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LIMONENE - A BIOMOLECULE WITH POTENTIAL APPLICATIONS IN REGENERATIVE MEDICINE

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Abstract

Limonene is a biomolecule that can be easily obtained from plant sources with remarkable biological effects. It is found in large quantities in citrus essential oils (concentrations up to 98%) and in moderate amounts in essential oils obtained from various species of geranium. The article presents the reasons why this compound may have potential uses in regenerative medicine, especially in dermatological applications, where these compounds or formulations which contain this compound, alone or via intermediary products which result from metabolization (such as perillyl alcohol) can accelerate wound healing, inhibit skin tumors development, or inhibit pathogenic microorganisms such as Staphylococcus aureus or Pseudomonas aeruginosa. Studies performed in vivo, on healthy persons who have tested in time the skin cleaning products which contain limonene or essential oils with limonene, were confirming that the products with Limonene do not give perturbation to normal skin microbiota. These data recommend Limonene as a potential candidate for regenerative medicine applications in the field of dermatology.

Key words*: limonene, Citrus essential oils, regenerative medicine.*

INTRODUCTION

Plant essential oils (EOs) are a mixture of monoterpenes, sesquiterpenes, and oxygenated derivatives. These are used in formulations with antimicrobial, immunomodulatory, antioxidant, antimicrobial, or antitumor properties (Aziz et al., 2018). The citrus genus belongs to the Rutaceae family and is widely important in the world economy. It includes about 17 citrus fruit species, including *Citrus reticulata Blanco* (mandarin orange, tangerine), *C. sinensis* L. (sweet orange), *C. aurantium* L. (bitter orange), *C. lemon* L. (lemon), and *C. paradise* (grapefruit) (Bora et al., 2020)**.** The fruit peel of citrus species contain vesicles with essential oil in which Limonene concentration can attain 97% (Voo et al., 2012; Dugo & Di Giacomo, 2002). A non-volatile fraction from peels contains sterols, fatty acids, waxes, coumarins, psoralens, and flavonoids (Dugo & Di Giacomo, 2012; Tranchida et al., 2012; Chi et al., 2013; Zulaikha et al., 2015). Preclinical studies achieved on lab animals have shown that when administered orally, Limonene is absorbed directly from the intestinal tract and transported to tissues, where it is metabolized to perillyl alcohol (POH), perilic acid, dehydroperyl acid, limonene 1,2 diol, and limonene 8.9 diol (Shojaei et al., 2014; Schmidt & Göen, 2018). One of these compounds, which occurs in the intermediate stages of metabolization, respectively POH, has a stronger antiproliferative effect than limonene, as demonstrated by studies performed on UACC62 melanoma tumor cells. Preclinical studies performed on lab animals with induced melanoma have shown that topical administration of limonene inhibits the development of tumor cells by inhibiting the farnesylation process and disrupting cell signaling by protein kinases (ERKs) (Shojaei et al., 2014). d'Alessio and collab in preclinical studies performed on lab animals with skin lesions induced mechanically or with TPA (12-o-tetradecanoylphorbol-13-acetate) have shown that when treating these wounds with formulations containing limonene or POH, the severity of the lesions is reduced. The continuation of these studies using in vitro

models by the same authors has shown that both d-Limonene and POH induce angiogenesis, accelerate wound healing, and inhibit the formation of proinflammatory cytokines (d'Alessio et al., 2014). Cytotoxicity studies performed *in vitro* by Alipanah and collab on melanoma tumor cell lines A-375 (ARRC CRL 1619), exposed at a different level of nanoformulations that contain Limonene or citrus essential oils (essential oils containing at least 1% limonene), showed that for concentrations between (75-1200 μg/mL, the viability of melanoma tumor cells decreased under 20%. The IC50 cytotoxicity values obtained for nanoformulations applied to melanoma cells in this study ranged from (13.53÷246) μg/mL (Alipananah et al., 2021).

MAIN COMPONENTS OF CITRUS PEEL ESSENTIAL OILS

Generally, many factors, including ripening stage, season, weather conditions, soil type, storage condition, genotype, method of extraction, and analytical process, significantly impact the quality and quantity of essential oil obtained from citrus peels. Citrus peel (CP) contains volatile aromatic compounds, which accounts for about 0.5-5% of the fresh weight of CP. The majority of CP contains monoterpene hydrocarbons, sesquiterpene hydrocarbons, oxygenated monoterpenes, and oxygenated sesquiterpenes. The more critical compound in EOs obtained from citrus plants is Limonene and its concentration in these bioproducts is (32-98)% (Table 1). Compared to citrus plants with thin peels, citrus fruits with thick peels such as sour orange, grapefruit, and bergamot, have a higher concentration of essential oils. The main essential oils found in the peels of mandarin orange, pummelo, sweet orange, grapefruit, and bitter orange were non-terpenoid ester and aldehyde derivatives. Mono and sesquiterpene hydrocarbons are widely distributed in the peels of yuzu, citron, key lime, and bergamot orange (González-Mas et al., 2019). Monoterpene hydrocarbons, such as limonene (92.6%), γ-terpinene (3.39%), β-pinene (1.55%), and α-pinene (1.55%), were found to be the most common essential oil components in orange peels (0.61%). Other EO components include oxygenated monoterpenes (linalool 0.31%), sesquiterpene hydrocarbons (α-humulene 0.08%), and oxygenated sesquiterpenes (cubebol 0.06% and α-sinensal 0.06%).

Table 1. Different concentrations of Limonene from different Citrus plant peels

Source of peel	Limonene content	References
C. sinensis	59.3%,	Oyedeji et al., 2020
C. reticulata	56.76%	Yi et al., 2018
	75.16%	Abdel-Aziz et al., 2019
	80.2%	Fouad, & da Camara, 2017
C. aurantiifolia	38.9%	Fouad, & da Camara, 2017
C. limon	48.48%	Sun et al., 2018
	70.95%;	Dănilă et al., 2018
	46.93%	Moosavy et al., 2017
	52.85%,	Yazgan et al., 2019
	57.65%	Caputo et al., 2020
C. paradisi	93.33%	Deng et al., 2020
C. grandis Osbeck	87.5%	Chen et al., 2018
C. grandis	87.9-90%	Tuan et al., 2019
C. medica	60.44%	Xing et al., 2019
C. bergamia	32.29%	Caputo et al., 2020)
C. myrtifolia	76.83%	Caputo et al., 2020)

Analysis performed by Gas Chromatography coupled with Mass Spectrometry (GC-MS) found eighteen compounds in *C. sinensis* peels. From these, monoterpene represents 63.95%, oxygenated monoterpenes 28.92%, and sesquiterpene 3.54%. The main compounds were limonene (59.3%), terpineol (8.31%), linalool (6.56%), and citronellol (6.21%) (Oyedeji et al., 2020; Yi et al., 2018). Studies performed on mandarin peels essential oil (MPEO) reveal the existence of twelve compounds. MPEO contained two forms of volatile compounds: monoterpene hydrocarbons (81.90%) and oxygenated monoterpenes (18.09%). Limonene was the most abundant monoterpene hydrocarbon in NPEO (75.16%**).** According to Fouad and da Camara, limonene was the key constituent of the *C. aurantiifolia* oil (38.9%) and *C. reticulata* oil (80.2%) (Fouad and da Camara, 2017). Citrus lemon oil (CLO) is a mixture of limonene, hesperidin, δ-gluconolactone, and other compounds extracted from the peels of *C. limon* (Sun et al., 2018). D-limonene (46.93%), γ-terpinene (16.89%), tri-

cyclen (6.67%), 1-β -pinene (4.69%), and 2-β -pinene (3.86%) were the major compounds of lemon peel essential oil (Moosavy et al., 2017). Dănilă and collab. made comparisons between the compounds found in CLO by two extraction methods respectively hydrodistillation (HD) and microwave-assisted extraction (MW). They found that a high quantity of limonene (75,57%) was obtained by extraction with MW (Dănilă et al., 2018). Yazgan et collab. have investigated the antimicrobial effects of nano-emulsified lemon essential oil and raw lemon essential oil on the food-borne pathogens and on the microorganisms implied in seafood spoilage. They found that D-limonene, p-cymene, and β-pinene, the main components of essential oil, are responsible for inhibiting the growth of these microorganisms (Yazgan et al., 2019). Investigation performed on the chemical composition of essential oils extracted from the peels of three citrus species (*C. lemon, C. myrtifolia*, and *C. bergamia*) reveal the existence of 30 components. The key components are limonene (57.65%), γ -terpinene (10.45%), β -pinene (9.31%), and citronellol (8.19%) (Caputo et al., 2020)**.** *Citrus myrtifolia* EO contains thirty-two compounds, and the major compounds were limonene (76.83 %), linalool (10.01%), and α-terpineol (2.66%). These findings were in agreement with results obtained by Plastina and collab. who identified limonene (54.3%) and linalyl acetate (22.9%) as the main constituents of EO (Plastina et al., 2018). The key constituents of EO obtained from *C. bergamia* are oxygenated monoterpenes (51.09%), linalool (33.64%), Limonene (32.29%), linalyl-acetate (9.22%), terpinene (6.39%), α -terpineol (4.62%), and α-pinene (4.29%). Xing et al. performed analysis on EO obtained from *C. medica*, by two extraction methods, respectively Ultrasound-assisted hydro distillation (UAHD) and Headspace solid-phase microextraction (HS-SPME)) have obtained a product with high concentration of limonene in the case of extraction with UAHD (Xing et al., 2019). A high concentration of limonene has been reported in the EO obtained from *C. paradise* peels (93.33%) (Deng et al., 2020). Differences regarding the limonene concentration in essential oils obtained from pomelo peel, are due to soil, climatic factors (temperature, rainfall), extraction method, and the part of the plant from which the EO was obtained. The EO obtained from *C. grandis* Osbeck peels, contains limonene (87.5 %,), myrcene (3.1%), β-pinene (2.7%), α-pinene (6.0 %) (Chen et al., 2018). Another study performed on EO obtained from pomelo peels, demonstrated that it contain Limonene (87.90-89.87%t), β-pinene (2.66-2.83%), and α-phellandrene (1.22-1.38% and low amounts of oxygenated hydrocarbons (0.63-0.89%) (Tuan et al., 2019).

BIOLOGICAL EFFECTS OF LIMONENE

Studies by Kohda and collab. on a model of the human epidermis have shown that when populations of *S. epidermitis* and *S. aureus* are inoculated on the surface of the epidermis, then *S. aureus* growth is inhibited and the release of proinflammatory cytokines are attenuated by the presence of *S. epidermitis* (Kohda et al., 2021). Regarding the effect of Limonene on commensal or pathogenic microorganisms, the studies are divided into two categories: there are numerous studies demonstrating the effectiveness of Limonene alone or in combination with other antibiotics on pathogenic microorganisms that cause skin diseases such as *S. aureus* or *P. aeruginosa*, (Costa et al., 2019; Gupta et al., 2021) and there are studies that have evaluated the effect of hygiene products containing limonene on the skin microbiota. Finally, studies on healthy human subjects with skincare products containing limonene or essential oils with limonene have shown that after exposure to the cleansing agent used, the skin microbiome returns to normal (Wallen Russel, 2019; Hwang et al., 2021). When using formulations with extracts of probiotic bacteria, plant extracts, and sources of Limonene, an abundance of commensal species such as *Cutibactyerium* sp., *Staphylococcus* sp. were found in the skin microbiome, while improving hydration and dermal texture.

Natural bioproducts which contain limonene, such as the EOs obtained from citrus plants, possess many biological activities (i.e. antimicrobial, antitumor, antiviral anti-inflammatory, and antioxidants) presented in the Table 2.

Antimicrobial activity of Limonene

The essential oils of mandarin have been demonstrated to possess potent antimicrobial

activity against different strains of bacteria and fungi (Ambrosio et al., 2017; Mitropoulou et al., 2017; Putnik et al., 2017; Rafiq et al., 2018, Yi et al., 2018). The mechanisms of action of mandarin EOs on microorganisms are due to Limonene and other compounds from it (da Silva Dannenberg et al., 2016), which penetrate the bacterial cell membrane by solubilizing its lipids, destroying it and making it permeable (Dhifi et al., 2016). Three factors are critical in the action of EOs across microbial strains (Djenane, et al, 2015). First, the antimicrobial activity of EO is determined by the functional groups present in their compounds (a major part of them belong to the class of terpenes). Second, some of the minor compounds from EOs are microbiologically effective**.** Thirdly, the presence of EO components can result in additional, adaptive, or even antagonistic antimicrobial activity. Three microorganisms are in connection with a common skin disorder like acne-related inflammation: *P. acnes, P. granulosum*, and *S. epidermidis*. These bacteria are common on the skin microbiome and are often isolated from acne lesions. Many commensal microorganisms (from the normal healthy skin microbiome) such as *S. epidermitis,* which normally stimulate keratinocyte production and limit the invasion of pathogens, (boosting innate immunity), occasionally become pathogenic, being involved in biofilms production. *Staphylococcus epidermitis* activate probiotic microorganisms due to secretion of polyethylene glycol dimethacrylate, with the effect of inhibiting the growth of *S. aureus* MRSA. The introduction of *Lactobacillus reuteri* into the intestinal microbiome (through drinking water) accelerates wound healing twice as fast due to the up-regulation of oxytocin (Johnson et al, 2018). *Propionbacterium acnes* usually produce protecting bacteriocins for other pathogens in sebaceous ducts and are responsible for inducing TLR2 and TLR4 expression in keratinocytes. Occasionally *P. acnes* can become pathogenic, with large populations being associated with the development of acne (Johnson et al, 2018). Moreover, the short-chain fatty acids produced by common skin microorganisms have antimicrobial properties; as a result, the incorporation of these metabolites into the skin treatment suppresses inflammations. Commensal microorganisms protect the epithelial surface against pathogens through competition, the production of antimicrobial peptides (AMP), and proteases. Proteases have a role in cell renewal processes, the production of biofilms, and QS (quorum sensing or quorum signaling). Quorum signaling represents the ability to detect and respond to the density of the microbial population through the secretion of signaling molecules called autoinductors (Bassler, 1999; Boxberger et al., 2021). Self-inducers can be self-inducing peptides for gram-positive microorganisms and N-acyl homoserine lactones (AHL) for gram-negative microorganisms. Normally the skin microbiota interacts with the host's immune cells (T cells) which are trained to respond to pathogenic microorganisms that can colonize the epithelium. Tests performed on lab animals have shown that the expression of 280 genes is modulated in response to increasing microbial populations. Many of the modulated genes have a role in the secretion of proinflammatory cytokines, signaling and localization of the T cells. Many of the commensal microorganisms produce metabolic compounds that have antimicrobial or antitumor effects. Microorganisms from the genus *Malassezia* produce indoles that inhibit yeasts and fungi; *S. epidermitis* is able of producing 6-N-hydroxyaminopurine, a compound that can provide protection against skin cancer (Bassler, 1999; Boxberger et al., 2021). Bioproducts obtained from *S. epidermitis* contain coagulase-negative inducing peptides that inhibit proteases and α-modulin phenol-soluble, secreted by *S. aureus,* which lead to proteolysis of the epidermis and cause skin damage (Williams et al., 2019). A self-inductor peptide (SYNVCGGYF) that inhibits *S. aureus* activity (Williams et al., 2019) has been identified in an isolate from *S. hominis*. These are just a few examples of QS that show how the commensal microbiome of human skin contributes to the homeostasis of the epithelial barrier, inhibiting the production of toxins by *S. aureus.*

S. epidermidis is resistant to current antibacterial reagents such as ciprofloxacin, ofloxacin, fusidic acid, and vancomycin. Infections caused by this microorganism are complicated further by its ability to form biofilms (Gavahian et al., 2012; Moradalizadeh et al., 2013)**.** In their

research, Dănilă and collab. have found that the synergistic role of the EOs mixture in inhibiting *S. epidermidis* biofilms is due to D-limonene (from *C. lemon* EO) and linalool (Dănilă et al., 2018). This study indicates that the mixtures of essential oils containing linalool and D-limonene could be suitable alternatives to antimicrobial reagents, for treating *S. epidermidis* or preventing *S. epidermidis* growth on viable tissues or medical products (Sun et al., 2018). Additionally, *C. lemon* has been shown to possess antibacterial properties against *Streptococcus sobrinus*, by inhibition of glucosyltransferase activity (Liu et al., 2020).

Antioxidant activity

Phenolic compounds, such as limonene, demonstrated antioxidant activity due to their ability of scavenger for the free radicals. Many studies established that limonene was able of ameliorating the effects of oxidative stress *in vitro* and *in vivo*. Limonene exhibit antioxidant activity in diabetic rats, by reducing the oxidative stress in lab animals that received Limonene in food for 45 days (Roberto et al., 2010; Murali et al., 2013; Bai et al., 2016). Similar findings were found *in vitro*, on the BALB/c cells line. As well, Limonene inhibits the expression of apoptotic proteins such as Bax and Bcl-2 which facilitates the apoptotic response to oxygen peroxide (Ahmad & Beg, 2013; Bacanli et al., 2015).

Anti-inflammatory effect

Citrus myrtifolia also known as chinotto is widely distributed as ornamental plant and is probably a mutation of sour orange (*C. aurantium*) (Liu et al., 2012)**.** The primary constituents of chinotto essential oil are limonene, linalool, linalyl acetate, and α-terpinene, which have a significant scavenging activity against DPPH and ABTS (Plastina et al., 2018)**.** Phytochemical studies revealed the presence of geranial, limonene, γ -terpinene, and others in the EOs of *C. Limon*, *C. aurantifolia*, and *C. limonia*. These bioproducts have anti-inflammatory activity, inhibits cell migration and cytokine formation caused by carrageenan. These results were also observed when equivalent concentrations of pure limonene were used. Additionally, *C. aurantifolia* was found to cause myelotoxicity in mice (Amorim et al., 2016)**.** The anti-inflammatory action of *C. lemon* and C. *limonia* (mandarin lime) EO is most likely due to their high limonene content, while the myelotoxicity of *C. aurantifolia* EO is most likely due to its high citral content (Amorim et al., 2016). In this regard, caution should be exercised when working with *C. aurantifolia* EO due to its toxicity. These findings suggest that the essential oils of *C. limon*, *C. aurantifolia*, and *C. limonia* have an important anti-inflammatory effect. Limonene do not have cytotoxicity *in vitro* on neutrophils isolated from the peritoneal cavity of BALB/c mice. When leukotriene B4 was used as a chemoattractant, limonene inhibited neutrophil migrating. *In vivo* assays demonstrated that oral pre-treatment with limonene decreased leukocyte infiltration and the level of TNF-α, whereas the IL-10 levels were unaffected. Limonene inhibited doxorubicin's cytotoxic effect in the kidneys if the food of lab animals was supplemented with Limonene (Kummer et al., 2013; Rehman et al., 2014). Osteoporosis is a common disease characterized by inflammation of the joints and cartilage deterioration. Rufino and collab. have demonstrated in their experiments that limonene exerts an anti-inflammatory effect on human chondrocytes (Rufino et al., 2015)**.**

Antitumor activity

Limonene has been shown to have anti-proliferative, apoptotic, and anti-carcinogenic properties. Chaudhary and collab. were examined the effects of limonene on the growth of skin tumors triggered by 7,12-dimethylbenz (a) anthracene (DMBA) and 12-O-tetradecanoylphorbol-13-acetate (TPA). They discovered that applying limonene to the mouse skin reduced significantly the effect induced by TPA (respectively edema, hyperplasia, cyclooxygenase-2 expression). Additionally, the treatment with limonene repaired the reduced glutathione, glutathione peroxidase, glutathione reductase, glutathione S-transferase, catalase, and malondialdehyde activity on the lab animals treated with TPA. The presence of limonene decreases significantly the tumors growth and incidence in skin oncogenesis as compared to DMBA/TPA-treated mice. Additionally, therapy with limonene increased the latency time for tumor growth from four to nine weeks. Limonene

therapy decreased the level of protein kinases as Ras and Raf in DMBA/TPA-induced tumors. Additionally, was observed and an increase of Bax expression in cancerous tissue of mice treated with limonene (Chaudhary et al., 2011).

Antiviral activity of limonene

Limonene is able to suppress viral replication of HSV-1 (Astani & Schnitzler, 2014), inhibit avian influenza (H5N1) (Nagy et al., 2018), and yellow fever virus replication (Gómez et al., 2013), being widely used in dermato-cosmetic formulations (Nazaroff & Weschler, 2004). Due to its savor and fragrance, limonene is generally used in foods, drugs, drinks, and industry (Sun, 2007; Bora et al., 2020)

LIMONENE TOXICOLOGICAL EVALUATION

Limonene is considered a non-toxic natural substance based on the level of lethal dose (LD50) and recurrent toxicity studies.

To assess the limonene safety, toxicological tests were performed *in vitro* and *in vivo*. When is ingested orally, limonene is spread to the liver, kidney, and bloodstream. Dermal exposure to high concentrations of limonene causes skin allergic reactions. This compound possesses irritant properties that differ according to its shape (D or L) and degree of oxidation. Limonene that has been oxidized was found to have the greater sensitizing ability. The oxidation products of limonene include limonene hydroperoxides, R carvone, limonene oxide, perillic acid, and limonene-1,8-diol (Kim et al., 2008)**.** The use of d-limonene in cosmetics is limited in Korea and the European Union (EU), where the peroxide content of limonene must be less than 20 mmol/L. Additionally, the EU Cosmetics Directive 76/768/EEC requires that limonene be listed as an ingredient if its concentration is greater than 0.001 % in "leave-on" products and 0.01 % in "rinse-off" products (Kim et al., 2013).

Table 2. Biological activities of Limonene

Activity	Type of model	Lab experimental models	References
Anti-oxidant properties	*in vitro* *in vivo*	Diabetes of lab animals induced by streptozotocin. Lymphocytes from BALB/c mice	Roberto et al., 2010; Murali et al., 2013; Ahmad & Beg, 2013; Bai et al., 2016
Anticancer properties		Neuroblastoma cell lines (SH-SY5Y); Cells lines initiated of biologic materials of from patients with cancer; Gastric carcinoma cell lines (MGC803); Colon cancer cells line (LS174T) Doxorubicin administrated lab animals	Russo et al., 2013 Miller et al., 2015 Zhang et al., 2014 Jia et al., 2013
Anti-inflammatory properties		BALB/c mice neutrophils; Zymosan-induced peritonitis Chondrocytes stimulated with IL-1β	Kummer et al., 2013 Rehman et al., 2014 Rufino et al., 2015
Antimicrobial properties	*in vitro* *in vivo*	The antimicrobial and antibiofilm efficacy of citrus EO, against acne-related pathogenic bacteria	Yi et al., 2018 Dănilă et al., 2018 Li et al., 2020
Wound healing properties	*in vitro* *in vivo*	Dermatitis Skin damage	A d'Alessio et al., 2014

Additionally, the EU Cosmetics Directive 76/768/EEC requires that limonene be listed as an ingredient if its concentration is greater than 0.001% in "leave-on" products and 0.01% in "rinse-off" products (Kim et al., 2013). The studies of Acute oral toxicity performed by Adams and collab. on Limonene indicated that the LD50 for this compound ranged between 4400 and 6600 mg/kg for rats and mice, while the chronic toxicity was 75 and 300 mg/kg in male and female rats. The chronic toxicity in male and female mice was 250 and 500 mg/kg respectively when Limonene was administrated through oral gavage (Adams et al., 2011). Limonene is registered as a commonly accepted safe (GRAS) material for synthetic flavours under the Code of Federal Regulations (CFR) (Kim et al., 2013). A recent study examined the amount of limonene used in cosmetic products, as well as its cumulative daily exposure to various human bodies. The study findings indicated that (69-95)% are due to the 280

fragrance and antiperspirants products which containing limonene. Additionally, the cumulative exposure to limonene was found to be highest in the face and neck area (Dornic et al., 2018). Studies *in vitro* performed by Rolseth and collab, which have compared the toxicity of limonene and limonene 1,2-epoxide on human lung fibroblasts, concluded that the Limonene is toxic. Additionally, they found that at limonene concentrations of 200-300 μM, the total growth of cells was inhibited. In this study the cell death occurred at concentrations of 350 μM limonene. Regarding mitochondrial activity, the limonene toxicity was significant at levels of 300-500 μM. Whole inhibition of mitochondrial activity was observed at concentrations of limonene above 500 μM (Rolseth et al., 2002). Anderson and collab. have evaluated the toxicity of limonene in the presence of ozone on differentiated epithelial tissue (MucilAirTM) and on epithelial cell line (A549). They discovered that the limonene inhibited cellular proliferation and altered the synthesis of pro-inflammatory cytokines (Anderson et al., 2013). Regarding the genotoxicity of limonene, Fernandez-Bedmar and collab. have investigated the genotoxicity, anti-genotoxicity, and cytotoxicity of citrus juices (lemon juice and orange juice) and their bioactive components (hesperidin and limonene) on *Drosophila melanogaster*. Their experiments revealed that all compounds were non-mutagenic at low concentrations and contributed to the improvement of life expectancy. Additionally, they reported that the limonene and hesperidin have anti-genotoxic properties, inhibiting the hydrogen peroxide H_2O_2 (Fernández-Bedmar et al., 2011).

Hepatotoxicity of limonene

Based on the hepatotoxicity of limonene, the liver was established as a target. Following oral administration of limonene, several researchers have reported the possibility of liver toxicity, after its metabolization. Ramos and collab. have investigated the possible hepatotoxic impact of limonene on Wistar rats. The animals have received between 25-75 mg limonene/kg for 30-45 days. During experiment, biochemical levels of serum alkaline phosphate (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were determined. The scientists reported that the rats treated with 75 mg limonene/kg/day for 45 days had significantly lower ALT levels in comparison with rats from control group (Ramos et al., 2015).

Neurotoxicity of Limonene

Animal studies have revealed the effects of limonene exposure on the central nervous system (CNS), but is still unclear if these findings indicate general toxicity. In studies of acute toxicity made on male mice, limonene epoxide was found to have analgesic and anxiolytic-like effects, (de Almeida et al., 2012). In male Wistar rats subjected to physical stress, the L-limonene has exerted antistress properties by inhibition of the $GABA_A$ receptors (GABA = γ aminobutyric acid) respectively by inhibiting the basal hypothalamic-pituitary-adrenal function (HPA) (Zhou et al., 2009). According to studies performed by Yun, limonene has sedative effects on rats and mice, by modulating dopamine release and serotonin neuronal activity. Additionally, limonene was shown to inhibit methamphetamine (METH)-induced hyperlocomotion (Yun, 2014).

CONCLUSIONS

Studies reported in the scientific literature show that the products which contain Limonene as an active compound (alone or as essential oils from different *Citrus* species) can accelerate wound healing, promote angiogenesis and decrease inflammation via suppressing pro-inflammatory cytokines production. Preclinical studies and studies performed in vitro reveal the ability of this compound to inhibit melanoma. This biomolecule exhibit antimicrobial activities on pathogenic microorganisms implied in skin disorder like *S. aureus* or *P. aeruginosa*. In combination with synthetic antibiotic reagents, Limonene increases the effectiveness of the antimicrobial activity. Studies performed on healthy humans, with cleaning skin products that contain Limonene (alone or as essential oils with Limonene), reveal that this compound does not affect the commensal skin microbiota. In the case in which the cleaning skin formulations contain probiotic and /or plant extract, then the population of skin commensal microbiota increases. Due to their biological effects, the

essential oils with Limonene represent the potential bioproducts which can be used in regenerative medicine. The new research can be initiated with these bioproducts, alone or in association with other drugs, to find new solutions for treating infections with microorganisms with antibiotic resistance or in association with a chemotherapeutic reagent, to suppress the tumor resistence.

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