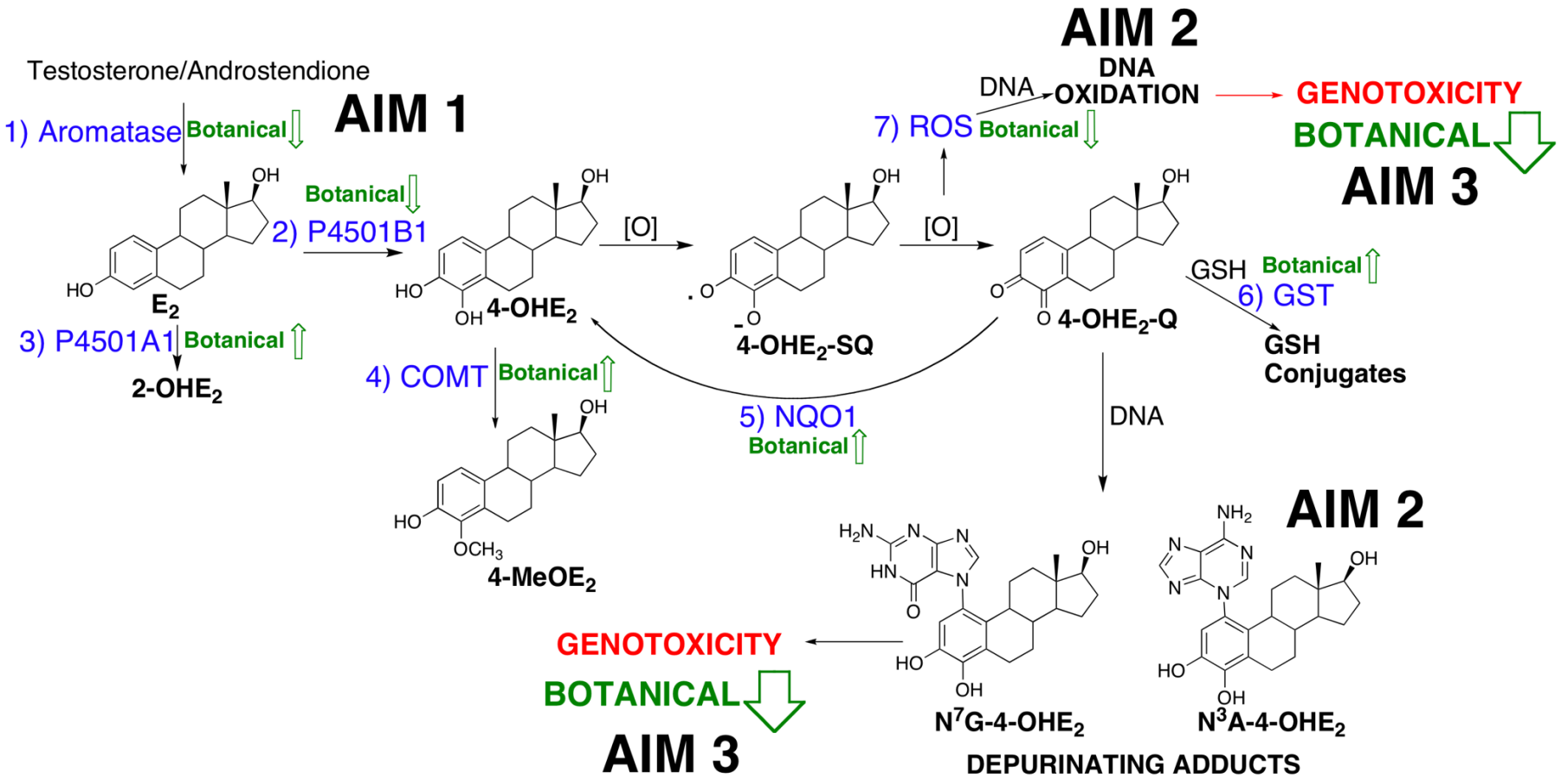
Guido Pauli, Shao-Nong Chen

- **Aim 1**: Chemodiversity Profiling of Bioactive Metabolites
- **Aim 2**: Metabolomic Standardization Methods
- **Aim 3**: Study of Botanical Synergy



Project 2: Botanical Modulation of Estrogen Carcinogenesis

Judy Bolton, Birgit Dietz



Project 3: Metabolism, Safety & Efficacy

Richard van Breemen, Dejan Nikolic

Aim 1: Metabolism & bioavailability of active compounds

- In vitro and in vivo metabolism
- Identification of enzymes responsible for metabolism
- Investigation of enzyme inhibition by botanicals
- Bioavailability in rats

Aim 2: Investigation of botanical-drug interactions

- Inhibition of phase I and phase II metabolism
- Induction of phase I and phase II enzymes
- Induction/inhibition of drug transporters
- Identification of compounds responsible for enzyme inhibition/induction and transporter inhibition/induction

Core B: Botanical Integrity

GF Pauli, SN Chen, DD Soejarto

Core C: Bioassay

Judy Bolton, Birgit Dietz

Core D: Analytical

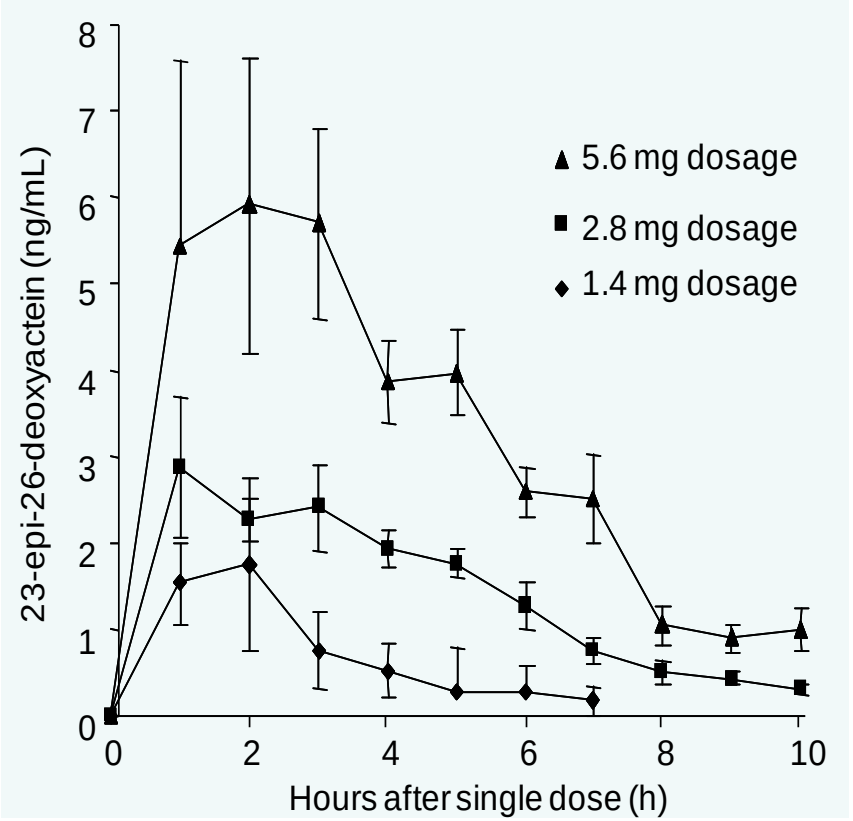
Richard van Breemen, Dejan Nikolic, David Lankin

- Botanical authentication and extraction
- Chemical and biological standardization
- Determination of mechanisms of action
- Identification of active constituents

Clinical Trials of Botanical Dietary Supplements at the UIC Botanical Center

- Phase I study of safety and pharmacokinetics of red clover. Piersen, *et al. Curr Med Chem*. 2004, *11*: 1361-74.
- Phase I clinical trial of safety and pharmacokinetics of black cohosh. van Breemen *et al. Clin Pharmacol Ther*. 2010, *87*: 219-25.
- Phase I investigation of safety and pharmacokinetics of hops. In progress.
- Phase II clinical trial of safety and efficacy of black cohosh and red clover. Geller *et al. Menopause,* 2009, *16*: 1156-66.

Concentration-time profiles of 23-epi-26-deoxyactein in human serum after oral administration of a black cohosh extract



Alternatives to Hormone Replacement Therapy: Screening Botanicals for Estrogens

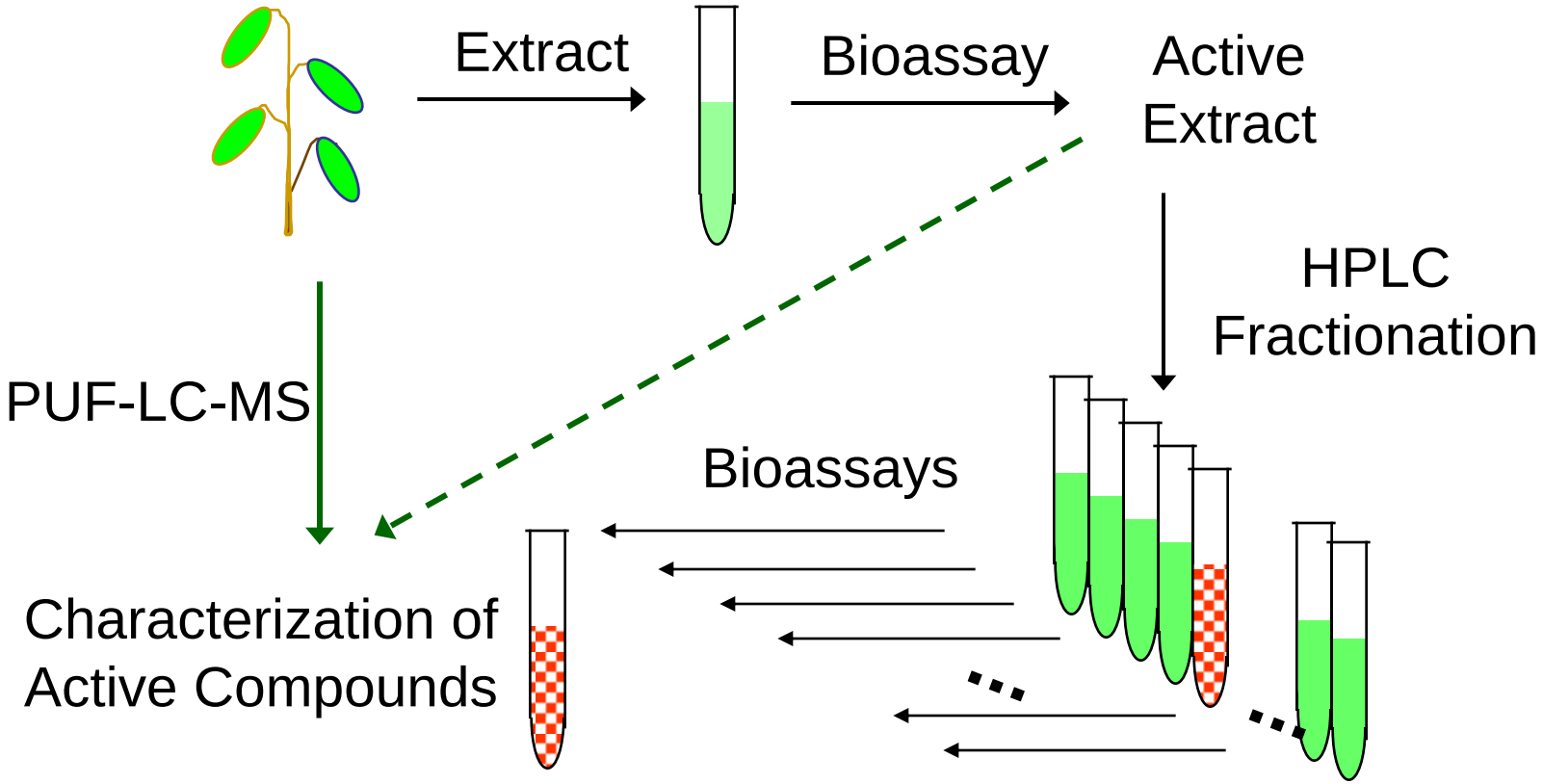
- Hormone replacement therapy (HRT) in women might increase risks of cardiovascular disease, breast cancer and dementia.
- Therefore, women are seeking safer alternatives to HRT.
- Many women are using botanical dietary supplements such as black cohosh, red clover or hops for the management of menopausal symptoms.
- The mission of the UIC/NIH Botanical Center is to investigate the safety and efficacy of botanical alternatives to HRT.
- Finding alternatives to HRT that prevent instead of causing cancer is a goal of the Center.

Hops: Promising Botanical for Postmenopausal Women's Health

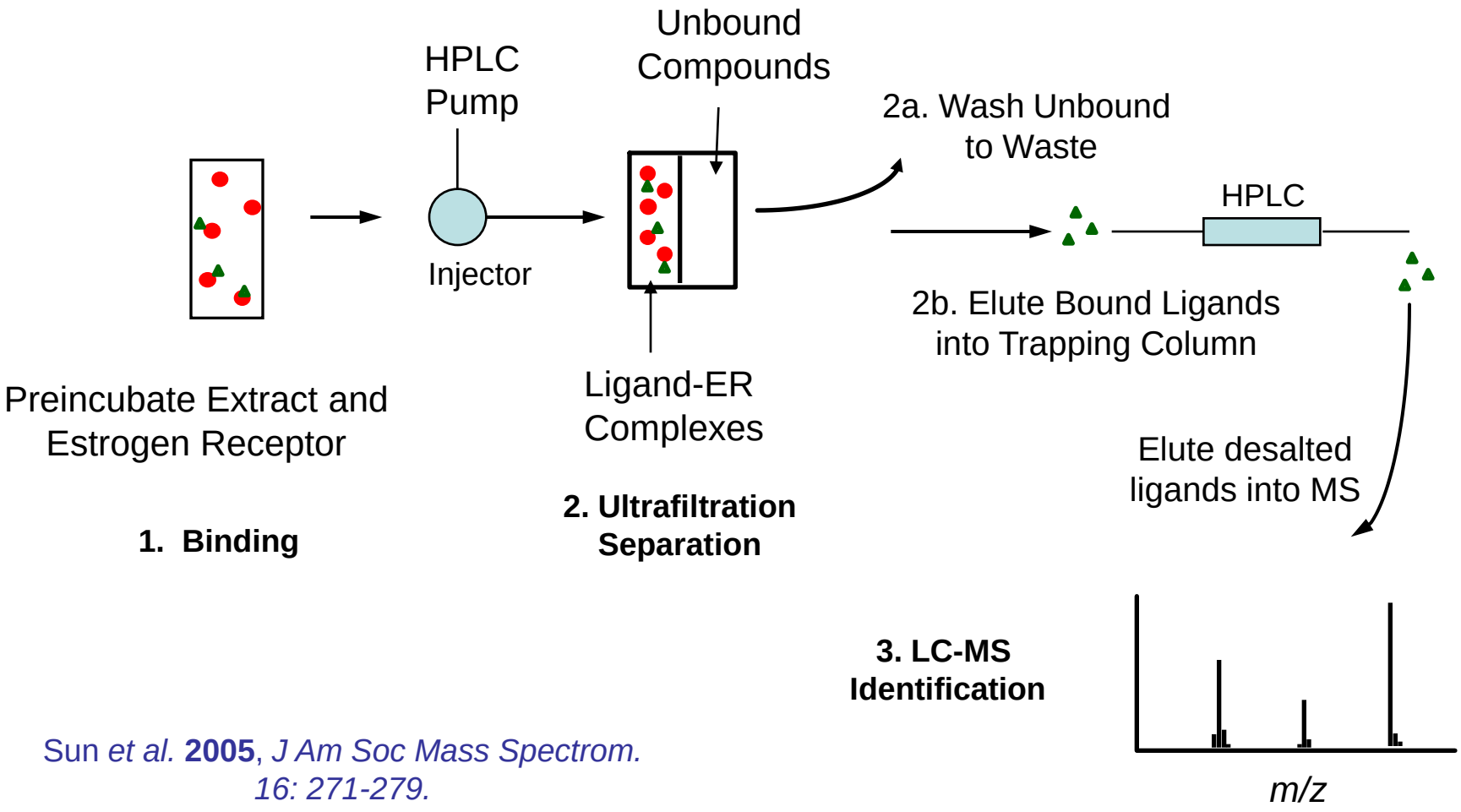
- Hops (*Humulus lupulus* L.) used during the brewing of beer as a preservative and flavoring agent.
- Contain the estrogen 8-prenylnarigenin. *J Agric Food Chem* 2005, *53*:6246-6253.
- Do not induce uterine weight. Might contain natural progestins. *Chem Biol Interact* 2008, *176*:30-39.
- Induce NQ01 activity. Active compound is xanthohumol. *Chem Res Toxicol* 2005, *18*:1296-1305.
- Inhibit catechol estrogen formation in MCF-10A cells. *Cancer Prev Res (Phila)* 2012, *5*:73-81.
- Under investigation for managing hot flashes In menopausal women.

Identification of Active Compounds in Botanicals

PUF-LC-MS vs Bioassay Guided Fractionation

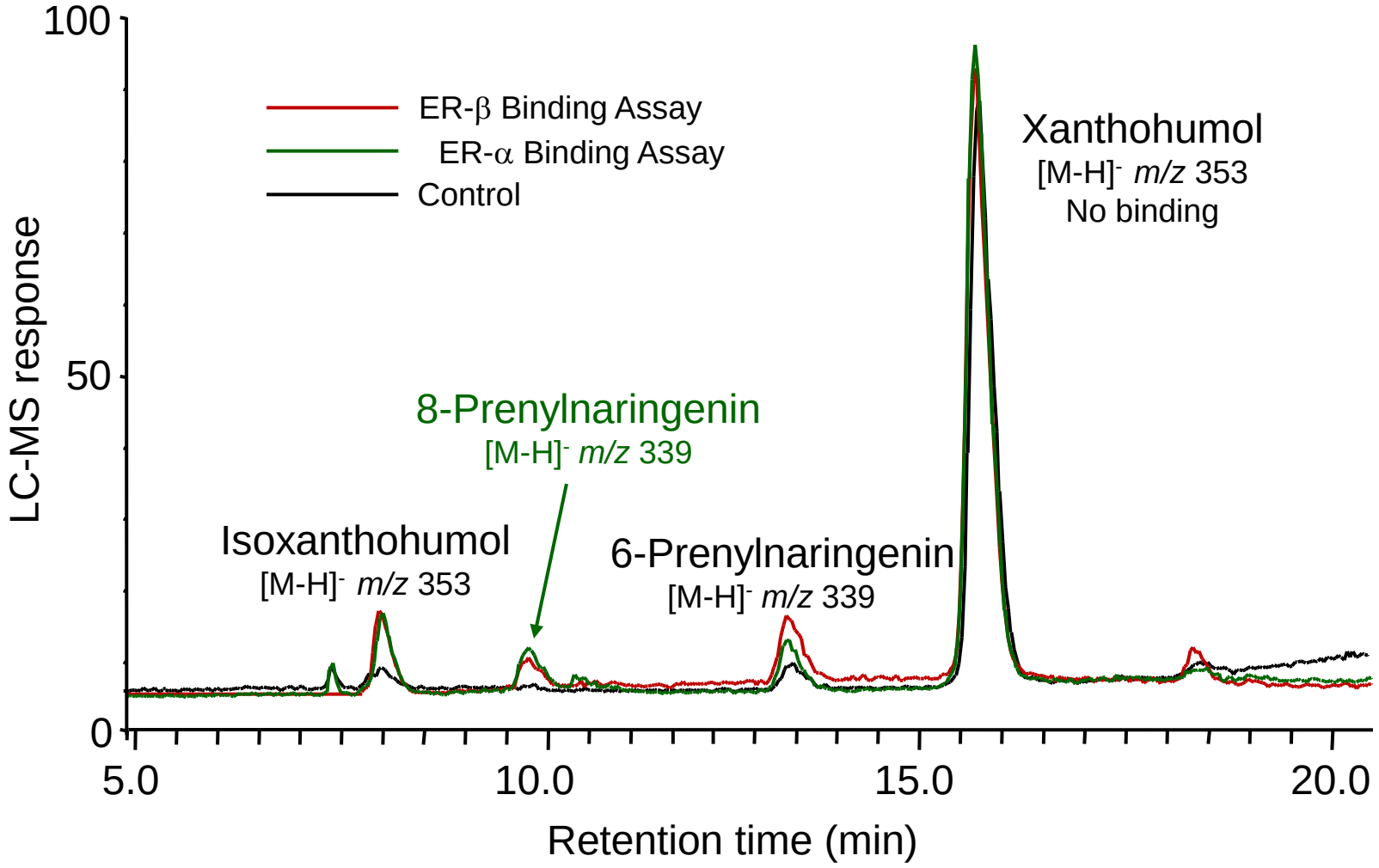


Pulsed Ultrafiltration-Mass Spectrometric Screening for Ligands of ER-α and ER-β



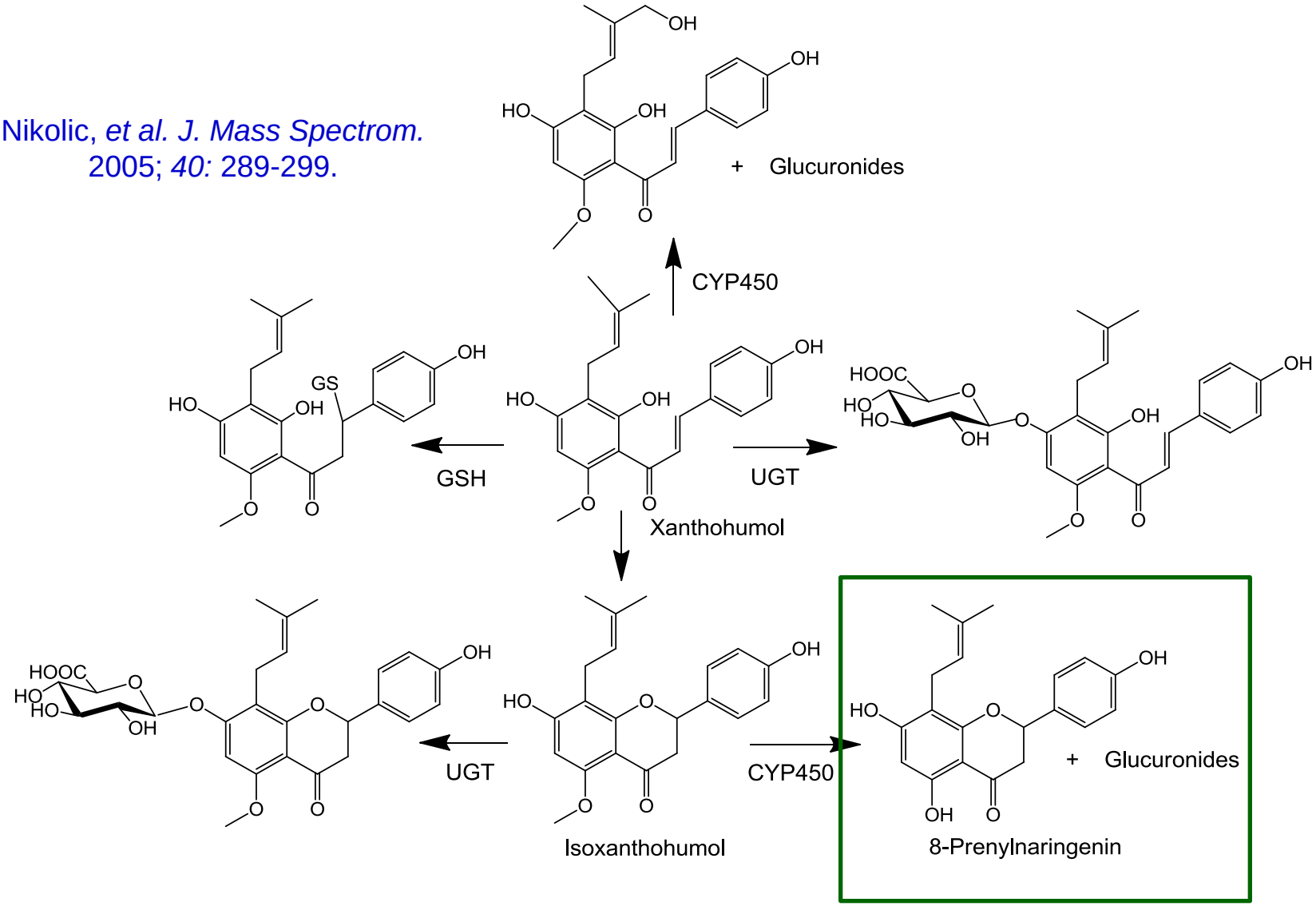
Sun *et al.* **2005**, *J Am Soc Mass Spectrom. 16: 271-279.*

Ultrafiltration LC-MS Assay of Hop Extract for Ligands to Estrogen Receptors

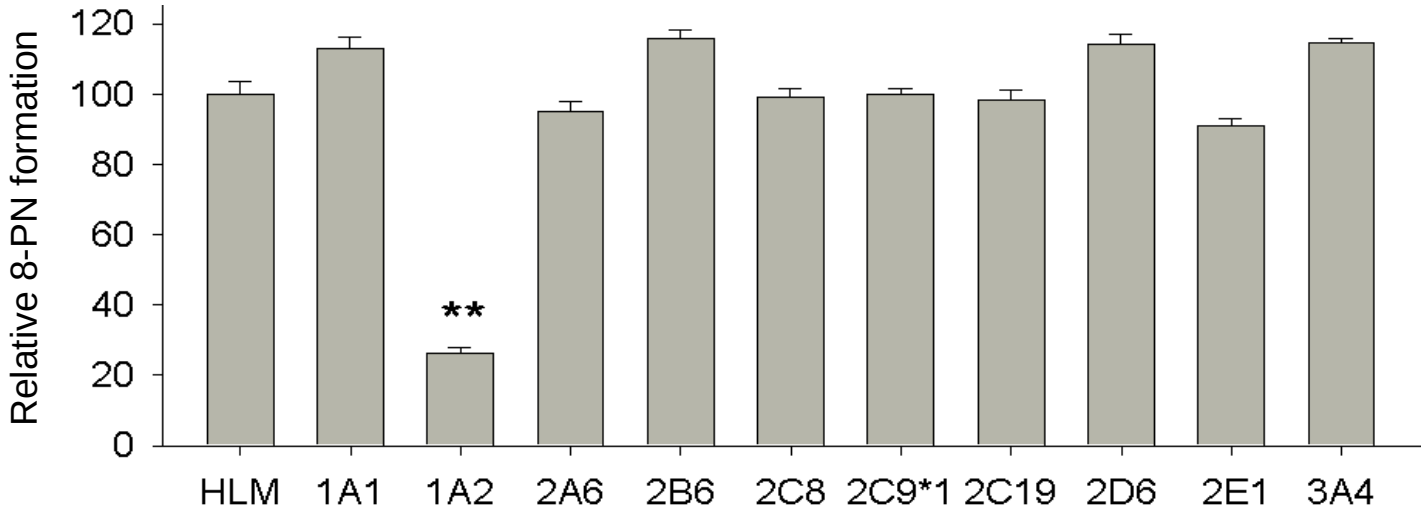


Metabolism of Xanthohumol Determined Using Human Liver Microsomes and Hepatocytes

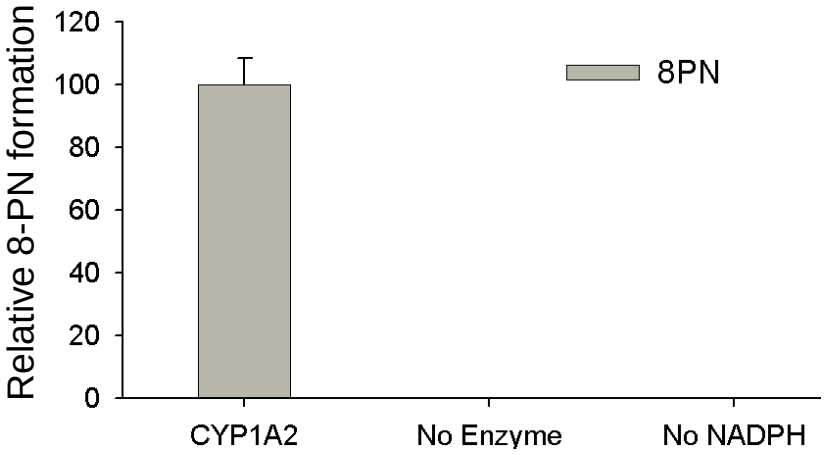
Nikolic, *et al. J. Mass Spectrom.* 2005; *40:* 289-299.



Identification of CYP450 Enzymes that *O*-demethylate Isoxanthohumol to Form 8-Prenylnaringenin

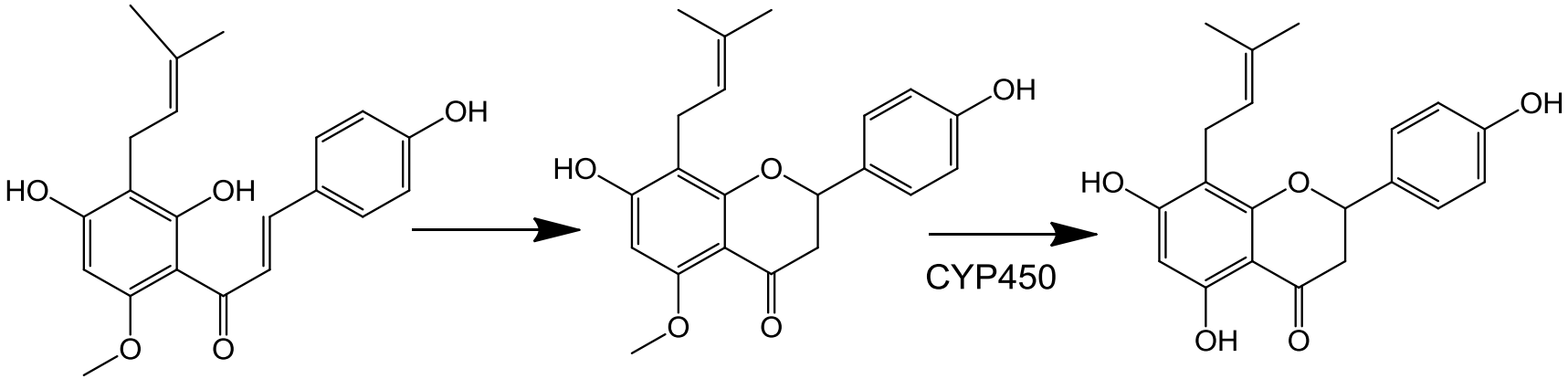


- mAb inhibitors of CYP450 enzymes were incubated with human liver microsomes, isoxanthohumol and NADPH.
- 8-Prenylnaringenin was measured using LC-MS.
- CYP1A2 *O*-demethylates isoxanthohumol to form 8-prenylnaringenin.



Nikolic *et al. J. Mass Spectrom.* 2005, *40*: 289-299.

Conversion of Xanthohumol to Isoxanthohumol and 8-Prenylnaringenin



Xanthohumol

Non-estrogen
Cancer chemoprevention agent via Keap1/Nrf2

Isoxanthohumol

Weak estrogen

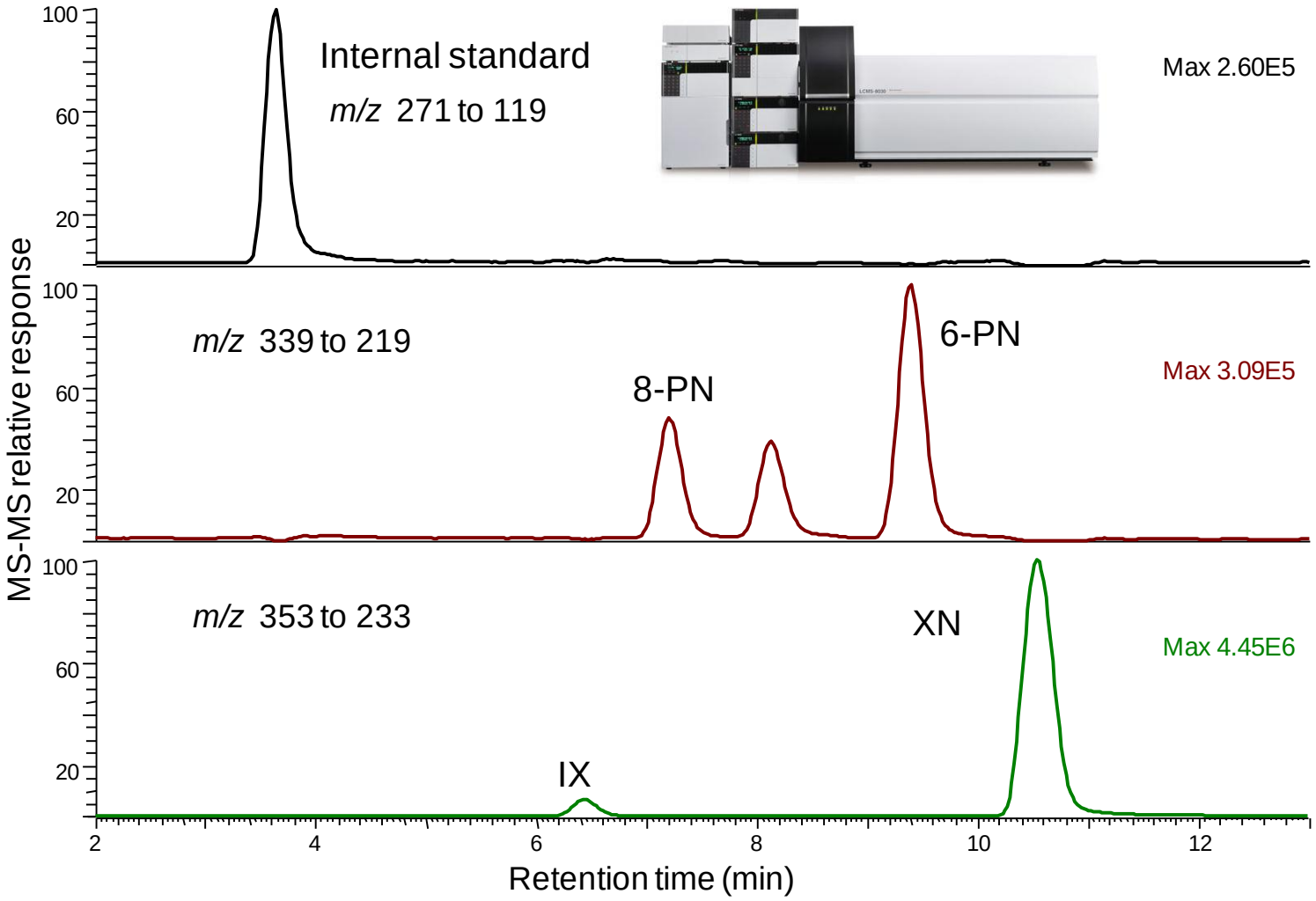
8-Prenylnaringenin

Potent estrogen

Although estrogenic 8-prenylnaringenin is only a minor constituent of hop products, it can be formed in vivo by hepatic metabolism of isoxanthohumol.

Nikolic D, Chadwick LR, Pauli GF, van Breemen RB. *J. Mass Spectrom.* 2005; *40:* 289-299.

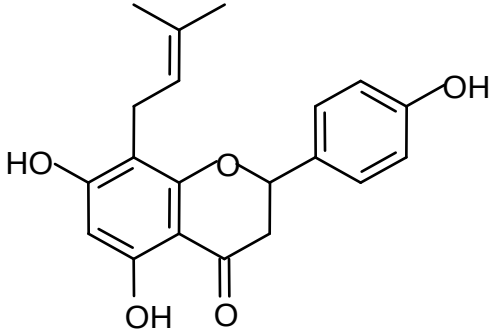
LC-MS-MS Analysis for Standardization of Hop Extract for Phase I Clinical Investigation



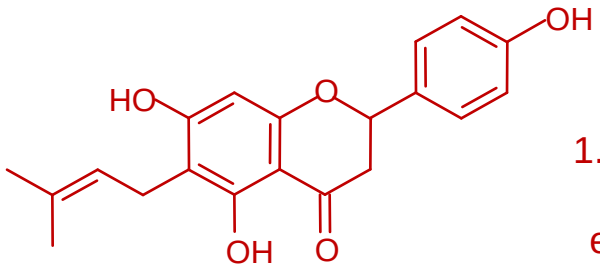
Thermo TSQ Quantum; YMC AQ 2 x 100 mm, 3 µm. Total analysis time 15 min.

Active Compounds Identified and Measured in Standardized Ethanol Extract of Hops

0.35% (wt)
estrogenic



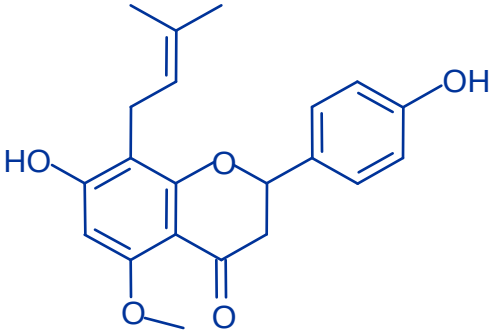
8-Prenylnaringenin



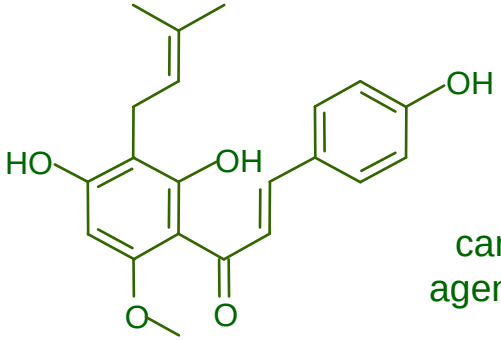
6-Prenylnaringenin

1.77% (wt)
weak
estrogen

1.07% (wt)
weak
estrogen



Isoxanthohumol



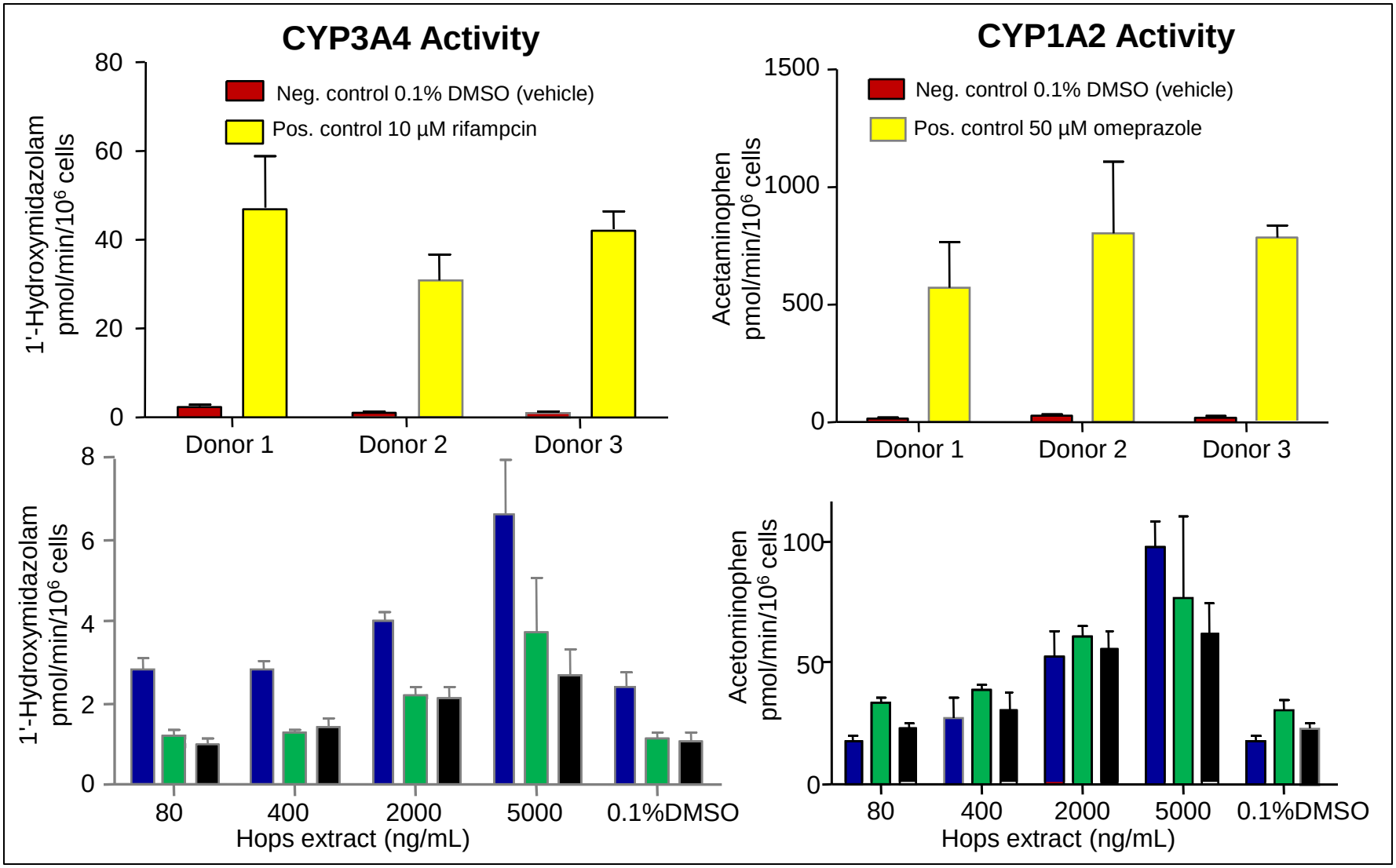
Xanthohumol

33.84% (wt)
cancer prevention
agent (non-estrogen)

Investigation of Possible Induction of Human Cytochrome P450 Enzymes by Hops

- Although hops (*Humulus lupulus* L.) are used to brew beer, little is known about possible interactions of hop extracts or their constituents with drug metabolizing enzymes.
- An *in vitro* assay utilizing human hepatocytes in cell culture was used to evaluate the potential for cytochrome P450 induction by hops.
- Cells were incubated with hop extracts or with vehicle only (control) to induce or inhibit drug metabolizing enzymes.
- Cytochrome P450 enzyme assays were carried out using standard substrates for the isoforms CYP3A4 and CYP1A2.
- UHPLC-MS-MS and SRM were used with the Nexera-8030 system to enhance the throughput, precision and accuracy of the enzyme activity assay.

CYP1A2 and CYP3A4 Activities of Human Hepatocytes after Treatment with Hops



Conclusion: Hops do not induce drug metabolizing enzymes.

Inhibition of Human CYP450s by Hops and Prenylated Flavonoids Isolated from Hops (*Humulus lupulus*)

- In addition to induction of drug metabolizing enzymes, inhibition of these enzymes is a common mechanism for drug-botanical interactions.
- The standardized hop ethanolic extract and active prenylated phenols were assayed for cytochrome P450 (CYP450) inhibition.
- When inhibition of CYP450 enzymes was observed, IC_{50} values (concentrations required to inhibit 50% enzyme activity) were determined for inhibitors of specific enzymes.

Screening of Prenylated Phenols from Hops and a Hop Extract for Inhibition of Cytochrome P450 Enzymes

	CYP1A2	2B6	2C8	2C9	2C19	2D6	2E1	3A4
				% Inhibition (1 µM) ± S.D.				
6-PN	< 10	< 10	< 10	< 10	< 10	< 10	< 10	< 10
8-PN	55.8 ± 0.7	< 10	32.2 ± 1.2	ND	43.8 ± 0.2	< 10	< 10	< 10
IX	< 10	< 10	87.7 ± 1.1	31.6 ± 8.0	51.0 ± 8.6	< 10	< 10	< 10
XN	< 10	< 10	47.8 ± 5.1	< 10	< 10	< 10	< 10	< 10
				% Inhibition (10 µM) ± S.D.				
6-PN	21.4 ± 0.8	35.7 ± 1.9	85.5 ± 1.2	63.8 ± 2.0	14.3 ± 0.1	< 10	< 10	< 10
8-PN	88.4 ± 1.6	34.7 ± 1.7	97.6 ± 0.1	93.0 ± 0.6	92.8 ± 5.5	< 10	< 10	31.4 ± 2.8
IX	48.2 ± 4.6	< 10	98.9 ± 0.1	82.1 ± 1.7	96.3 ± 1.5	< 10	< 10	< 10
XN	11.8 ± 1.1	43.9 ± 1.3	92.9 ± 0.4	69.7 ± 3.0	15.0 ± 1.9	< 10	< 10	< 10
Hop extract (5 µg/mL)	26.7 ± 8.1	36.4 ± 5.1	92.7 ± 0.4	88.1 ± 2.5	69.8±10.8	19.5 ± 2.5	13.7 ± 12.6	19.2 ± 2.9
4 Mix[1]	16.4 ± 0.7	14.9 ± 1.4	92.7 ± 0.6	75.9 ± 2.1	65.5 ± 6.8	20.8 ± 4.9	< 10	29.2 ± 2.3

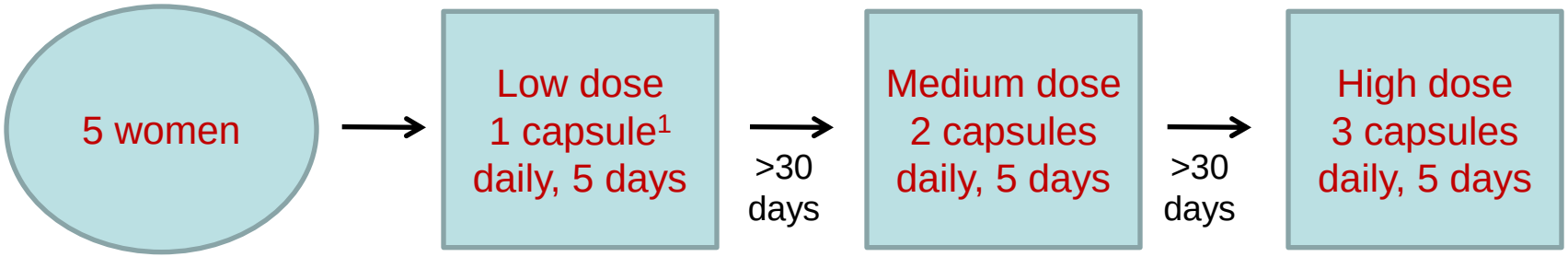
[1] Mixture of 6-PN, 8-PN, IX, and XN at concentrations identical to the 5 µg/mL hop extract

IC_{50} Values (μM) for the Inhibition of Cytochrome P450 Enzymes by a Hop Extract and Hop Prenylated Phenols

Compound	CYP1A2	CYP2C8	CYP2C9	CYP2C19
Hops	9.4 ± 0.6[1]	0.8 ± 0.1	0.9 ± 0.1	3.3 ± 0.7
IX	ND[2]	0.2 ± 0.1	2.1 ± 0.3	0.5 ± 0.1
8-PN	1.1 ± 0.2	0.6 ± 0.1	1.1 ± 0.2	0.4 ± 0.1
XN	ND	1.1 ± 0.1	3.3 ± 0.4	ND
6-PN	ND	1.9 ± 0.1	5.9 ± 0.7	ND

1. Data expressed as mean ± SD
2. ND: Not determined since ≤ 50% inhibition at 10 μM

Phase I hops Clinical Study Design

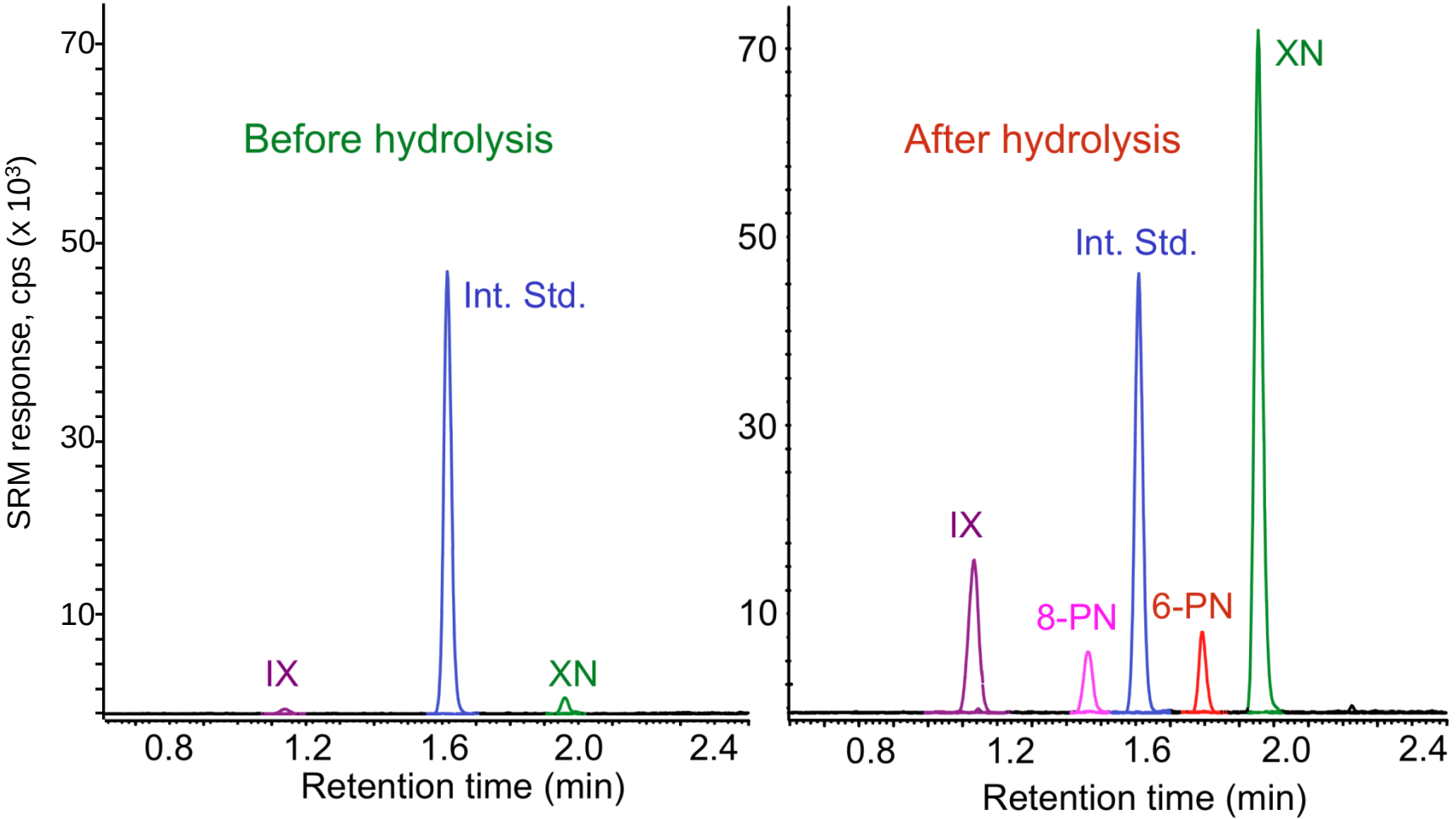


- Healthy women
- Post-menopausal
- Not on hormone replacement therapy
- No beer for 1 month before or during study

- Urine collected during first 24 h.
- Blood drawn hourly for first 24 h then daily through day 5.
- Women monitored for another 7 days for adverse effects.
- Urine and blood analyzed for effects on blood chemistry, hormone levels, etc.
- Urine and blood analyzed using UHPLC-MS-MS for 8PN, 6PN, IX, XN, and their conjugates in support of pharmacokinetics study.
- Data will be used for design of Phase II study of safety and efficacy.

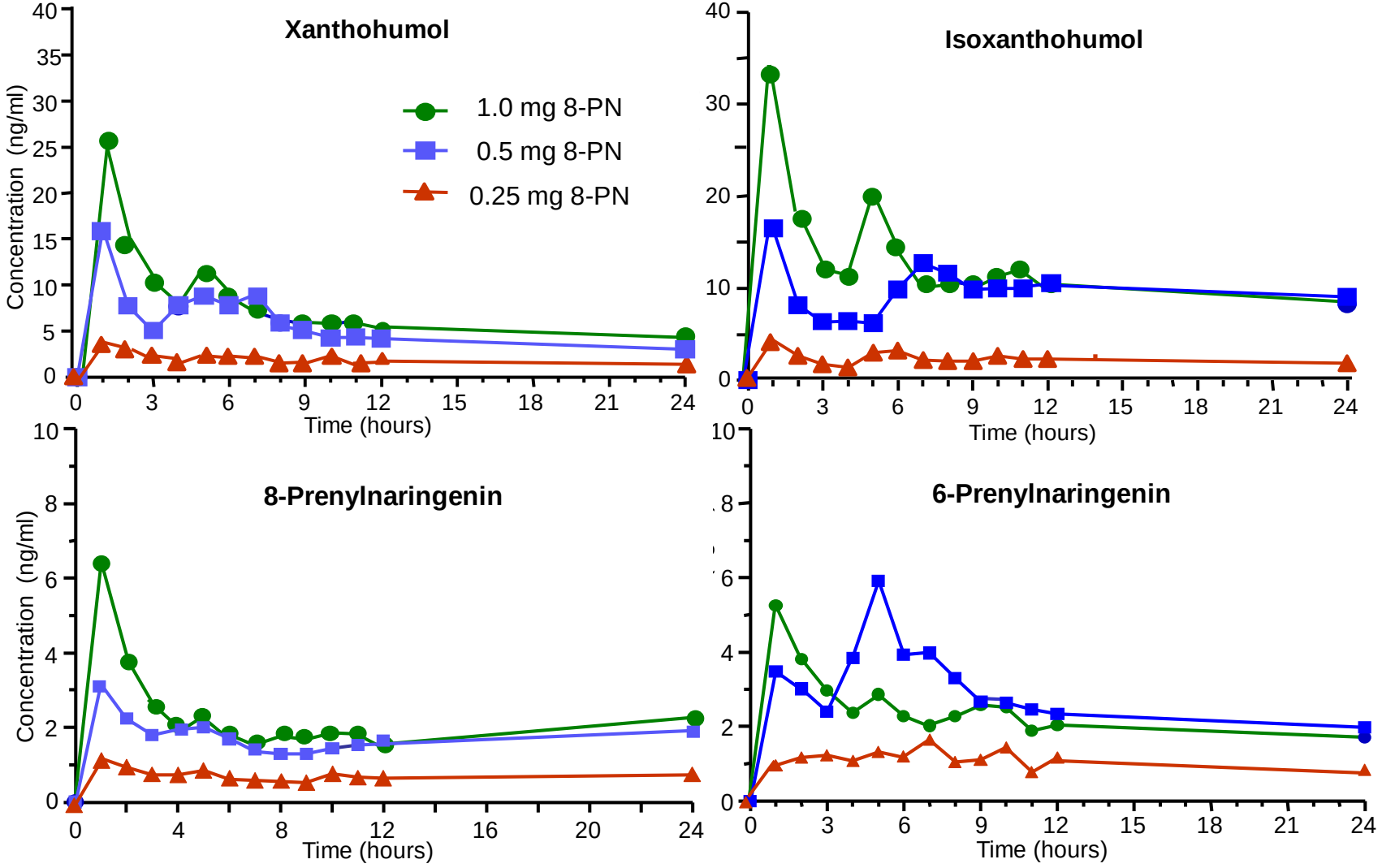
[1]**Each capsule was standardized to** 0.25 mg 8PN, 1.30 mg 6PN, 0.80 mg IX, 21.3 mg XN

UHPLC-MS-MS SRM Chromatograms of Hop Flavonoids in Serum 2 h after Administration of High Dose of Hop Extract



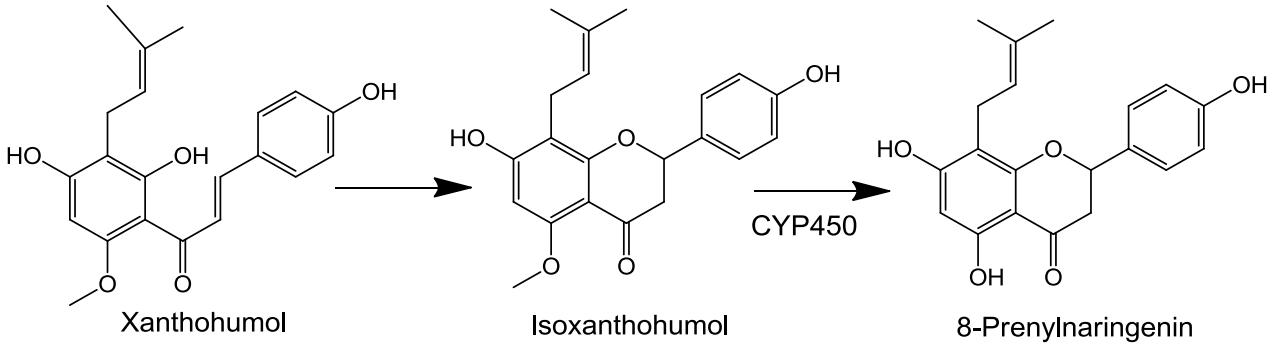
Concentrations of hop compounds increased after enzymatic deconjugation, indicating that most hop flavonoids in serum were conjugated.

Concentration-time Curves for Pharmacokinetics Evaluation of Hop Flavonoids in Human Serum



Assessment of Pharmacokinetics

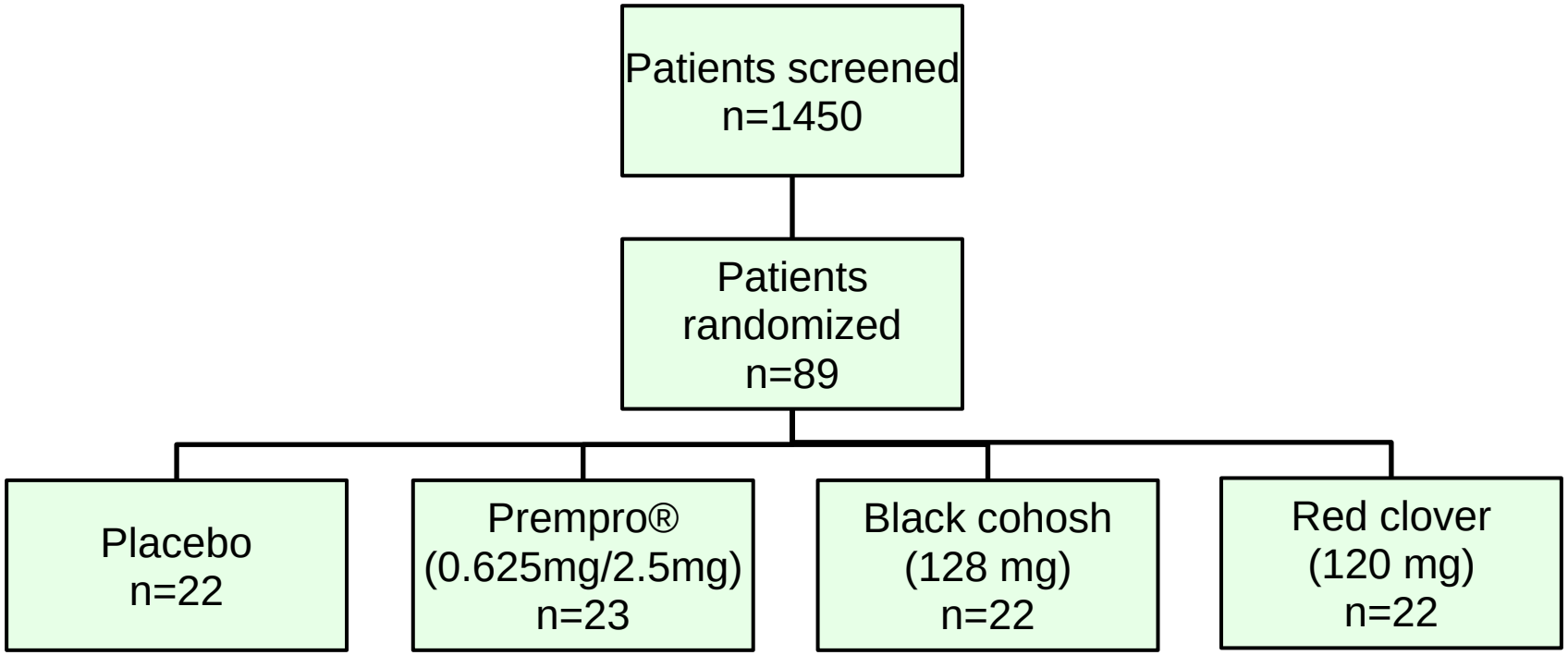
- WinNonlin 6.2 (Pharsight; Sunnyvale, CA)
- AUC, C_{max}, T_{max}, $T_{1/2}$, Vd/F, Cl/F were determined.
- Non-compartmental analysis (NCA) was used for pharmacokinetics modeling.
- Urinary excretion was ≤ 2% of XN, IX, 6PN, and 8PN
- Challenges
  - Interconversion of compounds
  - Enterohepatic recirculation



Pharmakokinetics of Hop Prenylated Flavonoids

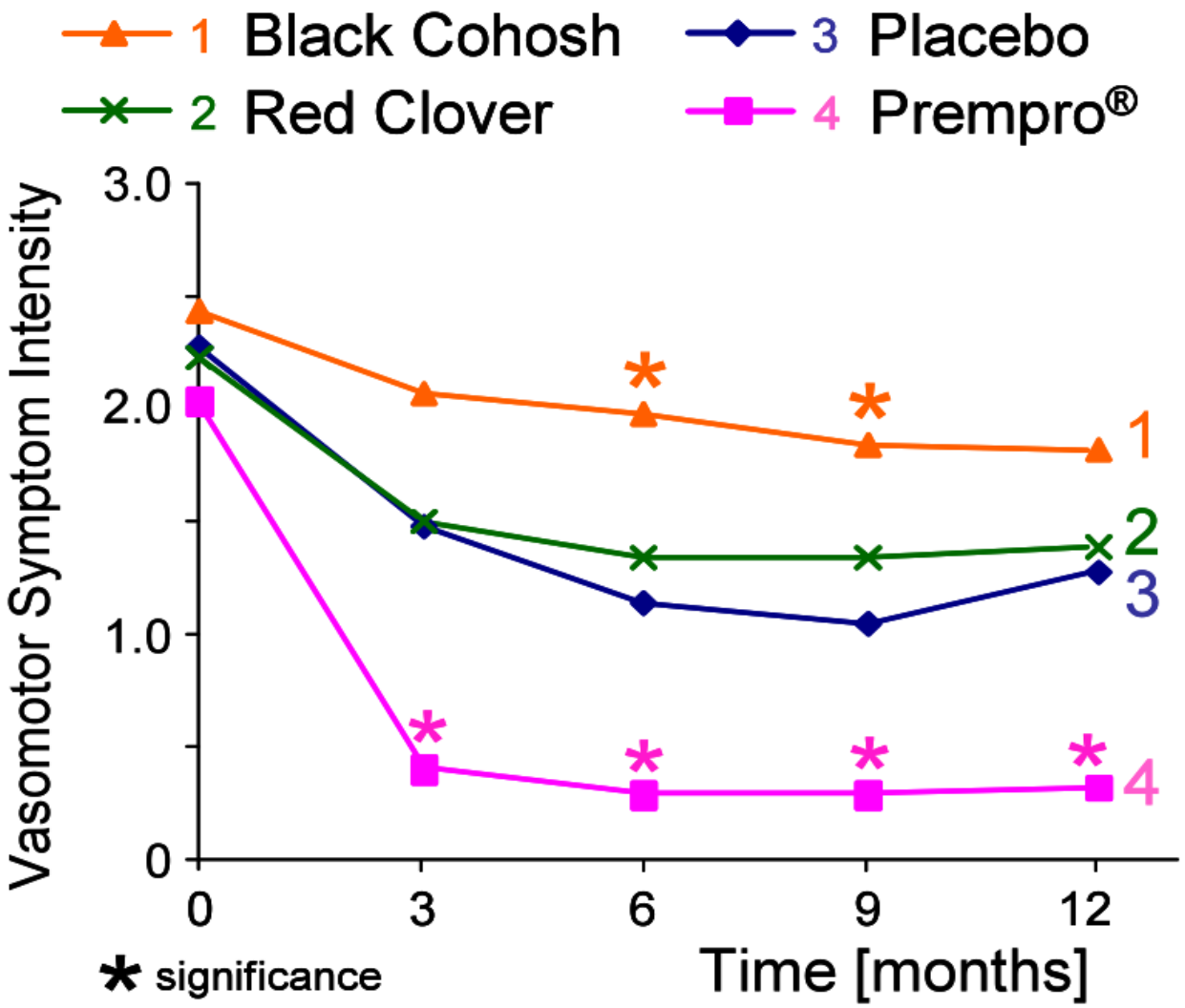
XN	Unit	Low Dose	Med Dose	High Dose
$AUC_{0\text{-}inf}$	h·ng/ml	73.2 ± 60.1	166.9 ± 62.1	322.6 ± 169.2
$T_{1/2}$	h	18.3 ± 5.3	9.5 ± 2.2	20.7 ± 12.7
Cmax	ng/ml	4.4 ± 3.0	22.2 ± 12.1	27.6 ± 8.9
IX				
$AUC_{0\text{-}inf}$	h·ng/ml	149 ± 86	418 ± 446	546 ± 301
$T_{1/2}$	h	27.5 ± 5.7	24.8 ± 24.3	19.9 ± 8.7
Cmax	ng/ml	5.7 ± 2.7	19.1 ± 15.5	37.6 ± 17.6
8PN				
$AUC_{0\text{-}inf}$	h·ng/ml	24.3 ± 8.9	69.2 ± 38.9	88.6 ± 52.6
$T_{1/2}$	h	>20	>20	>20
Cmax	ng/ml	1.4 ± 0.3	3.9 ± 2.6	6.7 ± 3.8

Enrollment and Randomization of Menopausal Women in a Phase II Trial of Safety and Efficacy of Black Cohosh and Red Clover vs. Prempro and



Primary outcome to be measured: Reduction of hot flashes

Phase II Clinical Trial Primary Outcome: Reduction of Hot Flashes in Postmenopausal Women



Geller et al., *Menopause* 2009, *16*: 1156-1166.

Summary

- Enzyme assays and mRNA measurements indicate that the hop clinical extract does not significantly induce CYP1A2 or CYP3A4 (according to FDA guidelines).
- The hop extract inhibited CYP2C8, CYP2C9 and CYP 2C19 with IC_{50} values of 0.8, 0.9, and 3.3 µg/mL, respectively.
- Isoxanthohumol (IX) was the strongest inhibitor of CYP2C8 with an IC_{50} of 0.2 µM.
- 8-Prenylnaringenin (8-PN) was the most effective inhibitor of CYP2C9 and CYP2C19 with IC_{50} values of 1.1 and 0.4 µM, respectively. 8-PN inhibited CYP1A2 with an IC_{50} of 1.1 µM.
- After rapid absorption in the Phase I study, prenylated hop phenols were conjugated so that only low levels of free flavonoids were observed in human serum.
- Enterohepatic recirculation and long half-lives were observed.
- In vivo studies are needed to evaluate significance of these inhibition data for hops and to investigate efficacy of hop extracts for the management of menopausal symptoms.

Conclusions

- The safety and efficacy of botanical dietary supplements used by menopausal women are under investigation using botanically authenticated, chemically and biologically standardized extracts prepared using GMP.
- Mechanisms of action are being determined, and synergy of constituents are under investigation.
- Safety studies include Phase I maximum tolerated dose and pharmacokinetic studies as well as evaluations of drug-botanical interactions such as induction and inhibition of drug metabolizing enzymes.
- Efficacy studies have included Phase II randomized, placebo-controlled clinical trials.

Acknowledgements

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- Shimadzu Instruments, Naturex and Pharmavite
- Harald Schwarz and Hopsteiner
- All the faculty, students and postdoctoral fellows of the UIC/NIH Botanical Center for Dietary Supplements Research, and especially the founding director, Norman R. Farnsworth.