

ANTI POLLUTION BENEFIT FROM BAICALINE

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ABSTRACT

The impact of pollution on skin quality is becoming a very important subject; especially in big cities where industrial activities bring the most part of known pollutants. Baicalin is a TCM polyphenol extracted from the genus *Scutellaria* widely known in Asia traditionally used for its detoxifying properties. We have evaluated the anti-oxidant properties of Baicalin towards a set of 4 different reactive oxygen species (ROO[•], HO[•], ¹O₂ and O₂^{-•}) and shown it has a very wide efficacy. Cigarette smoke in conjunction with UVA exposure was then used as a surrogate for urban atmospheric pollution. In a *in tubo* and *ex-vivo* protocols, Baicalin showed very good ability to protect sebum from this pollution mimic with a significant protection from 0.5% in formula. Overall, this study shows that Baicalin is efficient in protecting the outermost protection of the skin.

INTRODUCTION

Baicalin is a flavone widely used in the Traditional Chinese Medicine (TCM) as a liver - detoxifying agent. A growing set of studies conducted *in vitro* or on animal models, allowed to show Baicalin has strong anti-oxidant properties, alone or in mixture with other anti-oxidants such as Resveratrol, Vitamin C or Vitamin E [1-7]. Other works, using *in vitro* or animal models identified other biological effects of Baicalin: modulation of mitochondrial activity [8], tumor growth inhibition, mitigation myocardial infarction severity and suppression of induced contact dermatitis [9-11]. Atmospheric pollution has become a major concern, in many countries. Among pollutants are volatile elements such as NO₂, SO₂, O₃, CO, CO₂ and polycyclic aromatic hydrocarbons (usually expressed in ppb) and particulate matters (PM) in suspension (usually expressed in µg/m³). Air pollution is recognized to be a threat to human health being responsible for numerous premature deaths due to respiratory and cardiovascular diseases. Its impact on skin health is becoming a concern for many citizens of polluted cities in the world. It is known that, when combined with UV exposure, air pollution causes oxidative stress on the skin surface that may in turn impact living compartments of the skin.

MATERIALS AND METHODS

Baicalin (MMP Inc, Plainfield, USA).

Baicalin has been introduced at different concentration (0.2% ; 0.5%; 1%) in the aqueous phase, of a direct emulsion.

To succeed on it, first in water, preservatives, glycols, gelifiers and surfactants (glyceryl stearate, stearate) were dissolved, then emulsified with the oil phase. In the other second step, a second water phase was prepared containing baicalin at the desired level. Then added to the emulsion prepared. At the end, pH of emulsion has been adjusted to pH = 5.5.

The activity of Baicalin towards different forms of ROS: ROO[•] [12], ¹O₂ [13], HO[•] [14], O₂^{-•} [15] was evaluated though *in tubo* tests as described elsewhere.

Cigarette smoke plus UVA were used as a model for polluted atmosphere to evaluate the ability of Baicalin to prevent squalene (SQ) oxidation [16]. Briefly, solutions of squalene, cigarette smoke condensate and Baicalin at different concentrations were exposed to a dose of 5J/cm² UVA (Oriol UV solar simulator Newport, USA). Then, squalene and its oxidized product, squalene mono hydroperoxide (SQOOH) are measured by LC-DAD-MS/MS (Agilent).

Baicalin (0.2; 0.5 and 1%) was evaluated in an *ex-vivo* protocol versus placebo [16]. Sebum is collected from volunteer's forehead and Baicalin containing formula or its placebo is spread on top of it at 3mg/cm². Test samples are then exposed to polluted atmosphere mimicked by cigarette smoke and followed by 5J/cm² UVA. Squalene and its oxidized product, squalene mono hydroperoxide (SQOOH) are then measured by LC-DAD-MS/MS (Agilent).

1 ANTI-OXIDANT ACTIVITY OF BAICALIN, IN TUBO

The table below summarizes the IC₅₀ of Baicalin for the quenching of different forms of ROS (ROO[•], HO[•], ¹O₂ and O₂^{-•}). It shows that, Baicalin, apart from its moderate HO[•] quenching ability, displays a rather large anti-oxidant activity, at least against the three other studied forms of ROS (¹O₂, ROO[•], O₂^{-•}).

| Test | Targeted ROS | Baicalin | Reference |
|------------|------------------------------|-----------------------------|---------------------------------------|
| ORAC [8] | ROO [•] | 4759 µmol TE/g | Vitamin C 4456 µmol TE/g |
| SORAC [9] | ¹ O ₂ | IC ₅₀ = 195 ppm | Vitamin E IC ₅₀ = 230 ppm |
| HORAC [10] | HO [•] | 2592 µmol GAE/g | Ferulic acid 9868 µmol |
| SOAC [11] | O ₂ ^{-•} | IC ₅₀ = 250 U/mg | Vitamin C IC ₅₀ = 752 U/mg |

Table 1: Inhibition of the production of some ROS by Baicalin compared to reference compounds

2 BAICALIN PROTECTS AGAINST CIGARETTE SMOKE INDUCED SQUALENE PEROXIDATION IN TUBO.

The overall good anti-oxidants properties were confirmed in a model mimicking uncomplete burning particulate matter pollution. Indeed, when added into the reaction medium, Baicalin protected squalene from cigarette smoke + UVA induced squalene peroxidation with an IC₂₅ of 60,6 µg/mL.

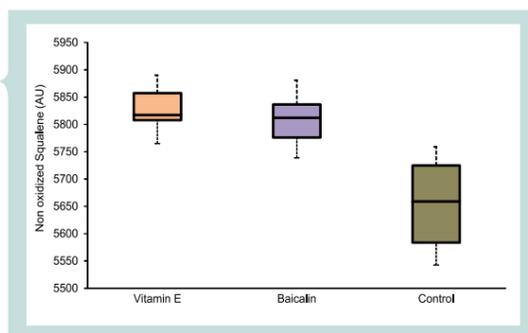


Figure 1: In-tubo evaluation of sebum peroxidation induced by cigarette smoke plus UVA. Solutions of squalene containing Baicalin or Vitamin E were mixed with cigarette smoke condensate and then exposed to 5J/cm² UVA. The resulting non oxidized squalene was quantified by LC-DAD-MS/MS and expressed in arbitrary unit.

As the solubility of Baicalin in Ethanol is poor, the experiment was performed at concentration of 0.1mg/ml instead of IC₅₀ of Vitamin E (0.15mg/mL). The results showed that residue of Squalene in treated working solutions of Baicalin and Vitamin E was similar. It can be concluded that Baicalin exhibited a comparable effect as Vitamin E (p-value = 0.774, independent samples t test).

3 BAICALIN PROTECTS AGAINST CIGARETTE SMOKE INDUCED SEBUM PEROXIDATION EX VIVO

So as to be closer to the real life situation, an *ex-vivo* test using sebum from the forehead of volunteer was performed. Dose effect formulations of Baicalin (0.2, 0.5, 1% Baicalin in formulations) were evaluated versus placebo for their ability to prevent human sebum from oxidation induced by cigarette smoke and UVA in formula.

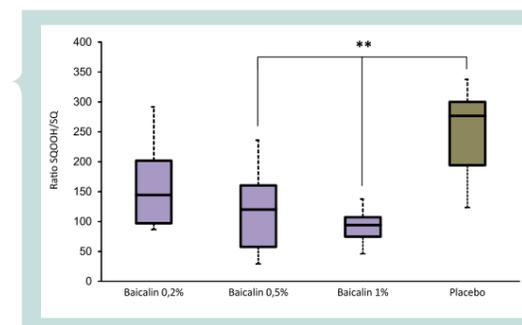


Figure 2: Ex-vivo evaluation of sebum peroxidation induced by cigarette smoke plus UVA. Sebum from the forehead of volunteer was spread with 3mg/cm² of different formula (Baicalin 0.2; 0.5 and 1% or Placebo) and exposed to cigarette smoke plus 5J/cm² UVA. The resulting squalene (SQ) and squalene hydroperoxides (SQOOH) were quantified by LC-DAD-MS/MS. The results are expressed in ratio of SQ on SQOOH. (** p< 0.0001; independent samples t test)

Squalene oxidation was evaluated as a bona fide surrogate for sebum oxidation. The results showed formula with 0.5% of Baicalin shows positive effect (p-value = 0.000 independent samples t test) versus placebo on anti-oxidation. (By IBM SPSS Statistics 23)

CONCLUSION

The results reported here confirm the overall good antioxidant properties of Baicalin. In models mimicking polluted atmosphere conditions, the efficacy of Baicalin was demonstrated against pollution induced oxidative stress. Based on these evaluations, Baicalin is a potent cosmetic ingredient to provide skin surface protection against global environmental stresses.

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