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Ocimum Tenuiflorum

Tulsi Bio Extractive®

Ayurvedic Power against Urban Pollution

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My plant extracts, naturally

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1 Summary

In India, Tulsi, also known as Holy Basil, is highly revered in the Ayurvedic medicinal system and supposed to help against various diseases due to its attributed antibacterial, antioxidant and anti-inflammatory properties. Tulsi is well-known in Southeast Asia as tea or spice but is also getting popular in the US and Europe .

Tulsi is *Ocimum tenuiflorum*, a synonym is *Ocimum sanctum*. In cosmetics, the INCIs Ocimum Tenuiflorum Extract and Ocimum Sanctum Leaf Extract both are known in the US, Japan and EU. The INCI Ocimum Sanctum Leaf Extract is also known in China.

Botanica let cultivate the Tulsi herb in Switzerland organic certified and manufactured the Tulsi Bio Extractive® using an in-house developed gentle extraction process which provides a concentrate of the extract.

HPTLC analysis identified rosmarinic acid as one of the main active compounds in the Tulsi Bio Extractive®. In addition, the antioxidant capacity and the UV absorbance of the Tulsi Bio Extractive® were analyzed. The results support its potential as cosmetic ingredient with antioxidant and UV protection properties.

Based on that properties, an *ex vivo* study with living human skin explants was conducted to determine the anti-pollution activity of two different concentrations of the Tulsi Bio Extractive®, i.e., 3% (C1) and 12% (C2). With both concentrations, the overexpression of Nrf2 (a marker for oxidative stress) was reduced. The reduction was stronger with C2 leading to Nrf2 levels of skin which was not exposed to pollutants at all. In addition, a reduction of the dosage of MDA (a product of lipid peroxidation due to oxidative stress) was shown but only significant for C2. C2 also showed a slight protective activity regarding cell viability.

Treatment with Tulsi Bio Extractive® C1 and C2 only (without pollution) showed a Nrf2 activation along with a reduction of MDA.

These results indicate that the Tulsi Bio Extractive® is a good anti-pollution agent and a protective aid against oxidative stress in general. It prepares the skin against oxidative stress via Nrf2 activation and finally leads to a reduction of the Nrf2 overexpression after pollutants exposure going along with reduced lipid peroxidation of the skin.

2 General Information

2.1 Origin

Indian Basil *Ocimum tenuiflorum (*synonym *Ocimum sanctum*), commonly known as **Tulsi**, Tulasi or **Holy Basil**, is a flowering perennial herb of the **family Lamiaceae**, indigenous to the Indian subcontinent and widespread throughout the Southeast Asian tropics, but is nowadays also cultivated in Europe and North America.

2.2 Characteristics

Tulsi is a herbaceous and perennial plant, which is typically 30 - 60 cm tall. Tulsi forms net-like roots, which are usually distributed in all directions. Compared to the well-known Mediterranean basil, the leaves of the Tulsi are serrated at its margins, somewhat darker and somewhat thinner. The leaves can get up to 6 cm long and 4 cm wide. The slightly pointed leaves are covered with fine glandular hairs. Tulsi is up-growing with a slightly brownish to reddish and mostly hairy stem, which lignifies from the base with increasing age. **Tulsi blooms between June and mid-September.** Five to six individual lip blossoms colored pink to purple usually sit on the ears of corn.

2.3 Cultivation

For cultivation, the Tulsi prefers sunny locations preferably sheltered from the wind. Full sunny locations should be avoided. The soil should be able to store moisture and nutrients well and be permeable.

Botanica has the **Tulsi** herb **cultivated in Switzerland** according to **controlled organic cultivation criteria**. In this way a high and continuous quality is guaranteed. After harvesting, the Tulsi herb is gently dried so that the ingredients remain intact.

2.4 Use

In India, Tulsi is highly revered as **medicinal plant** and used in the Ayurvedic medicinal system since thousands of years referred to as “Elixier of Life”. Tulsi is supposed to counter various diseases by attributed antibacterial activity, antianaphlylactic activity, anti-carcinogenic properties, wound healing effect, antioxidant and anti-inflammatory activities etc. [1,2]. Tulsi is asserted also to help against lifestyle-related chronic diseases like diabetes and all kinds of stress e.g. against metabolic stress through normalization of blood pressure or against psychological stress through positive effects on memory and cognitive function and its supposed anxiolytic and anti-depressant properties [3-5].

In cuisine, Tulsi is consumed as **tea or spice** especially in India and Southeast Asia for centuries but getting also more used in the European cuisine by chefs who like to experiment with spices. Usually the leaves are used. As kitchen herb it can be used for meat and fish dishes, rice pasta dishes or stews. Tulsi combines very well with ginger, cumin or chilli. Just like the other types of basil, Tulsi should not be cooked. It is recommended to pluck the leaves into small pieces and stir them into the dish after cooking. In contrast to the well-known Mediterranean basil, Tulsi can also be used dried. However, fresh leaves are slightly more tasty and show a slightly more intense peppery aroma.

For many years, Tulsi extracts are used in cosmetics (see e.g. EU 2006/257). The INCIs Ocimum Tenuiflorum Extract and Ocimum Sanctum Leaf Extract both are known in the US, Japan and EU. The INCI Ocimum Sanctum Leaf Extract is also known in China.

3 Manufacturing the Tulsi Bio Extractive®

The Tulsi Bio Extractive® is a concentrated extract of organic Tulsi herb from Switzerland. The Tulsi Bio Extractive® contains about 80% BIO glycerin and the rest are plant ingredients and water (for more details about composition, see specification).

It is manufactured according to a specific extraction process which was developed at Botanica and provides a **gentle extraction process** and a **concentration of the extract**.

For the production of that so-called 'C'G concentrate, a drug:extract ratio of 1:1 is used. The

manufacturing process consists of two stages. First, the drug is extracted with a circulating ethanol-water mixture. Secondly, the alcohol is evaporated and the plant ingredients are dissolved in a mixture of glycerin and water. The concentrated extract is therefore largely free of alcohol. From each production lot Botanica tests various quality parameters, e.g., appearance, density or the antioxidant capacity of the Tulsi Bio Extractive®. Further, each lot is microbiologically controlled.

4 Active Compounds

4.1 HPTLC Analysis

Tulsi contains several actives, hydrophilic polyphenols like rosmarinic acid and flavonoids and further lipophilic constituents like essential oils [1]. Botanica performed high performance thin liquid chromatography (HPTLC) analyses to determine the active compounds of the Tulsi Bio Extractive®.

4.2 Flavonoids

Flavonoids are widely distributed in plants. Flavonoids are polyphenols on the basis flavan and vary significantly in their chemical structure. In plants, flavonoids often contain one or more sugar residues like glucose, rhamnose, disaccharides etc. These derivatives are called glycosides. Their water solubility increases with the number of mono saccharides. Flavonoids are often yellowish - the Latin word *flavus* means yellow. Flavonoids have the capacity to absorb the most energetic solar wavelengths, i.e., UV-A and UV-B. Furthermore, flavonoids are known as **antioxidants** and **radical scavengers** and have **anti-inflammatory** and **antimicrobial activities** [6,7]. Therefore, flavonoids are used in cosmetics for anti-aging and sun protection products. Figure 1 shows the flavonoid profile of the Tulsi Bio Extractive® determined by HPTLC.

Figure 1. HPTLC Flavonoids of Tulsi Bio Extractive®

4.3 Rosmarinic Acid

As shown in Figure 2, HPTLC analysis identified rosmarinic acid as one of the main active compounds in the Tulsi Bio Extractive®. Rosmarinic acid is a water-soluble phenolic compound and an ester of caffeic acid. The chemical structure of rosmarinic acid is shown in Figure 3. Rosmarinic acid is known for its **antioxidant**, **anti-inflammatory** and **antimicrobial activities** according to in-vitro as well as in-vivo studies [8].

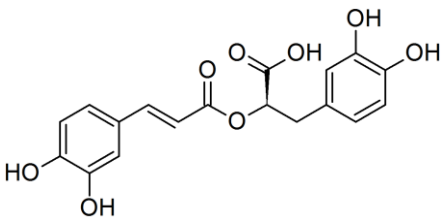

Figure 3. Chemical structure of rosmarinic acid

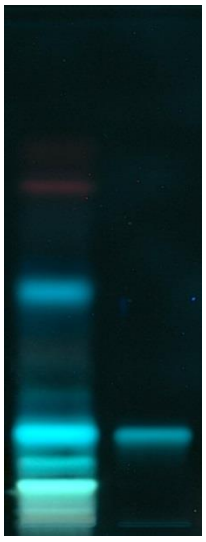

Figure 2. HPTLC Tannins of Tulsi Bio Extractive®

5 Dermatological Activities of Tulsi Bio Extractive®

5.1 Antioxidant Capacity

The antioxidant capacity (AOC) was measured based on Ph.Eur. 2.8.14. Gallic acid was used as reference standard and the AOC results expressed as mass of a polyphenol per sample volume. Since rosmarinic acid was identified as one of the main active compounds in the Tulsi Bio Extractive® and known for its antioxidant activity, the AOC results were expressed as rosmarinic acid .

The Tulsi Bio Extractive® CH 'C'G in Bio Glycerin-water P-00025582 has an antioxidant capacity of minimum 2 g/l expressed as rosmarinic acid.

5.2 UV Absorbance

The UV content of the solar spectrum is only 5%. It mainly consists of UV-A and little UV-B. UV-B is essential for vitamin D synthesis. The rest of the solar spectrum consists of visible light (50%) and infrared (IR), which is mainly Near IR (NIR or IR A). Over the whole spectrum, harmful reactive oxygen and nitrogen species are generated in the skin, partly via chromophores like melanin. Reactive species can create sunburn (mainly caused by UV-B), inflammation, skin aging (photo-aging), DNA damage etc. The same is valid for artificial light including blue light. Antioxidants reduce the amount of reactive species. Therefore, besides UV filter, antioxidants are recommended for dermo protection [9].

The UV absorbance spectrum of 0.2% Tulsi Bio Extractive® (diluted in water) was measured at Botanica and is shown in Figure 4. Based on the UV absorbance measured it is concluded that the Tulsi Bio Extractive® can be used in cosmetics for **anti-aging** and **sun protection** products.

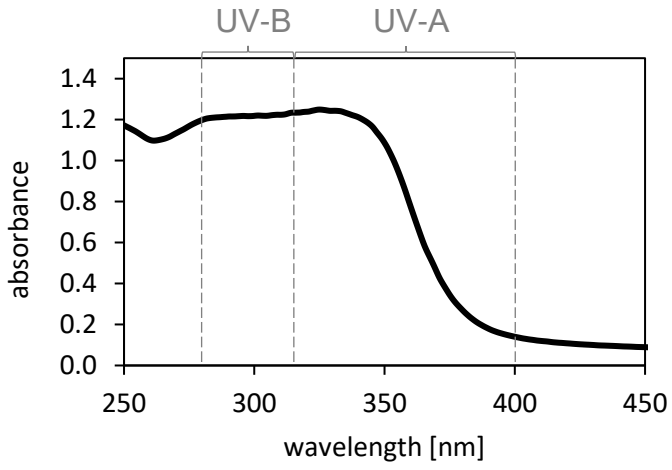

Figure 4. UV absorbance spectrum of 0.2% Tulsi Bio Extractive®

5.3 Anti-pollution Efficacy

5.3.1 Urban Pollution Threat

Of the world's population which live in cities, 92%, live in cities that do not comply with WHO air quality standards [10]. Urban pollution is a mixture of pollutants such as gases (CO_2, CO, SO_2, NO, NO_2, O_3), heavy metals, polycyclic aromatic hydrocarbons and particulate matter which cause oxidative stress for the skin and can lead to skin irritation, premature skin aging or in the worst case to skin cancer [11]. UV irradiation can accelerate the negative effect of urban pollution by catalyzing the formation of harmful free radicals [12].

Due to this negative effects of pollutants, anti-pollution cosmetics became a trend topic in the recent years.

The antioxidant and UV absorbance potential of the Tulsi Bio Extractive®, which was shown by HPTLC components analysis and spectrophotometry, respectively (see sections above), makes it a promising candidate as an anti-pollution agent.

An *ex vivo* study was conducted to test the anti-pollution activity of the Tulsi Bio Extractive®.

5.3.2 *Ex Vivo* Study Design

Skin explants originating from an abdominal plastic surgery of a 59-year-old Caucasian woman were treated with 3% Tulsi Bio Extractive® (C1) and 12% Tulsi Bio Extractive® (C2). Dilution of Tulsi Bio Extractive® C1 and C2 was done with water. In addition, a placebo sample containing the extract solvent only, i.e., glycerin-water, was also applied. Each treatment was performed on four replicates of skin explants.

As shown in Figure 5, after four days of treatment with Tulsi Bio Extractive® C1, Tulsi Bio Extractive® C2 or placebo, the skin explants were exposed to a concentrated pollutant mixture for 1.5 hour in order to mimic a pollutants exposure of an urban environment. The pollutant mixture was composed of heavy metals, hydrocarbons and diesel particulate matter based on actual measures of pollutants concentrations in Paris.

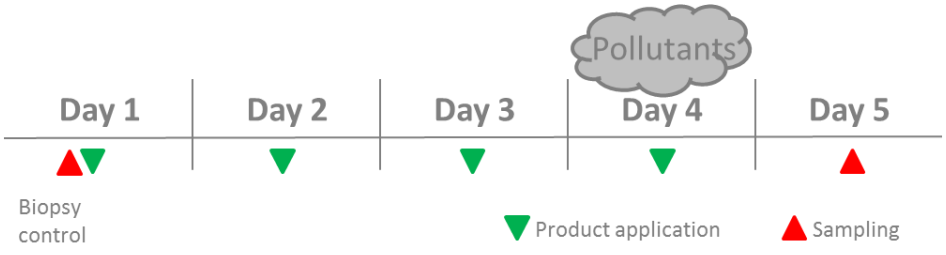

Figure 5. Schedule of the *ex vivo* study for which Tulsi Bio Extractive® was applied on skin explants to determine its anti-pollution activity

In addition, skin explants treated with pollutants only, skin explants with Tulsi Bio Extractive® C1 or C2 only (without pollutants) and skin explants without any treatment (without pollutants or product) were also sampled on day 5 and analyzed. On day 1, skin explants without any treatment were sampled for biopsy control.

Over the whole *ex vivo* study, the human skin explants were kept alive in terms of conserving a normal morphology, structure and metabolism using a specifically developed culture medium. In order to determine the effect of pollutants by pollution markers analysis, strong alterations of the cell viability are not aimed for. **The viability of the skin explants** was monitored by determining the cell viability of the epidermal and dermal structures by microscopial observation after Masson's trichrome staining.

After pollutants exposure, the skin explants were analyzed for Nrf2 activation. **Nrf2 is a key transcription factor** in the cellular response to oxidative stress [13]. The higher the oxidative stress, the more activated Nrf2 is detected by immunostaining. In addition, the dosage of malondiadehyde (MDA) was measured in the skin explants culture medium. **MDA is a product of lipid peroxidation** caused by free radicals induced by oxidative stress [14]. The higher the oxidative stress, the higher the MDA dosage.

5.3.3 Cell Viability

Histological analysis indicates a very slight alteration of the epidermis due to the pollutants mix exposure (Figure 6). This effect was shown to be inhibited by Tulsi Bio Extractive® C2. Tulsi Bio Extractive® C1 did not show this effect.

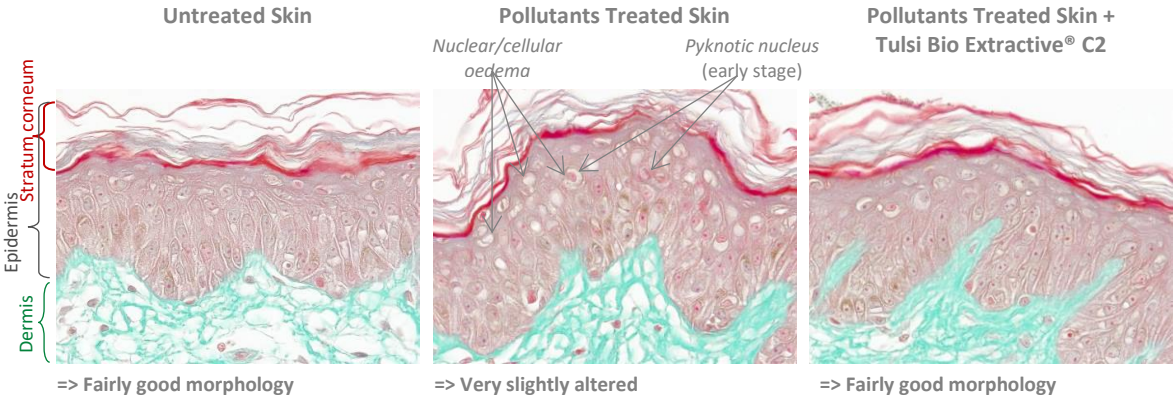

Figure 6. Viability analysis of untreated skin explants compared with skin explants treated with pollutants and Tulsi Bio Extractive® C2. Histological terminology: **Pyknotic nuclei:** Condensation of the nucleus content, the nucleus is retractable and hyper-stainable which indicates nucleus degeneration leading to cellular necrosis. **Nuclear/cellular oedema:** Swelling of the nucleus/cell due to an accumulation or excess of liquid.

5.3.4 Nrf2

As shown in Figure 7 below, increased amounts of activated Nrf2 were detected for skin explants treated with pollutants compared to the skin explants which were not exposed to pollutants. Activated Nrf2 clearly decreased for skin explants which were treated with Tulsi Bio Extractive® C1 and C2. **With Tulsi Bio Extractive®** C1 and C2, the **Nrf2** immunostaining results got **similar to skin explants which were not exposed to pollutants**.

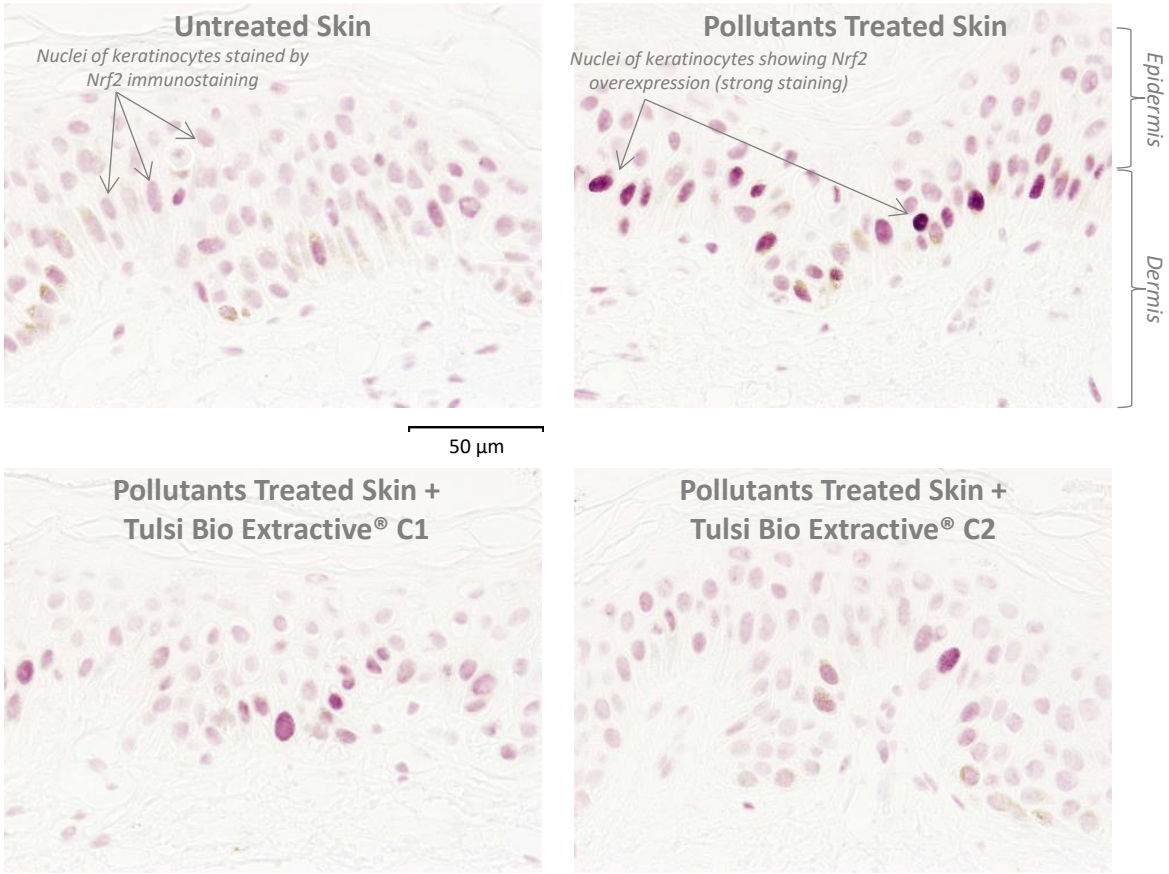

Figure 7. Nrf2 immunostaining analysis of human skin explants untreated and human skin explants treated with pollutants and/or the Tulsi Bio Extractive® C1 or C2

Figure 8 below shows the evaluation of Nrf2 immunostaining results of untreated skin explants and skin explants treated with placebo, Tulsi Bio Extractive® C1 or C2 and/or pollutants in a diagram.

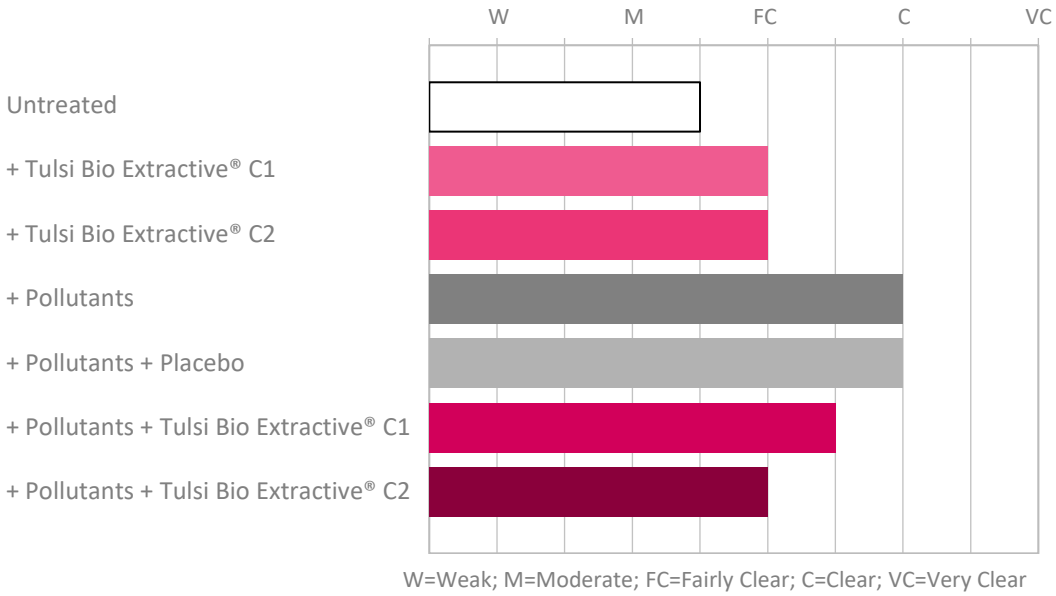

Figure 8. Evaluation of Nrf2 immunostaining results of human skin explants untreated, treated with Tulsi Bio Extractive®, placebo and/or pollutants

Treatment with **Tulsi Bio Extractive®** C1 and C2 **only (without pollutants) activated Nrf2** in the skin explants (Figure 8). Pollutants exposure led to an overexpression of Nrf2. With Tulsi Bio Extractive® C1, the Nrf2 overexpression could be reduced. Treatment with Tulsi Bio Extractive® C2 even inhibited the Nrf2 overexpression since the extent of immunostained Nrf2 is the same as of skin explants treated with Tulsi Bio Extractive® only. This pattern indicates a good protective mode of action of the Tulsi Bio Extractive® against pollution and against oxidate stress in general by **preparing the skin against oxidative stress via Nrf2 activation** and **finally reducing the Nrf2 overexpression caused by pollutants** exposure.

The placebo did not lead to a reduction of the Nrf2 overexpression.

5.3.5 MDA

The results of the MDA dosage in the skin explants culture medium are shown in Figure 9.

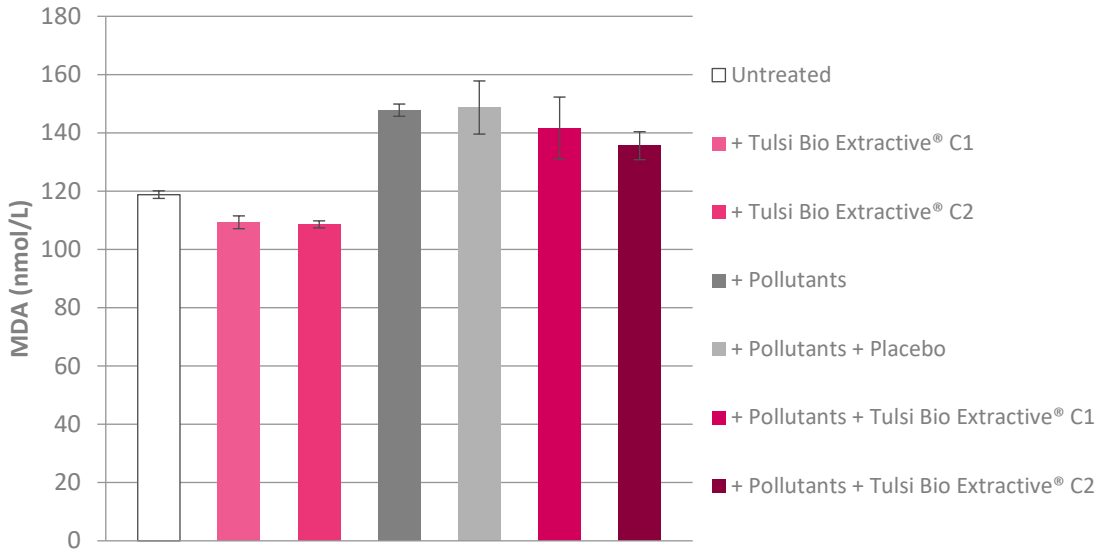

Figure 9. MDA (nmol/L) dosage in the skin explants culture medium

Treatment of skin explants with **Tulsi Bio Extractive®** C1 and C2 **only (without pollutants)** resulted in a **significant decrease of the MDA** dosage in the culture medium of 8% and 9%, respectively, compared with the MDA dosage in the culture medium of the untreated skin explants. This indicates a **reduction of the basal level of oxidative stress** of the skin explants by treating the skin with Tulsi Bio Extractive®.

After pollutants exposure, the MDA dosage in the culture medium significantly increased by 24% compared with the MDA dosage of the untreated skin explants. The MDA dosage in the culture medium of the placebo showed a slight non-significant increase of 1%. **Treatment with Tulsi Bio Extractive®** C1 resulted in a non-significant MDA decrease of 4% and treatment with Tulsi Bio Extractive® C2 resulted in a significant **MDA decrease** of 8% which indicates a protective effect against pollution.

The MDA dosage pattern supports the protective efficacy of the Tulsi Bio Extractive® against pollution and oxidative stress in general and is in line with the Nrf2 immunostaining results.

5.3.6 Conclusion

The *ex vivo* study with urban pollutants treated human skin explants proofed the anti-pollution efficacy of the Tulsi Bio Extractive® by reducing the overexpression of the oxidative stress marker Nrf2 and the dosage of MDA (a product of oxidative stress). The anti-pollution efficacy was shown to be stronger for C2 (12% Tulsi Bio Extractive®) compared to C1 (3% Tulsi Bio Extractive®) and led to Nrf2 levels of skin which was not

exposed to pollutants at all. In addition, C2 showed a slight protective activity regarding cell viability. Treatment with Tulsi Bio Extractive® C1 and C2 only (without pollution) activated Nrf2 and led to a reduction of MDA. These results indicate that the **Tulsi Bio Extractive®** is a good anti-pollution agent and a protective aid against oxidative stress in general. It **prepares the skin against oxidative stress via Nrf2 activation** and **finally** leads to a **reduction** of the **Nrf2 overexpression after pollutants exposure going along with reduced lipid peroxidation of the skin**.

The placebo sample containing the extract solvent only, i.e., glycerin-water, did not show any anti-pollution activity regarding cell viability, reduced Nrf2 expression nor reduced MDA dosage.

5.4 Summary of Dermatological Activities

Table 1 below provides an overview of the dermatological activities of the Tulsi Bio Extractive® based on the results described in the chapters above.

Table 1. Dermatological activities of the Tulsi Bio Extractive®

	Regeneration	Anti-aging	UV Protection	Skin Repair	Wound Healing	Anti-pollution
Flavonoids		x	x		x	x
Rosmarinic Acid		x			x	x
Antioxidant Capacity		x			x	x
UV Absorbance		x	x			
Cell Viability	x	x		x		x
Transcription Factor Nrf2		x	x	x	x	x
Lipid Peroxidation MDA	x	x		x		x

6 Acknowledgement

We would like to thank BIO-EC Laboratoire for performing an *ex vivo* study using living human skin explants for testing the anti-pollution activity of the Tulsi Bio Extractive®.

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