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Malus Domestica

baby pple extract

The sustainable solution from nature for sensitive skin





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1 Summary

Apple fruit (*Malus Domestica*) is a delicious fruit and consumed for centuries. Because of their health benefits "An apple a day keeps the doctor away!", apples are used as food, remedy and cosmetic ingredient. Apples contain dietary fiber, antioxidants and polyphenols, a lot of vitamins, minerals, fruit acids and carbohydrates. The composition of active substances varies depending on the ripening stage, immature apples providing other ingredients than mature ones. In immature apples there are more antioxidants like chlorogenic acid (polyphenol) and fruits acids like malic acid but less carbohydrates.

The "Baby apples" (immature, unripe or not fully developed apples) gently picked and sustainably harvested for the extract described in this paper have been extracted with Bio-Glycerine to an active cosmetic ingredient (Baby apple extract).

In order to identify the dermatological activity of Baby apple extract, an in vitro screening with different skin cells was carried out. The Baby apple extract stimulated following cell mechanisms: glucose uptake, transient receptor potential vanilloid type 1 antagonist as well as STAT3 activity and induced filaggrin genes, inhibited tyrosinase activity and showed an antioxidant activity.

The INCI *Malus Domestica* Fruit Extract is also known in China and Japan. According to the corresponding Cosmetic Ingredient Review (CIR), apple extracts are safe in the present practices of use when formulated to be non-sensitizing and non-irritating.

In cosmetics apple extracts are often used due to their antioxidant, acidifying or anti-inflammatory effects. Baby apple extracts can be used in, anti-ageing, whitening, anti-wrinkle, skin barrier recovery and sun care products among other cosmetic formulations.

2 Classification

Family:	Rosaceae
Genus:	Pyrinae
Species:	Domestica

3 General information on Baby apple

Apples (*Malus*) are the well-known fruits of *Malus Domestica*, which has synonyms like *Malus malus*, *Malus pumila*, *Malus sylvestris* and *Pyrus malus*. Apple trees are coming from the plant genus of the pome fruits (*Pyrinae*) in the *Rosaceae* family. The genus comprises about 42 to 55 species of deciduous trees and shrubs of the northern temperate zone in Europe,



Asia and North America. Additionally, a large number of often difficult to distinguish hybrids has emerged.

By far the best-known species in the world is the cultivated apple (*Malus Domestica*). The apple tree is a deciduous tree or shrub. The flowering time is depending on the variety usually between April and June. The flowers reach a diameter of 2 to 5 cm and are white, pink or red colored. The culture apple fruit consists of shell, pulp and the core casing.



During maturation the apples undergo different biochemical processes. The biochemical process in apples can be observed as changes in appearance, composition of ingredients like carbohydrates, fruit acid content, change of breathable aroma and colors. The first early ripe apples are harvested around the beginning of August, the last in autumn.

In the last years the apple market came under increasing pressure. Every year a record harvest is expected. In order to increase the quality of the crop, part of the growing apples are removed gently to allow remaining ones to develop and enlarge. We called these picked immature, unripe or not fully developed apples "Baby apple". They are not proper for consumption but they are sustainably processed in an active cosmetic ingredient.

4 Use

Apples (*Malus Domestica*) have been used and consumed for centuries. Their crisp flesh, intense, sweetish taste and the refreshing juiciness are responsible for the popularity of apples. The apple is mainly enjoyed fresh. It is also used for the preparation of salads (fruit, potato, herring, etc.), for apple strudel, cakes and pies of various kinds, for baked apples, raw food plates, etc. To fried liver with onions and mashed potatoes, the apple is a special culinary delight. Beside these, apples can be eaten as dried apple chips, jam, jelly, chutney or vinegar. It is also possible to make drinks such as apple juice, wine, most and schnapps. Pharmaceutical industry used apples to produce pectin.

Apples have always been appreciated and used in folk medicine, for example to treat or prevent scurvy, bleeding gums and teeth. Therefore, there are many sayings that refer to the apple, such as "An apple a day keeps the doctor away!". Because of its fruit acid content, the apple is also considered to be the "toothbrush of nature". A raw, grated apple is used in diarrhea, dyspepsia, nutritional disorders, especially in infants and toddlers. Apples are mentioned to help with weight loss, prevent asthma, protect against cancer, protect against cataracts, diabetes, Alzheimer disease, lipid oxidation, to cleanse the liver, restore the intestinal flora, lower risk for coronary heart disease and are good for the brain [1]. This is only a small selection of all the positive apple effects. Additionally, apple and parts of the apple extracts were used since years in cosmetic products.



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5 Content

Apple is a delicious fruit with the abundance of dietary fiber and antioxidants (mainly polyphenols). Apples contain various carbohydrates, healthy vitamins like vitamin A, B3, B5, B6, B9, C, E and K. Besides these, apples also contain many minerals such as potassium, calcium, iron, magnesium, phosphorus and zinc, fruit acids like malic acid, ascorbic acid, carbohydrates and polyphenols like chlorogenic acid.

The composition of active substances varies depending on the ripening stage, immature apples providing other ingredients than mature ones. In immature apples there are more antioxidants like chlorogenic acid (polyphenol), more starch and fruits acids like malic acid but less pectin and glucose (Fig. 3) [2].

Chlorogenic acid, malic acid, carbohydrates and antioxidant capacity concentration were measured in apples at different times of harvest as shown in Fig. 1.

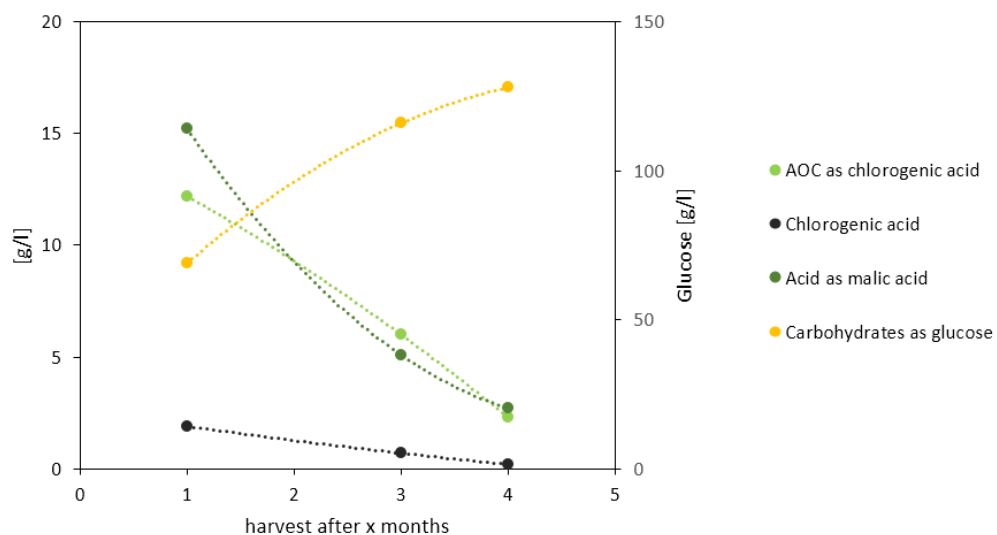


Fig 1. The results show that the amount of antioxidants and acids in the apples sinks during the ripening process in contrast to the amounts of carbohydrates. Hence, Baby apples contain more antioxidants and acids, but less carbohydrates than ripe fruits. (AOC-Antioxidant Capacity)





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5.1 Baby apple extract P-00025493 in Bio-Glycerine

To go into more details, main polyphenols and acids in Baby apple extract were analyzed. Chlorogenic acid is a water-soluble tannin (polyphenol). Tannins are widespread in plants like blueberry, pomegranate, and further preparations like wine, blackberry leaves, coffee and tea which have been consumed for centuries. They are known for their antioxidants and radical scavengers, antibacterial, antiviral, anti-inflammatory, astringent and toxin neutralizing effects [3, 4, 5] and they protect the skin against damaging effects of UV-radiation such as sunburn response and photo-ageing [5]. A UV-spectrum of the Baby apple extract shows UVA and UVB absorption.

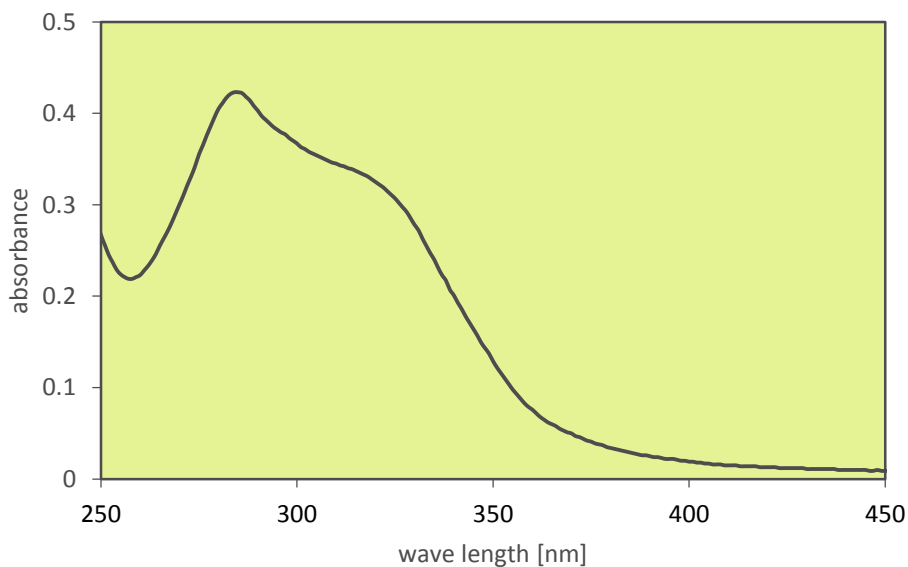


Fig 2. UV-spectrum of Baby apple extract P-00025493 in Bio-Glycerine shows that this extract absorbs UVA and UVB.

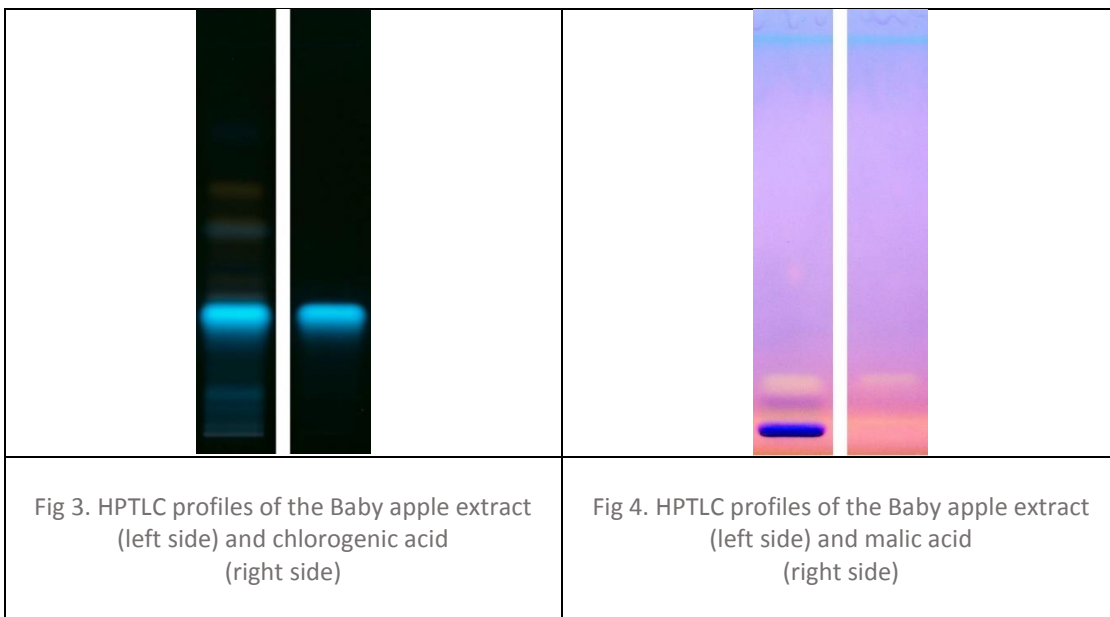
Malic acid is an important fruit acid. It is considered as the main acid in many fruits like apple, grape, quince etc. It occurs especially in unripe fruits and is used as food additive (E 296, EU 1995/2 and following) [7] even in organic products (EU 2008/889) [8] and generally recognized as safe (US 21 CFR 184.1069) [9]. Malic acid is mentioned to be able to increase skin cell renewal, and their ability to improve moisture content and reduce lines and wrinkles. Malic acid at 4 – 5% and pH 3 peels, softens and improves the skin structure, furthermore it depigments skin surface [10]. According to the corresponding Cosmetic Ingredient Review (CIR), malic acid is safe for use as pH adjuster.





This Baby apple extract was analyzed and both chlorogenic acid (polyphenol) and malic acid, the main α -hydroxy acid, were identified.

This Baby apple extract contain organic glycerin and is organic certified, hence it is conform with all natural and bio cosmetic labels. Furthermore, this extract is vegan suitable.



6 Dermatological activities of Baby apple extracts

Apple extracts have been used since decades by the cosmetic industry (see e.g. EU 1996/335) [11, 12]. In cosmetic products, apple extracts are often used due to their antioxidant, acidifying or anti-inflammatory effects. Additionally, they can be used in chemical peelings, anti-ageing and sun products among other things.

In order to identify the dermatological activity of Baby apple extract, an in vitro screening with different skin cells was carried out. It was shown that the Baby apple extract can activate different receptors or mechanisms in diverse skin cell types. The extract stimulated the following cell mechanisms: glucose uptake, transient receptor potential vanilloid type 1 (TRPV1) antagonist, tyrosinase activity and melanin synthesis, antioxidant activity, STAT3 activity and induced filaggrin genes. Some of these cell stimulations support each other. In the table below, these biological activations are assigned to specific skin enhancements. In the following chapters, we will only refer to glucose uptake, transient receptor potential vanilloid type 1 antagonist activity and tyrosinase inhibition.





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6.1 Baby apple extract enhances glucose uptake in keratinocytes

Glucose plays a role in the energy metabolism of keratinocytes (Fig. 5). It is associated with skin cell regeneration and renewal. Baby apple extract (BA Extract) induces glucose uptake in keratinocytes in a dose-dependent manner. BA Extract 0,1%, 0,2% and 1% concentrations demonstrate significant glucose stimulation in a dose-dependent manner. BA Extract concentration 0,1% stimulates the glucose uptake a bit more than positive control. These results may be useful in reversing dried, aged skin conditions to promote healthy regenerated skin.

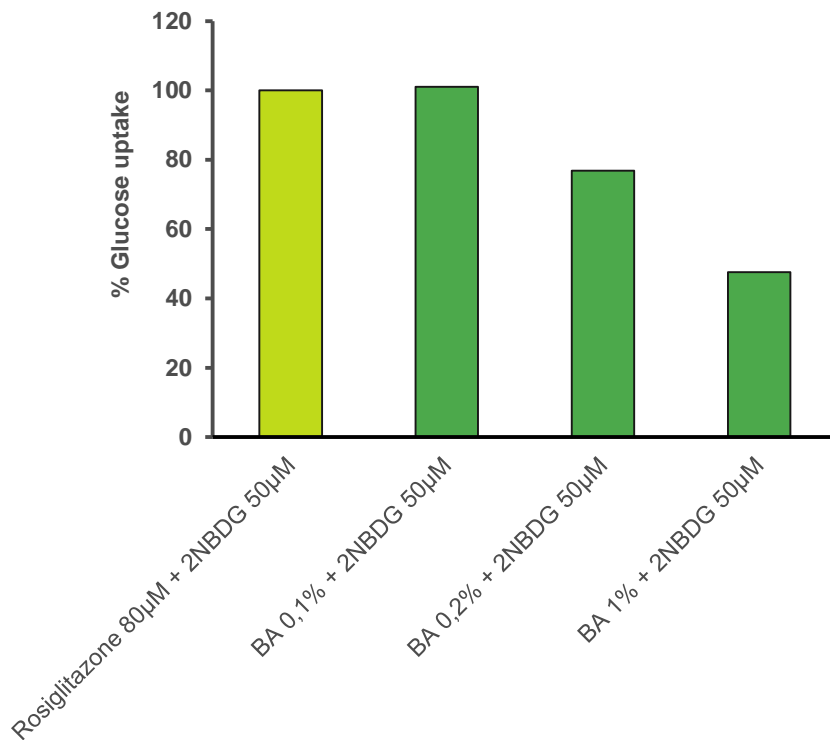


Fig 5. Cell treatment with Baby apple extract (BA Extract) showed increased glucose uptake in keratinocytes. Keratinocytes labeled with 2NBDG (2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino]-2-deoxy-D-glucose) and treated with the Baby apple extract or the positive control with Rosiglitazone for 24 h. Rosiglitazone is taken as 100% of glucose uptake.



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6.2 Baby apple extract enhances TRPV-1 antagonist in keratinocytes

Transient receptor potential vanilloid type 1 (TRPV1) is a non-selective cation channel activated by diverse obnoxious stimuli like capsaicin, low pH or heat. TRPV1 is widely expressed in skin tissues, and peripheral sensory nerve fibers. TRPV1 is associated with skin permeability barrier function, this suggests that modulation of TRPV1 might be beneficial for skin disorders with barrier damages [6]. Activation of TRPV1 releases neuropeptides, which results in neurogenic inflammation and is believed to contribute pruritus development. Baby apple extract is a TRPV1 antagonist. The antagonistic activity can be measured in a concentration dependent manner. BA Extract at 1% showed a better viability than controls. A TRPV1 antagonist has the potential to work as an anti-pruritic agent and inhibits skin irritation [12, 13, 14]. These findings indicate that this extract may be useful in sensitive skin, anti-ageing, anti-wrinkle, skin barrier recovery and sun care products [15, 16].

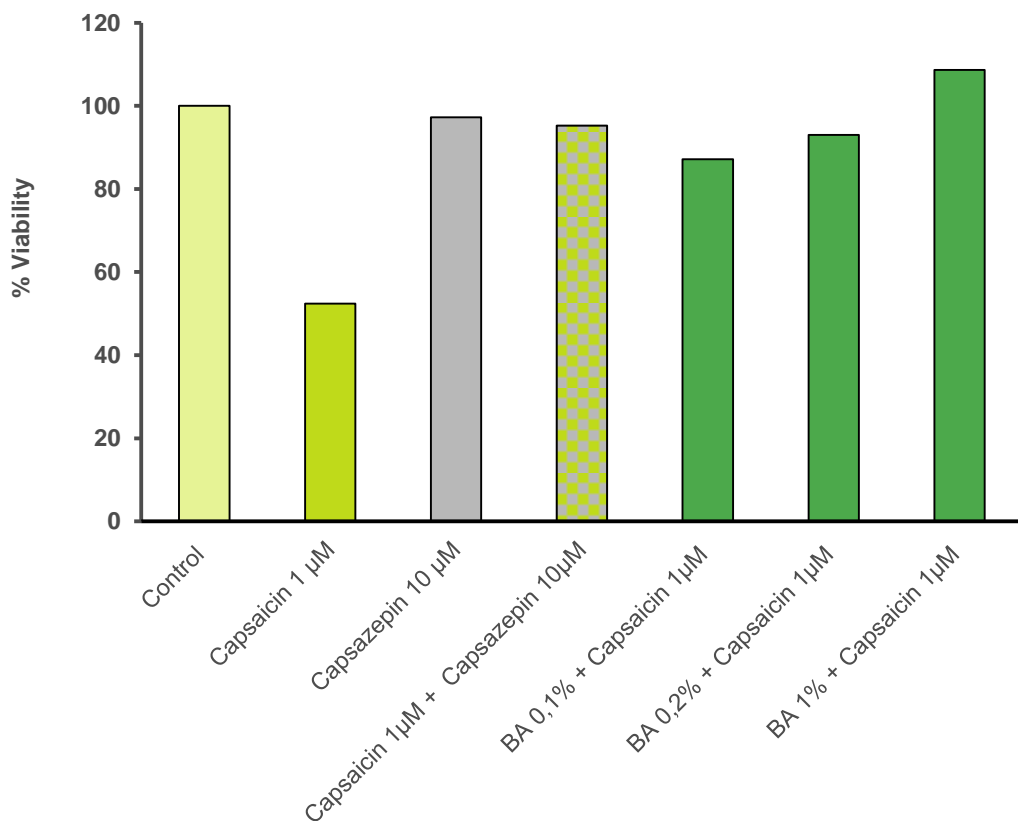


Fig 6. Cell treatment with Baby apple extract (BA Extract) showed antagonistic activities in a cell culture model. The cells are incubated with the BA Extract for 30 min and then stimulated with the agonist Capsaicin (1 µM). Capsazepine (10 µM) is used as the positive control of TRPV-1 antagonism. Untreated cells are defined as control and taken as 100%.



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6.3 Baby apple extract acts like tyrosinase inhibitor

Melanogenesis is responsible for the pigmentation of human skin, eye and hair. Beside melanin synthesis, numerous enzymatic catalyzed and chemical reactions are involved in the melanogenesis process. Enzymes such as tyrosinase and tyrosinase-related protein-1 and tyrosinase-related protein-2 play a major role in melanin synthesis. The downregulation of tyrosinase is the most prominent approach for the development of melanogenesis inhibitors.

All BA Extract concentration act on tyrosinase activity in a dose dependent manner. BA Extract (0,1% and 0,2%) inhibit tyrosinase only slightly. BA Extract (1%) showed a better decrease of tyrosinase activity than positive control Kojic acid.

The results regarding the tyrosinase activity complete the TRPV1 antagonist results. These data indicate that this Baby apple extract can be used to treat sensitive skin. In cosmetic formulation it may be able to have anti-ageing, whitening, and sun care effects.

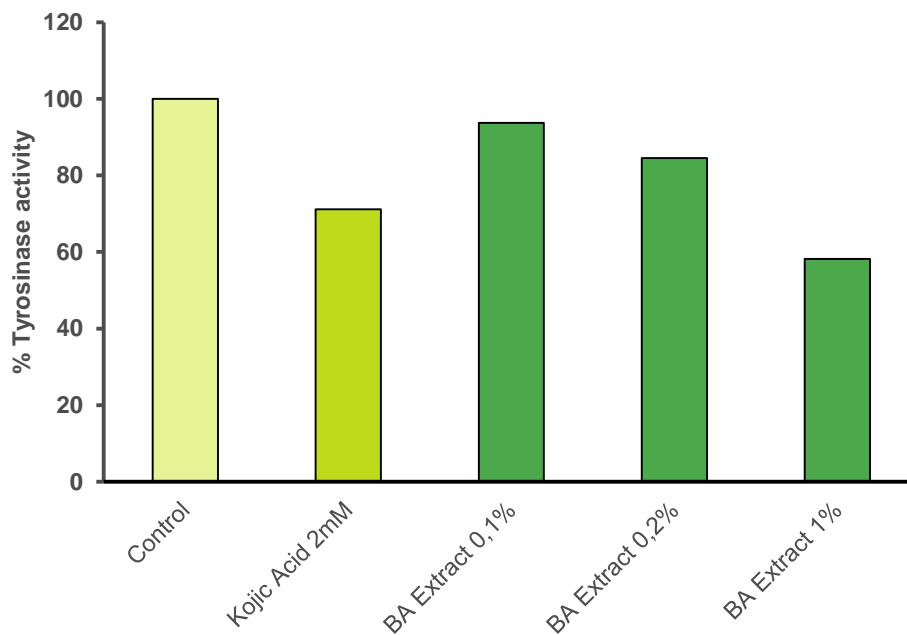


Fig 7. Cell treatment with Baby apple extract (BA Extract) showed decrease tyrosinase activity in melanocytes. Kojic acid is a known whitening agent and taken to be the positive control. Untreated melanocytes, control is taken as 100%.





7 Summary of dermatological Baby apple extract activities

Literature data confirm our product findings. A study by Son et al. mentioned that unripe green apples contain 10 times higher levels of tannins than ripe apples [17]. It was supposed that the antioxidant extract with astringent activity can reduce facial pore size and sebum secretion [17]. Synergistic photoprotective effects were observed which might be interesting to treat damaged skin [18], radical scavenging, antiproliferative [19] and anti-aging activities [20].

The high quantities of bioactive antioxidants in the Baby apple are responsible for its health-promoting effects which confirm the cosmeceutical value. The activations at the cellular level in vitro after addition of the Baby apple extract can trigger different positive effects on the skin (Table 1). In cosmetic formulations this Baby apple extract can be used due to its antioxidant, whitening, anti-ageing, anti-wrinkle, skin barrier recovery, sun care and sensitive skin care effects.

Table 1: Dermatological Baby apple extract activities

	Sensitive skin	Anti-ageing	Moisturizing	Sun care	Skin-lightening / Whitening	Anti-wrinkle	Hair growth
Glucose uptake		x	x		x		
TRPV-1 antagonist activity	x			x		x	
Tyrosinase activity	x			x	x		
Filaggrin gene induction			x	x			
STAT-3 activity							x

8 Acknowledgement

We would like to thank VivaCell Biotechnology GmbH and Sim Cosmetic Biotech for testing the Baby apple extract. Additional thanks also go to Dr. Silke Schagen for the support regarding communication.





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9 References

- [1] Jeanelle Boyer and Rui Hai Liu, Apple phytochemicals and their health benefits, *Nutr J.* 2004; 3: 5, <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC442131/pdf/1475-2891-3-5.pdf>.
- [2] De Vries JA, Voragen AGJ, Rombouts FM, Pilnik W, Extraction and purification of pectin from alcohol-insoluble solids from ripe and unripe apples., *Carbohydr Polym* 1:117–127, 1981.
- [3] Jung MK, Hur DY, Song SB, Park Y, Kim TS, Bang SI, Kim S, Song HK, Park H, Cho DH., Atopic Dermatitis via Suppression of Angiogenesis and TARC Expression in Nc/Nga Mice, *J Invest Dermatol.* 130(5):1459-63., 2010 May. .
- [4] Hoffmann J, Wölfle U, Schempp CM, Casetti F, Tannins from *Potentilla officinalis* display antiinflammatory effects in the UV erythema test and on atopic skin, *J Dtsch Dermatol Ges.* 2016 Sep;14(9):917-22.
- [5] P-Y Wu, C-C Huang, Yin Chu, Y-H Huang, P Lin, Y-H Liu, K-C Wen, Chien-Yih Lin,5 Mei-Chich Hsu,6 and Hsiu Chiang, Alleviation of Ultraviolet B-Induced Photodamage by *Coffea arabica* Extract in Human Skin Fibroblasts and Hairless Mouse Skin, *Int J Mol Sci.* 2017 Apr; 18(4): 782.
- [6] Yusuf N., et al., Photoprotective effects of green tea polyphenols, *Photodermatology, Photoimmunology & Photomedicine*, Volume 23, Issue 1, Pages 48–56, 2007.
- [7] https://www.fsai.ie/uploadedFiles/Reg1129_2011.pdf. s.u.
- [8] <http://eur-lex.europa.eu/eli/reg/2008/889/oj>
- [9] https://www.ecfr.gov/cgi-bin/text-idx?SID=20c990a3b374045d90cca0239ee15f3a&mc=true&tpl=/ecfrbrowse/Title21/21cfr184_main_02.tpl
- [10] GDCh Datenblätter zur Bewertung der Wirksamkeit von Wirkstoffen in kosmetischen Mitteln Hydroxysäuren https://www.gdch.de/fileadmin/downloads/Netzwerk_und_Strukturen/Fachgruppen/Lebensmittelchemiker/Arbeitsgruppen/kosmetik/hydroxys.pdf.
- [11] Yun JW, Seo JA, Jeong YS, Bae IH, Jang WH, Lee J, Kim SY, Shin SS, Woo BY, Lee KW, Lim KM, Park YH., „TRPV1 antagonist can suppress the atopic dermatitis-like symptoms by accelerating skin barrier recovery.“.
- [12] Rachel A. Gibson et al, „A Randomised Trial Evaluating the Effects of the TRPV1 Antagonist SB705498 on Pruritus Induced by Histamine, and Cowhage Challenge in Healthy Volunteers,“ p. <https://doi.org/10.1371/journal.pone.0100610>, Published: July 21, 2014.
- [13] Li DG, Du HY, Gerhard S, Imke M, Liu W, Inhibition of TRPV1 prevented skin irritancy induced by phenoxyethanol. A preliminary in vitro and in vivo study, *Int J Cosmet Sci.* 2017 Feb;39(1):11-16.
- [14] Jovanovic Z, Angabini N, Ehlen S, Mokos ZB, Subotic M, Neufang G, Efficacy and Tolerability of a Cosmetic Skin Care Product With Trans-4-t-butylcyclohexanol and Licochalcone A in Subjects With Sensitive Skin Prone to Redness and Rosacea, *J Drugs Dermatol.* 2017 Jun 1;16(6):605-610.
- [15] Yun JW1, Seo JA, Jeong YS, Bae IH, Jang WH, Lee J, Kim SY, Shin SS, Woo BY, Lee KW, Lim KM, Park YH., TRPV1 antagonist can suppress the atopic dermatitis-like symptoms by accelerating skin barrier recovery., *J Dermatol Sci.* 2011 Apr;62(1):8-15.
- [16] Schaffler K, Reeh P, Duan WR, Best AE, Othman AA, Faltynek CR, Locke C, Nothaft W., An oral TRPV1 antagonist attenuates laser radiant-heat-evoked potentials and pain ratings from UV(B)-inflamed and normal skin., *Br J Clin Pharmacol.* 2013 Feb;75(2):404-14..
- [17] Son DH, Nam MH, Hong CO, Seol HM, Yang JE, Kim YB, Kim CT, Lee KW, 5- α Reductase inhibitory effect and astringent activity of green apple rind extract on human keratinocytes and fibroblast cells, *Biosci Biotechnol Biochem.* 2013;77(4):714-21.
- [18] Bennet D, Kang SC, Gang J, Kim S, Photoprotective effects of apple peel nanoparticles., *Int J Nanomedicine.* 2014;9:93-108.
- [19] Cetkovic GS, Savatovic SM, Canadanovic-Brunet JM, Cetojevic-Simin DD, Djilas SM, Tumbas VT, Skerget M, Apple pomace: antiradical activity and antiproliferative action in HeLa and HT- 29 human tumor cell lines, *J BUON.* 2011 Jan-Mar;16(1):147-53.
- [20] Leu SJ, Lin YP, Lin RD, Wen CL, Cheng KT, Hsu FL, Lee MH, Phenolic Constituents of *Malus doumeri* var. *formosana* in the Field of Skin Care, *Biol Pharm Bull.* 2006 Apr;29(4):740-5.
- [21] <http://ernaehrung.gesunderwelt.de/polyphenole.html>.
- [22] www.chids.de/dachs/expvortr/589Apfel_Budde_Scan.pdf.
- [23] <https://patents.google.com/patent/US5587174A/en>.
- [24] Schürch C, Blum P, Züllli F, Potential of plant cells in culture for cosmetic application, *Phytochemistry Reviews*, October 2008, Volume 7, Issue 3, pp 599–605.