SYNERGIE SKIN
CLEAN SCIENCE

CLINICAL DATA – Camellia Sinensis (Green tea) leaf extract
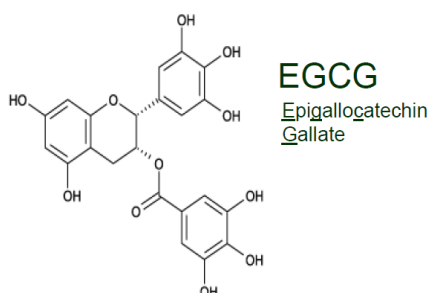
Major sources of dermal damage:

1. Exposure to Oxidative Stress
- Pollution, sunlight and other insults generate Reactive Oxygen Species (ROS) which disrupt biological processes essential for cellular survival

2. Increased levels of Proteolytic Enzymes
- Higher levels of MMP's (enzyme which metabolise ECM proteins such as collagen and elastin) are detected during ageing.

3. UV-Induced Pigmentation Disorders
- Age spots in mature skin are caused by cumulative UV-mediated damage.

Several Activities are necessary in order to provide good anti-ageing efficacy:

- Camellia Sinensis (Green Tea) Leaf Extract processed via sonic extraction, provides multifunctional anti-ageing benefits such as:
    1. Strong protection against oxidative stress
    2. Broad-spectrum protection against proteolytic damage
    3. Inhibits tyrosinase activity and melanin production
    4. Modulates inflammatory PLA_2 activity
- Camellia Sinensis (Green Tea) Leaf Extract also has proven stability and long-lasting efficacy

The technology

- Leaves of Green Tea are submitted to the sonic extraction process as part of manufacturing. No chemical additives (glycols, alcohols, etc.) are used during the industrial extraction process.
- Green tea extracts are among the most widely used ancient medicinal agents. They possess antioxidant, anti-inflammatory, wound healing and anticarcinogenic properties.
- Among the different polyphenolic catechins in Green Tea, Epigallocatechin gallate (EGCG) is the most abundant and effective.
- Green tea polyphenols are easily oxidised and lose their activity if not used immediately. The primary goal of topical formulations containing green tea extracts should be to maintain the stability of these beneficial components. This has been overcome using the sonic extraction method.

EGCG
Epigallocatechin Gallate
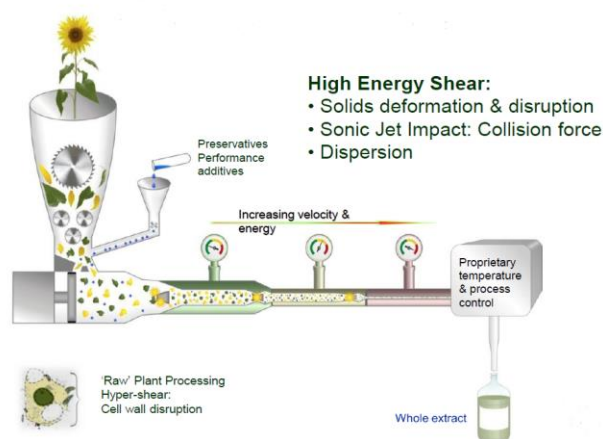
Efficacy 1. Inhibition of oxidative damage:

- Pollution, sunlight and other insults generate Reactive Oxygen Species (ROS) that disrupt biological processes essential for cellular survival. Two assays were used to evaluate the antioxidant activity of Camellia Sinensis (Green Tea) Leaf Extract.

a) UV-Induced Lipid Peroxidation Inhibition

- Camellia Sinensis (Green Tea) Leaf Extract was tested for its ability to inhibit oxidation of phospholipid liposomes exposed to UV light. Rates of oxidation were determined by measuring generation of malonaldehye, a toxic by-product of oxidized lipids.

b) Scavenging/quenching of Singlet Oxygen (SO):

- SO is generated from other reactive oxygen species at sites of inflammation, from peroxyl radicals such as those generated during UV-induced lipid oxidation and simple exposure of the skin to visible light by means of photosensitisation reactions.
- Camellia Sinensis (Green Tea) Leaf Extract was tested for its ability to prevent oxidation cause by SO. Reaction mixtures containing a photosensitising dye were exposed to visible light to generate SO. Oxidation of iodide by SO was measured spectrophotometrically.

Efficacy 1. Inhibition of oxidative damage: UV-induced lipid peroxidation.

Camellia Sinensis (Green Tea) Leaf Extract
Inhibition of UV-Induced Lipid Oxidation
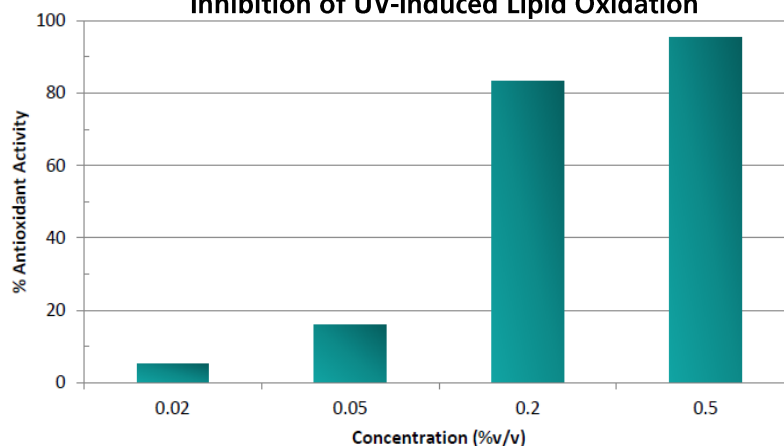
- Camellia Sinensis (Green Tea) Leaf Extract is significantly effective at low concentrations achieving almost complete inhibition of lipid oxidation at only 0.5%

Efficacy 1. Inhibition of oxidative damage: Scavenging/quenching of singlet Oxygen.

Camellia Sinensis (Green Tea) Leaf Extract
Scavenging/quenching of Singlet Oxygen
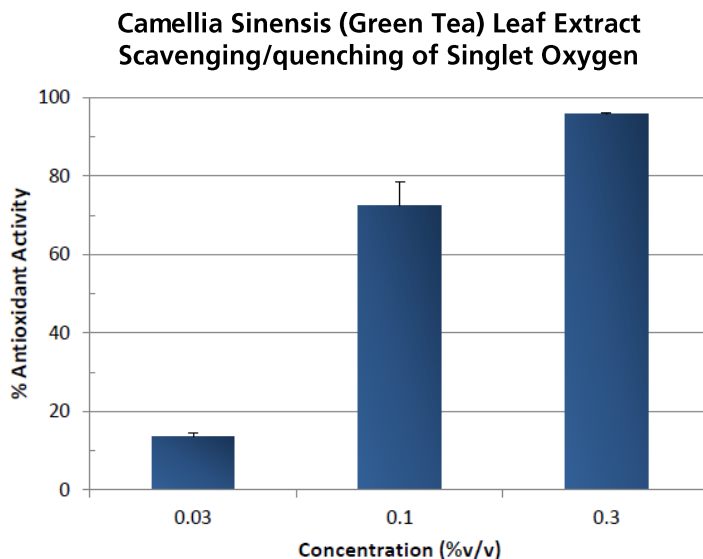
- Singlet Oxygen is one of the most aggressive Reactive Oxygen Species (ROS) and is especially damaging to mitochondria, the cell's energy source.
- Singlet Oxygen damages all classes of biological macromolecules, thereby compromising cell membrane structure, DNA integrity and protein functions.

Camellia Sinensis (Green Tea) Leaf Extract proves to be an effective Singlet Oxygen Scavenger!

- Batches of Camellia Sinensis (Green Tea) Leaf Extract of varying ages were tested for efficacy. The calculated EC50 values show only a minimal drop in potency over two years.

Camellia Sinensis (Green Tea) Leaf Extract
Batch Potencies for Singlet Oxygen Scavenging
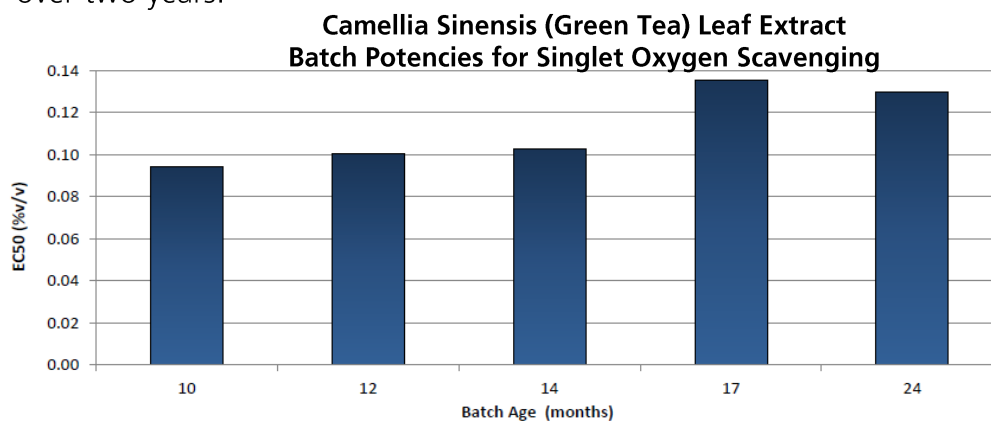
- Unlike most commercial green tea extracts, Camellia Sinensis (Green Tea) Leaf Extract provides long lasting antioxidant activity.

Efficacy 2. Protection of the Extracellular Matrix

The Extracellular Matrix (ECM) provides structural support to dermal cells (fibroblasts). ECM is mainly composed of proteins (e.g., collagen and elastin) and glycosaminoglycans. Camellia Sinensis (Green Tea) Leaf Extract was tested for its ability to inhibit proteases that damage ECM.
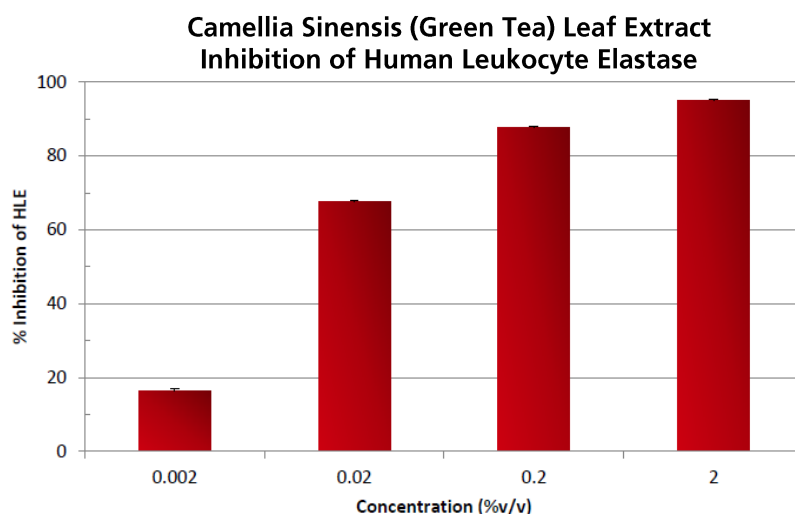
a) Inhibition of Human Leukocyte Elastase:

- This enzyme is a broad-spectrum serine protease released by infiltrating neutrophils during inflammation. It will attack and degrade most components of the ECM.
- Excessive Elastase activity is a key indicator of inflammation and is triggered by release of pro-inflammatory mediators (TNF-α, interleukins) from keratinocytes and other skin cells.

b) Inhibition of Matrix Metalloproteinases (MMP-1 and MMP-8)

- MMP's are released into the ECM by infiltrating white blood cells and also by resident skin cells in response to an inflammatory stimulus. MMP-1 and MMP-8 are especially damaging to type I, II and III collagens.

Efficacy 2. Protection of the Extracellular Matrix: Inhibition of Human Leukocyte Elastase:

Camellia Sinensis (Green Tea) Leaf Extract
Inhibition of Human Leukocyte Elastase

- With an EC90 of approximately 0.2%, Camellia Sinensis (Green Tea) Leaf Extract is a strong inhibitor of proteolytic damage.
- Batches of Camellia Sinensis (Green Tea) Leaf Extract of varying ages were tested for potency vs. human leukocyte elastase. The results were very consistent with the potency for fresh batches of Camellia Sinensis (Green Tea) Leaf Extract.

Elastase Inhibition by Camellia Sinensis (Green Tea) Leaf Extract
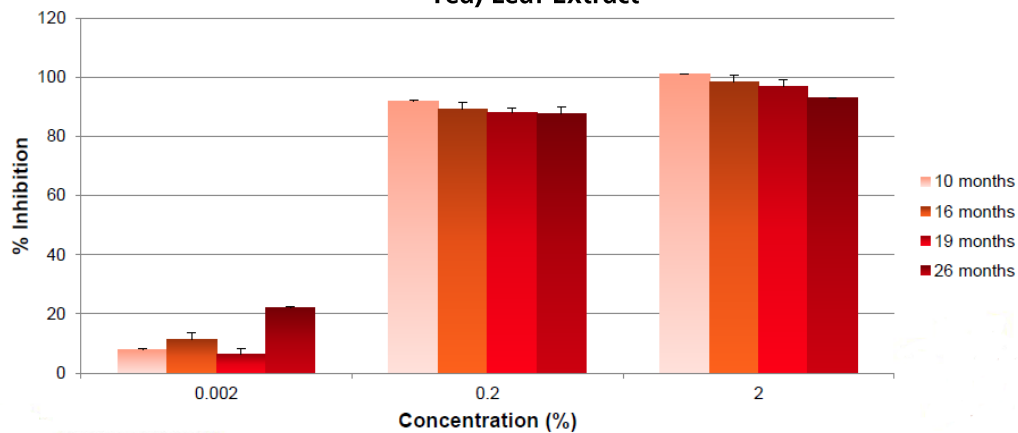
Efficacy 2. Protection of the Extracellular Matrix: Inhibition of MMP's

Camellia Sinensis (Green Tea) Leaf Extract

Camellia Sinensis (Green Tea) Leaf Extract
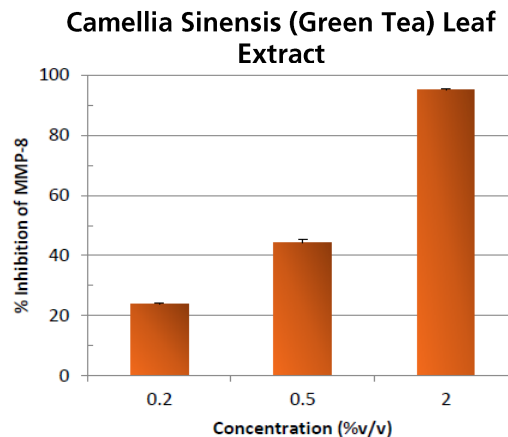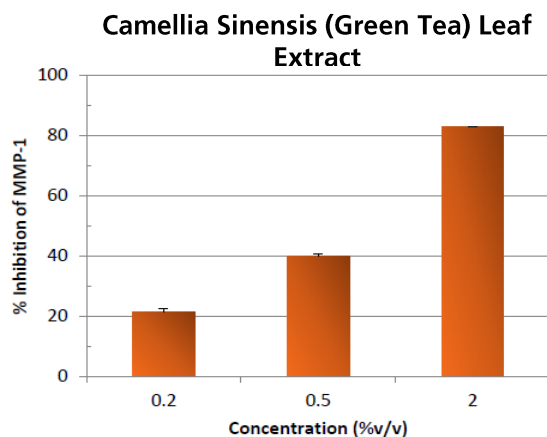
- Camellia Sinensis (Green Tea) Leaf Extract showed dose-dependent inhibition of MMP-1 & MMP-8.

Efficacy 3. Inhibition of Phospholipase A2 (PLA$_2$)

- PLA$_2$ enzymes are commonly found in mammalian tissues. Increased presence and activity of PLA$_2$ can lead to inflammation, itching and pain.
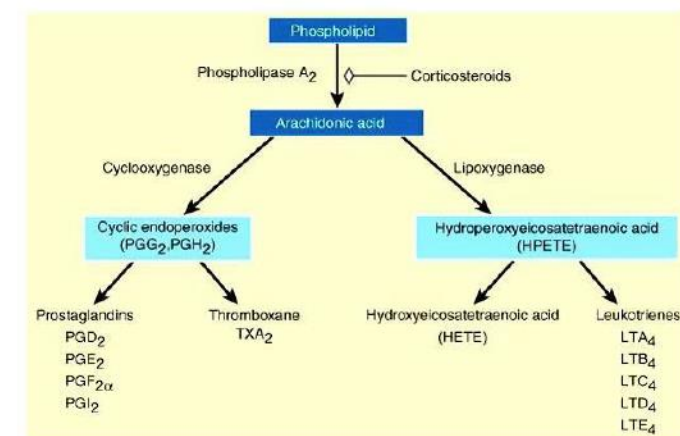
Schematic diagram of arachidonic acid metabolism. LT = leukotriene ; PG = prostaglandin; TXA$_2$ = thromboxane A$_2$.

- Inhibitors of PLA_2 are known to have anti-inflammatory efficacy. Camellia Sinensis (Green Tea) Leaf Extract effect on PLA_2 activity was assessed using the EnzChek PLA_2 Assay Kit.

Inhibition of PLA2 by Camellia Sinensis (Green Tea) Leaf Extract

EC_{50}: ~0.6%
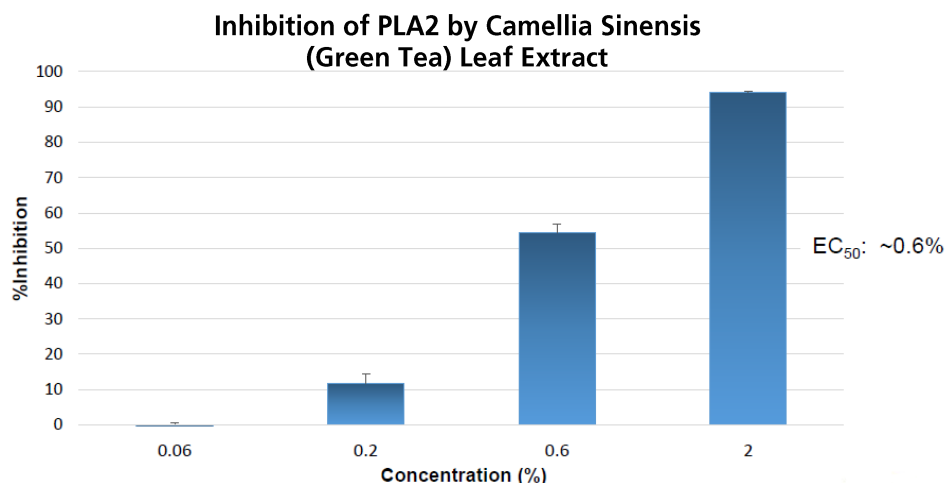
- Camellia Sinensis (Green Tea) Leaf Extract demonstrated dose-dependent inhibition of PLA_2.
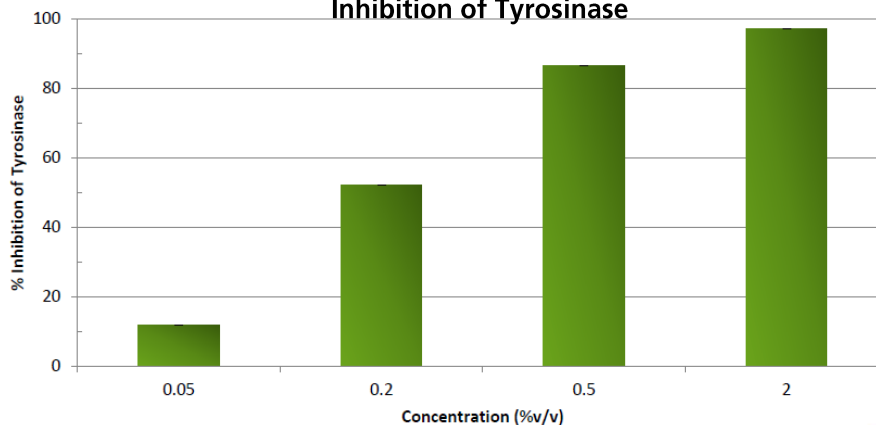
Efficacy 4. Inhibition of Tyrosinase

- Melanin production is dependent on initial conversion of tyrosine to DOPAquinone. This reaction is catalysed by the tyrosinase enzyme and is rate limiting for melanin production.
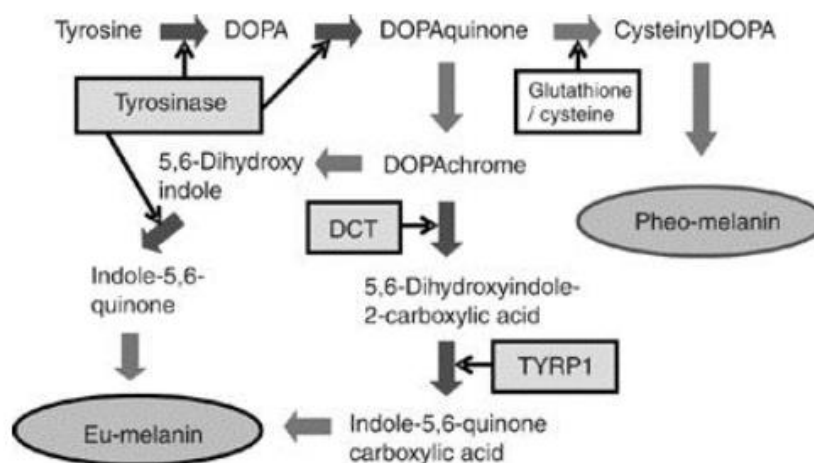
- Camellia Sinensis (Green Tea) Leaf Extract effect on tyrosinase activity was determined by measuring generation of DOPAchrome (OD_{495}) which is formed spontaneously following oxidation of tyrosine by tyrosinase.
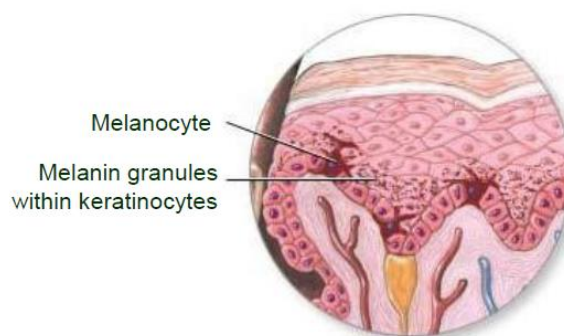
Camellia Sinensis (Green Tea) Leaf Extract
**Inhibition of Tyrosinase**

- Tyrosinase activity is efficiently inhibited by Camellia Sinensis (Green Tea) Leaf Extract.

Efficacy 5. Inhibition of Melanin Production

- Melanocytes produce melanin and transfer it to neighbouring keratinocytes in the basal layer of the skin's epidermis.

- B16-F10 melanocytes were cultured and treated with different concentrations of Camellia Sinensis (Green Tea) Leaf Extract, with non-treated cells used as control. Following 48h incubation, melanin levels were quantitated.

Camellia Sinensis (Green Tea) Leaf Extract
Concentration
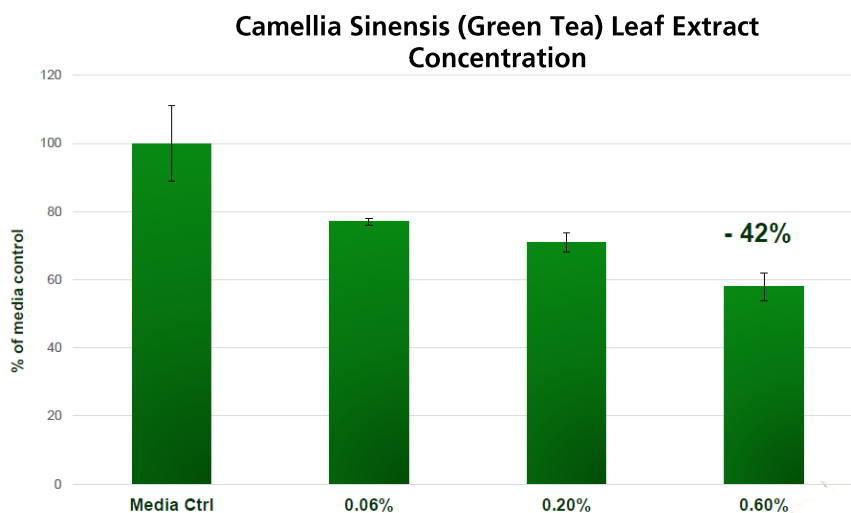
Conclusions

- Camellia Sinensis (Green Tea) Leaf Extract has multiple beneficial effects:
    - Efficient ROS scavenger
    - Strong inhibitor of Elastase and MMP's
    - Modulates inflammatory PLA_2 activity
    - Inhibits tyrosinase activity and melanin production.
- Unlike most commercial green tea extracts, Camellia Sinensis (Green Tea) Leaf Extract produced by sonic extraction, is a stable source of long-lasting antioxidant and anti-inflammatory activities.