Hibiscus-Eco

BOTANY

Hibiscus sabdariffa L. Different vernacular names for hibiscus are roselle, red sorrel, Jamaica sorrel, Indian sorrel and Guinea sorrel. It belongs to the family of Malvaceae. Herbaceous, annual or bi-annual plants, which grow up to 5 meters tall. The stem is robust; the leaves are dark green, oval, alternate, simple or divided into 3 lobes; the flowers grow solitary and axillary and are usually red. Hibiscus seems to be original from Central America and introduced into different tropical regions. This plant is currently cultivated in every sub-tropical region, especially in Sudan, Egypt, Thailand, Mexico and China.

The used parts of hibiscus are the flowers (calyces and capitula) collected at the beginning of the fruit season.

CHEMISTRY

The main components of hibiscus are anthocyanins, organic acids and carbohydrates.

Anthocyanins
These compounds are directly related to flavonoids, which can be found as free aglycons (anthocyanidins) or, more often, as glucosides (anthocyanosides).

They are coloured compounds, their colour varying with the pH. Anthocyanins (delphinidin, cyanidin) may reach up to 2% concentration.
Organic acids
Hibiscus contains a high organic acids percentage (15-30%), oxalic acid, tartaric acid, malic acid, succinic acid and hibiscic acid among them. Oxalic and tartaric acids constitute more than three quarters of the total acids present in hibiscus.

Carbohydrates
The plant as a whole is rich in carbohydrates (9.2-76.5%). However, the carbohydrate percentage reduces to about 3.3% in the flowers, where glucose is the most abundant sugar, followed by fructose and sucrose.

Hibiscus also contains mucilage and pectin, acid and neutral heterogeneous polysaccharides characteristic of plants, located in specialized cells (mucilage cells).

<table>
<thead>
<tr>
<th>Structure</th>
<th>Components</th>
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</thead>
<tbody>
<tr>
<td>ANTHOCYANS</td>
<td>delphinidin, cyanidin</td>
</tr>
<tr>
<td>ORGANIC ACIDS</td>
<td>oxalic, tartaric, malic, succinic, hibiscic</td>
</tr>
<tr>
<td>CARBOHYDRATES</td>
<td>Simple sugars, oligosaccharides, mucilages</td>
</tr>
</tbody>
</table>

TRADITIONAL USES
Hibiscus is the source of slightly acid, caffeine-free beverages (karkade). In the countries of origin, the flowers have been, and still are, widely used in the folk medicine as an antiseptic, aphrodisiac, astringent, cholangue, demulcent, digestive, diuretic, emollient, purgative, cooler, sedative, and tonic, among many other applications. Hibiscus and its derived extracts have an important inhibitory activity on the growth of fungus and yeasts. Additionally, they have a remarkable inhibitory activity on enzymes such as the acid phosphatase, the alkaline phosphatase, the glutamate-oxalacetate transaminase and the glutamate-pyruvate transaminase. Hibiscus flowers are currently included in different laxative infusion mixtures because of the osmotic effects of their organic acid contents, which make them hardly absorbable. It is also used to adjust the colour and flavour of different phytotherapeutic preparations.

COSMETIC PROPERTIES
Jonadet et al. 1990 studied the in vitro inhibitory activity on the enzymes ACE, elastase, trypsin and chymotrypsin, and the in vivo blood vessel-protective activity of two enriched fractions of hibiscus extract (anthocyans enriched fraction and flavones enriched fraction).

The flavones fraction was found to inhibit ACE. Both fractions inhibited elastase, as well as trypsin and chymotrypsin, to a lesser degree.

It was already known that the aqueous-extract of hibiscus had a relaxing effect on the smooth muscle fibbers and consequently exerted anti-hypertensive actions. However the underlying action mechanism had not been completely characterized.

Owolabi et al. 1995, demonstrated that these relaxing effects resulted from the inhibition of the calcium influx through adrenergic receptors by specific blockade of the agonist receptors. Additionally, a simultaneous inhibition of calcium mobilization in the intra-cellular deposits occurred.

Tseng et al., 1997 evaluated the antioxidant action of hibiscus on primary cultures of rat hepatocytes using tert-butylhydroperoxide (t-BHP). This later substance induces oxidative stress thus allowing insight into the action mechanism of antioxidants. The metabolism of t-BHP may follow two different pathways, through cytochrome P-450 (hepatocyte) or through hemoglobin (erythrocytes), and generates free radicals thus starting the oxidative process, which affects cell integrity and DNA.
Initiation of an oxidative process may be prevented by using substances that capture free radicals or substances that inhibit the enzyme xanthine oxidase.

In the present assay, the chloroform-soluble fraction (flavonoids) and the ethyl acetate-soluble fraction (phenolic compounds) of hibiscus were found to have great antioxidant activity. The chloroform-soluble fraction inhibited xanthine oxidase while the ethyl acetate-soluble fraction neutralized the free radicals generated during the assay. Both fractions inhibited LDH release (cytotoxicity) MDA formation (oxidation) and DNA damage.

Duhn et al. 1997 evaluated the antioxidant activity of three aqueous extracts, one of them of hibiscus. They evaluated the extracts’ lipid per oxidation inhibitory activity on linoleic acid, as well as on a liposome model, by following the thiobarbituric acid method.

The results showed that all of the three extracts had strong antioxidant activity and that such an activity was closely related to the polyphenolic substances contents in the evaluated extract.

Finally, Tee et al. 2002 assessed the antioxidant properties of hibiscus by comparing its activity with those of BHA and β-carotene. The results showed that the hibiscus extract had stronger antioxidant activity than BHA and β-carotene. In that study, the generation of conjugated diene compounds and of malonic dialdehyde were evaluated by using the thiobarbituric acid method.

The results showed that 200 ppm of a methanol extract of hibiscus were able to inhibit more than 85% of diene-conjugated compounds generation after 7 days incubation at 40° C. This activity was closely related to the extract’s contents of phenolic compounds, mainly the anthocyanins.

Müller et al. 1992 studied the chemical structure of the polysaccharides present in hibiscus in order to characterize their physiologic activity. Three fractions were identified; the two minor fractions consisted of arabinoses and arabinogalactans. The major fraction contained a structure similar to pectins, with lateral chains of galactose and arabinose. Additionally, the later fraction also contained large amounts of uronic acids and a minor proportion of neutral sugars.

This later fraction was demonstrated to participate in the immunostimulant activity of hibiscus extract.

**EFFICACY TEST**

**Enzymatic inhibition**

Elastase is an enzyme abundantly located in skin. Its main function is to catalyse elastin degradation. This enzyme is related to several pathologies involving large tissue destruction (e.g. psoriasis) and to inflammatory processes.

Elastin is a scleroprotein in the connective tissue. As illustrated by its name, it is the main responsible for skin elasticity. During inflammatory and aging processes, elastin is fragmented into soluble peptides and the dermis contents of this protein are reduced. The inhibition of elastase would therefore help maintaining skin protein levels and elasticity.

An assay to determine elastase inhibition by HIBISCUS-ECO has been carried out.

Elastase activity was assessed spectrophotometrically by measuring the amount of the peptide 4-nitroanilide generated after the action of a pancreatic elastase on the substrate.

1. **Experimental method**

   The substrate (N-Succinyl-Ala-Ala-Ala-p-Nitroanilide Sigma S-4760, lot 107H5003) degradation reaction starts when it is incubated together with the enzyme (Pancreatic Porcine Elastase, Roche, ref. 1027891, lot 85460424) and the substances to be tested in a bath at 37º C.

   The reaction has to be monitored for 60 minutes reading the absorbance at 410 nm every 10 seconds. The resulting values indicate the amount of free 4-nitroanilide.
Buffer Tris-HCl 0.2M pH=8.0 was used as the negative control. Elastatinal at a final concentration of 20 ng/ml in the assay medium was used as the positive control. The final concentration of HIBISCUS-ECO in the assay medium was 4%.

Maximal absorbance values were observed for those tubes corresponding to the negative control. Inhibition of the enzyme elastase generates smaller amounts of 4-nitroanilide and consequently, smaller absorbance values.

2. Results
The following graphic shows the values recorded for this assay.

It can be observed that HIBISCUS-ECO had an important inhibitory activity on elastase. An 81.57% inhibition was recorded.

In agreement with the present results, it can be concluded that HIBISCUS-ECO largely inhibits elastin degradation, which results in beneficial effects on skin such as the maintenance of elasticity and its consequent anti-aging effects, and a clear recovery of inflammatory processes involving this enzyme.

COSMETIC APPLICATIONS

- SKIN CARE: vitalising and rejuvenating treatments, special products to maintain skin tone and elasticity, anti-acne preparations, after-shave products
- BODY CARE: refirming and anti-cellulite products.
- HAIR CARE: conditioners and colour enhancing products.

RECOMMENDED DOSE

The recommended dose is between 0.1– 4.0%.
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