Chamomile-Eco

BOTANY

Chamomila recutita (L.) Rauschert*; Matricaria chamomilla* L.; *Matricaria recutita* L. Original from Central Europe, this plant typically grows at the edges of lanes and uncultivated fields. It is an herbaceous annual plant with an erect, branched stem bearing few leaves. Leaves are green, very narrow laciniae with a flat upper surface. Solitary head inflorescences (capitula) each with a convex, hollow receptacle, top the stems. A fringe of female white ligule florets surrounds each capitulum while the centre is covered by yellow tubular florets. Capitula are the parts used to obtain compounds employed in cosmetics and other industries such as pharmaceutics and veterinary. They are collected 3-4 times a year and dried in a shadowy ventilated place or at 35ºC maximum temperature.

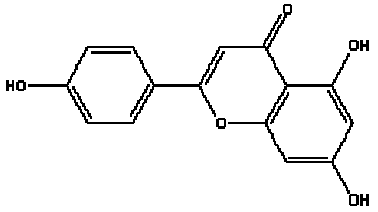
It used to grow abundantly in Greece and it was well known because of its particular perfume since the times of the Ancient Age. Noticeably, the empirical data reported by Dioscorides nineteen centuries ago have been confirmed by modern laboratory studies.

CHEMISTRY

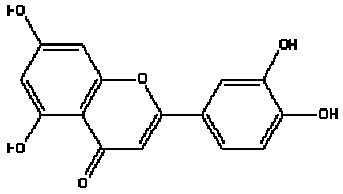
Flavonoids, coumarins, essential oils and **polysaccharides** are characteristic compounds in the chemical composition of Chamomile.

Flavonoids

The involved flavonoids (0.5-0.3%) belong to the type, in which the genins are flavones (apigenin, luteolin) and flavonols (quercetin, isorhamnetin, myricytin, patuletin). Remarkably apigenin-7-glucoside, a flavonoid present at a concentration of 0.45%.



Apigenin



Luteolin

Structure	Genins
FLAVONES	Apigenin, Luteolin
FLAVONOLS	Quercetin, Isorhamnetin, Myricytin, Patuletin

Coumarins
The involved coumarins (0.1%) derivate from the cinnamic acid. Noticeable compounds of Chamomile are Umbelliferone (7-hydroxycoumarin) and its methyl ester Herniarin.

Essential oil
Essential oil contents vary between 0.2 and 1.8 %. The blue colour of the freshly distilled essence is due to the high chamazulene percentage (1-15%). This later compound derivates from the colourless hydro soluble proazulene called matricine. During the essence distillation process, the chamazulene carbonic-acid (also called guaiazulene acid) is formed, subsequently decarboxylating to yield chamazulene.

Other components in the essential oil are (-)-α-bisabolol (10-15%), the bisabolol oxides A and B (10-25%), a cyclic ether (1-10%) and different hydrocarbons. Bisabolol and its derivatives are up to the 50% of the total essential oil.

Polysaccharides
These **polysaccharides** (10%) belong to the group of pectins with their main chain being α 1-4 bond to polygalacturonic acid and lateral chains being β 1-4 bond to xylose.

TRADITIONAL USES

Chamomile and Chamomile extracts are intended for skin, membranes and mucosa affections, eye, ear or throat inflammation and for runny nose. It is also used in erythema and as a skin and mucosa healer.

In the cosmetics, it is added to products for hair lightening, sun protection gels, products for sensitive, fatigued or irritable skin, anti-congestion tonics and as an essence for perfumes and soaps.

COSMETIC PROPERTIES

Cosmetic applications of Chamomile are based on the specific actions of its active compounds:

- **Flavonoids**: anti-inflammatory, vasodilator, sun protector and anti free radicals.
- **Essential oils**: anti-inflammatory, antiseptic, protector against ulcer and sun produced irritations.

Several studies have been performed to assess the **anti-inflammatory activity** of Chamomile extract. Carle K, et al. have collected those studies that evaluated the anti-inflammatory activity on the croton oil induced dermatitis of the rabbit ear. Indomethacin, hydrocortisone, phenylbutazone and acetylsalicylic acid were used as reference substances for these studies. The results showed that Chamomile exerts a potent anti-inflammatory action, the action of the flavonoid-containing Chamomile fraction being stronger. This activity was also evaluated for the genins of flavonoids isolated from Chamomile flowers. Apigenin and luteolin were found to be more active than indomethacin and phenylbutazone. The anti-inflammatory activity decreased in the following order:

apigenin > luteolin > quercetin > myricytin > apigenin-7-glucoside > rutin

Evaluating oedema formation and the degree of granulocyte infiltration assessed the anti-inflammatory effect of two genins derived from Chamomile, apigenin and luteolin. 18 hours lasting anti-inflammatory action and inhibition of the leukocyte infiltration were observed for these active compounds (Carle K, et al.).

The anti-inflammatory action seems to be due to:

- Inhibition of the histamine release.
- Blockade of the Ca^{2+} entry to the mast cells and basophiles and/or stabilization of the mast cell membranes.
- Anti free-radical action of the flavonoids and inhibition of the super oxide radical synthesis.

Flavonoids obtained from Chamomile with the genins apigenin, luteolin, patuletin and quercetin, show remarkable **vasodilator action** since they directly relax the blood vessels walls thus augmenting the blood flow to the skin.

Achterrath U, et al. assessed this later action using papaverine as a reference substance and found that the action was strong mainly due to apigenin. The activity decreases in the following order apigenin, quercetin, patuletin and luteolin.

Flavonoids have also the ability to **capture free radicals**, acting as inhibitors of the non-enzymatic lipid per oxidation and protecting the antioxidant defences of the organism (Rodríguez ML.).

Another important field of application of Chamomile extracts is that of **sun products**. According to Ramos MFS, et al., the characteristics that vegetal extracts must fulfil in order to be added to cosmetic formulations are: first, they have to absorb radiation between 290-320 nm with absorption maxima between 260-300 nm; second, they have to be stable under normal conditions of use of cosmetic products, in the presence of the other compounds in the formulation; finally, they should have no kind of toxicity.

Chamomile and its water-soluble extracts fulfil these characteristics and may be added to solar protection products since they improve the effects of physical and/or chemical solar filters (Malpede A.).

Chamomile essential oils have been used since the ancient times and exert two main functions, **anti-inflammatory and anti-ulcer** activity mainly due to the presence of chamazulene and α-bisabolol.

Jakovlev V. et al. evaluated this later activity on the carragenin-induced oedema. It was found that α-bisabolol obtained from Chamomile exerted stronger effect than that exerted by the synthetic active compound.

In the same study, the bisabolol oxides A and B were also observed to contribute to the anti-inflammatory activity.

Szelenyi I. et al. evaluated the anti-ulcer action of bisabolol on different experimental models. The ulcer process was inhibited and the tissue healing time was reduced for all of them. Issac O. Also observed a reduction of the healing time of burn tissue.

It has been suggested that the action mechanism is the local stimulation of prostaglandin synthesis, the protective action against mucosa ulceration and stimulation of renewed epithelial growth.

Vasodilator action of Chamomile essential oils has also been studied by Achterrath U. et al., who demonstrated this effect mainly due to bisabolol and to the bisabolol oxides to a lesser degree.

Finally, (-)-α-bisabolol has a remarkable **anti-bacteria** activity (Patri G. et al.).

Active compounds	Action
FLAVONOIDS	Anti-inflammatory, Vasodilator, Free-radical scavenger Sun protection
ESSENTIAL OILS	Anti-inflammatory, Anti-ulcer, Vasodilator, Anti-bacteria

EFFICACY TEST

Enzymatic inhibition

In cosmetics, the control of inflammatory processes is very important in order to influence on irritation and aging processes.
One method used to evaluate the efficacy of anti-inflammatory and anti-aging products is to determine their ability to inhibit the cyclooxygenase.
Cyclooxygenase is an enzyme present in most tissues. It catalyses the synthesis of inflammation agents such as prostaglandin, prostacyclin and tromboxane, from the arachidonic acid.
Inhibiting this enzyme reduces the synthesis of these agents thus impairing the emergence of an inflammation process.
An assay to evaluate the inhibition of the cyclooxygenase produced by **CHAMOMILE-ECO** has been carried out.

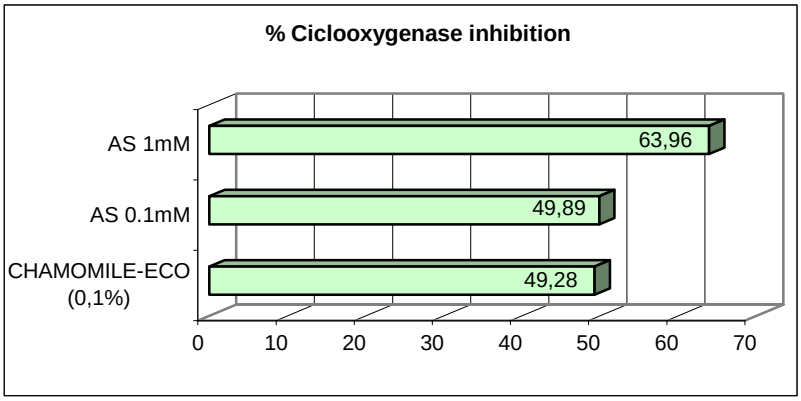
1. Experimental method

The study was carried out on primary cultures of human keratinocytes. After activating the cyclooxygenase with $CaCl_2$, the cultures were 24 hr incubated with the relevant substances in a concentration of 0.1%.

After incubation the prostaglandin (PGE2) contents were determined with an immunoassay. The keratinocytes culture medium was used as the negative control and acetylsalicylic acid (AS) was used as the positive control in concentrations of 1mM and 0.1mM respectively.

2. Results

The following graphic shows the values recorded for this assay.



It can be observed that **CHAMOMILE-ECO** exerts a 49,28% cyclooxygenase (COX) inhibition action in cultures of human keratinocytes.

COSMETIC APPLICATIONS

- SKIN CARE: tonic and decongestant products for sensitive and irritated skin.
- BODY CARE: treatments for tired legs and sensitive skin.
- Sun protector products and after-sun soothing products.
- HAIR CARE: repairing products for blond hair and treatments for scalp problems (rashes, itching).

ANALYSIS

1. Qualitative determination of flavonoids

- Reference solution
  0.05% solution of Rutin, Hyperoside, Chlorogenic acid and Ferulic acid in methanol. Apply 10 μL of this mixture.

- Sample preparation

  Weight 4g extract and dry by means of a rotavapor. Dissolve the extract again using 4 ml methanol and evaporate until 2 ml are left. If the resulting solution is not completely transparent, filter before applying on the plate. Apply 10 μL.
- Absorbent medium

  Silica gel 60 $F_{(254)}$ plate. Place the plate at 100ºC for 20 minutes before using. The plate length is 15 cm and the necessary time is 1 and a half hours approximately.
- Mobile phase

  Ethyl acetate / Formic acid / Glacial acetic acid / Water (100:11:11:26). The mobile phase can be used to develop 3 or 4 plates.

- Detection

  Spray on the plate with 4 or 5 ml of a 1% methanol solution of diphenylboroxiethylamine. Subsequently spray again with 4 or 5 ml of a 5% methanol solution of polyethylene glycol 4000. Place the plate at 100ºC for 2 minutes and evaluate the results under UV (365 nm).

  The zone corresponding to Rutin looks orange with Rf 0.4. The zone corresponding to Chlorogenic acid looks greenish blue with Rf 0.45, Hyperoside appears orange with Rf 0.55 and Ferulic acid appears blue with Rf 0.9.

2. Quantitative determination of apigenin-7-glucoside

Quantitative determination of Apigenin-7-glucoside by high performance liquid chromatography.

The chromatographic conditions used here were as follows:

- Apparatus: Hewlet-Packard 1050 with automatic injector and DAD (Diode Array detector).
- Column: Kromasyl C-18 5µm 250x4.6 mm.
- Cartridge: C18 solid phase extraction.
- Flow: 1.0 ml/min.
- Detection: 216, 268 y 334 nm.
- Injection volume: 10 and 20 µl.
- Column temperature: 40ºC
- Mobile phase: Water / Acetonitrile according to the gradient shown in the following table.

Time	H_2O	Acetonitrile
0	80	20
5	80	20
20	20	80
25	80	20

Quantification of the Apigenin-7-Glucoside in the sample is obtained by interpolation with a calibration curve, or else by comparing areas with the already known concentration of a standard (Apigenin-7-glucoside. Extrasynthèse). It must be taken into account that the recovery of Apigenin-7-Glucoside from the sample passing through the cartridge is 84%.

RECOMMENDED DOSE

The recommended dose is between 0.1 – 2.0%.

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