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Powerful Properties of Ozonated Extra Virgin Olive Oil

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Abstract

Extra virgin olive oil has been mainly produced and consumed in Mediterranean countries since ancient times; olive oil is one of the principal ingredients in the Mediterranean diet, and it constitutes the main source of nutritional fat. Aside from the high nutritional content of olive oil, it is also known for its cosmetic and therapeutic properties. In 1956, Thiers obtained satisfactory results in the treatment of scleroderma, stating that olive oil and its derivatives could be considered “a new group of therapeutic agents.” Hincky reported the beneficial properties of olive oil in the treatment of dry, senescent and sensitive skins. This has opened a new perspective for the use of the olive fruit, thus contributing to the increase in research about new applications. One such application is ozonized olive oil, which combines the properties of ozone with those of olive oil, to obtain a peerless compound. The composition of olive oil makes it a suitable vehicle for cutaneous absorption, as it is able to stabilize ozone, which is a highly reactive molecule. The oxidant power of ozone has interesting effects on microorganism and on wound healing.

Keywords: extra virgin olive oil, ozone, herbal medicine, antimicrobial activity, wound healing

1. Introduction

Herbal medicine is commonly used to treat skin disorders, and the ethnobotanical remedies are developed in different regions, based on local plants. In particular, two different systems, Ayurvedic herbs, established in India, and the traditional Chinese medicine, which uses the combination of different herbs, are known. In the occidental world, the use of herbal medicine is relative to purified extracts, often substituted for synthetic chemical drugs. In the last years, we assisted an intense return, in the occidental world, to herbal medicine, probably because we are living in the green revolution [1]. The use of vegetable raw materials in the preparation

of products for local application on the skin dates back to ancient times. The term *phytocosmetics*, from Greek *kosmesis*, which means adorn, and *phytos*, which means plant, is used to indicate the predominant and preferential use of botanical derivatives in cosmetic products. The vegetable field is an inexhaustible source of raw materials that, transformed by various processes, find many applications, both as functional substances and as excipients of the products. Contrary to some 50 years ago, when the cosmetic use of medicinal herbs was largely based on the mere observation of the traditional use, today, numerous scientific studies on the properties of plant-based drugs, as well as advanced knowledge on technical-scientific ones that allow to extract the active principles contained in the plants, are available. The herbal phytonutrient offers an enormous amount and heterogeneity of substances, which, by means of various extracellular processes, give rise to extracts with various functional applications. The active constituents of plants are in fact represented by a complex mixture of substances of different chemical nature (tannins, pectins, saponins, flavonoids, essences, fixed oils, etc.) whose concentration in the extract is essentially linked to the particular extraction process. The most widely used vegetable extracts are glycolic extracts, hydroalcoholic extracts, distilled water, dried extracts, oily extracts, essential oils, and vegetable oils. Vegetable oils are obtained by cold squeezing of plant drugs whose active ingredients are characterized by oily texture. The oil is predominantly made up of polyunsaturated fatty acids rich in triglycerides and also contains antioxidant substances, liposoluble vitamins, and the so-called insaponifiable fraction, a complex mixture of substances of extreme interest both from the dermatological and cosmetic field. Olive oil, coconut oil, wheat germ oil, borage oil, and almond oil are the main types of vegetable oils used in cosmetics. Compared to other types of oily ingredients, the advantage of using vegetable oils in cosmetics and cosmeceuticals lies primarily in their particular lipid composition, which is very close to the structure and function of that of the physiologic sebum present in the interstices and the surface of the corneal layer of the epidermis. The very high affinity to the skin sebum gives them an excellent ability to restore the physiological skin barrier by means of a protective, filmogenic, and emollient action. In this chapter, we describe the advantageous effects of ozonated olive oil in the treatment of skin disorders; in fact, olive oil is considered one of the most excellent foods for diet, but it has anti-inflammatory action, so it is used for skin disease. In the chapter, we report information about the safety and mechanism of action on microorganism and on wound healing.

1.1. Olive oil

Olive oil consists of glycerides, such as oleic, arachidic, palmitic, linoleic, and stearic acids, and of phenolic compounds. It is very important in the culinary use, but it has important applications in cosmetic and pharmaceutical fields. The olive tree *Olea europaea* is a common feature of the Mediterranean landscape, with the olive fruit and olive oil being the basic elements in the nutrition of civilizations around the Mediterranean basin for millennia. The principal reason is due to the tree's climatic requirements, which are found in limited areas of the earth's surface [2]. This longevous tree integrates and identifies economically, socially, and culturally with the inhabitants of this land and determines its rural landscape [3]. Even olive leaves have been used in popular medicine. The therapeutic and health properties of olive oil have been known for millennia so much that Hippocrates advised the juice of fresh

olives to cure mental illness and wraps to heal ulcers. During the Middle Ages and during the Renaissance, olive oil was used to cure gynecological infections and was considered useful in the treatment of heart disease, fever, and hypertension.

1.1.1. *Beneficial effects of olive oil: health properties*

Virgin olive oil has been and still is the subject of numerous studies that have attributed great properties to it, both in the field of health and in cosmetology. Various epidemiological studies have shown that the incidence of inflammatory, cardiovascular, and tumor illnesses is generally lower in Mediterranean European countries (such as Greece, Italy, and Spain) than in other western and northern countries [4]. This can be attributed to the high consumption of olive oil in the Mediterranean diet, which contributes to the daily requirement of vitamin E, essential fatty acids, and specific antioxidants, particularly represented by phenolic compounds and tocopherols. In addition, antioxidants have a primary role in resistance to oxidation and hence in the stability of olive oil and have been shown to exert numerous beneficial effects on the human body. The antioxidants' protective effect is mainly due to their ability to inhibit the action of oxygen free radicals, indicated by the acronym ROSs [5]. ROSs are highly reactive species represented by atoms or molecules with one or more electrons being dissipated, capable of generating the so-called oxidative stress. When the organism is subject to an increase in oxidative stress, an increase of F2-isoprostanes (IsoPs) in plasma levels and of urinary excretion is observed. IsoPs are a type of novel compound, structurally similar to prostaglandins, biosynthesized *in vivo* from the free radical-catalyzed peroxidation of arachidonate independent of the cyclooxygenases (COX) [6]. Oxidative stress seems to be the main reason for many chronic and degenerative diseases and skin aging. More specifically, ROSs induce (i) DNA mutations and protein alterations that are the basis of carcinogenesis [7]; (ii) oxidation of low density lipoproteins (LDL) involved in the formation of atherosclerotic plaques [8]; (iii) the onset of chronic intestinal inflammatory diseases, such as Crohn's disease [9]; (iv) probable onset of neurodegenerative diseases, such as Parkinson's disease [10]; and (v) cellular aging, by lipid peroxidation of the membranes, which become more permeable and less effective. All this evidence allows us to understand the importance of antioxidants, even from exogenous sources such as diet. The beneficial effects, and in particular the antitumor activity, of olive oil on human health are attributed to the high content of phenolic substances with high antioxidant power [11]. Phenolic compounds, in synergy with α -tocopherol and coenzyme Q, protect cells from oxidative damage by contrasting the toxic effects of ROS [12]. Through various epidemiological studies, the correlation between the consumption of virgin olive oil and the risk of onset of certain types of cancer has been demonstrated, such as breast [13], lung [14], colon [15], ovary [16], pancreas [17], and prostate cancer [18]. It has been shown that among the phenolic compounds, one of the most biologically active is hydroxytyrosol (3,4-DHPEA) that is able to inhibit the 5-lipoxygenase enzyme, by reducing the production of leukotriene B₄ in the leukocytes, originating from the metabolism of the eicosanoids [19]. In addition, hydroxytyrosol is able to inhibit *in vitro* the oxidation of LDL [20] and *in vivo* [21] the aggregation of platelets [22]. Some experimental studies have also shown that the phenolic extract of virgin olive oil and two isolated compounds, the dialdehyde form of hydroxytyrosol (3,4-DHPEA-EDA) and thiol (p-HPEA-EDA), are able to inhibit uncontrolled cellular

proliferation by blocking the cell cycle at G0/G1 and to induce apoptosis in some lines of cancer cells, as demonstrated for HL60 cells of promyelocytic human leukemia [23, 24]. However, compounds with greater biological activity are those containing the ortho-diphenol residues; it has been shown that 3,4-DHPEA and 3,4-DHPEA-EDA are more effective than p-HPEA and p-HPEA-EDA in protecting DNA from damage caused by oxidation [25]. In an *in vitro* study, by examining different virgin olive oil extracts, a chemoprotective effect was demonstrated on HL60 cell lines in relation to their composition but not to the total content of phenolic substances [26]. ROS production is also closely related to inflammatory processes in which the cyclooxygenase enzymes (COX-1 and COX-2), belonging to the oxidoreductase class, catalyze the conversion of arachidonic acid into prostaglandins. The p-HPEA-EDA, also called oleocanthal, has the ability to inhibit the activity of such enzymes and has a pharmacological effect similar to that of ibuprofen, which belongs to the class of nonsteroidal anti-inflammatory drugs [27]. It has also been shown that the consumption of olive oil may improve blood pressure regulation and cholesterol content in the blood; these events, together with the inhibition of platelet aggregation and the reduction of LDL oxidation, are important to prevent the onset of atherosclerotic plaques and, in general, cardiovascular pathologies [28, 29]. Olive oil also contains many monounsaturated fatty acids, including oleic acid, which is a key component of cellular membranes and can progressively replace polyunsaturated fatty acids. Membranes rich in monounsaturated fatty acids are more fluid and less subject to lipid peroxidation [30]. Some studies have also shown that regular intake of this food may result in a reduction in the risk of developing diabetes [31]. The therapeutic properties of olive oil include a laxative effect and stimulation of biliary function [32]. Finally, some studies on animal models have shown that the intake of olive oil can help to counteract the damage caused by epidermal ultraviolet radiation [33].

1.1.2. Dermatological and cosmetic properties

In recent years, in a number of fields, including cosmetics, there has been a renewed interest in materials of natural origin, particularly those of vegetable origin. Since ancient times, olive oil has been known not only for its high nutritional power but also for its cosmetic and therapeutic properties [34]. In 1971, Thiers was still pointing to its potential use in the cosmetic sector. To date, olive oil is certainly the most appreciated natural ingredient, alongside jojoba and avocado oils. The topical application of olive oil may be advised for its soothing action and its beneficial effects on eczema, surface wounds, and burns [35, 36]. In particular, the presence of phytosterols and triterpenoid compounds offers revitalizing and soothing properties for the skin. Vitamins E and A have an intense antioxidant action and have the ability to prevent irritation and aging of the skin, to help maintain its softness, smoothness, stability, and elasticity. As a result, in the cosmetic field, olive oil can be used to prevent signs of aging as a soothing emollient for dry skin and to strengthen hair [37]. Indeed, it is very often a component of lotions, lip balms, shampoos, bath oils, and massage oils. From a dermatologic point of view, olive oil has also proven to have antimicrobial activity, *in vitro*, against some positive and negative Gram and various types of fungi, including *Candida* spp. [38]. Some components of olive oil, especially certain aliphatic aldehydes, inhibit elastase activity; this enzyme is involved in the virulence process [39]. Olive oil is an important component of some

topical formulations used in the treatment of inflammatory and mycotic skin diseases [40]. The unsaponifiable fraction is rich in numerous active ingredients with sebum-regulating and moisturizing properties, as well as emollients; it can be a component of cosmetic products (in the form of creams, balms, gels, etc.) for the treatment of delicate, dry, and cracked skin. In fact, the unsaponifiable fraction is very useful in the case of particularly vulnerable skin, such as that of infants and children, or in the case of xerotic skin, such as that of the elderly. Skin hydration is above all important in the neonatal period and especially in premature infants: some clinical trials have been conducted to highlight the beneficial effects of emollient topical treatment [41, 42]. Furthermore, the unsaponifiable fraction exerts a good photoprotective effect on ultraviolet exposed skin: various studies have shown that the application of olive oil may reduce the incidence of skin epithelial tumors on UV-B-exposed mice compared to a control group [43, 44]. The unsaponifiable fraction can also be an additive in makeup products with the purpose of making them easier to apply, softer, and smoother. Butter contains high quantities of squalene (which is the most important constituent of sebum), waxes, and esters that guarantee high penetration of the skin. Butter is ideal for massages or as a vehicle for other active ingredients used in skin care. It acts also as an emollient and moisturizing agent, promoting skin elasticity and preventing the onset of wrinkles. Finally, it can be used as an additive in photoprotective products or in skin hygiene products due to its ability to neutralize aggressive detergent action.

An interesting and powerful way to use extra virgin olive oil is with ozone. The process of ozonization allows the properties of ozone gas to be combined with those of olive oil; the result is a peerless compound. Since ancient times, ozone has also been used in a large number of medical indications [45–49].

2. Ozone

Ozone is an oxygen derivative and is known primarily for its ecological role in the Earth's balance, absorbing most of the ultraviolet radiation from the sun and preventing it from reaching humans in a harmful way. It is an unstable gas that cannot be stored; in fact, it dissolves in very short time. Ozone is totally neutral to the human body, and in fact, it does not (i) modify pH, (ii) irritate skin or mucous membranes, (iii) damage hair or clothing, (iv) interact with drugs, and (v) cause allergic reactions. This molecule has been subjected to countless studies, and in particular, its strong oxidation capacity has been tested in order to underline its disinfectant and sanitizing properties principally applied as a disinfectant of drinking and waste water [45–47]. To this purpose, the dedicated design and construction of equipment for the production of gaseous ozone for air and water purification are increasing. But research into the properties of ozone has yielded promising results in biological applications, thus confirming the ozone activity in stimulating natural cell defenses and increasing their energy availability. Indeed, since ancient times, ozone has also been used in a large number of medical indications [48, 49]. Scientific studies have shown that ozone, while being highly unstable, can be trapped inside vegetable oils. These are composed of triglycerides in which saturated and unsaturated fatty acids are present, which have the ability to retain ozone, thus allowing

them to prolong their use. In addition, the greater the amount of unsaturated fats present, the greater the amount of ozone that will be retained [50]. Therefore, when extra virgin olive oil is ozonated, the produced product combines the beneficial properties of extra virgin olive oil with those of ozone. There are countless ozone-based products on the market, and in particular, in our laboratory, we have developed Bioxoil™, an ozonated extra virgin olive oil available in pharmacies. This product is exclusively made from olive cultivars from Puglia and Salento and the oil is ozonated by an innovative patented method (number: M2011A001045 titled: “Process for the ozonization of a vegetable oil”) that confers quality and efficiency in various fields of application; in particular, Bioxoil™ is indicated for the treatment of acne, herpes, psoriasis, fungal infections, bed sores, and wounds in general, due to its healing and disinfectant properties (**Figure 1**).

Bioxoil™ is produced from extra virgin olive oils from two local cultivars, *Ogliarola* and *Cellina*, in Salento (Apulia, Italy). The ozone reacts with unsaturated compounds through the known Criegee mechanism. The quality of extra virgin oil is very important to obtain a higher grade of ozonization; for this reason, during the process, it is necessary to control different physical and chemical parameters. Of these parameters, the most important is the temperature, and in fact, during the reaction, the temperature increases provoking an alteration of antioxidant content. The oils' peroxide content and acidity value that indicate the level of hydrolytic modification and primary and secondary oxidation of oil are analyzed and reported in **Table 1**. Peroxide index (PI) and acidity index (AI) after ozonization of oils increase with respect to relative controls. In particular, *Cellina*'s oil sample has an acidity index of 0.2% and a peroxide index of 12 mmol O₂ kg⁻¹, while related ozonated oil has an AI of 1.8% and a PI of 533 mmol O₂ kg⁻¹. *Ogliarola*'s oil sample presents an acidity index of 0.3% and a peroxide index of 13 mmol O₂ kg⁻¹, while respective ozonated oil has an AI of 1.3% and the PI increases to 677 mmol O₂ kg⁻¹.

In our experiments, the ability of ozone to react with olive oil and in particular with the carbon-carbon double bonds present in unsaturated fatty acids was demonstrated by gas liquid



Figure 1. Bioxoil™ products. The Bioxoil products have different applications. Bioxoil with a red label is indicated for bed sores; the sky blue label is indicated as soothing medication; the green label is indicated for herpes labialis; the pink label is indicated for acne; and the orange tag is indicated for mycosis.

Sample	Acidity index (AI), % (means \pm SD)	Peroxide index (PI), mmol O ₂ kg ⁻¹ , (means \pm SD)
<i>Ogliarola olive oil</i>	0.2 \pm 0.03	12 \pm 1.2
<i>Ozonated Ogliarola olive oil</i>	1.8 \pm 0.02	533 \pm 1.5
<i>Cellina olive oil</i>	0.3 \pm 0.02	13 \pm 1.5
<i>Ozonated Cellina olive oil</i>	1.3 \pm 0.01	677 \pm 1.6

Table 1. Physicochemical parameters of *Ogliarola* olive oil and *Cellina* olive oil after ozonization procedure.

Composition of fatty acids (%)	<i>Cellina</i>	<i>Cellina</i>	<i>Ogliarola</i>	<i>Ogliarola</i>
	Control	Ozonated oil	Control	Ozonated oil
Palmitic acid	12.45	11.91	11.89	11.30
Linolenic acid	5.57	2.19	4.96	2.06
Cis-oleic acid	70.27	39.82	67.00	41.25
Trans-oleic acid	8.22	6.68	7.88	7.2
Stearic acid	3.48	1.29	3.32	3.22
Nonanal	0	11.69	0	10.07
Nonanoic acid	0	1.36	0	1.98

Table 2. Composition of fatty acids in olive oil samples *Cellina* and *Ogliarola*.

chromatography (GLC). The composition of fatty acids of each olive oil and respective ozonated oil are analyzed by GLC. Data demonstrate that the amount of oleic acid decreases in both ozonized oil samples: in *Cellina*'s ozonated oil, we observed a 30% reduction, while in *Ogliarola*'s ozonized oil, the reduction was about 26% (**Table 2**). During the ozonization reaction, ozone etches mainly the double bond of acid oleic, the most abundant fatty acid in olive oil (about 80%). This explains the decrease in oleic acid and the contemporary appearance of new compounds, which are the reaction's products, among which are nonanal aldehyde and nonanoic acid, both compounds with nine carbon atoms (**Table 2**). In both cultivar ozonized oils, the composition of fatty acids changes, showing a gradual decrease in unsaturated fatty acids (C 18:1, C 18:2), with a gradual increase in ozone doses.

3. Biocompatibility of ozonated olive oil with skin

The skin is the largest organ of the body and is the major barrier between the inside and outside of our body. It is formed of two main layers: the epidermis, a thin outer portion, and the dermis, the connective tissue layer of skin. This portion is involved in the thermoregulation process, and the resident dermal fibroblasts secrete collagen, elastin, and substances that offer support and elasticity of the skin. The epidermis is subdivided into four layers: (i) the stratum

germinativum (SG) that provides the germinal cells necessary for the regeneration of epidermidis; (ii) the stratum spinosum (SS), in which the cells divided in the SG start to accumulate many desmosomes on their surface; (iii) the stratum granulosum (SGR), in which the keratinocytes accumulate dense basophilic keratohyalin granules that contain lipids, which help to form a waterproof barrier; and (iv) the stratum corneum (SC), the outermost layer, in which the cells are dead. The skin is constantly exposed to the environmental stress of pollutants or cigarette smoke, for example, so it is necessary for the wellness of the skin to preserve it from oxidative stress. To this purpose, there are a variety of antioxidants that include glutathione peroxidase, superoxide dismutase, catalases, and nonenzymatic low-molecular weight antioxidants such as vitamin E isoforms, vitamin C, glutathione (GSH), uric acid, and ubiquinol. Interestingly, the distribution of antioxidants in the SC follows a gradient with higher concentrations in deeper layers [51].

The biocompatibility of Bioxoil™ was investigated by MTT assay on fibroblast 3T3 and on keratinocytes HaCaT and compared with ozonated *dulcis* almond oils and nonozonated olive oil as controls. The MTT test values the cell metabolic activity and, in particular, measures the activity of oxidoreductase by reduction of the tetrazolium bromide dye in formazan. The *dulcis* almond oil was chosen because it is largely used in cosmetics, and as it is an oil, it is a possible vector of ozone. Data reported in **Figure 2** demonstrate the Bioxoil™ biocompatibility for both cell types. The viability of cells never significantly decreased in relation to the control and was the same as the nonozonated oil. Conversely, once ozonated, 10% of *dulcis* almond oil had a very significant negative effect on the viability of fibroblasts and keratinocytes but not when *dulcis* almond oil was nonozonated. Interestingly, the vitality test demonstrated that when these cells are exposed to a mixture of ozonated olive oil and 10% of ozonated *dulcis* almond oil (3:1), the adverse event induced by the ozonated almond oil alone was partially prevented (**Figure 2**).

3.1. Biological action of ozonides

By combining the beneficial properties of extra virgin olive oil with that of ozone, the ozonated extra virgin olive oil becomes very powerful for the topical treatment of acute and chronic skin lesions. The ozonides generated during the ozonization procedure possess many properties including a high germicidal activity on fungi, yeasts, viruses, and bacteria; activation of local microcirculation; stimulation of granulation and tissue growth; and revitalization of epithelial tissues.

3.1.1. Germicidal activity

The excessive consumption of antibiotics for the treatment of infectious diseases has fuelled the drug resistance phenomenon, *i.e.*, the microorganisms are resistant to antibiotics. Therefore, it is necessary to develop new molecules with antibiotic properties, preferably natural and non-synthetic. In fact, this research is divided into the field of synthetic molecules produced by the chemical/pharmaceutical industry and the field of natural active compounds, in which plant extracts are studied also by the use of green chemistry. The plants are able to withstand fungal and bacterial infections and therefore their secondary metabolites can be applied in the field

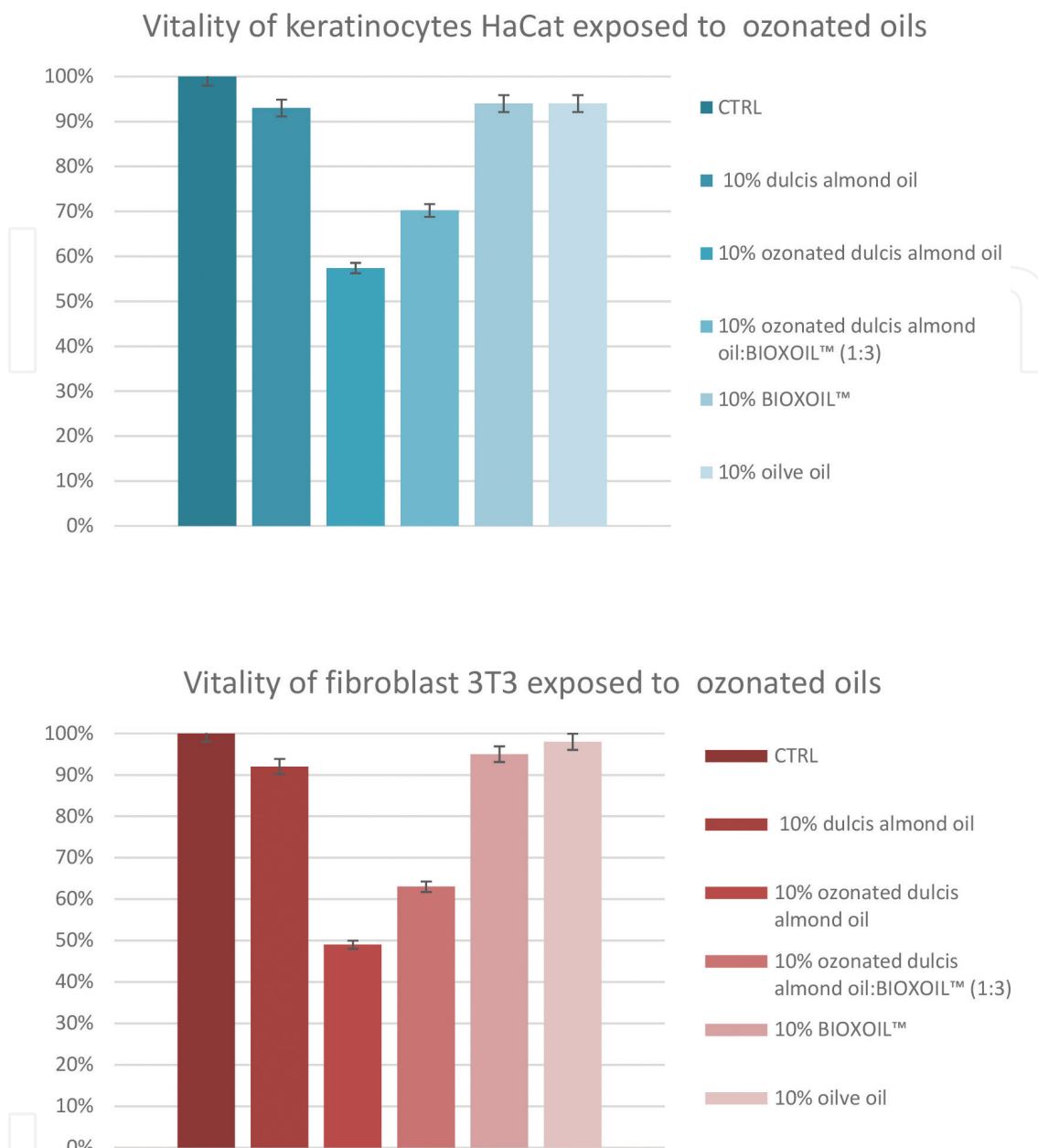


Figure 2. MTT assay on fibroblasts and keratinocytes.

of medicinal herbs. Maoz and Neeman in their research reported an antifungal action of olive oil due to the high content of oleic acid [52]. The ozonated oil increases this natural capacity, because ozone acts on microorganisms, thanks to its greater oxidizing power; in fact, it is able to break down the macromolecules that are the basis of the vital integrity of bacterial cells, fungus, protozoa, and viruses. The ozone in contact with the microorganism's lipoproteins forms H_2O_2 and the final products of lipid oxidation (LOP); H_2O_2 assures bacteriostatic and bactericidal activity, while final lipid oxidation products have induction activity and reactivate metabolic functions (**Figure 3**). Nagayoshi et al. [53] reported the capability of ozone to destroy Gram-positive and -negative microorganisms, and in particular, Gram-negative bacteria are more sensitive.

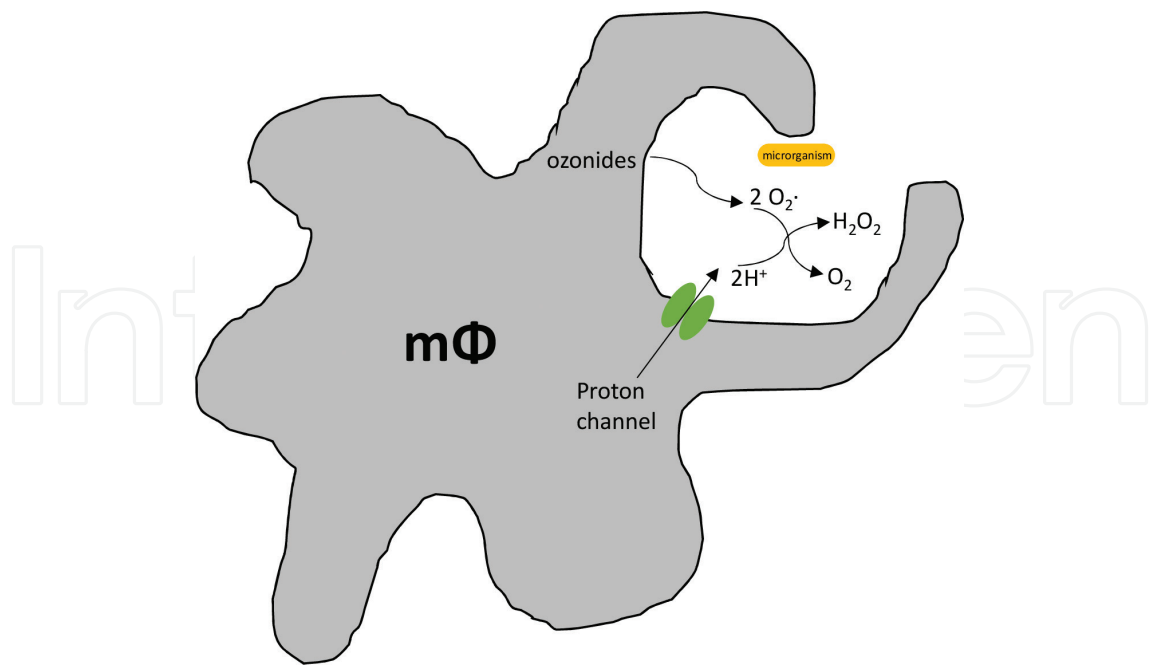


Figure 3. Molecular mechanism of antimicrobial activity of ozonides: the oxygen internalized from ozonides reacts with the proton/protons in order to form H_2O_2 that has a bacteriostatic and bactericidal activity.

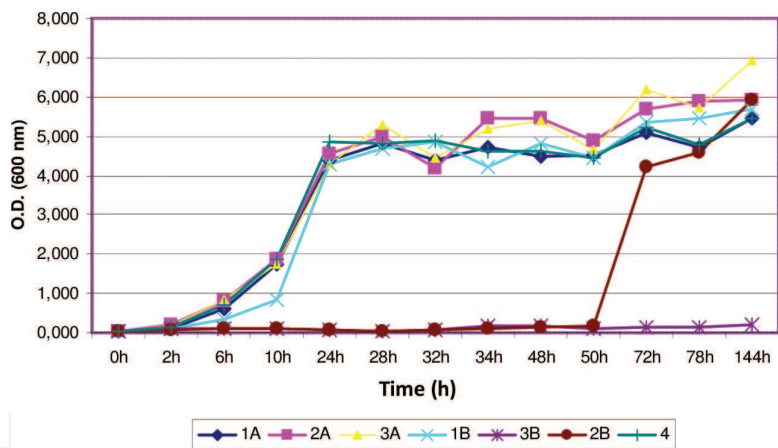


Figure 4. Growth curves of mycete *Epidermophyton floccosum* in liquid culture medium: 1A, 2A, and 3A are the positive controls with 12.5, 25, and 50 mg/ml of nonozonized olive oil, respectively; 1B, 2B, and 3B are ozonized olive oil samples with 12.5, 25, and 50 mg/ml of ozonized olive oil, respectively; 4 is the negative control.

For virus inactivation, a higher gas dosage than that required for bacteria is necessary. The ozone oxidates and subsequently inactivates the specific viral receptors used to bind the cell wall for virus invasion [54]. We tested the antibacterial activity of Bioxoil™ on mycete *Epidermophyton floccosum*, demonstrating its efficacy in the inhibition of mycete’s growth in liquid and agar medium; the bacteriostatic effect is obtained in the presence of 5 µl/ml of ozonated olive oil, and the bactericidal effect is obtained in the presence of 15 µl/ml (**Figure 4**).

The antibacterial activity of Bioxoil™ was also tested on *Staphylococcus aureus* and *Staphylococcus epidermidis*, and its efficacy in inhibiting microbes growing both in liquid medium culture and

in agar medium was demonstrated. We defined the minimal inhibitory concentration (MIC) of 50 mg·ml⁻¹ for *S. aureus* and of 25 mg·ml⁻¹ for *S. epidermidis*. This study confirms that ozonated olive oil has antimicrobial properties, which can be exploited for cutaneous infections, by slow release of O₃, which displays effective disinfectant and stimulatory activities that lead to rapid healing.

3.1.2. Biological activity

The ozonides easily penetrate the cell membrane and, thanks to their biological properties, stimulate skin cells and improve the tropism of the skin by promoting wound healing and repair of ulcers of various kinds; from the experiments, it was found that topical application of products based on ozonides has determined a considerable increase of fibroblasts resulting in increased production of collagen, glycosaminoglycans, and formation of elastic and reticular fibers. The healing of skin lesions includes complex movements like tissue hemorrhage, inflammation, re-epithelialization, granulation tissue, and finally remodeling. These events involve the coordination of many cell types and matrix proteins, which are important for the control of the various stages of tissue repair. Previous studies have shown that endogenous growth factors, such as fibroblast growth factor (FGF), growth factor derived from platelets (PDGF), the TGF-β factor, and vascular endothelial growth factor (VEGF) are important regulators in the healing of wounds [55]. They are released by macrophages, fibroblasts, and keratinocytes at the site of injury and participate in the regulation of re-epithelialization, formation of granulation tissue, collagen synthesis, and neovascularization [56–58]. The beneficial effects of ozone in the treatment of sores are due to the decrease of bacterial infection and the increasing oxygen tension in the wound. In the literature, it is reported that after exposure to ozone transcription factors are activated, such as NF-kB, and these are important regulators of the inflammatory response and tissue repair process [58]. In conclusion, the ozonated olive oil can accelerate acute cutaneous wound repair, by stimulation of dermal fibroblast, and the ozonized oil has shown to be effective against Gram-negative and -positive bacteria, mycetes, and viruses, so it can be used for the cure of infections.

4. Conclusions

The goal of pharmacological research has always been that of finding drugs that can cure diseases or soothe the pain that derives from them, and this research has evolved over the years in an extraordinary way both in the field of medical knowledge and scientific studies that are more and more powerful and sophisticated. Medicinal herbs, long popular in many parts of the world, are increasingly spreading in the western world and represent a large commercial market with an estimated annual growth of 25%, often replacing synthetic drugs. Among the medicinal herbs, particular interest is reserved to olive oil, called “yellow gold” by the Egyptians for its innumerable beneficial properties. Herbal trade today sees many products based on olive oil for both hygiene and personal care. In the field of oil-based products, particular interest is directed to products containing ozonated oil; these products are the result of the union of the beneficial properties of olive oil with those of ozone. Ozonated oil is the most practical, innovative, harmless, and noninvasive of the techniques of application developed in

the field of ozone therapy over the last 130 years. It has demonstrated interesting therapeutic results. The biological effects of ozone include antimicrobial activity (antibacterial, antiviral, and antifungal), antalgic action, and improved O₂ metabolism [59]. In this chapter, the possible applications of the Bioxoil™ products, already distributed in pharmacies and herbalist's shops, have been described; its production line includes five different products characterized by different concentrations of ozonides such as to allow the use for the most varied skin affections. Bioxoil with the highest content of ozonides is indicated for the treatment of bedsores at first and second stages, thanks to its anti-inflammatory and cicatrizing properties, Bioxoil with medium concentrations, but different from each other, is indicated for the treatment of herpes labialis, mycosis, and onychomycosis and for acne due to its antimicrobial action. Finally, Bioxoil with the lowest ozonide content is indicated to soothe contact or allergic irritations. The exceptional usability of the product and its completely natural origin offers a vast market.

Abbreviations

ROS	reactive oxygen species
IsoPs	F2-isoprostanes
COX	cyclooxygenase
LDL	low density lipoprotein
3,4-DHPEA	hydroxytyrosol
3,4-DHPEA-EDA	3,4-dihydroxyphenylethanol-elenolic acid dialdehyde
p-HPEA-EDA	2-(4-hydroxyphenyl)ethyl (4E)-4-formyl-3-(2-oxoethyl)hex-4-enoate
COX-1	cyclooxygenase 1
COX-2	cyclooxygenase 2
PI	peroxide index
AI	acidity index
SG	stratum germinativum
SS	stratum spinosum
SGR	stratum granulosum
SC	stratum corneum
GSH	glutathione
LOP	lipid oxidation
MIC	minimal inhibitory concentration

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