Proceedings of the 2nd Annual International Iridoid Research Symposium
Sponsored by TAHITIAN NONI INTERNATIONAL, INC.
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John introduced the mission statement of Tahitian Noni International, Inc. which is “To bless the whole earth with the natural goodness of TAHITIAN NONI BIOACTIVES, as we Tell the Story of lives transformed,” and how the study of iridoids is tied to the natural goodness of bioactives. John mentioned that iridoids have been studied for 50 years and there are more than 2,000 scientific publications about iridoids. Even today iridoids are not well known in the general population. Tahitian Noni is at the fore front of noni, bioactives and iridoid research and is committed to future research and promotion of bioactive iridoids.

2. Jeff Wasden, Vice President of Marketing, Tahitian Noni International, Inc., Provo, UT

Why Iridoids?

Noni’s medicinal heritage is as follows: It has been used for centuries in French Polynesia, used for a wide range of illnesses, brings the body into balance, and is safe to consume. Iridoids are the most abundant phytochemical in noni. The prevalence and bioactivity of iridoids in noni is leveraged in several Tahitian Noni International product lines, including beverages, dietary supplements, and cosmetics. Iridoids and other bioactives included in products must be safe, stable, standardized, sourced naturally, and scientifically proven. Tahitian Noni International wishes to increase iridoid scientific research, increase iridoid awareness and consumption.


Bioactivation of Deacetylasperulosidic Acid: Changes in Bioactivity

Upon ingestion, iridoids are hydrolyzed by β-glucosidase in the GI tract to more bioactive intermediates. These iridoid intermediates then may react further with other molecules to exert pharmacological effects, such as anti-inflammatory and hypotensive activities, to name just two examples. Deacetylasperulosidic acid (DAA) was isolated from noni fruit and hydrolyzed with β-glucosidase. The in vitro antioxidant activity of DAA and its hydrolyzed product were tested in three assays. The antioxidant activities, as measured in the ORAC, DPPH radical scavenging and Fe³⁺ reducing power assays, were greater with the hydrolyzed DAA than with DAA. These results reveal that the antioxidant activity of noni iridoids is also increased as they undergo digestion and metabolism. Iridoids have also been shown to increase the activity of antioxidant enzymes in the body, such as glutathione peroxidase and catalase. As noni juice has been shown to reduce the radicals metabolized by these enzymes, in vivo tests of the effect of DAA on these these enzymes should be conducted.
Structural Classifications of Iridoids and their Impact on Major Pharmaceutical Efficacies

Structurally iridoids are cyclopentano [c] pyran monoterpenoids consisting of 8 carbon, 9-carbon and 10-carbon skeletons. The 10- carbon iridoids have loganin and its related type, valeriana type and plumeria type structures. Other iridoid structures include secoiridoids, cerberidol analogs and other both pyran and cyclopentane rings rearranged iridoid derivatives. There are many common aroyl groups that are attached to the basic iridoid skeleton. Using a unique classification system all iridoids are grouped based on their structures. For example: Group 1 consists of 8-carbon iridoids with common substituents in the cyclopentane ring. Group 2a consists of 9-carbon iridoids with common substituents in the cyclopentane ring and the 9th carbon on C-4. Group 2b consists of 9-carbon iridoids with common substituents in the cyclopentane ring and the 9th carbon on C-8. Group 3 consists of 10-carbon iridoids. Group 4 are iridoid aglycones and derivatives. Noni iridoids have plumeria and other types of structures. Iridoids of other structures from various plant species are also described. Iridoids of valeriana and plumeria types usually have cytotoxic and antiprotozoal activities. Secoiridoids of the oleoside group have anti-diabetic activity. Harpagides, shanzhisides, ipolamiidosides and lamiridosides have antiviral activity. Aglycones of these iridoids are more active than the parent ones. Loganin and its seco compounds have anti-osteoporosis activity. Catalpols and harpagides have neuroprotective activity. Aucubins and geniposides have anti-inflammatory activity. Acylated catalpols have hepatoprotective and wound healing activities. Asperulosidic acid and scandoside Methyl ester have anti-melanogenesis activity. Alkyl swerosides e.g. hydramacrosides have anti-allergic activity. Cachinesides or its aglycones have anti-coagulative activity.

Study on the Potential Anti-cancer Activities of Iridoids through the Downregulation of Constitutive STAT3 Activation in Human Prostate Carcinoma DU145 Cells

Iridoids belong to a group of monoterpene compounds containing a cyclopentane ring, and in nature are found mostly as glycosides. They are considered to be the defense substances of several plant species including a variety of medicinal plants. Although many iridoids have been reported to exhibit anti-inflammatory and anti-cancer activities, their molecular targets/pathways are not fully understood. A review of signal transduction of cancer and how these signals can be regulated by enzymes, iridoids and anticancer drugs formed a basis for this study. In human cell transduction there are 127 human pathways with more than 8700 interactions. The present study is aimed to elucidate possible mechanism of the anti-
proliferative activity of the hydrolyzed-iridoid products (H-iridoids) through the STAT3 signaling pathways on several tumor cells, such as human prostate cancer DU 145 cells, breast cancer MDA-MB 231 cells, and chronic myelogenous leukemia U-266 cells. H-iridoids were obtained from five iridoid glycosides, namely aucubin, catalpol, geniposide, harpagoside, and geniposidic acid hydrolyzed with β-glucosidase. The effects of each H-iridoid on the cell viability and cell proliferation in tumor cells were measured by the MTT assay. No single iridoid glycoside and β-glucosidase exerted any cytotoxicity in the tumor cells; whereas H-iridoids had significant cytotoxic, anti-proliferative, and STAT3 inhibitory effects and revealed different potencies depending on their chemical structures. The phosphorylation levels of STAT3, its regulatory molecules, and apoptosis by H-geniposide treatment in DU145 cells were investigated by performing immunoblots and flow cytometry. Among the H-iridoids tested, H-geniposide inhibited constitutive STAT3 activation through inhibiting upstream JAK1 and c-Src. Consistent with STAT3 inactivation, H-geniposide down regulated the expressions of bcl-2, bcl-xl, survivin, and cyclin D1; this correlated with the accumulation of cells in the sub-G1 phase of the cell cycle, and the induction of apoptosis. The results indicate that geniposide exhibits potential anti-cancer activity by suppressing various down regulation steps of the STAT3 pathway and producing apoptosis of tumor cells. It is of interest how each iridoid exerts different sensitivities depending on tumor cell lines.

6. Dr. Simla Basar, Institute for Experimental and Clinical Pharmacology and Toxicology, University Clinic of the Hamburg-Eppendorf, Hamburg, Germany.

Occurrence of Iridoids in Noni Fruits and their Fate in the Body. Part 1 – Chemistry

The iridoid analysis of several types of noni from various islands of French Polynesia and the Indian Ocean was summarized with the iridoid content varying between 700 to 1400 mg /L. The iridoid content of several competitive noni juices was also compared and ranged between 150 to 1300 mg/L. Three samples contained no iridoids. Fermentation of noni fruit lowers the amount of iridoids. pH, temperature and solvents altered the HPLC detection of hydrolyzed iridoids glycosides. The results show to effectively detect hydrolyzed iridoids pH, temperature solvent composition and body fluids need to be to be considered and documented.

7. Professor Dr. Johannes Westendorf, Institute for Experimental and Clinical Pharmacology and Toxicology, University Clinic of the Hamburg-Eppendorf, Hamburg, Germany.

Occurrence of Iridoids in Noni Fruits and their Fate in the Body. Part 2 – Biology

Iridoids can be categorized as phytoalexins. Phytoalexins are toxic substances synthesized de novo by plants that accumulate rapidly at areas of pathogen infection or pesticide attack. Plants produce iridoids as a protection against predators. Iridoids also have beneficial bioactive properties such as immuno-stimulatory, anti-inflammatory, ergogenic, cognitive enhancing, anti-diabetic and others. Iridoids having both toxic activity and beneficial bioactivity have caused animals and humans to adapt. Animals and humans metabolize toxic iridoids into bioactive iridoid intermediates. Based on the previous report by Dr. Simla Basar and how iridoid detection
changes based on physical conditions Dr Westendorf explains how iridoids are metabolized in various parts of the human body. Because of the instability of the DAA-aglycone, hydrolyzed genipin was used for biological experiments. The reactivity of genipin with nucleotides and DNA suggests an influence on cell growth and genetic integrity. The induction of heat shock protein (Hsp-70) by TNJ was not observed in an experiment with two human subjects. In contrast, TNJ (0.1%) enhanced the heat tolerance of V79 cells in culture considerably. Iridoids were not detected in urine samples when TNJ was consumed. TNJ lowered relative lactate, but not glucose and pulse frequency levels of human volunteers in an ergometric bicycle study. This needs further analysis to see if iridoids are the bioactive component. Antinociceptive activity of the iridoid monotropein in hot plate test with mice was confirmed, while a similar test with DAA did not show the same effect yet with a DAA rich noni fruit concentrate there was an antinoceceptive effect.

8. Fumiyuki Isami, Administrative Director, Tahitian Noni International-Japan, Tokyo, Japan.

**Biotopical Effects of Morinda citrifolia Iridoids**

Iridoids are biosynthesized in cell cultures of *Morinda citrifolia*. There is still more work to be done. The biotopical effects of noni are anti-Inflammation, anti-ellastase, anti-collagenase and anti-tyrosinase. In an in-vitro test using human dermal fibroblasts, anthraquinones and noni fruit extract increased procollagen type I C-terminal peptide (PICP) and glycosaminoglycans (GAGs). A noni seed extract showed more potent DPPH radical scavenging anti-oxidant activity than noni fruit, noni leaf and a control. The same noni seed extract in an in-vitro test significantly inhibited human leukocyte elastase enzyme. Phytochemicals from noni fruit including iridoids had anti-tyrosinase activity or contributed to skin whitening by inhibiting melanin formation.


**Effects of Morinda citrifolia Extract and its Constituents / Iridoids on Blood Fluidity**

Life style factors such as smoking, poor diet and lack of exercise can adversely affect blood circulation and rheology. A noni fruit extract help control platelet aggregation and fibrinogen formation in a thrombosis model. The noni fruit extract in a dose dependent manner inhibited erythrocyte aggregation. The active compounds in the noni fruit included phenolic and terpenoids compounds, rutin inhibits platelet aggregation, quercetin inhibits platelet aggregation, ursolic acid inhibits platelet aggregation and inhibits erythrocyte aggregation, 3,3’-bisdemethylpinoresinol inhibits erythrocyte aggregation, americanin A inhibits erythrocyte aggregation. One of the active compounds in noni fruit that caused fibrinogenolysis activation was the iridoid, asperulosidic acid.

10. Dr Shixin Deng, Senior Research Scientist, Tahitian Noni International, Inc. American Fork, UT.

**Sodium Channel Inhibition of Tahitian Noni Bioactive Beverage and its Major Iridoid**

The sodium channel chemistry is explained and reviewed. The 3 states of the sodium channel are rest, open and inactivated states. There are sodium channel toxins that can open both gates
of the sodium channel causing sodium ion concentration increase in the cell. TN Original Bioactive at 5 mg/mL inhibits BTX-B toxin from binding to the sodium channel by 40%. DAA inhibits BTX-B toxin from binding by 36% at 100μg/mL. There was a dose dependent inhibition binding of BTX-B toxin by the noni fruit iridoid DAA.

11. Dr Zsolt Petendi, Pharmacist, Budapest, Hungary

Iridoids – A Pharmacist’s Prospective

The knowledge of pharmacists from the Old World are fused together from three parts: botany, chemisty, physiology. Iridoids can be found in many plants, with well-known and time-proven health, ethnobotanical and folk medicinal benefits. This plants have some common properties. They are well-known by and have been used hundreds to thousands of years by local inhabitants. They can tolerate harsh conditions from mechanical impacts, droughts etc. They have few adverse side effects, if any. There are very few fruits which contain iridoids, noni being one of them along with vaccinium species which includes blueberries. Iridoids are in the following plant families: Ericaceae, Gentianaceae, Lamiaceae, Loganiaceae, Menyanthaceae, Oleaceae, Plantaginaceae, Rubiaceae, Scrophulariaceae, Valerianaceae and Verbenaceae. There are more than 1200 scientific publications on iridoids. Iridoids are monoterpenes; they are synthethised in plants and animals from isoprene and they are often the intermediates in the synthesis of alkaloids. The human body synthesizes cholesterol and CoQ10 from isoprene. Natural rubber is also made from isoprene. An Argentine ant produces an iridoid associated with life. Live ants produce this iridoid and when an ant dies this iridoid disappears within an hour or two signaling death. This iridoid has been called the “scent of life.” Prof. Emile Bourquelot (1851-1921) at the Universiy of Paris said often that iridoids are playing a very important role in the healing of different illnesses.

12. Dr. Konstantin Eller, Head of the Food Analytical Chemistry Division, Russian Academy of Medical Science Institute of Nutrition, Moscow, Russia.

The comparison of legal frameworks for herbal biologically active dietary food supplements (BADFS) in USA, EC and Russian federation: regulation, registration, health claims (with particular focus on iridoids).

The regulations pertaining to dietary supplements in the USA and EC were compared. An updated to the regulations governing BADFS and fortified food products within the Custom Union of the Russian Federation, Belorussia and Kazakhstan include changes to Appendix 3. Also, the listing of the main biologically active substances, with the appropriate recommended (or adequate) daily intakes for adults, in the biologically active dietary food supplements (BADFS) is established by Annex 5 of the regulations. The biologically active substances included in Annex 5 are amino acids, vitamins, minerals, polyphenolics (flavones, iso-flavones, flavonols, flavanones, flavanoles, anthocyanins, OPC, phytoalexins, derivatives of dihydroxy-cinnamic acid etc.), olyunsaturated fatty acids (ω-3, ω-6), phytosterols, phytostanols, carbohydrates (mono-
, di-, oligo-, poly-sachharides), sugar polyols, aminosugars etc., alkaloids (caffeine, theobromine, synephrine, indol-3-carbinol etc.), and iridoids. The iridoids in Annex 5 are oleuropein (from olive leaves, olive oil, and olive leaf extract), harpagoside (from some spices and Devil’s claw), deacetylasperulosidic acid (from noni fruit, noni juice, and noni leaves), and asperulosidic acid (from noni fruit, noni juice, and noni leaves).


Simple Isolation Method of DAA and AA from Fruits of Morinda citrifolia (Noni)

A more rapid method to isolate DAA and AA from noni fruits has been developed. This method involves extracting dried noni fruits with water, followed by absorption onto activated charcoal. The charcoal is then desiccated and extracted with acetone. The acetone extract is further purified using a silica gel column. This method produces fairly high purity DAA and AA from 60% to 80% and is less time consuming.

14. Dr Il Moo Chang, Emeritus Professor, Natural Products Research Institute, Seoul National University, Seoul, Korea.

In vitro Obesity Model affected by Iridoids: Effects on Lipolysis and Fatty Acid Oxidation

Potential anti-obesity activity of several iridoids and their hydrolysis products were tested in vitro. Using 3T3 L1 cell cultures, the following bioassays were performed; lipolysis, secretion of aponeictin, and fatty acid oxidation. Initial low dose response indicates that higher concentrations should be evaluated.

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