Using zebrafish embryo as a model to screen the multiple efficacies of cosmetic ingredients

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Introduction:

Efficient and high through put efficacy testing technologies are of urgent need in the cosmetics industry worldwide. The global cosmetics market will reach up to approximately $429.8 billion by 2022, and the skin whitening market is about $23.0 billion by as of 2020 [1, 2]. Additionally, consumers also pay attention to cosmetics anti-ROS efficacy [3], skin rejuvenation and repair, related to anti-inflammation and pro-regeneration efficacies [4].
Zebrafish were proposed by the NIH as a novel vertebrate model to study human disease [5]. Zebrafish shares up to 82% of the ortholog sequence of human morbid genes [6], possesses advantages such as small size, rapid development, transparent embryo, easy maintenance, high fecundity and physiological similarity to mammals [5]. The skin of zebrafish shares several characteristics with humans, which allow zebrafish to be used as a vertebrate model for dermatological studies [7].
This study investigated potential “efficacies” of cosmetic ingredients using zebrafish embryos including anti-melanogenesis (whitening effect), anti-ROS (anti-aging effect), anti-inflammation (ache prevention effect) and pro-regeneration (wound healing and skin barrier recovery effect).

Materials & Methods:

Anti-melanogenesis assay
Zebrafish AB strain embryos of 6 hour-post fertilization (hpf) were exposed to sample solution for 48 h. Embryo pigment signal was measured using Imange J.
Anti-ROS assay
Zebrafish embryos of 72 hpf were exposed to sample solution for 24 h, stained with 1 μM H2DCFDA for 2 h, and measured ROS signal using Imange J.
Anti-inflammation assay
Zebrafish embryos of 72 hpf were exposed to 10 μM CuSO4 (inflammation inducer) and sample solution for 2 h, fixed with paraformaldehyde (158127; Sigma-Aldrich), stained with Sudan Black B (199664; Sigma-Aldrich), and counted neutrophil number along the lateral line.
Fin regeneration assay
Zebrafish embryos of 72 hpf anesthetized with tricaine (E10521; Sigma-Aldrich) were fin amputated, exposed to sample solution for 48 h and measured tail fin length.

Table 1. Investigated Cosmetics ingredients

INCI Name	Trade Name	Abbreviation
Nonapeptide-1	ZPC® Whiten016S	Nop-1
Diglucosyl gallic acid	Brightenyl®	Diga
3-O-Ethyl ascorbic acid	Gwhite ® VCE	Eaa
4-butylresorcinol	Mipaulic™ RS 10.0	4-Bur
4-butylresorcinol (nano encapsuled)	NanoActive BR	4-BuNa
Niacinamide	Niacinamide PC	Nico
a-Arbutin	Alpha-arbutin	a-Arb
Phenylethyl resorcinol	SymWhite®377	Phlr
Asorbyl tetraisopalmitate	NIKKOL VC-IP	Atip
Pogostemon cablin leaf extract	CB2-skin™ biofunctional	Pclex
Hydrolized Candida saitoana extract	CELLDETOX®	Hcsex
Acmella oleracea extract	Gatuline® Expression	Aoex
Salicylic acid	Beta-Hydroxyde™ ACSD	Sa
Fagus Sylvatica bud extract	Gatuline® RP	Fsbex
Hydroxypropyl tetrahydropyrantriol	Puri-xylane	Hpth
Palmitoyl tetrapeptide-7	MATRIXYL®3000	Pt-7
Cucurbita pepo L. var. styriaca extract	REFORCYL®-AION	Cpex
Olea europaea (olive) leaf extract	Eurol® BT	Olex
Squalane	Neossance™ Squalane	Squa
Anthyllis Vulneraria flower extract	Phytofleur™ Anthyllis GL	Avfex
Madecassoside	Madecassoside	Mcs

Results & Discussion:

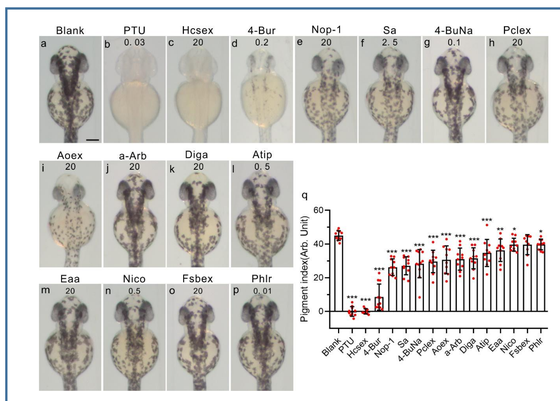

Fig. 1. Anti-melanogenesis effect of cosmetic compound in zebrafish embryos. a–q, representative images show the melanogenesis of zebrafish embryos exposed to corresponding cosmetics solutions. r, bar chart showing the pigment index of zebrafish embryos from panel a to q.

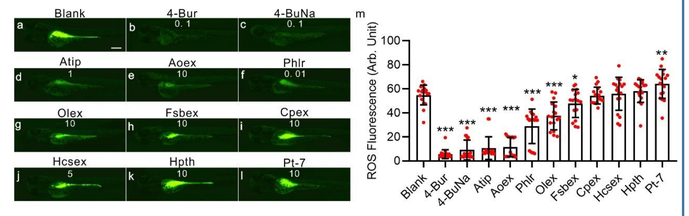

Fig. 2. Antioxidation effect of cosmetic compound in zebrafish embryos. a–l, representative images showing the ROS signals in zebrafish embryos exposed to corresponding cosmetics solution. m, bar chart showing the ROS fluorescence intensity of zebrafish embryos from panel a to l.

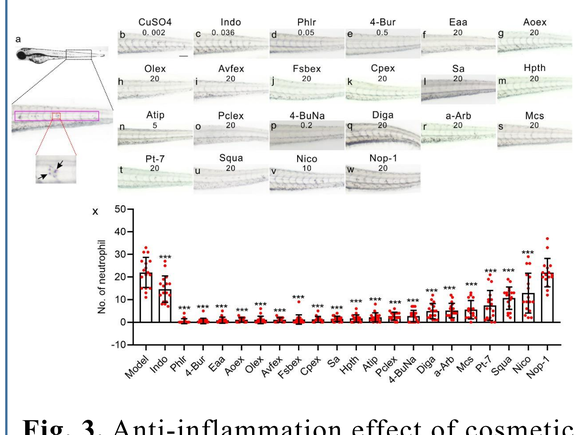

Fig. 3. Anti-inflammation effect of cosmetic compound in zebrafish embryos. a, representative image of 3 dpf embryos induced by $CuSO_4$ for 2 h. The middle panel is the magnification of the dash line boxed area of the zebrafish embryo, and the pink line bound area is approximately 10 cell diameters width near the lateral line and ten somites in length from anus. The neutrophils within the box were quantified. The black dots (black arrow) indicate neutrophils. b–w, representative images showing the neutrophils in zebrafish embryos exposed to different solutions.

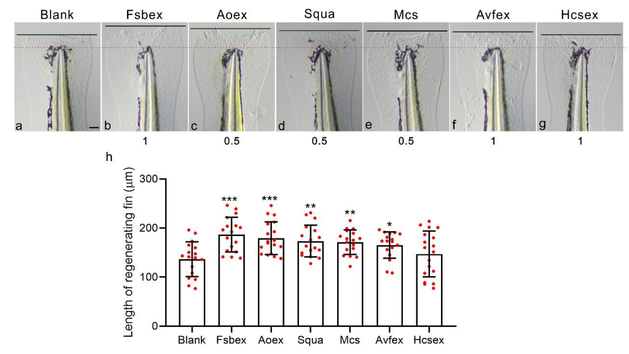

Fig. 4. Pro-regenerative effect of cosmetic compounds in zebrafish embryos. a -g, representative images showing the length of regenerating fin of zebrafish embryos exposed to corresponding cosmetics ingredients. h, bar chart showing the quantification of the length of regenerative fin in panel a-g.

Note: . The testing concentration (g/L) is found in the top portion middle section of each image. Scale bar: 1 mm. Asterisks on the top of each bar represent the significant difference observed compared to the blank control at $p < 0.05$ (*) and $p < 0.001$(***) using one-way ANOVA with LSD post hoc test.

Conclusions:

In this study, we evaluated the anti-melanogenic, anti-ROS, anti-inflammatory and pro-regeneration effects of several cosmetic ingredients using zebrafish embryos. Our results show that Fagus sylvatica bud extract and Acmella oleracea extract exhibit all four efficacies, while 4-butylresorcinol, phenylethyl resorcinol and asorbyl tetraisopalmitate exhibit three efficacies, and the remaining cosmetic ingredients demonstrate two efficacies. These results suggested that zebrafish embryos can be used to rapidly evaluate and screen the function of cosmetic ingredients. Therefore, zebrafish embryos exhibit great potential as a model for cosmetic industry applications.

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