PI-100-11

INKAAnatto

Rev1: 17/07/2014

INKA ANATTO is a unique Natural Ingredient, preservative free, organic certifiable, obtained from selected leaves of the Achiote (*Bixa orellana L.*), Amazonian plant used since immemorial time by the tribes of the region for skin protection.

INCI Denomination: **Propanediol (and) Water (and) Bixa Orllana Leaf Extract**

Description of the plant

Family: Bixaceae (Dicotiledonea)

Synonyms: *Bixa odorata, Bixa urucurana, Orellana americana*[1]

Other names: acote, achihuete (Quechua) achote, annato (English) anoto, apisiri (Chontaquiro) atase (Shipibo), bandor, bija, bixo.[2]

Annatto is a small tree or shrub with dense foliage up to 10 m long with short trunk of 20 to 30 cm of diameter; light and porous log with a whitish color. Leaves are alternate, heart-shaped, pointed, between 10 and 20 cm long and green on both sides, elongated petiole, presents 5 veins. Its flower is hermaphrodite, of a white to pink or violet color with 5 oval-shaped petals and calyx with 5 sepals. The fruit of 3-4 cm of length is an ovoid or tapered capsule, reddish coffee or yellow with small smooth thorns that encloses numerous polyhedral seeds inside, covered with fine red-orange pulp. The root is pivoting and of wide development.[3] [4]

[1] BRACK EGG, 1999, page 70

[2] idem

[3] BRACK EGG, 1999, p. 70

[4] DESMARCHELIER Y WITTING SCHAUS, 2000

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Distribution

Although it is original from Center and South America, this plant is geographically distributed in America from Mexico to Brazil and Northern Argentina, generally growing in warm zones. Today, it is found in India, China, Philippines, Brazil and Guyana.[5]

In Peru, the achiote was cultivated since pre-Hispanic times in the Departments of Cuzco (Quillabamba), Pasco (Oxapampa), Huanuco (Tingo Mariaa), Junin (Chanchamayo, Satipo), Loreto (Iquitos), Tumbes, Piura, Lambayeque, San Martin and Ucayali (Pucallpa).[6]

Traditional uses

All parts of the plant are used: the trunk for works in wood and as firewood; the bark for its fiber and for rigging; from the fleshy portion that surrounds the seeds, a coloring agent is extracted, which is used as ingredient in foodstuff[7]. It also has stimulating and digestive properties. The red dye is used on the skin and acts as insect repellent and also to avoid sunburns due to sun exposure. The achiote is considered as the best antidote against the *Manihot esculenta* (yuca or yuca brava or yuca amarga).

Regarding the medicinal use of the achiote leaf, the liquid of the ground leaves is used as antiemetic (against blood vomits) and antidiarrheal. The paste placed over the forehead and templesis cephalalgic. The infusion cures discomforts of the throat, respiratory conditions (such as bronchitis) and renal pains, dermal, vaginal and prostate inflammations. The aqueous maceration is applied to heal and cleanse wounds, against skin infections and for vaginal washings; also against conjunctivitis.

[5] SHILPI et al., (2006), p. 264

[6] INSTITUTO PERUANO DE SEGURIDAD SOCIAL, 1998

[7] Plantarum **1**: 512. 1753.

The Shipibos used the achiote leaves in incense to avoid erotic dreams.[8] Costa Ricans use the infusion of the leaves against baldness.[9]

Phytochemicals

The dry leaves produce an essential oil consisting of ishwarano (major component), bixaganeno, among others mono and sesquiterpenes; ellagic acid; flavonoids such as apigenin, luteolin together with its bisulfates and glucosides (7-epigenin bisulfate, 7-luteolin bisulfate, 8-hipolaetina bisulfate, apigenin glucoside, apigenin bisulfate, hipolaetina, cosmosiin), isoscutellarien, bixorelline and one steroidal sapogenin[10,11]. Other studies also report tannins[12].

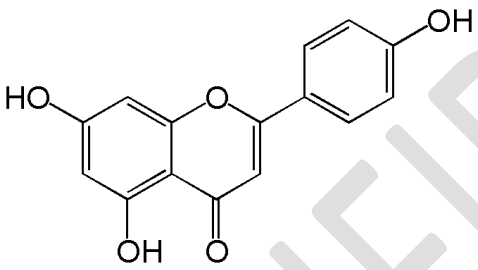


Fig 1: Apigenin

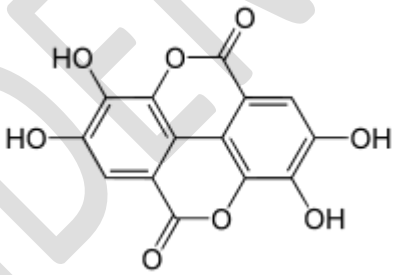


Fig 2:Ellagic Acid

Bioactivity

[8] BRACK EGG, 1999, p. 70
[9] DUKE 1993 in DUKE p. 107
[10] DANIEL M., 2006, pp. 138-139
[11] RAMÍREZ T., 2001
[12] SHILPI et al.,(2006), p. 267

Several activities were reported in the extracts, among them gastric anti-secretory, inhibition of prostaglandin synthetase, hypotensive, smooth muscle relaxant and hypoglycemic action[13] [14].
The cyto-protective effect of the gastric mucose is attributed to the inductive activity of the prostaglandins of the flavonoid compounds, tannins and carotenoids present in the leaves[15].

The methanol extract of the leaves, tested on mice had **analgesic** and sedative properties[16] against *Escherichia coli*, *Staphylococcus aureus* and *Shigella dysenteriae* [17, 18].

The alcoholic extract of *Bixa Orellana* leaves showed elevated **antioxidant** capacity using both the FRAP technique (1,38 mM/100 g dry weight) and the measurement against inhibition of DPPH vs. ascorbic acid and a moderate stimulant effect of the proliferation of primary cultures of fibroblasts of mice.[19, 20]

COSMETIC BENEFIT

ANTIOXIDANT AND SOOTHING

Phenolic compounds found in plants are believed to be effective in prevention of oxidative stress related diseases. An interest in antioxidants and natural foods continues to grow along with a commercial interest in plant-derived phenolic products. Flavonoids represent a family of plant compounds high in phenolic related antioxidant activity. Flavonoids are multi-active components used in common cosmetics primarily for antioxidant and soothing actions.

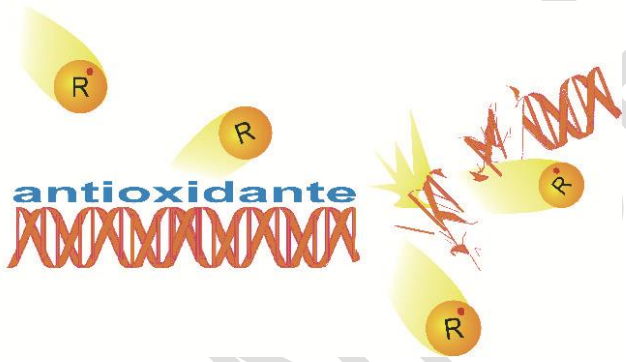
Flavonoids are naturally occurring compounds, widely distributed in fruits, vegetables, seeds, nuts, flowers and beverages such as tea and red wine. They are polyphenolic molecules, diverse in chemical structure and biochemical properties.

[13] DESMARCHELIER AND WITTING SCHAUS, 2000
[14] FLEISCHER T. C. et al., 2003
[15] HUAMÁN O. et al., 2007
[16] SHILPI et al., 2006, pp. 267-268
[17] CONABIO, 2010
[18] SHILPI et al., idem
[19] ENCISO J. et al., 2010
[20] SHILPI et al., p. 268

Flavonoids share the chromane ring with tocopherols, and similarly to these latter show strong antioxidant activities.

The free radicals are molecules provided with a potent oxidant capacity. Normally, they occur during metabolism. Also, the immunological system of the body creates them to neutralize virus and bacteria. Other sources of free radicals and oxidative stress include environmental factors such as pollution, cigarette smoke and certain pesticides.[21]

Under normal conditions, our body is able to maintain a balance between the free radicals that are generated or that infiltrate from the outside, and the systems that neutralize them. When the antioxidant defense is not one hundred percent efficient, the formation of free radicals increases; this is called oxidative stress: the tissues become attacked, producing an accelerated aging.



The free radicals attack especially the cellular membranes causing it destruction. These membranes are the delicate support of the genetic map of the cells, which nucleus contains the DNA. The integrity of this membrane protects the DNA and the life of our cells.

Fig 3: Antioxidant action

The **polyphenolic** compounds, among them the **flavonoids,** are substances present in nature with capacity to trap free radicals and thus delay or prevent the oxidation of other molecules such as proteins, fats, carbohydrates and nucleic acids (DNA and RNA). The protection to the DNA of the oxidative damage is a way in which the risk of cancer may be reduced.

[21] BLAKE S., 2007.

The free radicals may mediate in some inflammatory processes; thus, the antioxidant capacity of the flavonoids of INKA ANATTO **apigenin and luteolin** is also associated to its anti-inflammatory capacity.[22]

SKIN AGING PREVENTION

The process of skin ageing has been divided into two categories: Intrinsic and extrinsic ageing. Intrinsic skin ageing or natural ageing is caused by changes in elasticity of the skin over time. Extrinsic skin ageing is predominately a result of exposure to solar radiation (photoageing). UV exposure causes physical changes to the skin due to alterations that occur in the connective tissue via the formation of lipid peroxides, cell contents and enzymes, and reactive oxygen species (ROS).

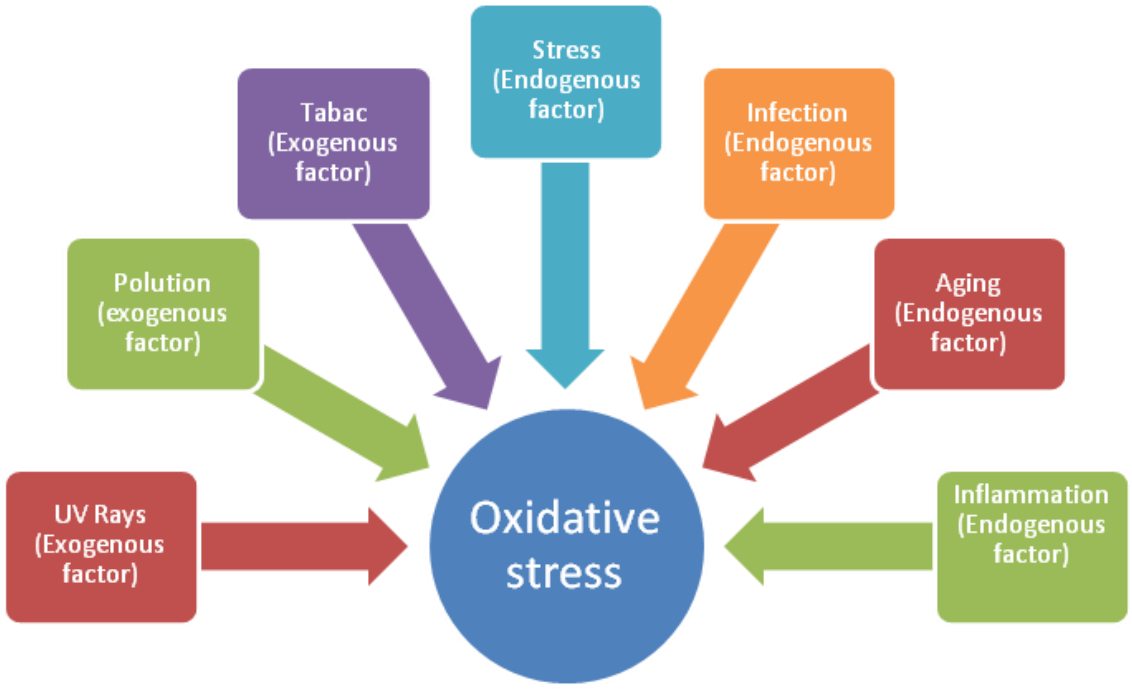


Fig 4: Skin aging process

Lipid peroxides can be metabolized to form secondary products which damage the extracellular matrix (ECM) while ROS are credited with involvement in the loss of skin elasticity and in diseases such as arthritis, diabetes and cancer. Biological systems need ROS for metabolic pathways and thus the body is capable of forming reactive

[22] K. RAJ NARAYANA, 2001.

species such as superoxide (O_2^-) and nitric oxide (NO). When ROS are overproduced, redox-active transition metal ions such as iron(II) or copper(II) can cause severe oxidative stress and thus damage tissues and the cellular DNA, protein, lipid and carbohydrate.

INKA ANATTO shows antioxidant properties to fight against the oxidative stress which leads to skin aging.

COLLAGEN PROTECTION

Eighty percent of skin dry weight is collagen which is responsible for the tensile strength of the skin. Elasticity is due to the elastin fiber network making up 2–4% of the ECM and glycoaminoglycans (GAG's) are involved in the hydration of the skin. Collagen fibres, elastin fibres and GAGs are produced by fibroblasts and are primarily affected by photoageing resulting in visible changes in the skin such as wrinkles, pigmentation and changes in thickness.

Efficacy Tests

EVALUATION OF ANTIOXIDANT ACTIVITY

Total Antioxidant Activity DPPH Assay

One of the most known methods of the evaluation of the antioxidant activity is the one that uses the 2,2-diphenyl-1-picrylhydrazyl (DPPH). This compound is a stable radical at room temperature and is reduced in presence of antioxidant compounds.

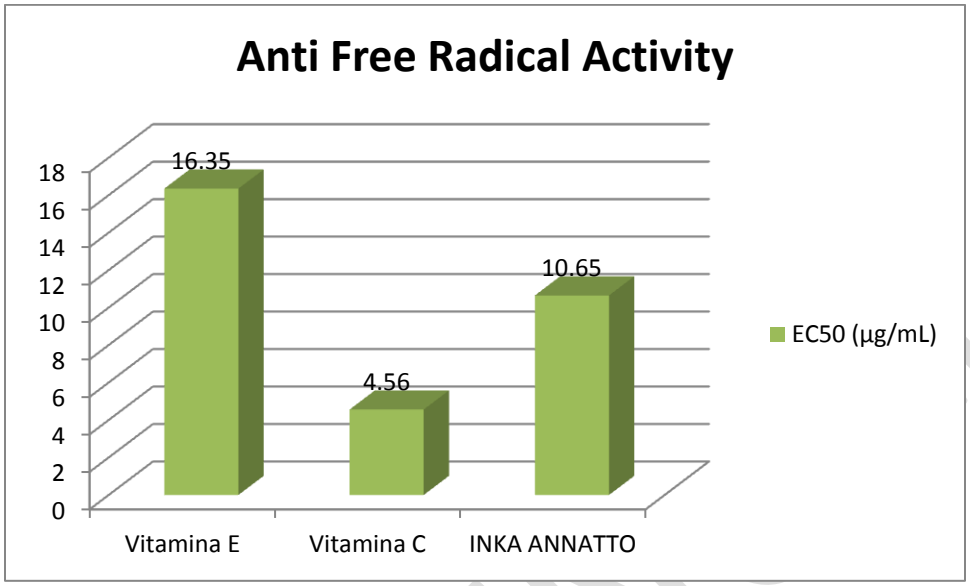


Fig 5: DPPH Test for INKA ANNATTO, Vit E and Vit C

INKA ANATTO has shown an important scavenging activity, with an EC50 of 10.65±0.48 µg/mL (n=3), superior than the one of Vitamin E (16.35±2.71µg/mL) and a little low than the one of Vitamin C (4.56±1.03 µg/mL).

TROLOX Assay

	TEAC average±
BHT	1.29 ± 0.04*
BHA	1.02 ± 0.04*
VITAMIN E	0.89 ± 0.01*
INKA ANATTO	0.52 ± 0.01

*[23]

The TEAC (Trolox equivalent antioxidant capacity) assay is based on scavenging of 2,2′ -azinobis-(3-ethylbenzothiazoline-6-sulfonate) radical anions ($ABTS^{\bullet-}$) In this method, an antioxidant was added to a solution pre-formed out of the $ABTS^{+\bullet}$ radical-cation and, within a fixed range of time, the $ABTS^{+\bullet}$ residual radical-cation was spectro-photometrically quantified.

Reducing Power

[23] Diego R. Merchán, et al. (2011)

The hydroxyl radical plays a significant role in the damage caused by the UV radiation and is more reactive towards the damage of the cellular constituents compared to the hydrogen superoxide and peroxide radicals.

The reducing potential of the INKA ANATTO was measured in its capacity to reduce the ion Fe^{3+} by the method of Hazra et al[24] and L-ascorbic acid was used as positive control. The results are showing in Figure 6.

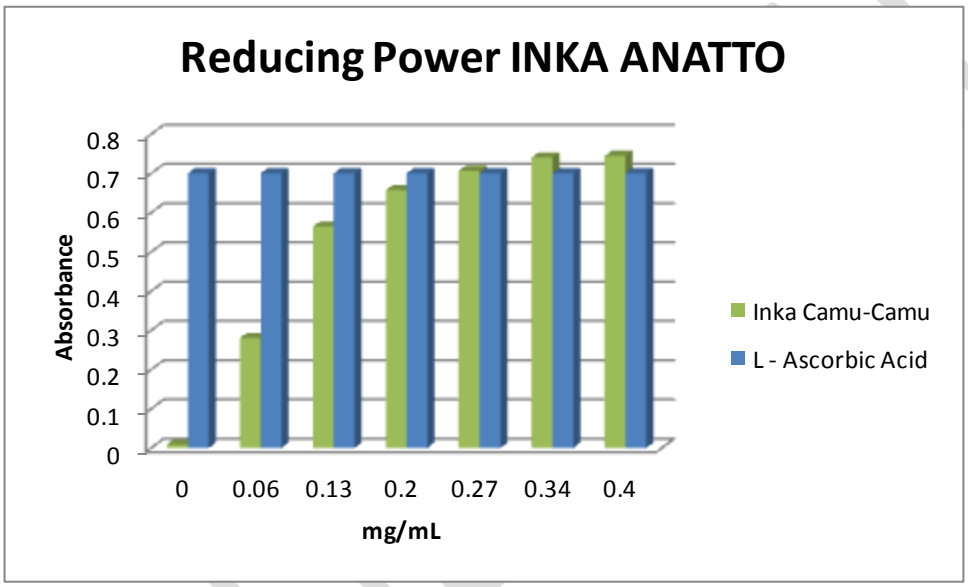


Fig 6: Reducing Power of INKA ANATTO

It is observed that from a concentration of 0.27 mg/mL, the reducing activity of the INKA ANATTO is equal to and even a little higher than the one of L-ascorbic acid.

ANTI - COLLAGENASE ACTIVITY

Collagenase from the bacteria*Clostridium histolyticum* (ChC) degrades Extracellular Matrix. This bacterial collagenase hydrolyses triple-helical collagen in both physiological conditions and *in vitro* conditions using synthetic peptides as substrates. In this study ChC was used to test INKA ANATTO for anti-collagenase activity.

[24] Hazra B. et al., (2008)

The assay employed was based on spectrophotometric methods according Thring et al[25]. Collagenase from *Clostridium histolyticum* was dissolved in buffer and the synthetic substrate *N*-[3-(2-furyl) acryloyl]-Leu-Gly-Pro-Ala (FALGPA) was dissolved in Tricine buffer to 2 mM. EGCG (epigallocatechin gallate), 250 µM (0.114 mg/mL) was used as a positive control.

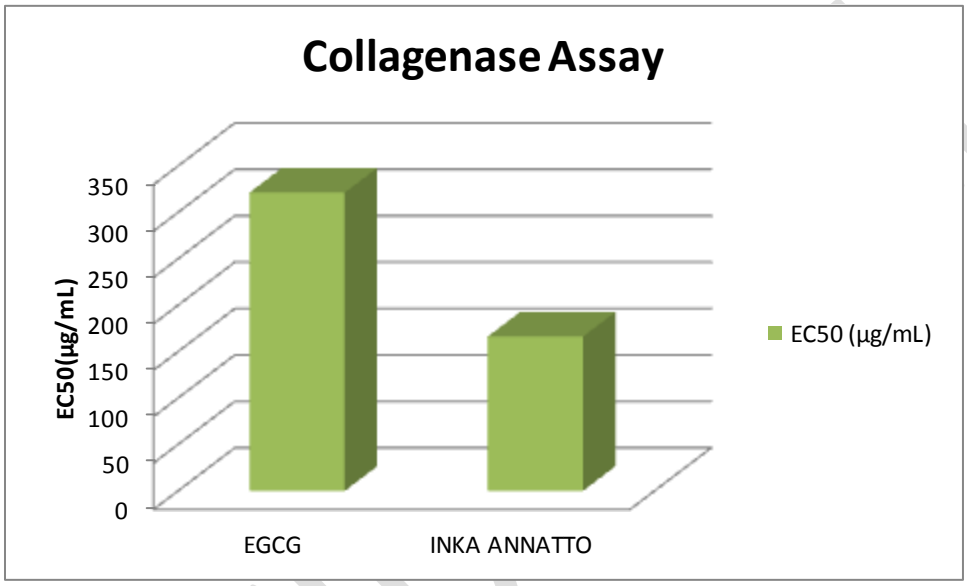


Fig 7: Collagenase Assay Inhibition

A very high anti-collagenase activity was exhibited by INKA ANNATTO with an IC50 of 166.23 ± 5.3, more over its activity was almost twice than the one of the positive control EGCG with an IC50 of 321.41 ± 10.65.

Ranks of the efficacies regarding collagen protection of some common plant extracts for comparison purpose are shown in fig. 8.

[25] Thring T et al.(2009)

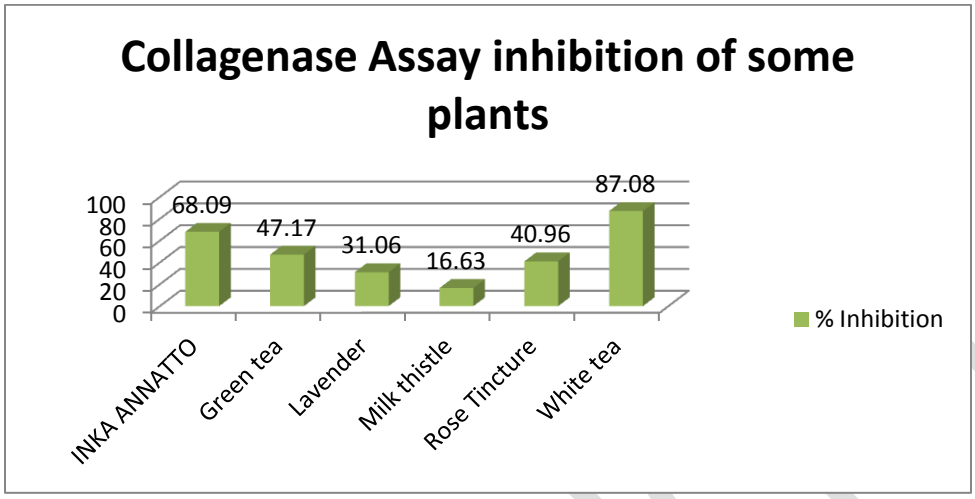
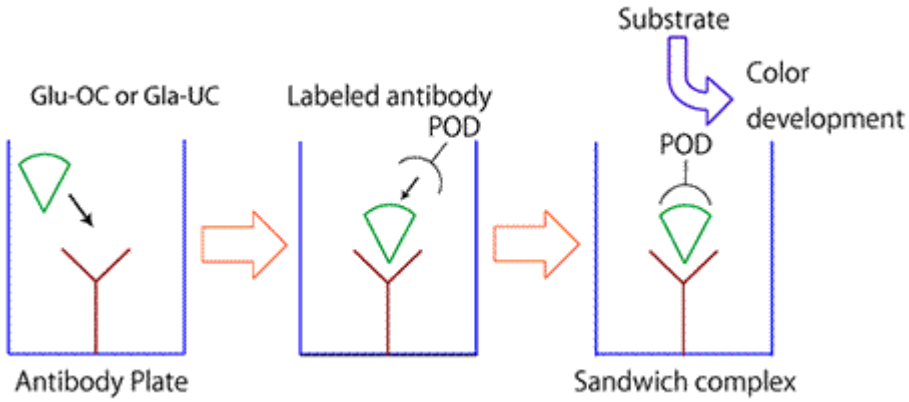


Fig 8. Collagenase Assay Inhibition of some plants

COLLAGEN TYPE I SYNTHESIS PROMOTION

To evaluate the amount of collagen type I synthesis that occurred upon exposure to the extract, collagen type I was quantitatively detected by using a procollagen type I C-peptide assay kit (Takara Bio, Japan).

Fibroblast cells were inoculated into 24-well plates ($5x10^5$ cells/well) and cultivated for 24 h. After culturing, the culture medium was changed to serum-free IMDM (Iscove's modified Dulbecco's medium) and cultivated for 24 h. The control group was cultivated without a compound. After culturing, the supernatant was collected from each well, and the amount of pro-collagen type I was measured with a pro-collagen type I C-peptide assay kit (Takara Bio, Japan).



As test result, **INKA ANATTO increased the expression of type I Collagen in 11.2% at concentration 25 µg/ml**

CONCLUSION

INKA ANATTO is remarkable innovative natural ingredient with Traditional Knowledge of use that can be employ to protect the skin against UV exposure and the Oxidative Stress produce as a result. INKA ANATTO could provide a good complementary action of the use of filters or anti-free radicals in sun care products.

It is indicated for:

- Skin Care. Products for sensitive skins
- Anti-aging treatments: Anti-free radical products. Protective products.
- Sun and after sun products.
- Capillary products.

Dose of use – Solubility - Preparation

DOSE OF USE: From 1 to 10%.

SOLUBILITY: Water-soluble.

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PREPARATION: INKA ANATTO is a product sensitive to light and humidity. Preferably, it will be incorporated into the preparations at the end of the manufacturing process and below 35°C.

Analytical Information (preliminary)

Aspect:	Homogeneous liquid
Odor:	Characteristic
Color:	Dark amber
Solubility in water:	Miscible
pH at 20°C:	5.0 – 7.0
Specific gravity, 20°C:	1.030 – 1.045

PRESERVATIVES: None

MICROBIOLOGY:

Total aerobic mesophilic count:	≤ 1000 ufc/ml
Total fungi and yeast count:	≤ 100 ufc/ml
Pathogens:	Absence

PRESERVATION: Store in airtight container, protected from light and humidity and at 15 – 25 °C.

If the original container is opened, it should be handled with special care in order to avoid a secondary microbiological contamination.

We provide our best knowledge about the subject; however, the formulator will have the responsibility to ensure the stability of the formulation by performing the necessary tests.

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- JP8231351 (A). For amelioration of skin condition such as dandruff, pimple and dermatitis.
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