



Skin Barrier Protection Botanical Antioxidant Standardized for Vitamin C Anti-aging

BACKGROUND

Nasturtium officinale, more commonly referred to as watercress, is an aquatic flowering and edible plant. The small, white and green flowers with hollow stems that float in water make watercress easily recognizable. Historically, watercress has been consumed globally for its unique peppery taste and also been used to treat various ailments. Today, watercress is making headway in the cosmetics industry as a popular superfood addition to skin care formulations. The use of superfoods became a quick leading trend, as consumers are just as concerned with what they put on their skin as they are with what they put in their bodies. The popularity of watercress as a superfood is attributed to its high nutritional content of vitamins and minerals, such as Vitamin C, Vitamin A, Vitamin B6, Riboflavin, Calcium, Manganese, and so on. Vitamins have been long associated with good health and trustworthiness by beauty consumers. The market is quite crowded with obvious sources for Vitamin C. By AC Watercress Extract SF being standardized for Vitamin C, this natural source of Vitamin C from a superfood will provide brand differentiation within the market. By doubling as an antioxidant and an anti-inflammatory, AC Watercress Extract SF aids in neutralizing harmful free radicals to keep fine lines and wrinkles at bay, and can help with problematic skin conditions caused by inflammation, such as acne and psoriasis. This sensational super green is just as good for your skin as it is for your body.

SCIENCE

AC Watercress Extract SF is standardized for 0.5 - 2.0% maximum Vitamin C content. Vitamin C is an essential nutrient, as the presence for Vitamin C is required for a range of essential metabolic reactions and is made internally by most organisms. Vitamin C functions as an antioxidant as it protects the body against oxidative stress and is a cofactor in several vital enzymatic reactions, such as the formation of collagen in the skin. This essential vitamin is the most abundant antioxidant in the skin. However, the levels of Vitamin C in the epidermis deplete with age and exposure to both UV irradiation and pollution. The topical application of Vitamin C is critical as our skin is unable to synthesize this power molecule on its own.

Code Number: 11907

INCI Name: Water & Nasturtium
Officinale Extract & Lactobacillus

Ferment

INCI Status: Conforms **REACH Status**: Compliant

CAS Number: 7732-18-5 & 84775-70-2 & 1686112-36-6 (or) 68333-16-4 **EINECS Number**: 231-791-2 & 283-899-4 & N/A (or) N/A

Origin: Botanical
Processing:
GMO Free
No Ethoxylation
No Irradiation
No Sulphonation

Additives:

Natural Antimicrobial: Lactobacillus

Ferment

Preservatives: None Antioxidants: None Other additives: None **Solvents Used**: Water

Appearance: Clear to Slightly Hazy Liquid, Pale Yellow to Amber Soluble/ Miscible: Water Soluble Microbial Count: <100 CFU/g,

No Pathogens

Suggested Use Levels: 1.00 – 10.00% **Suggested Applications**: Antioxidant, Anti-aging, Anti-

inflammatory

Benefits of **AC Watercress Extract SF:**

- Standardized for Vitamin C
- Homeostasis of Calcium Levels
- Antioxidant

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BENEFITS

AC Watercress Extract SF may be used to combine the benefits associated with the high content of phytochemicals watercress possesses, in addition to being standardized for Vitamin C, in a variety of personal care applications. Aging is inevitable, but with being able to protect our skin from UV exposure and other outside environmental factors, this ingredient provides the antioxidant protection consumers need to hold on to their youthfulness and skin elasticity for longer. It is commonly known that exposure to environmental stressors leads to the genesis of reactive oxygen species (ROS) that are the main contributors to the aging of the skin. An increase in ROS can increase the rate at which wrinkles and fine lines form. The number one way to prevent the signs of aging is to protect the skin from these external environmental factors everyday. By applying **AC Watercress Extract SF** to skin care formulations, that extra protection is added, keeping the skin smooth, elastic, and youthful for longer.

EFFICACY

Reactive oxygen species (ROS) are generated by normal cellular processes, environmental stress, and UV irradiation. Unfortunately, consumers are chronically exposed to these everyday external factors. ROS are detrimental to cellular structures and functional molecules as they act as strong oxidizing agents or free radicals. However, substances that provide antioxidant activity are able to protect the skin from these reactive oxygen species by controling autoxidation by interrupting the propagation of free radicals, or inhibiting the formation of free radicals. Vitamin C and Vitamin E are the main exogenous antioxidants and as previously stated, **AC Watercress Exract SF** is standardized for Vitamin C.

The oxygen radical absorbance capacity assay (ORAC) is a standard method used to assess antioxidant capacity. The assay quantitatively measures a sample's ability to quench free radicals that have the potential to react with and damage cellular components. **AC Watercress Extract SF** was compared to a set of Trolox® standards, a water soluble vitamin E analog with known antioxidant capabilities. As Figure 1 shows below, **AC Watercress Extract SF** exhibited antioxidant activity comparable to 200µM Trolox®.

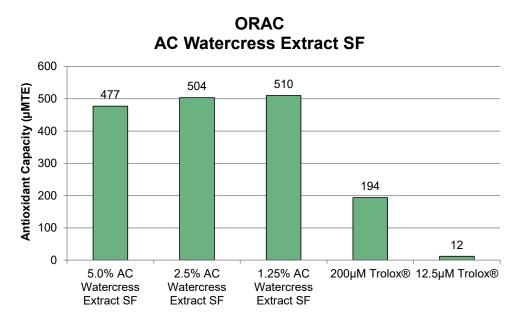


Figure 1. AC Watercress Extract SF Antioxidant Capacities.

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Low levels of intracellular oxidative stress are produced during normal physiological functions. However, UV irradiation, pollutants, foreign substances, and aging elicit unrestricted increases in reactive oxygen species (ROS). These deregulated augmentations in oxidative stress lead to an acceleration of DNA mutation, cellular senescence, advanced glycation end products, protein oxidation, and collagen degradation. Moreover, when intrinsic antioxidant capacities are reduced, such as during aging, an imbalance between pro- and anti-oxidant systems further accentuates these hallmarks of cellular aging, such as premature wrinkles, sagging, and the appearance of fine lines.

A ROS Scavenging Assay was conducted to assess the in-vitro effect of **AC Watercress Extract SF** to scavenge unnecessary oxidative stress in dermal fibroblasts. Attenuating excessive ROS preserves cellular homeostasis and blunts intrinsic and extrinsic age-related declines in skin cell function. Figure 2 below displays the effect of **AC Watercress Extract SF** on ROS scavenging.



Figure 2. The Effect of AC Watercress Extract SF on ROS Scavenging.

As shown in Figure 2, fibroblasts incubated with AntA, a known inducer of oxidative stress, elicited a 57% increase in ROS levels, compared to untreated fibroblasts. These data demonstrate the supraphysiologic level of ROS induced by AntA and the magnitude of ROS in fibroblasts is dynamic.

Conversely, fibroblasts treated with **AC Watercress Extract SF** at 0.01%, 0.1%, and 1.0% demonstrated 28%, 35%, and 37% reductions in ROS levels compared to the fibroblasts treated with AntA, respectively. These data demonstrate that **AC Watercress Extract SF** attenuates excessive oxidative stress, which may help to attenuate characteristics of cellular aging.

Intracellular calcium concentrations play a key role in skin health and immune response as it can help reduce inflammation with a direct effect on tissue repair and keratinocyte function. Calcium also helps maintain skin moisture levels and promotes the production of antioxidants. Keratinocytes, making up 90-95% of the epidermis, are a physical and chemical barrier for the skin by minimizing UV radiation damage, water loss, and microbial, viral, and parasitic invasion. Accordingly, keratinocytes must progressively proliferate, differentiate, and migrate to maintain skin barrier integrity and homeostasis. Provided keratinocyte proliferation, differentiation, and migration are calcium dependent, an increase in intracellular calcium levels can serve as a surrogate for improved skin health.

A Fluo-4 Direct™ Calcium Assay was performed to assess changes in intracellular calcium levels in **AC Watercress Extract SF**-treated human epidermal keratinocytes *in-vitro*. Human epidermal keratinocytes were seeded into a 96-well tissue culture plate and allowed to grow to confluency in complete media (CM). As experimental controls, complete media containing 1mM CaCl2 was utilized as a positive control to increase intracellular calcium levels, and 2mM EGTA in the presence of 1mM CaCl2 was utilized as a negative control to reduce the CaCl2-induced increases in intracellular calcium. Results are shown below in Figure 3.

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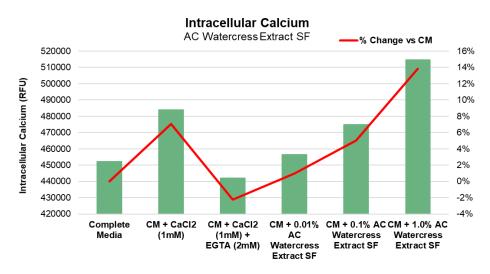


Figure 3. Effect of AC Watercress Extract SF on Keratinocyte Intracellular Calcium Levels.

Collectively, this data demonstrates that **AC Watercress Extract SF** increases intracellular calcium, an indicator of keratinocyte proliferation, differentation, and migration, which contributes to the maintenance of skin barrier integrity and homeostasis. Additionally, third party studies have shown that an increase in calcium increases immune responses, one being reducing inflammation. This led Active Concepts to test the antioxidant abilities of **AC Watercress Extract SF**.

An *in-vitro* Interleukin-6 ELISA study was conducted to assess the changes in IL-6 levels in **AC Watercress Extract SF** treated cultured human dermal fibroblasts. Interleukin-6 is a pro inflammatory cytokine known to play an active role in inflammation, immune response, bone metabolism, arthritis, neoplasia, and aging. IL-6 signals through the nuclear factor-kappa B pathway that results in the transcription of inflammatory mediators, including matrix metalloproteinase-1 (MMP-1). MMP's are responsible for breaking down the extracellular matrix and collagen in the skin, leading to wrinkles, fine lines, and the loss of skin elasticity. Reducing the levels of IL-6 and other inflammatory mediators is believed to slow down degradation of the skin matrix and possibly stimulate its replenishment. The results in Figure 4 below indicate that **AC Watercress Extract SF** exhibits anti-inflammatory effects on LPS-treated fibroblasts. The decrease in IL-6 production indicates a reduced inflammatory environment, which would slow the signs of premature aging.

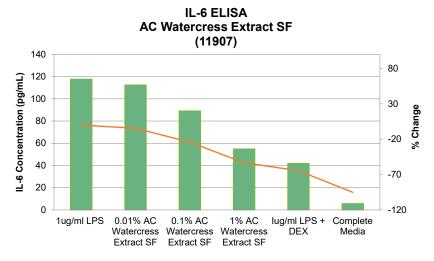


Figure 4. AC Watercress Extract SF-Treated Fibroblasts IL-6 Concentrations and Percent Change.

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CONCLUSION

With **AC Watercress Extract SF** being standardized for 0.5 - 2.0% maximum Vitamin C, this ingredient is able to topically enhance anitoxidant properties of the skin. As research has shown, antioxidants are able to combat the premature aging effects caused by ROS, such as wrinkles, fine lines, and sagging. However, there is much more to this ingredient than just Vitamin C benefits. By also being able to increase intracellular calcium, allowing for keratinocyte proliferation, differentation, and migration, **AC Watercress Extract SF** also aids in providing the skin with a calcium homeostatic environment. This allows for skin barrier integrity and homeostasis. Lastly, the ability of **AC Watercress Extract SF** to exhibit anti-inflammatory benefits also slows the aging process down by preventing the break down of collagen and soothing inflammatory responses. **AC Watercress Extract SF** is the perfect ingredient for formulators to add to a wide variety of skin care applications, providing the consumer with naturally derived anti-aging and anti-inflammatory benefits.



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