

PI-100-06

INKA Yaguar



INKA YAGUAR is a Natural Ingredient, preservative free, derived from selected leaves and stems of the *Oenothera rosea* (chupasangre), a wild harvest native plant from the Peruvian Andes. Traditionally the plant is used by the natives as poultices to cure blows and bruises.

INCI Denomination: Propanediol (and) Water (and) Oenothera Rosea Extract

Plant description: ^{1, 2}



Family: Onagraceae

Botanical name: *Oenothera rosea* L'Hér. ex Aiton

Synonyms: *O. psychrophila*, *O. purpurea* L., *O. rubra* C., *O. virgata* R y P. *Hartmannia rosea* G. Don, *Hartmania virgata* Spach.

Other names: Chupasangre (Perú) sanjuan, yawar chchungu, yawar ch'onqa, sanguinaria, hierba del golpe (Mexico), shullo amarillo (Ecuador)

Description:

Annual or perennial plant, with erect or procumbent stems, which reaches 50 cm of height, covered by pilosity. The leaves, alternate, of 2 - 5 x 1 - 2 cm, are oblanceolate to tightly oval. The flowers gather in an inflorescence. The calyx is formed by 4 sepals of 0.5 - 0.8 mm, with a greenish color. The corolla has 4 purple petals of 0.5 - 1 cm. The fruit is a capsule of 1.5 - 2.5 cm. In its interior, there are several seeds of elliptical or roundish contour. It blossoms from April to October.³

Distribution:

This plant is endemic from the United States, Mexico, Costa Rica, Guatemala, Bolivia, Ecuador, Peru, Argentina and Uruguay. In Peru, it can be found in the Upper Amazon Rainforest and in the Highlands, between 1500 y 4000 meters above sea level. It can be found as a wild plant.

¹ BRACK EGG A. (1999), page 351

² MÁRQUEZ-FLORES et al. (2009), page 12

³ MENÉNDEZ J., (2010).

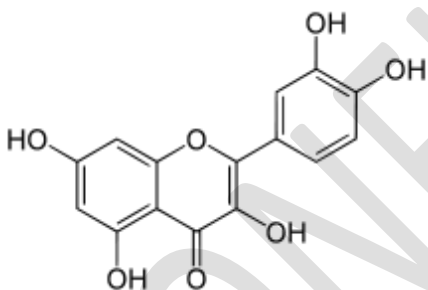
Traditional uses:

In Peru, the crushed leaves are used for wounds as well as in plasters against hits and bruises⁴, pneumonia and rheumatism; the root infusion is useful as anthelmintic and antitussive. Rubbing the skin with the alcoholic maceration of flowers and leaves soothes rheumatism.⁵

In the traditional Mexican medicine, it is used for the treatment of skin inflammatory, renal and bacterial ailments. The whole plant is used to cure internal inflammation or as poultice for topical treatment.^{6, 7} In Ecuador, the fresh leaves are used for liver and kidney discomforts.⁸



Phytochemicals:



Preliminary studies on hydroalcoholic extract of Yawar plant showed the presence of flavonoids as the major components, besides reducing sugars, tannins, coumarins and cardiotoxic glycosides. The glycosidic flavonoid called **quercetin** was identified in the most representative fraction.^{9 10}

Fig 1: Quercetin

Polyphenolic compounds, among them **flavonoids**, are substances that occur widely in the plant kingdom with very important roles for the survival of the plant. In the flowers, the colors of the flavonoids attract the pollinators. In the leaves, it is believed

⁴ CABIESES F., (2003).

⁵ BRACK EGG A., (1999), page 351

⁶ MÁRQUEZ-FLORES Y. et al., (2009), page 11

⁷ ANDRADE-CETTO A., (2009).

⁸ TENE V. et al., (2007)

⁹ MÁRQUEZ-FLORES et al., idem, page 13

¹⁰ GONZALES BELLIDO et al., (2000)

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that they protect them from pathogenic fungi and UV-B radiation; likewise, they are involved in energy transfer processes, such as photosynthesis.¹¹ Besides their function in plants, flavonoids are object of intense study due to their pharmacological activity.¹²

Bioactivity:

Research studies carried out on laboratory mice proved that aqueous and methanolic extracts of *Oenothera rosea* had an **anti-inflammatory** effect, without other toxic effects. This effect is attributed to the secondary metabolites detected in the extract, mainly flavonoids.^{13, 14}

COSMETIC BENEFIT:

ANTIOXIDANT

Free radicals are molecules provided with a potent oxidizing capacity. Free radicals generally occur during the 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.¹⁵

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 100% efficient, the formation of free radicals increases; this is called oxidative stress: tissues become attacked, producing an accelerated aging.

The skin in particular may decrease its capacity of self-repair, showing signs of deterioration such as lines, wrinkles, spots, and other signs of aging.

Flavonoids are potent antioxidants.¹⁶ Their capacity to quench free radicals allows them to delay or prevent the oxidation of other molecules such as proteins, fats, carbohydrates and nucleic acids (DNA and RNA). The fight against free radicals provides protection to the skin, but an additional benefit of the flavonoids (such as the

¹¹ LOCK O. et al., (2006).

¹² RAJ NARAYANA et al., (2001), pages 1-2.

¹³ MÁRQUEZ-FLORES Y. et al., idem, page 15

¹⁴ MECKES M. et al., (2004)

¹⁵ BLAKE, S. (2007).

¹⁶ RAJ NARAYANA et al., (2001), page 2

quercetin, a glycone of the quercetrin) is that they possess an effective inhibiting activity of the *matrix metalloproteinases* (MMP), enzymes that intervene in the degradation of the dermal collagen and which in states of imbalance (oxidative stress, inflammation, UV exposure) produce premature aging.¹⁷

SOOTHING

Free radicals may mediate in some inflammatory processes; thus, the antioxidant capacity of the flavonoids of Yawar, among them the quercetrin, is also associated to their anti-inflammatory activity.^{18 19} Besides, this compound shows antibacterial activity.²⁰

EFFICACY TESTS

TOTAL PHENOLIC CONTAIN

Phenolic compounds are commonly found in both edible and non edible plants, and they have been reported to have multiple biological effects, including antioxidant activity. The Total Phenolic contain was determined using Folin-Ciocalteu reagent as described by García *et al*²¹.

Raw materials	Total phenolics (mg of GAE/g dw)
Crowberry	50.8±1.0
Strawberry	17.7±0.2
Raspberry	23.9±0.2
Cranberry	21.2±0.7
Camomile	12.7±0.7
Thyme	17.1±0.2
INKA YAGUAR	15.9±0.4

Table 1. Total Phenolic contain in Plant Extracts²²

¹⁷ US 20080025930A1

¹⁸ US 20080025930A1

¹⁹ K. RAJ NARAYANA et al. (2001).

²⁰ VIJAYA K. (1996).

²¹ García Nava Manuel Alejandr, http://www.uaq.mx/investigacion/difusion/veranos/memorias-2007/56_IUAQGarciaNava.pdf (last access January 4th, 2011).

²² Antioxidant Activity of Plant Extracts Containing Phenolic Compounds. Marja P. Kähkönen, et. al. J. Agric. Food Chem. 1999, 47, 3954–3962.

Berries contained relatively high amounts of phenolics (12.4-50.8 mg/g GAE) (Tab 1). INKA YAGUAR contains comparative levels to some of them.

FREE RADICAL SCAVENGING EFFECT

The antioxidant potential of INKA YAGUAR was evaluated by using *in vitro* tests.

DDPH

Mensor *et al.*²³ method to measure antioxidant capacity of extracts *in vitro* involves the use of the free radical, 2,2-Diphenyl-1-picrylhydrazyl (DPPH). DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate their antioxidant activity. Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides.

In figure 2 you will see the result of DDPH analysis of INKA YAGUAR compared with Vitamin C and Vitamin E.

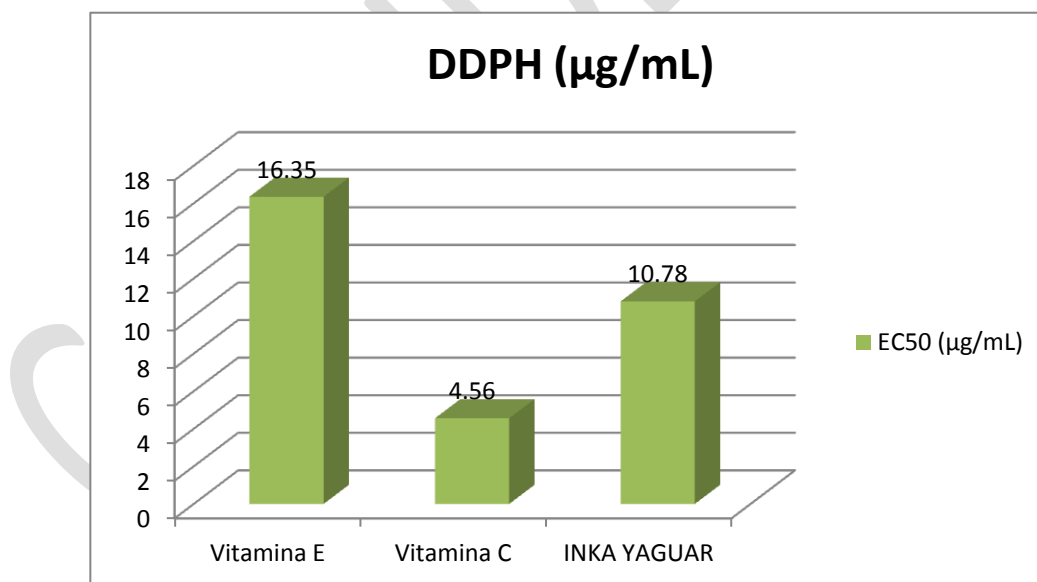


Fig 2. DDPH Assay of INKA YAGUAR

²³ Mensor L, *et al.* Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytoter. Res.* 2001,15:127-130

INKA YAGUAR shows an **important quenching activity** with an EC_{50} of 10.78 ± 0.05 $\mu\text{g/ml}$, between Vitamine C (EC_{50} 4.56 ± 1.03 $\mu\text{g/ml}$) and Vitamine E (EC_{50} 16.35 ± 2.71 $\mu\text{g/ml}$).

1. Reducing power determination

The reducing power of INKA YAGUAR was measured by its ability to reduce Fe^{3+} ion through Hazra et al method which used L-ascorbic acid as positive control.

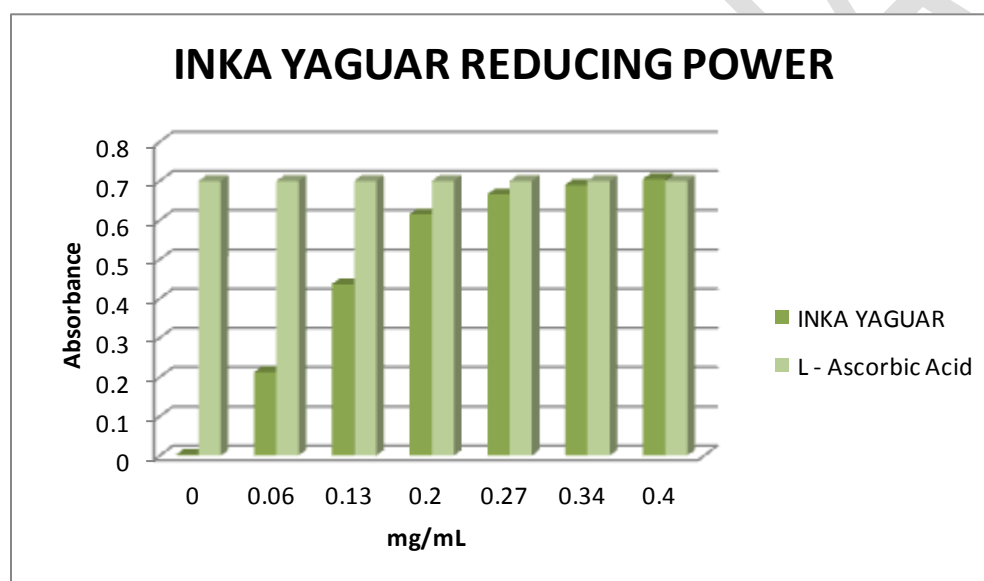


Fig 3. Reducing Power of INKA YAGUAR compared with L-Ascorbic Acid

INKA YAGUAR at concentrations of 0.27 – 0.4 mg/ml **shows a comparable ability of L-Ascorbic Acid to reduce Fe^{3+} .**

2. Collagen protecting effect

Studies have demonstrated that certain matrix metalloproteinases (MMP) are highly induced in photo aged skin, and they breakdown the dermal matrix proteins as collagen and elastin; this possibly leads to the skin damage and wrinkle formation. Therefore, the agents that inhibit collagenase activity may have beneficial effects for maintaining healthy skin.

We examined the inhibitory activities of INKA YAGUAR on collagenase from *Clostridium histolyticum* to establish its potential against skin inflammation and photoaging following the method described by Thring²⁴ *et al.* Epigallocatechin gallate (EGCG) was used as reference compound.

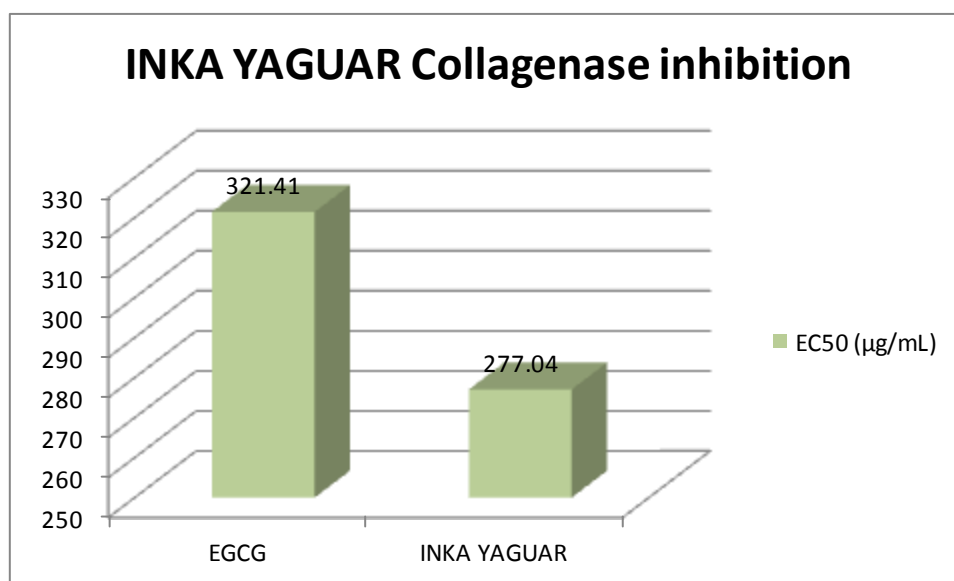


Fig 4: Collagen Protection of INKA YAGUAR

INKA YAGUAR shows a very important activity inhibiting collagenase, with an IC₅₀ of 194.89 ± 8.6 , even more has more activity compare with the control EGCG with an IC₅₀ of 321.41 ± 10.65 . Therefore we can assume that INKA YAGUAR **protects skin from collagenase induced damages.**

INHIBITORY EFFECT ON VIABILITY OF UV-B-EXPOSED SKIN CELLS.

To examine cell viability in UV-B-irradiated 3T3 human dermal fibroblasts for 24 h, the MTT reactive analysis was conducted, according Ji-Young Bae *et al.* method²⁵. When 3T3 fibroblast were pretreated with INKA YAGUAR at a concentration of 25 µg/ml, the product showed a protective effect up to 24.5%, against UV-B radiation.

²⁴ Tamsyn SA Thring *et al.* *BMC Complementary and Alternative Medicine* 2009,9:27

²⁵ Bae J-Y *et al.* *Exp. Dermatol.* 2010,19:e182-e190

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CONCLUSION

INKA YAGUAR is a Novel Natural Ingredient that can contribute to improve the appearance of prematurely aged skin. Its use in cosmetic products is a new way of protection against cellular oxidation. Inka Yaguar protects skin cells from oxidative damage that increases premature aging.

Dose of use – Solubility – Preparation

INKA YAGUAR is manufacture **without preservatives, without GMO organism** and without petroleum or animal derivatives.

It is indicated for:

- Anti-aging products
- Products for sensitive skin
- Sunscreens and after-sun products
- Products for personal care

DOSE OF USE: From 1 to 10%.

SOLUBILITY: Water-soluble.

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

Analytical Information

Aspect:	homogeneous liquid
Odor:	characteristic
Color:	Yellow to amber
Solubility in water:	miscible
pH at 20°C:	4.5 – 6.5
Specific gravity, 20°C:	1.020 – 1.045

PRESERVATIVES: None

MICROBIOLOGY:

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

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PRESERVATION: Store in airtight container, protected from light and humidity, 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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