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Anti-aging

Acer rubrum Bark Extract, the new Natural and Eco-responsible Anti-ageing IngredientObtained from a Coproduct of the Canadian Forest Industry

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ABSTRACT: Forest industry produces large amounts of coproducts such as barks that can be valorized through the extraction of bioactive molecules commonly called "extractives". The Canadian boreal forest biomass is a great sustainable source of new natural compounds that can be used to help maintaining or restoring skin health and fight against skin ageing. Red maple *(Acer rubrum)* is a famous Canadian tree species which has been widely used by native people for its medicinal properties. Its bark contains a high level of polyphenols that have already shown to be powerful antioxidants.

This study demonstrates that red maple bark extract is a new natural anti-ageing active with procollagen and proelastin properties. Indeed, this extract stimulates dermal regeneration by increasing collagen production while inhibiting collagenase enzyme synthesis that degrades collagen with age. Moreover, red maple bark extract increases elastin synthesis. Clinical studies reveal the complete and long-term anti-ageing efficacy of this polyphenolic extract with measured wrinkle amplitude reduction as well as visible effects on women proved by pictures before and after treatment with a cream containing the extract at 0.25 %. Moreover, red maple extract improves significantly several biomechanical parameters of the skin after only 28 days' use of the cream.

Introduction

The process of skin ageing is linked to intrinsic and extrinsic parameters [1]. Intrinsic skin ageing is the natural phenomenon caused by internal physiological factors leading to a loss of skin elasticity and a slowdown of metabolic activity. Extrinsic skin ageing is a result of environmental factors such as smoking, sun exposure, pollution, etc which increase the natural ageing process. Indeed, it leads to a destruction and loss of collagen (which provides skin firmness), of elastin (which supplies skin elasticity and rebound) and glycosaminoglycans (which keep the skin hydrated) and the appearance of roughness, uneven tone, brown spots, thin skin and deep wrinkles [2]. Although destruction of existing collagen is central to the deleterious changes observed in aged skin, failure to replace damaged collagen with newly synthesized material is also critical to the overall pathophysiology [3]. Indeed, the collagen content of skin is the net balance between collagen synthesis and collagen breakdown. With ageing, the synthesis of collagen decrease in human tissues and external factors stimulate the production of collagen-degrading enzymes. Collagenase is

one of the key collagen-degrading enzymes and considered as the main cause of skin ageing [4]. Direct inhibition of collagenase by plant compounds has been discovered as an effective approach to mitigate collagen breakdown in the skin [5]. When applied topically, plant extracts have also shown interesting properties for skin health. Several studies have highlighted the effect of natural compounds on dermal regeneration, reduction of wrinkles, etc. [6].

Trees, so far used for the production of lumber, pulp and paper, produce a large variety of molecules of secondary metabolites to fight against external aggressions such as phenolic compounds, terpenoids, saponins and alkaloids. These metabolites are easily extracted (hence their name, extractives) without severe treatments [7]. Their content and nature depend on tree species, part of the tree, geographical site of growth, genetics, harvest seasons and even the age of the tree. Extractives are responsible for the colour [8-10], the smell [11-13] and sometimes for their exceptional mechanical properties [14, 15]. According to their broad spectrum of biological and physical properties as well as their various chemical structures, multiple applications can be considered including skin care.

Also known as the Canadian Maple, the Red Maple tree *(Acer rubrum L.)* grows mainly in the Acadian peninsula, the Great Lakes area, the St-Lawrence valley and belongs to the Newfoundland boreal forest. Red Maple was widely used by Native Americans as a medicinal plant [16]. Bark infusion was used to treat various pains and diseases and was considered as a blood purifying medicine [17]. Red maple tree has also been recently added to the list of indigenous medicinal vascular plants of Quebec. However, few studies on the biological properties of Red Maple bark extracts have been performed so far. Two studies showed that bark and wood extracts presented antimicrobial/antibiotic and antifungal activity on several bacteria and pathogenic fungi responsible for human diseases and infections [18, 19]. A more recent study investigated the ROS/RNS scavenging activity of the extracts from various parts of A. rubrum including bark [20]. The various classes of polyphenols, along with their radical scavenging capacities examined by six *in vitro* tests, have been determined for the studied extract. Some years later, the same research group found that Red Maple Bark Extract (RMBE) can inhibit tyrosinase and elastase and demonstrates antimicrobial activity [21].

The high resistance of red maple's bark during all seasons in Quebec, its traditional medicinal uses, its high content in polyphenols and its great antioxidant action has inspired Bio ForeXtra laboratories to develop Borealine® Expert, a Red Maple Bark Extract (RMBE) for cosmetic applications to protect human skin from external aggressions. The barks are collected directly from Quebec sawmills as a coproduct of lumber production.

This present study showed that Borealine® Expert is a powerful active with a targeted action on different biological mechanisms involved in skin ageing. It guarantees great efficacy to stimulate the natural dermal regeneration of collagen and elastin, as well as powerful antioxidant and anti-inflammatory activities to fight oxidative stress and premature ageing. Finally, this new green active has also highlighted *in vivo* by a clinical study an improvement in skin elasticity and firmness, in addition to a long-term anti-wrinkle effect.

Materials

Red maple bark

Barks were collected at a Quebec sawmill that harvests red maple bark in a specific Quebec

region during a specific season. This sawmill is certified by Quebec government for its eco-responsible forest management.

Red Maple bark extract

RMBE is obtained by aqueous extraction of red maple bark *(Acer rubrum)*. Dried native extract is then dissolved in glycerin at a specific concentration to get a standardized extract in total phenol contents. *In vitro* testings were carried out on dried native extract powder.

RMBE formula for *in vivo* clinical studies

RMBE formula corresponds to a white basic cream containing Borealine Expert® at 0.25 %.

Methods

In vitro measurement of the collagenase inhibition

The evaluation of RMBE effect on collagenase inhibition was carried out using the standard method EnzChek Gelatinase/Collagenase (Molecular Probes). The extract was diluted in a buffer solution (0.5 M Tris-HCl, 1.5 M NaCl, 50 mM $CaCl_2$ and 2 mM sodium azide; pH 7.6). 1,10-phenanthroline monohydrate (Sigma 320056) was used as positive control. Extract (25, 50, 100 and 200 µg/mL) and positive control (18 µg/mL) were placed in a 96-well plate (80 uL). Collagen type 1 conjugated to fluorescein (20 µL) (MolecularProbes, D-12060) was added to all wells. Collagenase clostridium (Sigma, C0130) was also diluted in the reaction buffer to a final concentration of 0.1 U/mL and was added to the extract and positive control solutions (100 µL). The buffer solution was also added as a blank. The plate was then incubated at room temperature away from light for 2 hours. The fluorescence (λ excitation: 495 nm, λ emission: 515 nm) was measured using a VARIOSCAN fluorometer. Collected data were transferred to SigmaStat 3.5 software. The intergroup comparisons were performed using the Wilcoxon signed rank test ($P < 0.05$).

In vitro measurement of collagen production

The effect of RMBE on collagen production was evaluated using specific antibodies to type I Collagen. Once bound to collagen, the primary antibody is detected by a secondary antibody onto which a fluorophore is grafted. Human skin fibroblasts (WS1, ATCC CRL-1502) were seeded in 96-well microplates in a full decomplemented culture medium and incubated overnight at 37 °C and 5 % CO_2 to allow their adherence. Thereafter, the cells were incubated for 24 hours in the absence or presence of increasing concentrations of extract (12.5, 25, 50 and 100 µg/ml). The culture medium containing the extract was then removed and the cells were fixed with 95 % ethanol for 10 minutes. The cells were washed three times with PBS and permeabilized with 0.5 % Triton solution in PBS for 15 minutes. The cells were then incubated with the primary antibody (anti-collagen Calbiochem # 234167) in solution 1/50 in 3 % BSA overnight at 4 °C. After three washes with PBS, the secondary antibody (Cy™ 2 AffiniPure Goat Anti-Mouse IgG, Jackson ImmunoResearch, Inc. Laboratories. #115-225-003) was added in solution 1:50 in 1X PBS for an hour. The secondary antibody was then removed with three washes with PBS. The fluorescence emitted by the secondary antibody was then measured and pictures were taken with a fluorescence microscope Cytation3. Collected data were transferred in SigmaStat 3.5 software. The intergroup comparisons were performed using the Wilcoxon signed rank test ($P < 0.05$).

In vitro measurement of elastin production

The effect of RMBE on stimulating elastin production was assessed using specific antibodies elastin (anti-elastin, #21610, Abcam). Once bound to elastin, the primary antibody is detected by a secondary antibody to which is

grafted a fluorophore. Human skin fibroblasts (WS1, ATCC CRL-1502) were seeded in 96-well microplates in the middle of fully decomplemented culture and incubated overnight at 37°C and 5% CO_2 to allow their adherence. Thereafter, the cells were incubated for 24 hours in the absence or presence of increasing concentrations of extract solutions (12.5, 25, 50 and 100 µg/mL). The culture medium containing the extract solutions was then removed and the cells were fixed with 95 % ethanol for 10 minutes. The cells were washed three times with PBS and permeabilized with 0.5 % Triton solution in PBS for 15 minutes. The cells were then incubated with the primary antibody solution 1/50 in 3 % BSA overnight at 4°C. After three washes with PBS, the secondary antibody (Cy™ 2 AffiniPure Goat Anti-Mouse IgG, Jackson ImmunoResearch, Inc. Laboratories. #115-225-003) was added in solution 1:50 in 1X PBS for an hour. The secondary antibody was then removed with three washes with PBS. The fluorescence emitted by the secondary antibody was then measured and pictures were taken with a fluorescence microscope Cytation3. Collected data were transferred in SigmaStat 3.5 software. The intergroup comparisons were performed using the Wilcoxon signed rank test ($P<0.05$).

In vivo biometrological evaluation of the anti-ageing efficacy

RMBE formula was evaluated thanks to a clinical study lasting 56 days regarding its anti-ageing efficacy. The studied population was composed of 17 women, aged from 50 to 65 years with wrinkles and fine lines on the crow's feet grade from 4 to 6 according to Bazin® scale. Each subject applied the cream on the face twice daily.

In vivo measurements of anti-wrinkle effect

RMBE formula anti-wrinkle efficacy was measured with the 3D PRIMOS® Compact System at D0, D14 and D28. The maximum relief amplitude was performed by measure of the maximum height of the roughness profile. The parameter studied Rt is the difference from the highest peak to the deepest point within the total measurement section at time D14 and D28 compared to D0. A decrease in Rt characterises an anti-wrinkles effect. In the population targeted, 15 women completed the study. Pictures were taken with the Visia® system at D0, D14, D28 and D56 to see the effects of the formula on reducing wrinkles.

In vivo measurements of skin biomechanical properties

RMBE formula efficacy on the biomechanical properties of the skin was evaluated with Cutometer® on the following rheological parameters determining the viscoelastic properties of the skin:

- Uv: delayed elongation (viscoelastic component)
- Uf: final deformation (total deformation : elastic and viscoelastic)
- Ue: immediate elongation (purely elastic component)
- Ur: immediate withdrawal (tonicity)
- Ua: total recovery of the initial state

The analysed biomechanical parameters are the following:

Firmness

- R0 (Uf): A reduction of this parameter features a firming effect and a stronger skin.

Elasticity

- R2 (Ua/Uf): This parameter refers to the skin total elasticity. It is used along with R7 in order to evaluate the skin elasticity and ageing. As the value increases, skin is more elastic.
- R5 (Ur/Ue): This parameter refers to the net elasticity of the skin. R5 is a selection

parameter which quantifies the skin's ageing process. It represents the skin's ability to regain its shape after a deformation due to its elasticity, and that, independently of the skin thickness. This recuperation diminishes with age. Therefore, as the value increases, skin gains elasticity.

- R7 (Ur/Uf): This parameter refers to the skin's biological elasticity and its capacity to regain its initial position after deformation. Thus, as the value increases, skin is more elastic.

An increase of either one of these three parameters features improved skin elasticity and demonstrates the ability Boréaline Expert® has to diminish skin ageing signs.

- R1 (Uf-Ua) : elastic recuperation

A decrease of this parameter indicates skin elasticity improvement.

In the population targeted, 15 women completed this part of the study. The measures performed to D14 and D28 were compared to D0.

In vitro measurement of the collagenase inhibition

Fig. 1 shows the dose-dependent effect of RMBE on collagenase inhibition. At 100 µg/mL, RMBE inhibits 62 % of collagenase with a significative effect. Moreover, the inhibition efficacy of RMBE was greater than the positive control 1-10 phénantroline at 18 µg/ml.

The collagenase was incubated both with and without the presence of increasing concentrations of the extract. The results are expressed as a percentage of the collagenase activity inhibition compared with the enzyme only. The 1-10 phenantroline was used as a positive control. The data is significantly different from enzyme+substrate only; $P < 0.05$; Wilcoxon signed Rank Test (SigmaStat 3.5).

In vitro measurement of collagen production

Fig. 2 shows a dose-dependent effect of RMBE on collagen synthesis in human fibroblast with an increase of 100 % at 100 µg/mL. TGF-β (10 ng/mL) was used as positive control with a significant average increase of only 56%.

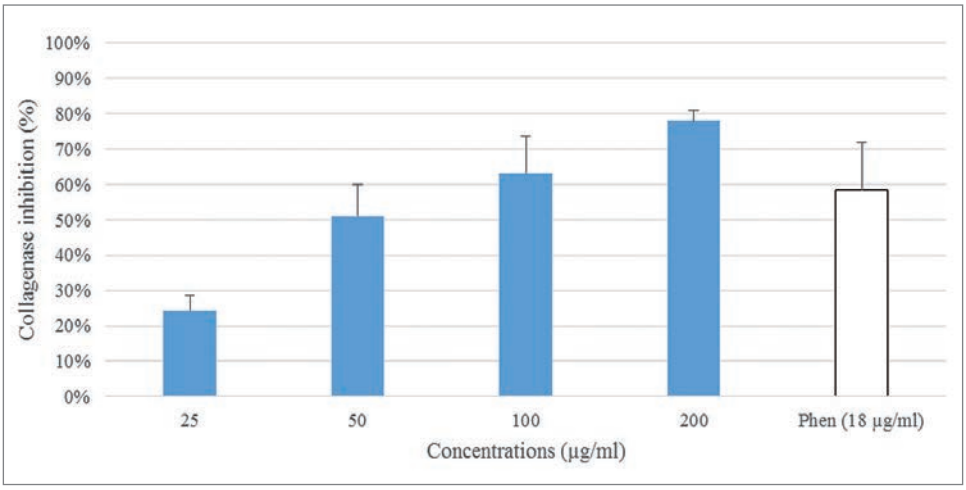

Fig. 1 Collagenase inhibition by Red Maple bark extract.

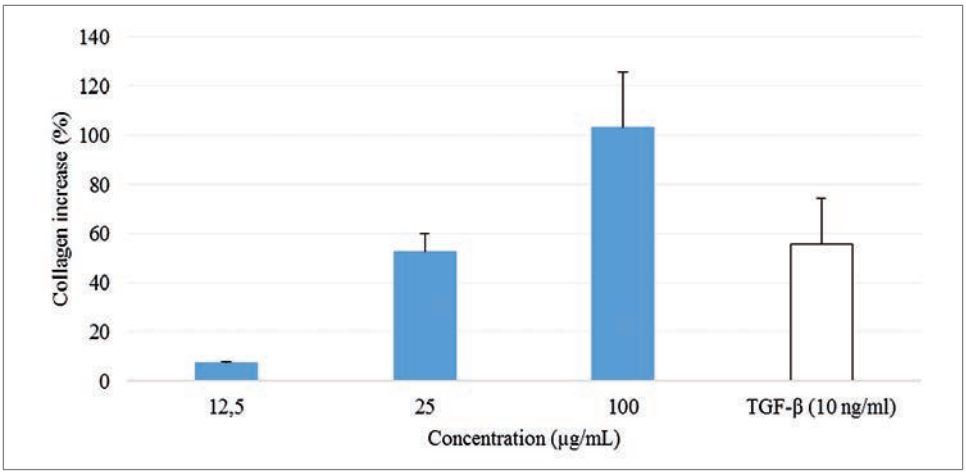

Fig. 2 Effect of RMBE on collagen production.

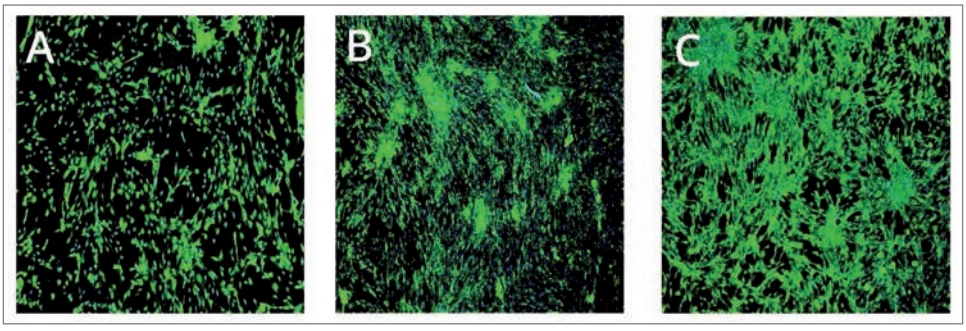

Fig. 3 Effect of RMBE on collagen stimulation.

Skin fibroblasts were incubated both with and without the presence of increasing concentrations of the extract. The cells were then fixed and incubated with an antibody against type 1 collagen. The results are expressed as a percentage of the collagen increase comparatively to untreated cells. TGF-β was used as a positive control. The data is significantly different from untreated cells; $P < 0.05$; Wilcoxon signed Rank Test (SigmaStat 3.5).

Fig. 3 highlights a high efficacy on collagen synthesis of RMBE at 100 µg/mL after 24 h. Clear collagen clusters can be observed on the picture B (in presence of RMBE) compared to the positive control (picture C).

Skin fibroblasts were incubated in absence (A) or in presence of 100 µg/mL of the extract (B). The cells were then fixed and incubated with an antibody against type 1 collagen. The cell nuclei was also labeled with DAPI. TGF-β (10 ng/ml) was used as a positive control

In vitro measurement of elastin production

Fig. 4 and 5 show the dose-dependant effect of RMBE on elastin stimulation. At 100 µg/mL, RMBE allows an increase of elastin production of 66 % after 24 h and 100 % after 48 h. The increase in elastin induced by 100 µg/mL of RMBE was visualized by fluorescent mi-

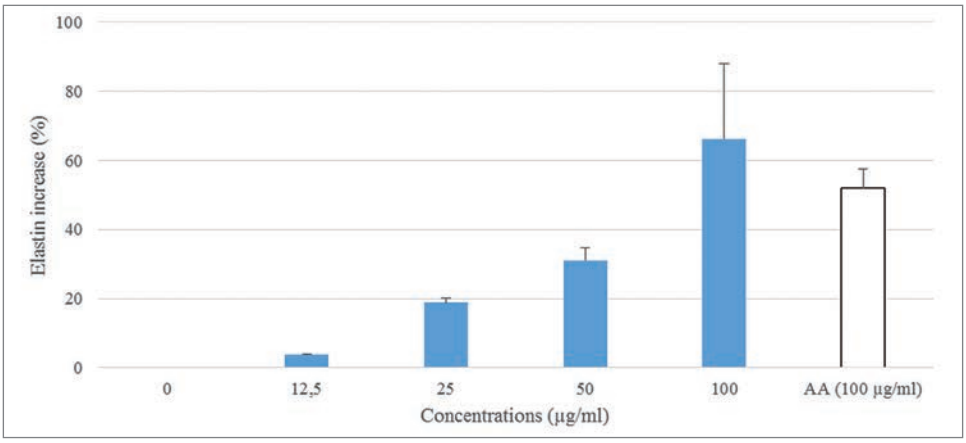

Fig. 4 Effect of RMBE on elastin stimulation after 24 h.

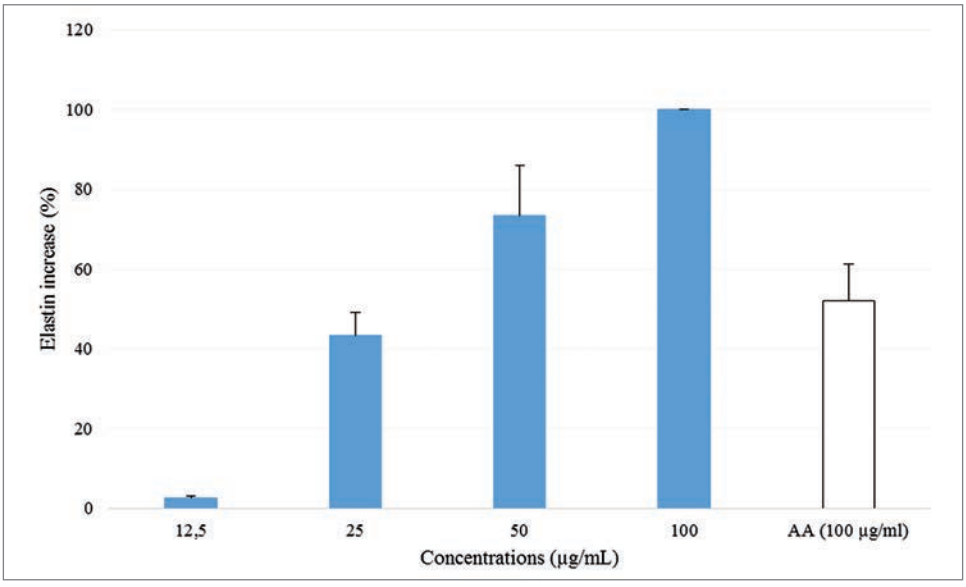

Fig. 5 Effect of RMBE on elastin stimulation after 48 h.

Fig. 6 Effect of RMBE on elastin stimulation.

croscopy. **Fig. 6** shows a higher fluorescence compared to the positive control effect.

Skin fibroblasts were incubated with and without the presence of increasing concentrations of the extract. The cells were then fixed and incubated with an antibody against elastin. The results are expressed as a percentage of the elastin increase after 48 h compared to untreated cells. Ascorbic acid (AA) was used as a positive control. The data is significantly different from untreated cells; $P < 0.05$; Wilcoxon signed Rank Test (SigmaStat 3.5).

Skin fibroblasts were incubated 48 h in absence (A) or in presence of 100 µg/mL of the extract (B). The cells were then fixed and incubated with an antibody against elastin. The cell nuclei was also labeled with DAPI. Ascorbic acid 100 µg/ml (AA) was used as a positive control (C).

Clinical studies: *in vivo* biometrological evaluation of the anti-ageing efficacy

In vivo measurements of the anti-wrinkle effect

The anti-wrinkle effect of RMBE formula on the skin surface parameter Rt representing the maximum amplitude relief was presented

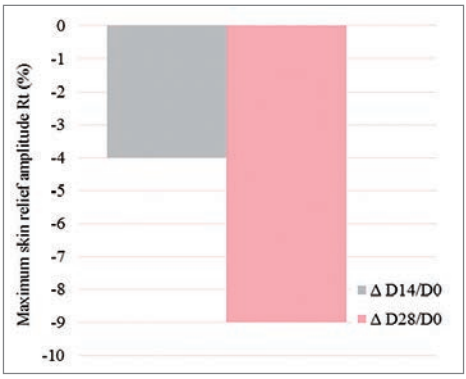

Fig. 7 Wrinkle efficacy of RMBE Formula.

in **Fig. 7**. After one month of using RMBE formula, Rt parameter has diminished significantly (9 %), deep wrinkles are corrected and smoothed compared with skin before treatment. This effect was observed on 73 % of the women.

It is important to point out that, besides the significant effect measured at day 28 by the 3D Primos, the anti-wrinkle efficacy of the cream containing Borealine Expert® was noticeable in the participating women on day 56. An average of 84 % of these women answered positively to questions about the anti-wrinkle on day 56. Pictures presented in **Fig. 8** show that wrinkles around the eye (crow's feet) were reduced after only one

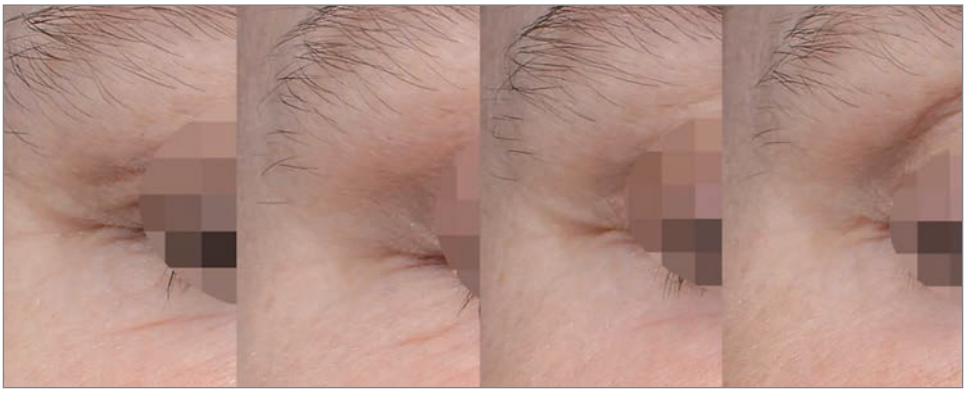

Fig. 8 Effect of Formula RMBE on wrinkles.

month of treatment and it continues after two months of use.

In vivo measurements of the skin biomechanichal properties

Positive effects on the skin's biomechanical properties show after only one month of using Borealine Expert® cream. Indeed, results indicate a significant improvement on all measured parameters. Skin firmness (R0) has significantly improved by 13 % after only 28 uses. This effect is observed on 82 % of women. The biological elasticity (R2) has also significantly improved by 18 % after 28 days. This was observed on 71 % of women. The net elasticity (R5) has significantly improved by 36 % after 28 days. This effect is observed on 82 % of women. The raw elasticity (57) has significantly improved by 49 % after 28 days. This effect is observed on 88 % of women. Elasticity (R1) is reduced by 31 % after using the cream for 28 days. This effect is observed on 71 % of women. Plasticity is reduced by 25 % after 28 consecutive days. This effect is observed on 65 % of women **(Fig. 9)**.

Discussion and Conclusion

Collagen makes up 70-80 % of the dry weight of the skin and gives the dermis its mechanical and structural integrity. Although destruction of existing collagen is, undoubtedly, central to the deleterious changes in aged skin, failure to replace damaged collagen with newly synthesized material is also critical to the overall pathophysiology [3].

Thus, a dual action on collagen production as well as on collagen degradation by MMP enzymes such collagenase 1 inhibitor is crucial. The present study has demonstrated that red maple bark extract is a powerful procollagen agent acting on both biological mechanisms.

In addition to collagen, elastin is a minor component of the dermis, but it also has an important function in providing the elasticity of the skin [22]. Synthetized by fibroblasts essentially during the growth period, it decreases with age and is replaced by inextensible collagen. The ageing process is engaged and

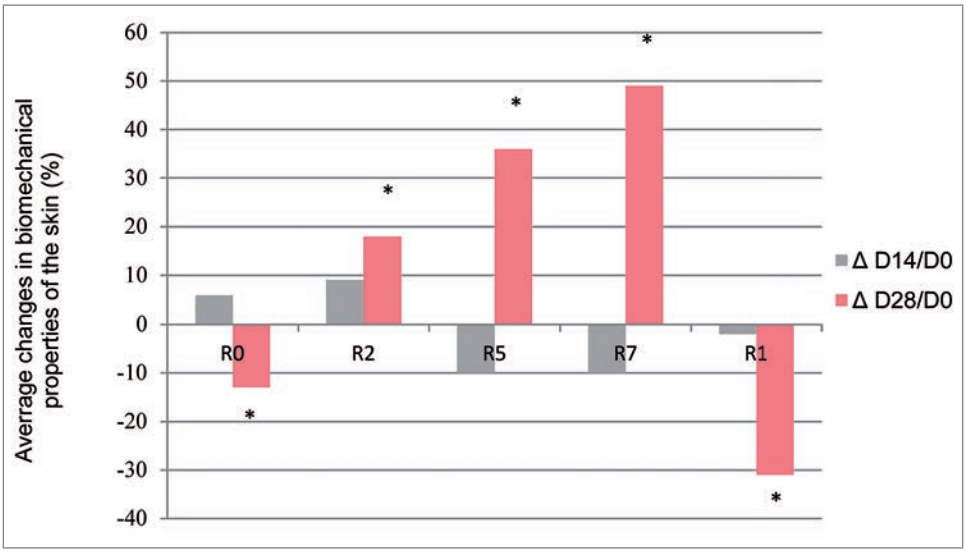

Fig. 9 Effect of RMBE Formula on the biomechanical properties of the skin.

wrinkles appear. Red maple bark extract acts as a great stimulator of elastin synthesis with an increase of 100 % after 48 h on cell model. The dual action of RMBE that helps to preserve the natural collagen in the skin as well as increase the production of natural elastin is the main biological mechanism that can explain its anti-ageing effect. Indeed, *in vivo* studies have shown a clear improvement in skin firmness, elasticity and plasticity, via measurements of various biomechanical parameters of the skin. During the clinical study, we observed a significant decrease in Rt parameter after only 28 days of use demonstrating a quick anti-wrinkle efficacy of the cream formulated with RMBE at 0.25 %. Pictures of women's faces before and after RMBE formula treatment highlight the visible anti-wrinkle effect with a decrease of crow's feet appearance and a long-term efficacy even after a two-month use of the formula.

In conclusion, this study demonstrates an innovative way to valorise the forest biomass through high added-value application of actives that are easily extracted using green solvents and eco-responsible technologies. Borealine Expert® is a global anti-ageing active that mitigates significantly oxidative stress and inflammageing while improving skin quality, firmness, elasticity and tone.

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References

[1] *Gilchrest B.A.,* (1996) A review of skin ageing and its medical therapy. Br J Dermatol 135 (6), 867-875.

[2] *Uitto J., Bernstein E.F.,* (1998) Molecular Mechanisms of Cutaneous Aging: Connective Tissue Alterations in the Dermis. Journal of Investigative Dermatology Symposium Proceedings 3 (1), 41-44.

[3] *Varani J., Dame M.K., Rittie L., Fligiel S.E.G., Kang S., Fisher G.J. et al.,* (2006) Decreased Collagen Production in Chronologically Aged Skin: Roles of Age-Dependent Alteration in Fibroblast Function and Defective Mechanical Stimulation. The American Journal of Pathology 168 (6), 1861-1868.

[4] *Fisher G.J., Kang S., Varani J. et al.,* (2002) Mechanisms of photoaging and chronological skin aging. Arch Dermatol 138 (11), 1462-1470.

[5] *Mukherjee P.K., Maity N., Nema N.K., Sarkar B.K.,* (2011) Bioactive compounds from natural resources against skin aging. Phytomedicine 19 (1), 64-73.

[6] *Svobodova A., Psotová J., Walterová D.,* (2003) Natural phenolics in the prevention of UV-induced skin damage. A review. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 147 (2), 137-145.

[7] *Anderson A.B.,* (1955) Recovery and utilization of tree extractives. Economic Botany 9 (2), 108-140.

[8] *Pandey K.K.,* (2005) A note of the influence of extractives on the photo-discoloration and photo-degradation of wood. Polym Degrad Stab 87, 375-379.

[9] *Dellus V., Scalbert A., Janin G.,* (1997) Polyphenols and colour of Douglas fir heartwood. Holzforschung 51 (4), 291-295.

[10] *Diouf P.-N., Merlin A., Perrin D.,* (2006) Antioxidant properties of wood extracts and colour

stability of woods. Annals of Forest Science 63, 525-534.

[11] *von Rudloff E.,* (1987) The Volatile Twig and Leaf Oil Terpene Compositions of Three Western North American Larches, Larix laricina, Larix occidentalis, and Larix lyallii. Journal of Natural Products 50 (2), 317-321.

[12] *Amusant N., Moretti C., Richard B., Prost E., Nuzillard J.M., Thévenon M.F.,* (2007) Chemical compounds from *Eperua falcata* and *Eperua grandiflora* heartwood and their biological activities againstwood destroying fungus (Coriolus versicolor). Holz Roh Werkst 65, 23-28.

[13] *Srinivasan S., Taylor A., Cooper P.A.,* (1998) Natural durability of tamarack (Larix laricina (Du Roi) K. Koch). Document – International Research Group on Wood Preservation (No. IRG/WP/98-0277) 5 pp.

[14] *Choong E.T., Achmadi S.S.,* (1991) Effect of extractives on moisture sorption and shrinkage in tropical woods. Wood and Fiber Science 23 (2), 185-196.

[15] *Hernandez R.E.,* (2007) Swelling properties of hardwoods as affected by their extraneous substances, wood density, and interlocked grain. Wood and Fiber Science 39 (1), 146-158.

[16] *Arnason T., Hebda R.J., Johns T.,* (1981) Use of plants for food and medicine by Native Peoples of eastern Canada. Can J Bot 59, 2189-2325.

[17] *Moerman D.,* ed. Medicinal Plants of Native America. ed. A. Arbor. Vol. 1 & 2. 1986, University of Michigan Museum of Anthropology.

[18] *Omar S., Lemonnier B., Jones N., Ficker C., Smith M.L., Neema C. et al.,* (2000) Antimicrobial activity of extracts of eastern North American hardwood trees and relation to traditional medicine. J Ethnopharmacol 73 (1-2), 161-170.

[19] *Ficker C.E., Arnason J.T., Vindas P.S., Alvarez L.P., Akpagana K., GbÃ©assor M. et al.,* (2003) Inhibition of human pathogenic fungi by ethnobotanically selected plant extracts. Mycoses 46 (1-2), 29-37.

[20] *Royer M., Diouf P.N., Stevanovic T.,* (2011) Poly phenol contents and radical scavenging capacities of red maple *(Acer rubrum L.)* extracts. Food Chem Toxicol.

[21] *Royer M., Prado M., García-Pérez M.E., Diouf P.N., Stevanovic T.,* (2013) Study of nutraceutical, nutricosmetics and cosmeceutical potentials of polyphenolic bark extracts from Canadian forest species. PharmaNutrition 1 (4), 158-167.

[22] *Oikarinen A.,* (1994) Aging of the skin connective tissue: how to measure the biochemical and mechanical properties of aging dermis. Photodermatol Photoimmunol Photomed 10 (2), 47-52.

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