Journal of Cosmetics, Dermatological Sciences and Applications, 2026, 16(2), 26-36
https://www.scirp.org/journal/jcdsa
ISSN Online: 2161-4512
ISSN Print: 2161-4105

In Vitro and *in Vivo* Demonstration of the Beneficial Effects of a *Caesalpinia sappan* Seeds Extract on Human Skin: From ROS Scavenging to Dark Spot Bleaching

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How to cite this paper: Fordiani, F. and Bourgeteau, V. (2026) *In Vitro* and *in Vivo* Demonstration of the Beneficial Effects of a *Caesalpinia sappan* Seeds Extract on Human Skin: From ROS Scavenging to Dark Spot Bleaching. *Journal of Cosmetics, Dermatological Sciences and Applications*, **16**, 26-36.
https://doi.org/10.4236/jcdsa.2026.162003

Received: December 17, 2025
Accepted: April 14, 2026
Published: April 17, 2026

Abstract

Background: *Caesalpinia sappan* seeds have recently shown beneficial effects on human health because of its content in anti-inflammatory and antioxidant compounds. Reasoning that these compounds could also display beneficial effects on human skin, we investigate the antioxidant power of a *Caesalpinia sappan* seeds extract and its ability to protect skin against oxidative attacks. Additionally, as inflammation is often related to oxidative bursts, we evaluate the potency of our extract to act on inflammation. Finally, as our *Caesalpinia sappan* seeds extract is particularly rich in 4-methyl-catechol, a well-known divalent cation chelator, we test its ability to reduce tyrosinase activity which depends on the presence of copper ions, and by this way to produce any significant whitening effect on human skin. **Materials and Methods:** In the first part of our study, intrinsic potential of our *Caesalpinia sappan* seeds extract was evaluated by using a paramagnetic radical, *i.e.* the DiPhenl Pricryl Hydrazyl (DPPH), which emits specific colorations following its oxidation state. For the second time, we took advantage of our original model of enrichment of epidermis cell cultures in progenitor cells to show the ability of our extract to protect progenitor cells against UV irradiations. Subsequently, the potency of our *Caesalpinia sappan* seeds extract to inhibit Lysyl Oxidase (LOX) activity was evaluated in an *in vitro* model composed by a purified human enzyme. Concerning the putative whitening effect of our *Caesalpinia sappan* seeds extract, it was assessed in several steps: 1) measurement of its inhibitory effect on tyrosinase activity in an *in vitro* acellular model calling for a mushroom enzyme, 2) measurement of its inhibitory effect on melanogenesis in a model of human normal melanocytes, and at last 3) assessment of its whitening effect, and notably its bleaching effect on dark spots, in an *in vivo* model calling for human

volunteers. **Results:** Our *Caesalpinia sappan* seeds extract strongly inhibited DPPH oxidation from 33.2 +/− 4.9% to 97.1 +/− 0.5% for *Caesalpinia sappan* seeds concentrations from 0.01 to 1% (v/v), respectively. As a consequence, *Caesalpinia sappan* seeds extract was able to efficiently protect cutaneous progenitor cells against UVB radiations from 51.1 +/− 1.7% to 86.2 +/− 3.8% of protection for *Caesalpinia sappan* seeds concentrations from 0.00015 to 0.0015% (v/v), respectively. LOX activity was significantly reduced by the *Caesalpinia sappan* seeds extract from 69.4 +/− 3.6 to 91.1 +/− 1.5% for concentrations from 0.01 to 1% (v/v), respectively. The activity of another tested copper-dependent enzyme, *i.e.* tyrosinase, was for its part, virtually totally inhibited by our *Caesalpinia sappan* seeds extract for concentrations as low as 0.5% (v:v). Subsequently, human melanogenesis was significantly inhibited by the tested extract: from 34.4 +/− 5.8% to 93.7 +/− 5.8% of inhibition for *Caesalpinia sappan* seeds extract concentrations comprised between 0.001 and 0.005% (v/v). Finally, the surface of dark spots on human volunteer's skin was significantly reduced by 22.2% after 28 days of use of a formulation containing 1% of our *Caesalpinia sappan* seeds extract. **Conclusion:** *Caesalpinia sappan* seeds not only display beneficial effects on human health through their actions on digestive tract but also can protect human skin against oxidative attacks and, thanks to their copper chelating activity, efficiently act against inflammatory bursts and significantly reduce skin dark spots coloration intensity.

Keywords

Stem Cells, Melanogenesis, Tyrosinase, LOX, Anti-Oxidant, *Caesalpinia sappan*, *In Vitro*, *In Vivo*, Inflammation, Dark Spots

1. Introduction

Caesalpinia sappan seeds were recently shown to display beneficial effects on human alimentary tract notably thanks to their anti-inflammatory and antioxidant properties [1] which are more probably linked to *Caesalpinia sappan* seeds content in 4-methyl catechol, a well-known divalent cation chelator [2]. Indeed, this type of chelator is able to reduce extra and intracellular available quantities of divalent cations such as Mg^{2+} and Ca^{2+}, which are essential to numerous cellular enzymes (such as catechol 2,3-dioxygenase or lipoxygenase) implicated in oxidant and inflammatory processes [3] [4].

In order to go further in the exploration of the beneficial effects of *Caesalpinia sappan* seeds on human health, we were then prompted to investigate the capabilities of a *Caesalpinia sappan* seeds extract to also protect human skin by assessing more deeply its anti-oxidant and anti-inflammatory properties. Additionally, as calcium-dependent enzymes are also implicated in the regulation of human melanogenesis [5], we also evaluated the ability of our *Caesalpinia sappan* seeds extract to act on melanin production to finally be able to reduce the anesthetic impact of human skin dark spots which are often linked to melanogenesis disorders

and/or aging (for a review, see [6]).

We then first clarified the intrinsic antioxidant potential of our *Caesalpinia sappan* seeds extract by using an acellular *in vitro* model calling for the well-known paramagnetic radical DiPhenyl Picryl Hydrazyl (DPPH) (for a review, see [7]). For the second time, we investigated if our *Caesalpinia sappan* seeds extract could display a sufficient antioxidant effect to protect skin progenitor cells against oxidative attacks (UV irradiations). To reach this goal, we developed an original model of human skin cell cultures enriched in progenitor cells by helping us of the work of 1) Goodell *et al*., which have published in 1996 a relatively simple method to select stem cells within murine blood [8], and of 2) Durand and Olive which have published in 1982 a result enabling a further simplification of the protocol of Goodell *et al.* [9]. Briefly, Goodell *et al.* used a dye called Hoescht 33342 to identify stem cells (only stem cells possess Multi-Drug Resistance—MDR—proteins allowing them to exclude the dye) and a cell-sorter to harvest them (stem cells are the unmarked ones). Durand and Olive showed in 1982 that Hoescht 33342 is finally cytotoxic for dividing cells. As a consequence, the introduction of Hoescht 33342 in monolayer cell cultures ends up killing non-stem cells after several replications.

Reasoning that an antioxidant effect could logically result in an anti-inflammatory one (for a review, see [10]), we then investigate the ability of our *Caesalpinia sappan* seeds extract to inhibit an enzyme notably implicated in epidermis inflammation processes, *i.e.* the lipoxygenase (LOX). In fact, even if LOX are key enzymes in the biosynthesis of a variety of signaling molecules implicated in the regulation of many essential biological functions, they are also thought to play a significant role in inflammatory skin diseases and cancer [11].

In a second part of our study, we reasoned that other calcium-dependent enzymes such as tyrosinase could also be inhibited by our *Caesalpinia sappan* seeds extract. As many melanogenesis disorders and/or aging could trigger the apparition of anesthetic dark spots on human skin (6), possibly leading to additional psychological problems [12]-[14], we were prompted to evaluate the efficacy of our extract to act on human skin melanogenesis regarding this "perhaps" more cosmetic considerations. We studied the ability of our *Caesalpinia sappan* seeds extract to inhibit tyrosinase in 1) an acellular *in vitro* model calling for a mushroom purified enzyme showing 98% of sequence homology with the human enzyme, and 2) an *in vitro* model of human normal melanocytes. Finally, we performed an *in vivo* test calling for human volunteers to achieve the demonstration of a significant bleaching effect of our *Caesalpinia sappan* seeds extract on human skin dark spots.

2. Materials and Methods

1) DPPH assay: Antiradical activities were determined via 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay to determine EC50 values. Briefly, 60 µM solution of DPPH was prepared by dissolving 1.5 mg of DPPH crystalline solid in 62.5 mL of analytical grade methanol and stored at 4˚C. For each tested condition 100 µL of test compound (*Caesalpinia sappan* seeds extract at final concentrations of 0.01;

0.1 and 1%; v/v) or reference standard solution were mixed with 100 µL of DPPH solution in a well of 96 well plates. After 30 min incubation in the dark at room temperature, the absorbance at 520 nm was recorded using a Spectrophotometer.

Reference standard compound being used was Trolox. A stock solution of 1 mg/mL was prepared by dissolving 2 mg of Trolox in 2 mL of ethanol. Then, serial dilution in distilled water were prepared.

For each concentration tested radical scavenging activity has been calculated using the formula bellow:

$$100 - \left[\frac{OD_{520} \textit{in the presence of test product}}{OD_{520} \textit{in the absence of test product}} \times 100 \right]$$

2) Stem cells protection assay: Primary human keratinocytes were isolated from an abdominal skin residue coming from plastic surgery. Cells were grown in Keratinocytes Growth Medium 2 (Promocell). Second passage keratinocytes were seeded into 96 well plates at a cell density of 5000 cells per well and were cultured at 37˚C in an atmosphere containing 5% of CO_2 until reaching around 80% of confluency. Hoescht 33342 at 1 µg/ml was incubated with cells for 1 hour. Keratinocytes monolayer were rinsed with PBS and incubated again for 6 days. During this period, cell medium was renewed every 2 days. *Caesalpinia sappan* seeds extract at 0.00015; 0.0005 and 0.0015% (v/v) was put in contact with cells for 24 hours and then cells were rinsed with PBS and irradiated with UVB at 30 mJ/cm^2 (this UV dose was determined following preliminary dose-dependence studies; data not shown). Keratinocytes were then incubated again with test compound at 37˚C. From this step every 2 days cell viability was measured in wells by using Alamar blue. Briefly, cells were incubated at 37˚C for 2 hours with 10-fold diluted Alamar blue and then optical density was measured at 560 nm and 600 nm. Cells monolayer were rinsed, and incubated again with test compound at 37˚C. The experimentation is stopped when clear difference of viability is noted between irradiated and non-irradiated control.

3) LOX activity assay: LOX activity assay was performed in an acellular *in vitro* model by using a dedicated assay kit commercialized by Mybiosource (San Diego, USA).

4) Tyrosinase activity assay (acellular *in vitro* model): Tyrosinase inhibition assays were performed with L-DOPA as substrate. The reaction mixture (200 µL) contained 50 µL of mushroom tyrosinase (100 U/mL), 100 µL of test compound solution (*Caesalpinia sappan* seeds extract at final concentrations of 0.5; 1 and 2%, v/v) and 50 µL of L-DOPA at 0.02% (w/v). Reaction between tyrosinase and L-DOPA was followed during 10 minutes after the addition of the L-DOPA by reading the absorbance of the incubation media at 490 nm. Kojic acid was used as a positive control.

5) Melanogenesis assay (cellular *in vitro* model):

(1) Materials: Human normal melanocytes were obtained from a 5 years old Caucasian donor. Melanocyte growth medium 2 PMA free (MGM 2 PMA free) was purchased from Promocell (Heidelberg, Germany). Penicillin and streptomy-

cin came from Dutscher (Bernolsheim, France). Kojic acid, Melanin and NaOH were acquired from Sigma-Aldrich (Saint louis).

(2) Cell culture: Normal human melanocytes were seeded in multiwell culture plates and cultured in MGM PMA free in a humidified incubator at 37˚C under a 5% CO_2/95% air atmosphere, until they reached confluence. Melanocytes were then incubated for a 72 hours period in the absence (control), or in the presence of Kojic acid at 250 µM or of increasing concentrations of our *Caesalpinia sappan* seeds extract (0.001; 0.0025 and 0.005%, v/v).

(3) Melanin quantification: At the end of the incubation period, the monolayers were rinsed with PBS. Cells monolayers were then lysed and melanin was solubilized using NaOH 1 N. Melanin was quantified in cell lysates by measuring optical density at 450 nm.

(4) Proteins measurement: Total proteins contained in the cell lysates were quantified, by using a spectro-colorimetric method (Bradford method [15]).

6) *In vivo* study (skin dark spots analysis): 20 healthy volunteers have applied twice a day a formulation containing 1% (v/v) of our *Caesalpinia sappan* seeds extract during 28 days. At D0 (before the application of any product) Surface of the spots was analyzed on the images by using a dedicated software.

7) Statistics: As indicated under each figure, results were statistically analyzed by a one way ANOVA analysis followed, when it was required, by a Holm-Sidak test or by using paired Student t-tests.

3. Results and Discussion

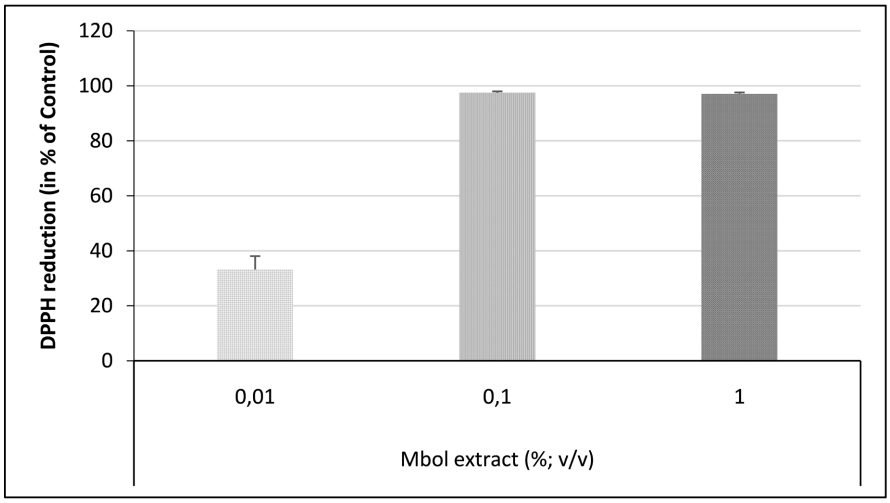
The recent publication of Sasongko *et al.* [1] in 2025 showing significant benefits of *Caesalpinia sappan* seeds on human health through its *per os* action, prompted us to investigate if *Caesalpinia sappan* seeds extracts could also display benefic actions in other human organs such as skin. As Sasongko *et al.* [1] strongly suggest that favorable effects of *Caesalpinia sappan* seeds are linked to its anti-oxidant properties, we start our study by evaluating the intrinsic anti-oxidant effect of our own *Caesalpinia sappan* seeds extract resorting to a paramagnetic radical, *i.e.* the Diphenyl Picryl Hydrazyl (DPPH). As shown in **Figure 1**, in the selected experimental conditions, our *Caesalpinia sappan* seeds extract display a significant and very powerful anti-oxidant effect: DPPH reduction raised 33.2; 97.5 and 97.1% for *Caesalpinia sappan* seeds extract concentrations of 0.01; 0.1 and 1% (v/v), respectively.

In the last decades, the number of researches concerning stem cells have grown in an exponential manner showing the growing interest of scientists for this promising "therapeutic" target. Epidermis progenitor cells so today consist in a very interesting target for the development of innovative and pertinent anti-aging products (for a review, see [16]). We then attempted to examine if our *Caesalpinia sappan* seeds extract could help human skin to slow its aging by at least protecting cutaneous stem cells against environmental aggressions such as UV-irradiations. To achieve this goal, we profit of our newly developed model of human normal keratinocytes cultures enriched in progenitor cells.

Rapidly, this model took advantage of the capability of stem cells to exclude

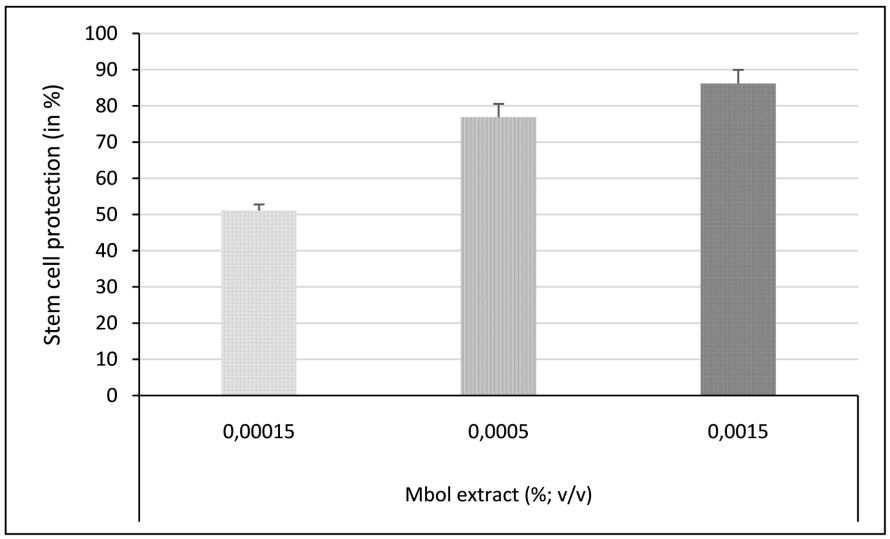
toxic agents thanks to their Multi-Drug Resistance proteins (MDR proteins), which are absent in the non-progenitor cells. As a consequence, when human normal skin cells were cultured in the presence of a genotoxic product, we obtained after several cell divisions, a culture enriched in the only cells which are able to exclude the genotoxic agent, *i.e.* the progenitor ones.

As shown in Figure 2, in the selected experimental conditions, our *Caesalpinia sappan* seeds extract at 0.00015; 0.0005 and 0.0015% (v/v) was able to significantly protect cutaneous progenitor cells against Uv-irradiations reducing their cytotoxic effect by 51.1; 76.9 and 86.2%, respectively.



***: Statistically different from the "not treated" condition ($p < 0.001$); One Way ANOVA analysis + Holm-Sidak test.

Figure 1. Anti-oxidant activity of *Caesalpinia sappan* seeds extract (DPPH assay).



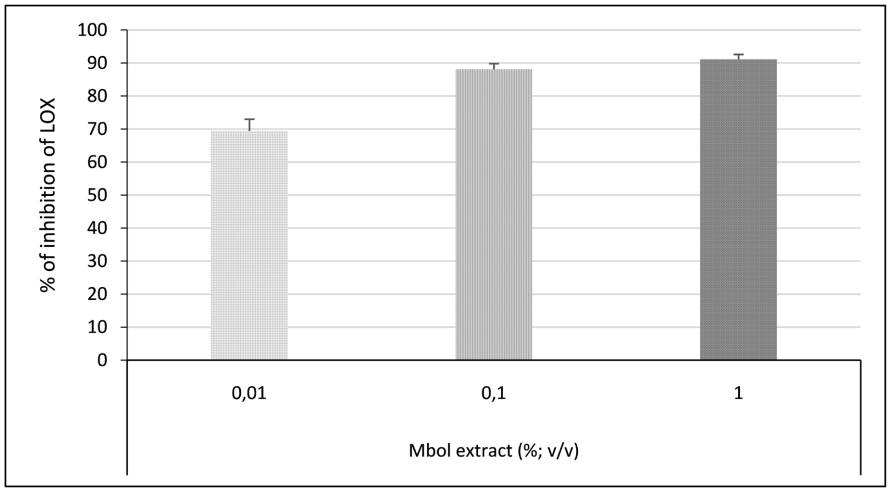
***: Statistically different from the "not treated" condition ($p < 0.001$); One Way ANOVA analysis + Holm-Sidak test.

Figure 2. Protective effect of *Caesalpinia sappan* seeds extract on cutaneous progenitor cells irradiated by UV.

These results clearly show that our *Caesalpinia sappan* seeds extract could provide a very efficient shield effect against deleterious effect of environmental aggressions such as UV radiations and the oxidative stress that they trigger. Skin stem cells capital could so be efficiently preserved ensuring a younger and healthier aspect to human skin.

As oxidative bursts are often linked to inflammatory situations, we then choose to test the ability of our *Caesalpinia sappan* seeds extract to inhibit enzymes implicated in pro-inflammatory responses via the generation of Reactive Oxygen Species (ROS), *i.e.* lipoxygenases (LOX) [17].

As shown in **Figure 3**, in the selected experimental conditions, our *Caesalpinia sappan* seeds extract at 0.01; 0.1 and 1% (v/v) significantly inhibited lipoxygenase by 69.4; 88.1 and 91.1%, respectively. This result so confirm that *Caesalpinia sappan* seeds could also constitute a good tool to soften inflammatory situations in human skin. Additionally, as LOX is also present in human intestine [18], it could be possible that the beneficial effect of *Caesalpinia sappan* seeds on human alimentary tract showed by Sasongko *et al.* in 2025, was at least partially due to an inhibitory effect on LOX.



***: Statistically different from the "not treated" condition ($p < 0.001$); One Way ANOVA analysis + Holm-Sidak test.

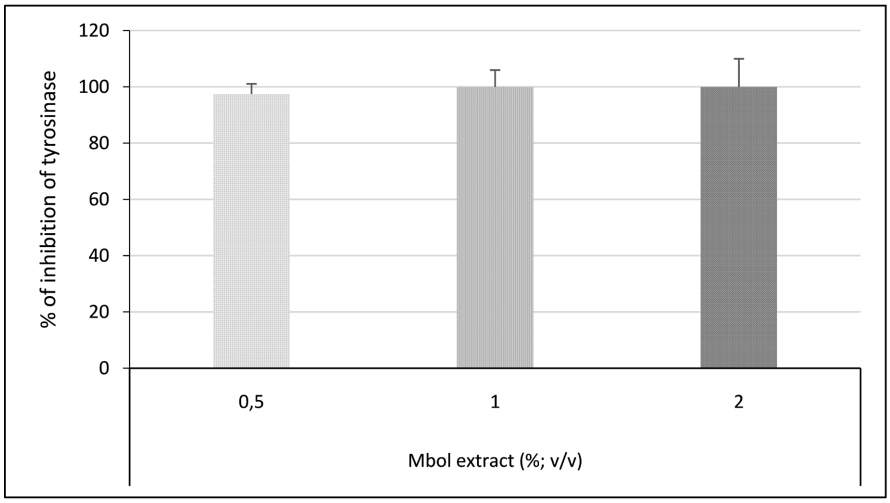
Figure 3. *Caesalpinia sappan* seeds extract effect on LOX activity.

In a more mechanistic way, as LOX activity needs divalent cations (for a review, see [19]), we can imagine that the 4-methyl catechol (a well-known divalent cation chelator [20]) contained in our *Caesalpinia sappan* seeds extract, could be responsible for the inhibition of this enzyme. Additionally, as divalent cations are implicated in oxidative reactions which not imply enzymes (we can for example mention the Fenton [21] and the Haber-Weis [22] reactions), it is also possible to suggest that the anti-oxidant activity of the 4-methyl catechol contained in our *Caesalpinia sappan* seeds extract, could also be understand in these terms.

In another part of our study, we reasoned that other divalent cation-dependent

enzymes could cover important functions in human skin homeostasis. It is notably the case of tyrosinase, the main enzyme of melanogenesis. In case a melanogenesis disorders notably, it could be useful to act on its activity to reduce esthetic impact of skin dark spots. We then choose to evaluate the ability of our *Caesalpinia sappan* seeds extract to modulate tyrosinase activity.

As shown in Figure 4, our *Caesalpinia sappan* seeds extract significantly and virtually totally inhibit tyrosinase activity for concentrations as low as 0.5% (v/v). More probably thanks to its content in 4-methyl catechol, our *Caesalpinia sappan* seeds extract could so also constitute a very good tool to fight against human melanogenesis disorders. As a consequence, and before to realize an *in vivo* study, we have decided to evaluate the ability of our *Caesalpinia sappan* seeds extract to reduce melanogenesis in a model of human normal melanocytes monolayers.



***: Statistically different from the "not treated" condition ($p < 0.001$); One Way ANOVA analysis + Holm-Sidak test.

Figure 4. *Caesalpinia sappan* seeds extract effect on tyrosinase activity.

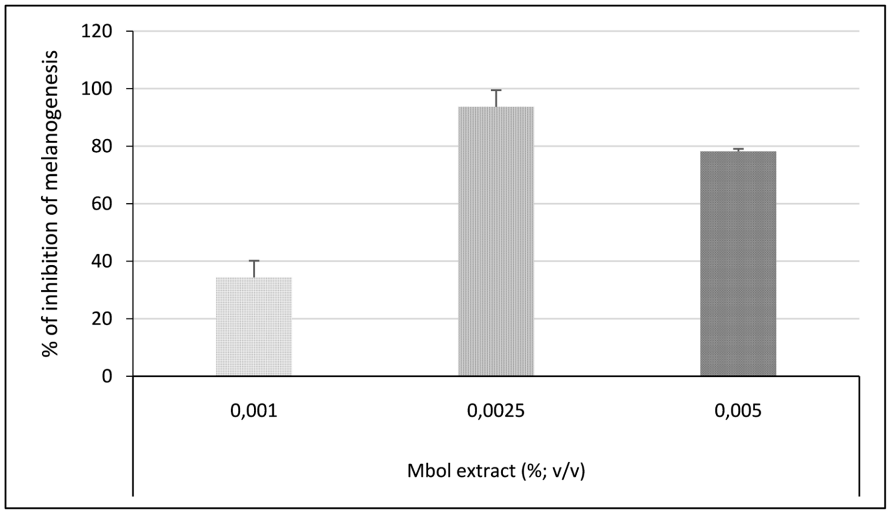
As shown in Figure 5, in the selected experimental conditions, our *Caesalpinia sappan* seeds extract significantly inhibited melanogenesis in a model of human normal melanocytes by 34.4; 93.7 and 78.2% at the following concentrations: 0.001; 0.0025 and 0.005%, respectively.

Reasoning that this effect may logically lead to a visible *in vivo* efficacy, we decided to perform an *in vivo* study calling for healthy volunteers showing dark spots on their faces.

As shown in Figure 6, the use twice a day of a formulation containing 1% of our *Caesalpinia sappan* seeds extract lead to a significant reduction of volunteer's face dark spot surface: −22.2%.

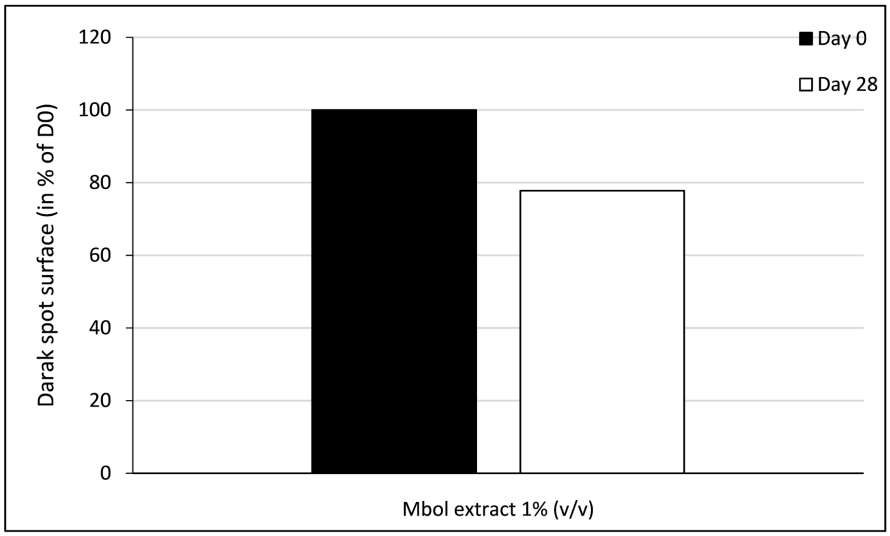
4. Conclusions

Taken together, our study results unambiguously show that *Caesalpinia sappan* seeds not only display beneficial effects on human health through alimentary



: Statistically different from the "not treated" condition ($p < 0.01$); One Way ANOVA analysis + Holm-Sidak test. *: Statistically different from the "not treated" condition ($p < 0.001$); One Way ANOVA analysis + Holm-Sidak test.

Figure 5. *Caesalpinia sappan* seeds extract effect on melanogenesis.



*****: Statistically different from the "not treated" condition ($p < 0.00001$); Paired Student t-tests.

Figure 6. Skin dark spot surface modification after treatment with a formulation containing 1% (v/v) of *Caesalpinia sappan* seeds extract.

tract, but could also benefit to other human organs such as skin. 4-methyl catechol content of our *Caesalpinia sappan* seeds extract seems to be largely implicated in its anti-oxidant effects and in its regulatory activity on inflammatory processes. The ability of this compound to chelate divalent cations is clearly responsible, at least in part, for the effects of our *Caesalpinia sappan* seeds extract on tyrosinase activity and human melanogenesis.

Caesalpinia sappan seeds extracts could so today consist in a very efficient tool to notably act on melanogenesis disorders and its esthetic impacts, but could also

help to preserve cutaneous stem cells capital in order to reach a younger and healthier skin, as suggested by our *in vitro* study.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Sasongko, H., Hedianti, D.P. and Safitri, L.I. (2024) *Caesalpinia sappan* Reduce Fever with an Anti-Inflammatory and Antioxidant Mechanism: A Review. *JPSCR*: *Journal of Pharmaceutical Science and Clinical Research*, **9**, 118-139. https://doi.org/10.20961/jpscr.v9i1.79562

[2] Konečný, L., Lomozová, Z., Karabanovich, G., Roh, J., Vávrová, K. and Mladěnka, P. (2024) Rapid Method for Screening of Both Calcium and Magnesium Chelation with Comparison of 21 Known Metal Chelators. *JBIC Journal of Biological Inorganic Chemistry*, **29**, 785-800. https://doi.org/10.1007/s00775-024-02078-6

[3] Wojcieszyńska, D., Hupert-Kocurek, K., Jankowska, A. and Guzik, U. (2012) Properties of Catechol 2,3-Dioxygenase from Crude Extract of Stenotrophomonas Maltophilia Strain KB2 Immobilized in Calcium Alginate Hydrogels. *Biochemical Engineering Journal*, **66**, 1-7. https://doi.org/10.1016/j.bej.2012.04.008

[4] Rouzer, C.A. and Samuelsson, B. (1987) Reversible, Calcium-Dependent Membrane Association of Human Leukocyte 5-Lipoxygenase. *Proceedings of the National Academy of Sciences of the United States of America*, **84**, 7393-7397. https://doi.org/10.1073/pnas.84.21.7393

[5] Solano, F. (2018) On the Metal Cofactor in the Tyrosinase Family. *International Journal of Molecular Sciences*, **19**, Article 633. https://doi.org/10.3390/ijms19020633

[6] Meunier, M., Bracq, M., Chapuis, E., Lapierre, L., Humeau, A., Bernard, S., *et al.* (2023) Targeting SDF-1 as an Efficient Strategy to Resolve Skin Hyperpigmentation Issues with *Himanthalia elongata* Extract. *Journal of Cosmetic Dermatology*, **22**, 383-394. https://doi.org/10.1111/jocd.15357

[7] Gulcin, İ. (2020) Antioxidants and Antioxidant Methods: An Updated Overview. *Archives of Toxicology*, **94**, 651-715. https://doi.org/10.1007/s00204-020-02689-3

[8] Goodell, M.A., Brose, K., Paradis, G., Conner, A.S. and Mulligan, R.C. (1996) Isolation and Functional Properties of Murine Hematopoietic Stem Cells That Are Replicating *in Vivo*. *The Journal of experimental medicine*, **183**, 1797-1806. https://doi.org/10.1084/jem.183.4.1797

[9] Durand, R.E. and Olive, P.L. (1982) Cytotoxicity, Mutagenicity and DNA Damage by Hoechst 33342. *Journal of Histochemistry & Cytochemistry*, **30**, 111-116. https://doi.org/10.1177/30.2.7061816

[10] Nakai, K. and Tsuruta, D. (2021) What Are Reactive Oxygen Species, Free Radicals, and Oxidative Stress in Skin Diseases? *International Journal of Molecular Sciences*, **22**, Article 10799. https://doi.org/10.3390/ijms221910799

[11] Krieg, P. and Fürstenberger, G. (2014) The Role of Lipoxygenases in Epidermis. *Biochimica et Biophysica Acta* (*BBA*)—*Molecular and Cell Biology of Lipids*, **1841**, 390-400. https://doi.org/10.1016/j.bbalip.2013.08.005

[12] Xue, T., Lu, Z., Zhang, W., Wang, Z., Shi, Y., Jiang, H., *et al.* (2022) Facial Erythema Due to Lupus Vulgaris and *Candida albicans* Infections: A Case Report. *Clinical*, *Cosmetic and Investigational Dermatology*, **15**, 1397-1402. https://doi.org/10.2147/ccid.s372359

[13] Scherdin, U., Bürger, A., Bielfeldt, S., Filbry, A., Weber, T., Schölermann, A., *et al.* (2008) Skin-Lightening Effects of a New Face Care Product in Patients with Melasma. *Journal of Cosmetic Dermatology*, **7**, 68-75. https://doi.org/10.1111/j.1473-2165.2008.00364.x

[14] Elvebakken, H.F., Christensen, I.B., Vedel, C. and Kjærulff, S. (2024) A Proof of Concept: Clinical Anti-aging Efficacy and Safety of *Lactiplantibacillus plantarum* LB244R® Applied Topically in a Double-Blinded Placebo-Controlled Study. *Journal of Cosmetic Dermatology*, **23**, 1233-1242. https://doi.org/10.1111/jocd.16138

[15] Bradford, M.M. (1976) A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Analytical Biochemistry*, **72**, 248-254. https://doi.org/10.1016/0003-2697(76)90527-3

[16] Lemaitre, J. (2024) Looking for the Philosopher's Stone: Emerging Approaches to Target the Hallmarks of Aging in the Skin. *Journal of the European Academy of Dermatology and Venereology*, **38**, 5-14. https://doi.org/10.1111/jdv.19820

[17] Kim, S.Y., Kim, T., Moon, K., Kim, T.J., Shin, D., Cho, Y.S., *et al.* (2008) Regulation of Pro-Inflammatory Responses by Lipoxygenases via Intracellular Reactive Oxygen Species *in Vitro* and *in Vivo*. *Experimental and Molecular Medicine*, **40**, 461-476. https://doi.org/10.3858/emm.2008.40.4.461

[18] Smelly, M. and Hawkey, C. (2003) Cox-Lox Inhibition: Current Evidence for an Emerging New Therapy. *International Journal of Clinical Practice*, **57**, 301-304. https://doi.org/10.1111/j.1742-1241.2003.tb10489.x

[19] Hammarberg, T., Reddy, K.V., Persson, B. and Rádmark, O. (2002) Calcium Binding to 5-Lipoxygenase. In: Honn, K.V., Marnett, L.J., Nigam, S., Dennis, E. and Serhan, C., Eds., *Eicosanoids and Other Bioactive Lipids in Cancer, Inflammation, and Radiation Injury*, 5., Springer, 117-121. https://doi.org/10.1007/978-1-4615-0193-0_19

[20] Sharma, H.K. and Vaidyanathan, C.S. (1975) A New Mode of Ring Cleavage of 2,3-dihydroxybenzoic Acid in *Tecoma stans* (L.). *European Journal of Biochemistry*, **56**, 163-171. https://doi.org/10.1111/j.1432-1033.1975.tb02219.x

[21] Rinnerthaler, M., Bischof, J., Streubel, M., Trost, A. and Richter, K. (2015) Oxidative Stress in Aging Human Skin. *Biomolecules*, **5**, 545-589. https://doi.org/10.3390/biom5020545

[22] Kehrer, J.P. (2000) The Haber-Weiss Reaction and Mechanisms of Toxicity. *Toxicology*, **149**, 43-50. https://doi.org/10.1016/s0300-483x(00)00231-6