In-depth study of a *Hibiscus sabdariffa* extract for Cosmetic applications

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Introduction

Hibiscus sabdariffa is a medical plant which belongs to the family of Malvacea [1]. *Hibiscus sabdariffa* is traditionally cultivated for its stem, leaves, calyces and seeds as all parts of this plant have medicinal applications [2]. The most interesting constituents of a therapeutic importance in this plant are the polysaccharides, organic acids and flavonoids in particular the anthocyanins [3]. More specifically, the water extracts of dried calyces are known to contain organic acids, polyphenols and anthocyanins [4] and possess free radical scavenging activities [5].

In the framework of the development of plant extracts for cosmetic applications, an aqueous extract of the calyces of *Hibiscus sabdariffa* was studied in-depth. First the amount of phenols, flavonoids and saponins were determined. Then the identification of some key flavonoids was carried out in this extract. Finally, this extract was tested in different *in vitro* assays to identify its potential for skin applications.

Materials & Methods

Chemical composition : Quantification of the different groups of natural products in the *Hibiscus sabdariffa* extract

The analysis of the *Hibiscus sabdariffa* extract was performed on a Thermo Scientific™ UltiMate™ 3000 UHPLC module (Chromeleon 7.2 software) connected to a photodiode array detector (PDA) and a charged aerosol detector (CAD) using a Purospher® Star RP-18e Hibar RT column (100 mm x 3.0mm id; Merck KGaA, Darmstadt).

Determination of the total content of phenols, flavonoids and saponins was performed with an Agilent Cary 60 UV-Vis spectrophotometer. HPLC-MS analyses were performed with a Thermo Scientific™ ISQ™ Single Quadrupole mass spectrometer coupled with a HESI source. Quantification of phenolic content was performed following the method of Singleton et *al.* [5]. Quantification of saponin content was performed following the method of Oludemi et *al.* [6]. Quantification of flavonoid content was performed following the method of Chang et *al.* [7]

Biological activities of the *Hibiscus sabdariffa* extract

All experiments related to the biological activity were evaluated on a Spark® 20M microplate reader (Tecan Group Ltd.).

- MMP activity assay

Inhibition of ten Matrix-Metalloproteinase (MMP) enzymes were quantified using a quenched fluorogenic peptide OmniMMP™ RED (EnzoLifeScience Inc., USA).

- Mitochondrial membrane potential assay

The mitochondrial membrane potential of adherent cells (HaCat) was quantified with the MITO-ID® Membrane Potential Kit (Enzo Life Sciences Inc., USA).

- Enzymatic COX-2 inhibition

Inhibition of enzymatic COX-2 was quantified using the fluorometric COX-2 inhibitor screening kit (BioVision, USA).

- TRPV-1 assay

This assay way perfomed with HEK - TRPV-1 cells (SB drug discovery, UK) stably transfected to over express the TRPV1 receptor. The FLIPR Calcium 5 Assay (Molecular devices, USA) was used, as per the manufacturer's instructions to assay TRPV1 antagonist activity.

Results & Discussion

Chemical composition of the extract

The chemical analysis of this extract showed that it contained 4.6 % (w/w) of phenolic compounds, 0.46% (w/w) of flavonoids and 3.5 % (w/w) of saponins (see Figure 1). The analyzed extract contained maltodextrin (40% in total) which had been added to help maintain a free-flowing powder characteristic.

A more thorough analysis of the Hibiscus sabdariffa water extract led to the unambiguous identification of 2 typical substances of *hibiscus sabdariffa*: chlorogenic acid and quercetin 3-O-sambubioside.

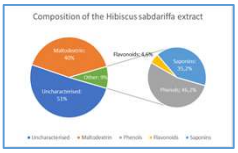

Figure 1: Composition of the Hibiscus sabdariffa extract

Biological evaluation of the *Hibiscus sabdariffa* extract

The aqueous extract of *hibiscus sabdariffa* was tested at 0.1% in different *in vitro* assays. First it was shown to inhibit *in vitro* the gelatinases MMP-2 by 79% and MMP-9 by 85%, the collagenase MMP-8 by 78%, MMP-13 by 51% and MMP-14 by 82% and the elastase MMP-12 by 68% (see Table 2). The MMP-2 and MMP-9 inhibition could be related to the presence of quercetin 3-O-sambubioside [8].

MMP subgroup	MMP type	% Inhibition	Effect
Collagenases	MMP-8 (Neutrophil collagenase)	78%	Protection of collagen type I
	MMP-13 (Collagenase-3)	51%	
	MMP-14 (MT1- MMP)	82%	
Gelatinases	MMP-2 (Gelatinase A)	79%	Protection of collagen type IV
	MMP-9 (Gelatinase B)	85%	
Elastase	MMP-12 (Metalloelastase)	68%	Protection of elastin

Table 2: Effect of an extract of *Hibiscus sabdariffa* (0.1%) on MMP activity

Mitochondrial MP activation vs. untreated control	%
Hibiscus Sabdariffa (0.1%)	51.58 %
NAM (0.061%) - Nicotinamide, positive control	14.75 %
CCCP (4µM) - Carbonyl cyanide *m*-chlorphenyl hydrazone, negative control	-38.14 %

Table 3: Effect of an extract of *Hibiscus sabdariffa* (0.1%) on the mitochondrial membrane potential

The aqueous extract of *hibiscus sabdariffa* improved the mitochondrial membrane potential by 52% (see Table 3).

The *Hibiscus sabdariffa* extract (0.1%) inhibited the cyclooxygenase COX-2 by 93% (see Table 4). This biological activity could be related to the presence of chlorogenic acid whose COX-2 inhibitory activity had been previously reported [9].

COX-2 inhibition vs. untreated	%
Hibiscus sabdariffa (0.1%)	93.33 %
Celecoxib (0.0017%) - COX-2 inhibitor	52.00 %

Table 4: Effect of an extract of *Hibiscus sabdariffa* (0.1%) on the COX-2 activity

Inhibition of TRPV1 activity vs. 0.6 µM Capsaicin	%
Hibiscus sabdariffa (1%)	25.38 %
Hibiscus sabdariffa (0.1%)	16.00 %
Hibiscus sabdariffa (0.01%)	9.04 %
Capsazepine (1µM) - positive control	94.02 %

Table 5: Effect of an extract of *Hibiscus sabdariffa* (0.01%, 0.1% and 1%) on the TRPV1 activity

Finally, our *Hibiscus sabdariffa* extract (0.1%) showed a dose-dependent inhibition of the TRPV1 receptor activation (see Table 5).

Conclusion

The aqueous extract of *Hibiscus sabdariffa* was found to possess a broad array of biological activities in the skin: it showed anti-aging activities as it protects the extracellular matrix, therefore preserving the youthful skin structure. It improved the mitochondrial membrane potential, maintaining the efficiency of the skin metabolism. An anti-inflammatory activity (COX-2) was demonstrated supporting the soothing of the skin. Finally, it inhibited the TRPV1 receptor activation, supporting the care of sensitive skin.

All these reported biological activities make the aqueous extract of the calyces of *Hibiscus sabdariffa* ideal for skin care applications.

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