# ECO-CONSCIOUS EXTRACTION PROTECTS PLANT EXTRACTS' INTEGRITY

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## Executive Summary

Natural plant-based ingredient development is experiencing an explosion in phyto-tech research. How can we advance extraction protocols and technologies to optimise harnessing and maintaining the integrity of natural molecules? Moving beyond isolating, purifying and standardising single phyto-compounds, we're learning more about the importance of compounds' synergistic relationships within a botanical specie's cell. Extracting, analysing and delivering natural molecules in their entourage, we gain a new understanding of what a species has to offer and the brilliance of nature's design. Looking to more eco-conscious extraction processes, we explore a micro and macro approach wherein resource-efficient extraction methods reduce our impact on the environment, as well as the botanical's molecular integrity.

## Introduction

The concept of eco-conscious or 'green' extraction employs novel techniques to extract bioactive compounds, using non-toxic, non-volatile, recyclable, bio-degradable solvents that are produced from renewable biomass sources.[1,2] These techniques are ideally more energy efficient; using relatively less solvent, less biomass and less extraction time to provide higher yields of a stable, 'true-to-nature' extract that preserves a plant's molecular integrity. With global debates raging around environmental sustainability, energy use, water conservation, and the preservation of precious resources, botanical extraction methods that can deliver high quality, stable phyto-compounds through the most resource-efficient means are a big part of reducing the Beauty Industry's environmental impact.

Traditional extraction techniques such as maceration, infusion, percolation and Soxhlet and steam distillation can be simple; however often require the use and removal of hazardous solvents, heat, additional filtration steps and are not time efficient.[3,4] Eco-conscious extraction techniques such as pressurised liquid extraction (PLE) and supercritical fluid extraction (SFE) offer eco-conscious, economical and quality advantages through reduced processing time, less solvent usage and conservative operative conditions that protect the molecular integrity of the raw material.[5]

Cellular Extraction (CE) is a PLE method that uses high pressure in cold water to deliver a botanical's hydrophilic or water soluble phyto-compound profile with as small an environmental impact as possible. Botanical evolution allows plants to adapt to their environment through the production of chemical compounds to support their survival.These extracts present the phyto-compounds in their natural entourage, stable and available to function in synergy, as nature intended. Research into this complex interplay between compounds is a challenging task as there are a great number of compounds still unknown and the combinations are many. Science has previously focussed on tools to reduce the complexity of natural products but with advances in analytics and a shifting focus to biomimicry or more 'natural' approaches to health and beauty, eco-conscious extraction techniques that can provide a natural, efficacious extract with as little environmental impact as possible are in demand.

## Examples of Extraction Methods for Phyto-compounds

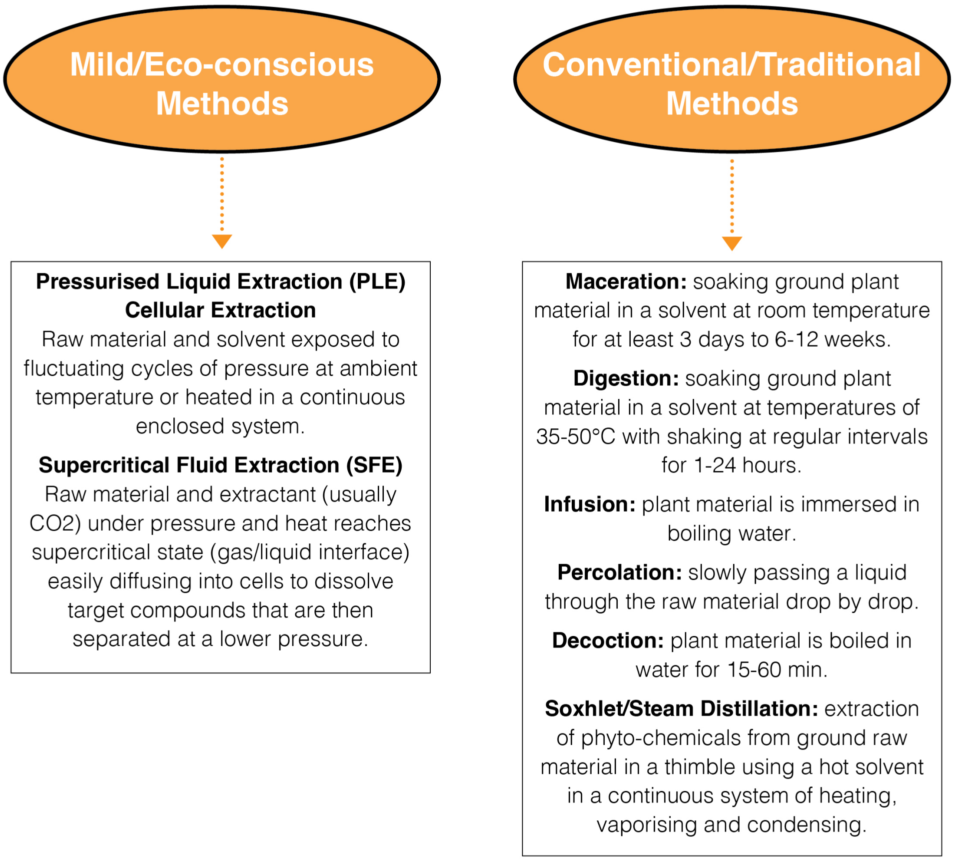


**Figure 1:** Classification of examples of eco-conscious and conventional extraction methods

## Energy

Energy consumption can be reduced through optimisation and intensification of extraction processes or energy recovery.[6] Traditional solid to liquid extraction techniques are considered to have a poor bioactive yield relative to the high energy input necessary for production, particularly when done in high volumes. The use of heating and vacuum evaporation over prolonged periods of time and the need to grind or shred the raw material in an additional pre-treatment step can significantly add to the energy usage total of the extraction process.[3] Non-conventional extraction methods such as PLE and SFE offer a greater bioactive yield relative to energy use through shorter processing times, reduced need for heating and cooling and reduced degradation of bioactive molecules.[3,5]

Cellular Extraction, is a highly efficient PLE process that relies on fluctuating cycles of pressure to rapidly move phyto-compounds across the cell membrane into a 'green' liquid extractant at ambient temperature. This extraction protocol does not require energy for heating or cooling during processing and storage. Energy consumption is offset through a decreased extraction time and a reduced need for pre-extraction processes such as homogenisation and dehydration and post-extraction processes such as filtration and centrifugation.[5] This fast, pressurised, ambient temperature extraction also protects the plant extract's molecular integrity through preservation of thermolabile compounds such as enzymes, anthocyanins and vitamin C. (See Figure 2 The process of Cellular Extraction)

SFE uses carbon dioxide, a non-toxic, non-flammable, inexpensive and freely available solvent that is a 'hybrid' between gas and liquid in its supercritical state. It is highly diffusive and able to transport small non-polar bioactive matter quickly and effectively, however energy loads are increased through the need for high pressure to convert $CO_2$ to a supercritical fluid and the equipment needed to pump/compress and cool the $CO_2$ stream.[5]

Although both extraction methods are considered 'eco-conscious', research analysis suggest that PLE has the least economic impact of the two.[5]

# The Process of Cellular Extraction

## The Revolutionary Process

CELLULAR EXTRACTION

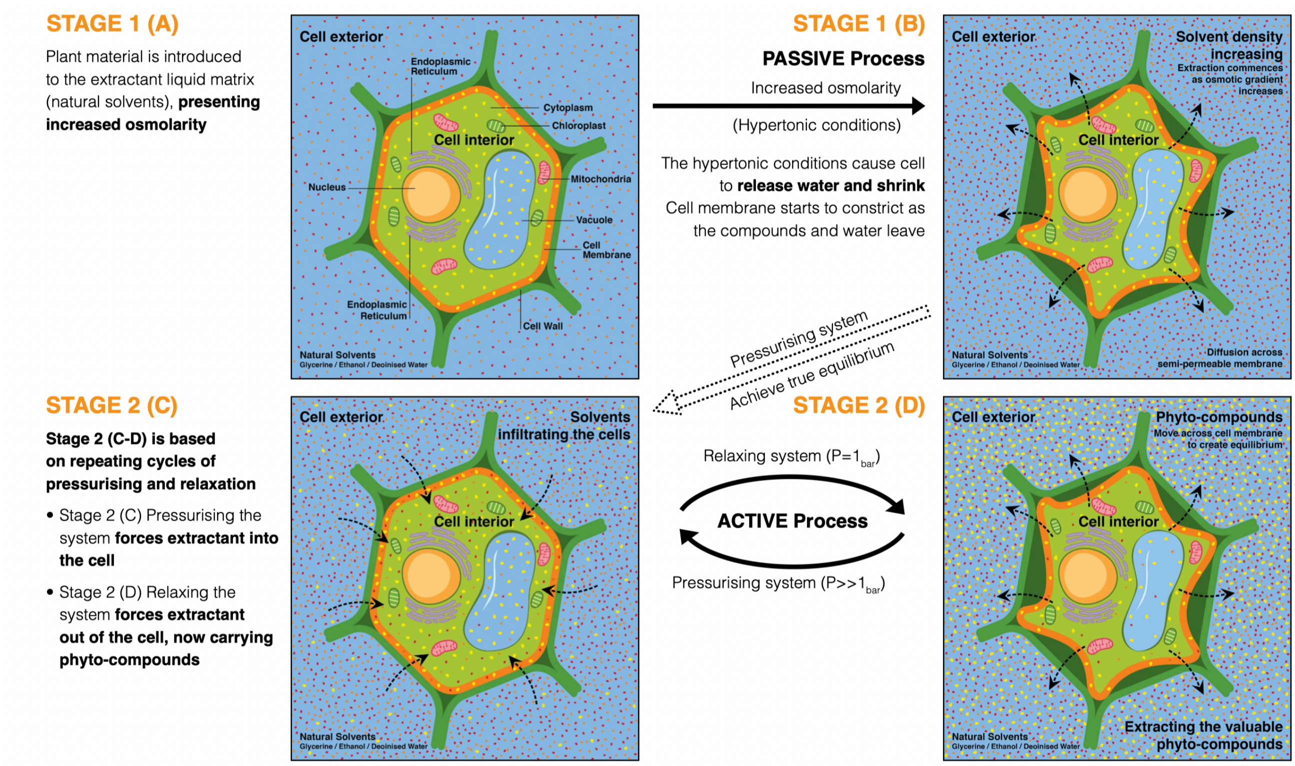


**Figure 2:** The process of Cellular Extraction. Fluctuating cycles of pressure rapidly dissolve phyto-compounds from the plant matrix into the solvent, maintaining plant extracts' integrity and extraction efficiency through reduced extraction time.

# Water

Water scarcity is a compelling topic globally and, as Australia is the driest inhabited continent, our substantial water footprint is particularly relevant in global terms. In Australia, agriculture is the largest consumer of freshwater, followed by industry then domestic sector usage.[7] In the 2020-21 period the Australian Bureau of Statistics recorded a 25% increase in total water consumption by industries and households and a 37% increase in agricultural water usage. This increased usage in the agricultural sector was due to the higher rainfall providing incentive for farmers to return to more water-intensive crops such as cotton and rice.[8] The Australian Government's Future Drought Fund provides continuous funding for drought resilience initiatives to help farmers and communities prepare for the impacts of drought through climate information, better planning and better practices in farming and land management to improve drought resilience. The $5 billion fund aims to improve the sustainability and resilience of critical agricultural natural resources such as fresh water, soil fertility and crop pollination in a changing climate.[26] Planting and sourcing of crops suited to specific agricultural regions is key to water conservation, as plants that are endemic have evolved to adapt to environmental conditions such as low rainfall, reducing the need for irrigation.

Rainwater harvesting can also provide an opportunity for industry to reduce its water footprint. With proper treatment, like purification and deionisation, this water can be used within the extraction process, eliminating chemicals like chlorine, which is used in preservation of the public water supply.

The use of raw materials from regenerative and water-wise growing practices such as aquaponics can reduce the demands on water resources and provide drought resilience for crop growers. Aquaponics cultivates plants, bacteria and fish together in a system that uses nutrient rich effluent to irrigate and fertilise hydroponic beds, leading to a more sustainable way of farming, with greater crop production in less space, up to 90% less water than conventional farming (dry cropping) and produce with an increase in beneficial phyto-nutrients and antioxidant potential.[9,10] One of NATIVE EXTRACT'S botanicals suppliers, Dom Smith, a Yuin man, is an indigenous farmer, agri-innovator and mentor based on Ngawait country in the South

Australian Murray River region. He is on a mission to create a platform for change, starting with his aquaponics and agribusiness, Pundi Produce at Monash, SA. Providing seasonal herbs and vegetables along with natives such as warrigal, rivermint, saltbush, sea parsley, bush tomato and wattle seed, Pundi Produce is Supply Nation registered and has a certificate in conservation and land management. His aquaponics system uses considerably less water, land and labour than traditional agriculture and doesn't rely on chemical fertilisers, pesticides or insecticides. Growing his Rivermint with aquaponics saves approximately 1000% water in comparison to dry cropping the same species (Smith 2022).

Cellular Extraction protocols use rain-harvested water, eliminating chlorine and fluorine from the final extract and reducing the use of public water by up to 1000L/day. This is especially important in the delivery of a 'true-to-nature' extract since chlorine has been shown to have effects on biological activity. The presence of chlorine is able to alter the intrinsic biological activity of natural molecules.[11] Moving to rain-harvested water also reduces the impact on filtration systems. Cartridge use in the reverse osmosis water system at NATIVE EXTRACTS has been reduced by half due to the reduced chemical load from rainwater as opposed to the public water supply.

## Natural Resources

An eco-conscious extraction policy requires the selection of sustainable varieties from renewable plant resources to ensure future supply and prevention of soil degradation. Cultivated plants, in contrast to wild-harvested, may also ensure a more predictable or consistent molecular outcome. Biodiversity can only be preserved through the use of controlled crops.[6] Foundations such as FairWild apply standard principles to wild plant collection operations, encouraging commitment to sustainable collection through limits on wild harvesting to preserve and protect ecosystems over the long term. Eco-conscious botanical sourcing is integral to NATIVE EXTRACTS' raw material selection. We aim to preserve and promote the health of our producer's eco-systems by only using botanicals that meet strict quality assurance criteria and are supplied with raw material information documents for commercially grown and organically certified biomass.

Using efficient, exhaustive extraction techniques allows for reduction in the amount of biomass required to make each extract and minimises the risk over harvesting. Pressurised liquid extraction can be automated to reduce the exposure of the less stable compounds to oxygen, light and heat, reducing the loss of valuable phytonutrients. Through increased pressure the solvent is forced to penetrate the matrix pores facilitating the extraction of target compounds and increasing yield.[5] For extraction of relatively heat stable compounds, pressurised liquid extraction allows for the option to use heat to increase total polyphenolic yield without excessive thermal degradation of compounds or the excessive loss of solvent experienced during conventional extraction.[12] This has multiple impacts throughout the cosmetic and supplement industry; reducing demand for raw material, reducing the resources required to produce the raw material and reducing extraction costs in comparison to conventional extraction methods.

Wastage of food and other botanical resources is an issue of intense global concern. According to the World Resources Institute one third of food is wasted and the carbon emissions generated globally through this wastage exceed those of all countries except the USA and China. Australia alone generated 7.3 million tonnes of food waste in 2016-2017.[5] This wastage also diminishes the precious water and land resources required for its production. According to the Australian Department of Climate Change, Energy, the Environment and Water, a massive 25% of water utilised in Australian agriculture is used to grow food that is wasted. Throwing away one burger is the equivalent water wastage of a ninety minute shower! The government has set a goal to halve this food wastage by 2030 through various actions to support the National Food Waste Strategy using education, innovation and investment in research and technology to create value from waste.[25] In addition to composting and energy recovery from waste is the practice of reprocessing waste into functional and therapeutic products for the cosmetic and food industries.

Exhausted bio-mass can be up-cycled through fermentation to produce bioethanol or a phenolic-rich post-biotic product that offers significant potential in the cosmetic and nutraceutical sectors. Phyto-compounds extracted from botanical waste can provide valuable co-products for the pharmaceutical, nutraceutical, food and cosmetic industries, increasing profit and reducing agri-food waste. The use of multiple or alternative plant parts can also add value and reduce the environmental cost. NATIVE EXTRACTS investigated the phytochemical profile of blueberry plants. The results demonstrated that the usually wasted plant parts such as unripe fruit, leaf and stem can contribute to the total phenolic profile of the plant, in this case introducing two valued compounds from the leaf, arbutin and myricetin. (See Table 1 below)

## Considering Alternative Plant Parts to Repurpose Crop Surplus & Waste

| **Blueberry Botanical Extract Compositional Analysis** | | | |
|---|---|---|---|
| Fruit (Unripe) | Fruit (Ripe) | Leaf | Stem |
| - | - | Hydroquinone (Arbutin) | - |
| - | - | Myricetin | - |
| - | Anthocyanins (pigment) | - | - |
| - | - | - | Procyanidins (Catechin/Epicatechin chains) |
| Catechin | - | - | Catechin |
| Epicatechin | - | - | Epicatechin |
| Chlorogenic acid | Chlorogenic acid | Chlorogenic acid | Phenolic acid (Potentially Chlorogenic acid) |
| Rutin | Flavone glycosides (Potentially Rutin) | Rutin | Rutin |
| Quercetin glycosides | Flavone glycosides (Potentially Quercetin) | Quercetin glycosides | Quercetin glycosides |
| Phenolic acid derivatives | Phenolic acid derivatives | Phenolic acid derivatives | Phenolic acid derivatives |

**Table 1:** Blueberry plant profile showing the similarities and variations in phenolic compounds for unripe and ripe fruit and leaf and stem extracts.
*Source: NATIVE EXTRACTS Pty Ltd & Govt Laboratory LCMS Compositional Analysis*

Another example of efficient use of resources to reduce wastage is the production of multiple finished products from one raw material. Table 2 shows the botanical extract methods and compositional analysis of Kakadu Plum fruit and seed. NATIVE EXTRACTS is able to maximise the potential of the fruit through diversifying from cellular extracts into concentrated liquid and powder extracts and seed oils. Cellular Extraction, an eco-conscious, energy efficient, PLE method uses 'green' solvents and provides an extraction matrix that preserves the entourage and the plant's molecular integrity. Vitamin C is an unstable compound that is easily oxidised and lost through exposure to heat, air and light. The high levels of natural vitamin C achieved in the extraction process are testament to the conservative operative conditions of the method to achieve a greater bioactive yield, reducing the demand for raw materials and the resources required to provide them.

## Kakadu Plum Plant Profiles - 1 Fruit : 4 Extractions

| **Kakadu Plum** *(Terminalia ferdinandiana)* **Botanical Extract Methods + Compositional Analysis** | | | |
|---|---|---|---|
| Fruit Cellular Extract | Fruit essenXce 5:1 | Fruit Powder 10:1 | Seed Oil |
| Natural vitamin C (Biological Ascorbic acid) | Natural vitamin C (Biological Ascorbic acid) 15 – 16,000mg/100g | Natural vitamin C (Biological Ascorbic acid) 10 – 14,000mg/100g | Palmitic acid |
| Gallic acid (GA) | Gallic acid (GA) | Amino acids | Stearic acid |
| Tryptophan | Tryptophan | Gallic acid (GA) | Oleic acid |
| Flavone C-glycoside | Flavone C-glycoside | Flavonoid C-gylcoside | Linoleic acid |
| Arginine + Asparagine | - | - | Palmitoleic acid |

**Table 2:** Kakadu Plum plant profile showing the similarities and variations in compositional analysis of four different extracts.
*Source: NATIVE EXTRACTS Pty Ltd.*

The use of suitable, eco-conscious extraction methods to extract from botanical waste helps to maximise the benefits of up-cycling by being selective, time and resource efficient and preserving the integrity of the active phyto-compounds. Bio-based solvents such as ethanol and glycerol are produced from renewable biomass sources such as wood, vegetable oils, fruit and starch and are classed as a renewable resource. Glycerol is also obtained as a byproduct of biodiesel production and organic glycerol can be obtained from non-GMO flaxseed. With a high solvent power and biodegradable, non-toxic properties, these solvents make excellent replacements for petrochemical solvents.[2,6]

Pharmaceutical formulations permit the presence of water, ethanol and glycerol in the final product. The extraction of hydrophobic or non-polar compounds is often done using nonpolar solvents such as hexane or chloroform, however these may leave toxic residue in the extract. The use of supercritical fluid extraction is an efficient, non-toxic extraction alternative for nonpolar compounds that has demonstrated an almost three fold increase in phenolic compound extraction in comparison to ethanolic extraction.[13] A solvent's power to extract the most compounds does not necessarily lead to the most efficacious extract. Kaczorova et al (2021) investigated the phenolic yield from two of the *Achillea* species using ethanol and the less polar solvent, chloroform. Although the yield of phenolic compounds was greater in the chloroform extract the ethanolic extract demonstrated the strongest antioxidant activity.[13]

Cellular Extraction uses polar, 'green' solvents such as glycerol and water to efficiently extract the water-soluble or polar compounds from sustainable botanical material, delivering their entourage, in the same ratio as they exist in nature. The phyto-compounds are presented in a glycerol matrix that not only supports the integrity of the compounds but provides its own cosmetic properties such as humectant, cell membrane maintenance and skin permeability.[14]

## Extraction Method Impact Comparisons

| Extraction Method | Energy Efficient | Time Efficient | Raw Material Efficient | Solvent Efficient | Yield Efficient |
|---|---|---|---|---|---|
| **CONVENTIONAL/TRADITIONAL METHODS** | | | | | |
| Maceration | ✗ | ✗ | ✗ | ✗ | ✗ |
| Digestion | ✗ | ✗ | ✗ | ✗ | ✗ |
| Infusion/Percolation | ✓ | ✗ | ✗ | ✗ | ✗ |
| Decoction | ✗ | ✓ | ✗ | ✗ | ✗ |
| Soxhlet Extraction | ✗ | ✗ | ✗ | ✓ | ✓ |
| **MILD/ECO-CONSCIOUS EXTRACTION METHODS** | | | | | |
| Pressurised Liquid Extraction | ✓ | ✓ | ✓ | ✓ | ✓ |
| Supercritical Fluid Extraction | ✗ | ✓ | ✓ | ✓ | ✓ |

**Table 3:** Traditional and Mild extraction methods rated for eco-conscious impact.

# Synergy, Entourage and Nutrient Regeneration Protecting Plant Extracts' Integrity

Whole botanical extracts are a rich mixture of the phytonutrients that protect plants from photosynthetic stress, oxidative damage and regulate physiological processes at the molecular level.[15] These bioactive compounds have a wide range of potential applications in the health and cosmetic industry,[16,17] used both as whole extracts and single compounds. The study of phyto-compounds is typically done through isolation and purification of single compounds, however this reductive analysis does not account for the complex, synergistic reactions that occur between phyto-compounds within their natural entourage. It is proposed that isolated and purified plant extracts may detract from the concept of eco-conscious extraction through losses in function and instability of compounds, the need for more raw material to reach therapeutic levels and more solvent during multiple extractions in addition to increased wastage.

Synergy occurs when the combined effects of compounds is greater than the sum of the effects of the individual compounds.[18] This can occur as the result of the combination of two or more species or within one plant extract's concert of unique constituents. Compounds work in synergy when active compounds within the combination potentiate the other compounds to give a benefit greater than expected. The entourage effect occurs when seemingly inactive compounds increase the activity of bioactive molecules.[19] Nutrient regeneration from an inactive or oxidised state back to a reduced or active state is thought to rely on the presence of enzymes and the supporting biochemistry present in the entourage.[20] Isolated compounds are not supported by a natural plant entourage and compounds such as vitamin C can be easily degraded in an aqueous medium, when exposed to oxygen and metal ions and at high pH. Such unstable compounds may require the addition of preservatives or stabilisers to extend the shelf life of a formulation.[27]

Changes in entourage integrity can be assessed through bioassays that measure antioxidant and/or anti-inflammatory potential. Various combinations of whole extracts, isolated compounds or crude extract fractions can be assessed for increases or decreases in potential. Recent research into the synergy between ginger and turmeric extracts demonstrated an optimal anti-inflammatory response using a specific ratio of ginger to turmeric at 5:2 (w/w). The same research also demonstrated a greater anti-inflammatory effect was achieved with a full spectrum extract of ginger and turmeric than was achieved through the isolation of compounds.[22]

Loss of entourage integrity becomes evident through investigations into the effects of fractionation and isolation of whole extracts. Research by Mathew et al. (2023) into the anti-inflammatory potential of *Backhousia myrtifolia* (Cinnamon Myrtle) extracts, demonstrates reduced anti-inflammatory effect in dichloromethane (DCM) fractions compared to a crude ethanolic extract after fractionation. Isolated compounds were found to have even lower activities than that of the DCM fraction.[24]

The growing body of evidence that has emerged through cannabis research presents a prime example of the potential for phyto-chemicals to exert these complex interactions. Use of isolated medicinal cannabinoids such as tetrahydrocannabinol (THC) and cannabidiol (CBD) are proving to be less efficacious than full spectrum cannabis extracts. Enhanced synergistic effects have also been observed between cannabinoids, such as a demonstrated increased reduction in mechanical hypersensitivity from an extract containing equal parts cannabigerol (CBG) and CBD. An entourage effect was observed when pure CBD was found to be ineffective against mechanical sensitivity but a complex hemp extract at the same CBD concentration was effective.[19] Terpenes have a broad synergistic effect and the presence of terpenes naturally present in the whole plant extracts of cannabis have been shown to potentiate analgesic, anti-inflammatory and sedative effects in vitro.[19,21]

Kakadu Plum plays an important role in Australian First Nation communities as a food and medicine and is associated with exceptionally high levels of natural vitamin C.[23] A NATIVE EXTRACTS hydrophilic Cellular Extract of Kakadu Plum further concentrated into its essenXce format (5:1) was analysed at 16% or 16,000mg/100g vitamin C. After 27 months it was shown to still contain 65% of the original vitamin C content. Although the mechanism for vitamin C preservation in the Kakadu Plum extract is not proven, it is theorised that the full suite of compounds present in the extract facilitates the means for a biological ascorbic acid regeneration pathway. It may be possible that in the presence of enzymes of the glutathione-ascorbate cycle and either NADH or FADH, oxidised ascorbic acid metabolites are reduced back to the active form of ascorbic acid. This would not be possible with a synthetic or an isolated extract of vitamin C in water. Figure 3 illustrates the mechanism of nutrient regeneration within a system in which vitamin C can be converted back to a non-oxidised form by glutathione or reductase enzymes.

## Nutrient Regeneration within a System

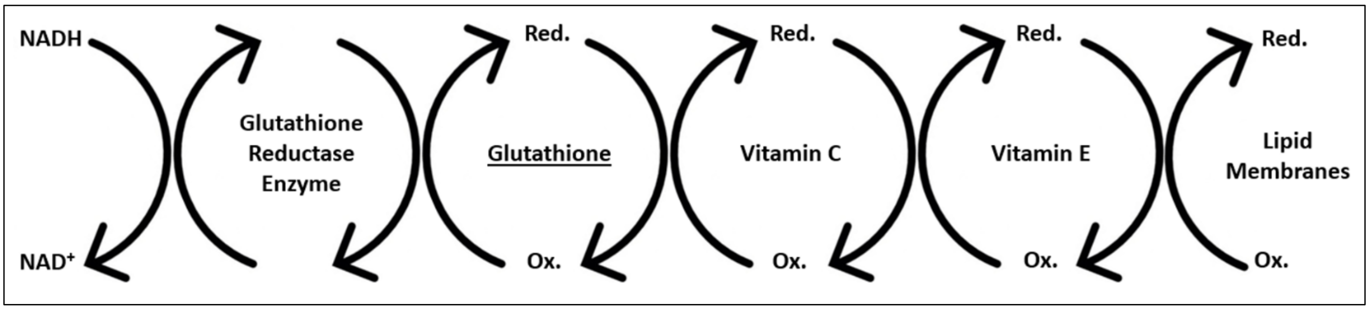


**Figure 3:** Basic schematic of the aqueous and membrane/lipid based antioxidant processes in the body and how nutrients can be regenerated from their oxidised (Ox.) state back into a non-oxidised or reduced state (Red.).[20]

## Research Advancing Our Understanding

As scientific technology evolves so too does the ability to learn more about the complex suite of compounds found in plant extracts and the interactions that occur between them.
Through a decade of research and discovery based on Cellular Extraction protocols, NATIVE EXTRACTS has been following and leading this development and created hundreds of plant profiles in order to learn more about the species hydrophilic entourage. Much of this data did not exist in the commercial space prior to the collection of these plant profiles. Through partnering with Analytical Research Laboratory at Southern Cross University we have seen progress in the identification of compounds grow significantly. Currently we are working with four leading Australian universities for further compound identification and investigating the anti-inflammatory and antioxidant potential of whole plant extracts and combinations of extracts or compounds that may have synergist effects. Research into the synergy or entourage effects of compounds within an extract or between blended extracts is an exciting and relatively new area that has the potential to provide more potent blends of extracts, thus reducing the raw material needed to achieve biological effects. The fragility of the relationships

between the compounds can be preserved through mild extraction methods such as Cellular Extraction that accommodate the complex interactions within botanical extracts.

As an example, in an extract concentrate of finger lime caviar we have been able to see the progress from the identification of 12 compounds by High Performance Liquid Chromatography with Mass Spectrophotometry (HPLC-MS) to 45 compounds using Quadrupole technology with time-of-flight and tandem mass spectrophotometry (Q-ToF MS/MS). There are still many compounds detected that are yet to be identified in both analysis methods, and the role of the lesser known compounds in the efficacy of the extract is still under investigation as science builds a respect and understanding of the complex nature of botanical chemistry.

## NE Finger Lime Cellular Extract Concentrate : Compound Identification by LCMS

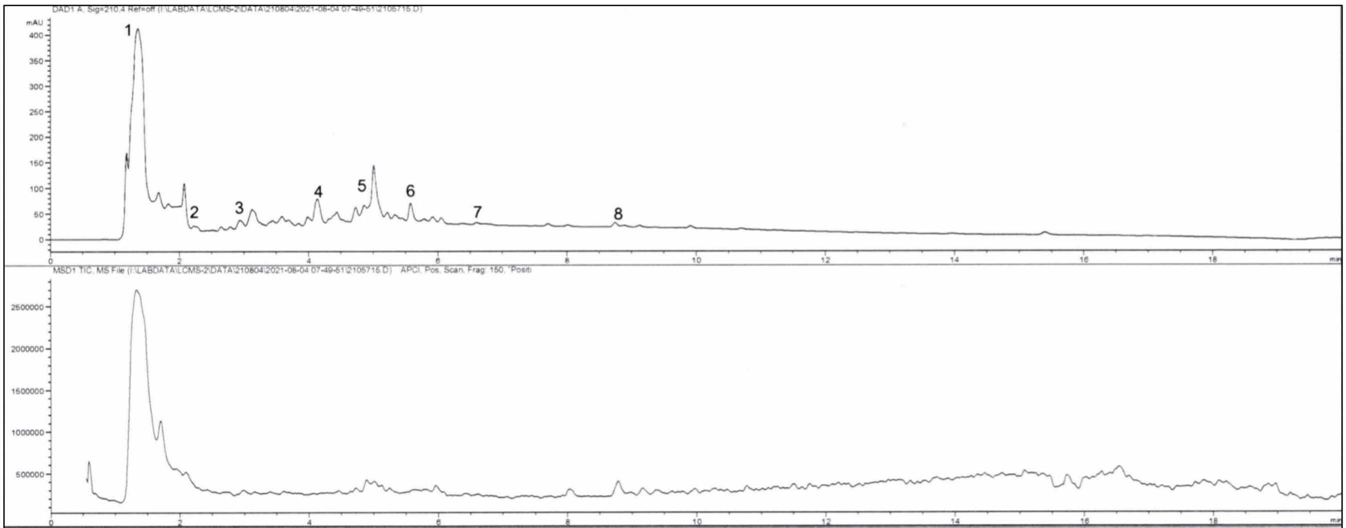


**TABLE 1. PEAK IDENTIFICATION**

| Peak # | RT (min) | Fragment ions [M+H] | Tentative ID (MW) |
|---|---|---|---|
| 1 | 1.1 - 1.8 | 116, 130, 223 | mixed peak - amino acids, orgaic acids |
| 2 | 2.1 | 188,205 | mixed peak - amino acid tryptophan m/z 205 |
| 3 | 2.9, 3.1 | | phenolic acid |
| 4 | 3.9, 4.1, 4.4 | | flavonoid derivatives |
| 5 | 4.7-5.0 | 303, 465; 317, 347, 509, 609, 611 | mixed peaks - flavone - rhamnetin, limocitrin, flavonone glcyosides |
| 6 | 5.6 | 565 | flavonoid C-glycoside |
| 7 | 6.5 | 335, 352 | byakangelicin |
| 8 | 8.4-10.0 | 261, 473, 515, 533 | methoxy coumarin derivative, liminoids |

**COMMENTS**

The HPLC-MS profile of the test sample NE210143-07 is given above with some major components from the plant extracts indicated. The peaks identified are a range of phenolic derivatveis including phenolic acids, flavone and flavonone glycosides, benzopyranone and methoxy coumarin derivatives, with terpenoid liminoid derivatives also present. 210 nm and MS TIC expanded overlays are attached.

**Table 4:** NE Finger Lime Cellular Extraction HPLC-MS trace and peak identification – 12 compounds identified.

## NE Finger Lime Cellular Extract Concentrate : Compound Identification by Q-ToF MS/MS

| Metabolites | | |
|---|---|---|
| 5-Hydroxy-2',4',7,8-Tetramethoxyflavone | 7-dihydroxy-2-(4-hydroxyphenyl)-6-methoxychromen-4-one | Acacetin |
| Chrysosplenetin B | Roseoside | Proline |
| Irigenin | Velutin | Maltol |
| C11H16O3_2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-6-hydroxy-4,4,7a-trimethyl-, (6S,7aR)- | Betaine | Niacinamide |
| Acaciin | C19H32O8_2-Butanone, 4-[3-(beta-D-glucopyranosyloxy)-4-hydroxy-2,6,6-trimethyl-1-cyclohexen-1-yl]- | Neoeriocitrin |
| Santin | Naringin | Eugenol |
| Adenosine | C18H28O3_8-{(1S,5R)-4-Oxo-5-[(2Z)-2-penten-1-yl]-2-cyclopenten-1-yl}octanoic acid | trans-Ferulic acid |
| C12H18O4_{(1R,2R)-2-[(2Z)-5-Hydroxy-2-penten-1-yl]-3-oxocyclopentyl}acetic acid | Isomaltulose | (3R,4S,5S,6R)-2-(6-hydroxy-2,6-dimethylocta-2,7-dienoxy)-6-(hydroxymethyl)oxane-3,4,5-triol |
| Choline | Hesperidin | (1R,3R,4S,5R)-3-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-1,4,5-trihydroxycyclohexane-1-carboxylic acid |
| 7-dihydroxy-6,8-dimethoxy-2-(4-methoxyphenyl)chromen-4-one | Rosmarinic acid | Uridine |
| Phenylalanine | L-Pyroglutamic acid | Diosmin |
| 2-(3,4-dihydroxyphenyl)-5-hydroxy-7-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-[[(2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxymethyl]oxan-2-yl]oxychromen-4-one | Maltose | 2-Piperidone |
| Rosmarinic acid | Dimethyl sulfoxide | L-Pipecolic acid |
| Isoleucine | 2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-6,8-dimethoxychromen-4-one | Nicotinic acid |
| 4-(2,6,6-Trimethyl-4-oxo-2-cyclohexen-1-yl)-2-butanyl beta-D-glucopyranoside | (2S,3S,4S,5R,6S)-6-[2-(3,4-dihydroxyphenyl)-5-hydroxy-4-oxochromen-7-yl]oxy-3,4,5-trihydroxyoxane-2-carboxylic acid | Adenine |
| Tri(butoxyethyl)phosphate | (2R,3R,4S,5S,6R)-2-[(2E)-4-hydroxy-3,7-dimethylocta-2,6-dienoxy]-6-(hydroxymethyl)oxane-3,4,5-triol | 2-methyl-3-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxypyran-4-one |

**Table 5:** NE Finger Lime Cellular Extraction UPLC-Q-ToF MS/MS compound identification – 45 compounds identified.

Untargeted profiling of phyto-compounds in hydrophilic extracts of fruits via UPLC-Q-ToF-MS/MS can yield valuable information regarding their chemical structure. The identification of these structures enables chemical profiling of novel compounds in hydrophilic extracts which can exhibit bioactivity as anti-inflammatory or antioxidant agents. In Figure 4, 280 phenolic compounds exhibiting bioactivity were profiled via UPLC-Q-ToF-MS/MS in a “flavonoid-rich fruit”. The ontology of some of these compounds include epigallocatechins, flavanones, flavones, flavonoid-3-O-glycosides, anthocyanins and phenolic acids.

## 280 Phenolic Compounds Exhibiting Bioactivity using UPLC-Q-ToF-MS/MS

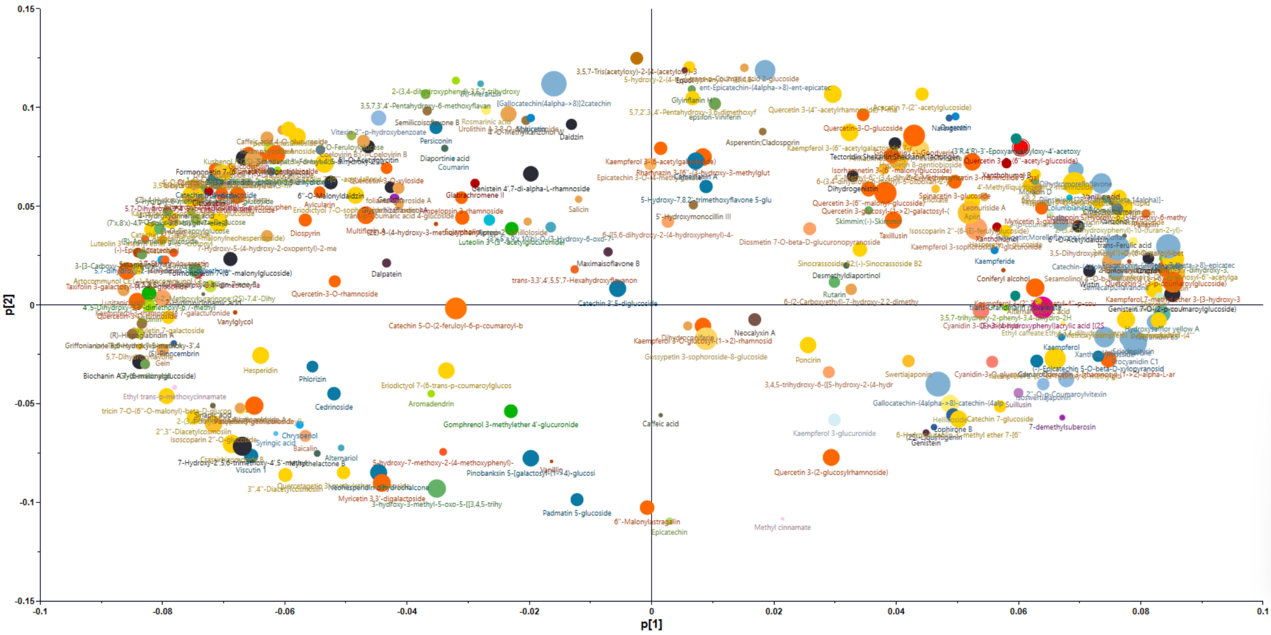


**Figure 4:** UPLC-Q-ToF-MS/MS analysis of a flavonoid rich fruit displayed using a Principal Component Analysis (PCA) loadings plot. The compounds are scaled for size, where smaller circles indicate low molecular weight phenolic compounds, and larger circles indicate high molecular weight phenolic compounds.

## 3-D Representation Illustrating the Ontology of a Flavonoid Rich Fruit

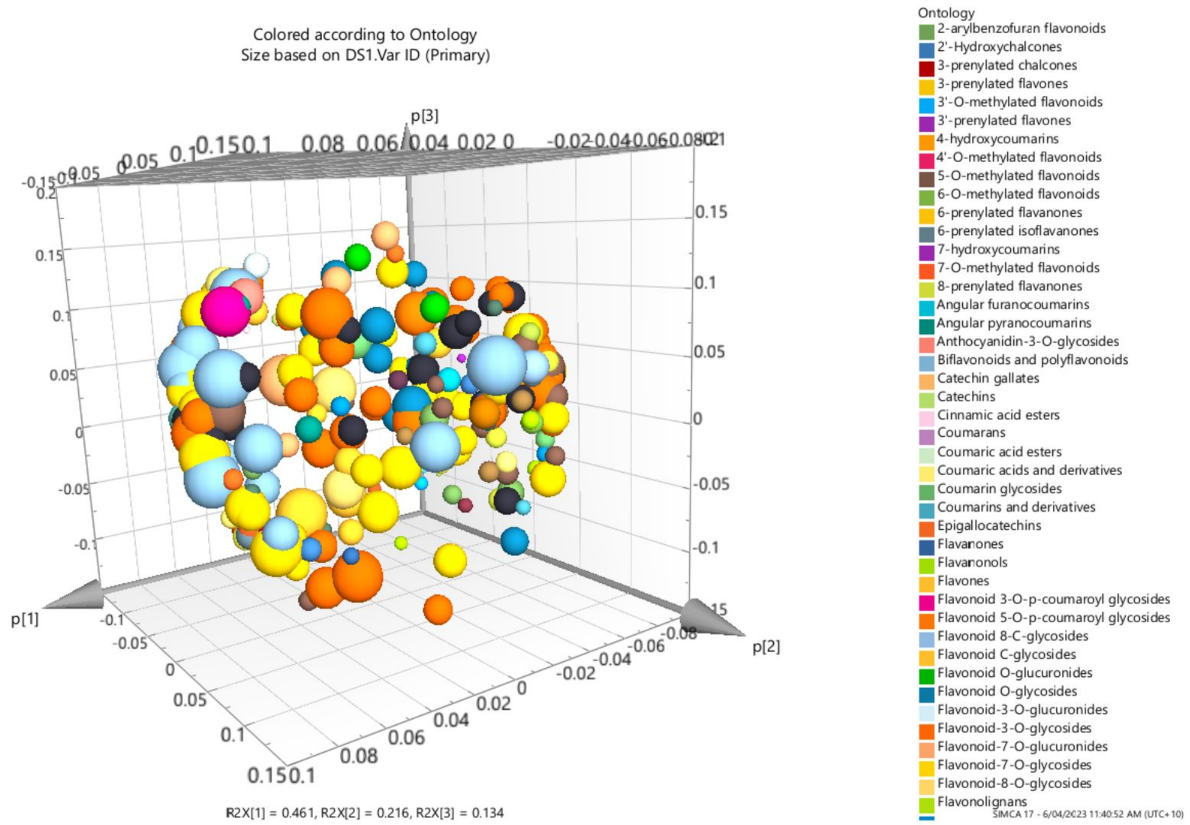


**Figure 5:** UPLC-Q-ToF-MS/MS analysis of a flavonoid rich fruit displayed using a Principal Component Analysis (PCA) loadings plot. The compounds are scaled for size, where smaller circles indicate low molecular weight phenolic compounds, and larger circles indicate high molecular weight phenolic compounds. Alternaive 3-D visualisation of the Ontology, colour coded.

## Conclusion

Botanical extracts are a rich source of bioactive compounds with a wide range of applications and are therefore much sought after in the pharmaceutical, complementary medicine and cosmetic industries. Current market forces are driven by consumers demanding more natural solutions to human problems, more sustainable and resource-wise options that are processed with minimal impact on the environment and responsible and ethical practices. The responsible sourcing, cultivation and extraction of these compounds is essential to preserve valuable resources and reduce wastage and carbon emissions. Pressurised liquid extraction methods such as Cellular Extraction offer the opportunity to use less solvent and less biomass to rapidly extract botanical phyto-compounds and preserve plant extracts' integrity in the ratios that nature intended, within their natural entourage.

NATIVE EXTRACTS has created a library of the hydrophilic entourage, following the science as interest and understanding of the complexities of phyto-chemical interactions evolves. Further research is needed to explore phyto-compound profiles and interactions therein. There are a great number of known and unknown compounds in botanicals that provide unlimited sources and possibilities to support cellular biochemistry and metabolism for human health and beauty using plant-based solutions for human problems.



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Dale has extensive analytical experience with a sound background in plant and food chemistry and microbiology, spanning the brewing and dairy industries, analytical chemistry and phytochemical research. As a Naturopath with a keen interest in the potential of phyto-compounds for human application, she is keen to explore the potential uses of Australian native and non-native flora with appropriate consideration of sustainability and the impact the food and cosmetic industries have on the environment.

In her role as Quantitative Team Leader at Analytical Research Laboratory at Southern Cross University, Dale was witness to the evolution of NATIVE EXTRACTS' hydrophilic plant profiling over the years and the increased complexity of their entourage analysis.

**Lisa Carroll**, CEO & Innovator NATIVE EXTRACTS Pty Ltd

Lisa Carroll was instrumental in the inception of NATIVE EXTRACTS (2012), it's direction, growth and communication of new discoveries and profiles of Australian natives, and connection to Indigenous and non-indigenous growers, University and 3rd part analytical services parterships. NATIVE EXTRACTS is recognised in the industry for it's breakthroughs and innovations in harnessing the natural vitamin C molecule in an aqueous format (public release 2013) and more recent advances in setting new benchmarks in concentration. Lisa has been pivotal in building new libraries of plant profiles of Australian native species and traditional botanicals. Dedicated to evolving the botanical extract to transparent, traceable natural ingredients delivering a "True to Nature" plant profile (water-soluble phyto-compounds, their derivatives etc), suspended in an entourage of supporting biochemistry, ready to formulate. She has been an influence behind the A(ustralian) Beauty trend, from ingredient through to consulting on brand development, and large scale multinationl Social Impact projects raising Frista Nation representaton in the supply chain.

Lisa has built a highly experienced technical team with expertise across multiple extraction processes, new technology, R&D, and analytical plant chemistry from acclaimed universities, with an emphasis on conscious manufacturing practices. She continues to launch world first ingredients, build strategic partnerships to commercialise ingredients under licence, expand libraries of botanical ingredients and delivery formats. She is passionate about supporting the growth of an inclusive Australian native primary industry, and "powered-by-nature" solutions for Cosmetics, Pharmaceutical, Nutraceuticals, Beverage sectors.